TOPOISOMERASE I INHIBITORS: CLINICAL STUDIES ON ORAL ADMINISTRATION AND/OR COMBINATIONS WITH CISPLATIN

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TOPOISOMERASE I REMMERS: COMBINATIES MET CISPLATIN EN/OF ORALE TOEDIENING

PROEFSCHRIFT

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de Erasmus Universiteit Rotterdam
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῾Απανθ᾽ ὁ μακρὸς κἀναρίθμητος χρόνος φύει τ᾽ ἄδηλα καὶ φανέντα κρύπτεται

De tijd -eindeloos en niet te tellen- brengt al wat onzichtbaar was aan het licht en doet het vervolgens weer in vergetelheid verzinken

Sophocles, *Aiax 646-647*

aan mijn ouders

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CONTENTS

Chapter 1

Introduction to the thesis

The topoisomerases were discovered in 1971, but it was not until the 1980s that the significance of these enzymes as potential therapeutic targets was appreciated [1]. Topoisomerase I plays a crucial role in the normal replication of DNA. In its physiological state in the chromosome, the DNA helix is supercoiled. Replication requires transient relaxation and unwinding of the parent DNA. In order to achieve this, transient cleavage of the DNA is required mediated by the formation of a cleavable-complex consisting of a covalent intermediate between topoisomerase I and DNA, allowing passage of the intact strand. The enzyme-bridged breaks are resealed afterwards. Topoisomerase I inhibitors stabilize the cleavable complex. thereby inhibiting the religation step [2-4]. This results in collision of the replication fork and, finally, in double strand breaks and cell death [5].

In the early 1970s, the parent compound camptothecin, an extract from the *Camptotheca acuminata*, an oriental tree, entered clinical studies [6-8]. Although some antitumor activity was observed, severe and unpredictable toxicities prevented further clinical development. It was not until the discovery of the mechanism of action of camptothecin, that numerous semi-synthetic camptothecin analogues were developed with a more predictable toxicity profile and better water-solubility and entered clinical trials. The first two of these, irinotecan and topotecan, were recently registered for different indications.

Preclinical studies with different topoisomerase I inhibitors showed more antitumor efficacy with prolonged low dose exposure to the drugs, and in animal models, low dose exposure resulted in less toxicity [9-19]. These preclinical findings were the stimulus for early clinical studies with low dose continuous infusion of topoisomerase I inhibitors in patients with advanced solid tumors [20-26]. However, the use of prolonged continuous infusion schedules is associated with the complications of the use of central venous catheters and the cost of administration is high. Aside from economic considerations, patients with advanced malignancies, when asked, preferred oral administration of cytotoxic drugs over the intravenous formulation, provided that no significant reductions in efficacy or duration of response would result from this mode of treatment [27]. The reasons for patients' preferences included convenience, current concern or previous difficulties with intravenous access lines, or preference to control the chemotherapy administration environment. An oral formulation would also provide a more convenient method for prolonged drug administration.

Considering their mode of action, topoisomerase I inhibitors may also interfere in processes involved in DNA repair [28-30].This renders them attractive for further

investigations in combination with other cytostatic agents, especially DNA-damaging agents. Preclinical studies have revealed synergism between topoisomerase I inhibitors and platinum-derivatives, topoisomerase II inhibitors and taxanes in a number of different human cancer cell lines and xenografts [31-45].
This thesis includes clinical and pharmacological studies on the oral administration of the novel topoisomerase I inhibitor 9-amino-20(S)-camptothecin and studies on the combination of cisplatin with the topoisomerase I inhibitors irinotecan and oral topotecan.

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Chapter 2

Topoisomerase I inhibitors: the relevance of prolonged exposure for present clinical development.

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SUMMARY

Topoisomerase I inhibitors constitute a new class of anticancer agents. Recently, Topotecan and irinotecan were registered for clinical use in ovarian cancer and colorectal cancer respectively. Cytotoxicity of topoisomerase I inhibitors is S-phase specific, and in vitro and in vivo studies have suggested that, for efficacy, prolonged exposure might be more important than short-term exposure to high concentration. Clinical development of those topoisomerase I inhibitors that have reached this stage is also focused on schedules aiming to achieve prolonged exposure. In this review, we summarize all published preclinical studies on this topic for topoisomerase I inhibitors in clinical development, namely 20-S-Camptothecin, 9-Nitro-Camptothecin, 9-Amino-Camptothecin, Topotecan, Irinotecan and GI147211. In addition, preliminary data on clinical studies concerning this topic are also reviewed. The data suggest that prolonged exposure may indeed be relevant for antitumor activity. However, the optimal schedule is yet to be determined. Finally, clinical data are yet too immature to draw definitive conclusions.

INTRODUCTION

Topoisomerase I-3' is a nuclear enzyme abundantly present in all eukaryotic cells [1]. Human topoisomerase I-3' is a monomeric 100 kDa polypeptide encoded by a single copy gene located on chromosome 20q12-13.2 [2]. Like all topoisomerases, topoisomerase I relaxes torsionally strained (supercoiled) duplex DNA. A tyrosine group of topoisomerase I becomes covalently bound to the 3'phosphate at the DNA break site (cleavable complex). To accomplish DNA relaxation, topoisomerase I introduces a single-strand nick in the phosphodiester backbone of the DNA, allows the intact strand to pass through the nick and then rejoins the nicked strand. DNA relaxation results from swiveling at this nick and plays an important role in DNA replication and RNA transcription. The enzyme-bridged breaks are then resealed by topoisomerase I (religation) [1,3-4].

Topoisomerase enzymes provide an essential function in solving topological problems encountered in DNA replication and DNA transcription. Topoisomerase may also be involved in recombinational processes and chromatin assembly, however their roles in these processes are less well defined [4].

As long ago as the 1970s camptothecin (CPT), an extract from the chinese tree *Camptotheca acuminata*, showed antitumor activity against several tumors. However,

In phase I and II studies, unpredictable severe toxicities occurred that led to the discontinuation of further development [5-9]. In the late 1980s studies revealed that camptothecin induced single strand DNA breaks in the presence of topoisomerase I, thus identifying this enzyme as a major target for the antitumor effect [10]. The cellular effects of camptothecin can be entirely attributed to its action on topoisomerase I as has been proven in genetic studies with yeast and mammalian cells [11-15]. Topoisomerase I cleavable complexes occur preferentially within expressed genes [4,16].

The lactone form of camptothecin (CPT) and all CPT-analogues appears to reversibly stabilise the cleavable complex, which results in single-strand DNA breaks and inhibition of religation in the presence of the drug. DNA synthesis is arrested in the presence of topoisomerase I inhibitors - religation does not occur, resulting in irreversible inhibition of DNA synthesis with double-strand DNA breaks. These events lead to the arrest of the cell cycle in the S/G_2 phase and ultimately cell death [17]. A S-phase specific cytotoxicity for topoisomerase I inhibitors has been observed, as S-phase cells are up to 1000-fold more sensitive than G_1 or G_2/M phase cells after brief exposure to the drug [18-20]. Analysis of the distribution of RNA polymerase molecules indicates that CPT-stabilized cleavable complexes block elongation by impeding the progression of the RNA polymerase molecules along the transcription unit [21]. Inhibition of RNA synthesis is rapidly reversible after removal of CPT from cultured cells, probably as a result of the dissociation of topoisomerase I cleavable complexes from transcription units. Thus, camptothecin demonstrates inhibition of DNA and RNA synthesis with fragmentation of nuclear DNA but, upon removal of the drug, nucleic acid synthesis inhibition and DNA fragmentation are reversible and, only at higher dose and longer exposure times, do these effects become irreversible [22-25]. Cytotoxicity of topoisomerase I drugs in the absence of detectable DNA synthesis has also been found in some cell lines such as human lymphocytes. The mechanism of this non-S-phase cytotoxicity is unknown but could be due to transcription inhibition [26]. Other effects of camptothecin analogues are induction of maturation in a variety of leukemia cell lines, expression of proto-oncogenes, and endo-nucleolytic DNA damage characteristic of apoptosis [27-33].

Topoisomerase I inhibitors were active both in slowly and rapidly proliferating tumors [34-35]. Sensitivity of tumor cells to these drugs is related to the topoisomerase I level, topoisomerase I catalytic activity and the interaction between topoisomerase I and its inhibitor, hence the importance of intracellular drug concentration.

Topoisomerase I is present at relatively high levels in both proliferating and quiescent cells, suggesting that its function may be independent of cellular growth rate. In proliferating cells, topoisomerase I mRNA levels are significantly higher than in quiescent cells. However topoisomerase I protein increased much less, which may be due to a shorter half-life of the protein in proliferating cells than in resting cells [36-37]. The catalytic activity of topoisomerase I also depends on the phosphorylation state of the enzyme, and phosphorylation has been shown to increase during mitogenic stimulation of quiescent cells [38].

The regulation of topoisomerase I is altered in neoplastic cells. Colon cancer cells, for example, contain a 5-16-fold higher level of topoisomerase I than normal colon mucosa cells [39-40].

Despite high levels of topoisomerase I some human tumor cell lines are nevertheless resistant to topoisomerase I inhibitors, which may be attributed to a low specific activity of this form of topoisomerase I [41-43]. The effect of topoisomerase I inhibitors on the enzyme can also be influenced by pointmutations or deletions within the topoisomerase I genes which affect protein or enzyme activity levels [44]. An absolute low level of topoisomerase I is another mechanism of resistance to topoisomerase I inhibitors [13,15,43,45].

In order to exert inhibitory effects, topoisomerase I inhibitors first have to enter tumor cells, while antitumor activity is only achieved with the lactone form of the compounds. This lactone form has a relatively short half-life and at physiologic pH, the hydroxyl moiety will predominate. Topoisomerase I inhibitors show readily reversible interaction with the target enzyme and do not form an intracellular reservoir [46-47]. Therefore exposure of only limited duration of tumor cells to the active lactone form of topoisomerase I inhibitors will be achieved in dose schedules with short-lasting infusions. Related to cell entry, Ma et al reported an ovarian cancer cell line which is resistant to topotecan and SN38 because of a decreased influx of the drug [48]. In a CPT-11 multidrug-resistant cell line, the cellular concentration of the drug appeared dependent on the plasma transmembrane potential [49].

P-glycoprotein overexpression does not influence the intra-cellular drug concentration of camptothecin, and many of its noncharged derivatives, MDR-1 overexpressing cells, are more resistant to the positively charged camptothecin derivative topotecan [18,50-51].

In vitro studies with topoisomerase I inhibitors suggest that cytotoxicity increases upon prolonged exposure to the drug. This review will further summarize the

preclinical and clinical studies of continuous or longterm exposure of topoisomerase I inhibitors in cancer research.

20-S-CAMPTOTHECIN

20-S-Camptothecin (20-S-CPT) has been identified as the active agent in the extract of the Camptotheca tree [5]. 20-S-CPT is water insoluble.
Stereochemistry and the positions of substituents have been found to be crucial in CPT and its analogues for the presence or absence of effects on topoisomerase I, indicating that the compounds interact with an asymmetrical receptor site on the enzyme or enzyme-DNA-complex [52]. The R-Camptothecin isomer has little or no effect on topoisomerase I, in contrast to the natural S-isomer which has a single asymmetrical carbon located at position 20.

Interaction with the receptor is influenced by configurational alterations causing little change in general chemical properties of topoisomerase I inhibitors but producing marked changes in topoisomerase I interaction [52].
The lactone form of the topoisomerase I inhibitor, which predominates at pH < 4.0, is the more potent inhibitor of the enzyme and therefore a much more potent antitumor agent than the inactive open ring compound.

Preclinical studies

In vivo studies

Antineoplastic and toxic effects in L1210 leukemia of intraperitoneal administration of the 20-S-CPT-sodium (20-S-CPT-Na^+) formulation were found to vary with the schedule of administration [53]). An intermittent schedule (day 1,5,9) of administration appeared superior on the resulting lifespan compared with any of the alternative treatment schedules studied, being a day 1-9 single daily dosing; dosing every 3 hours on day 1,5,9; every 3 hours on day 1 or dosing with a single dose on day 1 [54-55].

The sodium salt of 20-S-CPT is not the optimal formulation of administration. Prolonged administration of water-insoluble formulations of 20-S-CPT were recently studied in nude mice bearing human cancer xenografts. To test the efficacy of the lipophilic moiety 20-S-CPT was dispersed in intralipid 20% and injected intramuscularly (i.m.) at a dose of 0,1 mg/25 g body weight. The same formulation

was also administered orally and intravenously. Intravenous 20-S-CPT resulted in toxic deaths without inhibitory effects. Tested against 13 human cancer xenografts resistant to the most commonly available chemotherapeutic agents, 20-S-CPT given i.m. at a dose of 4 mg/kg twice weekly induced complete regression in the majority of the animals in 10 out of 13 xenografts. Only one melanoma and two colon cancers showed a poor response. Daily oral administration of 20-S-CPT at a dose of 4-8 mg/kg resulted in complete tumor regression in mice carrying SPA lung carcinoma. After 6 months of continuous treatment, regrowth was observed in 5 of the 7 xenografts, suggesting 20-S-CPT resistance under prolonged treatment [56]. 20-S-CPT given i.m. at a dose of 4 mg/kg twice daily also induced complete regression in BRO melanoma xenografts. In vitro cell proliferation of the same cell line was inhibited at a remarkably low concentration of 1 ng/ml, and it was demonstrated that a period of 20-24 h of drug exposure was required for complete growth inhibition [57]. In this model, 20-S-CPT i.m. (2 mg/kg) appeared to be the most effective mode of drug administration to induce tumor inhibition compared with i.v. or i.p. administration. 20-S-CPT at a dose of 2 mg/kg/day x2 intragastrically followed by one day of rest was more effective in inducing complete tumor inhibition than 1 mg/kg/day x5 (intragastrically) with two days rest [58].

Nude mice bearing intracranial human brain tumor xenografts were treated with intraperitoneal (i.p.) 20-S-CPT in different schedules. Single doses of CPT did not prolong survival, but CPT i.p. 2x/week for 6 weeks or daily oral 20-S-CPT induced 10 weeks survival in 40% or 60% of animals respectively [59]. In addition, 20-S-CPT administered intragastrically at an intermittent weekday schedule for 10 weeks was well tolerated and induced tumor responses in human cancer xenografts of malignant melanoma and colon carcinoma [60]. In order to bypass the insolubility of 20-S-CPT lactone, the compound can also be incorporated into a liposome-based delivery system for i.m administration. Release studies of liposomal-20-S-CPT show an initial rapid 50% loss of the drug in 4 hours, followed by a slow leakage of the remaining drug over a period of 20 hours. Complete tumor regression occurred after a single i.m. injection of this formulation at 10 mg/kg in nude mice xenografted with CLO breast carcinoma or BRO melanoma, with minimal host toxicity [61].

Lipid-complexed 20-S-CPT bypasses its insolubility and makes prolonged low dose exposure possible.

These preclinical studies suggest that intermittent intraperitoneal or more convenient daily oral administration of 20-S-CPT for a prolonged period is well tolerated and may have antitumor effects. Antitumor effects seem to be dose and

schedule dependent. The intramuscular or oral administration of camptothecin seem to enable protracted dose scheduling.

Clinical studies with camptothecin and prolonged exposure

Daily x5 i.v. administration

In the early 70s three phase I studies with intravenous administration of the sodium 20-S-camptothecin (20-S-CPT-Na^+) were performed in which 20-S-CPT-Na^+ (0.5-10 mg/kg) was administered as single i.v. bolus every 2-4 weeks. Myelosuppression with leucopenia and thrombocytopenia was the dose-limiting toxic effect. Diarrhea, reversible hemorrhagic cystitis and alopecia were observed at higher dose levels [6]. Muggia et al. studied i.v. 20-S-CPT-Na^+ at a once weekly and daily x5 schedule every 3 weeks. On the weekly schedule, dose-limiting toxicities were leuco- and thrombocytopenia, while hemorrhagic cystitis occurred in several patients who recieved multiple doses. Cumulative leuco- and thrombocytopenia were also dose-limiting with the daily x5 schedule, resulting in hemorrhagic cystitis in 3 of 17 patients [7]. Phase II trials with 20-S-CPT-Na^+ have been performed in patients with melanoma and advanced gastrointestinal carcinomas. Melanoma patients were treated with 20-S-CPT-Na^+ every 2 weeks [62]. Patients with gastrointestinal carcinoma were treated with either single dose 20-S-CPT-Na^+ (90-180 mg/m^2) every 3 weeks or a daily x5 schedule (11-55 mg/m^2/day) every 4 weeks [8]. Both treatment schedules showed equal toxicity. Because of severe and unpredictable myelo-suppression, hemorrhagic cystitis and diarrhea, the sodium salt formulation of camptothecin was then disregarded.

Prolonged exposure

However, results of the above mentioned preclinical studies recently renewed the interest in new formulations of camptothecin, and the drug is again undergoing phase I evaluation. 20-S-CPT in gelatin capsules administered orally once a day for 21 days followed by one week rest was studied in 52 patients. Doses were escalated from 0.3 - 15.4 mg/m^2/day [63]. DLT of 20-S-CPT over a three week period was diarrhea. Loose stools occasionally occurred in all patients at doses above 6.5 mg/m^2/day with a 32% incidence of persistent diarrhea. Anti-diarrheal medication generally solved this problem. The maximum tolerated dose was 8.7 mg/m^2/day. Chemical cystitis resulting in dysuria and occasional hematuria occurred in 20% of patients. It resolved within a week of drug discontinuation but sometimes reappeared with continued

administration. Only two extensively pretreated patients experienced severe hematologic toxicity, recovering within 10-14 days. In 12 patients, the oral administration of 20-S-CPT could be continued for 6-12 months, in 5 patients for more than 1 year. No long-term toxicities were reported. Partial responses occurred in two patients with breast cancer and two patients with melanoma, and one patient with non-Hodgkin lymphoma achieved a complete remission.

Thus it is possible to administer orally 20-S-CPT to patients with solid tumors for a long period of time without inducing long-term cumulative hematologic or non-hematologic toxicity. Presently 20-S-Camptothecin has entered a phase II study.

9-NITRO-CAMPTOTHECIN, 9-AMINO-CAMPTOTHECIN

9-nitro-camptothecin (9NC) is a semisynthetic derivative of the natural product camptothecin and is water-insoluble. 9NC is a precursor required for the synthesis of 9-amino-camptothecin (9AC) from CPT. 9NC is chemically more stable than 9AC, which is oxidized readily, generating toxic degradation products [58,64]. An additional finding is that 9NC is converted to 9AC by human cells of solid tissue of origin. Conversion of 9NC is less in hematopoetic cells. Cellular conversion of the lactone form of 9NC to 9AC is maximal in a slightly acidic environment (pH=6.0) [58,64]). Because of this relationship, results of preclinical and clinical studies of both compounds will be discussed under one heading.

Preclinical studies

In vivo studies

In vivo studies of 9NC and 9AC in the malignant melanoma BRO xenograft showed that, after 40 days of treatment with i.m. 9NC or 9AC at 4mg/kg twice a week, all engrafted mice were tumor free and did not experience significant toxicity. Growth inhibition of BRO cells in vitro occurred at a low 9NC concentration of 1 ng/ml and was complete after a period of 20-24 h of exposure [57].

Nude mice inoculated with three tumorigenic breast cancer cell lines developed complete tumor regression when treated with 9NC i.m. at a dose of 4 mg/kg twice a week [65]. No tumor regrowth nor toxicity occurred during prolonged 9NC administration.

Cell cultures of non-tumorigenic breast cancer cells (MDA-MB-134) and tumorigenic cells (MDA-MB-231) were exposed to 9NC. The non-tumorigenic cells accumulated in G_2/M without significant changes in S-fraction. Removal of 9NC from

the cultures of non-tumorigenic cells after 120 hours resulted in regrowth at a rate similar to untreated cells. In tumorigenic cells exposed to 9NC, there was a marked increase in cells containing a reduced DNA content and going into apoptosis. Removal of 9NC from the cultures of tumorigenic cells after 120 hours did not result in regrowth after 120 hours [66].

Experiments with 9NC and 9AC at an i.m. dose of 4 mg/kg twice weekly in various human breast cancer xenografts resulted in complete tumor regression but, regardless of 9NC continuation or discontinuation, tumorigenic MDA-MB-231 tumors regrew after a period of 50 days of complete tumor regression [67]. This indicates that drug resistance occurs.

Protracted i.v. administration of 9AC to mice innoculated with CLO human breast cancer cells was studied in various schedules. 9AC i.v. daily x3 every 21 days at dose levels of 0.75 and 1 mg/kg/day resulted in tumor regression, but, ultimately, with regrowth. This i.v. schedule had no inhibitory effect on tumor progression, unlike the i.m. 9AC 1mg/kg administration described earlier. A five day period of continuous 9AC administration followed by two days' rest was highly effective in tumor inhibition and regression even at a dose of 0.5 mg/kg/day. 9AC doses of 1 mg/kg/day or above were toxic for the animals. Intragastric administration of 9NC and 9AC was studied at different doses and schedules in mice with CLO xenografts. The optimal 9NC and 9AC dose and schedule was 1 mg/kg/day for 5 days followed by two days' rest. The authors conclude that, for practical reasons, oral administration is the route of choice for 9NC [67].

Intramuscular administration of 9NC 4mg/kg twice a week was efficacious in nude mice bearing human 2774 ovarian cancer [68]. Prolonged exposure of tumorigenic (2774) and non-tumorigenic (DUN) ovarian cancer cells in vitro to a concentration of 1 ng/ml of 9NC resulted in accumulation of non-tumorigenic cells in G_2/M and accumulation of tumorigenic cells containing reduced DNA content and going into apoptosis [69].

In a human melanoma xenograft model intramuscular administration gave the best antitumor effects of 9NC, 9AC and CPT. A dose schedule of 2 mg/kg/day x2 with one day rest compared to 1 mg/kg/day x5 with two days' rest was more efficacious for CPT and equally effective for 9NC [58].

Intragastric application of 9AC on a 5 day/week schedule for 3-6 weeks induced complete remission in subcutaneous human xenografts of malignant melanoma and non-small-cell lung carcinoma, and its efficacy was better than that of 20-S-camptothecin [70].

Two observations can be made on these preclinical studies: lower 9NC or 9AC concentrations applied for long periods of treatment are more effective in inducing apoptosis than higher concentrations for short periods. When 9NC initiates the process of apoptosis in tumorigenic cells, this is not reversible, even after removal of the drug. Non-tumorigenic cells are reversibly inhibited as long as drug exposure continues.

Route of administration and dose scheduling of 9NC and 9AC seem to be crucial for optimal antitumor responses. Prolonged or intermittent administration of a lower dose of these drugs is most efficacious.

Table 1. Topoisomerase-I-inhibitors continuous/prolonged administration in solid tumors. (9-AMINO-CAMPTOTHECIN)

Drug	Dose Schedule mg/m2	No. Pts	Cp-ss	MTD	DLT	Ref. Year
9-Amino-CPT i.v.	5-59 µg/m²/h 72h q 14d	48	0.9-10.6 nM	35 µg/m²/h	Neutro.	Dahut 1996
	47-74 µg/m² 72h q 14d + G-CSF			47 µg/m²/h	Neutro. Thrombo.	
9-Amino-CPT i.v.	-- 72h	19	--	--	Neutro.	Rubin 1994
9-Amino-CPT i.v.	36-62µg/m²/h 72h q 14d	18	2.23 ng/ml.	not (yet) reached	myelosupp.	Langevin 1996
9-Amino-CPT i.v.	6.2-9.4 µg/m²/h 21d q 28d	19	--	> 9.4 µg/m² /h	not yet reached.	Hochster 1996
9-Amino-CPT i.v.	17-25µg/m²/h 120h/wkx3 q 4wk	20	2.9 ± 1.6 (17 µg/m²/h)	not yet reached.	not yet reached.	Takimoto 1996

i.v.: intravenous; i.p.: intraperitoneal; p.o: oral; MTD: Maximum Tolerated Dose; DLT: dose limiting toxicity; Cpss:plasma-concentration steady state; *: chemotherapy-pretreated; **: non-pretreated; --: not stated; Neutro: neutropenia; Thrombo: thrombopenia

Clinical studies with prolonged or continuous exposure

72-hour infusion

Phase I studies of 9-amino-camptothecin in adult patients with solid tumors have been performed initially with continuous i.v. infusion over 72 hours [Table 1]. Leukopenia appeared to be the dose-limiting toxicity, together with modest thrombocytopenia. Nausea and vomiting, alopecia, stomatitis and diarrhea were less frequently reported [70-72]. Steady-state plasma concentrations increased linearly with the dose and ranged from 0.9-10.6 nM and correlated well with % decrease of granulocyte count [74]. In a similar phase I study in children, side-effects were similar, but the MTD in children exceeded that in adults [73].

Prolonged exposure

Phase I studies with longer infusion durations of 9-AC in adults are ongoing. A continuous i.v. infusion for 120 h weekly for 3 out of every 4 weeks is feasible, with DLT not yet being reached at the dose level of 20 $\mu g/m^2/h$. The resulting dose intensity is already higher than the dose intensity of the recommended phase II dose of 35 $\mu g/m^2/h$ over 72 h when given every 2 weeks [74].

The same holds for continuous infusion of 9AC for 21 consecutive days every 28 days [75]. The latter phase I studies suggest that with prolonged infusion a higher dose intensity of 9AC can be achieved. A phase I study with oral 9-NC given for 5 consecutive days every week revealed hematologic toxicity as being dose limiting. Non-hematologic toxicity was substantial with nausea/vomiting, diarrhea and hemorrhagic cystitis. An interesting level of antitumor activity was reported [76].

Further studies on prolonged dosing of oral 9NC and i.v. 9AC are presently ongoing.

In summary, dose intensities are higher for 9AC when administered with longer infusion duration. Oral administration of 9NC for 5 consecutive days gives substantial non-hematologic toxicity.

TOPOTECAN

Topotecan (TPT: 9-dimethylaminomethyl-10-hydroxycamptothecin) is a water-soluble potent camptothecin analogue with activity against various tumor types in *in vitro* and *in vivo* studies.

Preclinical studies

In vitro studies

In vitro effects of topotecan against cells from biopsy specimens of colorectal, breast, lung, ovarian, renal, gastric cancer and cancers of unknown primary origin were studied with 1 hour and with continuous exposure in a human tumor clonogenic assay. With 1-hour TPT exposure in vitro responses were seen in 10% and 25% of assessable tumor specimens at TPT concentrations of 1.0 and 10.0 ug/ml respectively. Response rates were 34% and 76% at concentrations of 0.1 and 1.0 ug/ml TPT with continuous exposure [77], suggesting that TPT was more active with long-term incubation. Continuous exposure of TPT in vitro showed an initial decrease of the active lactone form of TPT, followed by a stable ratio up to 72 hours, which corresponded to 19% of the initial value. The fraction of the lactone form during 1 hour exposure is not known, but nevertheless it is very likely that the concentration-time product (dose-intensity) is greater for continuous exposure than for 1 hour [77]. This implies that the time period of exposure to topotecan is an even greater determinant of cytotoxicity than anticipated.

In vivo studies

Different TPT schedules were studied in female CBA/CaJ immune-deprived mice engrafted with 7 colon carcinoma cell lines, 6 juvenile rhabdomyosarcomas and 3 osteosarcoma cell lines [78]. Initially, TPT was administered intraperitoneally (i.p.) using a schedule of 4 doses of TPT every 4 days (q4dx4 schedule). The maximum tolerated dose (MTD) with this schedule was 12.5 mg/kg per administration, and TPT caused significant regression in 4 of 5 rhabdomyosarcoma xenografts. Subsequently, the effect of TPT was studied as a daily x5 dose given for 3 consecutive weeks by oral gavage (2 mg/kg per administration) or daily x5 for 3 weeks intraperitoneally. Intraperitoneal administration was at least as efficacious as oral dosing but more toxic [78]. Intraperitoneal TPT 2 mg/kg per dose was lethal in > 15% of the mice, the MTD with intraperitoneal administration was 1.5-1.75 mg/kg/dose. The effect of prolonged topotecan administration was studied in two moderately responsive xenografts, Rh 12 rhabdomyosarcoma and VRC_5 colon adenocarcinoma. Mice bearing Rh 12 rhabdomyosarcoma xenografts were treated with TPT 2.0 or 1.75 mg/kg/dose/day x5 for three courses or a lower dose (1.25 mg/kg/dose) for up to twenty courses. The prolonged low dose regimen resulted in complete remission of all tumors without regrowth. The same effect was seen at an even lower dose level of

1.0 mg/kg/dose, also without significant toxicity. Mice with VCR_5 colon adenocarcinoma showed significant tumor reduction with prolonged oral administration of TPT at a dose of 1.0 mg/kg/dose x5 for 20 cycles. However, re-growth occurred after 16 weeks.

Additional studies with prolonged exposure schedules in mice bearing xenografts of colon adenocarcinoma, rhabdomyosarcoma and brain tumors showed less toxicity and better antitumor activity than dose-intensive short exposure schedules [79]. These in vivo studies show that oral administration is as efficacious as parental application, although the AUC is lower with oral administration. Furthermore prolonged intraperitoneal and oral (p.o.) TPT administration resulted in responses of xenografts not responsive to a short term parental intermittent high-dose schedule [78-79].

From these preclinical data, prolonged exposure to topotecan seems a treatment schedule with potential higher benefit with regard to antitumor activity.

Clinical studies with prolonged or continuous exposure

Daily x5 i.v. administration

Phase I studies with single i.v. bolus daily for 5 days repeated every 3-4 weeks, show a MTD of 1.5-2.5 mg/m2/day. The dose-limiting toxicity was myelosuppression, in particular neutropenia [80-82]. Non-hematologic toxicities were usually mild and reversible and consisted of nausea, vomiting, fatigue, alopecia, and sometimes diarrhea.

Phase II studies with this daily x5 TPT regimen every 21 days showed promising response rates in patients with small cell lung cancer (10-39%) and in pretreated patients with ovarian cancer, with response rates ranging from 9.5-25 % [83-89]. Other solid tumors, such as melanoma, colon carcinoma, head and neck cancer, renal cell carcinoma, cervix and prostate carcinoma, appear to be much less sensitive to this regimen [90-99]. In these phase II studies CTC grade III-IV neutropenia (32-81%) was reported as being the major toxicity. Thrombocytopenia CTC grade III-IV is infrequent. Anemia greater than CTC grade II was reported in 27-60%.

Table 2. Topoisomerase-I-inhibitors continuous/prolonged administration in solid tumors. (TOPOTECAN).

Drug	Dose schedule mg/m^2	No. Pts	Cp-ss.	MTD	DLT	Reference, year
Topotecan i.v.	2.5-5.0 24h q 3wk	15	--	4 mg/m^2/24h * 5 mg/m^2/24h **	Neutro. Thrombo.	Recondo, 1991
Topotecan i.v.	2.5-5.0 24h q 3wk	10	4-10 ng/ml	--	Neutro.	Reid, 1992
Topotecan i.v.	2.5-10.5 24h q 3wk	22	20 ng/ml	8.4 mg/m^2/24h	Neutro.	ten Bokkel Huinink, 1992
Topotecan i.p.	3-4 24h q 4wk	12	--	4 mg/m^2/24h	Neutro.	Plaxe, 1993
Topotecan i.v.	-/72h q 1wk	12	--	2 mg/m^2/72h	Neutro.	Sabiers, 1993
	-/72h q 2wk	7	--	2.6mg/m^2/72h	Neutro.	
Topotecan i.v. + G-CSF	10-15 24h q 3wk	13	--	4 mg/m^2 * 10 mg/m^2 **	Neutro. (+G-CSF: Thrombo.)	Abbruzzese, 1993
Topotecan i.v.	2.0-7.5 24h q 3wk	29	18.2 nM ± 3.7 nM	7.5 mg/m^2	Neutro. Thrombo.	Blaney, 1993
Topotecan i.v.	1.0-2.0 24h q 1wk	32	4.7-11.4 nM	1.75 mg/m^2/24h	Neutro.	Haas, 1994
Topotecan i.v.	0.75-1.9/day 72h q 3wk	27	3.1 ± 1.4 ng/ml	1.0 mg/m^2/d 1.3 mg/m^2	Neutro.	Pratt, 1994
Topotecan i.v.	0.17-0.68/day 120h q 3wk	14	5.5 ng/ml	0.68 mg/m^2/day	Thrombo.	Burris, 1994
	0.68-1.6/day 72h q 3wk	32	2.0 ng/ml	1.6 mg/m^2/day	Neutro.	
Topotecan i.v.	0.2-0.7 21d q 28d	44	--	0.53 mg/m^2/day	Thrombo+ Neutro.	Hochster, 1994
Topotecan i.v.	0.6 /day 21d	9	--	--	Neutro/ Thrombo.	Khater, 1995
Topotecan i.v.	0.4/day 21d q 28d	16	--	-- (phase II)	--	Hochster, 1996
Topotecan p.o.	0.8-1.1/day 21d q 28d	12 pediatric	--	0.8 mg/m^2/day	Thrombo.	Bowman, 1996

i.v.: intravenous; i.p.: intraperitoneal; p.o: oral; MTD: Maximum Tolerated Dose; DLT: dose limiting toxicity; Cpss: plasma-concentration steady state; *: chemotherapy-pretreated; **: non-pretreated; --: not stated; Neutro: neutropenia; Thrombo: thrombopenia.

Prolonged exposure

Continuous infusion of topotecan has been studied in various schedules: a 24 hour infusion weekly and every 3 weeks; a 72 hour infusion administered weekly, every 14 days and every 21 days; a 120 hour infusion every 3-4 weeks; a 21 day continuous infusion administered every 28 days. [Table 2].

In one study, TPT was administered intraperitoneally for 24 hours every 4 weeks [100]. Studies with continuous infusion of topotecan of 72 hours or more show mild non-hematologic toxicities (nausea, vomiting, alopecia). Dose-limiting toxicity is always leucocytopenia, more often with associated thrombocytopenia than with the daily x 5 i.v. bolus. Anemia requiring blood transfusions and thrombocytopenia with platelet transfusions are particular problems related to these schedules. In phase II studies in pediatric patients and adults with acute leukemia continuous infusion of TPT for 120 hours resulted in severe mucositis as DLT [101-102].

In a phase I study with continuous intravenous topotecan administration for 21 days every 28 days in 44 patients with solid tumors, the MTD was 0.53 mg/m^2/day, with myelosuppression as DLT [75]. The steady-state lactone TPT concentration was low, approximately 4 ng/ml. No consistent relationship was found between drug level and hematological toxicity. Partial tumor responses were noted in 2 patients with ovarian cancer, 1 patient with breast cancer, 1 patient with renal cell cancer and 1 patient with NSCLC [103]. Blood transfusions and platelet transfusions were necessary in 45% and 11% of patients respectively. The authors concluded that a 21-day infusion of TPT is generally well tolerated with minimal non-hematological toxicity. In a phase II study with this regimen in patients with progressive ovarian cancer after platinum containing chemotherapy, response rate was 37% and neutropenia was the major toxicity (31%). Blood transfusions needed to be given to 50% of patients [104]. Further phase II studies with the 21-day continuous infusion of TPT are ongoing.

The bioavailability of oral TPT varies from 32%-44% with relatively limited intrapatient variation [105-106]. Oral TPT was studied in paediatric patients with solid tumors in a phase I study with 2 different dose schedules. In one dose schedule TPT was administered orally every day for 21 days out of every 28 days. In the second schedule, oral TPT was given 5 days on and 2 days off for 15 total doses. In the 21-day schedule oral bioavailability was 46% ± 22% at 0.8 mg/m^2 and 34% ± 14% at dose level 1.1 mg/m^2. DLT of both schedules is thrombocytopenia, and myelosuppression is well correlated with systemic exposure to oral TPT [107]. Thus, in vitro studies show that time period of exposure to topotecan is an important

determinant of cytotoxicity. In vivo studies with human xenografts with prolonged administration of topotecan show better antitumor activity. In patients with solid tumors, continuous infusion of TPT is well tolerated, and tumor responses are being reported. Phase I studies with an oral formulation TPT in adult patients with solid tumors are ongoing.

IRINOTECAN (CPT-11)

CPT-11 (7-ethyl-10 [4-(piperidino)-1-piperidino]carboxyloxy-camptothecin) is a water-soluble analogue of camptothecin. CPT-11 has little inherent antitumor activity in vitro, but it is converted to SN-38, a metabolite that is 1000-fold more potent than the parent compound in vitro [108-109].

Preclinical studies

In vivo studies

CPT-11 has been studied in human tumor xenografts with chemorefractory colon carcinoma, chemoresponsive rhabdomyosarcoma, and sublines of rhabdomyosarcoma with in vivo resistance to vincristine, melphalan and topotecan, as well as with 3 paediatric brain tumors [79,110]. As a single i.v. administration at the MTD (50 mg/kg), CPT-11 had no inhibitory effect on any coloncarcinoma xenograft; however when administered for one cycle i.v. at a dose of 10-40 mg/kg/dose/daily x5 for 2 consecutive weeks it demonstrated significant activity against 5 of 8 colon carcinoma models, rhabdomyosarcomas and 2 xenografts (Rh18 rhabdomyosarcoma and VRC_5 colon adenocarcinoma), resistant in vivo to topotecan, were also highly responsive to this schedule [110]. To determine whether prolonged periods of treatment were more effective CPT-11 was administered as before as a daily x5 schedule for two weeks, but the cycles were repeated every 21 days for a total of 3 cycles. The MTD was 10 mg/kg/day. Complete regression of all VRC_5 colon tumors was achieved at 5-10 mg/kg/dose. CPT-11, given as a protracted schedule at 5 mg/kg/day, showed greater activity than a shorter intense therapy at 40 mg/kg/dose.

A single cycle of CPT-11 was only modestly active at a dose of 40 mg/kg in 4 of 25 Rh12 rhabdomyosarcoma xenografts whereas 3 cycles of therapy at 10 mg/kg/day, daily x5, resulted in complete regression of 12 of 13 tumors. Similar results were obtained in colon carcinoma and human brain tumor xenografts [110].

Thus, protracted therapy with low-dose CPT-11 had increased therapeutic efficacy as compared with more toxic short-term schedules.

Clinical studies with prolonged or continuous administration

In a phase I study with CPT-11 given as a 5-day continuous infusion every 3 weeks, the dose was escalated from 5-40 mg/m^2/day [111] [Table 3]. Dose-limiting toxicity consisted of CTC grade III-IV diarrhea. Toxic effects greater than CTC grade II included diarrhea (69%), nausea and vomiting (58%), leukopenia (25%), anemia (25%), thrombocytopenia (6%) and hepatic dysfunction (14%). Diarrhea was dose dependent, in contrast to the white blood cell nadir which was not dose dependent [111]. In another phase I study, CPT-11 was administered intravenously over 30 minutes for 3 consecutive days every three weeks. Both leucopenia and diarrhea were dose limiting at a dose of 115 mg/m^2/day [112]. In limited studies with low dose schedules of CPT-11 once daily x 3, once daily x 5 and twice daily x 7, antitumor responses were reported in patients with leukemia and lymphomas [113-114].

Table 3. Topoisomerase-I-inhibitors continuous/prolonged administration in solid tumors. (CPT-11).

Drug	Dose Schedule mg/m^2	No. Pts	Cp-ss	MTD	DLT	Ref. Year
CPT-11 i.v.	125-225 every other wk	20	--	≥ 200 mg/m^2	Not yet Reached	Rothenberg 1996
CPT-11 i.v. bolus	33-115/day 3d q 3wk	46	2034 ng/ml	115 mg/m^2	Neutro + Diarrhea	Catimel 1995
CPT-11 i.v.	5-40 /day 120h q 3wk	36	6.8-10.5 ng/ml (SN 38)	40 mg/m^2/day	Neutro + Diarrhea	Ohe 1992

i.v.: intravenous; i.p.: intraperitoneal; p.o: oral; MTD: Maximum Tolerated Dose; DLT: dose limiting toxicity; Cpss: plasma-concentration steady state; *: chemotherapy-pretreated; **: non-pretreated; --: not stated; Neutro: neutropenia; Thrombo: thrombopenia.

From small studies in ovarian and cervical cancer, it was suggested there were no significant differences between schedules concerning efficacy, but clearly these data need further confirmation [115-116]. Response rates in patients with NSCLC

treated with CPT-11 at a dose of 200 mg/m^2 every 3-4 weeks or 100 mg/m^2 weekly do not seem to differ [117-118]. In patients with solid tumors, the dose schedule apparently does not seem to be crucial in efficacy of the drug.

However, CPT-11 may have more efficacy when administered at lower doses for a longer time to patients with malignant lymphoma. An oral formulation of CPT-11 has been tested on a daily x5 schedule every 3 weeks with diarrhea and neutropenia as dose limiting toxicities [119].

GI147211

GI147211, (7-(4-methylpiperazinomethylene)10,11-ehtylene-dioxy-20-(S)-camptothecin) is a water-soluble analogue of camptothecin. The water solubilizing groups were introduced on position 7 in the B ring.

Preclinical studies

GI147211 appeared to have antitumor activity in vitro as well as in vivo studies [120]. In these studies, the dose schedule of twice a week administration for 5 weeks did not appear optimal. Recent data demonstrate that GI147211 is more active when administered at higher doses using an every 4 days schedule for a total of 3 doses [121]. Again, dose scheduling seems important for an optimal antitumor effect.

Clinical studies with prolonged or continuous administration

Daily x5 iv administration

Three phase I studies with intravenous GI14721 have been performed, two studies with a 30-minute GI147211 infusion once daily for five consecutive days every 3 weeks, a third study with GI147211 given as a 72-hour continuous infusion [122-124]. In all studies, AUC increased with dose in a linear fashion, and dose limiting toxicity consisted of leucocytopenia as well as thrombocytopenia. Non-hematological toxicity was mild and there was no diarrhea or hemorrhagic cystitis. Preliminary results of phase II studies show anti-tumor activity in ovarian cancer and small cell lung cancer [125].

Prolonged exposure

A phase I study with continuous infusion of GI147211 has been performed with doses ranging from 0.3-0.5 mg/m^2/day for 7,14 and 21 days. DLT reached at 0.5 mg/m^2/day consisted of neutropenia and thrombocytopenia. Non-hematologic toxicities CTC-grade ≥ II consisted of nausea, vomiting, dyspepsia, fatigue and diarrhea. Pharmacokinetics of GI147211 showed mean steady-state concentrations ranging from 0.1-0.35 ng/ml. The total body clearance was similar to the clearance with shorter infusions [126] [Table 4].

Table 4. Topoisomerase-I-inhibitors continuous/prolonged administration in solid tumors. (GI147211)

Drug	Dose Schedule mg/m^2	No. Pts	Cp-ss	MTD	DLT	Ref. Year
GI147211 i.v.	0.3-0.5/day 7-21d q 28d	38	0.1-0.35 ng/ml	0.5 mg/m^2/d x21	Neutro+ Thrombo.	Khater 1996
GI147211 i.v.	72h q 28d?	36	--	1.5 mg/m^2/d* 2.0 mg/m^2/d**	myelosupp.	O'Dwyer 1995

i.v.: intravenous; i.p.: intraperitoneal; p.o: oral; MTD: Maximum Tolerated Dose; DLT: dose limiting toxicity; Cpss: plasma-concentration steady state *: chemotherapy-pretreated; **: non-pretreated; --: not stated; Neutro: neutropenia; Thrombo: thrombopenia.

DISCUSSION AND CONCLUSION

Topoisomerase I inhibitors are a class of drugs with a broad antitumor activity, even against previously chemotherapy-resistant tumors. The issues concerning drugs scheduling are many, and one of the conclusions from all this review could be that there is no true consistency in the use of schedules and models in preclinical studies. It would be worthwhile to try to achieve this consistency in the development of drugs such as these. Clearly, many of the relevant questions on scheduling can already be answered in in vitro studies, such as the ones that have been performed with topotecan. With appropriate in vitro studies, one could easily mimic potential clinical application schedules. Following in vitro studies, in vivo studies could be performed taking the data from the in vitro studies into account. Obviously, long-term infusional application in animal models is difficult to achieve but, on the other hand,

many of the performed in vivo studies, because of their diversity, do not result in conclusive evidence. With a consistent approach in preclinical studies, one could also avoid the need to perform too many clinical studies on scheduling. We also recommend performing the clinical phase I and II studies with inclusion of pharmacokinetic/pharmacodynamic (PK/PD) relationship studies. A good example of this can be found in the yet unpublished study relating levels of topoisomerase I inhibitors to parameters such as decreased cleavable complex formation. Making use of the appropriate combinations of clinical studies with PK/PD studies, the number of studies necessary could easily be reducted. Also, such studies would answer the question of whether thresholds exist for the effect of topoisomerase I inhibitors in conjunction with exposure duration. The preliminary results from the above reviewed phase I and phase II studies indicate that prolonged administration with topoisomerase I inhibitors is feasible in patients with cancer. However, unfortunately, the optimal dose and schedule of the various agents available remain to be elucidated. Although preliminary results are encouraging and warrant further clinical exploration, the concept should still be considered investigational.

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Chapter 3

Pharmacokinetics and bioavailability of oral 9-aminocamptothecin capsules in adult patients with solid tumors

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ABSTRACT

Preclinical studies indicate enhanced antitumor activity of 9-amino-20(*S*)-camptothecin (9-AC) when administered in a manner that provides prolonged systemic exposure. In view of this observation, the pharmacokinetics and oral bioavailability of 9-AC PEG1000 capsules were evaluated in twelve patients with solid tumors. Patients were randomized to receive either 1.5 mg/m^2 of 9-AC orally on day 1 and 1.0 mg/m^2 of 9-AC i.v. on day 8 or *vice versa*. Serial plasma samples were collected upto 55 h after dosing, and analyzed for 9-AC by liquid chromatography. Plasma concentrations of the lactone and carboxylate forms of 9-AC rapidly reached an equilibrium, with the active lactone accounting for <10% of total drug at the terminal disposition phase. The drug demonstrated peak levels at 1.2 h and an overall bioavailability of 48.6±17.6% (range: 24.5-80.4%), indicating significant systemic exposure to the drug which may enable chronic oral treatment.

INTRODUCTION

9-Amino-20(*S*)-camptothecin (NSC 603071; 9-AC[2]) is a semisynthetic analogue of campto-thecin, a cytotoxic plant alkaloid derived from the bark and wood of the oriental tree *Camptotheca accuminata*, Decaisne [1]. The mechanism of action of 9-AC and struct-urally related derivatives is believed to stem from the unique propensity to inhibit mammalian DNA topoisomerase-I activity, causing stabilization of cleavable complexes during DNA replication, transcription and repair [2]. In the presence of ongoing DNA synthesis, this will lead to irreversible DNA damage with double-strand DNA breaks, S-phase cell-cycle arrest and, ultimately, cell death. Studies on structure-activity relation-ships have revealed that the terminal lactone ring of the camptothecins, which is in equilibrium with an open-ring carboxylate form at constant pH, is essential for this topoisomerase-I inhibition [3].

The high specificity in the mechanism of action of camptothecins for the S-phase in the cell cycle has led to the recognition that the compounds may require prolonged exposure in order to maximize the fractional cell kill. This finding has been confirmed experimentally in nude mice bearing human tumor xenografts given 9-AC, demonstrating the importance of maintaining a minimal threshold-drug level in plasma for at least 48 h [4]. Based on these preclinical data, several phase I clinical trials have been initiated with the drug administered using continuous i.v.-infusion schedules [5-8].

The availability of a clinically useful oral formulation of 9-AC would provide increased convenience for the administration of chronic-dosing regimens and the opportunity for cost-effective outpatient therapy [9]. Furthermore, preclinical evidence of efficacy and toxicity of 9-AC given orally to rodents and dogs is suggestive of significant gastro-intestinal absorption of the drug [10,11]. In the present study, we explored the bioavailability of 9-AC after oral administration in patients to provide a basis for further clinical development of the drug using chronic oral dosing.

PATIENTS, MATERIALS AND METHODS

Chemicals. Analytical reference material used for quantitation of 9-AC (lot #93LO7A) and camptothecin (lot #93K05A; internal standard) were supplied by Pharmacia Inc. (New Mexico, USA). Judged from reversed-phase HPLC (see below), the purity of 9-AC and camptothecin was higher than 99.0%. Perchloric acid was purchased from Baker (Deventer, The Netherlands) as a ready-to-use solution (70% (v/v)). All other chemicals were of analytical or HPLC grade, and originated from Rathburn (Walkerburn, UK). Drug-free human plasma was obtained from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands). Purified deionized water was prepared by the Milli-Q UF system (Millipore, Milford, MA, USA), and was used throughout.

Patients. All patients included in this study had a histologically or cytologically proven malignant solid tumor, for which no standard therapy was available. Eligibility criteria included the following: (*i*) age between 18 and 75 years; (*ii*) an Eastern Cooperative Oncology Group (ECOG) performance status <2; (*iii*) life expectancy of at least 12 weeks; (*iv*) adequate hematopoietic (absolute peripheral granulocyte count >2000 μL^{-1} and platelets >100,000 μL^{-1}), hepatic (total bilirubin within normal limits and SGOT, SGPT and alkaline phosphatase <2 times upper normal limits) and renal functions (creatinine <133 µM); and (*v*) all patient provision of informed written consent according to institutional guidelines before treatment.

Dosage Forms. 9-AC is a drug with a very unfavorable solubility profile in aqueous fluids. At acidic pH its solubility is very low (approximately 0.125 and 0.02 mg/mL at pH 1 and pH 3, respectively), while at pH values above 4 the opening of the lactone ring is occurring, resulting in a degradation product with low activity [3].

Since poor solubility characteristics may cause absorption and bioavailability problems, a semi-solid matrix formulation in hard gelatine capsules has been viewed as the first choice dosage form. The semi-solid matrices evaluated for development of the oral dosage form were prepared by mixing 9-AC with the carrier at a temperature higher than the melting point of the matrix. The molten dispersion was then filled into gelatine capsules under continuous stirring to enable the dosage form to be processed from the liquid phase. After filling, the melt was cooled at room temperature to obtain a drug dispersion at solid state.

The solubility of 9-AC in several carriers suitable for such technology was evaluated, and polyethylene glycols gave the most interesting results, with solubility ranging from 1.2 to 1.5 mg/mL (i.e. more than 50 times higher than an aqueous solution at pH 3). In subsequent experiments, the in vitro drug release performance was thoroughly investigated using polyethylene glycols of different molecular weight as vehicle (e.g. PEG400, PEG600 and PEG1000). Dissolution rate was determined with the basket apparatus, rotating at 100 r.p.m., in 0.001 N aqueous hydrochloric acid solution at 37°C. All experiments were performed under sink conditions. The results of these tests demonstrated that the fastest in vitro release could be attained with the semi-solid matrix formulation in PEG1000, with 100% of the drug dissolved within 30 min. The dissolution performance of this formulation was also superior to physical mixtures of the drug and either PEG400 or PEG20,000 (less than 80% of the drug dissolved after 90 min), or conventional capsule formulations, i.e. a powder formulation containing microcrystalline cellulose as filter/disintegrant and glyceryl palmitostearate as lubricant (less than 50% of the drug dissolved after 30 min).

Drug Administration. Eventually, for oral drug administration, 9-AC was supplied as hard gelatin capsules containing a yellow waxy mass with 0.25 (size N.4) or 1 mg (size N.2) of the active drug substance, with PEG1000 as excipient (Pharmacia & Upjohn, Pharmaceu-tical Development Department, Nerviano, Italy). Each patient received 1.5 mg/m^2 of 9-AC with 150-200 mL of water. For i.v. administration, 9-AC was provided as 1- or 2-mL vials containing 1 or 2 mg of lyophilized drug and a matrix of soybean phospholipids and mannitol as cryo-protectant (Pharmacia & Upjohn, Milan, Italy). The vials were reconstituted in a mixture of 20% dextrose, 0.9% sodium chloride and sterile water for injection (pH 3.5), and diluted further in the same diluent to obtain a concentration of about 20 µg/mL of 9-AC. Each patient received a single i.v. dose of 1.0 mg/m^2 through a peripheral venous-access device in 5 min.

All patients were randomized to receive 9-AC either orally on day 1 and i.v. on day 8, or *vice versa*. On both days of drug administration, patients had fasted for at least 8 h before, and 0.5 h after dosing. Patients were subsequently offered participation to a phase I study with chronic oral dosing of 9-AC.

Sample Collection. Serial blood samples (~4.5 mL) were collected in heparinized tubes by i.v. sampling from the arm opposite to the one in which the drug was infused. This was performed after oral administration at 0 (pre-dose), 20, and 40 min, and 1, 1.5, 2, 3, 5, 7.5, 11, 24, 28, 31, 48, 52 and 55 h. With the 5-min i.v. infusion, the sampling was performed at 0, 5, 15, 25 and 40 min, and 1, 2, 3, 5, 7.5, 11, 24, 28, 31, 48, 52 and 55 h after the start of infusion. Immediately after sampling, tubes were briefly immersed into an ice-bath kept at the bedside, and plasma was separated within 10 min by centrifugation at 3000 x *g* for 5 min (4°C) to prevent significant degradation of the lactone form. The supernatant was transferred to a clean tube and stored at -80°C, until the time of analysis within two weeks, which is sufficiently short to prevent potential stability problems [12].

Drug Analysis. 9-AC lactone and total drug (lactone plus carboxylate) concentrations in plasma were determined according to a validated reversed-phase HPLC method reported in detail previously by Loos *et al.* [12]. In summary, sample pretreatment for the lactone involved a solvent extraction of the analyte from 1-mL samples with 7.5 mL of acetonitrile-*n*-butylchloride (1:4, v/v) in glass screw-cap tubes containing 0.8 g of sodium chloride and 100 µL of 2.5 ng/mL of camptothecin (used as internal standard) in methanol-water-perchloric acid (500:500:1, v/v/v). After centrifugation for 5 min at 4000 x *g*, the organic layer was collected in a tube containing 50 µL of dimethylsulfoxide and evaporated at 50°C under nitrogen. To the residue 50 µL of methanol and 150 µL of perchloric acid-water (1:500, v/v) were added. Subsequently, the sample was transferred to a low-volume insert, and 150 µl were injected into the HPLC system. Sample preparation for 9-AC total involved vigorous mixing of 250 µL of plasma with an equal volume of 5% (v/v) perchlo-ric acid-methanol (1:1, v/v), followed by centrifugation for 5 min at 24,000 x *g*. The clear supernatant was transferred to a low-volume insert, and an aliquot of 200 µL was subjected to chromatography. The reversed-phase chromatographic system consisted of a constaMetric 3200 pump (LDC Analytical, Rivera Beach, USA), a

Waters 717Plus auto-sampler (Milford, USA) and a fluoriMonitor 4100 detector (LDC Analytical).

Compounds of interest were separated on an Inertsil ODS-80A stationary phase (150x4.6 mm ID; 5 µm particle size) from GL Science Inc. (Tokyo, Japan). The mobile phases were comprised of methanol-water (40:60 (v/v) for 9-AC lactone and 32.5:67.5 (v/v) for 9-AC total) with the pH adjusted to 2.1, and were delivered at 1.0 mL/min. Detection was performed at excitation and emission wavelengths of 370 and 450 nm, respectively. Peak detection was done with the Fisons ChromCard data analysis system (Milan, Italy).

Drug concentrations were determined from linear calibration curves, constructed in blank human plasma, by linear regression analysis of peak heights versus $1/X$. The mean percent deviation (accuracy) and precision (within-run and between-run) were always less than 10%. The lower limits of quantification were 50 pg/mL and 100 pg/mL for the lactone and total forms, respectively. The 9-AC carboxylate at each time point was quantitated indirectly from the difference in concentration of total and lactone forms.

Pharmacokinetic Analysis. Individual 9-AC plasma-concentration data were analyzed by both non-compartmental and compartmental analysis using the Siphar v4.0 software package (SIMED, Creteil, France). The AUC for 9-AC total was calculated by the linear-trapezoidal rule upto the last sampling point with detectable levels (C), with extrapolation to infinity ($AUC_{0-\infty}$) by the equation $AUC+C/k_{el}$, where k_{el} represents the terminal disposition rate constant. The latter term was calculated from the slope of data points in the final log-linear part of the drug concentration-time curve by weighted ($1/Y$) least-squares linear regression analysis. Maximum plasma concentration (C_{max}) and the time to maximum concentration (t_{max}) following oral administration were estimated by visual inspection of the semi-logarithmic plot of the concentration-time curve. The absolute oral bioavailability (F) expressed as a percentage was calculated by dividing the AUC_{oral} by the $AUC_{i.v.}$ normalized to dose ($F=[AUC_{oral}/AUC_{i.v.}]^* [dose_{i.v.}/dose_{oral}]^*100\%$), assuming a linear pharmacokinetic behavior during both study periods.

The apparent absorption rate constant (k_a) was estimated by the numerical point-area deconvolution technique [13]. Other pharmacokinetic parameters, including the terminal disposition half-life ($t_{1/2}(\gamma)$), the total body clearance (CL) and the volume of distribution (V_d) were calculated applying standard equations [14].

RESULTS

Twelve patients were enrolled onto this study (seven females and five males), with a median age of 60 years (range: 49-70), and a median ECOG performance score of 1. All patients completed the pharmacokinetic and oral bioavailability studies during both periods of drug administration, and subsequently participated to an ongoing phase I and pharmaco-kinetic study of oral 9-AC in a daily-times fourteen schedule. Except for one patient who experienced mild nausea, neither i.v. nor oral administration of 9-AC was associated with any adverse-effect, including myelosuppression and diarrhea.

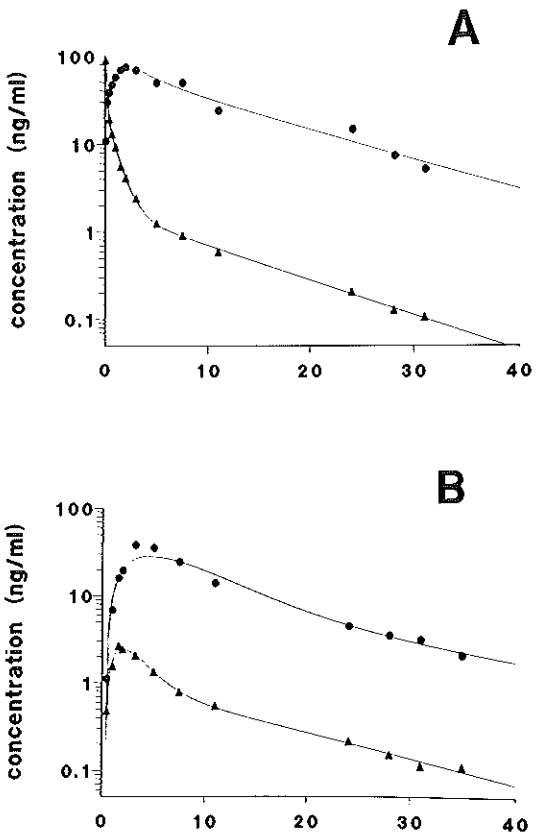

Fig. 1. Representative plasma concentration-time profiles of the lactone (triangles) and carboxylate (circles) forms of 9-AC after i.v. (**A**) and oral (**B**) drug administration of 1.0 and 1.5 mg/m^2, respectively. All curves were fitted to a three-compartment model.

The plasma concentration time profiles of the lactone and carboxylate forms of 9-AC after i.v. or oral administration were similar for the twelve patients studied, with typical examples shown in Figure 1. Concentrations of 9-AC lactone and carboxylate (calculated as the difference between the total drug and intact lactone) in plasma were always below the lower limit of quantitation of our HPLC assay (50 pg/mL) before administration of the second course (data not shown). Applying compartmental analysis for 9-AC lactone and carboxylate data, model discrimination was assessed by minimization of the sum of squares, dispersion of the residuals, visual inspection of fitted curves and Akaike's information criterion. Eventually, all concentration-time profiles were best fitted to a three-compartment model after zero-order input (lactone i.v. data only) or after extravascular bolus with lag time (t_{lag}) using the Powell minimization algorithm, and weighted least-squares analysis with the weighting factor of $1/Y$.

The two initial disposition phases of the intact lactone after the i.v. bolus were characterized by half-lifes of 6.6 and 38 min, while the terminal biological half-life was approximately 8 h [Table 1]. The conversion of 9-AC into the E ring-opened carboxylate species in plasma appeared from the first sample aquired, with about 50% of the total plasma concentration already present at 10 min after cessation of the 5-min infusion. The maximum concentration of 9-AC carboxylate occurred at 1.6 h, and the relative amount of this form increased until an apparent equilibrium was established at approximately 3 h, with the lactone accounting for 2.92±0.50% (mean ± SD; n=12) of 9-AC total concentrations. The AUC of the lactone was found to represent 9.1±3.4% of the total $AUC_{i.v.}$. The relatively low values of the volume of distribution at steady-state for 9-AC lactone and 9-AC carboxylate (92.9±25.5 L and 15.9±8.8 L, respectively), suggest that overall clearance is mainly dictated by actual elimination from the body, rather than extensive accumulation in peripheral tissues.

The absorption of 9-AC lactone after oral administration was associated with a lag time of 0.24±0.13 h, peak drug levels at 1.2±0.3 h and a mean absorption rate constant of 3.0±0.9 h^{-1} [Table 1]. Plasma levels of the carboxylate form exeeded the lactone component already at the first sampling time point (i.e. 20 min; Figure 1). Approximately 7.5 h after treatment, an apparent distribution equilibrium was achieved between the components, characterized by similar terminal rates of elimination at a ratio of lactone to carboxylate of 3.52±0.36%.

Table 1. Pharmacokinetic parameters of 9-AC lactone, 9-AC carboxylate and 9-AC total in twelve patients after i.v. (1.0 mg/m^2) and oral (1.5 mg/m^2) administration. Data represent mean values ± standard deviation.

	i.v.			*oral*		
Parameter	9-AC lactone	9-AC carboxylate	9-AC total	9-AC lactone	9-AC carboxylate	9-AC total
lag time (h)	-	0.01±0.01	-	0.24±0.13	0.31±0.11	-
C_{max}	83.1±48.6	57.6±18.6	140±33.6	6.82±2.98	35.4±19.2	42.2±11.1
t_{max}	-	1.59±0.45	-	1.17±0.33	2.39±1.05	-
AUC (ng.h/mL)	45.5±13.5	548±339	580±333	31.9±14.3	510±354	535±377
CL (mL/min)	732±210	87.4±59.0	52.9±29.1	-	-	-
k_a (h^{-1})	-	-	-	3.03±0.90	-	-
$t_{1/2}(\alpha)$ (h)	0.11±0.19	0.63±0.30	-	0.38±0.21	1.04±0.78	-
$t_{1/2}(\beta)$ (h)	0.64±0.11	1.79±0.91	-	1.13±0.59	2.83±1.96	-
$t_{1/2}(\gamma)$ (h)	7.91±2.14	11.7±3.39	-	12.6±4.20	12.4±6.48	-
V_d (L)	92.9±25.5	15.9±8.8	-	-	-	-
F (%)	-	-	-	48.6±17.6	-	62.5±24.9

Abbreviations: C_{max}: maximum plasma concentration; t_{max}: time to maximum concentration: AUC: area under the plasma concentration versus time curve; CL: systemic clearance; k_a: absorption rate constant; $t_{1/2}$: disposition half-life; V_d: volume of distribution; F: oral bioavailability

On average, the active lactone accounted for 7.1±3.2% of the total AUC_{oral}. Terminal disposition half-lifes for the lactone and carboxylate forms of the drug were estimated to be 12.6±4.20 h and 12.4±6.48 h, respectively, which is not significantly different from data obtained after i.v. administration. The mean percentage of the AUC extrapolated was <7%, justifying the use of compartmental methods for calculation of the bioavailability. The absolute oral bioavailability of 9-AC lactone averaged 48.6±17.6% (range: 24.5-80.4%), with an interindividual coefficient of variability of 36.2%. Calculations based on non-compartmental modeling of 9-AC total data resulted in slight overestimation of the oral bioavailability as a result of the disparity in the extent of lactone-carboxylate interconversion between i.v. and oral dosing [Table 1].

Significant linear correlations were observed between the AUCs of 9-AC total and that of the pharmacologically active 9-AC lactone after both i.v. (R=0.77) and oral (R=0.87) drug administration.

DISCUSSION

In view of preclinical data indicating enhanced antineoplastic activity of 9-AC when admi-nistered in a manner that provides prolonged systemic exposure [reviewed in 1)], the pharmacokinetics and oral bioavailability of the drug (formulated in PEG1000) were evaluated in twelve patients with various types of solid tumors. The i.v. and oral dosages were selected on the basis of toxicological data in dogs [10], which is the most sensitive species to 9-AC, so as to be safe, yet allowing pharmacokinetic analysis after both routes of administration. The overall bioavailability of this formulation of 9-AC averaged 48.6±17.6% (range: 24.5-80.4%). Compared to other camptothecin analogues, including topotecan [bioavailability (F)=30.0%] [15], 7-(4-methylpiperazinomethylene)-10,11-ethyl-enedioxy-20-(*S*)-camptothecin (GI147211; F=11.3%) [16] and irinotecan (F=12-21%) [17], 9-AC has a higher oral bioavailability, which may be an advantage with potential pharmacodynamic importance.

Similar to all other camptothecin analogues in clinical development, 9-AC can undergo a reversible, pH-dependent hydrolysis in which the closed lactone form is converted to the open carboxylate form and *vice versa* [3]. In the present study, we observed that both after i.v. and oral drug administration interconversion of the lactone and carboxylate rapidly reached an *in vivo* equilibrium with the

pharmacologically active lactone accounting for less than 10% of total drug at steady state. Combined with a significantly reduced volume of distribution for the carboxylate form, this suggest that there is preferential uptake of 9-AC lactone in peripheral tissues at early times, thereby accelerating predominance of the carboxylate in blood. This hypothesis is in line with available data indicating that a closed lactone form is also an important structural requisite for (passive) diffusion across cell membranes in addition to succesful interactions with topoisomerase-I [3].

The finding that the carboxylate form is the predominant species in plasma is also consistent with previous reports demonstrating preferential affinity of 9-AC carboxylate for human serum albumin as compared to the corresponding lactone, shifting the equili-brium hydrolysis toward the former species [18]. The significant degree of interpatient variability with regard to the oral bioavailability of 9-AC in the present study might thus be related in part to differences in albumin levels in individual patients. More importantly, the coefficients of variation of the AUC after i.v. and oral administration were not significantly different, suggesting that oral delivery is not associated with increased interpatient differences in systemic exposure.

The steady-state lactone to total-AUC ratio in plasma was of the same order after i.v. and oral drug administration. Although this ratio for 9-AC is the lowest relative to all other camptothecin analogues [4], recent findings indicated that 9-AC lactone is the predominant species within tumors, and that the carboxylate may itself have a role in inhibiting cell growth by a mechanism other than the ability to bind topoisomerase-I [11]. The clinical implication of differences in the lactone to total-AUC ratio for camptothecin analogues with respect to antitumor efficacy remains, therefore, unclear at present. Although in our study the AUC_{oral} of 9-AC total was significantly correlated with that of 9-AC lactone, measurement of systemic exposure to both the lactone and carboxylate forms separately still is pivotal to our understanding of the pharmacology of 9-AC, until a well-designed study shows 9-AC total to be predictive of outcome (e.g. toxicity or efficacy).

Disappearance of 9-AC from the central plasma compartment was characterized by terminal disposition half-lifes of approximately 12 h that were not significantly different between administration routes for both drug forms. These results contrast with the reported biphasic elimination of 9-AC lactone with a half-life of 4.5±0.5 h when administered as a 72-h i.v. infusion [7], which may relate to limited sampling-time points precluding observation of the third pharmacokinetic compartment. However, the values of the total plasma clearance, viz. 33.0±9.8 L/h/m^2 (present study) *versus* 24.5±7.3 L/h/m^2 [7], are quite similar, probably because the fractional

AUC associated with the terminal disposition phase(s) has a minor contribution to the AUC extrapolated to infinity.

The present results are inconsistent with recent data from a clinical phase I and pharmacokinetic study of a colloidal-dispersion formulation of 9-AC administered orally [19]. In that study, dose escalation was discontinued as a result of poor bioavailability and apparent saturable absorption of the drug, although the absolute bioavailability of this formulation in dogs suggested a value of ~20% (unpublished data, Pharmacia & Upjohn, Milan, Italy). Although the disparity in bioavailability data may relate to differences in absorption of 9-AC from the two formulations, our own data from oral dose escalation with the present PEG1000 formulation indicate, however, that the power to detect a linear dose-AUC_{oral} relationship is mainly limited by the significant degree of interpatient variability in pharmacokinetics (to be published elsewhere).

In conclusion, we have characterized the pharmacokinetics and bioavailability of 9-AC administered orally to patients with solid tumors. The bioavailability of oral 9-AC illustrates significant systemic exposure to the drug, which may enable chronic oral treatment. The high degree of interpatient pharmacokinetic variability observed in the present study, however, suggest that 9-AC administration may be associated with substantial interpatient differences in drug response. At present, a clinical phase I and pharmacokinetic study with oral 9-AC is in progress to assess the pharmacokinetic-dynamic relationships and its clinical utility.

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Chapter 4

Phase I and pharmacologic study of oral [PEG-1000] 9-aminocamptothecin in adult patients with solid tumors

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ABSTRACT

Purpose: 9-Amino-20(S)-camptothecin (9-AC) is a specific inhibitor of topoisomerase-I. Recently, a bioavailability of approximately 48% for the oral PEG-1000 formulation was reported. We conducted a phase I and pharmacokinetic study of the oral PEG-1000 formulation of 9-AC to define the maximum-tolerated dose (MTD), toxicity profiles, pharmacokinetic-dynamic relationships, and preliminary antitumor activity in patients with solid tumors.

Patients and Methods: Patients were treated with oral [PEG-1000] 9-AC given once a day for 7 or 14 days at doses ranging from 0.25 to 1.1 mg/m²/day; cycles were repeated every 21 days. For pharmacokinetic analysis, plasma sampling was performed on day 1 and 6 or 8 of the first course using a validated high-performance liquid chromatographic assay.

Results: Thirty patients entered into the study; three patients were not assessable for toxicity and response. Twenty-seven patients received a total of 89 courses. The dose-limiting toxicities (DLTs) were myelosuppression and diarrhea at a dose of 1.1 mg/m²/day for 14 days. Pharmacokinetics showed a substantial interpatient variation of the area under the plasma concentration-time curve (AUC) of 9-AC. The intrapatient variability was extremely small. A significant correlation was observed between the percentage decrease in WBC count and the AUC of 9-AC lactone (r^2 = 0.86). One partial response was noted in a patient with metastatic colorectal cancer.

Conclusion: Dose limiting toxicities in this phase I study of oral 9-AC daily x 14 every 21 days were myelosuppression and diarrhea. The recommended dose for phase II studies is 0.84 mg/m²/day. In view of the substantial interpatient variability in AUC and the availability of a limited sampling model, a pharmacokinetic guided phase II study should be considered.

INTRODUCTION

9-Amino-20(S)-camptothecin (9-AC, NSC 603071, IDEC-132) is a semisynthetic analog of camptothecin. Like camptothecin, 9-AC is a specific inhibitor of topoisomerase-I. Topoisomerase-I is a nuclear enzyme that can relax the torsional strain of supercoiled DNA, which is necessary for DNA replication, RNA transcription and DNA recombination [1]. This is achieved by forming a covalent adduct between topoisomerase-I and the DNA, termed "the cleavable complex". This catalytic intermediate involves single-strand breaks, which allow the DNA molecule to rotate

around the intact DNA strand at the cleavage site and leads to relaxation of the DNA molecule. These enzyme-bridged breaks are then resealed by topoisomerase-I. Camptothecin analogs stabilize the cleavable complex, thus preventing resealing of the topoisomerase-I-mediated single-strand break. Cytotoxicity of camptothecin analogs is specific to the S-phase of the cell cycle.

Unlike other camptothecin analogs (topotecan and irinotecan), 9-AC is poorly soluble in water. In preclinical studies, 9-AC demonstrated activity against human colon, prostate, breast, non-small cell lung cancer and melanoma tumor xenografts [1-5]. Preclinical *in vivo* data suggested that duration of exposure to 9-AC above a certain threshold concentration (10 nmol/L=3.6 ng/mL) and frequency of administration were essential for antitumor activity [6,7]. Therefore, initial phase I studies using the intravenous formulation of 9-AC focused on schedules with prolonged infusion duration of 24 to 72 hours [8-12]. Dose limiting toxicities (DLT) consisted of neutropenia, thrombocytopenia and diarrhea. A more convenient method for prolonged drug administration might be the use of an oral formulation. 9-AC can be administered orally as a colloid dispersion (CD) or as gelatine capsules in polyethylene glycol (PEG) 1000 (PEG-1000). Given orally to rodents and dogs both the CD and PEG 1000 formulation of 9-AC retained the antitumour activity [13] (data on file, Pharmacia/Upjohn, Milan, Italy). In dogs, the oral bioavailability of the CD formulation was 13 % (range 4.5-26%) compared with 10% of the PEG-1000. Recently, a phase I study of the CD formulation of 9-AC administered orally 5 days a week every 2 weeks was completed. Diarrhea was the DLT at a dose level of 0.2 mg/m^2 [14].

In the present report, we describe a phase I study of oral 9-AC using the PEG-1000 capsule formulation given once a day for 7 to 14 days repeated every 21 days.

PATIENTS AND METHODS

Patient Selection

Patients with a histologically confirmed diagnosis of a malignant solid tumor refractory to standard forms of therapy were eligible. Other eligibility criteria included the following: age 18 - 75 years; Eastern Co-operative Oncology Group (ECOG) performance status $\leq$ 2; estimated life expectancy $\geq$12 weeks; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosourea or mitomycin); no previous therapy with other camptothecins and/or intensive ablative regimens; and

adequate hematopoietic (absolute peripheral granulocyte count ≥ 2000/mm^3 and platelet count ≥ 100 x 10^9/L), hepatic (bilirubin within normal limits, and serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase ≤ 2.5 times normal limit) and renal (serum creatinine concentration < 133 µmol/L) function. Specific exclusion criteria included significant gastrointestinal dysfunction that could alter absorption or motility, and chronic treatment with corticosteroids. Concomitant administration of H_2-antagonists, antacids, proton pump inhibitors and non-steroidal anti-inflammatory drugs were avoided. If necessary, their time of administration was at least 3 hours after the intake of the study drug. All patients gave written informed consent before study entry.

Treatment and Dose Escalation

9-AC was supplied as hard gelatine capsules that contained 0.10, 0.25 or 1 mg of the active drug and PEG-1000 as excipient, and were stored at room temperature. A detailed description of the constituents and preparation of the oral dosage form will be presented elsewhere. Capsules were taken once a day with a glass of water after an overnight fasting at least 30 minutes before a milk-free, non-fat breakfast. Patients were treated on an outpatients basis. The daily dose of 9-AC was provided in separate boxes, which each daily dosing clearly identifiable by the patient. Patients were instructed to record their daily amount of capsules taken, the time of administration, and the timing in relation to breakfast. Compliance with the scheduled treatment was assessed at the end of each course by counting the used and returned capsules of 9-AC in relation to the record kept by the patient for the given cycle.

The starting dose of 9-AC, 0.25 mg/m^2 given orally once a day for 7 days, was 1/3 of the maximum tolerated dose (MTD) in dogs. The total oral dose was rounded off at 0.25 mg. Courses were to be repeated every 21 days. Because prolonged drug administration might be essential for antitumor activity of 9-AC, the duration of the therapy was first extended from 7 to 14 days at the second dose level. Further dose escalations were based on the prior dose level toxicity. If no toxicity (excluding alopecia, fatigue, nausea and vomiting) was observed at the previous dose level, then a 50% dosage increment was allowed. However, if toxicity was observed, a dose escalation of 15-40% (which was determined by the worst significant toxicity) was prescribed. At least three patients were entered at each dose level. The MTD

was defined as one dose level below the dose that induced DLTs during course 1, which were defined as National Cancer Institute common toxicity criteria (NCI-CTC) grade 4 granulocytopenia for at least 5 days or occurring during treatment, grade 4 thrombocytopenia, complicated grade 3 or 4 granulocytopenia and/or non-haematological toxicity ≥ grade 3 (grade 2 for neurotoxicity), excluding fatigue, nausea and vomiting, in two of six patients [15]. If neutropenia or thrombocytopenia grade 4 and/or non-haematological toxicity ≥ 3 (grade 2 for neurotoxicity) occurred during treatment days, 9-AC administration was stopped immediately. Intrapatient dose escalation was not allowed. If a patient encountered DLT, the dose of 9-AC was decreased with one dose level at re-treatment. The treatment was resumed when the neutrophil count had recovered to ≥ 2000/mm^3 and the platelet count to ≥ 100 x 10^9/L.

Treatment assessment

Before initiating therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell count (CBC) including WBC differential, and serum biochemistry, which involved sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, γ-glutamyl transferase, glucose and uric acid, were performed, as were urinalysis, ECG and chest x-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the CTC criteria, and serum chemistry. CBC count was determined twice weekly. Tumor evaluation was performed after every two courses according to the World Health Organization (WHO) criteria for response. Patients were taken off protocol at the onset of disease progression.

Sample Collection and Drug Analysis

For pharmacokinetic analysis, 10 blood samples (~4 mL) were obtained from an indwelling i.v. canula and collected in vials containing lithium heparin as anticoagulant. The samples were taken immediately before dosing and at 20 and 40 min, and 1, 2, 3, 5, 7½, 11, and 24 hours after administration of the drug on days 1 and 6 (7-day schedule) or days 1 and 8 (14-day schedule) of the first course. All samples were centrifuged immediately after sampling and the plasma supernatant was snap-frozen at -20ºC to prevent degradation of the 9-AC lactone form.

Concentrations of 9-AC lactone and 9-AC total (i.e. lactone *plus* carboxylate) drug forms in plasma were determined according to a validated reversed-phase high-performance liquid chromatographic (HPLC) assay with fluorescence detection, described in detail elsewhere [16]. The lower limits of quantitation were 50 pg/mL for 9-AC lactone and 100 pg/mL for 9-AC total using 1-mL and 0.25 mL volumes, respectively, for sample clean-up and analysis. The percentage deviation from nominal values and the intra- and interassay variability were always less than 10%.

Pharmacokinetic and Pharmacodynamic Data Analysis

The plasma concentration-time curves were analyzed using the pharmacokinetic software package Siphar *version* 4.0 (SIMED, Creteil, France), by determination of slopes and intercepts of the plotted curves with multiexponential functions. Initial parameter estimates were obtained by an automated peeling-algorithm procedure, with an integrated numerical algorithm based on the Powell method to minimize any objective function by the following criteria:

$$F = \sum_{i=1}^{n} [(Y_{oi} - Y_{ci}) / \sigma_{yi}]^2$$

where n is the number of observations, Y_{oi} and Y_{ci} are Y observed and calculated values, respectively, for the *i*-th observation, and σ_{yi} is the standard deviation for the *i*-th observation. The statistical best fit was determined by application of (*i*) Akaike's information criterion with the χ^2 test to discriminate between models, and (*ii*) the coefficient of correlation, defined as the ratio of the standard deviation computed using the variance-covariance matrix and the parameter value. Both weighted least squares and extended least squares methods were evaluated to estimate model parameters minimizing the sum of squared differences between experimental and computed values and the log-likelihood function. The drug disposition half-lives ($t_{1/2}$) and the area under the plasma concentration-time curve (AUC) were determined on the basis of the best fitted curves, whereas the peak plasma concentration (C_{max}) and the time to the peak plasma concentration (T_{max}) were determined graphically. The observation of multiple peaks in the kinetic profile of 9-AC total [17] limited application of conventional compartmental analysis. Therefore, the AUC of 9-AC total was estimated using the experimental values (trapezoidal rule) with extrapolation to infinity using the terminal elimination rate constant, defined as the slope of the final 3 to 4 data points of the log-linear concentration-time plot.

Pharmacokinetic/pharmacodynamic relationships between 9-AC kinetic parameters and hematological toxicity associated with drug administration were evaluated using the Siphar (SIMED) and NCSS [Number Cruncher Statistical Systems *version* 5.0 (1992); Dr. Jerry Hintze, East-Kaysville, UT] computer programs. Within individual patients, myelosuppression was described as the continuous variable, consisting of percentage decrease in white blood cell count (WBC), absolute neutrophil count (ANC) and platelet count (PLT) and CTC myelotoxicity grade as the discrete variable. The relative haematological toxicity was defined as: % decrease = (pretherapy value - nadir value)/ (pretherapy value)*100. Only the first course of each patient was taken into consideration to avoid potentially confounding bias due to cumulative toxicity. All data were fitted to a sigmoidal maximum effect (E_{max}) model based on the modified Hill equation, as follows:

$$E=E_0+E_{max}*[(KP^{\gamma})/(KP^{\gamma}+KP_{50}^{\gamma})].$$

In this equation, E_0 is the minimum reduction possible, fixed at a value of 0, E_{max} is the maximum response, fixed at 100 (continuous variables) or 4 (discrete variable), KP is the pharmacokinetic parameter of interest, KP_{50} the value of the pharmacokinetic parameter predicted to result in half of the maximum response, and γ is the Hill constant describing the sigmoidicity of the curve. Models were evaluated for goodness of fit by minimization of sums of the squared residuals and by reduction of the estimated coefficient of variation for fitted parameters. Significance of the relationships was assessed by construction of contingency tables with subsequent χ^2 analysis.

RESULTS

A total of 30 patients entered the study. Patients characteristics are listed in Table 1. All patients were eligible, but three patients were considered not assessable for toxicity and response because they were taken off protocol on their own request before completing the first course without any notable toxicity at that time. Therefore, 27 patients were assessable for toxicity and response. The majority of the patients were either asymptomatic or had only mild symptoms. All patients except two had received prior chemo- and/or radiotherapy. The most common tumor type was colorectal cancer. The total number of assessable courses was 89. The median

number of courses per patient was two (range 1 to 10). Dose levels studied were 0.25 mg/m^2/d for 7 days, and 0.25, 0.40, 0.60, 0.84, 1.0 and 1.1 mg/m^2/d for 14 days. Two treatment cycles had to be discontinued early because of toxicity; one patient treated at a dose of 0.60 mg/m^2/day x 14 experienced fatigue grade 3 during his second cycle and discontinued therapy after 10 days. In a second patient, treated at 1.1 mg/m^2/day x 14, therapy was discontinued after 10 days during the first cycle because of neutropenia and thrombocytopenia grade 4.

Table 1. Patient characteristics

Characteristic		No. of Patients
No. Entered		30
No. Assessable		27
Age, years		
Median	60	
Range	29-73	
Sex		
Female		12
Male		15
Performance status		
Median	1	
Range	0-2	
Tumor type		
Colorectal		13
Ovarian		4
Sarcoma		2
Mesothelioma		2
Lung (non-small cell)		2
Miscellaneous		4
Previous treatment		
Chemotherapy		13
Radiation		2
Chemotherapy and radiation		10
None		2

Hematological toxicity

A combination of thrombocytopenia and neutropenia complicated by fever was the dose limiting toxicity of 9-AC at a dose of 1.1 mg/m²/d in with this schedule. Dose reduction to 1.0 mg/m²/d also resulted in DLT. Overall, the haematological toxicity was relatively mild [Table 2]. with neutropenia occurring mainly in the second and third week and thrombocytopenia in the third week after the start of treatment [Figure 1]. Grade 3 to 4 neutropenia was observed in 10 of 89 courses (11%). It was complicated by neutropenic fever in 4 patients. Thrombocytopenia was mild, being grade 3-4 in only 6% of the cycles, all in conjunction with neutropenia. Despite the limited severity of myelosuppression, treatment had to be delayed in 23% of the courses due to prolonged myelosuppression. One patient was taken off study because of persisting leucocytopenia after two weeks of treatment-delay. Two patients required dose reductions after experiencing dose-limiting toxicity. A marked inhibition of erythropoiesis was observed. The percentage of patients requiring erythrocyte transfusions was 55 % during 22 of 89 courses.

Table 2. Haematological toxicity (worst per cycle)

9-AC	nr pts/	Leukocytes				Granulocytes				Platelets				Hb		
mg/m²/d	cycles	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
0.25 x 7	3/9	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0.25 x 14	3/6	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0
0.4 x 14	3/5	0	0	0	0	1	0	0	0	0	0	0	0	2	1	0
0.6 x 14	3/14	5	6	0	0	6	2	0	0	5	0	0	0	1	0	0
0.84 x 14	8/33	11	2	1	1	3	2	1	1	1	1	0	1	9	4	1
1.0 x 14	6/18	3	6	1	3	3	2	3	3	4	3	2	0	6	5	0
1.1 x 14	3/4	0	2	0	2	1	1	0	2	2	0	0	2	0	3	1

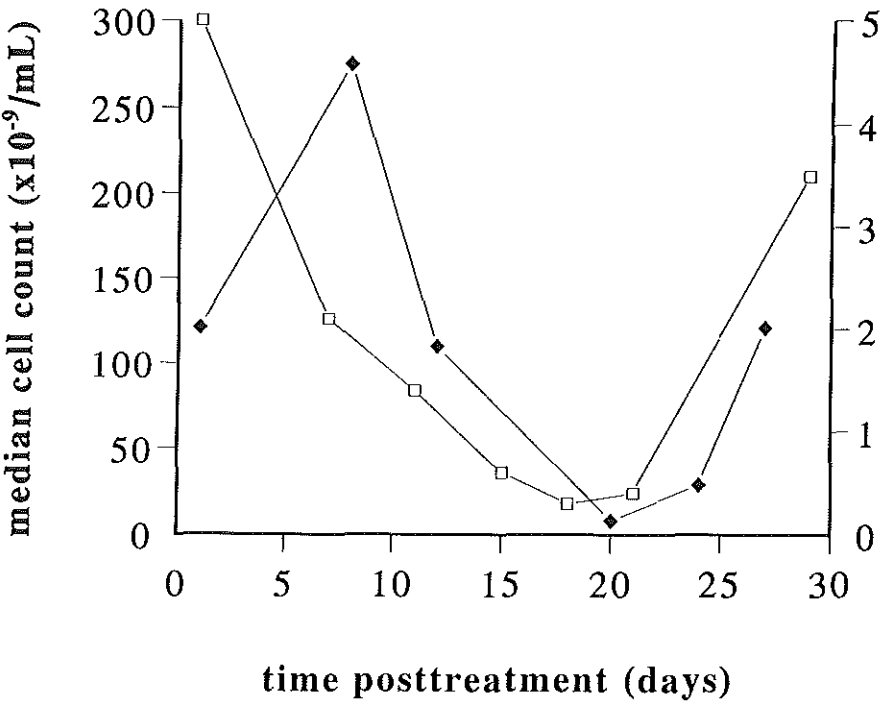

Fig. 1. Median ANC (open symbols; left y-axis) and platelet count (closed symbols; right axis) as a function of the time post treatment in patients experiencing grade 4 myelotoxicity

Nonhematological toxicity

One patient treated at a dose of 9-AC of 1.0 mg/m²/d experienced grade 4 diarrhoea. Grade 1-2 diarrhoea was observed in 25 of 89 courses (28%) and seemed to be dose-related [Table 3]. The median day of onset of the diarrhoea was day 12 (range 1-23). Diarrhoea lasted for a median duration of 3 days (range 1-23) and was self-limiting in most patients. Two patients, who required treatment for diarrhoea responded to a low dose loperamide regimen. Mild to moderate (CTC grade 1-2) nausea and vomiting occurred in 48% and 36%, respectively, of the courses. Anti-emetic therapy consisting of low dose metoclopramide (20 mg t.i.d.) was sufficient in most patients. Other side effects were alopecia (30%), mucositis (9%) and fatigue (56%). The latter was partly associated with anaemia as symptoms subjectively reduced after transfusion.

Table 3. Non-hematological toxicity (worst per cycle)

9-AC mg/m²/d	nr pts/ cycles	Nausea 1	2	3	Vomiting 1	2	3	4	Diarrhea 1	2	3	4	Fatigue 1	2	3
0.25 x 7	3/9	5	0	0	3	1	0	0	2	0	0	0	2	2	0
0.25 x 14	3/6	3	3	0	2	0	0	0	2	1	0	0	4	0	0
0.4 x 14	3/5	2	2	0	3	0	0	0	1	0	0	0	4	1	0
0.6 x 14	3/14	2	2	0	1	1	0	0	2	0	0	0	0	1	1
0.84 x 14	8/33	13	1	0	9	2	0	0	4	0	0	0	14	3	3
1.0 x 14	6/18	7	1	1	8	0	0	0	8	4	0	1	11	3	0
1.1 x 14	3/4	0	2	0	2	0	0	0	1	0	1	0	0	1	0

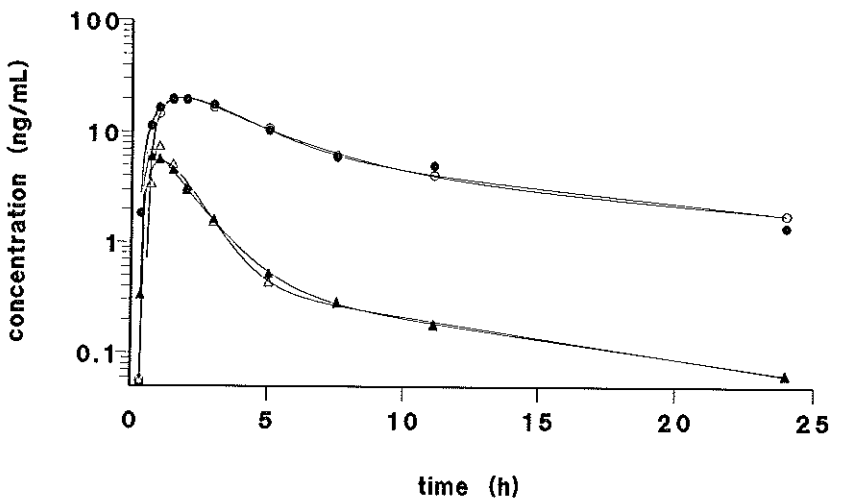

Fig. 2. Representative plasma concentration-time of 9-AC lactone (triangles) and 9-AC total (circles) measured on day 1 (open symbols) and day 8 (closed symbols) of the first treatment course in a single patient following oral administration of 9-AC at a dose level of 0.84 mg/m² in a daily-times fourteen schedule

Pharmacokinetics and Dynamics

Full kinetic data were obtained on day 1 and 6 (7-day schedule) or 8 (14-day schedule) from 29 patients following the administration of 9-AC. Pharmacokinetics could not be determined in three courses as a result of limited sample availability or significant chromatographic interference in the drug assay by an unknown compound.

The plasma concentration-time profiles of 9-AC were similar for all patients studied, with a representative example shown in Figure 2. The absorption of 9-AC lactone after oral drug administration was associated with a lag time of 0.302±0.063 hours (mean±SD; n=29) and maximum peak drug levels at 1.05±0.149 hours. The conversion of 9-AC lactone into the ring-opened carboxylate species in plasma could be demonstrated from the first sample acquired, and peaked at 2.50±0.68 hours after dosing. Eventually, the 9-AC carboxylate accounted for 90.9±3.32% of 9-AC total concentrations, indicating a clear predominant interconversion of lactone to carboxylate. In the majority of patients, concentrations of 9-AC lactone and 9-AC carboxylate, calculated as the difference between total drug and intact lactone, were still above the lower limit of quantitation of our HPLC assay (50 pg/mL) before administration of the drug on the second day. However, the kinetic data and recorded AUC values for the following days of administration were similar to those achieved the first day in the same patient [Table 4]. The resulting intrapatient variability, expressed as the coefficient of variation, in AUC and peak drug levels was extremely small and averaged 8.67% for 9-AC lactone and 10.9% for 9-AC carboxylate. The interpatient variability in the observed pharmacokinetics was large, with coefficients of variation in AUC values as high as 89.5% for 9-AC lactone and 99.0% for 9-AC carboxylate. Elimination of 9-AC from the central plasma compartment was characterised by decay in an apparent tri-exponential manner based on conventional compartmental modelling using weighted least-squares analysis with a weighting factor of 1/Y. The estimated terminal elimination half-life was relatively constant in all subjects, exhibiting mean values of 6.83±2.51 hours for 9-AC lactone and 7.56±1.73 hours for 9-AC carboxylate, and was not dependent on the dose of 9-AC.

Table 4. Summary of the plasma pharmacokinetic parameters of 9-AC lactone and 9-AC carboxylate.

		9-AC lactone					*9-AC carboxylate*				
dose ($mg/m^2/d$)		*n*	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng*h/mL)	$t_{1/2}(\gamma)$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng*h/mL)	$t_{1/2}(\gamma)$ (h)	
0.25	day 1	6	1.06±0.35	2.09±0.82	6.88±4.76	5.22±4.22	2.60±1.19	9.43±3.50	90.8±48.2	6.36±3.24	
	day 8	6	1.15±0.38	2.32±0.83	9.48±3.85	11.8±11.8	3.21±1.42	12.2±4.74	177±127	8.55±5.49	
0.40	day 1	3	0.79±0.15	7.94±7.39	16.2±14.5	8.65±1.25	2.20±0.60	11.8±5.76	89.0±49.4	6.59±2.82	
	day 8	3	1.25±0.22	5.40±3.80	17.4±15.5	6.12±2.16	2.18±0.61	12.1±5.53	137±105	7.04±3.12	
0.60	day 1	4	0.94±0.11	3.43±0.60	8.15±0.76	3.16±0.62	1.63±0.23	14.5±6.93	102±45.1	4.98±1.74	
	day 8	3	0.99±0.01	3.79±0.41	8.69±0.70	5.21±0.96	2.09±0.84	10.2±2.14	83.9±34.1	5.00±2.12	
0.84	day 1	6	0.98±0.27	4.61±1.51	12.9±3.97	10.3±6.77	2.01±0.49	15.2±4.88	174±39.4	9.61±3.73	
	day 8	5	1.03±0.26	4.14±1.66	11.7±2.98	6.80±3.07	2.32±0.62	15.4±1.66	162±36.3	7.23±2.23	
1.0	day 1	7	0.99±0.26	11.0±7.46	31.2±19.1	5.10±2.68	2.36±1.19	25.5±12.7	357±265	8.62±3.56	
	day 8	6	1.10±0.23	8.34±4.43	31.2±27.0	4.55±2.22	2.01±0.50	25.3±16.9	315±327	7.09±2.43	
1.1	day 1	3	1.00±0.01	12.2±6.95	49.3±29.7	6.76±2.26	3.67±0.94	41.8±19.6	578±311	10.1±2.29	
	day 8	3	1.36±0.25	11.8±6.13	48.5±23.8	8.24±1.63	3.72±0.96	37.1±14.7	592±304	9.59±2.85	

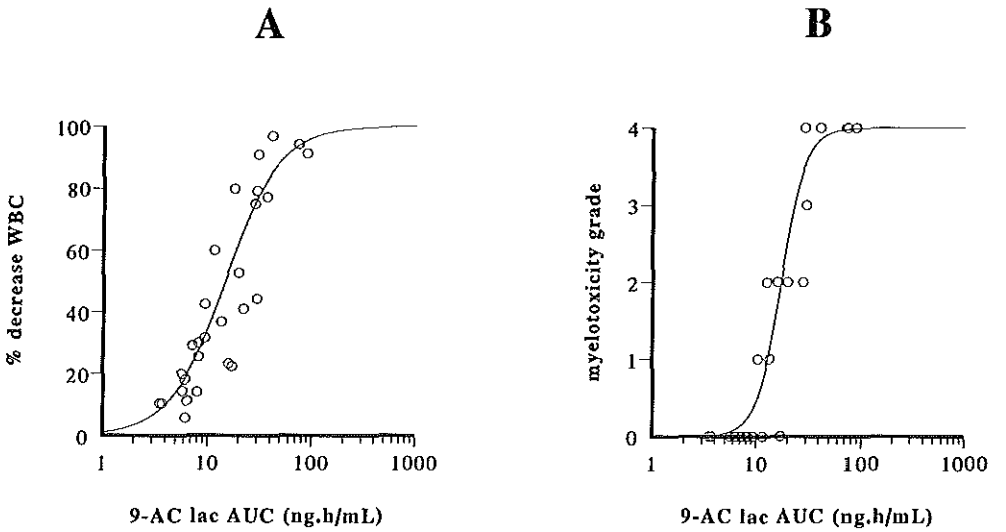

Fig. 3. Correlation between 9-AC area under the plasma concentration-time curve (AUC) of the lactone drug form and the percentage decrease in white blood cell count (WBC) at nadir of the first treatment course (**A**) or the worst observed myelotoxicity grade (**B**). The lines represent the fitting of the data to a sigmoidal maximum-effect model.

Sigmoidal maximum effect modelling of pharmacokinetic and haematological toxicity data revealed that the AUC of 9-AC lactone was significantly correlated (P<0.001) with the percentage decrease in WBC [coefficient of correlation (r^2)=0.86; Figure 3A] and the worst observed myelotoxicity grade according to CTC criteria (r^2=0.93; Figure 3B). In the latter case, the cut-off AUC value associated with development of any myelotoxicity grade 2 or worse was 17.3 ng*h/mL, using the Hill equation and data shown in Figure 3B. Significant correlations were also observed between the AUC of 9-AC lactone and the percentage decrease in neutrophil count (r^2=0.66) and platelet count (r^2=0.83). Pharmacokinetic/pharmacodynamic correlations based on linear and non-sigmoidal maximum effect models were less predictive, as were models based on 9-AC carboxylate or 9-AC total kinetics (data not shown).

Responses

One partial response was observed at a dose of 1.0 mg/m²/d for 14 days in a patient with metastatic colorectal cancer involving the liver lasting for 28 weeks. Disease stabilisation was noted in 6 patients with colorectal, breast, ovarian, and bile duct cancer, and cancer of the appendix lasting for a median of 28 weeks (range 21-50 weeks).

DISCUSSION

Topoisomerase-I inhibitors are a class of drugs with a broad antitumour activity and they form an important addition to the presently available classes of agents. 9-AC is a semisynthetic analogue of the parent topoisomerase-I inhibitor camptothecin. In preclinical studies 9-AC demonstrated activity against several human tumour cell lines and xenografts. In vitro experiments and in vivo studies with human xenografts revealed a better anti-tumour effect with prolonged exposure to topoisomerase-I inhibitors [2-3, 18-22]. Therefore, initial phase I studies using the intravenous formulation of 9-AC focused on schedules with prolonged infusion duration. The availability of an oral formulation of 9-AC for clinical use would enable a more convenient method of prolonged drug administration and provide the opportunity for cost-effective outpatient therapy. Recently, the bioavailability of 9-AC formulated as gelatine capsules in polyethylene glycol [PEG-1000] was explored after oral administration using 9-AC at a dose of 1.5 mg/m² [17]. After oral delivery 9-AC is rapidly absorbed with an overall bioavailability (F) of 48.6±17.6 % (range 24.5-80.4%), indicating significant systemic exposure to the drug. This compares favourable to the oral bioavailability of topotecan (F=30.0%) [23], GI147211 (F=11.3%) [24] and irinotecan (F=12-21%) [25], which may be an advantage with potential pharmacodynamic importance.

The dose limiting toxicity of the oral administration of [PEG-1000] 9-AC given for 14 days every 3 weeks was a combination of thrombocytopenia, febrile neutropenia and diarrhoea. Overall, the haematological toxicity was relatively mild. Grade 3-4 haematological toxicity was observed in 11.2% of the courses and consisted of neutropenia which in 5 of 10 courses occurred in conjunction with thrombocytopenia grade 3-4. Despite the mild myelosuppression, treatment had to be delayed in 23% of the cycles due to prolonged myelosuppression. In contrast, after oral administration of topotecan in different schedules, treatment had to be delayed due

to slow recovery from myelosuppression in only 0-7% of the cycles [26,27]. However, oral administration of 9-nitrocamptothecin also resulted in prolonged myelosuppression requiring treatment delay in 12-25% [28]. Anaemia is a well documented side effect of treatment with topoisomerase I inhibitors, especially of topotecan [26, 27, 29-31]. In our present study anaemia ≥ CTC-grade II occurred in 16.8% of the cycles despite red blood cell transfusions.

Diarrhoea is also a well known side effect of camptothecin and its derivatives. However, the types of diarrhoea appear to differ. Irinotecan administered intravenously induces an acute as well as a delayed type of diarrhoea. The acute diarrhoea seems to be related to a release of vasoactive compounds, whereas the delayed type of diarrhoea is related to the degree of glucuronidation of the irinotecan metabolite SN-38 in the bile. Oral administration of 20-S-camptothecin [32], 9-nitrocamptothecin [28], topotecan [26,27] and 9-AC in the colloid dispersion formulation [14] induced diarrhoea in 24-54% of the cycles. Especially prolonged oral administration (21 days) of topotecan resulted in severe diarrhoea in 22%, which could not be controlled with loperamide. In our present study diarrhoea grade 1-2 was observed in 28% and grade 3-4 in 2% of the cycles. In most patients diarrhoea consisted only of several loose stools not requiring any therapy. Local intestinal effects of camptothecin and its derivatives seem to be responsible for the diarrhoea. However, the exact mechanism is yet unknown. Other non-haematological toxicities were mainly mild.

In the present study, 9-AC demonstrated linear and dose-independent pharmacokinetics over the dose range studied, with the area under the concentration versus time curve (AUC) increasing from 8.18 ± 3.84 to 48.9 ± 26.8 ng*h/mL. Interpatient variability in the concentrations of 9-AC at each of the sample-time points, as well in the AUC was large, with values for the coefficient of variation (CV) as high as 99%. In contrast, intra-patient variability in AUC and peak drug levels was extremely small (CV <10%), indicating that repeated exposure to 9-AC does not result in drug accumulation or alteration of the kinetic profile. These findings are inconsistent with the data reported by Mani et al [14]. In their phase I study of a colloidal-dispersion formulation of 9-AC administered orally, dose-escalation was discontinued because of poor bioavailability and apparent saturable absorption of the drug. The difference in bioavailability may be related to differences in absorption of the two formulations of 9-AC. However, the significant degree of interpatient variability in the pharmacokinetics of 9-AC limits the power to detect a linear dose-AUC relationship especially when only a limited number of patients are studied.

Sigmoidal maximum effect modelling of the pharmacokinetic and pharmacodynamic data of the present study revealed a significant correlation of the AUC of 9-AC lactone with the percentage decrease in leucocyte (r =0.86), granulocyte (r =0.66) and platelet count (r =0.83). Recently, a limited-sampling model was developed for reliable and accurate prediction of the systemic exposure to 9-AC after oral drug administration [33]. By measuring 9-AC plasma concentrations at 3 hour and 11 hour after drug dosing, AUCs of total 9-AC and 9-AC lactone could be predicted. Since the determination of the lactone and lactone plus carboxylate forms of 9-AC in plasma was simplified by using a reversed-phase high-performance liquid chromatography [16], application of the proposed model in clinical routine has become possible. This may enable us to optimise the treatment for any given patient. After determination of the target AUC, treatment can be adjusted on the basis of individual pharmacokinetic characteristics.

Topoisomerase-I inhibitors are S-phase specific drugs. In preclinical studies a better anti-tumour effect was noted after prolonged exposure to the active lactone form of 9-AC above a threshold concentration of 10 nmol/L (3.6 ng/mL) [6,7]. In previous studies of the intravenous administration of 9-AC only in the schedule studying the 24-hour infusion of 9-AC once weekly for 4 weeks every 5 weeks the concentration of 9-AC lactone at steady state reached this threshold value at the recommended dose for phase II studies of 1.65 mg/m²/week [11]. In our present study, concentrations of 9-AC lactone ≥ 10nmol/L were achieved on every treatment day (day 1-14) at the dose level 0.84 mg/m²/day. This might be of therapeutical advantage.

In the present study using the PEG-1000 formulation of 9-AC, administered orally for 14 days every 3 weeks, one partial response was observed in a patient with metastatic colorectal cancer. In another five patients disease stabilisation was achieved. Whether this schedule is the most optimal has to be investigated. Other phase I studies exploring different schedules should be performed.

In conclusion, in this phase I study with oral administration of [PEG-1000] 9-AC given once a day for 14 days, repeated every 3 weeks, the DLT is myelosuppression and diarrhoea. The recommended dose for phase II studies is 0.84 mg/m²/day. However, in view of the substantial interpatient variation in AUC and the availability of a limited sampling model, a pharmacokinetically guided phase II study should be considered.

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Chapter 5

Prediction of the systemic exposure to oral 9-amino-20(S)-camptothecin using single-sample analysis

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ABSTRACT

The purpose of this study was to develop and validate limited-sampling strategies for prediction of the area under the plasma-concentration time curves (AUCs) of the lactone and total (i.e. lactone plus carboxylate) forms of the novel topoisomerase-I inhibitor 9-amino-20(*S*)-camptothecin (9-AC). Complete pharmacokinetic curves for both drug species were obtained from 32 patients who received the drug orally in a clinical phase I setting at dose levels ranging from 0.25 to 1.10 mg/m^2. The concentrations of the lactone and carboxylate forms of 9-AC in plasma were measured by HPLC. Using data from 20 randomly-selected patients, forward-stepwise multivariate-regression analysis was employed to generate modeling strategies incorporating data from 1, 2 or 3 plasma samples. The simultaneous optimal prediction of both 9-AC lactone and 9-AC total AUCs was obtained with sample-time points at 0.33, 3.0 and 11.0 h after drug dosing. Validation of the models on an independent data set, comprising data of the remaining 12 patients demonstrated that 9-AC lactone and 9-AC total AUCs could be predicted sufficiently unbiased and precise using one and two time points: {AUC (ng.h/mL) = 7.103*C_3 + 4.333} for 9-AC lactone and {AUC (ng.h/mL) = 9.612*C_3 + 13.77*C_{11} – 44.11} for 9-AC total, where C_3 and C_{11} represent the 9-AC plasma concentrations in ng/mL at 3 h and 11 h after drug dosing. Application of the proposed models will be valuable in the determination of 9-AC popula-tion pharmacokinetics and permits treatment optimization for patients on the basis of individual pharmacokinetic characteristics through restricted drug monitoring in clinical routine.

INTRODUCTION

9-AC (NSC 603071) is a synthetic derivative of the cytotoxic plant alkaloid camptothecin, that does not produce hemorrhagic cystitis associated with the parent compound [1-3]. The mechanism of action of 9-AC involves stabilization of a cleavable complex between the intranuclear enzyme topoisomerase-I and DNA, thereby inhibiting resealing of enzyme-mediated single-strand breaks required for DNA replication and RNA transcription [4-6]. In preclinical studies, complete remissions have been obtained with 9-AC in nude mice bearing human-tumor xenografts resistant to common antineoplastic agents [7-10]. These animal studies further demonstrated that a prolonged duration of exposure and a higher frequency of administration were necessary to maximize drug efficacy.

Although many schedules of drug administration for camptothecin analogues have been evaluated [1-11], the optimal schedule and route of administration of 9-AC have not yet been defined. Based on favorable results of preclinical studies of 9-AC in mice given on an intermittent-protracted intragastric or oral schedule [12-14], and our observation of significant intestinal absorption of the drug in patients [15], we recently performed a clinical phase I study of oral 9-AC in a daily-times fourteen schedule [16]. The dose-limiting myelotoxicity in that study was demonstrated to be significantly correlated with the AUC of the closed-lactone form of 9-AC, which suggests that kinetic-dynamic relationships of the drug may be important for future dosing strategies [17]. In addition, we have shown that 9-AC delineates dose-independent pharmacokinetics with substantial interindividual differences in the C_{max} as well as in the AUC with both i.v. and oral drug administration [17], further indicating that tailoring 9-AC dosage to a patient's individual needs could be of crucial importance. Accurate estimation of the AUC of 9-AC, however, requires analysis of 9-12 samples after drug administration, which is in general considered inconvenient and expensive. In view of these problems inherent to the drug, it was the aim of the present report to investigate the utility of limited-sampling strategies for prediction of the systemic exposure to oral 9-AC. These strategies would eventually enable estimation of the risk of hematological toxicity and/or convenient use of adaptive-controlled dosing, using a limited number of samples drawn on the first day of oral 9-AC chemotherapy.

PATIENTS, MATERIALS AND METHODS

Patients and Treatment

The pharmacokinetic models were developed and validated in 32 patients with a histologically or cytologically proven malignant solid tumor, that participated in a phase I and pharmacokinetic evaluation of 9-AC given in a repeated-oral schedule [16]. Eligibility criteria included the following: (*1*) age between 18 and 75 years; (*2*) an Eastern Cooperative Oncology Group (ECOG) performance status <2; (*3*) life expectancy of at least 12 weeks; (*4*) adequate hematopoietic (absolute peripheral granulocyte count >2000 μL^{-1} and platelets >100,000 μL^{-1}), hepatic (total bilirubin within normal limits, and aspartate amino-transferase, alanine aminotransferase and alkaline phosphatase <2 times upper normal limits) and renal functions (creatinine: <133 μM); and (*5*) all patient provision of informed written consent according to guidelines of the institutional review board before treatment.

9-AC was provided by Pharmacia & Upjohn (Milan, Italy) as hard gelatin capsules in a matrix containing PEG1000 as excipient (see: Sparreboom *et al* [15] for descriptive characteri-stics of the dosage form). The drug was given with 150-200 mL of water by single-daily oral administration for 14 days at 0.25 mg/m^2 (*n*=8), 0.40 (*n*=3), 0.60 (*n*=3), 0.84 (*n*=7), 1.0 (*n=7)* or 1.1 mg/m^2 (*n*=3). Treatment cycles were repeated every 21 days, and all patients had fasted at least 8 h before and 0.5 h after 9-AC administration.

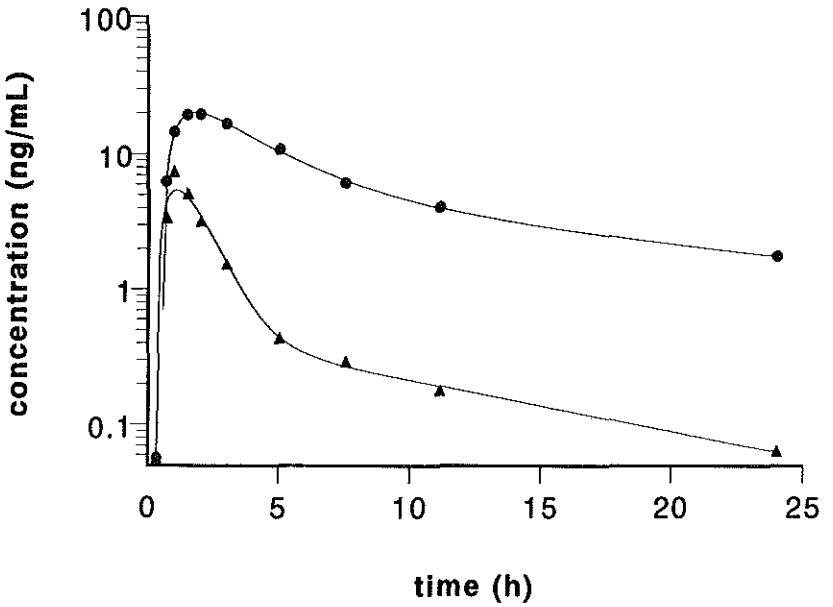

Fig. 1. Representative plasma concentration-time profiles of 9-AC lactone (triangles) and 9-AC total (circles) measured on day 1 the first treatment course in a single patient following oral administration of 9-AC at a dose level of 0.84 mg/m^2 in a daily-times fourteen schedule. Pharmacokinetic curves were fitted to a tri-exponential equation using the Siphar v4.0 computer program (SIMED, Cresteil, France), assuming a three-compartment model for the distribution and elimination of the drug.

Pharmacokinetic Analysis

Blood sampling for 9-AC pharmacokinetic analysis was performed on day 1 of the first chemotherapy course, making 32 pharmacologic data sets evaluable for analysis. Heparinized blood samples were drawn from an indwelling cannula at 0 (predose), 0.33, 0.66, 1, 2, 3, 5, 7.5, 11, and 24 hours after dosing. Determination of 9-AC lactone and 9-AC total (i.e. lactone plus carboxylate) plasma concentrations

was performed by reversed-phase HPLC with fluorescence detection as described in detail previously [18]. The lower limits of quantitation using 1-mL samples were 50 pg/mL and 100 pg/mL for the lactone and total forms, respectively, with the percent deviation (accuracy) and precision of the assay being always less than 10%.

Individual 9-AC lactone concentration-time data were fitted to a tri-exponential equation after extravascular bolus with lag-time using the Powell-minimization algorithm and weighted ($1/y$) least-squares regression analysis, using Siphar v4.0 (SIMED, Creteil, France) as described [15]. The AUC for 9-AC total from time zero to the last measurable level (C_{last}) was calculated by the trapezoidal rule. Extrapolation to infinity was obtained by dividing C_{last} by the elimination rate constant (k_{el}), estimated by a log-linear fit of the terminal phase. The terminal elimination half-life ($t_{1/2}(\gamma)$) was estimated by $\ln 2/k_{el}$. C_{max} was estimated by visual inspection of the semi-logarithmic plot of the concentration-time curve. Interpatient and intrapatient variability in pharmacokinetic parameters was assessed by the coefficient of variation, expressed as the ratio of the standard deviation and the observed mean. Pharmacokinetic parameters were Gaussian distributed as judged by normality plots and the Kolmogorov-Smirnov test.

Model Development and Validation

Limited-sampling models were constructed on a training-data set that comprised 20 complete pharmacokinetic curves from randomly assigned patients. Using this data set, the 9-AC concentrations at each time point (independent variable) were correlated with the corresponding AUC (dependent variable) by univariate linear-regression analysis, as assessed by Pearson's correlation coefficient (r), to find the interval with the optimal single-sample time point. Forward stepwise multivariate-regression analyses were performed to include one or two additional sample-time points, if necessary. Backward-elimination regression analysis, the F-test statistic and the coefficient of determination were used to select the optimal modeling strategy. The models obtained with the training-data set were validated on an independent data set composed of 12 pharmacokinetic curves from the remaining patients. The predictive performance of the developed models was evaluated on the basis of bias (%MPE) and precision (%RMSE), as described [19]. Pearson's correlation coefficient (r) was used to rank the concordance between measured and predicted pharmacokinetic parameters. Differences in patient demographics and pharmacokinetics between the training and validation-data set were evaluated with the two-sided Student's t-test or the Fisher exact probability test, if required. All statistical

computations were performed with the software package Number Cruncher Statistical System (NCSS v5.x; J.L. Hintze, Kaysville, UT, 1992), running on an IBM-compatible computer.

Table 1. Patient demographics

Characteristics	Training set	Validation set
No. of patients	20	12
Age (years)	63 (54-74)[a]	55 (39-74)[a]
Sex (female/male)	8 : 12	7 : 5
ECOG Performance Status	1 (0-1)[b]	1 (0-2)[b]
Primary tumor		
Ovarian	2	3
colo-rectal	8	3
pancreas	1	1
lung (non-small cell)	1	1
breast	1	1
miscellaneous	7	3

[a] mean value with range in parenthesis; [b] median value with range in parenthesis

Table 2. Univariate correlation of 9-AC lactone and 9-AC total concentrations at each sample time point with the corresponding AUC in the training-data set.

Time point	9-AC lactone		9-AC total	
	n	*r*	*n*	*r*
0.33	14	0.389	17	0.241
0.67	19	0.733	20	0.571
1.0	20	0.854	20	0.628
1.5	20	0.849	20	0.754
2.0	20	0.841	20	0.862
3.0	20	0.959	20	0.989
5.0	20	0.921	20	0.943
7.5	19	0.717	20	0.943
11	19	0.699	20	0.931
24	12	0.514	19	0.899

Abbreviations: *n*: number of data sets with complete pharmacokinetic curves; *r* = Pearson's correlation coefficient

RESULTS

Thirty-two patients with various types of solid tumors were entered in a phase I and pharmacokinetic study with 9-AC given orally. Patients were randomly divided in a training-data set (20 patients) and a validation-data set (12 patients) [Table 1]. There were no significant differences in baseline patient characteristics or pharmacokinetic parameters between the two cohorts (not shown). There was large interpatient pharmacokinetic variability in the concentrations of 9-AC at each of the sample-time points, as well as with the AUC, with values for the coefficient of variation up to 99% [17]. The 9-AC lactone and total concentrations at each of the sample-time points were correlated with the AUC using the training-data set by univariate-regression analysis [Table 2]. Overall, Pearson's correlation coefficients ranged from 0.241 to 0.989, with the best correlations observed at the 3-h sample-time point for both the lactone and total forms of the drug, which was thus considered the most informative variable. The measured drug concentrations were subsequently subjected to multivariate-regression modeling, with a restriction to models with one or two additional time points. In the bivariate models, sample-time couples with the highest correlation and lowest %RMSE were composed of drug concentrations at 3 h and 0.33 h, and 3 h and 11 h for AUCs of the lactone and total respectively [Table 3]. Strategies for estimation of the 9-AC total AUC employing more conveniently timed samples were all associated with significantly worse predictive ability as compared to the modeling that included the 3 and 11 h samples. For example, models based on inclusion of the 5 and 7.5-h samples demonstrated correlation coefficients of only 0.969 and 0.971 with corresponding %RMSE values as high as 63.4% and 62.2%, respectively. The best models with three-time points included strategies with addition of the 11-h and 0.33-h concentration. In the training-data set, all models demonstrated little bias with the absolute value of the %MPE ranging from 0.10 to 0.64% of the measured AUC [Table 3].

Concentrations of 9-AC for use in univariate and bivariate models to predict the terminal elimination half-life showed poor correlation coefficients ($r \leq 0.01$) combined with low accuracy (%RMSE≥46.1%), and hence were not used in the limited-sampling model validation. We also considered the use of 9-AC lactone concentrations for prediction of 9-AC total AUC or 9-AC carboxylate AUC, as we had previously demonstrated a significant linear relationship ($r=0.87$) between 9-AC lactone and 9-AC total AUCs, with the drug administered orally at a dose level of 1.50 mg/m^2 [15].

Table 3. Limited-sampling models for prediction of 9-AC lactone AUC (ng.h/mL) and 9-AC total AUC (ng.h/mL) in patient plasma.

Modeling-strategy		Training set			Validation set		
		r	%MPE	%RMSE	*r*	%MPE	%RMSE
9-AC lactone							
A	$AUC = 7.103*C_3 + 4.333$	0.959	-0.10	8.75	0.943	-0.71	8.18
B	$AUC = 1.031*C_{0.3} + 6.212*C_3 + 4.858$	0.982	+0.21	5.11	0.972	-0.15	8.24
C	$AUC = 1.381*C_{0.3} + 6.711*C_3 - 1.731*C_{11} + 4.135$	0.989	+0.21	4.55	0.977	-0.23	6.05
9-AC total							
D	$AUC = 16.12*C_3 - 55.54$	0.989	-0.30	35.6	0.854	-7.8	30.1
E	$AUC = 9.612*C_3 + 13.77*C_{11} - 44.11$	0.991	-0.38	13.4	0.941	-4.8	14.5
F	$AUC = 1.561*C_{0.3} + 9.776*C_3 + 1.571*C_{11} - 45.79$	0.993	+0.64	11.1	0.942	-4.9	15.1

Abbreviations: *r* = Pearson's correlation coefficient; %MPE = percentage mean predictive error; %RMSE = percentage root mean-squared predictive error; AUC: area under the plasma concentration-time curve; $C_{0.3}$, C_3 and C_{11}: plasma concentrations of 9-AC in ng/mL at 0.33, 3.0 and 11 h, respectively, after oral drug administration.

In both cases, the best models included three sample-time points with acceptable correlation coefficients (up to r=0.91), but in the end were rendered highly inaccurate, with values for the %RMSE ranging from 106 to 288%.

Prospective evaluation of the proposed models was performed in the validation-data set composed of the remaining 12 patients. In the case of 9-AC lactone, all three models had minor bias (%MPE range: -0.71 to -0.23%) and excellent precision (%RMSE range: 6.05 to 8.24%), indicating that the addition of a second and third variable did not substantially improve the model [Table 3]. Furthermore, a strong linear correlation was observed between the measured and predicted 9-AC lactone AUCs in all models [Figure 2A]. There was some bias noticed in the single-point model for prediction of 9-AC total AUC with an absolute value of the %MPE of 7.8%, accompanied by a %RMSE of ≥30%. Validation of the bivariate and trivariate models, however, resulted in predictions of 9-AC total AUC that were sufficiently unbiased and precise to warrant clinical application [Table 3 and Figure 2B].

As in all validated models computations were made without dose-normalization, we also performed an additional analysis with the univariate and bivariate models by including dose in milligrams per square meter of body-surface area (mg/m^2) in the AUC prediction. For both 9-AC lactone and 9-AC total models, similar results were obtained with and without dose as additional variable, as indicated by equivalent correlation coefficients and values for the %RMSE of 8.24 *versus* 8.12 and 14.5 *versus* 16.2, respectively, for lactone and total forms in the two-sample models.

DISCUSSION

In the present study we have shown that several limited-sampling strategies can be developed for reliable and accurate prediction of the systemic exposure to 9-AC after oral drug administration. Using stepwise forward regression analysis, univariate and bivariate models for independent estimation of 9-AC lactone AUC values based on one and two sample-time points, respectively, were developed and tested for the statistical best fit. Results of models using three time points did not show improved results in terms of bias and precision. In view of logistical and economical reasons, the single-sample strategy is clearly preferred to those using two samples, particularly with respect to application in large-scale studies of population pharmacokinetics, which require methods that are both accurate and practical in a daily routine.

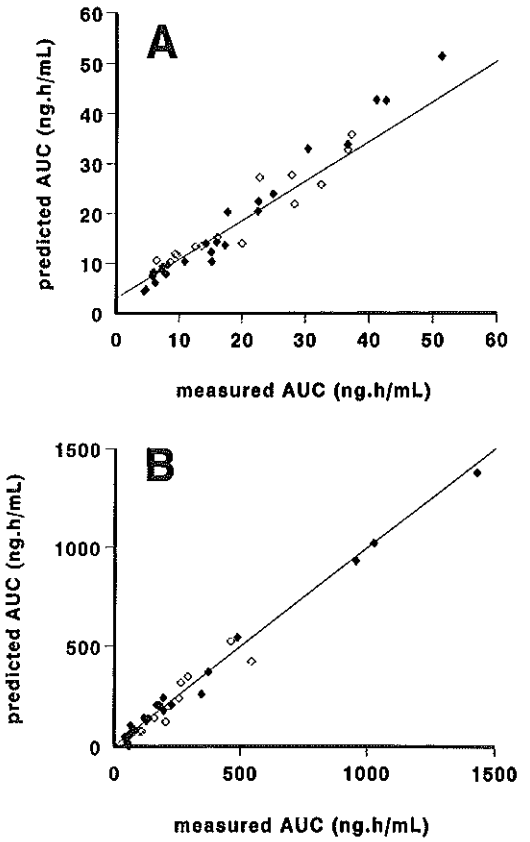

Fig. 2. Correlation between the measured 9-AC lactone AUC (**A**) or 9-AC total AUC (**B**) and the AUC predicted from modeling-strategies incorporating 1 or 2 plasma samples for lactone and total forms, respectively, in the training-data set (closed symbols) and the validation-data set (open symbols). Pearson's correlation coefficients in the training and validation-data set were, respectively, 0.959 and 0.943 (**A**), and 0.991 and 0.941 (**B**). The solid line represents the line of identity.

Pharmacokinetic studies with camptothecin analogues, including 9-AC, were previously shown to be complicated by a chemical, pH-dependent instability of the terminal E-lactone ring of the compounds, generating a ring-opened carboxylate, which is over 1000-fold less active as an inhibitor of topoisomerase-I [20]. The importance of this non-enzymatic hydrolysis reaction of the lactone moiety in the pharmacology and toxicology of 9-AC is not yet fully understood. The clinical

pharmacokinetics of the lactone and total (i.e. lactone *plus* carboxylate) forms of 9-AC has been extensively studied in patients receiving the drug by intravenous infusion over 72 h [21-24]. From these studies, pharmacodynamic correlations have been suggested between the 9-AC lactone steady-state concentration in plasma and the degree of leukocytopenia. We have recently observed similar relationships for 9-AC lactone AUC and myelotoxicity with the drug administered orally [17], which is in line with the lactone being the pharmacological active species of the drug. Applying a limited-sampling strategy, questions of 9-AC pharmacodynamic outcome relating to lactone-carboxylate interconversion could be answered conveniently in prospective studies. It is noteworthy, however, that model prediction for 9-AC total AUC was less precise than that of models for the lactone, including slight bias in the validation-data set toward underestimation of the AUC with all three strategies. Nevertheless, the best model for prediction of the 9-AC total AUC (including two sample-time points) still can be considered acceptable and clinically useful.

In recent years, limited-sampling strategies have also been developed for several other antineoplastic agents [25], including the camptothecin analogues irinotecan (CPT-11; Campto) [26-32] and topotecan (Hycamtin) [33-34]. In some of these models, drug-dose levels as measured in milligrams per square meter of body-surface area (mg/m^2) are included in the AUC estimate, by dose-normalization of each patient's pharmacokinetic data to a constant dose. The rationale for this procedure is to be able to discriminate between variability in dose and interindividual variation in pharmacokinetics as the primary cause for variability in measured concentrations. To test whether the administered dose would improve the validity of the presented models, this parameter was also included in the single and two-sample strategies by multivariate-regression analysis. For both approaches, correlation coefficients remained unchanged, whereas the %MPE and the %RMSE in the training as well as the validation-data set of the two-sample approach slightly decreased by an absolute maximum of 0.4%. Thus, the use of the dose in mg/m^2 as an additional independent variable did not contribute significantly to prediction of the AUC for oral 9-AC. This conclusion is consistent with our recent observation that oral 9-AC delineates linear and dose-independent pharmacokinetics within the examined dose interval, *viz.* 0.25-1.5 mg/m^2 [17].

The presented models have proven both valid and acceptable in terms of bias and precision in a heterogeneous group of cancer patients given 9-AC over a wide range of dose levels. Furthermore, our current finding of extremely low intrapatient variability in oral 9-AC pharmacokinetics indicates that the models are valid also for

prediction of the AUC with repeated administration of the drug. The clinical significance and the ultimate utility of the models, however, remain to be explored in future studies. In addition, use of the models in chemotherapy regimens other than the one investigated in the current study should be done with caution, as the potential for pharmacokinetic interactions between 9-AC and co-administered drugs, e.g. phenytoin, phenobarbital and/or valproic acid [35].

In conclusion, the feasibility and validity of prediction of the systemic exposure to oral 9-AC using limited-sampling strategies were demonstrated. The optimal strategies included an univariate model with one sample-time point at 3 h for 9-AC lactone AUC, and a bivariate model with two sample-time points at 3 h and 11 h for 9-AC total AUC. Application of the proposed models will be valuable in the determination of 9-AC population pharmacokinetics and investigat-ions on the clinical implications of the 9-AC lactone-carboxylate interconversion with regard to pharmacodynamics. In addition, with the strategies routine drug monitoring is feasible, thereby allowing treatment optimization for a given patient on the basis of individual pharmacokinetic characteristics. This could be achieved after oral drug administration of an appropriate starting dose of 9-AC (e.g. the maximum-tolerated dose in a 14-day schedule of 1.0 mg/m^2) by measuring the 9-AC lactone plasma concentration at 3 h after drug dosing. Using the limited-sampling model and the linear-regression relationship between drug dose and AUC [17], the optimal dose leading to the target AUC determined according the toxicity considered acceptable, can then be calculated.

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Chapter 6

Clinical pharmacokinetics of encapsulated oral 9-aminocamptothecin in plasma and saliva

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ABSTRACT

Objective: To study the pharmacokinetics and pharmacodynamics of the novel topoisomerase I inhibitor and antitumor agent, 9-amino-20(*S*)-camptothecin (9-AC), in patients with solid tumors after repeated oral dosing.

Methods: Thirty-two cancer patients received oral 9-AC formulated in capsules with polyethylene glycol-1000 as excipient at doses that ranged from 0.25 to 1.5 mg/m^2/day. Serial plasma and saliva samples were obtained on days 1 and 6 or 8 of the first cycle and analyzed for the lactone and carboxylate forms of 9-AC by HPLC.

Results: 9-AC demonstrated linear and dose-independent pharmacokinetics, with extremely small intrapatient kinetic variability (coefficient of variation: <10%). However, interpatient variability in plasma pharmacokinetics was large (coefficient of variation:99%). The relative extent of lactone to carboxylate interconversion was large (>90%), and predictable from individual pretreatment serum albumin values (p=0.0099). The 9-AC concentration ratio in plasma and saliva was strongly patient dependent, and highly variable around a mean value of <0.8, suggesting that saliva is an unreliable matrix for kinetic monitoring. The area under the curve of the lactone form of 9-AC was significantly correlated with the dose-limiting hematological toxicity (p<0.001).

Conclusion: Our data indicate that the large interindividual pharmacodynamic variability in response to 9-AC is mainly caused by a variability in kinetic characteristics, suggesting that a kinetic-dynamic guided study design is warranted in future clinical investigations.

INTRODUCTION

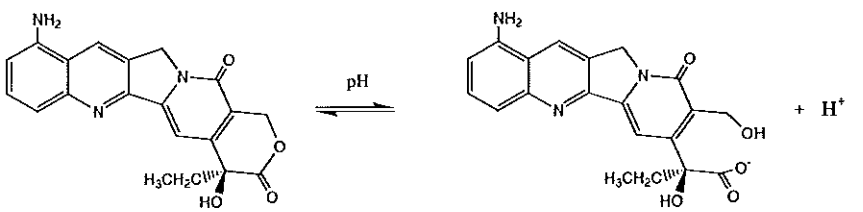
In the early 1970s, camptothecin, a plant alkaloid extract from the bark and wood of the Chinese tree *Camptotheca acuminata*, was demonstrated to possess antineoplastic activity [1]. Analogs of camptothecin belong to a family of anticancer agents with a unique mechanism of action, which is based on reversible inhibition of DNA topoisomerase I [2-3].

Despite the cytotoxicity of the compound further development was halted because of a number of severe and unpredictable side-effects observed in early clinical trials [4-6]. The subsequent search for less toxic analogs of camptothecin resulted in the discovery of irinotecan, topotecan, 9-amino-20(S)-camptothecin (9-AC), 9-nitrocamptothecin, DX-8951f and GI147211. The first two of these, irinotecan and

topotecan, were recently registered for the treatment of colorectal and ovarian cancer, respectively.

In aqueous solutions, camptothecins are unstable and undergo a rapid, pH-dependent, non-enzymatic hydrolysis of the terminal lactone ring to form the more water-soluble, ring-opened carboxylate form [Figure 1] [7]. The presence of the intact terminal lactone ring is thought to be essential for the topoisomerase I inhibition [8]. The closed lactone ring predominates at acidic pH, whereas in human plasma, the equilibrium between these two species greatly favors formation of the carboxylate form, partly because of the physiologic pH and the preferentially binding of this form to albumin [9,10]. The ratio of the lactone form to the total drug concentration at steady state in plasma is different for each camptothecin analog, which might have important pharmacokinetic and pharmacodynamic implications.

In preclinical studies 9-AC demonstrated activity against human colon, breast, prostate, non-small cell lung cancer and melanoma xenografts [11-14]. Preclinical *in vivo* data suggested that duration of exposure to 9-AC lactone above a certain threshold concentration (10 n*M*) and frequency of administration were essential for antitumor activity [15,16].



lactone form carboxylate form

Fig. 1. Chemical structure and pH dependent interconversion of 9-AC lactone and 9-AC carboxylate.

Based on its preclinical activity, 9-AC appeared to merit evaluation as an antineoplastic agent. To mimic the preclinical studies, initial Phase I studies using the intravenous formulation of 9-AC focused on schedules with prolonged infusion duration of 24-120 hr [17-21], or a continuous infusion for 21 days every 4 weeks [22]. Pharmacokinetic data obtained during these studies showed marked interpatient variability. Steady state plasma concentrations of 9-AC lactone greater than 10 n*M* were achieved only in the Phase I study of the 24-hour infusion of 9-AC at the dose recommended for further Phase II studies (i.e. 1.65 mg/m^2). When 9-AC was administered as a 72-hr infusion once every 2 or 3 weeks, the maximal tolerated dose was 35-54.2 μg/m^2/hr. The dose limiting toxicity consisted of neutropenia in combination with thrombocytopenia and correlated to the steady state 9-AC lactone concentration.

Recently, we reported that 9-AC demonstrated rapid absorption in humans after oral delivery with an overall bioavailability of approximately 50% [23]. In the present report, we present a comprehensive analysis of the plasma pharmacokinetics of the lactone and carboxylate forms of 9-AC in cancer patients receiving the drug orally over a wide range of dose levels, with special focus on pharmacokinetic-pharmacodynamic characteristics. In order to assess the clinical usefulness of salivary monitoring of 9-AC for kinetic modeling, paired plasma and coinciding unstimulated saliva samples were collected in a limited number of patients.

PATIENTS AND METHODS

Patient population

The patients, from whom pharmacokinetic curves were obtained, participated in an oral bioavailability study of 9-AC and/or in a Phase I trial of oral 9-AC administered daily for 7-14 consecutive days every 3 weeks. Treatment plans and detailed clinical profiles have been documented elsewhere [23]. Eligibility criteria included a histologically or cytologically confirmed diagnosis of a solid malignant tumor not amenable to established forms of treatment. All patients had an adequate hematopoietic (absolute peripheral granocyte count ≥2.0 x 10^9/L and platelet count ≥100 x 10^9/L), hepatic (bilirubin within normal limits, and serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase ≤2.5 times normal limit) and renal (serum creatinine <133 μ*M*) function. Other eligibility criteria included the following: age between 18 and 75 years; Eastern Cooperative Oncology

Group performance status ≤2; estimated life expectancy ≥12 weeks; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas or mitomycins); no previous therapy with other camptothecins and/or intensive ablative regimens. Specific exclusion criteria included significant gastrointestinal dysfunction that could alter absorption or motility, and chronic treatment with corticosteroids. Concomitant administration of H_2-antagonists, antacids, proton-pump inhibitors and non-steroidal anti-inflammatory drugs were avoided. All patients gave written informed consent before study entry.

Treatment plan and Dose Escalation

The oral formulation of 9-AC was supplied by Pharmacia & Upjohn (Nerviano, Italy) as hard gelatin capsules that contained 0.10, 0.25 or 1 mg of the active drug and polyethylene glycol-1000 (PEG1000) as excipient [23], and were stored at room temperature.

A detailed description of the preparation and rationale for composition of the formulation has been described earlier [23]. Patients received 9-AC orally with 150-200 mL of water at dose levels of 0.25, 0.40, 0.60, 0.84, 1.0 or 1.1 mg/m^2/day for 7 or 14 consecutive days, or as a single dose of 1.5 mg/m^2. Intrapatient dose escalation in the phase I trial was not permitted. Weekly evaluation of the patients included a clinical history, physical examination, toxicity assessment according to common toxicity criteria (CTC), and serum chemistry. A complete blood cell count with differentiation was determined twice weekly.

Sample collection and drug analysis

Serial blood samples were collected in heparinized tubes from an indwelling venous catheter at 0, 0.33, 0.67, 1, 1.5, 2, 3, 5, 7.5, 11 and 24 hours after administration on days 1 and 6 or 8 of the first treatment course. In a limited number of patients, unstimulated saliva samples were obtained at coinciding time points. In one patient with a malignant pleural effusion additional pleural fluid sampling was performed to assess the influence of the pleural effusion on the plasma pharmacokinetics. Immediately after sampling, aliquots of plasma (separated at 4°C), pleural fluid and saliva were frozen at -80°C, and analyzed later for 9-AC lactone and 9-AC total drug (i.e. lactone *plus* carboxylate) with use of a validated reversed-phase high-performance liquid chromatographic method as described previously [24].

Drug concentrations in patient plasma samples were calculated using interpolation of the corresponding regression analysis. Specimens with drug levels exceeding the upper range of the calibration curve were reanalyzed upon appropriate dilution with drug-free plasma. Saliva and pleural fluid samples were diluted 4-fold in drug-free plasma and analyzed with use of the same analytical assay, with minor modifications. The lower limits of detection of the assays were 0.05 ng/mL in plasma and 0.4 ng/mL in pleural fluid and saliva.

Data analysis

Plasma-concentration data were analyzed by both non-compartmental and compartmental analysis using the Siphar version 4.0 software package (SIMED, Créteil, France). The model-independent pharmacokinetic parameters included the maximum plasma concentration (C_{max}) and the time to reach the peak concentration (t_{max}). Initial parameter estimates were obtained by an automated peeling algorithm based on the Powell method to three compartments, which yielded the best statistical fit as determined by Akaike's information criterion and the *F*-test. The AUC values were calculated based on the best fitted curve, as were the disposition half-lives ($t_{1/2}$). The apparent absorption rate constant (k_a) was obtained through numerical point-area deconvolution. Relationships between the AUC and pharmacodynamic outcome were evaluated with (log-)linear and (non-)sigmoidal-maximum effect modeling using Siphar and NCSS version 5.0 (Dr.Jerry Hintze, East Kayesville, UT).Within individual patients, myelosuppression was described as the continuous variable, consisting of percentage decrease in white blood cell count (WBC), absolute neutrophil count (ANC) and platelet count (PLT). The relative hematological toxicity was defined as : % decrease = (pretherapy value- nadir value)/(pretherapy value)*100. Only the first course of each patient was taken into consideration to avoid potentially confounding bias due to cumulative toxicity. All data were fitted to a sigmoidal maximum effect (E_{max}) model based on the modified Hill equation, as follows: $E=E_0+E_{max}*[(KP^{\gamma})/(KP^{\gamma}+KP_{50}^{\gamma})]$. In this equation, E_0 is the minimum reduction possible, fixed at a value of 0, E_{max} is the maximum response, fixed at 100, KP is the pharmacokinetic parameter of interest, KP_{50} the value of the pharmacokinetic parameter predicted to result in half of the maximum response, and γ is the Hill constant describing the sigmoidicity of the curve. Models were evaluated for goodness of fit by minimisation of sums of the squared residuals and by reduction of the estimated coefficient of variation for fitted parameters.

Significance of the relationships were assessed by construction of contingency tables with subsequent χ^2 analysis.

Table 1. Patient characteristics

Characteristic		No. of patients
No. Entered		32
No. Assessble for toxicity		30
Age, years		
Median	59	
Range	29-74	
Sex		
Female		14
Male		16
Performance status		
Median	1	
Range	0-2	
Tumor type		
Colorectal		14
Ovarian		5
Sarcoma		2
Mesothelioma		2
Lung (non-small cell)		2
Miscellaneous		5
Previous treatment		
Chemotherapy		16
Radiation		2
Chemotherapy and radiation		10
None		2

RESULTS

Demographic characteristics of all 32 patients who had blood sampling for pharmacokinetic analysis are shown in Table 1. Ten patients completed the oral bioavailability study and subsequently participated in the phase I and pharmacokinetic study of oral 9-AC. Hence, pharmacokinetic data were obtained in 42 courses. Plasma pharmacokinetics could not be determined in 1 course on day 1 and in 2 courses on day 8 as a result of limited sample availability or significant chromatographic interference in the drug assay by an unknown compound.

Twenty-seven patients were assessable for pharmacodynamic analysis and 30 patients were assessable for toxicity.

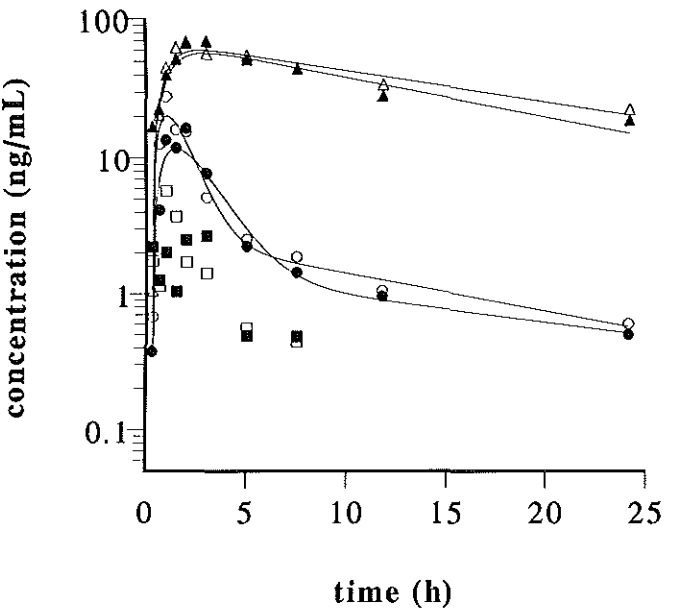


Fig. 2. Representative concentration-time profiles of 9-AC lactone (circles) and 9-AC total (triangles) in plasma and in saliva (rectangle) measured on day 1 (open symbols) and day 8 (closed symbols) of the first treatment course in a single patient following oral administration of 9-AC at a dose level of 0.84 mg/m²/day in a daily-times fourteen schedule. All pharmacokinetic curves were fitted to a tri-exponential equation assuming a three-compartment modal for the distribution and elimination of the drug.

The plasma concentration-time profiles of 9-AC lactone and 9-AC carboxylate were similar for all patients studied, with representative examples shown in Figure 2. The pharmacokinetics of both species of 9-AC could be best described with a three-compartment model. The kinetic parameters obtained by means of this model are presented in Tables 2 and 3.

Table 2. Model-dependent pharmacokinetics of 9-AC_{lac} after oral drug administration of 9-AC.

Dose (mg/m^2/day)	0.25	0.40	0.60	0.84	1.0	1.1	1.5
n	6	3	4	6	7	3	12
k_a (1/h)	ND	ND	ND	ND	ND	ND	3.03±0.90
t_{lag} (h)	0.33±0.13	0.32±0.05	0.38±0.14	0.28±0.05	0.29±0.05	0.17±0.13	0.24±0.13
t_{max} (h)	1.06±0.35	0.79±0.15	0.94±0.11	0.98±0.27	0.99±0.29	1.00±0.01	1.17±0.33
C_{max} (ng/mL)	2.09±0.82	7.94±7.39	3.43±0.60	4.61±1.51	11.0±7.46	12.2±6.95	6.82±2.98
$t_{1/2}(\alpha)$ (h)	0.25±0.20	0.13±0.11	0.23±0.07	0.24±0.11	0.24±0.09	0.36±0.07	0.38±0.21
$t_{1/2}(\beta)$ (h)	0.68±0.40	0.82±0.33	0.54±0.20	0.73±0.33	0.76±0.51	0.82±0.16	1.13±0.59
$t_{1/2}(\gamma)$ (h)	5.22±4.22	8.65±1.25	3.66±0.55	10.3±6.77	5.10±2.68	6.76±2.26	12.6±4.20
$AUC_{0-\infty}$ day 1 (ng.h/mL)	6.88±4.76	16.2±14.5	8.15±0.76	12.9±3.97	31.2±19.1	49.3±29.7	31.9±14.3
$AUC_{0-\infty}$ day 8 (ng.h/mL)	9.48±3.85	17.4±15.5	8.69±0.70	11.7±2.98	31.2±27.0	48.5±23.8	ND
intra %CV of AUC	7.31	7.31	4.44	9.74	4.96	6.48	ND
inter %CV of AUC	69.2	89.5	89.2	30.7	61.1	60.3	44.8
% of 9-AC_{total} AUC	7.18±2.33	12.9±4.20	8.76±3.49	6.98±1.72	10.2±4.94	8.67±3.22	7.30±3.22

Abbreviations: *n*, number of patients; k_a, absorption rate constant; t_{lag}, lag time; t_{max}, time to peak plasma levels; C_{max}, maximum plasma concentrations; $t_{1/2}(i)$, half-life of the *i*-th disposition phase; AUC, area under the plasma concentration *versus* time curve; CV, coefficient of variation.

Table 3. Model-dependent pharmacokinetics of 9-AC_{car} after oral drug administration of 9-AC.

Dose (mg/m^2/day)	0.25	0.40	0.60	0.84	1.0	1.1	1.5
n	6	3	4	6	7	3	12
t_{lag} (h)	0.41±0.20	0.34±0.04	0.39±0.13	0.31±0.02	0.35±0.15	0.34±0.06	0.31±0.11
t_{max} (h)	2.60±1.19	2.20±0.60	1.63±0.23	2.01±0.49	2.36±1.19	3.67±0.94	2.39±1.05
C_{max} (ng/mL)	9.43±3.50	11.8±5.76	14.5±6.93	15.2±4.88	25.5±12.7	41.8±19.6	35.4±19.2
$t_{1/2}(\alpha)$ (h)	0.55±0.50	0.40±0.30	0.20±0.20	0.18±0.26	0.24±0.38	0.96±0.18	1.04±0.78
$t_{1/2}(\beta)$ (h)	1.78±0.67	1.12±0.44	1.00±0.69	1.07±0.55	0.78±0.51	4.26±1.60	2.83±1.96
$t_{1/2}(\gamma)$ (h)	7.50±3.96	6.59±2.82	4.98±1.74	9.61±3.73	8.62±3.57	10.1±2.29	12.4±6.48
$AUC_{0-\infty}$ day 1 (ng.h/mL)	90.8±48.2	89.0±49.4	102±45.1	174±39.4	357±265	578±311	510±354
$AUC_{0-\infty}$ day 8 (ng.h/mL)	177±127	137±105	83.9±34.1	162±36.3	315±327	592±304	ND
intra %CV of AUC	13.9	27.7	1.63	8.52	6.49	10.5	ND
inter %CV of AUC	53.1	55.5	44.3	22.6	99.0	53.9	69.4
% of 9-AC_{total} AUC	92.8±2.33	87.1±4.20	91.2±3.49	93.0±1.72	89.8±4.94	91.3±3.22	92.7±4.15

Abbreviations: *n*, number of patients; t_{lag}, lag time; t_{max}, time to peak plasma levels; C_{max}, maximum plasma concentrations; $t_{1/2}(i)$, half-life of the *i*-th disposition phase; AUC, area under the plasma concentration *versus* time curve; CV, coefficient of variation.

The absorption of 9-AC lactone after oral administration was rapid with a lag time of 0.29±0.07 hr (mean ± SD; *n*=41), maximum peak drug levels at 0.99±0.12 hr, and a mean absorption rate constant of 3.03±0.90 h^{-1}. In the first plasma samples the ring-opened carboxylate form of 9-AC was already detectable. Maximal plasma concentrations of 9-AC carboxylate were reached at 2.41±0.64 hr after dosing. Eventually, the 9-AC carboxylate accounted for 91.1±2.11% of 9-AC total drug concentrations, indicating a clear predominant conversion of lactone to carboxylate. Elimination of 9-AC from the central plasma compartment was characterized by a decay in an apparent tri-exponential manner based on conventional compartment modeling using weighed least-squares analysis with a weighting factor of 1/*Y*. The mean values for the linear segments of 9-AC lactone were $t_{1/2}$ (α): 0.26 hr (range, 0.13-0.38 hr), $t_{1/2}$(β): 0.78 hr (range, 0.54-1.13 hr) and $t_{1/2}$(γ): 7.47 hr (range, 3.66-12.6 hr). No significant quantitative differences were observed between the decay kinetics of 9-AC lactone and 9-AC carboxylate.

The kinetic data and recorded AUC values for the following days of administration were similar to those achieved the first day in the same patient [Tables 2 and 3]. Hence, the resulting intrapatient variability in AUC and peak drug levels, expressed as the coefficient of variation, was extremely small and averaged 8.67% for 9-AC lactone and 10.9% for 9-AC carboxylate. The interpatient variability in the observed pharmacokinetics, however, was large, with coefficients of variation in AUC values as high as 89.5% for 9-AC lactone and 99.0% for 9-AC carboxylate.

Over the total dose range studied, 9-AC lactone and 9-AC total demonstrated linear and dose-independent pharmacokinetics [Figures 3*A* and *B*, respectively]. No significant relationship was observed between the AUCs of 9-AC total and that of the pharmacologically active species, 9-AC lactone (not shown). The interpatient variation in the equilibrium ratio of 9-AC lactone and 9-AC carboxylate could be explained in part by a individual differences in pretreatment serum albumin levels, for which a significant correlation with the AUC ratio of 9-AC lactone and 9-AC carboxylate could be demonstrated (r=0.471, p=0.0099). This finding clearly indicates that separate monitoring of 9-AC lactone and 9-AC carboxylate concentrations is mandatory to relate drug levels to pharmacodynamic outcome in patients treated with oral 9-AC.

Salivary drug monitoring was evaluated as an option for determining the AUC of 9-AC lactone. The 9-AC lactone concentration ratio in plasma and unstimulated saliva was strongly patient-dependent and highly variable around a mean value of ~1.4, suggesting that saliva is an unreliable matrix for pharmacokinetic analysis of

this drug [Figure 2]. To determine the impact of a pleural effusion on the pharmacokinetics of 9-AC, plasma and pleural effusion samples were obtained for drug analysis in a single patient with a malignant pleural effusion. The mean pleura versus plasma concentration ratio of 9-AC lactone was 4.95%±2.32 (mean ± SD; range, 0.7-6.8%).

These data indicate that pleural effusion does not constitute a major pharmacokinetic compartment for this drug.

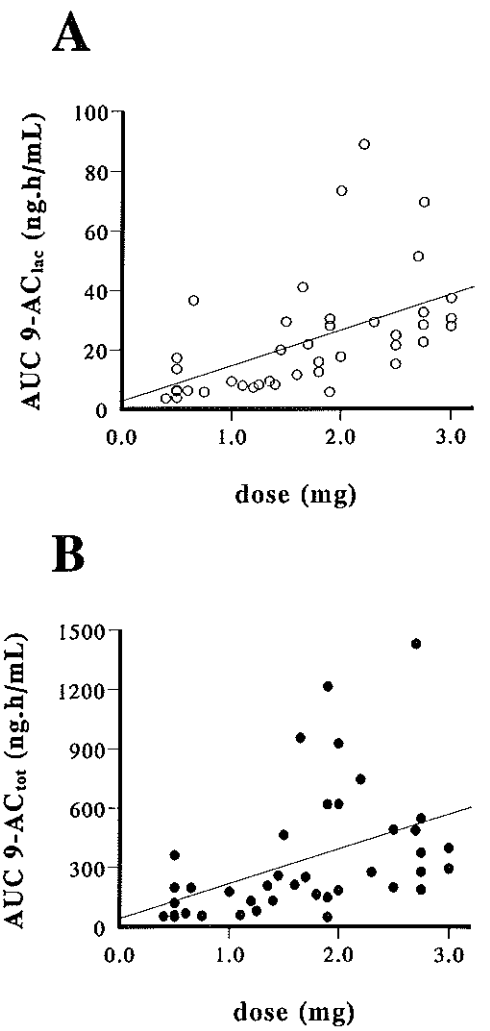


Fig 3. Absolute dose of 9-AC plotted versus AUC of 9-AC lactone (**A**) and 9-AC total (**B**).

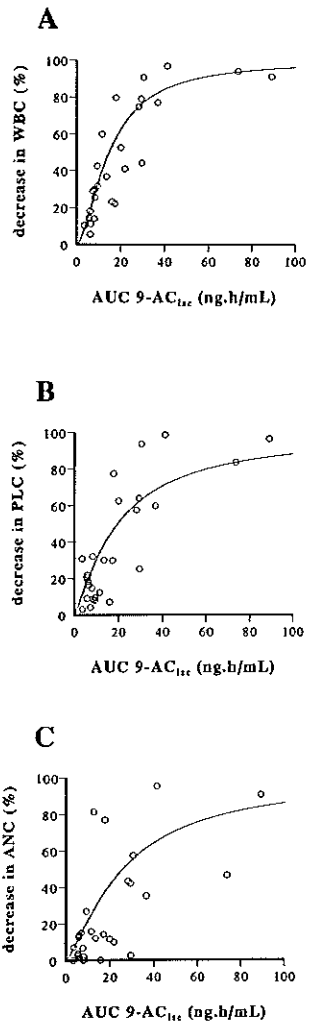


Fig. 4. Correlation between the area under the plasma concentration-time curve (AUC) of 9-AC lactone and the percentage decrease in white blood cells (WBC) at nadir of the first treatment course **(A)**, in platelets (PLC) **(B)** and in neutrophiles (ANC) **(C)**. The lines represent the fitting of the data to a sigmoidal maximum-effect model.

The pharmacokinetic data obtained from 27 patients were plotted against the percentage decrease in white blood cell count (WBC), platelet count (PLT) and absolute neutrophil count (ANC), at nadir relative to the pretreatment value. Four different models, based on linear, log-linear, maximum effect (E_{max}), and sigmoidal

E_{max} fitting, were compared for their ability to describe the data. Using sigmoidal E_{max} modeling of the pharmacokinetic and hematological toxicity data significant correlations between the AUC of 9-AC lactone and the percentage decrease in WBC (r=0.86; p<0.001; Fig. 4*A*), percentage decrease in PLT (r=0.83; p<0.001; Fig. 4*B*) and percentage decrease in ANC (r=0.66; p<0.001; Fig. 4*C*) could be demonstrated. In addition, the worst observed myelotoxicity grade according to common toxicity criteria (CTC) in the entire patient population correlated with the AUC of 9-AC lactone (r=0.93; p<0.001; not shown). The development of any myelotoxicity grade 2 or worse was associated with an AUC of 9-AC lactone ≥17.3 ng*h/mL, using the Hill equation and data shown in Fig. 3*B*. Pharmacokinetic/pharmacodynamic relationships based on (log-)linear and non-sigmoidal E_{max} models were less predictive, as were models based on 9-AC carboxylate or 9-AC total (not shown).

DISCUSSION

Topoisomerase I inhibitors are of great clinical interest because of their unique mode of action, their important antitumor activity and the high expression of the enzyme in various human tumor types. 9-AC, a semisynthetic analog of camptothecin revealed a broad antitumor activity in preclinical studies. Initial Phase I studies focused on schedules with prolonged infusion duration. In order to facilitate the prolonged drug administration, an oral formulation was developed. 9-AC can be administered orally as a colloid dispersion (CD) or as gelatine capsules in PEG1000. In dogs the mean oral bioavailability of the CD formulation was 13% (range, 4.5-26%), as compared to 10% of the PEG1000. Both formulations retained their antitumor activity after oral administration. Recently the Phase I study on the oral administration of the CD formulation of 9-AC, 5 days per week, every 2 weeks was completed [25]. Diarrhea was the dose limiting toxicity at a dose level of 0.2 mg/m^2. PEG1000 9-AC was previously shown to demonstrate rapid intestinal absorption in patients after oral delivery, with an overall bioavailability (F) of 48.6±17.6%. This compares favorable to other camptothecin analogs, including topotecan (F=30.0%) [26], 7-(4-methyl-piperazinomethylene)-10,11-ethylenedioxy-20(*S*)-camptothecin (GI147211; F=11.3%) [27], and irinotecan (F=12-21%) [28]. The terminal half-life ($t_{1/2}$) of 9-AC was shown to be 12.6±4.20 hr, which is substantially longer than that of topotecan (range, 2.35-5.91 hr) [26] and GI147211 (range, 6.85±3.13 hour) [27]. Another difference between topoisomerase I inhibitors constitutes the AUC ratio of the active lactone and the total drug of the parent drug. The conversion of 9-AC

lactone into the ring-opened species in plasma could be demonstrated from the first sample acquired (i.e. at 0.33 hr). At equilibrium, the 9-AC carboxylate accounted for 91.1±2.11% of 9-AC total drug concentrations.

This compares unfavorable to the percentages present in the ring-opened form at equilibrium observed in patients for topotecan (~50%) [26], GI147211 (~60%) [27], and irinotecan and its active metabolite SN-38 (~65% and ~35%, respectively) [29]. These figures underscore the important differences in pharmacokinetics between camptothecin analogs.

Our results of the Phase I study with oral 9-AC capsules indicated that the drug could be administered in a 14-day schedule repeated every 3 weeks with tolerable and manageable toxicity [30]. The dose-limiting toxicities were a combination of thrombocytopenia *plus* neutropenia complicated by fever and diarrhea occurring at a dose level of 1.1 mg/m^2/day. Other side effects were mild to moderate (CTC grade 1 to 2) and consisted of nausea, vomiting, alopecia, mucositis and fatigue. Although 9-AC demonstrated a linear pharmacokinetic behavior over the entire dose range studied, we observed that the AUC of 9-AC lactone was a better indicator for the observed hematological toxicity than the dose. The intrapatient variability in AUC and peak drug levels was extremely small and averaged less than 10% for 9-AC lactone. However, the interpatient variability in the concentrations of 9-AC at each of the sample-time points as well as in the AUC was large, with values for the coefficient of variation as high as 99%. In this study, the high variability in lactone to carboxylate interconversion was significantly related to individual differences in pretreatment serum albumin levels. Although our results need to be confirmed in a larger number of patients, they tend to indicate that higher protein levels will result in a more profound binding of 9-AC carboxylate, thereby further diminishing the effective concentration of the active species of the drug. In all, these data indicate that classical drug dosing based on body-surface area alone is unlikely to be effective in minimizing interpatient differences in systemic exposure to oral 9-AC.

The pharmacokinetics of 9-AC were clearly related to the pharmacodynamic outcome, (i.e. hematological toxicity). The sigmoidal E_{max} model was found most appropriate to fit the kinetic data to the observed myelosuppression. The best correlation was obtained with the AUC of 9-AC lactone, the exposure to the active drug. Considering this pharmacokinetic-pharmacodynamic relationship, a target AUC for 9-AC lactone can be defined according to the grade of toxicity that is considered to be acceptable in future studies. If hematological toxicity graded 2 or less is defined

as acceptable, then the target AUC of 9-AC lactone is 17.3 ng*h/mL, using the Hill equation and data shown in Figure 3*A*.

For pharmacokinetic and pharmacodynamic analysis frequent blood sampling is inevitable. In order to evaluate salivary drug monitoring of 9-AC as an alternative to drug monitoring in plasma, the concentrations and AUC of 9-AC total and lactone were measured in unstimulated saliva samples in 5 patients during this study. The 9-AC concentration ratio in plasma and unstimulated saliva proved to be strongly patient-dependent and highly variable, suggesting that saliva is an unreliable matrix for pharmacokinetic analysis of 9-AC. Similar results were obtained recently for pharmacokinetic analysis of CPT-11 and SN-38 in saliva demonstrating large interpatient variability in plasma/saliva ratios [3].

Recently, we developed a limited-sampling model for reliable and accurate prediction of the systemic exposure to 9-AC after oral drug administration, using only one time blood sample taken at 3 hr after drug dosing [32]. In order to further diminish the interpatient variability in drug exposure in future studies, a pharmacokinetic guided approach may be considered. After oral administration of an appropriate starting dose of 9-AC (i.e. 1 mg/m^2), the 9-AC lactone plasma concentration can then be measured at 3 hr after drug dosing. Using the limited-sampling model and the linear-regression relationship between drug dose and AUC [Figure 3], the optimal dose leading to the target AUC, determined according to the toxicity considered acceptable, can be calculated.

This procedure may prove valuable in reducing interpatient variation in exposure to 9-AC, and will enable us to optimize the treatment for any given patient by combining maximally achievable doses with tolerable toxicity during treatment. This strategy seems to be interesting both in further Phase II studies using the 14 day administration schedule and in Phase I studies with different schedules of administration. At present, the clinical applicability of this concept is under investigation at our institute with 9-AC given orally in a daily times five weekly or three-weekly schedule.

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Chapter 7

The development of combination therapy involving camptothecins: A review of preclinical and early clinical studies

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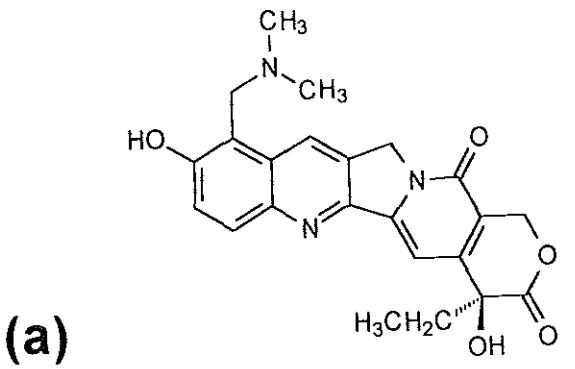
INTRODUCTION

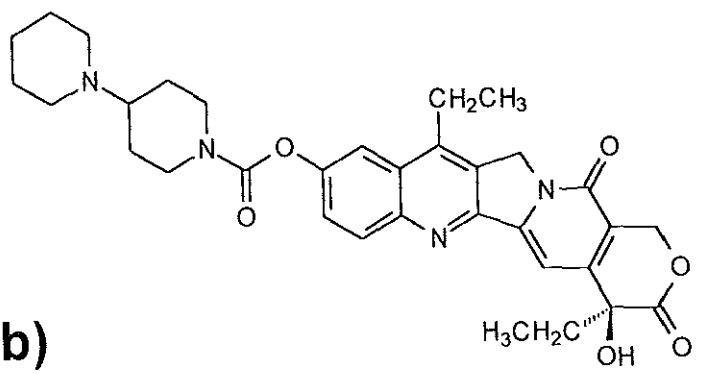
Camptothecin analogues are a family of anticancer agents with a unique mechanism of action, which is the reversible inhibition of DNA topoisomerase I [1,2]. DNA topoisomerases are essential nuclear enzymes involved in multiple nuclear functions such as chromosomal recombination, DNA repair, transcription and chromatin assembly. Topoisomerase I inhibitors exert their cytotoxic action through the stabilisation of the topoisomerase I - DNA complex, the so-called cleavable complex. This results in collision of the DNA replication fork, finally leading to irreversible DNA double-strand breaks and cell death [3,4]. Topoisomerase I inhibitors are of great clinical interest because of their unique mode of action, their important antitumour activity as single agents in a broad spectrum of tumour types [5-30] and the high expression of the enzyme in various human tumour types [31-34]. In addition, topoisomerase I inhibitors may also interfere with the processes involved in DNA repair [35,36,37]. The latter renders them attractive for further investigations on combination therapies especially involving those with DNA-damaging agents. Preclinical studies have revealed synergism between topoisomerase I inhibitors and drugs such as cisplatin, topoisomerase II inhibitors and paclitaxel in a number of different human cancer cell lines and xenografts. This paper reviews the development of combination therapy with topoisomerase I inhibitors with a focus on the two topoisomerase I inhibitors (irinotecan and topotecan [Figure1]) that are presently registered for clinical use.

Platin-derivatives

Prominent clinical activity has been demonstrated for topotecan and irinotecan in several malignancies in which platin-derivatives are also highly effective. The ability of camptothecin-analogs to inhibit topoisomerase I mediated DNA functions suggests they might interfere with processes involved in DNA repair and enhance cytotoxicity when combined with DNA damaging agents. The different toxicity profiles of platin-derivatives and topoisomerase I inhibitors further support the potential use of these agents in combination.



(a)



(b)

Fig. 1. Chemical structures of topotecan (A) and irinotecan (CPT-11) (B)

Preclinical studies

Interactions of topoisomerase I inhibitors with platin-derivatives have been studied *in vitro* and *in vivo*. The combination of irinotecan with cisplatin (CDDP) was synergistic in human small cell lung cancer- [38,39], oesophageal cancer- [40], and ovarian cancer cell lines [41,42], in the human T-cell leukaemia cell line MOLT-3 [43] and in a human tumour xenograft of bladder cancer [44]. Additivity was observed in the human ovarian cancer cell line 2008 [45] and in human tumour xenografts of oesophageal, gastric and colon cancer [46].

Also for the combination of topotecan and cisplatin the interaction varied with the cell lines examined. Synergism was observed in teratocarcinoma, glioma, ovarian cancer, small cell lung cancer and non-small cell lung cancer cell lines [47-51] and in a IGROV-1 tumour xenograft model [49].

Bissery et al. studied the combination of irinotecan with oxaliplatin in human osteogenic sarcoma xenografts and found only an additive effect when both drugs were administered simultaneously [52]. Sequence-dependency of the cytotoxicity of the combination of topoisomerase I inhibitors and platin-derivatives was also studied by various other groups. Rowinsky et al. found no sequence dependent difference in cytotoxicity for cisplatin and topotecan in the A 549 human lung cancer cell line [50]. Maliepaard et al. however observed a strongly schedule-dependent effect, with synergy increasing when topotecan or irinotecan were preceded by the platin-derivative [42]. In contrast to the above mentioned osteosarcoma model data, the combination of irinotecan and oxaliplatin studied in a HT 29 human colon carcinoma cell line demonstrated synergism when the drugs were given simultaneously [52,53]. Supra-additivity in this model was found with any sequence of administration [54].

The mechanism of interaction between the topoisomerase I inhibitor and cisplatin was studied by Zeghari and Goldwasser [54,55]. The topoisomerase I inhibitor delayed the reversal of CDDP-induced DNA interstrand cross-links (ISCs) without modifying the formation of ICSs [53-55]. No alteration in cleavable complexes formation or reversion was observed. Simultaneous treatment prolonged the DNA and RNA synthesis inhibition produced by either drug alone. At higher concentrations cisplatin (150 µM) enhanced the topoisomerase I inhibitory activity determined by relaxation of supercoiled *Escherichia coli* DNA [38] possibly by creating nicks or gaps with 5'-phosphate termini and thereby facilitating the stabilisation of the cleavable complexes by the topoisomerase I inhibitor [56].

These preclinical data on drug-interactions between topoisomerase I inhibitors and platin-derivatives seem to suggest the potential of a sequence dependent effect.

Where observed, synergism might at least partly be explained by interference of the topoisomerase I inhibitor in the repair of CDDP-induced DNA interstrand cross-links.

Phase I studies

Phase I studies on the combination of irinotecan (CPT-11) and CDDP were performed using different schedules of drug administration with a clear focus on fractionated dose schedules for both agents. A once every three weeks administration schedule for the two drugs has not yet been investigated. In all studied schedules irinotecan administration preceded that of CDDP. The details of these studies are shown in Table 1 [57-66]. Although recent recommendations are that in such trials pharmacokinetics of both drugs should be studied to exclude interactions, this was only done partly in one of these trials. Moreover, in interpreting these studies we have to bear in mind that apart from one, they were all performed in Japan. Single agent study results on irinotecan suggest that Japanese patients may be more susceptible to irinotecan than Caucasians. Whether this is a result of a true pharmacogenetic difference, or whether these results reflect a different approach by the investigators is unclear.

Table 1. Phase I studies on the combination of irinotecan (CPT-11) and CDDP

Day of Administration		Cycle Interval (wks)	G-CSF	Tumour type	DLT	Recommended dose (mg/m^2/day)		Ref.
CPT-11	CDDP					CPT-11	CDDP	
1, 8, 15	1	4	-	NSCLC	Diarrhoea, neutropenia	60	80	57
1, 8, 15	1	4	-	(N)SCLC	Diarrhoea	80	60	58
1, 8, 15	1	4	-	cervix ca	Neutropenia	60	70	59
1, 8, 15	1, 8, 15	4	-	NSCLC	Neutropenia	60	33	60
1, 15	1	4	-	Gastric ca	Neutropenia	70	80	61
1,8	1, 8	4	-	(N)SCLC	Diarrhoea, neutropenia	50	60	62
1	1-5	4	-	NSCLC	Neutropenia	100	20	63
1, 8, 15, 21	1, 8, 15, 21	6	-	Various	Neutropenia Diarrhoea	50	30	64
1, 8, 15	1	4	+	NSCLC	Diarrhoea,	80	80	65
31	1-5	4	+	NSCLC	neutropenia	160	20	66

wks: weeks

Throughout the studies the major dose limiting toxicities were neutropenia and diarrhoea. Grade 4 neutropenia was observed in 35% of the patients with the nadir around day 20 (range 8-29) and recovery in most patients by day 29 (range 24-39). These neutropenic episodes were only in 12% complicated by fever (4% of all patients). By adding G-CSF to schedules with neutropenia as the dose limiting toxicity, a 33 and 60% increase in dose intensity of irinotecan could be achieved [65,66]. Diarrhoea is a difficult clinical problem associated with the use of irinotecan. It may involve early onset abdominal cramping and flushes, suggestive of release of vasoactive compounds, and delayed onset secretory diarrhoea. There is a correlation with this late onset diarrhoea and the biliary excretion of SN-38, the active metabolite of irinotecan, as determined by the extent of SN-38 glucuronidation in plasma [67]. The acute diarrhoea responds to treatment with atropine. Whenever secretory diarrhoea developed immediate therapy with loperamide was started, rendering the diarrhoea manageable. Other side effects consisted of nausea and vomiting, alopecia and mucositis. Major responses were observed in all treatment schedules in patients with non-small cell lung cancer, small cell lung cancer, gastric, head and neck and cervix carcinoma (response rates reported: 35-80%). Although the latter data are of limited value in view of the small sample sizes, the combination of irinotecan and cisplatin seems to be highly active.

Pharmacokinetics were only studied by Masuda et al. [56] and Sumiyoshi et al. [68]. The former observed a marked increase in the mean peak plasma concentration and the area under the concentration-time curve (AUC) of SN-38 after a modest increase in the dose of irinotecan, suggesting a potential pharmacokinetic interaction. However, recently the metabolic ratio of the conversion of irinotecan to SN-38 ($AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$) [Figure 2] was considered to be a reliable parameter to determine any pharmacokinetic interaction. Comparison of the metabolic ratio of the combination therapy to the single agent therapy with irinotecan did not reveal any difference, indicating no apparent interaction [69-72]. These data lend further support to the recommendation to include pharmacokinetics in combination chemotherapy phase I studies.

The dose-intensity of irinotecan that could be achieved in combination with cisplatin varied from 25-60 % of the single agent dose. Higher dose-intensities were reached when irinotecan was combined with a single administration of cisplatin. As stated a once every 3 weeks administration schedule for both drugs has not yet been investigated. Further studies on patients in Europe and the USA have to be awaited for a better insight in the tolerability of the combination of irinotecan and cisplatin.

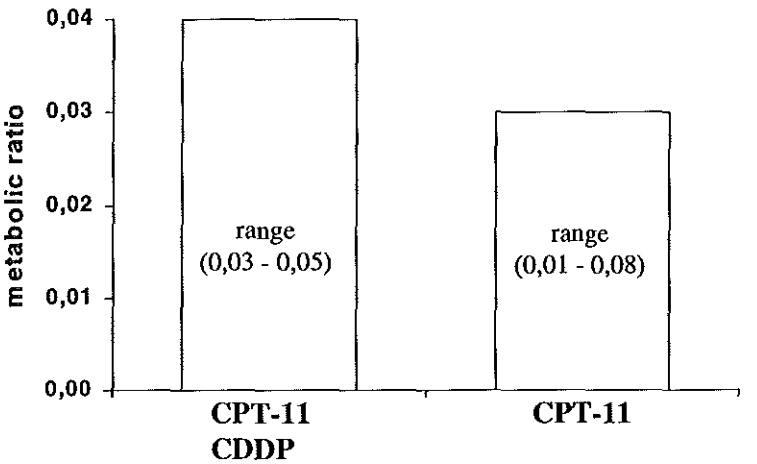


Fig. 2. Metabolic ratio AUC_{SN-38}/AUC_{CPT-11} of the conversion of irinotecan to SN-38 in irinotecan single-agent therapy and combination therapy with cisplatin

The combination of irinotecan, cisplatin and vindesine was also found feasible. Dose limiting toxicities again consisted of neutropenia and diarrhoea. The recommended doses for phase II studies are either irinotecan 37.5 mg/m²/day d 1 and 8 in combination with cisplatin 100 mg/m² day 1 and vindesine 3 mg/m²/day d 1 and 8, or irinotecan 80 mg/m²/day d 1 and 8 combined with cisplatin 60 mg/m² d1 with the same dose of vindesine [73].

Sugiura et al. studied the combination of irinotecan and carboplatin [74]. Carboplatin was administered on day 1 at a fixed dose of 300 mg/m², with escalating doses of irinotecan on day 1, 8 and 15 every 4 weeks. Dose limiting toxicities consisted of neutro- and thrombocytopenia and diarrhoea. The recommended dose of irinotecan for phase II studies with this combination is 50 mg/m² d 1, 8 and 15. Adding G-CSF seemed to enable somewhat higher doses of irinotecan [75]. Cvitkovic et al. studied the combination of oxaliplatin (L-OHP) and irinotecan in colorectal cancer [76]. L-OHP was given as a 2-hour infusion followed after 1 hour by irinotecan given over 30 minutes, once every 3 weeks. Dose limiting toxicities

consisted of neutropenia and diarrhoea. Other side effects included a.o. peripheral neuropathy, nausea and vomiting. Recommended doses for phase II studies are oxaliplatin 110 mg/m² in combination with irinotecan 200 mg/m². Pharmacokinetic data show no drug interaction. The AUC of irinotecan, SN-38, SN-38G and L-OHP were comparable with single agent data [77].

Table 2. Phase I studies on the combination of topotecan and CDDP.

Day of administration		Cycle interval (wks)	G-CSF	DLT	Recommended dose (mg/m²/day)		Ref.
Topotecan	CDDP				Topotecan	CDDP	
1-5	1	3	-	Neutropenia thrombocytopenia	Not feasible		78
1-5	1	3	-	Neutropenia	1.0	50	79
1-5	1	3	-	Neutropenia, thrombocytopenia	0.75	50	50
1-5	1-5	4	-	Neutropenia	not feasible		80
1-5	1	3	+	Neutropenia, hyperbilirubinemia	1.0	75	79
1-14 civ	1	4	-	Not yet reached	0.4	≥75	81

Wks: weeks; civ: continuous intravenous infusion.

Combining topotecan with cisplatin also required considerable dose reduction as compared to the single agent dose. Several schedules of administration were studied. The details are summarised in Table 2 [50,78-81]. Dose limiting toxicities consisted of neutropenia and thrombocytopenia. Attempts to only marginally increase the dose of either topotecan or CDDP rapidly resulted in unacceptable toxicity. In the study of Miller et al. further dose escalation of CDDP seemed feasible after addition of G-CSF [79]. However, in a subsequent phase II study using this schedule in patients with small cell lung cancer (extended disease) unacceptable toxicity was encountered [82]. This fact stresses the importance of patient selection, since patients with SCLC are more vulnerable for developing infectious complications in case of chemotherapy induced prolonged neutropenia. Considering the definition of dose-limiting toxicity in this study, these side effects could have been anticipated. In the study performed by Rowinsky et al. no further dose escalation was possible after addition of G-CSF [50]. Non-haematological toxicity was usually mild to moderate, consisting of nausea and vomiting, diarrhoea, mucositis, fatigue and alopecia. Other organ toxicities were not reported. In preclinical studies sequence dependency was

noted for the combination of CDDP and topotecan. Unfortunately the potential importance of sequence dependency in the clinical setting has only been investigated by Rowinsky et al. [50]. In their study topotecan was administered as a 30-minute infusion daily for five days and cisplatin was given either before topotecan on day 1 or after topotecan on day 5, alternating in the same patient. CDDP given before topotecan induced significantly more and severe neutropenia and thrombocytopenia than the alternate sequence. Pharmacokinetic studies suggested that the differences in toxicity were due, in part, to lower topotecan clearance when CDDP preceded topotecan administration [Figure 3].

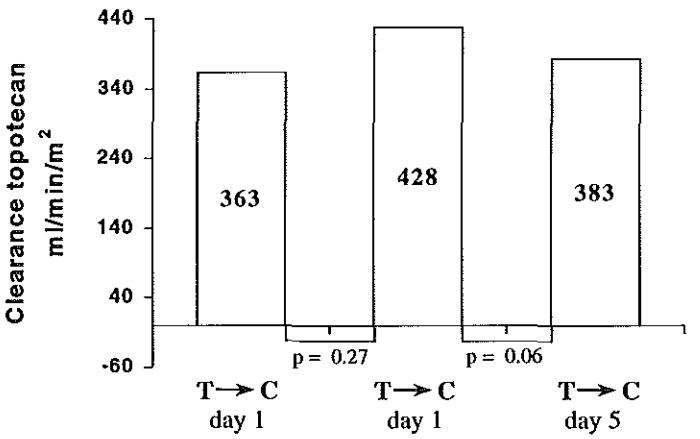


Fig 3. Clearance of topotecan on day 1 in sequence topotecan day 1-5, cisplatin day 5 (T ⟶C) and cisplatin day 1, topotecan day 1-5 (C ⟶ T).

Also, maximal interactions between topoisomerase I-DNA adducts and polymerase molecules involved in repair from cisplatin induced DNA damage might play a role. In theory this could result in an increase in both antitumour activity and toxicity due to a decrease in repair, if cells were exposed to alkylating agents early in the course of topotecan treatment. These effects however, were not measured.

Heideman et al. [83] studied the combination of topotecan and carboplatin in paediatric solid tumours. Carboplatin was administered at a fixed target AUC of 6.5 mg/ml.min followed by a continuous infusion of topotecan for 72 hours, courses

repeated every 4 weeks. Dose limiting toxicity consisted of neutropenia and thrombocytopenia at a topotecan dose of 0.5 mg/m²/day. The recommended dose of topotecan for phase II studies is 0.4 mg/m²/day. Pharmacokinetic data could not reveal any interaction between topotecan and carboplatin.

Both the preclinical and clinical data underscore the necessity to evaluate sequence dependent effects of combination therapy already in phase I investigations. Further investigations are needed to determine whether the sequence causing more side effects, is also the more potent one. To assess any pharmacokinetic interaction between the drugs administered, in all phase I studies involving combination regimens pharmacokinetic and pharmacodynamic data should be collected.

Combinations with other alkylating agents

In preclinical studies the combination of topotecan with DNA alkylating agents (melphalan, bis[chloroethyl]nitrosurea and 4-hydroperoxycyclophosphamide) at low-to-intermediate levels of cytotoxicity revealed cytotoxic effects that were less than additive in most cases. However, nearly additive or synergistic effects were observed with the same drug combinations at high levels of cytotoxicity (i.e., at ≥ 90% inhibition of colony formation) [47].

The combination of topotecan with cyclophosphamide was studied in patients with refractory cancer and patients with refractory leukaemia [84-86]. Miller et al. treated patients with refractory leukaemia with cyclophosphamide 40 mg/kg day 1 followed by a continuous infusion of topotecan day 2-6 at a dose of 1.5 and 1.8 mg/m² [84]. Dose limiting toxicity had not yet been reached at the time of publication of the abstract. Preliminary data suggest activity of this combination. A different schedule was studied in paediatric patients with malignant solid tumours [85]. A fixed dose of cyclophosphamide 250 mg/m²/day was followed by topotecan in escalating doses, each given as a 30-minute infusion daily for 5 days repeated every 3 weeks. At the starting dose of topotecan (0.75 mg/m²/day) dose-limiting neutropenia was observed. Dose reduction of topotecan to 0.60 mg/m²/day also resulted in dose-limiting toxicity. Addition of filgrastim allowed the escalation of topotecan to the 0.75 mg/m²/day dose level, which is the dose recommended for further phase II studies. However, patients treated at this dose level experienced substantial anaemia and thrombocytopenia requiring platelet transfusions in 53% of the courses and RBC transfusions in 50%. Also 20% of treatment courses at the recommended dose level were complicated by febrile neutropenia. Pharmacokinetic data indicated no alteration of topotecan

kinetics. Responses were reported in patients with a Wilms'tumor, neuroblastoma, rhabdomyosarcoma and osteosarcoma. Interestingly, the responding patients were all pre-treated with either ifosfamide or cyclophosphamide. Murren et al. combined a fixed dose of cyclophosphamide 600 mg/m² d 1 with escalating doses of topotecan given as a 30-minute infusion for 5 consecutive days every 3 weeks in patients with refractory solid tumours [86]. Doses of topotecan ranged from 0.5-1.2 mg/m²/day. Reversible neutropenia was dose limiting at a dose of topotecan of 1.0 mg/m²/day. Other side effects were anaemia, thrombocytopenia, fatigue and weight loss. The recommended dose for phase II studies for pre-treated patients is topotecan 0.75 mg/m²/d d 1-5 without growth factor support and 1.0 mg/m²/d d 1-5 if G-CSF is added. Cyclophosphamide did not appear to alter the pharmacokinetics of topotecan. Significant increases in topoisomerase I concentration were identified in peripheral-blood mononuclear cells after the administration of cyclophosphamide on day 1, followed by a significant decrease during the 5-day course of treatment with topotecan. This means that biochemical changes in cells induced by exposure to camptothecins can be measured in vivo. These effects can be used to monitor pharmacodynamic interactions in combination therapies with camptothecins and to determine the optimal scheduling of this class of agents.

In 24 patients with clear cell carcinoma of the ovary, who progressed during platinum-based chemotherapy or relapsed within 6 months after the end of platinum-based chemotherapy, irinotecan 140 mg/m² infused i.v. over 4 hours on day 1 and 15 was combined with intraperitoneal mitomycin-C 7 mg/m² on day 1 and 15 every 4 weeks. This schedule was never appropriately tested in a phase I study. Therefore it is impossible to conclude if patients were appropriately dosed. In 11% of the courses, grade 3 diarrhoea was observed. Other side effects were acceptable. Despite the dose issue, 11 patients showed a response [87].

Topoisomerase II inhibitors

Preclinical studies

It has been shown in preclinical studies that cross-resistance to topoisomerase I and II inhibitors is unusual in resistant cell lines [88,89]. Alterations in the regulation of one topoisomerase are often compensated by alterations in the other [90]. The expression of topoisomerase II is enhanced in topoisomerase I-deficient cells [91] and cell lines with deficient topoisomerase I activity appear to be more sensitive to

treatment with topoisomerase II inhibitors [92]. This led to the assumption that the combination of a topoisomerase I and II inhibitor might lead to synergistic cytotoxicity. Preclinical studies on the combination of camptothecins and topoisomerase II inhibitors however, revealed that their interaction was cell line and sequence dependent. Concomitant administration resulted in antagonism in HL-60 human progranulocytic leukaemia cells [93], U 251 glioma cells [94], HT-29 human colon cancer cells [95] and in SW 480 colon carcinoma xenografts [96]. In other cell lines additive or even synergistic cytotoxicity was noted [43,94,97]. Also in a first study combining 9-aminocamptothecin with etoposide in human glioblastoma cells a synergistic effect was noted [97]. Sequence dependent cytotoxic effects were studied combining several topoisomerase I and II inhibitors. The outcome varied with the cell lines studied [98,99]. However, when the administration of the topoisomerase II inhibitors, etoposide or adriamycin, was preceded by the administration of a topoisomerase I inhibitor in most cell lines and xenografts a synergistic effect was noted [46,96,100-103]. This might be explained by an increase in topoisomerase II α levels observed after inhibition of topoisomerase I [46,104,105] and an increase in the S-phase cell population, which might enhance the sensitivity to topoisomerase II inhibition [43,46,93,95,96,102]. These results seem to suggest that concurrent administration of topoisomerase I and II inhibitors is unlikely to be beneficial. Clearly, appropriate clinical studies are required to formally examine whether or not sequential administration of these agents offers any advantage over monotherapy [106].

Phase I studies

Several phase I studies combining irinotecan and etoposide (VP-16) were performed [Table 3] [107-110]. The combination of both drugs was only feasible with growth factor support suggesting at least additive myelotoxicity. Dose limiting toxicity consisted of a combination of neutropenia, thrombocytopenia and diarrhoea. Further common side effects consisted of nausea and vomiting, alopecia, reversible elevation of transaminases and bilirubin. The elevation of transaminases and bilirubin correlated with the grade of the observed haematological toxicity [107]. This may suggest that liver impairment may prolong bone marrow exposure leading to more pronounced and prolonged myelosuppressions. Otherwise, liver impairment might co-segregate with myelosuppression as a sign of severe toxicity. Since both drugs

are metabolised in the liver, the administration of irinotecan and etoposide on consecutive days may have caused these hepatic toxicities via any drug-drug interaction. Ando et al. investigated the effect of the sequence of drug administration on the observed toxicity [107]. Patients were treated with irinotecan d 1-3 followed by treatment with etoposide d 4-6 (arm A) or with etoposide d 1-3, followed by irinotecan d 4-6 (arm B). G-CSF was given to all patients daily on days 7-17. No remarkable difference in toxicity was observed between the two treatment arms, although the AUC of irinotecan and SN-38 were significantly higher when this drug followed the administration of etoposide. In a study combining irinotecan and etoposide on day 1-3 a drug interaction was also suggested. The Cmax and AUC of etoposide were significantly higher on day 3 as compared to day 1 [108]. Clearly all further studies on the combinations of these two drugs should be paralleled by pharmacokinetics in order to elucidate the background of these apparent but unexplained interactions.

Table 3. Phase I studies on the combination of topoisomerase I and II inhibitors

Day of administration		Cycle interval (wks)	G-CSF	DLT	Recommended dose (mg/m^2/day)		Ref.
CPT-11	VP-16				CPT-11	VP-16	
1-3	4-6	4	+	Neutropenia, thrombocytopenia, diarrhoea	Not feasible		107
1-3	1-3	3-4	+	Diarrhoea	60	60	108
1, 8, 15	1-3	4	+	Neutropenia, diarrhoea	80 70 pre-treated	80 80	109 110
1, 8, 15	1-3	4	+	Neutropenia, diarrhoea	70	80	
Topotecan	VP-16				Topotecan	VP-16	
1-5	6-8	3-4	-	Mucositis	1.5	100	111
1-3 civ	4-8		-	Mucositis, diarrhoea,	0.85	100	112
1-3 civ	7-9	4	-	hyperbilirubinemia	0.17 pre-	100	114
1-5	6-12 bid po		-	Neutropenia Neutropenia	treated 1.0	40	113
Topotecan	Doxo				Topotecan	Doxo	
1-3 civ	5	3-4	-	Neutropenia	0.35	45	115
1-3 civ	5	3-4	+	Neutropenia, thrombocytopenia	0.75	45	115

Wks: weeks, civ: continuous intravenous infusion, po: orally, bid: twice a day, Doxo: doxorubicin

The combination of topotecan and etoposide was studied in patients with solid tumours and in patients with haematological malignancies [Table 3] [111-114]. In patients with solid tumours the dose-limiting toxicity consisted of neutropenia [113,114]. In general, the haematological toxicity encountered was more severe than expected. The AUC of topotecan correlated with the decrease in white blood cell (WBC) count (r^2=0.65) [113]. As expected in view of schedule and known half-lifes, topotecan and etoposide pharmacokinetic parameters were similar to those reported in single agent studies. Eckardt et al. determined tumour topoisomerase II levels in 5 patients with accessible solid tumours [105]. Samples were taken before the start of the therapy, after the last topotecan administration and after the last etoposide administration. In one patient with colon cancer topoisomerase II levels were markedly increased after administration of topotecan and decreased again after administration of etoposide. This patient demonstrated a resolution of ascites. In the other four patients no pattern of modulation of topoisomerase I or II levels was observed.

In patients with acute myeloid leukaemia dose-limiting toxicities consisted of mucositis, hyperbilirubinemia and peripheral neuropathy [111,112]. Grade 4 neutropenia and thrombocytopenia was seen in all courses. Topoisomerase I and II levels were measured in leukaemic blasts before and during the treatment with topotecan. Both levels decreased during therapy compared to pre-treatment values. No correlation was found between the decline in topoisomerase I and clinical tumour response [111,112].

Combinations of camptothecins with doxorubicin were also studied. For topotecan this involved a 72-hour continuous infusion followed by bolus doxorubicin on day 5. Cycles were repeated every 3-4 weeks [115]. Unexpectedly dose-limiting toxicity (neutropenia) was already encountered at the first dose level but with the addition of G-CSF a further dose increase was feasible. The recommended dose for phase II studies is topotecan 0.35 mg/m²/day continuous infusion on day 1-3 without G-CSF or 0.75 mg/m²/day with G-CSF, followed by doxorubicin 45 mg/m². No alterations in drug clearance were found as compared to single agent data. In ten patients paired bone marrow aspirates were obtained before and after treatment with topotecan. In 7 of 10 patients, an increase in the proportion of CD34+ cells in S-phase was noted 24 hours after the end of topotecan infusion. This increase may in part be responsible for the unexpected haematological toxicity, since topoisomerase II inhibitors such as doxorubicin are most cytotoxic to cells in S phase when the topoisomerase II enzyme levels are the highest [93].

Saotome et al. performed a pilot study on the combination of irinotecan 25 mg/m²/day given on day 1 and 2 and doxorubicin 40 mg/m² given on day 3, cycles repeated every 3 weeks in 12 patients with relapsed malignant lymphoma refractory to doxorubicin containing chemotherapy or relapsing after doxorubicin containing regimens. Major toxicities included leucocytopenia (≥ WHO grade 3: 67%), thrombocytopenia (25%), and diarrhoea (8%). Four patients (33.3%) achieved a complete response [117].

At present, based upon the above data, it appears that combining topoisomerase I and II inhibitors is difficult for a variety of reasons. In general these combinations yield much toxicity, especially myelotoxicity, which renders it impossible to administer enough drug and enough courses of treatment to expect an important level of antitumour activity.

Antimetabolites

Preclinical studies

Since irinotecan and 5-FU are two drugs active in metastatic colorectal cancer and irinotecan exerts its cytotoxic activity in both chemotherapy-naïve and pre-treated patients, combination therapy with 5-FU seems interesting. However, in preclinical studies simultaneous incubation of HT-29, HCT-8, HT-29R1, HT-29R24 colon cancer, A549 lung cancer and human T-cell leukaemia MOLT 3 cell lines with irinotecan/SN-38 or topotecan and 5-FU revealed antagonistic or only additive effects [43,47,118-122] as did simultaneous treatment in a HT-29 colon cancer xenograft model. Sequential incubation, with 5-FU preceding irinotecan resulted in either antagonism [120,121] or synergism [118,123] depending on the cell lines studied. In case of synergism, 5-FU seemed to augment the accumulation of irinotecan in the cell and to increase topoisomerase I levels [118,122]. When irinotecan incubation preceded 5-FU most often synergism was observed [118-120,124]. Irinotecan did not modify 5-FU uptake by the cell. However, the combination of 5-FU and irinotecan induced a higher thymidylate synthase inhibition compared to 5-FU alone [124]. In a HT-29 nude mice xenograft model tumour relapse was preceded by an increase in thymidylate synthase activity and a decrease in achieved topoisomerase I inhibition [124].

Aschele et al. studied the combination of Tomudex and SN-38 in HCT-8 colon cancer cell lines [125]. Synergism was encountered with sequential short-term

exposure with any sequence of administration. However, the magnitude of potentiation was greater when SN-38 was given first and when a higher relative dose of Tomudex was used [125].

Combinations of topotecan and methotrexate or cytarabine revealed cytotoxic effects that were less than additive [47]. For irinotecan antagonism was observed in combination with methotrexate [43]. The combination of irinotecan with cytarabine showed a synergistic effect [43].

Phase I studies

Numerous studies were initiated to assess the feasibility of the combination irinotecan and 5-FU. Salz et al. conducted a phase I study on irinotecan administered over 90 minutes day 1, 8, 15 and 22 every 6 weeks in combination with 5-FU and leucovorin [126]. The first 5-FU and leucovorin were administered on day 2. On days 8, 15 and 22, irinotecan infusion was immediately followed by leucovorin and then 5-FU. For the second 6-week cycle, leucovorin was administered first, followed by 5-FU and then irinotecan, which permitted comparison of the pharmacokinetics of irinotecan in the different sequences of administration. Dose limiting toxicity was neutropenia. Although diarrhoea was common, it was rarely dose limiting. Other side effects consisted of nausea and vomiting (> grade 2 only in 1/27 patients), fatigue and alopecia. Among the 38 patient with colorectal cancer, six partial responses (16%) were seen in this predominantly 5-FU-refractory patient population. The recommended dose for phase II studies is irinotecan 125 mg/m^2 in combination with 5-FU 500 mg/m^2 and leucovorin 20 mg/m^2 days 1, 8, 15 and 22, cycles repeated every 6 weeks. The co-administration of 5-FU had no substantial effect on the pharmacokinetics of irinotecan or SN-38, which contrasts the results reported by Sasaki et al.. They suggested that 5-FU reduced the degree of conversion of irinotecan to SN-38, possibly by interference with the function of carboxylesterases that catalyse this conversion [127]. However, their pharmacokinetic data were obtained in a relatively small number of patients. Also the pharmacokinetic data obtained by other investigators revealed no difference in irinotecan kinetics with the co-administration of 5-FU, whatever the order of administration [128-130]. Benhammouda et al. studied the combination of irinotecan and 5-FU in a 3-weekly schedule [130]. Dose limiting toxicity was haematological. The recommended doses for phase II studies are irinotecan 300 mg/m^2 day 1 and 5-FU 375 mg/m^2/day iv bolus day 1-5 q 3-4 weeks.

Table 4. Phase I studies on the combination irinotecan (CPT-11) and 5-FU and leucovorin.

Days of administration			Cycle interval (weeks)	RR colorectal cancer	DLT	Recommended dose (mg/m^2/day)			Reference
5-FU	LV	CPT-11				5-FU	LV	CPT-11	
1,8,15,2	id	id	6	6/38	Neutropenia	500	20	125	126
2		1	3-4		Neutropenia	375		300	130
1-5	2-5	1			Not reached	250	20	≥200	131
2-5		1	3-4		Not reached	400		≥225	132
1-7 civ		1,15		5/19	Not reached	600		≥150	129
3-8 civ		1	3		Febrile neutropenia,	250		350	133
1-14 civ					Diarrhoea, Mucositis				
	1	1	weekly x6 followed	15/24	Diarrhoea	2600	500	80	134
1,24 h			by 1 wk rest						
	1	1	2	8/31	Not reached	400 bolus	200	≥200	135
1,						600 civ			
bolus+									
22 h civ									

Id: idem, civ: continuous intravenous infusion, h: hour, LV: leucovorin, RR: response rate

Several phase I studies combining irinotecan and 5-FU in different schedules are still ongoing, but the preliminary reported data have not revealed any dose limiting side effects [Table 4] [126,129-135]. Thus, these preliminary results suggest that the combination of irinotecan and 5-FU is well tolerated.

Another approach to combine irinotecan and 5-FU consists of the alternating administration of both agents. Two schedules were studied; irinotecan 350 mg/m² 90 min d1 and 5-FU 425 mg/m² immediately after leucovorin 20 mg/m² d 22-26, every 6 weeks [136], and irinotecan 100 mg/m² weekly x 4 followed by a 2-week rest, after which 5-FU 425 mg/m² and leucovorin 20 mg/m² are both given for 5 days, the full cycle repeated every 10 weeks [137].

In these alternating regimens similar toxicities were observed as with either therapy given alone. Overall response rates of 26 %(18/70 pts) and 31 % (9/29 pts) were achieved in patients with colorectal cancer.

These latter studies add further evidence that irinotecan can be easily combined with various 5-FU regimens. This has prompted the initiation of a phase III study comparing such a combination with high dose infusional 5-FU alone in patients with colorectal cancer.

A phase I study on the combination irinotecan and tomudex, another regimen of potential interest in colorectal cancer, was recently completed, but data have not yet been reported [138].

In patients with acute leukaemia, not responsive to standard therapy, the combination of topotecan and cytarabine was studied [139]. Topotecan was given as a 30-minute infusion daily with cytarabine 1 g/m²/d, both for 5 days. Oropharyngeal mucositis was dose limiting. Liver function test abnormalities occurred frequently, but in most cases were reversible. All patients developed neutropenic fever that required treatment with broad-spectrum antibiotics. The recommended phase II dose of topotecan is 4.75 mg/m²/d for 5 days in high-risk patients (PS 2, or > one prior chemotherapy regimen) and 7.0 mg/m²/d for low-risk patients. This is three to five times the recommended dose for topotecan as single agent in patients with solid tumours. During therapy the percentage of apoptotic cells in blood and bone marrow was determined, and the cell cycle distribution of the leukaemic cells was studied. Forty-eight hours after the first administration of topotecan the maximal number of apoptotic cells was seen in the blood. Patients with a high S-phase fraction either before treatment or following cytarabine were more likely to achieve bone marrow aplasia. This result is in line with the fact that both drugs are most active in S-phase.

Taxanes

Only limited preclinical data are available for the combination of paclitaxel or docetaxel and topoisomerase I inhibitors. The combination of irinotecan or topotecan with paclitaxel resulted in less than additive cytotoxicity in several cell lines [47]. In breast cancer xenografts a good antitumour efficacy was noted for the combination of irinotecan and docetaxel [140].

Since paclitaxel and topotecan both demonstrated activity in women with recurrent and cisplatin-refractory ovarian cancer, the feasibility of combining these agents was pursued. Topotecan, administered as a 30-minute infusion day 1-5, was combined with paclitaxel both studied as a 3-hour [141] or a 24-hour infusion [142]. When paclitaxel was given as a 3-hour infusion before the administration of topotecan, dose-limiting toxicity consisted of neutropenia. The recommended dose for phase II studies is paclitaxel 80 mg/m^2 day 1, combined with topotecan 1.0 mg/m^2/d d 1-5, cycles repeated every 3 weeks. With addition of filgrastim, the dose of paclitaxel could be further escalated to 230 mg/m^2 in combination with the same dose of topotecan. DLT consisted of a combination of haematological toxicity (especially neutropenia) and neuromuscular toxicity. Other non-haematological toxicity was usually mild, and included nausea and vomiting, diarrhoea, mucositis, myalgia and arthralgia. The other phase I study was performed in patients with recurrent or refractory ovarian cancer, and paclitaxel was given over 24-hour combined with topotecan d 1-5, again with cycles repeated every 3 weeks [142]. Dose-limiting toxicity consisting of neutropenia, was already encountered at the first dose level (topotecan 0.75 mg/m^2/d d 1-5, paclitaxel 135 mg/m^2 d1). Even after addition of filgrastim no further dose escalation was possible due to unacceptable haematological toxicity (neutro- and thrombocytopenia). The recommended dose for further phase II studies is topotecan 0.75 mg/m^2/d d1-5 and paclitaxel 135 mg/m^2 with G-CSF support. Non-haematological toxicity consisted of mild nausea and vomiting, mucositis and diarrhoea. In this study the issue of sequence dependent toxicity was also addressed. No difference in toxicity was observed whether paclitaxel preceded the administration of topotecan or was given on day 6, after the administration of topotecan. Also no pharmacokinetic interaction between paclitaxel and topotecan was observed. Given the magnitude of toxicological differences between the 3- and 24-hour paclitaxel schedules, particularly with respect to neutropenia, it seems most appropriate to select the 3-hour paclitaxel infusion for further combination studies with topotecan. It results in less myelosuppression and allows higher doses of topotecan and paclitaxel to be combined.

Of concern, in a subsequent phase II study in patients with extended-stage small cell lung carcinoma using the combination of paclitaxel as a 3-hour infusion with topotecan, unexpected severe haematological toxicity was encountered. Three of the 13 treated patients died of treatment related sepsis [82]. In patients with small cell lung cancer neutropenic fever and infection are related to the duration of grade 4 neutropenia. Considering the definition of DLT in the phase I study, grade 4 neutropenia lasting more than 7 days, this toxicity could have been anticipated in this patient population. Moreover, lung cancer patients are known to be more prone to developing post-obstruction infections. In view of this, dose levels for further studies should be chosen depending on the patient population and the risk of prolonged and/or febrile neutropenia deemed acceptable. Given the magnitude of the haematological effects, both of the 3-hour and the 24-hour paclitaxel/topotecan regimens, and the requirement for hematopoietic colony-stimulating factor support further careful evaluation of these regimens is necessary.

Another phase I study assessed the toxicity of the combination of paclitaxel on day 1 as a 3-hour infusion with topotecan as a continuous infusion over 14 days once every 3 weeks [143]. Dose limiting toxicity consisted of neutropenia. In pre-treated patients the dose recommended for further studies is paclitaxel 135 mg/m^2 in combination with a daily topotecan dose of 0.3 mg/m^2. In chemotherapy-naïve patients further dose escalation is ongoing.

Docetaxel given 3-weekly was studied in combination with irinotecan in a weekly and a 3-weekly schedule. Docetaxel 60 mg/m^2 given as a 60-minute infusion could be combined with irinotecan 250 mg/m^2 given over 90 minutes, once every 3 weeks. Dose limiting toxicity was febrile neutropenia. No pharmacokinetic interaction between the two drugs was observed [144]. In the study combining irinotecan on day 1,8 and 15 with docetaxel day 2 every 4 weeks [145] DLT was encountered at the dose level combining docetaxel 50 mg/m^2 and irinotecan 60 mg/m^2 and consisted of neutropenia and diarrhoea. Additional dose levels are under study to determine the recommended dose of this combination for further phase II studies.

Phase II studies

The results of published phase II studies in which combination chemotherapy with topoisomerase I inhibitors was used to treat patients with solid tumours, are summarised in Table 5 [87,146-153]. Response rates of 31-54% have been reported

in previously untreated patients with advanced non-small cell lung cancer using the combination of cisplatin with irinotecan.

Table 5. Phase II studies on combinations of irinotecan and topotecan in various tumour types

Tumour type	Therapy scheme (mg/m^2/day)	No patients	RR %	Reference
First line				
NSCLC	CPT-11 160 d1 CDDP 20 d1-5 + G-CSF 2µg/kg d6-21 q 4 weeks	41	54	146
	CPT-11 60 d1, 8, 15 CDDP 80 d1 q 4 weeks	52	31	147
	CPT-11 60 d1-3 VP16 60 d1-3 + G-CSF 50 µg/m^2/d d4-17 q 3 weeks	61	21.3	148
	Topotecan 1.25 d1-5 CDDP 75 d1 + G-CSF 5µg/kg d6-16 q 4 weeks	22	14	149
	Topotecan 1.0 d1-5 Paclitaxel 190 d1 + G-CSF 5µg/kg d6-16 q 4 weeks	61	24	149
SCLC	CPT-11 80/60 d1, 8, 15 CDDP 60 d1 q 4 weeks	75	78	150
Cervical	CPT-11 60 d1, 8, 15 CDDP 60 d1 q 4 weeks	30	68	153
Gastric cancer	CPT-11 70 d1, 15 CDDP 80 d1 q 4 weeks	29	59	151
Second line				
Gastric cancer	CPT-11 70 d1, 15 CDDP 80 d1 q 4 week	15	27	151
Ovarian cancer	CPT-11 50/60 d1, 8, 15 CDDP 50/60 d1 q 4 weeks	18	54	152
clear cell ca	CPT-11 140 d1, 15 Mitomycin-C i.p. d1, 15 q 4 weeks	24	46	87

i.p. intraperitoneal, RR: response rate

The major side effects consisted of myelosuppression (≥ grade 3: leukopenia 13-23%, neutropenia 22-46%, thrombocytopenia 12-18%) and diarrhoea (≥ grade 3: 17-25%) [146,147]. A high percentage of patients required reduction of the irinotecan dose especially due to non-haematological toxicity. This regimen also seems to be highly active in patients with small cell lung cancer yielding a overall response rate of 84% and a complete response rate of 29%. During the latter study the dose of irinotecan in the first course had to be reduced because of severe haematological toxicity, diarrhoea and/or liver toxicity in three of the initial 10 patients [150]. High response rates have also been achieved using the combination of CDDP and irinotecan in patients with gastric carcinoma [151]. In first line therapy a response rate of 59% was noted, in second line this was 27%. The median duration of response was 118 days (range 41-253+). The encountered toxicity was acceptable (grade 4 neutropenia: 22%, ≥ grade 3 diarrhoea: 7%). The same combination used as first line chemotherapy also yielded a response rate of 68% in patients with recurrent or advanced cervical cancer [153]. A phase II study using the same combination in patients with relapsed or metastatic ovarian cancer revealed a response rate of 54% [152]. Because of neutropenia, the administration of irinotecan on day 15 was skipped in 31% of the patients receiving irinotecan at 60 mg/m^2. Therefore, during the study the dose of irinotecan was reduced to 50 mg/m^2.

The efficacy of the combination of topotecan 1.25 mg/m^2/d d 1-5 and cisplatin 75 mg/m^2 d 1 (arm A), and topotecan 1.0 mg/m^2/d d 1-5 and paclitaxel 190 mg/m^2 d 1 (arm B), both with addition of G-CSF and administered once every 4 weeks, was evaluated in a randomised phase II study in 83 patients with non-pre-treated advanced non-small cell lung cancer [149]. Neither treatment arm showed activity (arm A response rate 14%, arm B 24%) that can be considered superior to the activity of currently available regimens. Excessive toxicity was encountered for the combination of cisplatin and topotecan; grade 4 neutropenia 62% and thrombocytopenia 76%, ≥ grade 3 non-haematological toxicity 43%. Neither regimen was recommended for further study. Also, combination chemotherapy with the concurrent administration of irinotecan 60 mg/m^2/d d 1-3 and etoposide 60 mg/m^2/d d 1-3 every 3 weeks with G-CSF support revealed only modest efficacy against metastatic non-small cell lung cancer in first line therapy (response rate 21%, median duration of response 141 days, range 62-299 days) [148]. The toxicity appeared manageable, and consisted of neutropenia (≥ grade 3: 39%, complicated by infection in 15/24 patients), anaemia (≥ grade 3: 8%), diarrhoea (≥ grade 3: 16%), elevation of

transaminases and bilirubin (≥ grade 3: 5%) and interstitial pneumonitis. Since preclinical studies stressed the potential importance of sequential administration of topoisomerase I inhibitors with various other classes of agents, further studies are required to determine the antitumour effect of these combinations.

CONCLUSION AND FUTURE PERSPECTIVES

Topoisomerase I inhibitors are an important addition to the presently available classes of drugs. Relatively recently irinotecan was registered for use in colorectal cancer patients and topotecan for use in second line chemotherapy for ovarian cancer. Irinotecan can be combined with antimetabolites and taxanes at relatively high doses. The dose intensity reached in combination with platin-derivatives seems to depend on the administration schedule used. In schedules combining irinotecan with a single administration of CDDP, a higher dose intensity could be achieved. Combinations of topoisomerase I and II inhibitors yielded much toxicity, especially myelotoxicity, mucositis and diarrhoea. Even after addition of G-CSF the gain in dose intensity was limited. However, growth factor support seems only warranted if the antitumour activity of a combination therapy is enhanced by achieving a higher dose intensity. Further studies are necessary to evaluate the efficacy of these combination regimens. Combination therapy with topotecan required even more dose reductions as compared to the single agent dose. The reason for this particular difference between topoisomerase I inhibitors is not yet known.

The sequence of drug administration in the combinations influenced the severity of the observed side effects. This might in part be explained by pharmacokinetic interactions, by up-regulation of topoisomerase IIα, by a change in cell cycle distribution of the tumour and blood progenitor cells and/or by the interaction between topoisomerase I inhibitor and DNA-adducts. Clearly, all future trials should be paralleled by pharmacokinetics in order to evaluate the existence of pharmacological interactions and sequence dependent effects of a combination therapy. Further investigations are also needed to determine whether the more toxic sequences are also the more cytotoxic. Since it is less desirable to address this issue in phase III studies, it would be worthwhile to have these studies preceded by randomised phase II studies in order to exclude a major impact of drug-sequences on antitumour activity.

In order to reduce the side effects and maximise the dose intensity of the combination therapy, other schemes of administration of the drugs should be

evaluated. I.e. the administration once every three weeks of both CDDP and irinotecan, the reversed sequence of administration of topotecan in combination with CDDP, paclitaxel, etoposide and doxorubicin, and schemes with different time intervals between the administration of the drugs.

Considering the antitumour activity, especially the combination of irinotecan and cisplatin seems to be promising in patients with non-small cell and small cell lung cancer, gastric cancer and ovarian cancer. Optimal utilisation of irinotecan in combination with 5-FU, with or without leucovorin, in patients with metastatic colorectal cancer will require further studies in chemonaive patients. The impact of irinotecan as adjuvant therapy has yet to be explored.

Additional large studies in patients with ovarian cancer are planned that will determine the contribution of topotecan when used as part of front-line combination therapy with e.q. paclitaxel and cisplatin.

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Chapter 8

Phase I and pharmacological study of oral topotecan and intravenous cisplatin: sequence dependent hematologic side-effects

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ABSTRACT

Background and Purpose: In *in vitro* studies synergistic cytotoxicity for the combination of topotecan and cisplatin and the existence of a sequence dependent effect was reported. Recently an oral formulation of topotecan became available for clinical studies. This phase I and pharmacological study was performed to assess the feasibility of the combination of oral topotecan and cisplatin, the pharmacokinetic interaction, and sequence dependent effects.

Patients and Methods: Topotecan was administered orally daily for five days in escalating doses and cisplatin was given at a fixed dose of 75 mg/m² as a 3-hour infusion either before topotecan on day 1 (CT) or after topotecan on day 5 (TC) once every 3 weeks. Patients were treated in a randomized cross-over design.

Results: Forty-nine patients entered the study; one patient was not eligible. The CT sequence induced significantly more severe myelosuppression than the alternate sequence, and resulted in MTD at a topotecan dose of 1.25 mg/m²/dx5. In the reversed sequence (TC), MTD was encountered at a topotecan dose of 2.0 mg/m²/dx5. DLT consisted of a combination of myelosuppression and diarrhea. Pharmacokinetics of topotecan and cisplatin were linear over the dose range studied. No sequence dependent effects were observed in the pharmacokinetic parameters of topotecan. In addition, topotecan did not influence the protein binding of cisplatin and the platinum-DNA adduct formation in peripheral leukocytes in either sequence.

Conclusion: The recommended dose for phase II studies in selected patients is oral topotecan 1.25 mg/m²/day x 5 preceded by cisplatin 75 mg/m² day 1 once every 3 weeks, and topotecan 2.0 mg/m²/day followed by the same dose cisplatin on day 5. No indication of a mutual pharmacokinetic interaction could be discerned. The antitumor efficacy of both administration schedules should be evaluated in a randomized phase II study.

INTRODUCTION

Topotecan (9-dimethylaminomethyl-10-hydroxycamptothecin, Hycamtin®) is a water-soluble topoisomerase I inhibitor (camptothecin). DNA topoisomerase I is a nuclear enzyme, involved in cellular replication and transcription. It exists in two forms in a pH dependent dynamic balance between the closed lactone ring (active) form and the carboxy acid (inactive) form. By forming a covalent adduct between topoisomerase I and DNA, named the cleavable complex, topoisomerase I inhibitors

interfere with the process of DNA breakage and resealing during DNA synthesis. The stabilized cleavable complex blocks the progress of the replication fork resulting in irreversible DNA double-strand breaks leading to cell death [1-3]. Based on their mechanism of action, synergy was suspected for the combination of topoisomerase I inhibitors and DNA damaging agents such as cisplatin. Preclinical studies confirmed this hypothesis. However, the observed interaction seemed to depend on the cell line studied and the schedule of administration of topotecan and cisplatin used [4-10]. When topotecan was preceded by cisplatin, synergy was increased compared to concomitant incubation with both drugs in the IGROV-1 ovarian cancer cell line and the MCF7 cell line [5,6]. Also in the clinical setting drug sequencing seems to be important [10].

To date, topotecan has demonstrated prominent activity in several malignancies, most notable in ovarian [11-16], small cell lung carcinomas [17-19], and hematological malignancies [20-23], in which cisplatin is also highly active. Recently, an oral formulation of topotecan became available, which is a more convenient method of drug administration. The oral formulation has a bioavailability of 32-44% [24,25] with moderate intrapatient variability. The maximally tolerated dose for oral topotecan, administered for five days every 21 days as a gelatin capsule, has been determined as 2.3 mg/m²/day with myelosuppression, in particular neutropenia, as the dose limiting toxicity (DLT) [26]. Non-hematological toxicities were generally mild and not dose limiting, including fatigue, anorexia, nausea, vomiting and diarrhea. In ovarian- [27] and small cell lung cancer [28] randomized studies suggest the oral formulation is equivalent to the intravenous formulation.

Against this background, we initiated a phase I study in which patients were treated in a randomized cross-over design to determine the maximum tolerated dose of oral topotecan given daily for 5 days combined with cisplatin 75 mg/m² i.v. administered either on day 1 or day 5 every 21 days, to describe and quantitate the toxicities of the combination and to determine whether the sequence of topotecan and cisplatin administration has any influence on the observed toxicity or the pharmacokinetic interaction between the drugs.

PATIENTS AND METHODS

Patient Selection

Patients with a histologically or cytologically confirmed diagnosis of a malignant solid tumor resistant to standard forms of therapy were eligible. Other eligibility

criteria included the following: age between 18-75 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas or mitomycin C); no previous therapy with topoisomerase I inhibitors ; adequate hematopoietic function (absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L and platelet count ≥ 100 x 10^9/L), renal function (creatinine clearance ≥ 60 mL/min) and hepatic (total serum bilirubin ≤ 1.25 x upper normal limit and serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) ≤ 2.0 x upper normal limits, in case of liver metastasis serum ASAT and ALAT ≤ 3.0 x upper normal limits) function. Specific exclusion criteria included the existence of gross ascites and/or any gastrointestinal condition that would alter absorption or motility. All patients gave written informed consent before study entry.

Dosage and Dose Escalation

Escalating doses of oral topotecan were combined with a fixed dose of cisplatin, 75 mg/m², administered intravenously over 3 hours once every 3 weeks. The starting dose of topotecan was 0.75 mg/m²/day for 5 consecutive days, which is 33% of the recommended dose of oral topotecan, when administered as a single agent. Dose escalation was based on the prior dose level toxicities. At least three patients were treated at each dose level. If one of three patients experienced dose limiting toxicity (DLT), three additional patients were entered at that dose level. The maximum-tolerated dose (MTD) was defined as one dose level below the dose that induced DLTs in 3 out of 6 patients during the first course in any sequence, which were defined as NCI-CTC grade 4 neutropenia lasting for five days or more, or complicated with fever requiring hospitalization, grade 4 thrombocytopenia and/or non-hematological toxicity ≥ grade 3 (grade 2 for renal toxicity), excluding nausea. Intrapatient dose escalation was not permitted. If a patient encountered DLT, the dose of topotecan was decreased one dose level at re-treatment. The treatment was resumed when the neutrophil count had recovered to ≥ 1.0 x 10^9/L and the platelet count to ≥ 100 x 10^9/L. A maximum of six cycles was administered to each individual patient.

Drug Administration and Sequencing

In the first part of the study, patients were randomly assigned at study entry to one of two treatment groups. Six patients were treated at each dose level.

Group A. In the first treatment course, patients received cisplatin as a 3-hour infusion diluted in 250 mL of hypertonic saline [3% (w/v) sodium chloride] on day 1, immediately followed by the oral administration of topotecan (sequence CT), which was given for five consecutive days on an empty stomach, at least 10 minutes before meals. In the second course, the sequence of administration of topotecan and cisplatin was reversed, starting with topotecan for 5 days and administering cisplatin on day 5 3-hours after the last oral administration of topotecan at the same doses (sequence TC).

Group B. Patients received the two treatment courses in reversed order.

The third and following courses were administered using the least toxic sequence, with in the third course a 24 hour interval between the administration of cisplatin and topotecan to study the pharmacokinetics of both drugs to rule out the possibility of any pharmacokinetic interaction.

In the second part of the study, after determination of the MTD in the most toxic sequence, further dose escalation of topotecan was pursued in the reversed sequence. Patients were then enrolled to receive that single sequence with a 24-hour interval between the administration of topotecan and cisplatin in the second course only.

In all patients pre-medication consisted of ondansetron (8 mg i.v.) combined with dexamethasone (10 mg i.v.) administered 30 min before the start of the cisplatin infusion. To prevent cisplatin-induced renal damage, the administration of cisplatin was preceded by the infusion of 1000 mL of a mixture of 5% (w/v) dextrose and 0.9% (w/v) sodium chloride over 4 hours, and followed by another 3000 mL with the addition of 20 mM potassium chloride and 2 g/L magnesium sulphate applied over 16 hours. Topotecan capsules containing either 0.25 or 1.00 mg of the active compound were supplied by SmithKline Beecham Pharmaceuticals (Harlow, UK). Cisplatin (Platosin®) was purchased as a powder from Pharmachemie (Haarlem, The Netherlands).

Treatment Assessment

Before therapy a complete medical history was taken and a physical examination was performed. A complete blood count (CBC) including WBC and differential, and serum biochemistry, which included sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine transferase (ALAT), γ-glutamyl transferase,

glucose and uric acid, were performed, as was creatinine clearance. Weekly evaluations included history, physical examination, toxicity assessment according to the CTC criteria, and serum chemistry. CBC was determined twice weekly. Tumor evaluation was performed after three courses in the first part and after every two courses in the second part of the study according to the World Health Organisation (WHO) criteria for response. Patients were treated for at least three cycles of therapy in the first part and two cycles in the second part of the study unless disease progression or unacceptable toxicity was encountered.

Sample Collection for Pharmacokinetics

Blood samples for pharmacokinetic analysis were obtained during the first 3 treatment courses until the MTD was reached in the most toxic sequence. Hereafter samples were only taken in the first 2 courses. Blood sampling for topotecan pharmacokinetics was performed on the first and fifth day of drug dosing, whereas for cisplatin pharmacokinetics sampling was performed on the day of administration (days 1, 5 or 6, dependent on the schedule). At the doses recommended for further study, additional topotecan pharmacokinetics were performed on day 2 of the first treatment course. Blood was withdrawn from a vein in the arm opposite to that used for drug infusion, and collected in 4.5-mL glass tubes containing lithium heparin as anticoagulant. For analysis of topotecan kinetics, samples were obtained at the following time points: prior to dosing, and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after administration of topotecan. Immediately after sampling, tubes were briefly immersed into an ice bath kept at the bedside, and plasma was separated within 10 min by centrifugation at 3000 × *g* for 5 min (4°C). Next, 250-µL aliquots of the plasma supernatant were added to 2.0-mL polypropylene vials (Eppendorf, Hamburg, Germany) containing 750-µL of ice cold (-20°C) methanol. After mixing on a vortex-mixer for 10 s, samples were stored at -80°C until the day of analysis.

Blood samples for measurement of cisplatin concentrations were obtained immediately before infusion; at 1, 2, and 3 hours after start of the infusion; and 0.5, 1, 2, 3, and 18 hours after the end of the infusion. Sample volumes were 4.5 mL each except at predose and 1 and 18 hours after infusion, which were 21 (3×7) mL each. Immediately after sampling plasma was separated by centrifugation at 3000 × *g* for 10 min. Next, 500-µL aliquots of the plasma supernatant were added to 1.0-mL of ice cold (-20°C) ethanol. After mixing on a vortex-mixer for 10 s, samples were stored at -80°C until the day of analysis.

Pharmacokinetic Assays

Samples for topotecan kinetics were analyzed by a reversed-phase high-performance liquid chromatographic (HPLC) method, as described [29], that allowed simultaneous determination of the lactone and the hydrolyzed ring-opened carboxylate forms. Prior to drug analysis, samples were removed from the freezer and centrifuged for 5 min at 23000 × *g* (4°C), A volume of 100 µL was transferred to a clean microtube containing 400 µL phosphate buffer. Of this mixture, a 200-µL volume was used for analysis. The HPLC system consisted of a constaMetric 4100 solvent delivery system (LDC Analytical, Riviera Beach, FL), a Waters 717Plus autosampler (Bedford, MA), and a Jasco FP 920 fluorescence detector (Jasco). Chromatographic separations were achieved at 35°C on a Shandon Hypersil BDS column (100×3 mm, internal diameter; 3 µm particle size) from Applied Science (Breda, The Netherlands), with a mobile phase composed of 10 mM aqueous potassium dihydrogen phosphate containing 22% (v/v) methanol and 0.2% triethylamine, with the pH adjusted to 6.0 (orthophosphoric acid). The mobile phase was filtered [0.45-µm Millipore HA filters (Milford, MA)] and degassed by ultrasonication. The flow rate was set at 0.7 mL/min, and the column effluent was monitored at excitation and emission wavelengths of 381 and 525 nm, respectively with the emission band width set at 40 nm. Peak detection was performed with the Fisons ChromCard data analysis system (Milan, Italy). Drug concentrations in unknown samples were determined by interpolation on linear calibration curves, constructed in blank human plasma, by least-squares linear regression of peak heights *versus* 1/*x*. The mean percentage deviation from nominal values (accuracy) and precision (within-run and between-run variability) were always <15%. The lower limit of quantitation for both the lactone and carboxylate forms were 100 pg/mL.

Non-protein bound and total cisplatin concentrations in plasma were determined by a validated analytical procedure based on measurement of platinum atoms by flameless atomic-absorption spectrometry (AAS) as described [30,31]. For measurement of unbound cisplatin, 500-µL aliquots of plasma were extracted with neat 1000 µL ice-cold ethanol in a 2-mL polypropylene vial. After a 2-hour incubation at -20°C, the supernatant was collected by centrifugation at 23,000×*g* for 5 min (4°C), and transferred to a clean vial. A volume of 600 µL was evaporated to dryness under nitrogen at 60°C, and the residue reconstituted in 200 or 600 µL water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride by vigorous mixing. A volume of 20 µL was eventually injected into the AAS. For determination of total

cisplatin, a 100-µL volume of plasma was added to 900 µL water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride, followed by vortex-mixing for 10 s. Of this solution, a volume of 20 µL was injected into the AAS. Samples were analyzed on a Perkin Elmer Model 4110 ZL spectrometer with Zeeman-background correction using peak area signal measurements at a wavelength of 265.9 nm and a slid width of 0.7 nm [30,31]. The injection temperature was set at 20°C. Platinum DNA adduct levels in peripheral leukocytes were determined as described [32], with modifications [33]. Following DNA isolation from buffy coat preparations, samples were digested with DNAse I and zinc chloride and injected into the furnace using a 4-times multiple sampling feature of the Perkin Elmer AAS. The cisplatin DNA-adduct levels were expressed as picogram platinum per microgram DNA (pgPt/ µg DNA).

Pharmacokinetic Data Analysis

Individual plasma concentrations of topotecan were fit to a two-compartment model, using the software package Siphar v4.0 (SIMED, Creteil, France). The concentration-time profiles were obtained after zero-order input, with weighted least-squares analysis applying a weighting factor of $1/y$. The topotecan area under the plasma concentration-time curve (AUC) was determined for both the lactone ($AUC_{(L)}$) and carboxylate forms ($AUC_{(c)}$) on the basis of the best fitted curves. The apparent plasma clearance ($CL/f_{(L)}$) of topotecan lactone was calculated by dividing the dose administered (expressed in free base equivalents) by the observed AUC. The terminal disposition half-life [$T_{1/2}(z)$] of topotecan was calculated as $\ln 2/k$, where k is the terminal elimination rate constant (expressed in h^{-1}). The peak plasma concentrations (C_{max}) and the time to peak plasma concentration (T_{max}) were determined graphically from the (observed) experimental values. The ratio of the systemic exposure of topotecan lactone to total drug (L/T ratio) was defined as $AUC_{(L)}/[AUC_{(L)}+AUC_{(c)}]$.

Kinetic profiles of CDDP were obtained similarly using a two-compartment linear model with extended least-squares regression analysis as reported earlier [34]. The AUC of cisplatin was calculated to the last sampling time point with detectable drug levels (C_{last}) by the linear trapezoid method and extended to infinity by addition of C_{last}/k_{term}, where k_{term} is the slope obtained by log-linear regression of the final plasma concentration values.

Statistical Considerations

Pharmacokinetic parameters for all compounds are reported as mean values ± S.D. The difference in pharmacokinetic parameters between sequences was evaluated statistically using a paired Student's t-test. Probability values (two-sided) of less than 0.05 were regarded as statistically significant. All calculations were performed using the statistical packages NCSS version 5.X (J.L. Hintze, Kaysville, UT) and STATGRAPHICS Plus version 2.0 (Manugistics Inc., Rockville, MA).

Table 1. Patient characteristics

Characteristic		No. of Patients
No. Entered		49
No. Assessable		47
Age, years		
Median	57	
Range	28-70	
Sex		
Female		19
Male		28
Performance status		
Median	1	
Range	0-1	
Tumor type		
Head/Neck		11
(N)SCLC		10
ACUP		10
Cervical		3
Miscellaneous		13
Previous therapy		
Chemotherapy		9
Radiation		12
Chemotherapy and radiation		8
None		18

RESULTS

Forty-nine patients entered this study between January 1997 and February 1999. Patient characteristics are listed in Table 1. One patient was not eligible due to reduced renal function at the time of study entry, 1 patient was not assessable for toxicity because of the occurrence of a cerebrovascular accident after two days of treatment with topotecan and was taken off study. Forty-seven patients were assessable for toxicity and 45 patients for response.

The majority of the patients was either asymptomatic or had only mild symptoms. Nineteen patients were female and 28 were male. Twenty-nine patients had received prior chemo- and/or radiotherapy. The most common tumor type was head and neck cancer. Dose levels of topotecan studied were 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.3 mg/m²/day, respectively. The total number of assessable courses was 175. The median number of courses per patient was 4 (range 1-6).

Both myelosuppression and diarrhea were the principal DLTs of this regimen. Seven patients required dose reductions after experiencing dose-limiting toxicity. Once dose reduction had taken place, the courses in these patients were evaluated for toxicity at the lower dose level.

Table 2. Hematological toxicity (worst per cycle) and drug sequencing

Topotecan mg/m²/day	Sequence of drug administration	No. of cycles	Neutropenia 3	Neutropenia 4	Thrompbocytopenia 3	Thrompbocytopenia 4
0.75	CT	9	1	1	1	0
	TC	19	0	0	0	0
1.0	CT	7	2	3	1	0
	TC	22	1	0	0	0
1.25	CT	17	7	5	4	1
	TC	17	1	1	0	0

CT: cisplatin followed by topotecan; TC: cisplatin preceded by topotecan

Hematological toxicity and drug sequencing

The severity of the observed hematological toxicity was clearly dependent on the sequence of drug administration. At each dose level studied, both neutropenia and thrombocytopenia were more severe when cisplatin administration preceded the

administration of topotecan (CT sequence) [Table 2] reflected in both a significantly lower nadir and percentage decrements in neutrophil and platelet counts in this sequence [P=<0.00001 (neutropenia), P=<0.00001 (thrombocytopenia)]. At the dose level of 1.0 mg/m² in the sequence CT, two out of six patients experienced neutropenia grade 4 lasting for 5 days or more. By protocol definition, these patients were judged as having DLT. At the next dose level combining topotecan 1.25 mg/m²/day with cisplatin 75 mg/m² in the sequence CT, of the six patients treated, one patient experienced neutropenic fever and another patient had neutropenia grade 4 lasting for more than 5 days in combination with diarrhea grade 3 and vomiting grade 4. Four additional patients were treated at this dose level. One of these patients had DLT because of a neutropenia grade 4 lasting longer than 5 days. Another patient, who formally was ineligible due to reduced renal function at study entry, experienced grade 4 neutropenia and thrombocytopenia in the second course, and died as a result of the complications of this toxicity. Of importance, most of the DLTs were uncomplicated and/or manageable, in some cases with supportive medication (loperamide). Since 3 out of 9 (4 out of 10 taking into account the ineligible patient) patients experienced DLT, which on the other hand was still manageable, no further dose escalation was pursued. The protocol defined MTD as the dose level below that level at which 3/6 patients experienced DLT, but since at the dose level combining cisplatin 75 mg/m² day 1 with by topotecan 1.25 mg/m²/day day 1 to 5 the toxicities were manageable, they could be regarded as being not dose limiting. This dose level is considered the recommended dose for this sequence, but only in non- or marginally pretreated patients in good physical condition and under strict conditions of control. In all other patients the recommended dose is cisplatin 75 mg/m² day 1 followed by topotecan 1.0 mg/m²/day, day 1-5. Phase II clinical trials will determine which of these doses offer the better benefit/risk ratio in a wider clinical setting.

After the determination of the recommended dose in the sequence CT, dose escalation of topotecan continued in the reversed sequence. At the dose level 1.25 mg/m² one out of six patients experienced DLT consisting of neutropenic fever in this sequence. At the dose level 1.5 mg/m² no DLT was observed. One patient had a neutropenia grade 4 lasting for 5 days or more and vomiting grade 3 at dose level 1.75 mg/m² in the sequence TC. At the dose level combining topotecan 2.0 mg/m²/day with cisplatin, no DLT occurred in the initial 3 patients. It was decided to escalate the dose of topotecan to the dose recommended for use as a single agent, 2.3 mg/m²/day. Of the first three patients, only one patient developed DLT (diarrhea

grade 4). However, three of the four additional patients treated at this dose level, were considered to have DLT, on the basis of vomiting grade 4 (1 patient), diarrhea grade 4 (1 patient), neutropenia grade 4 lasting for 5 days or more (2 patients) and thrombocytopenia grade 4 (1 patient). Thus, combining topotecan 2.3 mg/m²/day and cisplatin 75 mg/m² was not considered feasible. Seven additional patients were treated at dose level 2.0/75 mg/m². Four of these patients developed DLTs: vomiting grade 4 (2 patients), diarrhea grade 3 or 4 (3 patients) although manageable with loperamide therapy, neutropenia grade 4 lasting for 5 days or more (2 patients), and thrombocytopenia grade 4 (1 patient), resulting in DLT in 4 out of 10 patients. Since most of these DLTs were uncomplicated and/or manageable, this dose could still be considered feasible in selected patients. Thus, in full accordance to the recommendations for the sequence CT, the recommended dose of topotecan is 2.0 mg/m²/day combined with cisplatin 75 mg/m² for the sequence topotecan followed by cisplatin. Since it was felt that these doses are only feasible in non- or minimally pretreated patients in good physical condition under strict medical surveillance, it was decided to expand the dose level combining topotecan 1.75 mg/m² to six patients. One of these patients experienced grade 4 vomiting in the first course. No other DLTs were observed.

Table 3. Hematological toxicity (worst per cycle) in sequence topotecan followed by cisplatin

Topotecan	No.	Neutropenia		Thrombocytopenia	
mg/m²/day	pts/cycles	3	4	3	4
0.75	7/19	0	0	0	0
1.0	6/22	1	0	0	0
1.25	6/17	1	1	0	0
1.5	3/13	3	1	0	0
1.75	7/17	2	3	2	0
2.0	12/36	11	2	1	2
2.3	7/17	8	3	1	1

Overall, the hematological toxicity was relatively mild [Table 2 and 3]. Grade 3 to 4 neutropenia was observed in 55 of 175 courses (31%). It was complicated by neutropenic fever in only 4 patients. The onsets of neutropenia and thrombocytopenia were relatively late. The nadir of the neutrophils usually occurred

around day 19 (range 4-30) after the start of the treatment and lasted for median 5 days (range 1-15) [Fig. 1]. Thrombocytopenia was mild, being grade 3-4 in only 8% of the cycles, all in conjunction with neutropenia. Despite the limited severity of myelosuppression, treatment had to be delayed in 34% of the courses due to prolonged myelosuppression. A marked inhibition of erythropoiesis was observed. The percentage of patients requiring erythrocyte transfusions was 72%, in 74 of 175 courses.

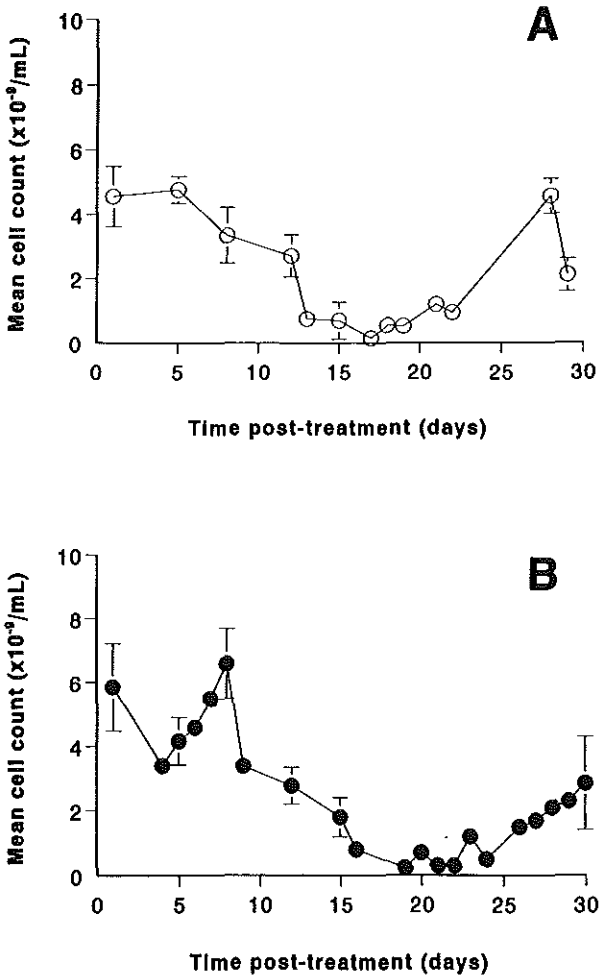

Fig. 1. Absolute neutrophil counts of patients experiencing grade 3 or 4 neutropenia during the first course at the recommended dose levels: sequence CT (panel **A**) topotecan 1.25 mg/m^2/day; sequence TC (panel **B**) topotecan 2.0 mg/m^2/day. Data represent mean values (symbol) ± S.D. (error bars)

Non-hematological toxicity

Gastrointestinal toxicity was mild to moderate [Table 4] and generally comparable to the toxicities that result from similar single agent doses of topotecan and cisplatin. No sequence dependent effects were noted. Nausea grade 2 or 3 was observed in 60 of 175 courses (34%) and vomiting grade 3 or 4 in 15 of 175 courses (9%). Both were in time related to the administration of cisplatin. Diarrhea grade 3 or 4 was encountered in 7 cycles (4%) and had a median day of onset on day 8 (range 7-14) and a median duration of 4 days (range 2-10). The diarrhea was self-limiting or resolved after low dose loperamide therapy in all but two patients, who were hospitalized for i.v. rehydration.

Table 4. Non-hematological toxicity (worst per cycle) in both sequences

Topotecan	nr	Nausea			Vomiting				Diarrhea				Fatigue		
mg/m²/day	pts/cycles	1	2	3	1	2	3	4	1	2	3	4	1	2	3
0.75	7/28	18	3	0	9	1	0	0	0	0	0	0	11	6	0
1.0	7/29	16	7	1	6	18	0	1	5	1	0	0	7	5	0
1.25	9/34	12	11	2	4	8	1	1	11	3	1	0	12	4	0
1.5	3/13	4	2	0	0	0	0	0	2	0	0	0	8	1	0
1.75	7/17	4	9	3	9	2	2	2	4	0	0	0	3	6	0
2.0	12/36	15	12	3	10	9	3	2	8	6	1	2	9	13	1
2.3	7/17	7	5	3	8	5	0	3	2	1	0	3	4	2	1

Consistent with the profile of cisplatin 75 mg/m², seventeen patients developed nephrotoxicity grade 1, and 4 patients grade 2 after a median of 2 cycles (range 1-6). Peripheral neurotoxicity grade 1 was encountered in 18 patients. Twenty patients had mostly reversible ototoxicity grade 2 (tinnitus) and 2 patients ototoxicity grade 3 after receiving median 2 cycles (range 1-6). One patient at dose level 2.3 mg/m² developed grade 4 bilirubinemia, due to obstruction of a biliary stent. One patient with a nasopharyngeal cancer, treated at dose level 2.0 mg/m² developed progressive dyspnoe accompanied by fever during the second course. An X-ray of the chest revealed interstitial enhancement with a reticulonodular pattern, especially more prominent at the bases. The pulmonary function demonstrated reduced lung volumes [forced expiratory volume in 1 sec 1.28 L (51% of normal), total lung capacity 2.21 L (42% of normal)]. Bronchoscopy revealed no abnormalities. Despite therapy with

antibiotics and low dose corticosteroids, the patient's condition worsened and it was decided to perform an open lung biopsy. Pathological examination revealed interstitial fibrosis with a marked infiltration with eosinophils, which was considered related to topotecan treatment. The patient was treated with high dose corticosteroids resulting in an amelioration of the symptoms.
Other side effects were mucositis (8 % of cycles), alopecia (19 patients grade 1, 7 patients grade 2), and fatigue.

Anti-tumor activity

Five patients achieved a partial response. The tumor types included, head and neck cancer, non-small cell and small cell lung cancer, ACUP and pancreatic cancer. The duration of the responses were 20, 21, 22, 26 and 30 weeks. Twenty-nine patients showed disease stabilization.

Table 5. Effect of drug sequence and interval time on the pharmacokinetics of topotecan and cisplatin at a topotecan dose of 0.75 mg/m^2/dx5 and a single fixed cisplatin dose of 75 mg/m^2. Data are mean values ± S.D.

	3-hour interval (n = 6)		24-hour interval (n = 6)	
Parameter	CT (d1)	TC (d1)	CT d1/2)	TC (d1/2)
Topotecan				
C_{max} (ng/ml)	2.05 ± 0.96	1.97 ± 0.55	3.01 ± 1.85	2.86 ± 1.10
$AUC_{(L)}$ (ng.h/ml)	7.52 ± 2.51	6.18 ± 1.56	9.20 ± 3.45	9.89 ± 3.09
$CL/f_{(L)}$ (L/h/m^2)	111 ± 34.0	131 ± 38.3	96.3 ± 40.8	83.5 ± 25.2
L/T ratio	0.36 ± 0.04	0.33 ± 0.08	0.33 ± 0.03	0.33 ± 0.02
Cisplatin				
AUC_{fu} (µg.h/ml)	2.30 ± 1.17	2.29 ± 0.20	3.24 ± 0.27	3.07 ± 0.51
CL_{fu} (ml/min)	817 ± 463	747 ± 177	823 ± 52.2	765 ± 139
AUC_{tot} (µg.h/ml)	33.3 ± 10.5	29.6 ± 4.12	46.5 ± 1.26	45.3 ± 4.08
A_{max} (pg/µg DNA)	2.91 ± 2.33	1.93 ± 1.28	2.41 ± 0.81	3.20 ± 2.78

Abbreviations: C_{max}, peak plasma level; AUC, area under the plasma concentration-time curve; CL/f, apparent clearance; L/T ratio, topotecan lactone to total drug AUC ratio; fu, unbound platinum fraction; tot, total platinum fraction; A_{max}, peak platinum DNA adduct levels in peripheral leukocytes.

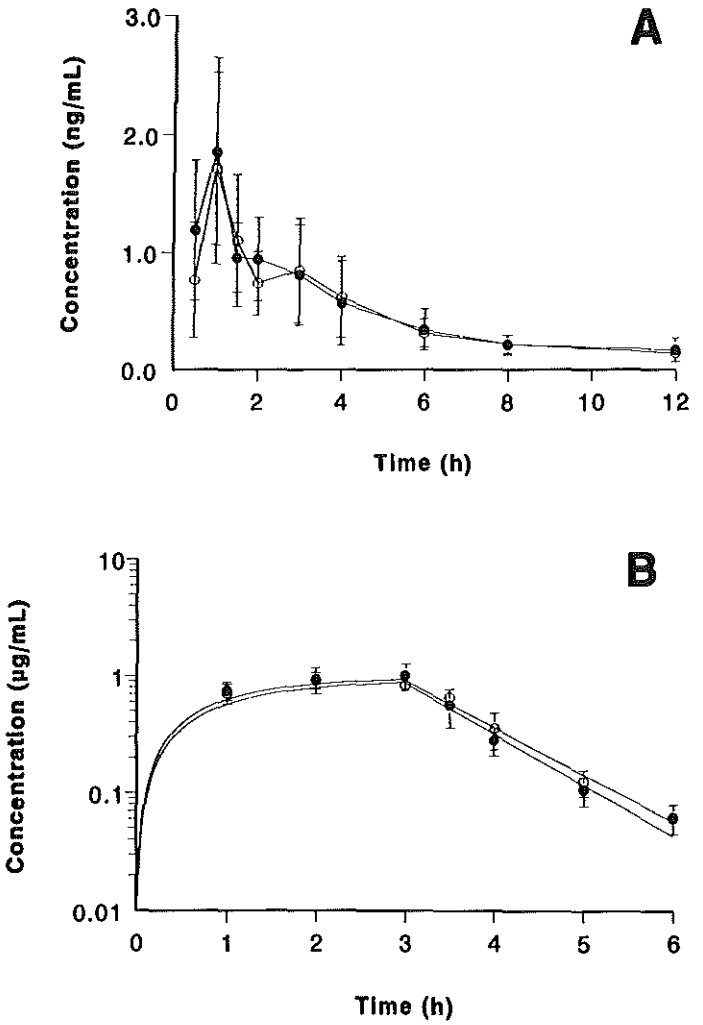

Fig. 2. Plasma concentration-time curves of topotecan lactone (closed symbols: sequence CT; open symbols: sequence TC, panel **A**) and unbound cisplatin (closed symbols: sequence CT; open symbols: sequence TC, panel **B**) in 6 patients treated with topotecan 0.75 mg/m²/day and cisplatin 75 mg/m². Data represent mean values (symbol) ± S.D. (error bars).

Topotecan and Cisplatin Pharmacokinetics

The possible effect of drug sequence on the pharmacokinetics of topotecan and cisplatin was investigated in the first 18 patients, that were randomized in a cross-over design for the administration sequence. These patients were all treated at the fixed cisplatin dose of 75 mg/m^2 and topotecan doses of 0.75, 1.0 or 1.25 mg/m^2/dx5. Table 5 lists the main pharmacokinetic parameters from a compartmental analysis of the two drugs, with topotecan given at 0.75 mg/m^2/dx5. The sequence of drug administration did not significantly influence the disposition of topotecan lactone, indicating mean (±SD) AUC values of 7.52±2.51 and 6.18±1.56 ng.h/mL (P=0.31) using the CT and TC sequence, respectively [Fig. 2].

The apparent clearance (CL/f) of topotecan lactone was clearly dose-independent in the range of 0.75 to 1.25 mg/m^2, similar to single agent data, and not significantly different between study courses (107±33.0 (CT) *vs* 109±53.5 L/h/m^2 (TC); P=0.38, paired Student's t-test). Similarly, the lactone to total drug AUC ratio (L/T ratio) was independent of the sequence and averaged 0.36±0.04 (CT) *vs* 0.33±0.08 (TC). Topotecan pharmacokinetic parameters obtained on the fifth administration day were essentially similar to the data from day 1 (Data not shown). In order to rule out a potential effect of the interval time between drug administration, kinetic data were obtained from the first 18 patients (3 receiving the CT sequence and another 3 receiving the reversed order at each dose level) treated with a topotecan dose of 0.75, 1.0 or 1.25 mg/m^2/dx5. Data of unpaired analysis in these patients indicated that a change of the interval time to 24 hours had no significant influence on any of the studied parameters (P>0.05, Mann-Whitney's U-test; Table 5). The peak plasma levels and the plasma clearance of unbound cisplatin were also independent of the drug sequence with a 3-hour or a 24-hour interval time between administration [Table 5]. Over the 3 dose levels studied, the cisplatin clearance was not dependent on the topotecan dose, and averaged 823±52.2 (CT) *vs* 765±139 mL/min (TC) (P=0.19) with the 3-hour interval time [Fig. 2]. Similarly, sequence and topotecan dose had no influence on the protein binding of cisplatin (overall mean: 93.1±2.8%) and on the peak platinum DNA-adduct levels in peripheral blood leukocytes [4.58±4.12 (CT) *vs* 5.72± 4.66 pg Pt/µg DNA (TC) across all 3 dose levels; P=0.55].

To further assess the effects of cisplatin administration and drug sequence on topotecan pharmacokinetics, all additional patients enrolled in the study had complete sampling performed, with the exception of 3 patients treated at the 2.0

mg/m^2/dx5 topotecan dose and 1 patient (only second course missing) at the 2.3 mg/m^2/dx5 dose. A summary of the topotecan pharmacokinetic data from the first course is provided in Table 6. In both sequence groups, substantial interpatient variability in kinetic parameters was apparent, with more than 2-fold variation in AUC values, although mean values were correlated to the administered dose (Spearman's ρ(rho)=0.76; TC sequence). There were no significant differences in any of the parameters between the topotecan dose levels (P>0.05, Kruskal-Wallis' test), consistent with a linear and dose-independent behavior of the compound. Pharmacokinetic parameters between sequences were again not significantly different, with overall mean apparent topotecan clearances of 109±40.2 (CT) *vs* 118 L/h/m^2 (TC). Parameters between the day of topotecan dosing were not significantly different as indicated by the ratio of the topotecan lactone AUC measured on days 1 and 5 (Data not shown), although the mean ratios slightly deviated from 1.0 probably as a result of a minor topotecan accumulation during the consecutive treatment days. Pharmacokinetic data obtained during the second treatment course, again with sampling performed on days 1 and 5, were essentially similar to the first course (not shown).

Table 6. Summary of topotecan pharmacokinetics during the first course during the first course as a function of treatment cohort. Data are mean values ± S.D.

Topotecan mg/m^2/dx5)	n	$C_{max\,(L)}$ (ng/ml)	$AUC_{(L)}$ (ng.h/ml)	$CL/f_{(L)}$ L/h/m^2	L/T ratio
CT sequence					
0.75	6	2.05 ± 0.96	7.52 ± 2.51	111 ± 34.0	0.36 ± 0.04
1.00	6	2.26 ± 0.73	8.38 ± 1.93	126 ± 28.4	0.39 ± 0.05
1.25	10	4.10 ± 1.72	14.5 ± 4.42	99.0 ± 46.0	0.42 ± 0.07
Overall mean		-	-	**109 ± 40.2**	**0.40 ± 0.06**
TC sequence					
0.75	6	1.97 ± 0.55	6.18 ± 1.56	131 ± 38.3	0.33 ± 0.08
1.00	6	3.88 ± 2.43	8.27 ± 4.47	95.8 ± 63.8	0.35 ± 0.16
1.25	6	4.33 ± 2.43	14.7 ± 5.64	102 ± 48.3	0.38 ± 0.05
1.50	3	4.73 ± 1.67	14.6 ± 2.94	107 ± 19.0	0.40 ± 0.07
1.75	3	6.87 ± 5.74	18.2 ± 6.11	106 ± 29.0	0.37 ± 0.05
2.00	6	5.92 ± 3.10	18.4 ± 6.74	131 ± 64.6	0.38 ± 0.04
2.30	7	3.72 ± 1.00	18.4 ± 6.15	145 ± 60.1	0.39 ± 0.07
Overall mean		-	-	**118 ± 55.0**	**0.37 ± 0.09**

Abbreviations: Cmax: peak plasma level; AUC: area under the plasma concentration-time curve; CL/f: apparent clearance; L/T ratio: topotecan lactone to total drug AUC ratio; n: number of patients studied.

The effect of the topotecan dose on the disposition of unbound and total cisplatin in plasma during the first treatment course is shown in Table 7. None of the pharmacokinetic parameters between the sequences and the various topotecan dose levels was significantly different. The overall mean plasma clearances of unbound cisplatin were 713±345 (CT) *vs* 877±152 mL/min (TC) (P=0.31).

Table 7. Effect of topotecan dose on the pharmacokinetics of cisplatin during the first treatment course at a single fixed cisplatin dose of 75 mg/m^2. Data are mean values ± S.D.

Topotecan mg/m^2/dx5)	n	AUC_{fu} (µg.h/ml)	CL_{fu} (ml/min)	AUC_{tot} (µg.h/ml)	CL_{tot} (ml/min)
CT sequence					
0.75	6	2.30 ± 1.17	817 ± 463	33.3 ± 10.5	88.1 ± 45.1
1.00	6	2.64 ± 1.27	623 ± 310	41.5 ± 6.51	58.6 ± 13.5
1.25	10	2.97 ± 1.12	962 ± 660	37.9 ± 4.39	56.3 ± 7.72
Overall mean		**2.74 ± 1.12**	**713 ± 345**	**38.1 ± 8.44**	**67.7 ± 31.1**
TC sequence					
0.75	6	2.71 ± 0.20	925 ± 177	37.0 ± 4.12	67.5 ± 9.87
1.00	6	2.87 ± 0.35	833 ± 143	35.5 ± 4.45	67.3 ± 11.5
1.25	6	2.58 ± 0.45	874 ± 183	41.6 ± 9.79	55.6 ± 14.8
1.50	3	2.75 ± 0.46	919 ± 174	32.9 ± 3.54	75.3 ± 8.06
1.75	3	3.22 ± 0.27	722 ± 75.0	47.6 ± 4.28	48.8 ± 4.36
2.00	6	3.13 ± 0.50	815 ± 87.0	37.1 ± 6.44	69.7 ± 11.3
2.30	7	2.58 ± 0.23	975 ± 130	36.1 ± 4.89	69.8 ± 7.30
Overall mean		**2.79 ± 0.38**	**877 ± 152**	**37.9 ± 7.16**	**65.4 ± 12.9**

Abbreviations: AUC: area under the plasma concentration-time curve; CL: clearance; fu: unbound platinum fraction; tot: total platinum fraction; n: number of patients studied.

At the recommended doses for further clinical studies, *viz.* 75 mg/m^2 cisplatin followed by 1.25 mg/m^2/dx5 topotecan (CT) and 2.0 mg/m^2/dx5 topotecan followed by 75 mg/m^2 cisplatin (TC), plasma sampling was also performed on day 2 to ensure that the topotecan disposition did not alter before day 5. Paired analysis showed that all relevant parameters were essentially similar between days of drug administration in both sequences [Table 8], although in the (less myelotoxic) TC sequence, the topotecan lactone peak plasma level and AUC values were slightly higher on day 2 as compared to days 1 and 5. This is most likely caused by the small number of patients studied (n=4 on day 2), in combination with large intrapatient and interpatient variability in topotecan kinetics.

Table 8. Topotecan and cisplatin pharmacokinetics at the recommended doses during the first treatment course: (1) 75 mg/m^2 cisplatin followed by 1.25 mg/m^2/dx5 topotecan and (2) 2.00 mg/m^2/dx5 topotecan followed by 75 mg/m^2 cisplatin. Data are mean values ± S.D.

	(1) CT sequence (n = 10)			(2) TC sequence [n = 6 (T) or 7 (C)]		
Parameter	day 1	day 2*	day 5	day 1	day 2*	day 5
Topotecan						
C_{max} (ng/ml)	4.10 ± 1.72	3.31 ± 2.31	3.50 ± 2.25	5.11 ± 3.50	7.52 ± 3.55	6.16 ± 3.30
$AUC_{(L)}$ (ng.h/ml)	14.5 ± 4.42	13.8 ± 4.22	14.4 ± 4.42	15.9 ± 8.79	24.6 ± 8.24	22.6 ± 6.10
L/T ratio	0.40 ± 0.03	0.39 ± 0.07	0.40 ± 0.08	0.36 ± 0.02	0.37 ± 0.04	0.41 ± 0.05
Cisplatin						
AUC_{fu} (µg.h/ml)	2.97 ± 1.12	-	-	-	-	3.13 ± 0.50
CL_{fu} (ml/min)	962 ± 660	-	-	-	-	815 ± 87.0
AUC_{tot} (µg.h/ml)	37.9 ± 4.39	-	-	-	-	37.1 ± 6.44
A_{max} (pg/µg DNA)	3.12 ± 6.52	-	-	-	-	2.34 ± 2.67

* Topotecan pharmacokinetic parameters on day 2 were only available from 4 patients. Abbreviations: C_{max}: peak plasma level; AUC: area under the plasma concentration-time curve; L/T ratio: topotecan lactone to total drug AUC ratio. fu: unbound platinum fraction; tot: total platinum fraction; CL: clearance; A_{max}: peak platinum DNA adduct levels in peripheral leukocytes.

DISCUSSION

Both cisplatin and topotecan have broad antitumor activity. Because topoisomerase I inhibitors might interfere in the repair of cisplatin induced DNA interstrand cross-links, there has been considerable interest in the effects of combining these classes of drugs. Preclinical studies indicated that the observed interaction depended on the cell line studied and the sequence of drug administration. The potential importance of sequence dependence for the combination of cisplatin and the intravenous formulation of topotecan in the clinical setting was studied by Rowinsky et al., revealing enhanced myelosuppression when cisplatin administration preceded topotecan [10]. Recently, an oral formulation of topotecan with a bioavailability of 32-44% became available, which is a more convenient method of drug administration [24,25]. The reported phase I study was performed to explore the influence of alternate sequences of oral topotecan in a daily times five schedule and cisplatin on the observed side-effects and pharmacokinetic behavior of both drugs and to determine the maximum tolerated dose of topotecan in combination with cisplatin 75 mg/m^2 once every 3 weeks in both sequences.

Both neutropenia and diarrhea were the DLTs of oral topotecan combined with cisplatin in this schedule. Other toxicity was usually mild to moderate and consisted of nausea and vomiting, mucositis, fatigue, neuro- and nephrotoxicity and alopecia.

Myelosuppression was significantly more severe when cisplatin preceded topotecan administration. This observation is in accordance with the data reported for the combination of the intravenous formulation of topotecan and cisplatin [10]. The onset of the neutropenia was relatively late with a median day of onset of the nadir on day 19 (range 4-30). These data are in line with the data reported by Miller et al. [35]. The combination of topotecan, administered intravenously on day 1 to 5, with cisplatin on day 1 resulted in a neutrophil nadir around day 12 (range 8-25). Compared to the median time to neutrophil nadir of 12 days (range 9-15) for single agent oral topotecan [25] and day 9 (range 6-10) [12] for single agent intravenously administered topotecan, the nadir in our study was delayed. This resulted in treatment delay due to prolonged myelosuppression in 34% of the courses. Despite grade 3 or 4 neutropenia was observed in 31% of the courses, the incidence of neutropenic fever was only 2%.

The doses in this sequence we can recommend for phase II studies are, cisplatin followed by topotecan, are oral topotecan 1.25 mg/m^2/day day 1 to 5 and cisplatin 75 mg/m^2, but only in non- or minimally pretreated patients in good clinical condition and

under strict medical surveillance. The recommended dose of topotecan in this schedule is 54% of single agent oral topotecan in a daily times five schedule [26]. In other phase I studies, combining cisplatin 50 mg/m² on day 1 with topotecan as a 30-min infusion daily for 5 consecutive days, neutropenia and thrombocytopenia constituted the principal toxicities. The recommended dose of topotecan for further trials was 0.75 to 1.0 mg/m²/day combined with cisplatin 50 mg/m², accounting for 50-66% of the single agent intravenous dose of topotecan [10,35].

For the reversed sequence, the recommended doses are oral topotecan 2.0 mg/m²/day day 1 to 5 followed by cisplatin 75 mg/m². This constitutes a topotecan dose of 87% of the single agent dose. However, as indicated, it is recommended that the use of topotecan and cisplatin at these doses should be limited to patients similar to those studied in this trial, i.e., untreated or minimally pretreated patients with a good performance status under strict medical surveillance. This sequence of drug administration was also studied for the combination of the intravenous formulation of topotecan for 5 consecutive days in escalating doses and cisplatin 50 mg/m² in an alternating schedule with carboplatin, cisplatin, teniposide and vincristine in patients with small-cell lung cancer [36]. Preliminary data indicate that it is feasible to combine i.v. topotecan 1.5 mg/m²/day, the recommended dose of single-agent topotecan, with cisplatin 50 mg/m². Thus, the observed hematological toxicity is sequence dependent both for the intravenous and the oral formulation of topotecan in combination with cisplatin, resulting in a higher dose intensity of topotecan when administered before cisplatin.

The observed pharmacokinetic parameters of the lactone and the carboxylate form of topotecan demonstrated linear and dose independent behavior over the total dose range studied and were similar to single agent data [37] and also comparable to the data obtained in the schedule with 24 hour interval between the administration of topotecan and cisplatin in our study, indicating no apparent pharmacokinetic interaction between topotecan and cisplatin. The sequence of drug administration also had no influence on the pharmacokinetics of topotecan at the dose levels administered, neither on day 1, 2 or 5. This is in contrast with the reported reduction of the clearance of intravenously administered topotecan observed after preceding cisplatin administration [10]. Also the ratio of topotecan AUC of lactone to total drug correspond very well with data of a previous study in which oral topotecan was administered as a single agent [37] and did not vary with the sequence of drug administration.

The plasma clearance and volume of distribution of unbound cisplatin as well as the AUC up to the last measured time point of total cisplatin in plasma indicated no significant influence of topotecan on the protein binding and plasma disposition of cisplatin. Preclinical studies indicated that the reversal of cisplatin-induced DNA interstrand cross-links was delayed by concomitant incubation with a topoisomerase I inhibitor [38,39], without modifying their formation. However, in our study the values of the maximal platinum DNA-adduct formation in peripheral leukocytes and the area under the DNA-adduct versus time curve were consistent with single agent data [34], and were independent of the drug sequence. Although the preclinical observations might not be extrapolated to the clinical setting, it is possible that the extreme variability in platinum DNA-adduct values would not allow any small alteration in adduct formation to be observed even if it was present.

Based on the available data, the importance of the sequence of drug administration and the enhanced toxicity observed when cisplatin is followed by topotecan can not simply be extrapolated to the antitumor activity of the combination. However, a sequence dependent effect on antitumor activity can not be ruled out. Further randomized phase II studies in patients with topotecan sensitive tumor types are needed to elucidate the importance of drug sequencing and possible cytotoxic interaction, and the potential relevance of the higher dose intensity of both drugs, that can be achieved when the less toxic sequence of drug administration is used.
In conclusion, the recommended dose for phase II studies in selected patients is oral topotecan 1.25 mg/m²/day for 5 consecutive days combined with cisplatin 75 mg/m² on day 1, once every 3 weeks, or topotecan 2.0 mg/m²/day day 1 to 5 followed by the same dose cisplatin on day 5. No pharmacokinetic interaction could explain the enhanced myelosuppression observed in the sequence CT.

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Chapter 9

Phase I study of 3-weekly irinotecan combined with cisplatin once every 3 weeks in patients with advanced solid tumor

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ABSTRACT

Background and Purpose: Irinotecan and cisplatin show a broad antitumor activity as single agents, and synergism in preclinical investigation. This phase I and pharmacokinetic study was performed to assess the feasibility of the combination in a 3-weekly schedule, the pharmacokinetic interaction, and possible sequence dependent effects. Also the influence of additional G-CSF on the hematological toxicity was assessed.

Patients and Methods: Patients who had received no more than one prior combination chemotherapy regimen or two single-agent regimens were entered. Treatment consisted of irinotecan given as a 90-minutes infusion followed by cisplatin as a 3-hour infusion on day 1, cycles repeated once every 3 weeks. After determination of the MTD, the sequence of administration was reversed. In a separate cohort of 6 patients the influence of the administration of G-CSF on the experienced hematological toxicity and dose intensity was assessed. Irinotecan doses ranged from 175-300 mg/m^2 and cisplatin doses from 60-80 mg/m^2.

Results: Fifty-two patients entered the study; one patient was not eligible, two patients were not assessable for response. Twenty-five patients were pretreated, 26 were non-pretreated. Fifty-one patients received a total of 223 courses. DLT was a combination of neutropenic fever, diarrhea and fatigue at a dose level combining irinotecan 300 mg/m^2 with cisplatin 80 mg/m^2. Neutropenia was common (grade 3-4, 68%). Pharmacokinetics of irinotecan were linear over the dose ranged studied. No sequence dependent side effects were observed. Tumor responses included three complete responses and eight partial responses.

Conclusion: The recommended doses for phase II studies are irinotecan 260 mg/m^2 combined with cisplatin 80 mg/m^2 once every 3 weeks for chemonaive patients in good physical condition. For other patients irinotecan 200 mg/m^2 combined with cisplatin 80 mg/m^2 is recommended.

INTRODUCTION

Irinotecan (CPT-11, Campto®) is a water-soluble camptothecine analog. Camptothecine analogs are a family of anticancer agents with a unique mechanism of action, which is based on the reversible inhibition of DNA topoisomerase I [1,2]. Topoisomerase I inhibitors are of great clinical interest because of their important

antitumor activity as single agents in a broad spectrum of tumor types [3]. In addition, topoisomerase I inhibitors may also interfere with the processes involved in DNA repair [4-6] and enhance cytotoxicity when combined with DNA damaging agents. The different toxicity profiles of platinum-derivatives and topoisomerase I inhibitors and the lack of cross resistance further support the potential use of these agents in combination. In preclinical studies, the combination of irinotecan and cisplatin was shown to be synergistic in several human tumor cell lines and human xenograft tumor models [7-13]

Until now phase I studies on the combination of irinotecan and cisplatin focused on fractionated dose schedules for both agents [14-24]. The dose-intensity of irinotecan that could be achieved in these schedules in combination with cisplatin varied from 25-60% of the single agent dose. The higher dose-intensities were reached when irinotecan was combined with a single administration of cisplatin. Throughout these studies the major dose limiting toxicities were neutropenia and diarrhea. Other side effects consisted of nausea and vomiting, alopecia and mucositis. Major responses were observed with all treatment schedules in patients with non-small cell lung cancer, small cell lung cancer, gastric-, head and neck- and cervical carcinoma [14-30]. In Europe, the recommended schedule of administration of irinotecan single agent is an every three week schedule at the dose of 350 mg/m^2. Although there is no consensus yet, there appears to be a tendency among physicians to favor this 3-weekly schedule, not because of a believe in higher activity, but because of patient convenience. Randomized studies comparing the two schedules are presently ongoing. In the present report, we describe a phase I study on the combination of irinotecan and cisplatin, both administered intravenously once every 3 weeks.

PATIENTS AND METHODS

Patient Selection

Patients with a histologically or cytologically confirmed diagnosis of a malignant solid tumor refractory to standard forms of therapy were eligible. Other eligibility criteria included the following: age between 18-70 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas or mitomycin C); no previous therapy with topoisomerase I inhibitors or platin-derivatives; not treated with more than one prior

combination regimen or two single agent regimens; no major surgery within 28 days prior to inclusion; and adequate hematopoietic (absolute neutrophil count (ANC) ≥ 2.0 x 10^9/L and platelet count ≥ 100 x 10^9/L), renal (serum creatinine concentration ≤ 135 µmol/L or creatinine clearance ≥ 60 mL/min) and hepatic function (total serum bilirubin ≤ 1.25 x upper normal limit and serum aspartate aminotransferase and alanine aminotransferase ≤ 3.0 x upper normal limits, in case of liver metastasis total serum bilirubin ≤ 1.5 x upper normal limit and serum aspartate aminotransferase and alanine aminotransferase ≤ 5.0 x upper normal limits). Specific exclusion criteria included unresolved bowel (sub)obstruction and chronic diarrhea or chronic colic disease and symptomatic peripheral neuropathy > grade 1 according to the NCI Common Toxicity Criteria (NCI-CTC). All patients gave written informed consent before study entry.

Treatment and Dose Escalation

All patients were hospitalized for the combined treatment with irinotecan and cisplatin. The starting dose of irinotecan was 175 mg/m² combined with cisplatin 60 mg/m² based on the dose intensity of both agents achieved in the weekly schedules (irinotecan 45 mg/m²/week) [15]. Doses of irinotecan and cisplatin were escalated according to a planned schedule [Table 1], combining irinotecan 175-300 mg/m² with cisplatin 60-80 mg/m². After determination of the maximal tolerable dose of irinotecan in combination with cisplatin 80 mg/m², the dose of cisplatin was diminished to 60 mg/m² and combined with the dose of irinotecan that produced dose limiting toxicities when combined with cisplatin 80 mg/m² in order to determine the feasibility of this dose level. The courses were to be repeated every three weeks.

Table 1. Dose escalation schedule

Dose level	Dose (mg/m²)	
	CPT-11	CDDP
A	175	60
B	200	60
C	200	80
D	230	80
E	260	80
F	300	80
G*	300	60
H*	reversed sequence	

*G: this level is explored if level F constitutes the MTD.

*H: reversed sequence at recommended dose level.

Preceding the infusion of the cytotoxic drugs, patients were prehydrated with an infusion of 1000 mL of dextrose/saline given over 4 hours. In the first part of the study, irinotecan was administered intravenously (i.v.) diluted in 250 mL of 0.9% sodium chloride solution over 90 minutes on day 1. Subsequently, cisplatin was given as a 3-hour i.v. infusion, diluted in 250 mL 3.0 % saline followed by 2000 mL of dextrose/saline infused over 8 hours and another 1000 mL of dextrose/saline with the addition of 20 mmol KCl and 2 g $MgSO_4$ per liter, infused over the following 8 hours to avoid cisplatin-induced renal damage. Prior to the administration of irinotecan, patients received anti-emetic therapy consisting of ondansetron 8 mg i.v. combined with dexamethasone 10 mg i.v.. In case of severe acute cholinergic symptoms, i.e. acute diarrhea, 0.25 mg atropine was administered subcutaneously. For CPT-11 induced delayed type diarrhea, high dose loperamide therapy was administered orally consisting of a starting dose of 4 mg at the first episode of diarrhea followed by 2 mg every 2 hours for at least 12 hours. The patient was allowed to stop loperamide only after a 12-hour diarrhea free interval. If the diarrhea persisted for more than 24 hours despite the recommended loperamide treatment, a 7 day prophylactic oral antibiotic therapy (ciprofloxacine 500 mg b.i.d.) was added since severe diarrhea is considered a risk factor for febrile neutropenia. At least three patients were to be entered at each dose level. If dose limiting toxicities (DLTs) were seen in 1 of 3 patients, a further 3 patients were to be entered at that dose level. The maximum-tolerated dose (MTD) was defined as one dose level below the dose that induced DLTs during the first course, which were defined as NCI-CTC grade 3 or 4 neutropenia complicated with fever, grade 4 thrombocytopenia and/or non-hematological toxicity ≥ grade 3 (grade 2 for renal toxicity and grade 4 for vomiting lasting for more than 3 days), excluding nausea, in three or more of six patients. Intrapatient dose escalation was not allowed. If a patient encountered DLT, the dose of irinotecan and cisplatin was decreased one dose level at re-treatment. The treatment was resumed when the neutrophil count had recovered to ≥ 2.0 x 10^9/L and the platelet count to ≥ 100 x 10^9/L.

In the second part, after reaching MTD, the sequence of administration of irinotecan and cisplatin was reversed, administering cisplatin prior to irinotecan at the MTD to determine sequence dependent side effects and/or pharmacokinetic interaction. The hydration and anti-emetics were administered in the same way as in the first part. If no DLT was encountered, further dose escalation was foreseen.

In the third part, the influence of the administration of G-CSF (lenograstim) 150 µg/m²/day s.c. with a maximum of 263 µg/day on the experienced hematological

toxicity was assessed in six patients at a dose level recommended for further studies. Since at the dose levels studied the nadir of the neutrocytopenia occurred in the second and third week after treatment, it was questioned whether the administration of G-CSF during day 3 to 13 would be sufficient to prevent neutrocytopenia. In order to study the effect of the timing of the administration of G-CSF on the observed neutrocytopenia, the administration of G-CSF during day 3 to 13 in the first cycle was compared to administration during day 6 to 16 in the second cycle in the same patient.

Treatment Assessment

Before therapy a complete medical history was taken and a physical examination was performed. A complete blood count (CBC) including WBC differential, and serum biochemistry, which involved sodium, potassium, calcium, phosphorus, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, glucose and uric acid, were performed, as was creatinine clearance. Weekly evaluations included history, physical examination, toxicity assessment according to the CTC criteria, and serum chemistry. CBC was determined twice weekly. Tumor evaluation was performed after every two courses according to the World Health Organisation (WHO) criteria for response. Patients were treated for at least two cycles of therapy unless disease progression or unacceptable toxicity was encountered.

Pharmacokinetic Studies

Blood samples for pharmacokinetic analysis (total blood volume 129-154 mL) were obtained only during the first treatment cycle. Blood was drawn from a vein in the arm opposite to that used for drug infusion, and collected in 10-mL heparinized tubes. For analysis of irinotecan kinetics, samples were obtained at the following time points: before infusion; 0.5, 1, and 1.5 hours during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32, 48, and 56 hours after infusion. The tubes were briefly immersed into an ice bath kept at the bedside, and plasma was separated within 10 min by centrifugation at 4°C for 5 min at 3000 *g* on a tabletop centrifuge, to prevent continued degradation of the lactone forms. The supernatant was transferred to a clean tube and stored at -80°C, until the time of analysis. Samples for measurement of cisplatin concentrations were obtained immediately before infusion; 1, 2, and 3

hours after start of infusion; and 0.5, 1, 2, 3, 4, and 24 hours after completion of the infusion.

Plasma samples were assayed for irinotecan and SN-38 according to a validated reversed-phase high-performance liquid chromatographic method reported in detail previously [31,32]. The mean overall extraction efficiencies for irinotecan and SN-38 ranged between 83.0 and 99.1%. The percentage deviation from nominal values, and the inter- and intra-assay precision for each compound were always less than 12%. The lower limit of quantitation of CPT-11 and SN-38 (total drug forms) was 2 ng/mL.

Non-protein bound and total cisplatin concentrations in plasma were determined by atomic absorption spectrometry according to the method of Reed et al [33], with modifications as described [34,35].

Individual plasma concentrations of irinotecan and SN-38 were fit to a three-compartment model using Siphar v4.0 (SIMED, Creteil, France) as described [36]. The area under the plasma concentration-time curve (AUC) was estimated by least-squares fitting using weighting of $1/y$. Total body clearance of irinotecan was calculated by dividing the dose administered by the observed AUC.

Kinetic profiles of cisplatin were obtained similarly using a one- or two-compartment linear model with extended least-squares regression analysis as reported earlier [37].

RESULTS

Fifty-two patients entered this study between May 1996 and September 1998. Patient characteristics are listed in Table 2. One patient was not eligible because of elevated liver enzymes at entry of the study, all other patients were assessable for toxicity and forty-nine patients were assessable for response. The majority of the patients were either asymptomatic or had only mild symptoms. Eighteen patients were women and thirty-three were men. Twenty-five patients had received prior chemo- and/or radiotherapy. At the highest dose levels studied patients were carefully selected for study entry and most of them were non-pretreated. The most common tumor type was colorectal cancer. The total number of assessable courses was 223. The median number of courses per patient was 4 (range 1-10).

Table 2. Patient characteristics

Characteristic		No. of Patients
No. entered		52
No. assessable		51
Age, years		
Median	52	
Range	37-69	
Sex		
Female		18
Male		33
Performance status		
Median	1	
Range	0-1	
Tumor type		
Colorectal		22
(A)CUP		8
Head/Neck		5
Lung (non-small cell)		4
Miscellaneous		12
Previous therapy		
Chemotherapy		17
Radiation		7
Chemotherapy and radiation		1
None		26

No dose-limiting toxicities were observed at the first two dose levels [Table 3 and 4]. Dose-limiting toxicities were reported for the first cycle at the following dose-levels: neutropenic fever (one patient) at dose level C; neutropenic fever and diarrhea grade 4 (one patient) and nephrotoxicity (creatinine grade 2, one patient) at dose level D, neutropenic fever (two patients) and fatigue grade 3 (one patient) at dose level F, diarrhea grade 3 and 4 (two patients) and neutropenic fever (one patient) at dose level G, diarrhea grade 3 (one patient) and neutropenic fever (one patient) at dose level H.

Eleven patients required dose reductions after experiencing dose-limiting toxicity. Once dose reduction had taken place, these patients were evaluated for toxicity at the lower dose level.

Table 3. Hematological toxicity (worst per cycle)

Dose level	CPT11/CDDP mg/m²/3 wks	nr pts/cxs*	Neutropenia		Thrombocytopenia	
			3	4	3	4
A	175/60	6/27	2	10	1	0
	175/80	3/11	1	10	0	0
B	200/60	4/10	4	2	0	0
C	200/80	14/43	13	18	0	2
D	230/80	8/35	15	9	0	0
	230/60	2/6	2	2	0	0
E	260/80	6/25	7	14	0	0
F	300/80	4/8	3	4	0	0
G	300/60	6/13	6	3	0	0
H*	260/80	6/14	5	7	0	0

*Cxs:cycles. Several patients experiencing DLT required dose reductions to the dose levels combining 175/80 and 230/60. Once dose reduction had taken place, patients were evaluated for toxicity at the lower dose level. H: reversed sequence of drug administration.

Hematological toxicity

Neutropenia was the major hematological side effect in both sequences of drug administration without the addition of G-CSF [Table 3]. Grade 3 to 4 neutropenia was observed in 131 of 193 courses (68%) and was already present from the first dose level onwards. It was complicated by neutropenic fever in only 9 courses (5%). The nadir usually occurred around day 18 (range 7-23) after start of the treatment and lasted for median 7 days (range 2-22). Thrombocytopenia was mild, being grade 3-4 only in 1% of the cycles. Treatment had to be delayed in 32% of the courses due to prolonged myelosuppression. No cumulative toxicity was observed. One patient was taken off study because of persisting leukocytopenia after two weeks treatment delay. Eleven patients required dose reductions after experiencing dose-limiting toxicity. Details on the total dose of irinotecan actually delivered at each dose level are listed in Table 4. At the dose level recommended for further study combining irinotecan 260 mg/m² with cisplatin 80 mg/m² a median relative dose intensity of 100% was achieved (range 100-50%).

Table 4. Absolute dose of irinotecan delivered at each dose level.

Dose level CPT-11/CDDP mg/m²/3 wks	irinotecan mg/m²/week median (range)		% of planned dose median (range)
	cycle 1	all cycles	all cycles
A 175/60	58	58	100
B 200/60	67 (67-50)	50 (67-44)	75 (100-66)
C 200/80	67 (67-50)	50 (67-35)	75 (100-52)
D 230/80	77 (77-57)	67 (77-57)	87 (100-74)
E 260/80	87 (87-65)	87 (87-43)	100 (100-50)
F 300/80	75 (100-75)	84 (100-57)	84 (100-57)
G 300/60	100 (100-68)	68 (100-40)	68 (100-40)
H 80/260	87 (87-65)	87 (87-57)	100 (110-66)

Since non-hematological toxicities prevailed at the recommended dose level for phase II studies, combining irinotecan 260 mg/m² with cisplatin 80 mg/m², it was not deemed rational to add G-CSF to this dose level. In stead it was decided to study the influence of additional G-CSF at the dose level combining irinotecan 200 mg/m² with cisplatin 80 mg/m², at which dose level the principal toxicity was neutropenia, on the experienced hematological toxicity and achieved dose intensity in 6 patients. Administration of G-CSF resulted in a reduction in both duration and grade of myelosuppression [Table 5] resulting in a relative dose intensity of 100%. No difference was observed whether G-CSF was given on day 3-13 or 6-16 after the start of the treatment (results not shown).

Table 5. Comparison of toxicity in patients treated with or without additional G-CSF

CPT11/CDDP mg/m²/2 wks	G-CSF	nr pats/cxs	Diarrhea				Neutropenia				Achieved relative dose intensity all cycles
			1	2	3	4	1	2	3	4	
200/80	-	14/43	21	3	0	0	4	6	13	18	75% (100-52%)
200/80	+	6/30	19	3	0	0	1	3	2	1	100% (100-75%)

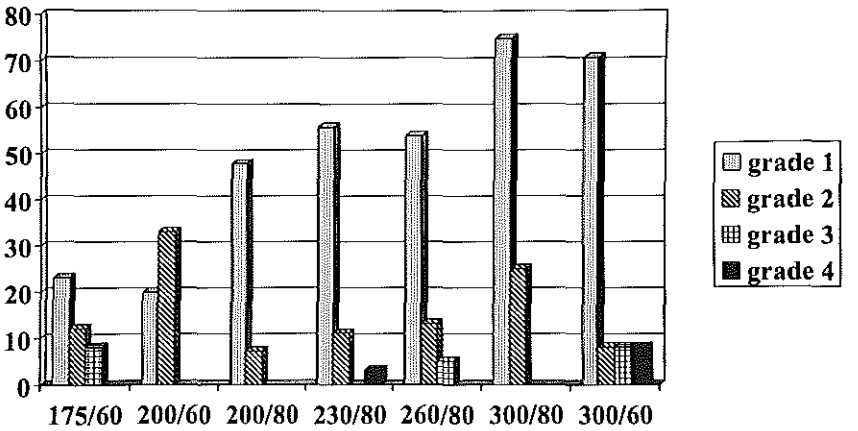

Fig. 1. Percentage of cycles complicated with diarrhea related to the dose level of cisplatin and irinotecan administered.

Non-hematological toxicity

Gastrointestinal toxicity was the most prominent non-hematological adverse effect, including nausea, vomiting and diarrhea. Diarrhea was one of the dose-limiting toxicities of this combination regimen. The incidence of diarrhea increased from 42% at the first dose level to 93-100% at the dose levels combining 300 mg/m^2 irinotecan with 60-80 mg/m^2 cisplatin [Figure 1]. Grade 3 or 4 diarrhea was observed in 8 of 193 courses (4%) and occurred despite the use of a high dose loperamide regimen. Treatment consisted of supportive care with i.v. administration of fluids and electrolytes. Grade 1 or 2 diarrhea occurred in 61% of the cycles, being more protracted at the higher dose levels. The median day of onset of diarrhea (all grades) was day 8 (range 2-26) and recovery was observed by day 9 (range 3-27). Nine patients received prophylactic therapy with ciprofloxacin because of late diarrhea persisting for more than 24 hours. No cumulative intestinal toxicity was observed.

Grade 3 nausea and grade 3 or 4 vomiting were observed in 36 (16%) of the cycles, but were transient and were no reason for dose reduction or withdrawal. One patient treated at the dose level combining irinotecan 300 mg/m^2 with cisplatin 80 mg/m^2 experienced grade 3 fatigue during the first course. Grade 2 or 3 fatigue was observed in 6 out of 22 courses at the dose levels involving irinotecan 300 mg/m^2.

Other side effects were mucositis (grade 1-2: 7%), acute cholinergic-like syndrome (15%) and alopecia (grade 2: 55%). In only 11 patients (22 cycles) the acute cholinergic-like syndrome required treatment with atropine (0.25 mg s.c.). Administration of atropine sulfate could in all cases prevent or reduce the symptoms. There was no evidence for severe hepatic or pulmonary toxicity in any of the patients that was treatment-related, and there was no evidence of potentiation of the renal and neurologic toxicity of cisplatin by irinotecan. The sequence of drug administration did not have a major influence on the observed toxicity. No treatment-related deaths were observed.

Antitumor Activity

Three patients achieved a complete response: one patient with a small cell bladder cancer, who was treated with subsequent radiotherapy, one patient with a metastatic colorectal cancer and in a third patient with a metastatic basaloid rectal cancer the residual tumor mass was surgically removed, revealing no viable tumor at histological examination (pCR). The duration of the complete responses are 3.5 month, 18$^+$ months and 19$^+$ months. Eight of the 51 patients exhibited partial responses. The tumor types included 2 colorectal cancer, 2 head and neck cancer, 2 NSCLC, ACUP, and cancer of the stomach. Eight patients showed minor responses and another 20 had disease stabilization.

Pharmacokinetics

Full kinetic data following the administration of irinotecan were obtained on day 1 to 3 from 45 patients and of cisplatin from 46 patients during the first course. Over the total dose range studied, the area under the plasma concentration-time curve (AUC) and the peak plasma concentrations of irinotecan increased from 17.2±7.08 (mean±SD) to 32.1±8.69 μM.h and from 3.94±1.25 to 6.55±1.91 μM, respectively, consistent with a linear pharmacokinetic behaviour. Marked interpatient variability in the AUC for irinotecan and SN-38, with a >2-fold variation in irinotecan AUC, was observed at

each dose level. However, mean values of the AUC of irinotecan and SN-38 increased proportionally with the dose of irinotecan administered. Elimination of irinotecan and SN-38 from the central plasma compartment was characterized by a decay in an apparent tri-exponential manner based on conventional compartmental modeling using weighted least-squares analysis with a weighting factor of $1/Y$. Cisplatin pharmacokinetics could best be described with a two-compartment model. The plasma clearance of unbound cisplatin was 1.05±0.27 L/min (mean±SD, n=46; range, 0.526-2.42 L/min). Results of the pharmacokinetic part of the study are reported in full separately.

DISCUSSION

Phase II studies have shown that irinotecan apart from antitumor activity in colorectal cancer, also has activity in non-small cell and small cell lung cancer, cervical, gastric and ovarian cancer and lymphoma, tumor types in which cisplatin also exerts substantial activity. The different toxicity profiles and the lack of cross resistance further support the use of these agents in combination. Preclinical studies demonstrated that combining irinotecan, and its major active metabolite, SN-38 with platinum-derivatives resulted in synergistic cytotoxicity in several human tumor cell lines and human xenograft tumor models. Previous phase I studies on the combination of irinotecan and cisplatin used various schedules of administration with an emphasis on fractionated dosing. In all studies irinotecan preceded the infusion of cisplatin. Preclinical data, however, on drug interactions between topoisomerase I inhibitors and platinum derivatives seem to suggest the potential of a sequence dependent effect. The potential importance of sequence dependence in the clinical setting has only been investigated for the combination of cisplatin and topotecan, revealing enhanced myelosuppression when cisplatin administration preceded topotecan. Pharmacokinetic data suggested that the differences in toxicity were due in part, to lower topotecan clearance when cisplatin was given before topotecan [39].

The reported phase I study was performed to assess the feasibility of combining cisplatin and irinotecan in a 3-weekly schedule, to determine the MTD and the side-effects of the combination, to investigate sequence dependent effects and to study the impact of G-CSF administration on the observed hematological toxicity at the dose recommended for further studies.

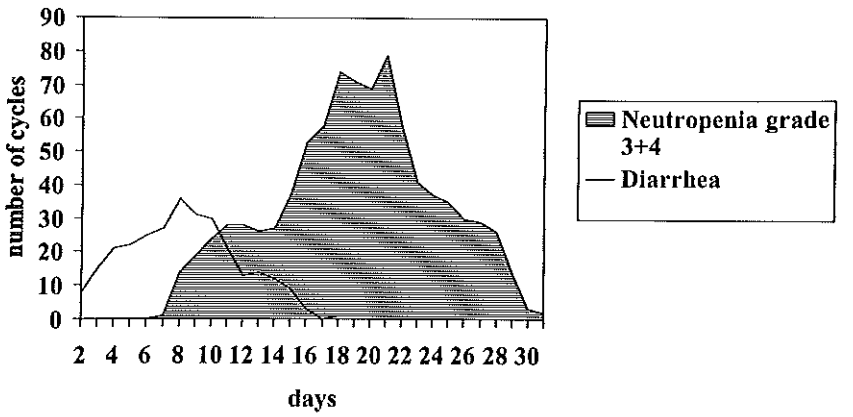

Fig. 2. Concomitant period of neutropenia grade 3 and 4 and any grade diarrhea at all dose levels.

The dose-limiting toxicity in our study was a combination of neutropenic fever, fatigue and diarrhea at the dose-level combining irinotecan 300 mg/m² with cisplatin 80 mg/m². Dose reduction of cisplatin to 60 mg/m² also resulted in DLT. At all dose levels grade 3-4 neutropenia was observed ranging from 44% of the cycles at the first dose level to 85% at the higher dose levels, with a median duration of the nadir of 7 days. The median time to neutrophil nadir was 18 days (range, 7-23) at all dose levels. Compared to the median time to neutrophil nadir of 8 days (range, 5-28) after treatment with single agent irinotecan at a dose of 350 mg/m² every three weeks [41], the nadir in our study was delayed. In regard of the high percentage of grade 3 and 4 neutropenia observed, the incidence of neutropenic fever was strikingly low. The concomitant occurrence of neutropenia and diarrhea, indicative for a damaged intestinal mucosa, seemed to predestine patients treated with irinotecan single agent

to the development of neutropenic fever. The delayed neutrophil nadir in our study reduced the period of overlapping of neutropenia and diarrhea [Figure 2] and might reduce the risk of patients for the development of neutropenic fever. The hematological toxicity is comparable to the toxicity observed in a phase II study combining irinotecan 60 mg/m² day 1, 8 and 15 with cisplatin 60 mg/m² day 1 every 28 days (neutropenia grade 3 or 4 77% of the cycles, thrombocytopenia grade 3 or 4 12%) [27].

Diarrhea was also considered a dose limiting toxicity. However, grade 3 or 4 diarrhea was only observed in 4% of the cycles but in these instances it occurred despite the vigorous administration of loperamide. In the phase I studies combining the weekly administration of irinotecan with cisplatin, diarrhea was not dose limiting. However, Kudoh reported in a subsequent phase II study an incidence of diarrhea of 76% of the patients, with grade 3 or 4 diarrhea occurring in 19% [27]. Single agent therapy with irinotecan at a dose of 350 mg/m² once every 3 weeks is complicated by diarrhea grade 3 or 4 in 22% of the cycles [40,41]. The incidence of grade 3 and 4 diarrhea in our study compares favourable to these data. No relationship could be demonstrated between the occurrence of the delayed onset diarrhea and the biliary index of SN-38 (data not shown).

The sequence of drug administration had no apparent influence on severity and frequency of the observed side effects.

Since the non-hematological side effects dominated at the recommended dose level combining 260 mg/m² irinotecan with 80 mg/m² cisplatin, it was not considered advantageous to add G-CSF to this dose level. However, since this dose level is only feasible in highly selected (WHO PS 0), chemonaïve patients, it was decided to expand the number of patients treated at the dose level combining irinotecan 200 mg/m² and cisplatin 80 mg/m² and to study the effect of additional G-CSF on the observed hematological toxicity and achieved dose intensity at that dose level. Considering the median time to neutrophil nadir of 18 days at all studied dose levels, it was questioned whether the early administration of G-CSF on day 3 to 13 adequately could prevent neutropenia. In order to study the effect of the timing of the administration of G-CSF on the hematological toxicity, each patient received G-CSF on day 3 to13 in the first course and subsequently on day 6 to 16 in the second course. The use of G-CSF resulted in a reduction of the grade and the duration of the myelosuppression, enabling optimal administration of both agents without treatment delay. The timing of G-CSF administration had no apparent influence on the reduction of the hematological toxicity.

In our study, the actually achieved median dose of irinotecan and cisplatin administered at the recommended dose level (irinotecan 260 mg/m² and cisplatin 80 mg/m²) during all cycles was 84 mg/m²/week and 27 mg/m²/week, respectively and at the dose level combining irinotecan 200 mg/m² and cisplatin 80 mg/m², 50 mg/m²/week and 27 mg/m²/week, respectively. This compares favourably to the reported planned dose intensity in previous phase I trials studying the fractionated administration of irinotecan in combination with cisplatin taking into account the data on absence of prior chemotherapy and on the performance score of the patient population in those trials which seem similar compared to ours [15-24]. In these studies only the planned dose intensity of irinotecan and cisplatin was reported and varied, respectively, between 25 to 60 mg/m²/week and 15 to 30 mg/m²/week [Figure 3].

In the present study, irinotecan and SN-38, the active metabolite, demonstrated linear and dose-independent pharmacokinetics over the dose range studied comparable with single agent data [37, 43]. Even though we did not study either agent alone, our data do at the very least suggest there is no apparent interaction. This is in contrast with the results reported by Masuda *et al.*, who described an unexpected rise in plasma SN-38 levels after only a slight increase in the irinotecan dose. However, comparison of the metabolic ratio of the conversion of irinotecan to SN-38 of the combination therapy to the single agent therapy, which was recently considered to be a reliable parameter to determine a pharmacokinetic interaction, did not reveal any difference indicative for a pharmacokinetic interaction. Also the pharmacokinetic data of cisplatin in our study were comparable with single agent data. Reversing of the administration sequence of irinotecan and cisplatin did not seem to have any influence on the pharmacokinetic data. To determine more precisely whether the sequence of drug administration has an impact on the pharmacokinetic or metabolic interaction between irinotecan and cisplatin, a study is currently being conducted in which patients are treated in a cross-over design with cisplatin either given before or after the administration of irinotecan.

Antitumor responses to the combination of irinotecan and cisplatin were observed in a variety of tumor types. Remarkable were the complete responses achieved in a patient with a small cell carcinoma of the bladder and a rectal basaloid cell carcinoma.

Planned dose intensity in comparison with other schedules

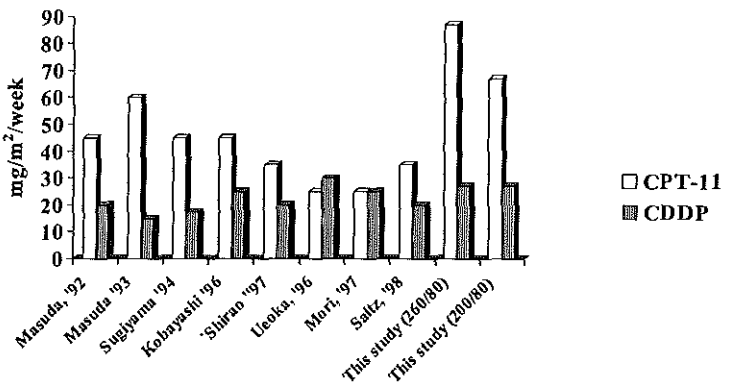

Fig. 3. Planned dose intensity in mg/m²/week of irinotecan (□) and cisplatin (■) at the recommended doses for phase II studies in comparison to other schedules.

In conclusion, in this phase I study on the combination of irinotecan and cisplatin administered once every 3 weeks, the DLT is a combination of neutropenic fever, diarrhea and fatigue. The recommended doses for phase II studies are irinotecan 260 mg/m² and cisplatin 80 mg/m² in non-pretreated patients in good physical condition (WHO PS 0). In other patients, doses of irinotecan 200 mg/m² combined with cisplatin 80 mg/m² should be considered. Addition of G-CSF at this dose level substantially reduces the hematological side effects and should be considered if one aims to optimise dose-intensity.

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Chapter 10

Drug administration sequence does not change pharmacodynamics and kinetics of irinotecan and cisplatin

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ABSTRACT

In this study, 11 patients with solid tumors were randomized to receive irinotecan (CPT-11; 200 mg/m²) as as a 90-min i.v. infusion, immediately followed by cisplatin (CDDP; 80 mg/m²) as a 3-h i.v. infusion in the first course and the reversed sequence in the second course or vice versa. No significant differences in any toxicity were observed between the treatment schedules (decrease in absolute neutrophil count, 74.4 ± 18.3 *versus* 80.3 ± 18.0%; P = 0.41). CPT-11 lactone clearance was similar to singel agent data and not significantly different between study courses (60.4 ± 17.1 *versus* 65.5 ± 16.3 liter/h/m^2; P = 0.06. The kinetic profiles of the major CPT-11 metabolites SN-38, SN-38 glucuronide, 7-ethyl-10-[4-*N*-(5-aminopentanoid acid)-1-piperidino[carbonyloxycamptothecine, and 7-ethyl-10-[4-*N*-(1-piperidino)-1-amino]carbonyloxycamptothecine were also sequence independent (P ≥ 0.20. In addition, CPT-11 had no influence on the clearance of nonprotein-bound CDDP (40.8 ± 16.7 *versus* 50.3 ± 18.6 liter/h/m^2; P = 0.08) and the platinum DNA-adduct formation in peripheral leukocytes in either sequence (1.94 ± 2.20 *versus* 2.42 ± 1.62 pg Pt/µg DNA; P = 0.41). These data indicate that the toxicity of the combination CPT-11 and CDDP is schedule independent and that there is no pharmacokinetic interaction.

INTRODUCTION

Topoisomerase I inhibitors have demonstrated important antitumor activity as single agents in various tumor types. Their mechanism of action suggests that they might interfere in processes involved in DNA repair and might enhance cytotoxicity when combined with DNA-damaging agents. Interactions of topoisomerase I inhibitors with platinum-derivatives have been studied *in vitro* and *in vivo*, and the interaction observed for the combination of irinotecan (CPT-11) and CDDP varied with the cell line studied [1-4]. Preclinical data also seemed to suggest the potential of a sequence-dependent effect, with synergy increaing when CDDP preceded CPT-11 incubation as compared with concomitant exposure to both drugs in various cell lines [2]. However, the sequence-dependent cytotoxicity of the combination of topoisomerase I inhibitors and platinum-derivatives also seemed to vary with the cell line studied and the schedule used [1,2].

In general, the design of effective combination chemotherapy regimens requires adequate attention to possible drug interactions at the pharmacokinetic and/or pharmacodynamic level. Until now, the importance of drug sequencing for the combination of topoisomerase I inhibitors and platinum-derivatives has clinically only been investigated for the combination of topotecan and CDDP [5,6,]. When CDDP was administered before a 5-day schedule of topotecan, significantly more and severe hematological toxicity was encountered than with the alternate sequence. Pharmacokinetic studies suggested that the differences in toxicity in part were due, in part, to a slower topotecan clearance when CDDP preceded topotecan [5].

In all phase I studies on the combination of CPT-11 and CDDP, CPT-11 administration preceded that of CDDP [1, 7-13], and pharmacokinetic data were only scarcely obtained [8]. Against this background, we initiated a study in which patients were treated in a randomized cross-over design to determine whether the sequence of CPT-11 and CDDP administration has any influence on the observed toxicity or is related to any pharmacokinetic interaction between the drugs.

MATERIALS AND METHODS

Eligibility Criteria

Patients with a histologically or cytologically confirmed diagnosis of a malignant solid tumor refractory to standard forms of therapy were eligible. All patients had adequate hematopoietic (absolute neutrophil count $\geq 2.0 \times 10^9$/L) and platelet count $\geq 100 \times 10^9$/L), renal (serum creatinine concentration $\leq$ 135 µmol/L or creatinine clearance $\geq$ 60 mL/min) and hepatic (total serum bilirubin $\leq$ 1.25 x upper normal limit and serum aspartate aminotransferase and alanine aminotransferase $\leq$ 3.0 x upper normal limits; in case of liver metastasis: total serum bilirubin $\leq$ 1.5 x upper normal limit and serum aspartate aminotransferase and alanine aminotransferase $\leq$ 5.0 x upper normal limits) function. All patients gave written informed consent before study entry.

Treatment Plan and Drug Administration

Patients were randomized to one of two treatment groups. In **group A**, patients received CPT-11 as a 90-min i.v. infusion at a dose of 200 mg/m^2 on day 1, immediately followed by the infusion of CDDP at a dose of 80 mg/m^2 as a 3-hour i.v.

infusion diluted in 250 ml of sodium chloride 3% (w/v) on day 1. Doses were selected based on experience obtained in a preceding phase I study. In the second course, the sequence of administration of CPT-11 and CDDP was reversed, administering CDDP before CPT-11 at the same doses. In case a patient encountered neutropenic fever or grade 3 or 4 non-hematological toxicity (except nausea and vomiting), the dose of CPT-11 was reduced to 175 mg/m^2 and CDDP was reduced to 60 mg/m^2 in the second course.

Group B. Patients received the two treatment cycles in reverse order. In all patients, premedication consisted of ondansetron 8 mg i.v. combined with dexamethasone 10 mg i.v. administered 30 min before the start of the chemotherapy. The administration of chemotherapy was followed by the infusion of 2000 ml of dextrose/saline applied over 8 hours and another 1000 ml of the same solution infused over the following 8 hours to avoid CDDP-induced renal damage.

Pharmacokinetic Sampling and Analysis

Blood samples for pharmacokinetic analysis were obtained during the first and second treatment cycle (total blood volume 283 ml). Blood was drawn from a vein in the arm opposite to that used for drug infusion and collected in 10-ml heparinized tubes. For analysis of CPT-11 kinetics, samples were obtained at the following time points: before infusion; 0.5, 1, and 1.5 hours during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32, 48, and 56 hours after infusion. Samples for measurement of CDDP concentrations were obtained immediately before infusion; 1, 2, and 3 hours during infusion; and 0.5, 1, 2, 3, 4, and 24 hours after infusion.

Plasma samples were assayed for total drug forms of CPT-11 and metabolites and the lactone forms of CPT-11 and SN-38, according to validated reversed-phase HPLC methods reported previously [14,15]. Nonprotein-bound and total CDDP concentrations in plasma, and platinum DNA-adduct levels in leukocytes were determined by flameless atomic absorption spectrometry [16].

Individual plasma concentrations of CPT-11 and its metabolites were fitted to a three-compartment model using Siphar v4.0 (SIMED, Creteil, France) as described [17]. Metabolic ratios for the various irinotecan metabolites were calculated as defined by Rivory et al [18], and included the relative extent of conversion of CPT-11 to SN-38 (i.e. $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$), the relative extent of glucuronidation of SN-38 (i.e. $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$), and the relative extent of metabolism (i.e. $AUC_{APC\ or\ NPC}/AUC_{CPT\text{-}11}$). Kinetic profiles of CDDP were obtained similarly using a one- or two-compartment model with extended least-squares regression analysis as reported

earlier [16]. The CDDP DNA-adduct levels in leukocytes were expressed as pg of platinum/μg DNA (pgPt/μgDNA).

Statistical Considerations

Pharmacokinetic parameters for all compounds are reported as mean values ± S.D. Differences in pharmacodynamic and pharmacokinetic parameters between sequences were evaluated statistically using a paired Student's t-test and the 95% confidence limits for the mean difference using Number Cruncher Statistical System version 5.X (Dr. Jerry Hintze, Kaysville, UT) and STATGRAPHICS Plus version 2.0 (Manugistics Inc., Rockville, MA). The power to discern potentially clinically relevant differences in the test parameters >30% (π) was determined at $\alpha = 0.05$ and previous pharmacological data. Probability values (two-sided) of <0.05 were regarded as statistically significant.

RESULTS

Toxicity and pharmacodynamics

A total of 11 patients, 6 males and 5 females with a median age of 59 years (range, 41-66) and a median performance score of 1 (range, 0-1), was accrued to the study. However, 1 patient was taken off study after receiving one course of chemotherapy because of deterioration of his condition due to disease progression. The predominant tumor type was colorectal cancer (n=7), and the main toxicity consisted of neutropenia (grade 3 or 4 in both sequences was observed in 6 of 10 cycles). Four patients encountered neutropenic fever (first course, n=2; second course, n=2) in the sequence CDDP→CPT-11, and 1 patient (first course only) in the sequence CPT-11→CDDP, which required dose reductions for the second course in three cases (CPT-11→CDDP, n=2; CPT-11→CDDP, n=1). Hence, only 8 patients received the planned dose in the sequence CPT-11→CDDP, compared to 9 patients in the reversed suquence. Paired analysis of hematological pharmacodynamic parameters indicated, however, that drug-sequencing had no significant influence on the observed myelotoxicity [Table 1], including the percent decrease in absolute neutrophil count (π=0.93). The severity and incidence of nonhematological toxicities, including nausea (grade 2 or 3, n=4 (CPT-11→CDDP *versus* n=4 (CDDP→CPT-11)), vomiting (grade 3 or 4, n=1 *versus* n=2), diarrhea (grade 3 or 4, n=0 *versus* n=1), and were also sequence independent.

Table 1. Summary of hematological pharmacodynamics

Data were obtained from patients after treatment with a 90-min i.v. infusion of CPT-11 at a dose level of 200 mg/m^2 given either before (CPT-11→ CDDP; first course) or after CDDP at a dose level of 80 mg/m^2 (CDDP→ CPT-11; seocnd course) or *vice versa*. The relative hematological toxicity (*i.e.*, the percentage decrease in blood cell count) was defined as: %decrease = [(pretherapy value-nadir value)/(pretherapy value)] x 100%. Data represent mean values ± SD.

Parameters	CPT-11→ CDDP	CDDP→ CPT-11	CL (∂)[a]	P[b]
Leukocytes				
Nadir (x10^9/l)	2.54 ± 1.15	2.23 ± 1.29	-1.70 and 1.08	0.50
%decrease WBC	65 7 ± 20.9	69.6 ± 18.1	-19.6 and 0.75	0.61
Neutrophils				
Nadir (x10^9/l)	0.93 ± 0.85	0.89 ± 0.67	-0.83 and 0.75	0.87
%decrease ANC	74.7 ± 18.3	80.3 ± 18.0	-15.1 and 26.5	0.41
Platelets				
Nadir (x10^9/l)	219 ± 57.6	198 ± 61.2	-47.8 and 7.0	0.09
%decrease PLC	34.3 ± 18.8	38.8 ± 25.4	-18.5 and 27.5	0.55

[a] CL (∂), 95% confidence limits for the mean difference; ANV, absolute neutrophil count; PLC, platelet count.

[b] Paired Student's *t* test.

Table 2. Summary of dose-normalized pharmacokinetic parameters of CPT-11 lactone

Data were obtained from 10 cancer patients after treatment with a 90-min i.v. infusion of CPT-11 at a dose level of 200 mg/m^2 given either before (CPT-11→ CDDP; first course) or after CDDP at a dose level of 80 mg/m^2 (CDDP→ CPT-11; second course) or *vice versa*. In three second courses, the CPT-11 and CDDP doses were reduced to 175 mg/m^2 and 60 mg/m^2, respectively, due to severe toxicity encountered in the first course (CPT-11→ CDDP, n=1; (CDDP→ CPT-11, n=2). All parameters were calculated by compartmental analysis, and data represent dose normalized (to 200 mg/m^2) mean values ± SD.

Parameter	CPT-11→ CDDP	CDDP→ CPT-11	CL (∂)[a]	P^b
C_{max} (μM)	1.89 ± 0.393	1.74 ± 0.449	-0.55 and 0.34	0.60
$t_{1/2}(\alpha)$ (h)	0.180 ± 0.146	0.167 ± 0.097	-0.17 and 0.13	0.79
$t_{1/2}(\beta)$ (h)	1.70 ± 0.81	1.98 ± 0.85	-0.46 and 1.03	0.87
$t_{1/2}(\gamma)$ (h)	12.2 ± 2.85	12.3 ± 2.63	-2.08 and 2.48	0.20
AUC (μM.h)	6.13 ± 2.07	5.58 ± 1.86	-1.01 and 0.87	0.89
CL (L/h/m^2)	60.4 ± 7.1	65.5 ± 16.3	-9.31 and 13.9	0.66
MRT (h)	7.98 ± 2.07	7.46 ± 1.53	-1.43 and 1.05	0.39
V_{ss} (L/m^2)	477 ± 131	483 ± 166	-279 and 121	0.74

[a] CL (∂), 95% confidence limits for the mean difference; C_{max} maximum concentration; $t_{1/2}(i)$, half-life of the *i*-th disposition phase; CL, total body clearance; MRT, mean residence time.

[b] Paired Student's *t* test.

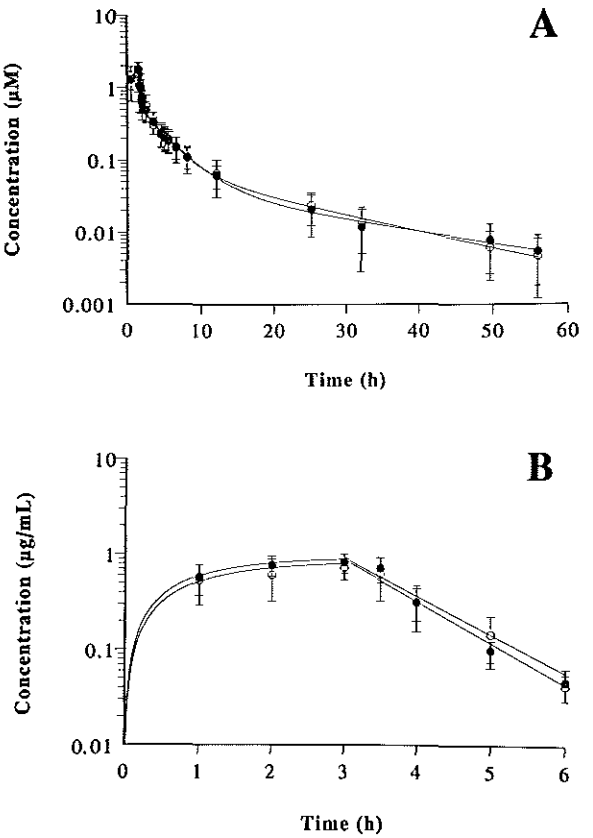

Fig. 1. Plasma concentration-time curves of CPT-11 lactone (panel A) and unbound CDDP (panel B) in 10 patients given CPT-11 beofre (closed symbols) or after CDDP (open symbols). Data represent mean values (symbol) ± S.D. (error bars).

Pharmacokinetics

The pharmacokinetics of CPT-11 and its metabolites SN-38, SN-38G, APC, and NPC could best be described by a three-compartmental model [Figure 1A], in line with previous findings [17]. Elimination of CPT-11 was characterized by a decay in an apparent tri-exponential manner and indicated no significant difference between suquences (Table 2). Analysis of the 10 paired, dose-normalized AUCs of CPT-11 lactone in both sequences demonstrated no significant differences, indicating that

treatment with CDDP immediately before CPT-11 did not alter the clearance of CPT-11 lactone (π=0.99). The AUC ratios of CPT-11 lactone to total drug was 0.37±0.07 and 0.35±0.14, whereas for SN-38 this ratio was 0.67±0.15 and 0.62±0.27, in the sequences CPT-11→CDDP and CDDP→CPT-11, respectively [Table 3].

Table 3. Summary of dose-normalized pharmacokinetic parameters of total CPT-11 and metabolites (lactone plus carboxylate forms)

Data were obtained from 10 cancer patients after treatment with a 90-min i.v. infusion of CPT-11 at a dose level of 200 mg/m^2 given either before (CPT-11→ CDDP; first course) or after CDDP at a dose level of 80 mg/m^2 (CDDP→ CPT-11; second course) or *vice versa*. In three second courses, the CPT-11 and CDDP doses were reduced to 175 mg/m^2 and 60 mg/m^2, respectively, due to severe toxicity encountered in the first course (CPT-11→ CDDP, n=1; (CDDP→ CPT-11, n=2). All parameters were calculated by compartmental analysis, and data represent dose normalized (to 200 mg/m^2) mean values ± SD.

Parameter	CPT-11→ CDDP	CDDP→ CPT-11	CL (∂)[a]	*P*[b]
CPT-11				
$t_{1/2}$ (h)	12.9 ± 3.52	11.4 ± 2.27	-4.22 and 1.03	0.25
AUC (μM.h)	16.6 ± 6.52	17.3 ± 8.66	-1.36 and 2.16	0.20
AUC_L/AUC_T	0.37 ± 0.07	0.35 ± 0.14	-0.094 and 0.076	0.62
SN-38				
$t_{1/2}$ (h)	25.5 ± 7.6	21.9 ± 3.9	-4.09 and 0.95	0.81
AUC (μM.h)	0.30 ± 0.17	0.29 ± 0.14	-0.23 and 0.19	0.19
AUC_L/AUC_T	0.67 ± 0.15	0.62 ± 0.27	-0.017 and 0.019	0.84
SN-38G				
$t_{1/2}$ (h)	21.4 ± 6.19	22.7 ± 3.71	-3.20 and 5.78	0.88
AUC (μM.h)	5.39 ± 3.60	5.50 ± 3.50	-2.32 and 2.56	0.53
APC				
$t_{1/2}$ (h)	10.1 ± 4.17	9.05 ± 2.60	-3.35 and 1.29	0.23
AUC (μM.h)	3.91 ± 2.73	4.04 ± 2.48	-0.81 and 1.06	0.34
NPC				
$t_{1/2}$ (h)	5.32 ± 2.86	6.05 ± 3.64	-1.52 and 3.65	0.75
AUC (μM.h)	0.32 ± 0.37	0.22 ± 0.23	-0.42 and 0.043	0.35

[a] CL (∂), 95% confidence limits for the mean difference; $t_{1/2}$, half-life of the terminal disposition phase; AUC_L, AUC of the lactone form; AUC_T, AUC of total drug

[b] Paired Student's *t* test.

The mean values for the apparent terminal half-lives of SN-38 and SN-38G, APC and NPC were similar in both sequences of drug administration [Table 3]. In addition, the relative extent of conversion of CPT-11 to SN-38 was not influenced by the administration sequence (0.047±0.018 *versus* 0.046±0.027, *P*=0.92), and neither was

the relative extent of glucuronidation of SN-38 (9.12±5.22 *versus* 9.01±6.99, *P*=0.87). No sequence dependence was observed in the metabolism of CPT-11 to APC or NPC, as estimated from the relative extent of metabolism (APC: 0.23±0.10 *versus* 0.23±0.08, *P*=0.77 and NPC: 0.012±0.011 *versus* 0.011±0.009, *P*=0.14).

CDDP pharmacokinetics could best be described with a two-compartment model [Figure 1B], as described [16]. The total body clearance and the V_{ss} of unbound CDDP were the same in both sequences (π=0.64), indicating no influence of the drug sequence on the protein binding of CDDP (Table 4). The platinum-DNA adduct levels in leukocytes peaked consistently at 1 hour after the end of infusion, and showed wide interpatient variability (Table 4). Administration of CPT-11 before CDDP resulted in a mean value of 1.94±2.20 pgPt/μ DNA, that was comparable with 2.42±1.62 pgPt/μ DNA observed in the reverse sequence (*P*=0.41).

Table 4. Summary of dose-normalized pharmacokinetic pharmacokinetic parameters of CDDP

Data were obtained from 10 cancer patients after treatment with a 3-h i.v. infusion of CDDP at a dose level of 80 mg/m^2 given either after (CPT-11→ CDDP; first course) or before CPT-11 at a dose level of 200 mg/m^2 (CDDP→ CPT-11; second course) or *vice versa*. In three second courses, the CDDP and CPT-11 doses were reduced to 60 mg/m^2 and 175 mg/m^2, respectively, due to severe toxicity encountered in the first course (CPT-11→ CDDP, n=1; (CDDP→ CPT-11, n=2). All parameters were calculated by compartmental analysis, and data represent dose normalized (to 80 mg/m^2) mean values ± SD.

Parameter	CPT-11→ CDDP	CDDP→ CPT-11	CL (∂)[a]	*P*[b]
C_{max} (μg/mL)	0.84±0.30	0.77±0.25	-1.03 and 1.17	0.36
$t_{1/2}$ (h)	0.73±0.23	0.49±0.15	-0.62 and 1.10	0.074
AUC_{fu} (μg.h/mL)	2.35±0.83	2.01±0.66	-0.25 and 0.95	0.10
AUC_{tot} (μg.h/mL)	39.8±12.9	35.8±6.4	-1.84 and 9.96	0.58
CL (L/h/m^2)	40.8±16.7	50.3±18.6	-20.2 and 1.05	0.081
V_{ss} (L/m^2)	35.5±14.3	30.0±11.6	-0.47 and 11.5	0.083
A_{max} (pg Pt/μg DNA)	1.94±2.20	2.42±1.62	-1.55 and 0.59	0.41

[a] CL (∂), 95% confidence limits for the mean difference; C_{max} maximum concentration; $t_{1/2}$(el), half-life of the terminal disposition phase; AUC_{fu}, AUC of unbound CDDP; AUC_{tot}, AUC of total CDDP; CL, total body clearance; A_{max}, maximum CDDP DNA-adduct level in leukocytes.

[b] Paired Student's *t* test.

DISCUSSION

This study was performed to explore the influence of alternate sequences of CPT-11 and CDDP on the observed side-effects and pharmacokinetic behavior of both drugs.

Using a randomized cross-over design for the administration sequence, no substantial differences in toxicity were observed between the two treatment schedules. The pharmacokinetics of the lactone form of CPT-11 revealed a substantial degree of interpatient variability, in line with previous observations [17]. In addition, the observed kinetic parameters of CPT-11 were similar to single agent data [18], indicating no apparent interaction between CDDP and CPT-11. The sequence of drug administration had also no influence on the pharmacokinetics of CPT-11 and its metabolites SN-38 and SN-38G and the CYP-450 3A4-mediated metabolites APC and NPC at the dose levels administered. This contrasts the reduction of topotecan clearance observed previously in patients after CDDP administration [5]. Our findings, however, are consistent with previous *in vitro* studies, indicating that CDDP had no statistically significant effect on the carboxylesterase-mediated bioactivation of CPT-11 to SN-38 using human hepatic microsomes [19]. In that study, there was also a lack of protein-binding site displacement of CPT-11 by CDDP in a clinically relevant concentration range [19].

The total body clearance and the V_{SS} of unbound CDDP, as well as the plasma AUC of total CDDP, indicated no significant influence of CPT-11 (or its metabolites) on the protein binding and plasma disposition of CDDP. The values of the maximal platinum DNA-adduct formation in peripheral leukocytes and the area under the DNA-adduct *versus* time curve were consistent with single agent data [16], and were independent of the drug sequence. In preclinical studies, however, topoisomerase I inhibitors delayed the repair of platinum-induced DNA interstrand corss-links without modifying their formation [1]. Although there is no formal proof that this preclinical observation also applies to the clinical situation, it is possible that the small patient population studied, in combination with the extreme variability in platinum DNA-adduct values, would not allow any alteration to be observed even if it were present.

In our study, CDDP was immediately administered at the end of the CPT-11 infusion, or vice versa in the alternate sequence. The lack of sequence dependence in the kinetic profiles of the two drugs in this schedule does not necessarily indicate that any reciprocal pharmacokinetic interference will be absent when the administration interval is increased. In this respect, it is noteworthy that CDDP can

modulate specific CYP-450 mRNA levels and may alter hepatic drug metabolism in vivo [20], a mechanism that has recently been proposed to account for drug interactions between CDDP and paclitaxel [21] or CDDP and etoposide [22]. In view of the major role of CYP-450 isozymes in CPT-11 metabolism and disposition [17], drug interactions with CDDP cannot be excluded a priori in case of alternative schedules of administration.

In conclusion, no sequence dependent side-effects between CPT-11 and CDDP could be demonstrated in this study, nor an indication of a mutual pharmacokinetic interaction. On the basis of these findings and the conflicting data on the mechanism of drug interaction between topoisomerase I inhibitors and platinum-derivatives in preclinical models, no clear preference in administration sequence can yet be formulated.

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Chapter 11

Pharmacokinetic, metabolic and pharmacodynamic profiles in a dose escalating study of irinotecan and cisplatin

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ABSTRACT

Purpose: To investigate the pharmacokinetics and pharmacodynamics of irinotecan and cisplatin administered once every three weeks in a dose-escalating study in patients with solid tumors.

Patients and Methods: Fifty-two cancer patients were treated with irinotecan administered as a 90-minutes infusion at doses ranging from 175-300 mg/m^2 followed by cisplatin as a 3-hour i.v. infusion at doses ranging from 60-80 mg/m^2. After reaching maximum tolerated dose, the sequence of drug administration was revised. For pharmacokinetic analysis serial plasma samples were obtained on day 1-3 of the first cycle. Forty-five patients were evaluable for irinotecan pharmacokinetics and forty-six patients for cisplatin.

Results: Irinotecan and cisplatin demonstrated linear pharmacokinetics comparable with single agent data suggesting an absence of pharmacokinetic interaction. SN-38G constituted the major plasma metabolite of irinotecan, whereas NPC was only a very minor metabolite in plasma, possibly indicating a rapid conversion of NPC to SN-38. The terminal elimination phases of SN-38 and SN-38G were similar, and relatively delayed compared to the elimination of irinotecan. Maximal DNA adduct formation did not significantly differ from single agent data. The percentage decrease in WBC was significantly related to the AUCs of the lactone form of irinotecan (P=0.0245) and SN-38 (P=0.0123). The severity of diarrhea was not significantly related to the AUCs of irinotecan and SN-38, nor to the systemic glucuronidation rate of SN-38.

Conclusion: There was no apparent pharmacokinetic interaction between irinotecan and cisplatin in this study. Reversion of the administration sequence of the drugs did not seem to have any influence on the pharmacokinetics. The incidence and severity of delayed type diarrhea was not related to any of the studied parameters.

INTRODUCTION

Irinotecan and other camptothecine analogs reversibly inhibit DNA topoisomerase I. The ability of camptothecine-analogs to inhibit topoisomerase I-mediated DNA functions suggests they might interfere with processes involved in DNA repair and enhance cytotoxicity when combined with DNA damaging agents. Several preclinical studies demonstrated a sequence-dependent cytotoxicity for the combination irinotecan/SN-38 and platinum-derivatives in vitro, with synergy increasing when

irinotecan was preceded by the platinum-derivative [1]. However, the interaction observed was not consistent, which indicates that it may be cell type dependent [2]. Where observed, synergism might at least partly be explained by interference of the topoisomerase I-inhibitor in the repair of cisplatin-induced DNA interstrand cross-links [3,4].

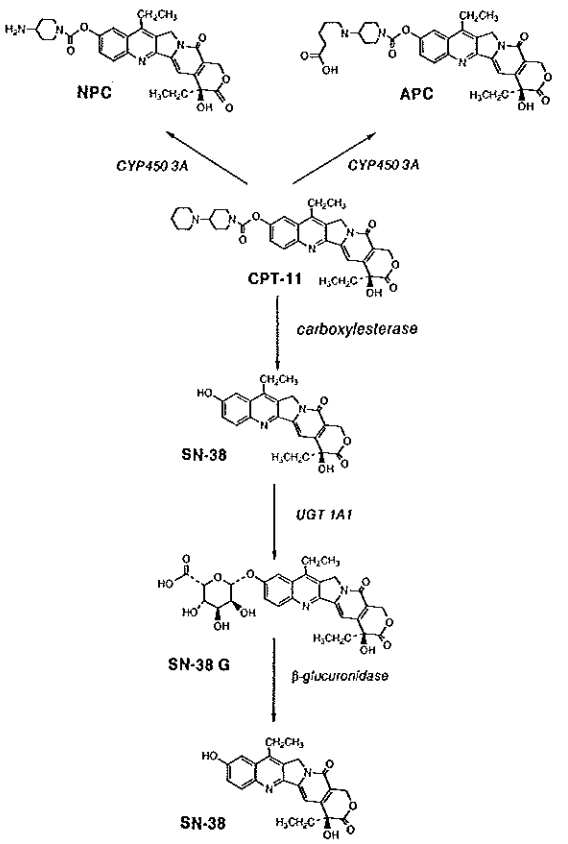

Fig. 1. Chemical structures of irinotecan (CPT-11) and its four major human metabolites.

Until now, phase I studies on the combination of irinotecan and cisplatin focused on fractionated dose schedules [5-14]. In all studied schedules irinotecan administration preceded that of cisplatin. Pharmacokinetic data were only obtained in two phase I trials [6,15], in a limited number of patients with contradicting results concerning the existence of a drug interaction.

After intravenous administration, irinotecan is converted to its active metabolite SN-38 by a carboxylesterase. Carboxylesterase activity has been characterized in serum [16], liver [17], small intestines [18] and tumor tissues [19,20]. SN-38 undergoes further metabolism to an inactive β-glucuronide derivative [21,22], which is present in significant concentrations in plasma, urine and bile [21,23].

Another pathway of irinotecan metabolism constitutes cytochrome P-450 3A-mediated oxidation of the terminal piperidine group on the C-10 side chain resulting in the formation of 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecine (APC), and 7-ethyl-10-[4-*N*-(1-piperidino)1-amino]-carbonyloxycamptothecine (NPC) [24,25] [Fig 1].

In the present report, we present a comprehensive analysis of the plasma pharmacokinetics of the lactone and total forms of irinotecan and its four major metabolites and cisplatin in cancer patients, who were treated in a phase I dose-finding study with irinotecan and cisplatin i.v. once every three weeks.

PATIENTS AND METHODS

Patient Population

The patients, from whom pharmacokinetic curves were obtained, participated to a phase I study. Eligiblity criteria included a histologically or cytologically confirmed diagnosis of a malignant solid tumor refractory to standard forms of therapy. All patients had an adequate hematopoietic (absolute neutrophil count $\geq 2.0 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L), renal (serum creatinine concentration $\leq$ 135 µmol/L or creatinine clearance $\geq$ 60 mL/min) and hepatic (total serum bilirubin $\leq$ 1.25 x upper normal limit and serum aspartate aminotransferase and alanine aminotransferase $\leq$ 3.0 x upper normal limits, in case of liver metastasis total serum bilirubin $\leq$ 1.5 x upper normal limit and serum aspartate aminotransferase and alanine aminotransferase $\leq$ 5.0 x upper normal limits) function. All patients gave written informed consent before study entry. Results of the clinical part of this study will be reported separately [26].

Treatment Plan and Drug Administration

Three or more patients were treated at each dose level. In the first part of the study, the administration of irinotecan preceded the infusion of cisplatin. In the second part, after reaching MTD for the sequence irinotecan followed by cisplatin, the sequence of administration of irinotecan and cisplatin was reversed, administering cisplatin prior to irinotecan at the MTD to determine sequence dependent side effects and/or pharmacokinetic interactions in a subsequent cohort of patients. Irinotecan doses ranged from 175-300 mg/m² and cisplatin doses from 60-80 mg/m².

Irinotecan (CPT-11, Campto®; Rhône-Poulenc Rorer, Antony, France) was provided as a concentrated sterile solution (20 mg/mL) in a 5-mL vial. This was diluted before use with 250 mL 0.9% sodium chloride solution. The drug was administered i.v. over 90 minutes.

Cisplatin (Platosin®; Pharmachemie, Haarlem, The Netherlands) was supplied as a powder and was dissolved in 250 mL 3.0 % saline and was given as a 3-hour i.v. infusion.

Premedication consisted of a 5-hydroxytryptamine-3 receptor antagonist given i.v. (ondansetron 8 mg) combined with dexamethasone 10 mg i.v. administered 30 minutes before the start of the chemotherapy. The administration of the chemotherapy was followed by the infusion of 2000 mL of dextrose/saline applied over 8 hours and another 1000 mL of dextrose/saline infused over the following 8 hours to avoid cisplatin-induced renal damage.

Experimental Studies

All blood samples (total blood volume 129-154 mL) for pharmacokinetic analysis were obtained only during the first treatment cycle. Blood was drawn from a vein in the arm opposite to that used for drug infusion, and collected in 7-mL heparinized tubes. For analysis of irinotecan kinetics, samples were obtained at the following time points: before infusion; 0.5, 1, and 1.5 hours during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32, 48, and 56 hours after infusion. The tubes were briefly immersed into an ice bath kept at the bedside, and plasma was separated within 10 min by centrifugation for 5 min at 3000 *g* on a tabletop centrifuge at 4°C, to prevent continued degradation of the lactone forms. The supernatant was transferred to a clean tube and stored at -80°C, until the time of analysis. Samples for measurement of cisplatin concentrations were

obtained immediately before infusion; 1, 2, and 3 hours during infusion; and 0.5, 1, 2, 3, and 18 hours after the end of the infusion.

Plasma samples were assayed for lactone and total drug forms of irinotecan and SN-38 according to a validated reversed-phase high-performance liquid chromatographic method reported previously [27]. Briefly, for measurement of the lactones, aliquots of plasma were spiked with the internal standard, camptothecine, and extracted with a mixture of acetonitrile-n-butyl chloride (1:4, v/v). After centrifugation, the clear supernatant was evaporated to dryness under nitrogen, and reconstituted in mobile phase. Sample clean-up for total drug forms was a simple protein precipitation with aqueous perchloric acid-methanol (1:1, v/v), which results in quantitative conversion of the carboxylate to lactone forms. Chromatography was carried out on a column packed with Hypersil ODS material (5 µm PS, 100x4.6 mm ID, Applied Science Group, Breda, The Netherlands) using isocratic elution with methanol-0.1 *M* ammonium acetate containing 0.01 *M* tetrabutylammonium sulphate (35:65, v/v; pH 5.5) . The flow rate was set at 1 mL/min and the eluent was monitored fluorimetrically at excitation and emission wavelengths of 355 and 515 nm, respectively. For quantitative determination of total concentrations SN-38G, APC, and NPC, samples were re-analyzed using a modified mobile phase with decreased organic modifier content to ensure sufficient selectivity and analyte separation [28]. The determination of each compound was based on chromatographic retention times and peak area measurements in comparison with injected standards, typically over a range of 0.5 to 200 ng/mL. Calibration curves were prepared in drug-free plasma, and fitted by a least-squares regression function with proportional weighting using the Lotus v2.4 package (New York, NY). The mean overall extraction efficiencies for irinotecan and the metabolites ranged between 83.0 and 99.1%. The percentage deviation from nominal values, and the inter- and intra-assay precision for each compound were always less than 12%.

Non-protein bound and total cisplatin concentrations in plasma, and cisplatin DNA-adduct levels in leukocytes were determined by flameless atomic absorption spectrometry according to the method of Reed et al [29], with modifications as described [30,31].

Pharmacokinetic and Pharmacodynamic Analysis

Individual plasma concentrations of irinotecan and its metabolites were fit to a three-compartment model using Siphar v4.0 (SIMED, Creteil, France) as described [32]. The volume of distribution at steady state (V_{ss}), the α, β, and γ rate constants, and the area

under the plasma concentration-time curve (AUC) were estimated by least-squares fitting using weighting of $1/y$. The percentage of the AUC extrapolated to infinity was always less than 15% for all compounds. Total body clearance of irinotecan was calculated by dividing dose administered by the observed AUC. Metabolic ratios for the various irinotecan metabolites were calculated as defined by Rivory et al [33]. The relative extent of the conversion (REC) of irinotecan to SN-38 was estimated as AUC_{SN-38}/AUC_{CPT-11}, the relative extent of metabolism (REM) of irinotecan to APC or NPC as $AUC_{APC\ or\ NPC}/AUC_{CPT-11}$ and the relative extent of glucuronidation (REG) of SN-38 as AUC_{SN-38G}/AUC_{SN-38}.

Kinetic profiles of cisplatin were obtained similarly using a one- or two-compartment model with extended least-squares regression analysis as reported earlier [34]. The AUC of cisplatin was calculated to the last sampling period (C_{last}) by the linear trapezoid method and extended to infinity by addition of C_{last}/k_{term}, where k_{term} is the slope obtained by log-linear regression of the final plasma concentration values. The cisplatin DNA-adduct levels in leukocytes were expressed as picogram of platinum per microgram of DNA (pgPt/μgDNA).

Kinetic-dynamic relationships were evaluated using the Siphar and NCSS (v5.X; Dr. Jerry Hintze, Kaysville, UT) programs, and were rated for goodness of fit by minimization of sums of squared residuals and by construction of the estimated coefficient of variation for fitted parameters. Significance of the relationships was assessed by construction of contingency tables with subsequent Chi-square analysis. Within individual patients, hematological toxicity was described as a continuous variable defined as a percentage decrease at nadir in white blood cell count (WBC), absolute neutrophil count (ANC) and platelet count (PLT), whereas diarrhea was a discontinuous variable defined by a CTC grade. The pharmacodynamics of hematological toxicity was evaluated by four different models, based on linear, log-linear, maximum effect (E_{max}), and sigmoidal E_{max} modeling of irinotecan or metabolite AUC values based on a modified Hill equation, as described [35]. Relationships between the incidences of diarrhea and irinotecan pharmacokinetics were assessed by estimation of systemic SN-38 glucuronidation rates, expressed as a biliary index. This index was calculated as the product of the AUC of irinotecan and the ratio of the AUCs of SN-38 and SN-38G [36,37], $AUC_{CPT-11} \times AUC_{SN-38}/AUC_{SN-38G}$.

Statistical Considerations

Pharmacokinetic parameters for all compounds are reported as mean values ± standard deviation. Variability in parameters between the various irinotecan dose levels was evaluated by the Kruskal-Wallis statistic followed by a Dunn's test to determine group differences. Interpatient differences in kinetics was assessed by the coefficient of variation, expressed as the ratio of the standard deviation and the observed mean. The relationships between the AUC ratios of lactone and total drug and the AUCs and the administered dose level were analyzed by means of Pearson's or Spearman's correlation coefficient, respectively, and linear-regression analysis. In case of diarrhea, patients were ranked in two cohorts with either severe (graded ≥3) or mild (graded ≤2) toxicity, and analyzed for differences by a nonparametric Mann-Whitney *U*-test. Probability values (two-sided) of less than 0.05 were regarded as statistically significant. All statistical calculations were performed using NCSS and STATGRAPHICS *Plus* version 2.0 (Manugistics Inc., Rockville, MA).

RESULTS

Patient Characteristics and Toxicity

A total of 52 patients, 18 female and 33 male with a median age of 52 years (range 37-69), and a median performance status of 0 (range 0-1), were entered in the pharmacological part of the phase I study The predominant tumor type was colorectal cancer (22 patiens). One patient was not eligbile. Forty-five patients were evaluable for the complete pharmacokinetics of irinotecan and metabolites and forty-six patients for cisplatin. Thirty-nine patients were assessable for the pharmacokinetic-pharmacodynamic analysis.

In all patients, the main hematological toxicity was neutropenia with both sequences of drug administration. No indications of cumulative myelotoxicity were noted.

Gastro-intestinal toxicity was the most prominent non-hematological side effect, including nausea, vomiting and diarrhea. Grade 1-2 diarrhea was observed in 61% of the cycles, grade 3-4 diarrhea occurred in 4%. The percentage of patients experiencing diarrhea increased with the irinotecan dose. Other side-effects were fatigue, mucositis, alopecia and acute cholinergic syndrome. No significant sequence-dependent differences in side-effects were observed.

Response could be evaluated in 49 patients. Three complete responses were observed and eight patients exhibited a partial response. Disease stabilization was noted in 28 patients.
Results of the full phase I study are reported in full separately.

Pharmacokinetics

The plasma concentration-time profiles of each compound (parent drug and metabolites SN-38, SN-38G, APC and NPC) were similar for all patients studied, with representative examples shown in Figure 2. The pharmacokinetics of irinotecan and its metabolites could best be described with a three-compartment model. The kinetic parameters obtained by means of this model are presented in Tables 1 to 3. Maximal plasma concentrations of SN-38, SN-38G, APC and NPC were reached at 1.71, 2.12, 2.75 and 2.11 hour, respectively. The ratio of irinotecan AUC of lactone to total drug was 31.3±8.1% (mean±SD; n=46; range, 18.9-48.2), whereas for SN-38 this ratio was 62.6±20.6% (range, 36.1-88.0). Elimination of irinotecan and its metabolites was characterized by a decay in an apparent tri-exponential manner based on conventional compartment modeling. The mean values for the linear segments of irinotecan lactone were $t_{1/2}(\alpha)$: 8.64 min, $t_{1/2}(\beta)$: 1.49 and $t_{1/2}(\gamma)$: 11.2 hours. The mean values for the terminal disposition half-lives of SN-38 and SN-38G were 23.5 and 23.7 hours, respectively, and were markedly different from those observed for irinotecan total, APC and NPC, being 12.1, 10.9 and 7.35 hours, respectively.

Over the total dose range studied, the AUC and the peak plasma concentrations of irinotecan increased from 17.2±7.08 (mean±SD) to 32.1±8.69 µM.h and from 3.94±1.25 to 6.55±1.91 µM, respectively, consistent with a linear and dose-independent pharmacokinetic behavior. The AUC of the lactone forms of irinotecan and SN-38 were significantly correlated to their respective total drug AUCs (CPT-11: r=0.86 & P<0.001; SN-38: r=0.92 & P<0.001).

Substantial interpatient variability in pharmacokinetic parameters was apparent, with a >2-fold variation in irinotecan AUC, although mean values were strongly correlated to dose (Spearman's r=0.78). No significant differences in dose-normalized pharmacokinetic parameters were observed between the irinotecan dose levels (P>0.05; Kruskal-Wallis).

Alternation of the administration sequence of irinotecan and cisplatin did not seem to have any influence on the pharmacokinetic data [Tables 1 and 2].

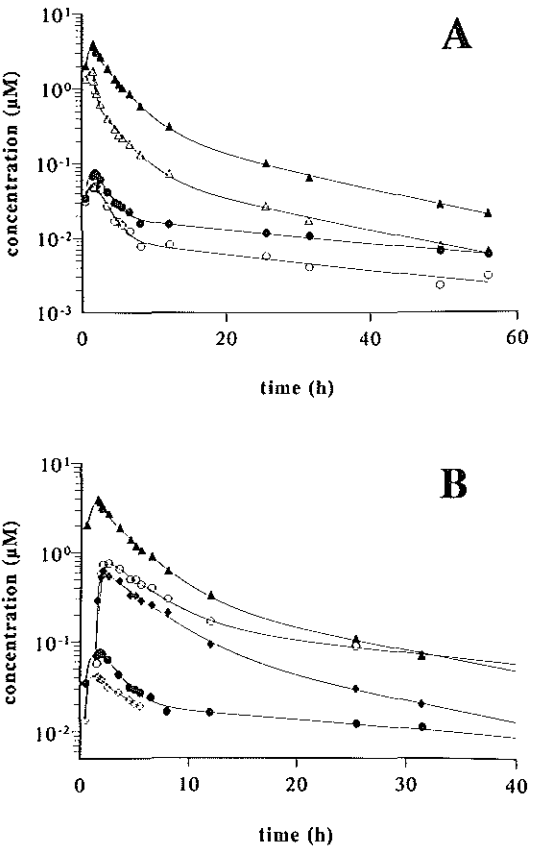

Fig. 2. Representative plasma concentration-time curves of irinotecan lactone (Δ) and total drug (▲) and of SN-38 lactone (○) and total drug (●) [panel A] and irinotecan (▲), SN-38 (●), SN-38G (○), APC (◆), and NPC (◊) [panel B] in a single patient given irinotecan at 200 mg/m².

Table 1. Pharmacokinetic parameters of irinotecan lactone (mean ± SD)

Cohort*	No. of patients	C_{max} (μM)	$t_{1/2}(\alpha)$ (min)	$t_{1/2}(\beta)$ (h)	$t_{1/2}(\gamma)$ (h)	AUC (μM.h)	CL (L/min)	CL (L/h/m²)	V_{ss} (L)
175/60	3	1.98±0.84	5.52±5.51	1.51±1.23	8.72±1.23	5.22±2.10	2.09±0.70	65.7±21.2	604±145
200/60	3	1.88±0.46	17.9±15.8	3.41±1.74	16.7±5.26	7.54±1.48	1.53±0.41	47.3±10.4	871±58.8
200/80	11	1.84±0.34	7.82±5.16	1.60±0.66	14.9±9.13	6.40±1.18	1.75±0.36	55.0±8.95	776±218
230/60	7	2.46±0.70	10.1±9.33	2.43±1.67	11.2±3.22	8.06±1.89	1.63±0.66	52.1±14.8	623±238
260/80	6	2.06±0.58	8.74±9.70	1.52±0.58	12.9±3.82	6.54±2.03	2.16±0.50	75.1±24.4	855±195
300/80	4	3.24±1.11	4.51±2.80	1.37±0.45	10.3±1.84	12.19±4.88	1.60±0.60	49.3±18.5	601±237
300/60	6	3.07±0.77	5.06±3.91	1.53±0.54	13.8±2.62	11.02±3.91	1.68±0.60	51.0±13.4	807±322
80/260	6	2.53±0.48	9.45±7.96	2.00±1.48	11.1±2.21	8.58±3.02	1.70±0.46	58.0±18.4	801±300

*Values in parentheses indicate irinotecan dose (mg/m²) and cisplatin dose (mg/m²), respectively, for cohorts 1-7, or cisplatin dose (mg/m²) and irinotecan dose (mg/m²), respectively, for cohort 8. Abbreviations: C_{max}, maximum concentration; $t_{1/2}$, disposition half-life; AUC, area under the curve; CL, total body clearance; V_{ss}, steady-state volume of distribution.

Table 2. Pharmacokinetic parameters of irinotecan total (mean ± SD)

Cohort*	No. of patients	C_{max} (μM)	$t_{1/2}(\alpha)$ (min)	$t_{1/2}(\beta)$ (h)	$t_{1/2}(\gamma)$ (h)	AUC (μM.h)	CL (L/min)	CL (L/h/m²)	V_{ss} (L)
175/60	3	3.94±1.25	7.77±10.1	2.26±0.61	10.6±1.54	17.2±7.08	0.63±0.22	20.1±6.67	273±77.0
200/60	3	4.44±0.10	6.61±3.27	2.56±0.79	13.3±3.67	27.5±6.26	0.43±0.13	13.3±3.51	221±31.8
200/80	11	4.30±0.67	28.7±31.7	1.98±0.43	14.5±7.12	20.1±3.26	0.56±0.13	17.5±3.16	301±112
230/60	7	5.41±0.80	24.7±44.4	1.99±0.72	10.5±2.19	27.0±5.12	0.47±0.14	15.2±3.14	209±47.0
260/80	6	4.45±1.09	50.1±56.5	1.63±0.58	13.6±4.84	21.3±8.57	0.69±0.21	23.8±8.18	344±108
300/80	4	7.63±1.95	8.52±2.40	1.87±0.36	11.7±2.00	43.2±18.1	0.46±0.17	14.2±5.95	204±49.7
300/60	5	6.55±1.91	12.6±16.5	2.07±0.56	12.3±2.18	32.1±8.69	0.55±0.15	17.0±3.64	274±74.3
80/260	6	4.62±1.04	14.3±24.3	1.78±0.62	10.3±1.87	24.9±7.54	0.57±0.13	19.4±5.31	267±60.5

*Values in parentheses indicate irinotecan dose (mg/m^2) and cisplatin dose (mg/m^2), respectively, for cohorts 1-7, or cisplatin dose (mg/m^2) and irinotecan dose (mg/m^2), respectively, for cohort 8. Abbreviations: C_{max}, maximum concentration; $t_{1/2}$, disposition half-life; AUC, area under the curve; CL, total body clearance; V_{ss}, steady-state volume of distribution.

The average REC of irinotecan to SN-38 ranged from 0.01 to 0.07. Over the dose range studied, no significant correlation between REC and irinotecan dose could be established. No dose-dependence of the extent of metabolism of irinotecan to APC, as estimated from the REM, was observed. The elimination phases of irinotecan and APC were consistently parallel. Also, no significant dose-dependence of the REG of SN-38, which ranged from 4.5 to 32.0 was noted. The elimination phases of SN-38 and SN-38G were also consistently parallel and the terminal half-lives of these compounds were significantly correlated.

Cisplatin pharmacokinetics could best be described with a two-compartment model. The kinetic parameters obtained by means of this model are presented in Table 4. The plasma clearance of unbound cisplatin was 1.05±0.27 L/min (mean±SD, n=46; range, 0.526-2.42 L/min). The pharmacokinetic behavior of cisplatin across all irinotecan dose levels was highly consistent with previously published values obtained with cisplatin used as a single agent. In addition, the CL and Vsss data of unbound cisplatin and the AUC of total cisplatin in plasma indicate no significant influence of irinotecan on the protein binding of cisplatin. The platinum-DNA adduct levels in leukocytes peaked consistently at 1 hour after the end of the cisplatin infusion, and showed wide interpatient variability. Mean values of 2.60±1.59 pgPt/μDNA (n=9), and 8.13±7.87 pgPt/μDNA (n=29) were observed at the cisplatin doses of 60 and 80 mg/m^2, respectively, and were not significantly altered by an increase in the irinotecan dose.

Administration of cisplatin before irinotecan resulted in a mean value of 9.04±5.38 pgPt/μDNA (n=6), that was not significantly different from 6.77±6.47 pgPt/μDNA (n=6) observed in the reversed schedule. The number of responses was too limited to establish a meaningful relationship between platinum-DNA adduct formation in leukocytes and the likelihood of tumor response.

Table 3. Pharmacokinetic parameters of irinotecan metabolites (mean ± SD)

Compound	Parameter	175 (n=3)*	200 (n=14)	230 (n=7)	260 (n=12)	300 (n=9)
SN-38	t_{max} (h)	1.67±0.17	2.08±0.15	1.63±0.05	1.59±0.12	1.63±0.23
	C_{max} (μM)	0.075±0.046	0.090±0.023	0.090±0.023	0.11±0.044	0.13±0.051
	$t_{1/2}(\gamma)$ (h)	19.0±1.28	23.8±7.70	19.9±1.53	20.9±3.15	29.1±13.8
	AUC (μM.h)	0.24±0.11	1.14±0.36	1.76±0.31	0.73±0.53	1.15±0.47
	REC	0.01	0.05	0.07	0.03	0.03
SN-38G	t_{max} (h)	2.03±0.03	2.32±0.21	2.30±0.20	1.89±0.27	2.08±0.27
	C_{max} (μM)	0.46±0.21	0.48±0.27	0.57±0.13	0.70±0.37	0.78±0.22
	$t_{1/2}(\gamma)$ (h)	24.6±13.5	23.5±10.6	20.8±3.16	21.5±6.57	27.9±10.3
	AUC (μM.h)	7.67±4.75	8.01±2.95	8.00±1.22	8.02±6.27	12.5±5.90
	REG	32.0	7.0	4.5	11.0	10.9
APC	t_{max} (h)	2.25±0.25	2.86±0.54	4.04±0.54	2.16±0.32	2.45±0.46
	C_{max} (μM)	0.47±0.13	0.52±0.29	0.90±0.27	0.73±0.39	0.83±0.51
	$t_{1/2}(\gamma)$ (h)	8.12±2.88	15.1±2.30	9.62±0.82	8.67±1.45	13.0±3.06
	AUC (μM.h)	6.48±3.65	5.90±1.20	6.84±1.48	7.37±5.28	9.54±6.92
	REM	0.38	0.27	0.25	0.35	0.26
NPC	t_{max} (h)	2.03±0.03	2.66±0.47	1.92±0.09	1.72±0.13	2.20±0.54
	C_{max} (μM)	0.051±0.005	0.057±0.020	0.076±0.008	0.053±0.021	0.083±0.081
	$t_{1/2}(\gamma)$ (h)	4.55±2.07	9.67±5.12	5.38±0.44	4.16±2.74	8.01±5.28
	AUC (μM.h)	0.22±0.13	0.73±0.33	0.39±0.07	0.36±0.26	0.52±0.43
	REM	0.01	0.03	0.01	0.02	0.01

*Values indicate irinotecan dose (mg/m^2). Abbreviations: t_{max}, time to maximum concentration; C_{max}, maximum concentration; $t_{1/2}(\gamma)$, terminal disposition half-life; AUC, area under the curve; REC, $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$; REM, $AUC_{APC}/AUC_{CPT\text{-}11}$; REG, $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$.

Table 4. Pharmacokinetic parameters of unbound and total cisplatin (mean ± SD)

Cohort*	No. of patients	Free cisplatin C_{max} (μg/mL)	$t_{1/2}(\beta)$ (h)	AUC (μg.h/mL)	CL (L/min)	Total cisplatin C_{max} (μg/mL)	$t_{1/2}(\beta)$ (h)	AUC (μ.h/mL)
175/60	3	0.69±0.32	0.60±0.09	1.87±0.75	1.26±0.63	1.87±0.67	45.1±6.97	84.5±30.0
200/60	3	0.47±0.28	0.70±0.35	1.33±0.45	1.63±0.63	1.75±0.52	38.9±3.38	82.0±27.1
200/80	11	0.79±0.10	1.12±0.71	2.69±0.38	0.98±0.19	2.73±0.32	50.8±26.6	141±52.8
230/80	7	1.04±0.24	1.31±0.72	3.08±0.59	0.88±0.22	2.96±0.49	52.3±21.0	161±58.5
260/80	6	0.89±0.16	1.30±0.46	3.00±0.41	0.84±0.06	2.85±0.32	69.2±30.2	181±57.5
300/80	4	1.10±0.18	2.00±1.16	3.13±0.79	0.89±0.21	3.09±0.11	41.0±15.3	140±52.3
300/60	6	0.78±0.09	1.02±0.38	1.98±0.19	1.02±0.11	2.15±0.38	42.6±10.5	90.0±21.0
80/260	6	1.08±0.24	1.06±0.79	2.78±0.66	0.92±0.25	3.13±0.45	38.2±8.07	123±32.0

*Values in parentheses indicate irinotecan dose (mg/m^2) and cisplatin dose (mg/m^2), respectively, for cohorts 1-7, or cisplatin dose (mg/m^2) and irinotecan dose (mg/m^2), respectively, for cohort 8. Abbreviations: C_{max}, maximum concentration; $t_{1/2}(\beta)$, terminal disposition half-life; AUC, area under the curve; CL, total body clearance.

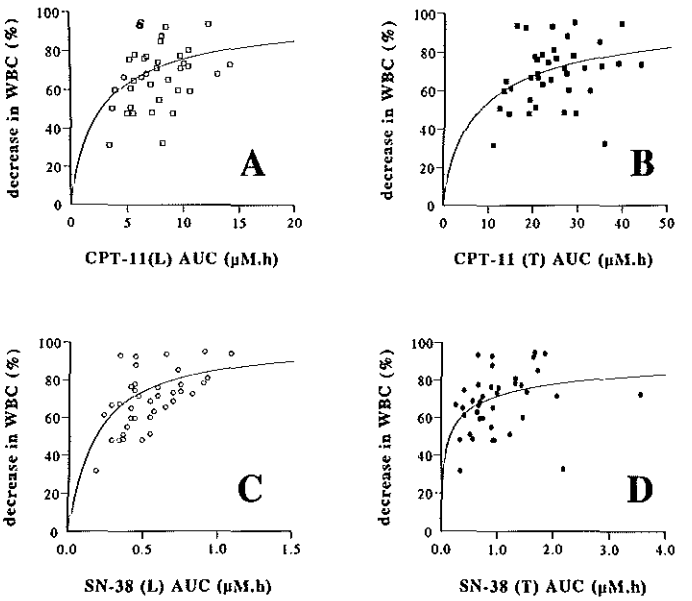

Fig. 3. Relationships between CPT-11 lactone (**A**) and total (**B**) AUC or SN-38 lactone (**C**) and total (**D**) AUC and the percent decrease in white blood cell count (WBC) at nadir of the first treatment course. The lines represent the fitting of the data to a sigmoidal maximum-effect model.

Pharmacodynamics

The pharmacokinetic data obtained from 39 patients were plotted against the percentage decrease in white blood cell count (WBC), platelet count (PLT) and absolute neutrophil count (ANC), at nadir relative to the pretreatment value. Four different models, based on linear, log-linear, maximum effect (E_{max}), and sigmoidal E_{max} fitting, were compared for their ability to describe the data. The percentage decrease in ANC and PLT was not significantly related to the AUC of any compound (neither lactone nor total drug) in any of the models tested. The percentage decrease in WBC, however, was significantly related using a sigmoidal maximum effect model to the AUC of irinotecan lactone [EC_{50}=2.77 μM (CV=36.2%); γ=0.90 (CV=39.6%); *P*=0.0245], AUC of SN-38 lactone [EC_{50}=0.182 μM (CV=31.5%); γ=0.85 (CV=32.8%); *P*=0.0123], and to a lesser extent also to AUC of irinotecan total drug [EC_{50}=8.04 μM

(CV=44.1%); γ=0.82 (CV=43.5%); *P*=0.0442] and AUC of SN-38 total drug [EC_{50}=0.157 μM (CV=69.6%); γ=0.49 (CV=41.9%); *P*=0.0317] [Figure 3] [38]. These data indicate that the hematological toxicity was mainly related to the pharmacological active lactone form of both irinotecan and SN-38.

The severity of diarrhea was not significantly related with AUC of irinotecan lactone (*P*=0.467), AUC of irinotecan total drug (*P*=0.680), AUC of SN-38 lactone (*P*=0.683), or AUC of SN-38 total drug (*P*=0.613). Biliary index in patients with diarrhea CTC-grade 0-2 (2033±1124; *n*=32) or CTC-grade 3-4 (1917±577.8; *n*=3) were also not significantly different (*P*=0.519; Mann-Whitney *U*-test).

DISCUSSION

This is the first pharmacokinetic study of irinotecan to incorporate the analysis of the plasma pharmacokinetics of the four major metabolites of irinotecan identified to date (viz. SN-38, SN-38G, APC and NPC) and cisplatin in cancer patients, who were treated in a phase I dose-finding study with irinotecan and cisplatin administered i.v. once every three weeks.

Over the total dose range studied, the AUC and the peak plasma concentrations of irinotecan demonstrated linear and dose independent behavior similar to single agent data, indicating no apparent pharmacokinetic interaction. The ratio of irinotecan and SN-38 AUC of lactone to total drug agree very well with data of a previous study in which irinotecan was administered as a single agent in a small patient population (viz. 36.8±3.5% and 64.0±3.4% for irinotecan and SN-38, respectively [39]). Reversion of the administration sequence of irinotecan and cisplatin did not seem to have any influence on the pharmacokinetic data for both compounds. However, our data are limited and the impact of the sequence of drug administration on the pharmacokinetic or metabolic interaction between irinotecan and cisplatin has to be further evaluated.

Quantitatively, the major plasma metabolite of irinotecan was SN-38G. This observation is different from the data reported by Rivory et al., who noticed APC to be the major plasma metabolite [33]. This difference may be explained partly by the prolonged sampling time applied in the present study. By obtaining samples at 24, 32, 48 and 56 hour after the end of the infusion of irinotecan, the $t_{½}(\gamma)$ of irinotecan and SN-38G could be calculated more accurately, revealing a $t_{½}(\gamma)$ of SN-38G of 23.5 hour and a $t_{½}(\gamma)$ of APC of 11.6 h resulting in a higher AUC of SN-38G compared to

the AUC of APC. These results underscore the importance of the application of appropriate kinetic models with sufficient sampling time points for the accurate estimation of concentration-time profiles.

The parallel decline of NPC and APC with the parent drug suggest that their elimination is rate-limited by the formation of the metabolites. We observed that NPC was only a very minor metabolite of irinotecan in plasma of our patients, and accounted for only approximately one thirtieth of total circulating drug. Also, the metabolite was only detectable in plasma during times of relatively high concomitant parent compound concentrations. This finding sharply contrasts with recent in vitro observations by Dodds and co-workers [25]. Furthermore, when pooled human hepatic microsomes were incubated with irinotecan, NPC was identified to be the prevailing biotransformation product of the oxidative metabolism of irinotecan [25], which is catalyzed by cytochrome P-450 3A. From these data, one would expect a large fraction of irinotecan to be metabolized to NPC in vivo. In vitro incubations of NPC with both human liver microsomes or hepatic carboxylesterase revealed, however, that NPC may be a possible precursor of SN-38 through enzymatic cleavage of the 4-N-(1-piperidino)-1-amino group at C10 (see Figure 1) [25]. Thus, the low plasma AUC of NPC observed in our patient population might indicate a rapid and virtually complete conversion of NPC to SN-38. Obviously, we can not exclude other possible explanations. In this respect, it is noteworthy that the terminal disposition phases of SN-38 and SN-38G were very similar, and relatively delayed compared to the elimination of irinotecan and the cytochrome P-450-mediated metabolites. Hence, the conversion of NPC to SN-38 may be partly responsible for the prolonged terminal half-lives of SN-38 and SN-38G aside from the enterohepatic recirculation of these metabolites. In addition, after hepatobiliary and/or intestinal secretion of irinotecan and NPC, the re-absorption of both compounds and their subsequent conversion to SN-38 may also contribute to the extended disposition phases of SN-38 and its glucuronide conjugate.

Cisplatin pharmacokinetics were comparable with single agent data [34]. Although preclinical studies indicated that the reversal of cisplatin-induced DNA interstrand cross-links was delayed by concomitant incubation with a topoisomerase I inhibitor [3,4], maximal DNA adduct formation did not differ from single agent data with the method used [34].

The pharmacodynamic analysis revealed only a significant correlation between the percentage decrease in WBC and the AUC of the active lactone form of irinotecan and SN-38. Surprisingly no correlation was found between the percentage

decrease of the ANC and the AUC of any compound. Previous studies have shown a correlation between the systemic SN-38 glucuronidation rates, expressed as the biliary index and the incidence of delayed type diarrhea [36,37]. Although biliary index data for our patients with diarrhea graded 0-2 are similar to those reported recently [viz. median: 2228], values for patients experiencing grade 3 or 4 diarrhea were lower [viz. median: 5499 (ibit.)]. A similar discrepancy has been reported recently by Canal et al in a large group of patients receiving single agent irinotecan at a dose of 350 mg/m^2 [39]. The relatively low incidence of grade 3 and 4 diarrhea during our phase I study and the substantial interpatient variability in plasma pharmacokinetic parameters may partly be responsible for this discrepancy. Recently however, in a study on the metabolism and urinary and fecal excretion of irinotecan, we observed an unexpectedly high fecal concentration of SN-38 accompanied by a virtual disappearance of SN-38G [32] in patients treated at a dose level of 200 mg/m^2.

These findings may reflect the conversion of SN-38G to SN-38 under influence of endogenous and bacterial β-glucuronidase in the intestines. Although the exact mechanism of the observed delayed type diarrhea after administration of irinotecan is still unknown, it is thought to be related to the exposition of the intestinal tract to SN-38. Interindividual differences in fecal β-glucuronidase activity could play a role in the observed variation in irinotecan-induced intestinal side-effects and may form a possibility to modulate the experienced toxicity. This concept is presently under further investigation in our institute.

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Chapter 12

Liquid chromatographic determination of Irinotecan and three major metabolites in human plasma, urine and feces

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ABSTRACT

A new simple reversed-phase high-performance liquid chromatographic method was developed for the determination of irinotecan (CPT-11) and four metabolites in human plasma, urine and feces homogenate. The metabolites of interest were 7-ethyl-10-hydroxycamptothecin (SN-38), its β-glucuronide derivative (SN-38G), 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (RPR 121056A; also referred to as APC) and 7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxycamptothecin (RPR 132595A; also referred to as NPC). Sample-pretreatment from the various biological matrices involved a rapid protein-precipitation with simultaneous solvent extraction of 250 µl aliquots of sample with 500 µl of methanol-5% (w/v) aqueous perchloric acid (1:1, v/v). Separation of the compounds of interest was achieved on an analytical column packed with Hypersil ODS material (100x4.6 mm I.D., 5 µm), and isocratic elution with a mixture of methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). The column effluent was monitored at excitation and emission wavelengths of 355 and 515 nm, respectively. Results from a four-day validation study indicated that this single-run determination allows for simple, simultaneous and rapid quantitation and identification of all analytes with excellent reliability. The described procedure permits the analysis of patient samples, and will be implemented in future studies to investigate the complete metabolic fate and disposition of CPT-11 in cancer patients.

INTRODUCTION

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; CPT-11) [Figure 1] is a promising semisynthetic derivative of the topoisomerase-I poison 20-(*S*)-camptothecin with increased aqueous solubility [1-3]. Interesting response rates have been documented in patients with a variety of malignancies, including colorectal cancer, gynecologic cancers and refractory cervical cancer [4,5].

In recent years, the clinical pharmacokinetic behavior of CPT-11 has been the subject of intensive investigation. Along with these studies, the elucidation of the metabolic fate of CPT-11, its potential clinical therapeutic use, and its toxic properties were investigated. These studies revealed that CPT-11 is rapidly hydrolyzed by carboxylesterases to form the highly active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38) [6-11], which is subsequently conjugated by liver uridine-

diphosphate glucuronosyltransferase (isoform 1.1) to an inactive β-glucuronide derivative (SN-38G) [12-18]. Another pathway of CPT-11 metabolism consists of an oxidative attack at the terminal piperidine group on the C_{10}-side chain, presumably mediated by cytochrome P-450 3A4, generating a major metabolite that was recently identified as 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyl-oxycamptothecin (RPR 121056A) [19,20]. Although this compound was demonstrated to have weak inhibitory activity of cell growth *in vitro*, the potential contribution of this and other metabolites to biological effects is still unknown [19,21].

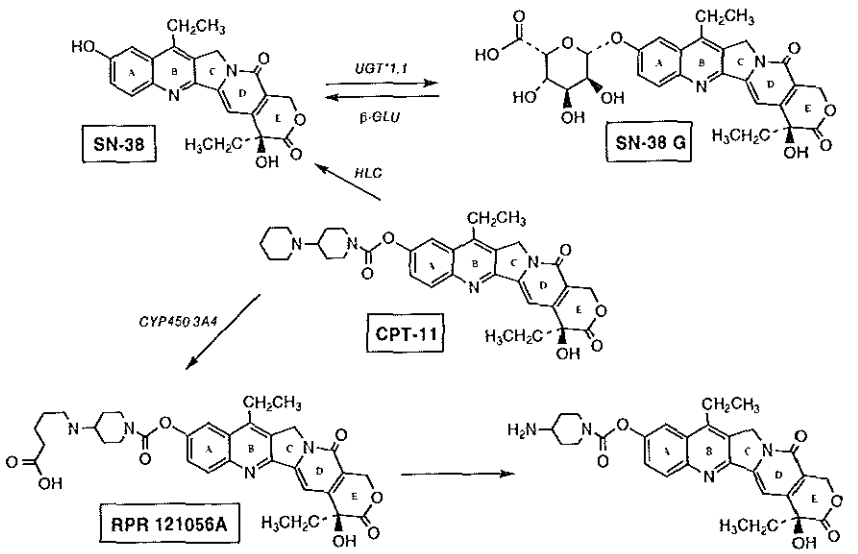

Fig. 1. Chemical structures of irinotecan (**CPT-11**; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) and four major human metabolites: (*a*) **SN-38** (7-ethyl-10-hydroxycamptothecin); (*b*) **SN-38G** (7-ethyl-10-[3,4,5,-trihydroxy-tetrahydro-pyran-2-carboxylic acid]camptothecin); (*c*) **RPR 121056A** (7-ethyl-10-[4-*N*-(5-amino-pentanoic acid)-1-piperidino]carbonyloxycamptothecin; (*d*) **RPR 132595A** (7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxycamptothecin.

Preliminary examination of elimination pathways for CPT-11 have indicated that less than 50% of the delivered dose is excreted unchanged into the urine and bile, with a minor contribution from both SN-38 and SN-38G [15]. Hence, establishment of a complete mass balance for this drug, and determination of the metabolic fate and disposition of CPT-11 in humans is urgently required. To facilitate such studies, we now report on development, validation and use of a reversed-phase high-performance liquid chromatogra-phic (HPLC) method for the quantitative determination of CPT-11 and its major metaboli-tes in human plasma, urine and feces. The method is a modification of our previously reported procedure applied for the analysis of CPT-11 lactone *plus* CPT-11 carboxylate levels in plasma, and involves a rapid and highly selective protein-precipitation step for sample clean-up [22]. A pilot study in a cancer patient receiving the drug by a 90-min intravenous infusion was included to confirm the suitability of the method for clinical use.

EXPERIMENTAL

Chemicals and reagents. CPT-11 hydrochloride (batch: KO16) and reference standards of its metabolites SN-38 hydrochloride (batch: LIE783), SN-38G trifluoroacetate (batch: YEO265), RPR 121056A hydrochloride (batch: EBO1143 [23]) and RPR 132595A trifluoroacetate (batch: YEO304) were supplied by Rhône-Poulenc Rorer (Vitry-sur-Seine Cedex, France). A purity of ≥94.0% for each compound was confirmed by analytical HPLC as described [22]. All other chemicals and HPLC solvents were of the highest grade available commercially. Milli-Q-UF quality water was used throughout (Millipore, Milford, MA, USA). Blank specimens of human plasma, urine and feces were obtained from healthy volunteers.

Sample extraction. Frozen plasma or urine samples were thawed in a water-bath and were homogenized by vortex-mixing. Next, 250 µl aliquots of plasma or 250 µl of plasma-diluted urine (1:1, v/v) were transferred to clean polypropylene microtubes (Eppendorf, Hamburg, Germany) containing 500 µl of methanol-5% (w/v) aqueous perchloric acid (1:1, v/v). The tubes were capped and mixed for 5 min on a multi-tube mixer, and then centrifuged at 24,000 *g* for 5 min. The upper aqueous layer from plasma and urine extracts was diluted 2-fold and 10-fold, respectively, in methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). Finally, diluted extracts were transferred to limited-volume inserts, and 100 to 200-µl aliquots were subjected to chromatography. Feces specimens were accurately weighed and homogenized in 5 volumes of 5% (w/v)

perchloric acid in water using five 1-min bursts of an Ystral X1020 tissue homogenizer (Dottingen, Germany) operating at 20,500 r.p.m. Next, aliquots of the feces homogenate were centrifuged at 24,000 *g* for 10 min, the resulting supernatant diluted with one volume of drug-free human plasma, and processed further as described above for urine samples.

Equipment and chromatographic conditions. Chromatographic analyses were performed using a constraMetric 4100 pump, an autoMetric 4100 autosampler and a fluoriMonitor 4100 fluorescence detector (LDC Analytical, Rivera Beach, USA). The analytical column used was packed with Hypersil ODS material (100x4.6 mm I.D., 5 µm) from LC Service (Emmen, The Netherlands), and protected by a LiChroCART 4-4 endcapped (RP-18) pre-column from Merck (Darmstadt, Germany). The column temperature was maintained at 50°C by using a Spark Holland Model SpH99 HPLC-column oven (Meppel, The Netherlands). The fluorescence detector operated at excitation and emission wavelengths of 355 nm and 515 nm, respectively, which yielded the optimum signal to noise ratio for all compounds. The mobile phase consisted of a mixture of methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid), and was delivered at a flow rate of 1.0 ml/min. The mobile phase was filtered and degassed before use, and was prepared fresh for each run.

Calibration. Separate standard stock solutions of CPT-11 and metabolites were prepared at 1.0 mg/ml in dimethyl sulfoxide (DMSO) and were stored in polypropylene at -80°C. A mixture of all five compounds as the free lactone forms was obtained in methanol-0.01 *M* hydrochloric acid (40:60, v/v) by dilution of the standard solutions in DMSO, yielding a final concentration of 20 µg/ml for each standard. This solution was subsequently diluted serially with an appropriate volume of methanol-0.01 *M* hydrochloric acid (40:60, v/v) to obtain 1-ml aliquots. Next, plasma, urine and feces homogenate standards were prepared on the day of sample analysis by transferring 25 µl of the diluted stock mixtures into 975 µl of the biological matrix (i.e. plasma, urine or feces homogenate) to prepare a series of standards ranging from 2 to 200 ng/ml in plasma, or 100 to 5000 ng/ml in urine and feces homogenate. Acquisition and integration of HPLC data was performed with the Chrom-Card data analysis system (Fisons, Milan, Italy), running on an ICW chromatographic workstation. Calibration curves were made by linear least-squares regression analysis of peak areas *versus* 1/(nominal concentration)^2 using the Lotus *v2*.4 software package (Lotus Development Corporations, New York, NY, USA).

Method validation. Method validation was performed according to the guidelines recorded in the conference report on 'Analytical Method Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies' [25], with minor modifications as described by us previously [22]. All validation runs were performed on three (urine and feces) or four (plasma) consecutive days, and included a calibration curve processed in duplicate, and sets of quality control (QC) samples in quintuplicate analyzed with repeated freezing and thawing. QC samples were prepared in all biological matrices at concentrations which fell within all four quartiles of the corresponding standard curve range. The accuracy (or percent deviation from the nominal concentration) and intra- and inter-assay (or within-day and between-day) precision were calculated by one-way analysis of variance (ANOVA) using the Number Cruncher Statistical System *v*5.0 package (Dr J.L. Hinze, East Kaysville, USA).

Patient samples. The patient studied participated in a clinical phase I and pharmacokinetic study of CPT-11 in combination with the anticancer drug, cisplatin, in various non-hematological malignancies [24]. The CPT-11 dose of 300 mg/m^2 was delivered as a 90-min intravenous infusion, followed by a 3-h intravenous infusion of cisplatin at 80 mg/m^2, with treatment cycles repeated every three weeks. The protocol was approved by the Institutional Review Board of the Rotterdam Cancer Institute (Rotterdam, The Netherlands) and written informed consent was obtained prior to treatment.

Blood samples were collected in lithium heparin-containing glass tubes before and during the 90-min CPT-11 infusion (0.5 h, 1 h, and 1 h 25 min after initiation), and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32 and 48 h after the end of the CPT-11 infusion. The samples were centrifuged instantaneously for 5 min at 3000 *g* at ambient temperature, and the plasma supernatant was snap-frozen at -80°C. The renal and hepato-biliary excretion of CPT-11 and metabolites was studied by obtaining complete collections of urine and stools for the duration of the study (48 h). Urine samples were diluted immediately with one volume of drug-free human plasma, and 1.0-ml aliquots were kept frozen in poly-propylene microtubes (Eppendorf). After collection, feces specimens were stored instantly at -80°C until later analysis.

RESULTS AND DISCUSSION

Analytical procedure. The reversed-phase HPLC method in this study had been developed initially for the quantitative determination of CPT-11 and SN-38 in human

plasma samples [22]. Our data from stability studies indicated that lactone forms of CPT-11 and SN-38 were unstable after storage at room temperature or at 37°C [22], necessitating rapid freezing of clinical samples after blood collection to prevent continued hydrolysis into the carboxylate forms. In view of the limited clinical applicability of methods based on estimation of lactone levels only, total (i.e. lactone *plus* carboxylate forms) concentrations of the drug and its metabolites were measured in the present study instead.

Using our previous HPLC method, several major chromatographic peaks in addition to CPT-11 and SN-38 were detected in processed samples obtained from patients after CPT-11 treatment that were not present in pre-infusion specimens. One of these peaks was determined to be a β-glucuronide conjugate of SN-38 (SN-38G), a known metabolite of CPT-11 [14,15], based on treatment of the sample with β-glucuronidase. In the present study, we observed that the remaining unknown peaks in our clinical plasma samples could be identified with the aid of pure reference standards as 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxy-camptothecin (RPR 121056A; also referred to as APC [19,23; figure 1]) and 7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxycamptothecin (RPR 132595A; figure 1).

Sample pretreatment of total drug forms in plasma was initially performed by protein precipitation using a mixture of 5% (w/v) perchloric acid-methanol (1:1, v/v), with direct injection of the supernatant into the HPLC system. This sample handling, however, resulted in an unusual chromatographic behavior of compounds RPR 121056A and SN-38G, with separation into two peaks of comparable peak-area proportion (data not shown). As suggested by Rivory and Robert, this artefact may relate to self-association of the carboxylate forms of the drugs with dimerization due to the formation of hydrogen bonds between carboxyl functions in organic solvents [26]. Peak separation of compounds RPR 121056A and SN-38G was efficiently removed by a 2-fold dilution of the plasma extract prior to chromatography in a mixture of methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutyl-ammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). This sample handling was also chosen for its optimal elimination of endogenous interference, while maintaining a high extraction efficiency for all compounds.

In order to ensure sufficient selectivity and analyte separation in our new assay, we have slightly modified the mobile phase composition as compared to the earlier method by decreasing the organic modifier content from 35 to 30%. This change in mobile phase, however, resulted in poor accuracy and precision for the two late-eluting compounds (CPT-11 and SN-38), due to severe tailing bands (asymmetry

factor (A_s)≥2.0), arising from secondary retention effects on the reversed phase column. This chromatographic distortion was particularly evident at the low end of plasma-calibration curves (i.e. below 100 ng/ml). Although this problem could be overcome in part by the use of a (less retentive) gradient elution, we noticed the occurrance of severe baseline drift during the chromatographic run. Therefore, concentrations of CPT-11 and SN-38 in plasma were quantitated after re-injection of the sample using the previously developed mobile phase [22].

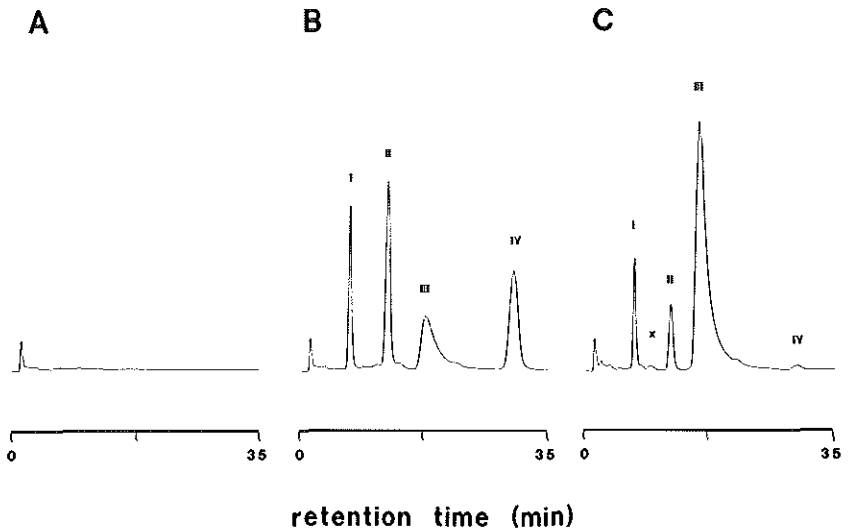

Fig. 2. HPLC chromatograms of **(A)** a blank human plasma sample; **(B)** a spiked human plasma sample (200 ng/ml of each compound); and **(C)** a patient plasma sample taken 30 min after i.v. infusion of irinotecan at a dose level of 300 mg/m^2. Based on chromatographic behavior and spectrometric properties relati-ve to pure reference standards, peaks labeled I, II, III, IV, and V were identified as **SN-38G** (t_R=7.08 min), **RPR 132595A** (t_R=9.52 min), **RPR 121056A** (t_R=12.7 min), **CPT-11** (t_R=17.9 min) and **SN-38** (t_R=29.8 min), respectively.

Modification of our plasma assay for urine samples was easily achieved by addition of the unknown specimens to drug-free human plasma and further processing as if they were human plasma samples. In order to allow convenient

sample-handling of fecal specimens, several homogenization and extraction procedures were evaluated. Our preliminary data indicated that upon standing at room temperature, SN-38G was highly unstable in feces homogenated in (weakly acidic) aqueous media. The disappearance of SN-38G in such matrices was completed within 1 h (data not shown), and was accompanied by a concomitant increase in the SN-38 peak area, suggesting sustained activity by β-glucuronidase from the intestinal microflora present in feces. The procedure reported by Li and Zhang [26] for measurement of 10-hydroxy-camptothecin in feces, based on EDTA and proteinase K treatment of the sample at 37°C, also proved unfavorable. Among various other procedures tested, homogenization and simultaneous extraction with 5% (w/v) perchloric acid was shown to result in the optimal combination of sufficient sample stability and acceptable analyte recovery (see below).

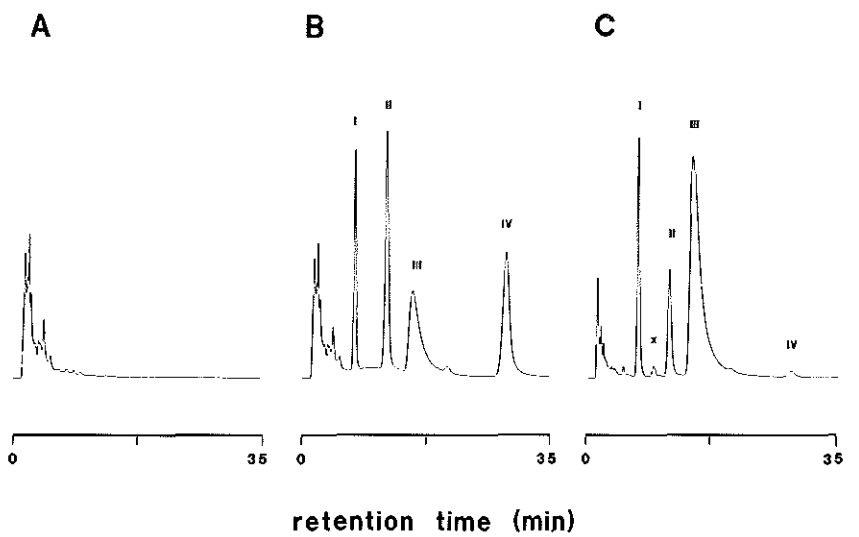

Fig. 3. HPLC chromatograms of (**A**) a blank human urine sample; (**B**) a spiked human urine sample (1000 ng/ml of each compound); and (**C**) a patient urine sample collected during the first 0-5 hours after i.v. infusion of irinotecan at a dose level of 300 mg/m^2. Based on chromatographic behavior and spectrometric properties relative to pure reference standards, peaks labeled **I**, **II**, **III**, **IV**, and **V** were identified as **SN-38G** (t_R=6-.83 min), **RPR 132595A** (t_R=8.82 min), **RPR 121056A** (t_R=10.9 min), **CPT-11** (t_R=14.3 min) and **SN-38** (t_R= 27.3 min), respectively.

Criteria for selection of the reversed-phase analytical column and the fluorescence-detection wavelength couple were as described previously [22].

Method validation. Chromatograms of blank and spiked human plasma, urine and feces homogenate samples are shown in figs. 2-4. The selectivity for the analytes is shown by the sharp and symmetrical resolution of the peaks, with no significant interfering peaks for all compounds in drug-free specimens, obtained from five different individuals. The retention times for SN-38G, RPR 132595A, RPR 121056A, CPT-11 and SN-38 were 6.95±0.21, 9.15±0.49, 11.8±1.27, 16.1±2.55 and 28.6±1.77 min, respectively, with the overall chromatogra-phic run time established at 35 min. Applying the peak height in combination with a weight factor of $1/x^2$, linear calibration curves were obtained over the concentration ranges tested for all compounds (except CPT-11 and SN-38 in plasma), i.e. 10-400 ng/ml for plasma (mean r=0.9982), and 100-5000 ng/ml for urine (mean r=0.9987) and feces homogenate (mean r=0.9991).

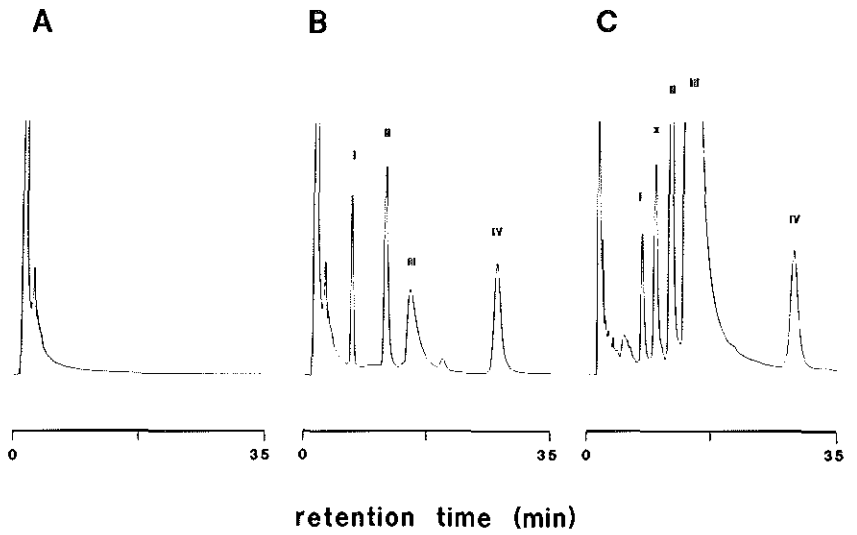

Fig. 4. HPLC chromatograms of **(A)** a blank human feces sample; **(B)** a spiked human feces sample (5000 ng/ml of each compound); and **(C)** a patient feces sample collected during the first 0-5 hours after i.v. infusion of irinotecan at a dose level of 300 mg/m^2. Based on chromatographic behavior and spectrometric properties relative to pure reference standards, peaks labeled I, II, III, IV, and V were identified as **SN-38G** (t_R=6-.92 min), **RPR 132595A** (t_R=9.08 min), **RPR 121056A** (t_R=11.1 min), **CPT-11** (t_R=14.4 min) and **SN-38** (t_R= 26.9 min), respectively.

Table 1. HPLC validation characteristics of CPT-11 and four major metabolites in human plasma samples

Compound	nominal (ng/ml)	n[a]	observed (ng/ml)	deviation (%)	precision (%) within-day	between-day
SN-38G	10	20	10.16	+1.55	8.60	([b])
	25	20	25.98	+3.93	1.69	1.75
	150	20	150.0	± 0.00	1.47	2.53
	300	20	295.5	-1.49	4.76	([b])
RPR 132595A	10	20	10.09	+0.92	4.08	4.86
	25	20	25.82	+3.27	1.90	4.28
	150	20	152.3	+1.55	1.58	4.26
	300	20	312.7	+4.23	3.04	6.46
RPR 121056A	10	20	10.16	+1.57	5.89	4.88
	25	20	26.86	+7.45	1.65	6.27
	150	20	156.9	+4.58	1.64	6.48
	300	20	312.9	+4.29	2.57	6.14
CPT-11[c]	2	20	2.135	+6.75	14.3	4.25
	10	20	9.213	-7.87	3.38	3.08
	75	19	73.01	-2.60	2.27	2.35
	150	20	155.4	+2.93	1.75	2.46
	750	20	752.1	+0.28	1.75	2.22
SN-38[c]	2	20	2.006	+0.32	12.4	([b])
	10	20	10.96	+9.57	2.52	2.89
	75	19	82.43	+9.90	2.56	2.62
	150	20	168.3	+12.2	2.08	2.79
	750	20	809.9	+7.98	1.58	2.78

[a] number of replicate observations of each concentration in four separate validation runs.

[b] no additional variation was observed as a result of performing the assay in different runs.

[c] data from De Bruijn, *et al.* [22]; mobile phase composition: methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (35:65 (v/v).

Table 2. HPLC validation characteristics of CPT-11 and four major metabolites in human urine samples

Compound	nominal (ng/ml)	n[a]	observed (ng/ml)	deviation (%)	precision (%) within-day	between-day
SN-38G	100	14	99.61	-0.39	13.6	([b])
	400	15	404.9	+1.22	2.10	3.47
	2500	15	2520	+0.79	1.40	1.18
	4500	15	4444	-1.25	4.83	1.53
	4500[c]	15	4358	-3.16	5.08	2.39
RPR 132595A	200	14	196.4	-1.80	5.13	1.89
	400	15	361.1	-9.72	1.25	4.92
	2500	15	2201	-11.8	1.51	2.10
	4500	15	3992	-11.3	0.92	3.39
	4500[c]	15	3736	-16.9[d]	1.93	0.38
RPR 121056A	200	14	204.7	+2.37	4.51	4.17
	400	15	401.4	+0.35	0.94	2.50
	2500	15	2533	+1.31	1.34	2.60
	4500	15	4597	+2.17	4.64	2.29
	4500[c]	15	4313	-4.16	1.98	3.14
CPT-11[c]	200	14	200.1	+0.06	6.82	6.91
	400	15	379.8	-5.06	4.78	4.12
	2500	15	2543	+1.74	1.82	2.11
	4500	15	4819	+7.09	4.34	2.78
	4500[c]	15	4141	-7.97	2.98	2.57
SN-38[c]	100	14	98.76	-1.24	6.08	7.38
	400	15	418.5	+4.61	1.32	7.98
	2500	15	2497	-0.11	1.08	0.99
	4500	15	4514	+0.31	0.90	2.12
	4500[c]	15	4238	-5.82	5.76	9.70

[a] number of replicate observations of each concentration in three separate validation runs.
[b] no additional variation was observed as a result of performing the assay in different runs.
[c] quality control sample diluted 10-fold prior to chromatography.
[d] percent deviation outside the acceptable 85-115% range for accuracy [25].

Table 3. HPLC validation characteristics of CPT-11 and four major metabolites in homogenized human feces samples

Compound	nominal (ng/ml)	n[a]	observed (ng/ml)	deviation (%)	precision (%) within-day	between-day
SN-38G	100	15	99.19	-0.81	6.36	5.49
	400	15	392.1	-1.97	1.24	1.58
	2500	15	2557	+2.27	1.50	2.07
	4500	15	4637	+3.05	1.36	2.00
	4500[c]	15	4568	+1.51	1.32	0.98
RPR 132595A	200	15	205.5	+2.77	2.21	1.36
	400	15	407.3	+1.81	3.40	5.73
	2500	15	2585	+3.40	0.82	1.93
	4500	15	4758	+5.73	0.84	1.93
	4500[c]	15	4612	+1.03	1.68	([b])
RPR 121056A	200	15	202.1	+1.04	1.73	2.20
	400	15	403.8	+0.95	0.97	0.46
	2500	15	2604	+4.14	1.27	1.41
	4500	15	4796	+6.59	1.02	1.93
	4500[c]	15	4341	-3.54	1.17	1.92
CPT-11[c]	200	15	200.4	+0.22	5.81	4.20
	400	15	408.3	+2.08	2.59	1.96
	2500	15	2732	+9.26	1.76	0.99
	4500	15	5179	+15.1[d]	1.28	2.37
	4500[c]	15	3953	-12.2	4.48	3.51
SN-38[c]	100	15	105.3	+5.31	6.37	2.23
	400	15	416.8	+4.20	1.93	0.99
	2500	15	2654	+6.15	0.96	2.80
	4500	15	4805	+6.78	1.11	2.45
	4500[c]	15	4300	-4.45	2.09	1.23

[a] number of replicate observations of each concentration in three separate validation runs.
[b] no additional variation was observed as a result of performing the assay in different runs.
[c] quality control sample diluted 10-fold prior to chromatography.
[d] percent deviation outside the acceptable 85-115% range for accuracy [25].

Validation data of the analytical methods in terms of accuracy (percent deviation) and precision for spiked plasma, urine and feces samples are shown in Tables 1, 2 and 3, respectively. The accuracy for all analytes showed values ranging within ±12.2% (plasma), ±16.9% (urine) and ±15.1% (feces) of the nominal values. The intra- and inter-assay variability as assessed by one-way ANOVA varied upto 14.3% and 7.98%, respectively, in the various biological matrices [Tables 1-3].

The extraction recovery for each compound was determined at six different concentrations in three (urine and feces) or four (plasma) analytical runs by comparing peak areas of samples prepared in the appropriate biological matrix with those for non-processed samples prepared in the mobile phase. The mean overall extraction efficiency for CPT-11 were 85.3±5.3% in plasma, 89.2±12.4% in urine and 101.5±9.3% in feces homogenate. Recoveries observed for the metabolites ranged between 82.6 and 113.6% [Table 4], and were not significantly different from CPT-11 (Student's *t*-test, $P>0.05$), suggesting that concentrations of these compounds can be estimated using the CPT-11 calibration curve in the absence of pure reference compounds.

Table 4. Mean recovery of CPT-11 and four major metabolites in plasma, urine and homogenized human feces extracts

Compound	plasma recovery (%)	n[a]	urine recovery (%)	n[a]	feces recovery (%)	n[a]
SN-38G	95.0 ± 4.3	48	98.9 ± 11.2	35	110.5 ± 8.3	36
RPR 132595A	87.6 ± 3.5	48	96.2 ± 4.8	36	97.1 ± 3.0	36
RPR 121056A	82.6 ± 4.2	48	90.7 ± 4.6	36	100.5 ± 5.1	36
CPT-11	85.3 ± 5.3[b]	47	89.2 ± 12.4	35	101.5 ± 9.3	36
SN-38	99.3 ± 9.2[b]	47	98.1 ± 6.5	35	113.6 ± 4.7	36

[a] total number of replicate observations of three or four separate validation runs.

[b] data from De Bruijn, *et al* [22]; mobile phase composition: methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (35:65 v/v).

Pharmacokinetic studies. The suitability of the developed methods for clinical use was demonstrated by the determination of CPT-11 and its metabolites in biological specimens obtained from a 58-year old male with advanced gastric adenocarcinoma, treated with irinotecan at a dose level of 300 mg/m^2. Preliminary analyses were

carried out in duplicate in the presence of both blank and spiked plasma, urine and feces samples. Examples of the patient's sample trace are shown in figs. 2C (plasma), 3C (urine) and 4C (feces). Distinct peaks were obtained for CPT-11 and the four metabolites in all matrices, with RPR 132595A only detectable in 8 out of the 17 plasma samples assayed. The plasma concentration *versus* time profiles for the various compounds are shown in Figure 5.

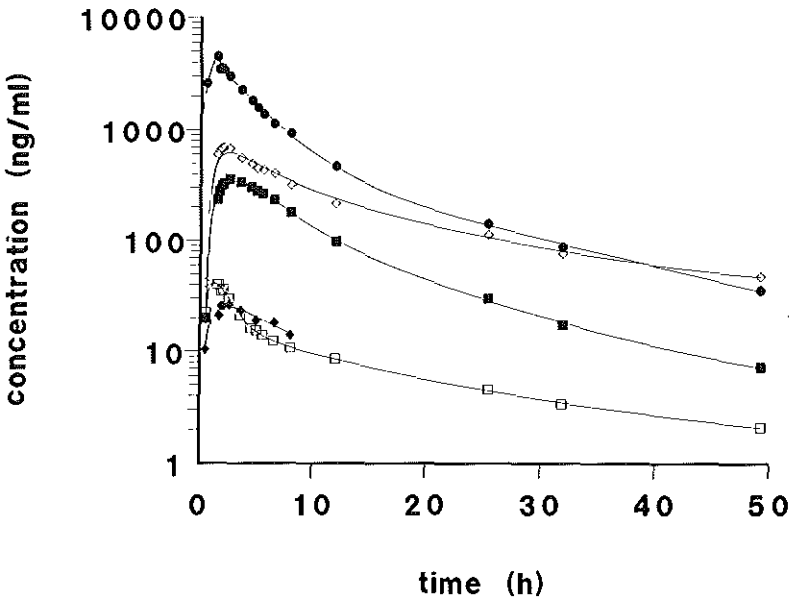

Fig. 5. Plasma concentration *versus* time profile of **CPT-11** (O) and its metabolites **SN-38G** (◊), **RPR 132595A** (♦), **RPR 121056A** (■) and **SN-38** (□) in a single patient given irinotecan by a 90-min i.v. infusion at a dose level of 300 mg/m^2.

All pharmacokinetic curves could be fitted to a tri-exponential equation using the Siphar *v*4.0 software package (SIMED, Creteil, France), assuming a three-compartment model for the distribution and elimination processes. The area under the plasma concentration-time curve (AUC), calculated upto the last sampling point with detectable levels with extrapolation to infinity, for unchanged CPT-11 was 43.7 μ*M*/h, whereas that of total metabolites was 31.8 μ*M*/h, indicating an apparent predominance of the parent drug.

Disappearance of CPT-11 from the central plasma compartment was characterized by a terminal elimination half-life of approximately 12 h, which is within the same range as described for this compound previously [5]. The elimination phases of SN-38 and SN-38G showed a parallel decline with a prolonged half-life as compared to CPT-11 and the two cytochrome P-450-mediated metabolites, which may reflect differences in binding affinity for plasma proteins. The time course of the cumulative urinary and fecal elimination demonstrated that the excretion pattern was virtually completed within the first 15 h (data not shown). The total cumulative urinary excretion was approximately 40%, with unchanged CPT-11 and SN-38G as the main excretion products. Fecal excretion of unchanged CPT-11 also constituted a major route of elimination, with unconjugated SN-38 being the main metabolite.

In conclusion, we have developed and evaluated liquid chromatographic methods for measuring CPT-11 and four major metabolites in human plasma, urine and feces. The methods were shown to meet the current requirements as to validation of bioanalytical methodologies, providing good accuracy and precision. The described methods permit the analysis of patient samples, and will be implemented in future studies to further investigate the metabolic fate and disposition of CPT-11 in cancer patients.

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Chapter 13

Irinotecan (CPT-11) metabolism and disposition in cancer patients

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ABSTRACT

The objective of this study was to determine the metabolic fate and disposition of the antitumor camptothecine derivative irinotecan (CPT-11). Ten patients with histologic proof of malignant solid tumor received 200 mg/m^2 of CPT-11 as a 90-min i.v. infusion, followed by a 1.5-hour i.v. infusion of cisplatin (60 or 80 mg/m^2). Plasma, urine and feces were collected for 56 hours and analyzed for the parent drug and all four metabolites positively identified to date, viz. SN-38, its β-glucuronide conjugate (SN-38G), 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecine (APC) and 7-ethyl-10-[4-*N*-(1-piperidino)-1-amino]-carbon-yloxycamptothecine (NPC), by a specific reversed-phase high-performance liquid chromatographic assay.

A three-exponential decline was observed in plasma for all compounds, with a clear predominance of the parent drug [25.6±5.71 μ*M*.h (CPT-11) versus 15.8±3.51 μ*M*.h (total metabolites)]. Total urinary excretion was 28.1±10.6% of the dose, with unchanged CPT-11 and SN-38G as the main excretion products. Whereas renal clearance of SN-38 was only a minor route of drug elimination, fecal concentrations of this compound were unexpectedly high (on average 2.45% of the dose), suggestive of intestinal hydrolysis of SN-38G by bacterial β-glucuronidase. CPT-11 and the other metabolites could also be identified from fecal extracts, with overall a very minor contribution of the cytochrome P-450-mediated compounds NPC and APC. Surprisingly, fecal excretion accounted for only 24.4±13.3% of the dose, leading to a total excretion of approximately 52%. These data indicate that half of the dose in urine and feces may constitute some further unknown nonextractable or nonfluorescent metabolites. The findings from this study should be of importance as a guide to further therapeutic evaluation of this drug.

INTRODUCTION

The topoisomerase-I poison irinotecan (CPT-11) is one of the most promising antitumor agents to have entered clinical trials in recent years, displaying a broad range of clinical activity against several neoplastic disorders, including gynecologic cancers and 5-fluorouracil refractory colorectal cancer (reviewed in Refs. [1,2]). Structurally, CPT-11 is unique among camptothecine drugs, because of a bulky [1,4'-bipiperidine]-1'-carboxylate side chain located at the C-10 position, with an ethyl-group at C-7 [Fig. 1]. The former side chain can be cleaved enzymatically by a carbo-

xylesterase converting enzyme that generates the active metabolite SN-38 [3] [see: Fig. 1].

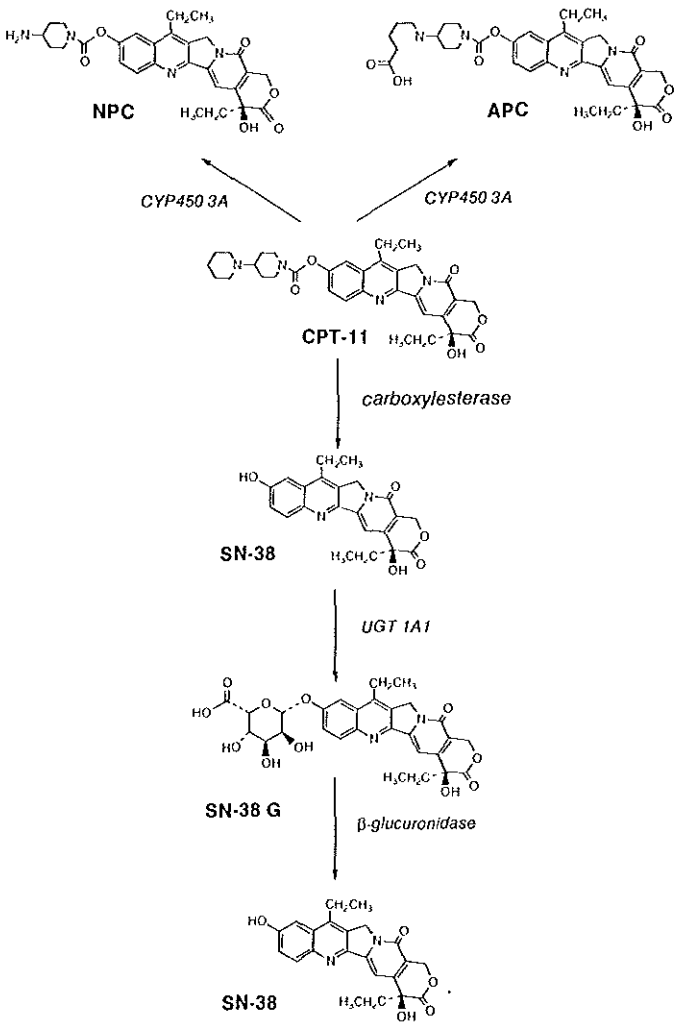

Fig. 1. Chemical structures of irinotecan (**CPT-11**) and four human metabolites: (*a*) **SN-38** (7-ethyl-10-hydroxycamptothecine); (*b*) **SN-38G** (SN-38 β-glucuronide); (*c*) **APC** (7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecine) (*d*) **NPC** (7-ethyl-10-[4-(1-piperidino)-1-amino] carbonyloxycamptothecine). *Abbreviations:* UGT 1A1, human uridine diphosphate glucuronosyltransferase isoform 1A1; CYP450 3A, cytochrome P450 isoform 3A.

Because SN-38 is over 100-fold more potent as a topoisomerase-I inhibitor in various *in vitro* systems, CPT-11 is thought to function *in vivo* as a prodrug of SN-38. Peripheral converting enzyme activity in animals has been characterized in serum [4], liver [5] and small intestine [6], and preliminary evidence indicates that carboxylesterase activity within the tumor may also be an important factor in drug activity [7,8]. SN-38 undergoes further metabolism, mediated by uridine-diphosphate glucuronosyltransferase 1A1, to an inactive β-glucuronide derivative [9-11].

Another pathway of CPT-11 metabolism consists of a cytochrome P-450 3A-mediated oxidation of the terminal piperidine group on the C-10 side chain, which gives rise to formation of several compounds [12,13]. The structures of the major metabolites of CPT-11 resulting from this pathway have recently been established by Rivory and co-workers as APC [14] and NPC [15] [see: Fig. 1]. Other, minor metabolites have not yet been positively identified. Although APC was recently shown to be less biologically active than SN-38 in *in vitro* culture, the contribution of this and other metabolites to biological effects *in vivo* is still unknown. In addition, there have been no reports on the quantitative urinary and fecal elimination of NPC and APC. There is thus an urgent need to establish a mass balance for CPT-11 and to determine the complete metabolic fate of this drug in humans. Thus, in the present study, we have examined the plasma disposition, metabolism and urinary and fecal excretion pathways of CPT-11 in a group of patients with solid tumors using a recently developed analytical method based on HPLC that detects all metabolites currently identified [16,17].

MATERIALS AND METHODS

Patients and Treatment. The metabolism and disposition of CPT-11 were studied in 10 adult patients participating in a phase I study of CPT-11 in combination with cisplatin in various nonhematological malignancies [18]. All patients had a histologically confirmed diagnosis of a malignant solid tumor refractory to standard forms of therapy, and had an adequate hematopoietic, hepatic and renal function at the time of study. All patients had no more than one prior combination chemotherapy regimen or two single agent regimens, excluding prior treatment with CPT-11 or other topoisomerase I inhibitors and platinum derivatives. Vials that contained 40 or 100 mg of CPT-11 (as a hydro-chloride trihydrate form) formulated in *d*-sorbitol and a lactic acid-sodium hydroxide buffer system of pH 3.5-4.5 were provided by Rhône-Poulenc Rorer (Antony Cedex, France). The CPT-11 dose of 200 mg/m^2 was

administered as a 90-min i.v. infusion, followed immediately by a 3-hour i.v. infusion of cisplatin (60 or 80 mg/m^2). Premedication was uniform for all patients, and consisted of (*i*) ondansetron (8 mg i.v.) or tropisetron (3 mg i.v.) and (*ii*) dexamethason (10 mg i.v.) just prior to start of the CPT-11 infusion and repeated after 24 hours. The clinical protocol was approved by the Rotterdam Cancer Institute Review Board, and all patients signed informed consent forms before entering the study.

Sample Collection. In each patient, sufficient plasma, urine and feces was obtained before CPT-11 administration to evaluate possible interfering peaks in the HPLC analysis. Blood samples for analysis of CPT-11 and its metabolites were obtained at the following time points: before infusion; 0.5, 1, and 1.5 hours during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32, 48, and 56 hours after the end of infusion. All blood samples were drawn from a vein in the arm opposite to that used for CPT-11 infusion. Samples were collected in glass tubes containing lithium heparin, and centrifuged immediately for 5 min at 3000 x *g* (4°C) to yield plasma, which was stored frozen in polypropylene vials (Eppendorf, Hamburg, Germany) at -80°C until the time of analysis. Complete urine collections were obtained for the duration of the study during hospitalization (i.e. up to 56 hours after start of drug administration), and 0.5-mL aliquots were diluted 1:1 (v/v) with drug-free human plasma and stored frozen in microtubes. Complete collections of feces were also obtained in polystyrene containers, and stored immediately at -80°C to prevent continued degradation of SN-38G [17]. Weighted feces samples were homogenized individually in 5 volumes of 5% (w/v) of aqueous perchloric acid using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany) operating at 20,500 r.p.m. Aliquots of the feces homogenates were diluted with human plasma prior to further sample processing as described above for urine.

Determination of CPT-11 and Metabolites. Pure reference standards of CPT-11 hydrochloride trihydrate (batch: KO16) and the metabolites SN-38G trifluoroacetate (batch: YEO265), NPC trifluoroacetate (batch: YEO304), APC hydrochloride (batch: EBO1143; Ref. [19]) and SN-38 hydrochloride (batch: LIE783) were kindly supplied by Rhône-Poulenc Rorer, and were used as received. CPT-11 and SN-38 in plasma were measured by a previously described HPLC method [16], whereas for simultaneous quantitation of CPT-11 and SN-38G, APC and SN-38 in urine and feces the methodology was further modified as described [17]. The latter method proved also suitable for concurrent analysis of NPC.

Briefly, 250-µL aliquots of plasma, plasma-diluted urine or feces homogenate were acidified with 500 µL of a mixture of methanol-5% (w/v) aqueous perchloric

acid, to enable estimation of total, i.e. lactone plus carboxylate, concentrations of the drug and its metabolites simultaneously. The samples were rocked on a multitube vortex-mixer for 5 min, followed by centrifugation at 24,000 x *g* for 5 min (4°C). The clear supernatant from the extracts was diluted 2 to 10-fold with methanol-0.1 M ammonium acetate containing 10 mM tetrabutylammonium sulphate (3:7, v/v), adjusted to pH 5.3 with hydrochloric acid. The diluted extracts were transferred to limited-volume inserts, and 100 to 200-µL aliquots were injected into the HPLC system. The system was composed of a constaMetric 4100 solvent delivery system, an autoMetric 4100 autosampling device and a fluoriMonitor 4100 fluorescence detector (LDC Analytical, Riviera Beach, CA). Chromatographic separations were achieved using a Hypersil ODS column (100x4.6 mm ID, 5 µm PS; LC Service, Emmen, The Netherlands), protected by a LiChroCART guard column (4x4 mm ID, 5 µm; Merck, Darmstadt, Germany). The column temperature was maintained at 50°C using a SpH99 column oven (Spark Holland, Meppel, The Netherlands). Injected samples were isocratically eluted with methanol-0.1 M ammonium acetate containing 10 mM tetrabutylammonium sulphate [35:65 (v/v) for CPT-11 and SN-38 in human plasma only, or 30:70 (v/v) for other compounds in plasma, urine and feces homogenate]. The mobile phase was delivered at 1.0 mL/min, and the column effluent was monitored at excitation and emission wavelengths of 355 and 515 nm, respectively. The detector signal was processed with a Chrom-Card data analysis software system implemented on an IBM personal computer running under Microsoft Windows 3.0 (Fisons, Milan, Italy). The qualitative and quantitative determination of each compound was based on HPLC retention times and peak area measurements, respectively, in comparison with injected standards, typically over a range of 2 to 200 ng/mL (CPT-11 and SN-38) and 10-400 ng/mL (SN-38G, NPC and APC) in plasma, or 100 to 5000 ng/mL in urine and feces homogenate. Calibration curves were prepared in blank samples of the appropriate biological matrix and fitted by a least-squares linear regression function with proportional weighting using the Lotus *version* 2.4 software package (Lotus Development Co, New York, NY). The mean overall extraction efficiencies for CPT-11 were 85.3±5.3% in plasma, 89.2±12.4% in urine and 90.0±5.0% in feces homogenate. Recoveries observed for the metabolites ranged between 82.6 and 99.3%, and were not significantly different from CPT-11. The percent deviation from nominal values, and the between-run and within-run variabilities at various concentration levels for each compound were always <15%.

Pharmacokinetic Data Analysis. The plasma concentration-time curves were analyzed using the pharmacokinetic Siphar *version* 4.0 (SIMED, Créteil, France), by

determination of slopes and intercepts of the plotted curves with multi-exponential functions. Initial parameter estimates were obtained by an automated peeling-algorithm procedure, with an integrated numerical algorithm based on the Powell method to minimize any objective function by the following criteria:

$$F=\sum_{i=1}^{n} [(Y_{oi}-Y_{ci})/\sigma_{yi}]^2$$

where n is the number of observations, Y_{oi} and Y_{ci} are Y observed and calculated values, respectively, for the *i*-th observation, and σ_{yi} is the standard deviation for the *i*-th observation. The statistical best fit was determined by application of (*i*) Akaike's information criterion with the χ^2 test to discriminate between models, and (*ii*) the coefficient of correlation, defined as the ratio of the standard deviation computed using the variance-covariance matrix and the parameter value. Both weighted least-squares and extended least-squares methods were evaluated to estimate model parameters minimizing the sum of squared differences between experimental and computed values and the log-likelihood function. The drug disposition half-lives ($t_{1/2}$), area under the plasma concentration-time curve from zero time to infinity (AUC), total body clearance (CL) and steady-state volume of distribution (V_{ss}) were determined on the basis of the best fitted curves, whereas the peak plasma concentration (C_{max}) and the time to the peak plasma concentration (T_{max}) were determined graphically.

Cell Cultures. The human colon carcinoma cell line WiDr and the ovarian adeno-carcinoma cell line IGROV-1 were grown and maintained in RPMI medium (Brunschwig, Amsterdam, The Netherlands). Cells were kept in continuous logarithmic growth at 37°C in a humidified atmosphere in 5% CO_2/95% air in media supplemented with 10% (w/v) of heat-inactivated bovine calf serum (Hyclone, Logan, UT), 100 U/mL of penicillin, 100 µg/mL of streptomycin and 2 mM freshly added *L*-glutamine (all from Life Technologies, Gaithersburg, MD). Exponentially grown cells were trypsinized and plated (2000 cells/well) in 96-well culture plates (Costar Co, Cambridge, MA), 48 hours before drug exposure. CPT-11 and the metabolites were dissolved separately in dimethylsulfoxide to obtain concentrations of ~2 µg/mL (SN-38) or ~20 µg/mL (CPT-11, SN-38G, NPC and APC). The compounds were added to the cells by serial dilution in the medium, followed by an incubation period of 5 days. After fixation with 10% (w/v) of aqueous trichloroacetic acid, inhibition of cell proliferation was assessed using sulphorhodamine B staining as described [20], with minor modifications [21]. Each compound was tested in quadruplicate in at least

three independent experiments. Cell survival was plotted relative to controls incubated in medium in the absence of drug.

RESULTS

Complete pharmacokinetic studies were performed in 10 patients entered onto a phase I clinical trial of CPT-11 given as a 90-min i.v. infusion in combination with cisplatin [18]. Full clinical toxicities and treatment responses will be reported in detail elsewhere. The group consisted of 6 males and 4 females ranging in age from 36 to 66 years. Each patient had a refractory solid malignancy, with colorectal cancer being the predominant disease type, present in 7 out of 10 cases. The median clinical chemistry values for all 10 patients included a total bilirubin level of 8 μM (range, 7-11), serum creatinine level of 87 μM (range, 72-115), SGOT and SGPT of 19 units/L (range, 15-42) and 18 units/L (range, 13-27), respectively, and total protein concentrations of 73 g/L (range, 69-78). Only two of the participants in the study were previously treated according to conventional protocols, typically with a 5-fluorouracil containing chemotherapeutic regimen.

Analytical Method. In order to gain a preliminary insight into the composition of the CPT-11 metabolites in plasma, urine and feces, samples from patients were analyzed by our initial HPLC procedure [16]. This assay procedure was subsequently modified, as described [17], so that base-line resolution of all chromatographic peaks observed could be achieved [Figs. 2A (plasma), 2B (urine) and 2C (feces)]. The retention times of the suspected metabolites and of pure reference standards of SN-38G, NPC, APC and SN-38 were identical in all matrices (means ± S.D.: 6.95±0.21, 9.50±0.92, 11.8±1.27 and 28.6±1.77 min, respectively). Attempts to obtain positive structural identification of the metabolites by tandem mass spectrometry and nuclear resonance spectroscopy have not yet been successful because of interference from endogenous, non-fluorescent, polar urinary and fecal constituents. Analysis of the HPLC chromatograms did not reveal any other major compounds with similar characteristics that might represent CPT-11 metabolites in any matrix. In addition, treatment of whole-sample aliquots or isolated metabolite peaks with 1000 units of β-glucuronidase (EC 3.2.1.31) failed to release the parent drug or CPT-11 metabolite, with the exception of SN-38 from its hydrophilic glucuroconjugate, as predicted earlier [17].

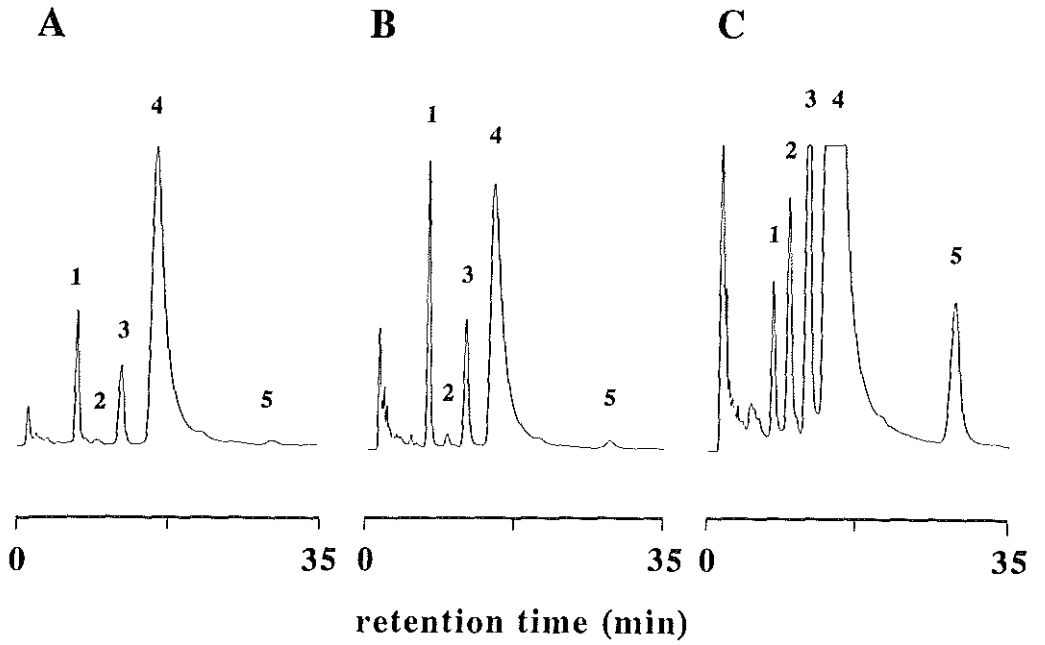

Fig. 2. Reversed-phase HPLC tracing with fluorescence detection at excitation and emission wavelengths of 355 and 515 nm of **(A)** a 250 µL plasma extract taken from a sample 30 min after i.v. infusion of CPT-11, **(B)** a 125 µL urine extract from a sample collected during the first 0-5 h after i.v. infusion of CPT-11 and **(C)** a 25 µg fecal extract from a sample collected during the first 0-7 h after i.v. infusion of CPT-11. All samples were obtained from a female with colorectal cancer receiving CPT-11 at a dose level of 200 mg/m^2. Chromatographic peaks represent **(1), SN-38G; (2), APC; (3), NPC; (4) CPT-11** parent drug; **(5), SN-38.**

Plasma Disposition. The plasma concentration *versus* time profiles of CPT-11 and the metabolites were similar for the 10 patients studied, with a typical example shown in Fig. 3. All the concentration-time profiles were best fitted simultaneously to a three-compartmental model after zero-order input using the Powell minimization algorithm, and weighted least-squares analysis with the weighting factor of 1/*Y*. The mean plasma pharmacokinetic parameters for CPT-11 and the metabolites, as calculated by this tri-exponential model, are listed in Table 1.

Table 1. Summary of pharmacokinetic parameters of CPT-11 and metabolites in plasma

Parameter	CPT-11	SN-38G	NPC	APC	SN-38
T_{max} (h)	1.52±0.11	2.32±0.21	2.66±0.47	2.86±0.54	2.08±0.15
C_{max} (μ*M*)	3.90±0.484	0.475±0.265	0.057±0.019	0.524±0.292	0.090±0.023
$t_{1/2}(\alpha)$ (h)	0.197±0.273	0.720±0.320	0.382±0.208	1.07±0.335	0.684±0.183
$t_{1/2}(\beta)$ (h)	2.35±0.64	2.11±0.41	0.85±0.48	2.92±0.96	1.34±045
$t_{1/2}(\gamma)$ (h)	13.5±2.06	23.5±10.6	9.67±5.12	15.1±2.30	23.8±7.70
AUC (μ*M*.h)	25.6±5.71	8.01±2.95	0.731±0.329	5.90±1.20	1.14±0.357
CL (L/h/m^2)	14.0±3.15	-	-	-	-
MRT (h)	10.7±0.62	-	-	-	-
V_{ss} (L/m^2)	138±24.0	-	-	-	-

Data were obtained from 10 cancer patients after the first treatment course of a 90-min intravenous infusion of CPT-11 at a dose level of 200 mg/m^2. The parameters were calculated by compartmental analysis and data represent mean values ± S.D.

Abbreviations: T_{max}, time to maximum concentration; C_{max}, maximum concentration; $t_{1/2}(i)$, half-life of the *i*-th disposition phase; AUC, area under the plasma concentration-time curve; CL, total body clearance; MRT, mean residance time; V_{ss}, volume of distribution.

Plasma concentrations of CPT-11 decreased rapidly immediately after cessation of the infusion, followed by a more prolonged terminal phase with a half-life of approximately 13.5 hours, which is within the same range as described for this compound previously [22]. The concentration-time course of the two cytochrome P-450-mediated metabolites NPC and APC followed the same general pattern as the parent drug, although concentrations were always well below corresponding CPT-11 levels. Consequently, the relative plasma AUC values of CPT-11 were more than 35 and 4 times greater than that of NPC and APC, respectively. The terminal half-life estimate for NPC could not be determined accurately in some patients due to constraints in sensitivity of the HPLC procedure (lower limit of quantitation: 10 ng/mL), but was overall still within the same range as that observed for CPT-11 and APC.

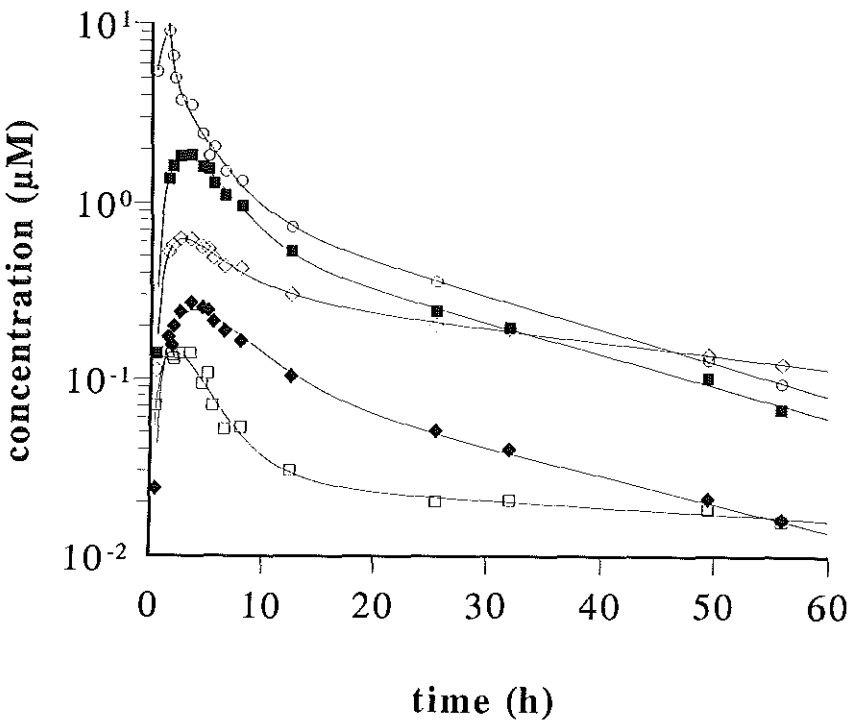

Fig. 3. Representative plasma concentration *versus* time profile of **CPT-11** (○) and its metabolites **SN-38G** (◊), **NPC** (♦), **APC** (■) and **SN-38** (□) in a single patient given CPT-11 by a 90-min i.v. infusion at a dose level of 200 mg/m^2.

All metabolites consistently peaked within one to two hours after start of the i.v. infusion, with APC predominating up to approximately 25 hours post-infusion (Fig. 3). At this time, SN-38G became increasingly more important due to a 1.75-fold increased terminal elimination half-life relative to CPT-11 and APC. As a result of this extended elimination phase, which was also observed for unconjugated SN-38, SN-38G was the principal metabolite detected in plasma in most patients. Overall, however, the mean AUC value for unchanged CPT-11 was more than 40% larger than the summation of the AUCs of the measured metabolites.

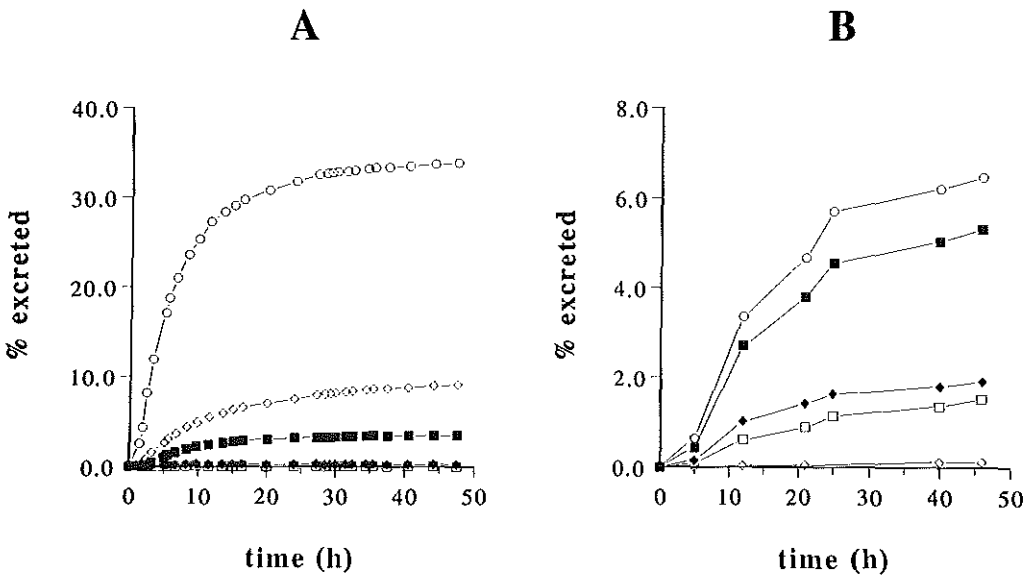

Fig. 4. Representative urinary (**A**) and fecal (**B**) excretion *versus* time profiles of **CPT-11** (○) and its metabolites **SN-38G** (◊), **NPC** (♦), **APC** (■) and **SN-38** (□) in a single patient given irinotecan by a 90-min i.v. infusion at a dose level of 200 mg/m^2.

Urinary and Fecal Recovery. The time course of the cumulative urinary and fecal elimination of CPT-11 and its metabolites for a representative patient is depicted in Fig. 4A and 4B, respectively. The urinary excretion pattern was virtually identical in all 10 patients, with approximately 30% (range, 10.7-40.1%) of the dose

excreted in the first 15 hours and only little after this time. The time course of the fecal excretion was more variable, with most of the compounds excreted from 5 to 24 hours after the CPT-11 infusion in 9 out of 10 patients. Data of fecal excretion from one patient were excluded in the pharmacokinetic calculations because of incomplete stool collection. Although patients were kept in the study for only ~56 hours after start of drug administration, the cumulative excretion in either urine or feces is unlikely to have changed after this time period.

Table 2. Cumulative urinary and fecal excretion of CPT-11 and metabolites

Compound	fe_{urine} (%)	fe_{feces} (%)
CPT-11	20.9±7.38	14.9±10.3
	(12.4-33.4)	(5.68-25.9)
SN-38G	3.39±2.31	0.317±0.306
	(0.86-7.13)	(0.103-0.678)
NPC	0.308±0.198	1.68±0.200
	(0.181-0.735)	(1.49-1.90)
APC	2.80±1.68	5.08±1.98
	(0.853-6.06)	(2.28-6.88)
SN-38	0.389±0.260	2.45±1.16
	(0.075-0.807)	(1.45-3.77)
Total compounds	28.1±10.6	24.4±13.3
	(11.8-42.2)	(11.0-38.0)

Data were obtained from 10 (urine) or 9 (feces) cancer patients after the first treatment course of a 90-min intravenous infusion of CPT-11 at a dose level of 200 mg/m^2. The data represent mean values ± SD, with ranges in parentheses.

Abbreviation: fe, percent of the absolute CPT-11 dose excreted as indicated drug.

The total cumulative urinary excretion of CPT-11 and the metabolites accounted for 28.1±10.6% (mean ± S.D.) of the dose in the 10 patients. Surprisingly, fecal excretion represented only 24.4±13.3% of the dose (Table 2), leading to a total

excretion of approximately 52% of the dose. As in plasma, unchanged CPT-11 could be distinguished in urine and feces of all patients as the predominant species.

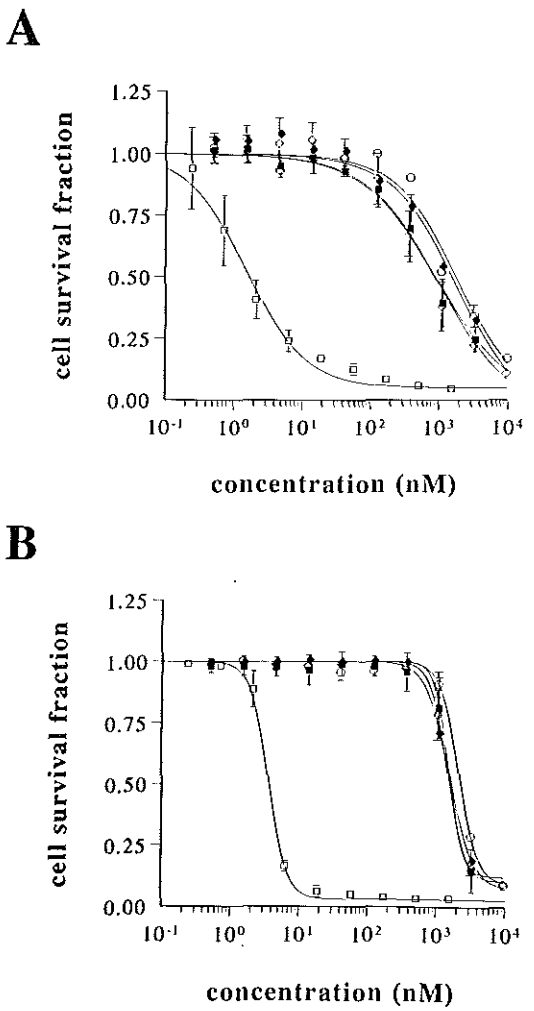

Fig. 5. Cell survival curves of the IGROV-1 ovarian adenocarcinoma (**A**) and the WiDr colon carcinoma cell lines (**B**) after a 5-day continuous exposure to different concentrations of CPT-11 (O), SN-38G (◊), NPC (♦), APC (■) or SN-38 (□), as assessed by the sulphorhodamine B assay. Data are presented as mean values (symbols) ± S.D. (bars) of at least three independent experiments performed in quadruplicate.

SN-38G was the major metabolite in urine, consistent with the highly polar nature of the glucuronic acid group and increased aqueous solubility with subsequent rapid renal excretion. Whereas renal clearance of SN-38 was only a minor route of drug elimination, fecal concentrations of this compound were unexpectedly high with, on average 2.45% of the dose excreted unconjugated in the feces. The two cytochrome P-450-mediated metabolites NPC and APC were mainly excreted in the feces, reaching mean total percentages of 1.68 and 5.08% of the dose, respectively, probably due to their lower water solubility favouring a hepatobiliary secretion pathway.

***In Vitro* Cytotoxicity.** Preliminary insight into the cytotoxic properties of the metabolites relative to CPT-11 and SN-38 was obtained by the exposure of the IGROV-1 ovarian carcinoma and the WiDr colon carcinoma cell lines to various concentrations of each test compound for a period of 5 days. Similar to the recent data obtained for APC in an analogous cell growth inhibition assay using the KB human epidermoid cell line [14], all metabolites (i.e. SN-38G, NPC and APC) were found to be weak inhibitors of cell proliferation [Fig. 5]. The mean concentration of each compound to produce a 50% inhibition of normal cell growth (IGROV-1 and WiDr cell lines, respectively) were 1850 and 2210 n*M* for CPT-11, 1010 and 1520 n*M* for SN-38G, 1860 and 1610 n*M* for NPC, 930 and 1580 n*M* for APC, and 1.54 and 3.63 n*M* for SN-38. Overall these data indicate that SN-38G, NPC and APC are up to 1000-fold less potent than the active metabolite SN-38. This is in line with previous data [14] and structure-activity studies demonstrating loss of biological activity with (bulky) substitutions at the C-10 of the camptothecine structure [23].

DISCUSSION

In the present study we have described for the first time the human pharmacokine-tics of CPT-11 and metabolites in plasma, urine and feces. The data complement previous knowledge of the clinical pharmacology of CPT-11 and have important practical implications for its optimal use. Previous studies of CPT-11 metabolism in cancer patients were able to recover only 25-50% of the administered dose in urine and bile, expressed as the summation of CPT-11, SN-38 and SN-38G, during the first 48 hours post-infusion [24]. This gave rise to the hypothesis that a substantial portion of the dose is eliminated through other metabolic and (intestinal) secretory pathways. The use of a recently developed specific analytical method [17]

helped to resolve this uncertainty, at least in part, by making it possible to simultaneously measure all four principal metabolites identified to date, viz. SN-38, SN-38G, NPC and APC. Although the CPT-11 infusion was followed by cisplatin administration in this study, important pharmacokinetic drug interactions are not very likely, because (*i*) in human liver microsomal experiments cisplatin had no statistically significant effect on CPT-11 metabolism [25], and (*ii*) comparison of the pharmacokinetics and metabolism of CPT-11 in clinical combination therapy with cisplatin to single agent therapy did not reveal an apparent kinetic interaction [26].

Of the greatest importance for the antitumor activity of CPT-11 treatment is the disposition of CPT-11 and its potentially active metabolites in plasma. The pharmacokine-tic model presented here accurately describes the plasma concentration *versus* time profile of both CPT-11 and all four metabolites simultaneously. The disappearance of CPT-11 and SN-38 from the central plasma compartment was characterized by terminal elimination half-lives of approximately 13.5 and 23.5 hours, respectively, which are in good agreement with recent data from Rivory, *et al.* obtained with the drug administered as a single agent [22]. Previously, the use of simpler noncompartmental or linear bi-exponential models to describe CPT-11 disposition have consistently underestimated the elimination phase and failed to yield dose-independent parameter estimates [27,28]. Thus, the results of this study emphasize the need to apply appropriate kinetic models with sufficient sampling-time points for the accurate estimation of complete CPT-11 (and metabolite) concentration-time profiles. In our patients, we observed that the total amount of NPC and APC in plasma accounted for only approximately one fourth to one thirtieth of the total CPT-11 AUC. Also, the metabolites were only detectable in plasma during times of relatively high concomitant CPT-11 concentrations. The parallel decline of NPC and APC with the parent drug suggests that metabolite elimination is formation rate-limited. Terminal elimination phases of SN-38G and unconjugated SN-38 were also very similar, as observed previously with CPT-11 administered as a 90-min i.v. infusion [9], and declined at a constant SN-38G to SN-38 ratio of approximately 7. The basis for the delayed elimination of these metabolites relative to CPT-11 is not completely understood, but may involve (saturable) rate-limited elimination processes or differences in binding affinity for plasma proteins in addition to preferential enterohepatic recycling of SN-38 and conversion of CPT-11 to SN-38 by carboxylesterases during intestinal reabsorption. Overall, the plasma AUC of the metabolites constituted only ~40% of the total AUC, indicating a clear predominance of the parent drug. The potential contribution of

CPT-11 metabolites to biological effects, however, depends on the intrinsic activity of the biotransformation products and their concentrations at the active sites. Our *in vitro* studies have shown that all metabolites are many-fold less potent than the pharmacologically active species, SN-38. However, before drawing any conclusions on the contribution of CPT-11 metabolites (other than SN-38) to antitumor activity, it will be essential to determine their plasma protein binding [preliminary data are reported in Ref. [29]]. The expected lower plasma binding of the metabolites compared with that of SN-38, which is 94-96% bound, principally to human serum albumin and gamma globulin, could denote a greater ability to interact with topo-isomerase-I or other essential binding sites.

The cumulative urinary excretion of unchanged CPT-11, SN-38 and SN-38G of 20.9, 0.389 and 3.39%, respectively, agrees very well with the data of previous studies in which CPT-11 was administered without cisplatin [24,30-33]. In addition, only small amounts of the major cytochrome P-450 metabolites APC and NPC were detected in urine. The mean renal clearance of CPT-11, i.e. the product of the dose-fraction excreted unchanged in urine and total body clearance, was estimated to be 2.93 L/h/m^2. This value is less than the glomerular filtration rate in humans, presumably due to binding of CPT-11 to plasma proteins (~58-68% in the studied concentration range[5]), and suggests that CPT-11 is neither reabsorbed nor actively secreted into the tubular lumen to a great extent. It also indicates that as much as ~80% of the overall clearance can be attributed to nonrenal processes, including metabolic degradation of CPT-11. Part of the nonrenal elimination was accounted for by fecal excretion of unchanged CPT-11 and the four metabolites as previously suggested [17], leading to a total recovery of ~50% of the dose. This is highly surprising, as it would suggest that half of the dose in urine and feces may constitute some further unknown nonextractable or, more likely, nonfluorescent metabolites. These may include compounds formed after decarboxylation of the carboxylate form of CPT-11, metabolites resulting from oxidation of the camptothecine nucleus, or from combinations of these pathways, as described by Lokiec, *et al.* [12]. Further investigation is required to quantitate the contribution of the formation of these compounds and potentially other, as yet unidentified metabolites, to the overall renal and nonrenal clearance. Chemical or microflora-induced degradation of CPT-11 within the gut lumen following biliary or intestinal secretion, as observed with anthracycline antineoplastic drugs [34], is unlikely to play an important role in the overall drug elimination, in view of the extended stability of this compound in feces homogenates [17]. In addition, concentrations of CPT-11 in sweat, pleural fluid and

saliva were recently shown to be fairly lower than corresponding plasma concentrations [35,36], suggesting that excretion into these fluids are of subordinate importance.

As predicted by earlier studies [12,17], there was no indication of glucuronic acid conjugates of CPT-11 or any other metabolite (except SN-38) in either urine or feces. This may be related to the large size of these molecules relative to SN-38, preventing them from interaction with the glucuronosyltransferases. However, great caution has to be exercised with respect to the fecal metabolites, as endogenous (biliary or enteric) and bacterial β-glucuronidase expressed by the intestinal microflora may together with other enzymes have led to modification of the CPT-11 metabolites excreted by this route. Indirect evidence for this was obtained from the observation of unexpectedly high fecal concentrations of SN-38 accompanied by a virtual disappearance of SN-38G in all 9 patients. This notion has been described previously by Kaneda and Yokokura with CPT-11 administered to rats [37].

The finding of increased local concentrations of the active metabolite SN-38 released from SN-38G in the intestines may have considerable ramification with respect to the clinical use of CPT-11. The major dose-limiting toxicities of CPT-11 include diarrhea and, to a lesser extent, myelosuppresssion [1]. The diarrhea is characterized by an unexpected onset and significant incidence (~60-70%), and does not respond adequately to conventional antidiarrheal agents. Although the mechanism for the observed toxicity is still controversial, it is thought to be related to structural and functional injuries to the intestinal tract that result from the mitotic inhibitory activity of SN-38 [38]. In line with this hypothesis, it is of interest to note that the only patient in our study experiencing delayed diarrhea graded >2 (NCI common toxicity criteria) had also the highest fecal excretion of unconjugated SN-38 and the lowest fecal SN-38G to SN-38 excretion ratio. Previous studies designed to establish pharmacokinetic-dynamic relationships for CPT-11 have shown a correlation between systemic SN-38 glucuronidation rates, expressed as a biliary index (i.e. the product of the plasma AUC of CPT-11 and the ratio of the plasma AUCs of SN-38 to SN-38G) and diarrheal incidences [30,33]. However, similar to recent findings by Canal, *et al.* [39], a such relationship was not observed in our patient population[6]. In all, these data suggest that consideration of interindividual differences of fecal β-glucuronidase activity would assist in deriving more accurate prediction of CPT-11-induced intestinal side effects, and may provide a basis to modulate the experienced toxicity. This need is intensified in light of recent findings of Takasuna and co-workers that cotreatment of CPT-11 with baicalin, an inhibitor of β-glucuronidase [40], or

penicillin plus streptomycin [38] markedly ameliorated the severity of diarrhea in rats. A clinical trial to evaluate the effects of pretreatment with the antibiotic neomycin before the administration of CPT-11 is in progress in our institute.

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Chapter 14

Sparse-data set analysis for irinotecan and SN-38 pharmacokinetics in cancer patients co-treated with cisplatin

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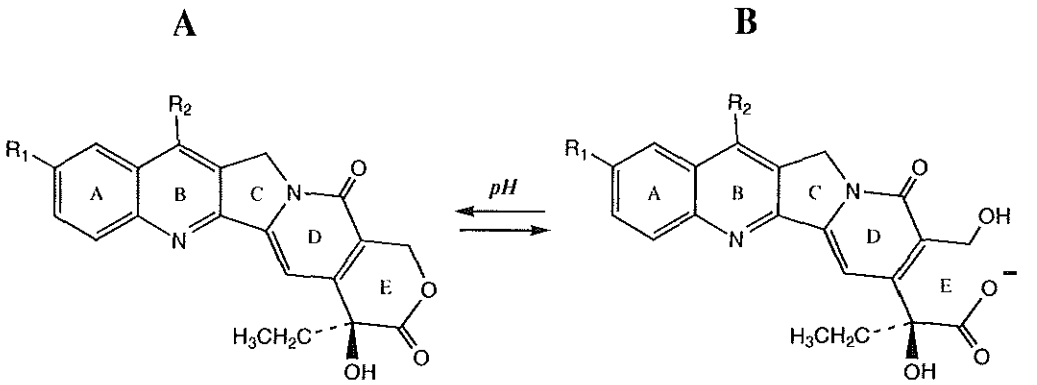
ABSTRACT

The clinical pharmacokinetics of the antineoplastic agent irinotecan (CPT-11) is associated with substantial interpatient variability. The degree to which this variability in CPT-11 exposure impacts upon the response and toxicity of the drug has not yet been properly determined. In general, the area under the plasma concentration-time curve (AUC) is an appropriate indicator of exposure, but requires collection of upto 17 timed blood samples. This presents difficulties if large-scale population samplings are required. The present study involved the development and validation of a strategy to estimate the AUCs of the lactone and total (i.e. lactone *plus* carboxylate) forms of CPT-11 and its active metabolite SN-38 from a limited number of blood samples in patients co-treated with cisplatin. Using data from 24 patients, univariate and multivariate regression analyses were employed to generate the models. The best predictive models for simultaneous estimation of CPT-11 and SN-38 AUCs were obtained with three time points at 0.5 h, 1.67 h and 5.50 h after start of the 90-min i.v. infusion of CPT-11. The models were tested separately in another group of 24 patients receiving the same combination treatment. This validation set demonstrated that CPT-11 and SN-38 AUCs after standard dose administration could be predicted sufficiently unbiased and precise with three timed samples to warrant clinical application.

INTRODUCTION

The antineoplastic agent irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecine) is a promising water soluble semisynthetic derivative of camptothecine, a plant alkaloid from the Chinese tree *Camptotheca acuminata* [1,2]. The drug displays potent antitumor activity against a variety of tumors, and has recently been approved for the treatment of refractory colorectal and ovarian carcinomas in several countries [3]. The mechanism of action of CPT-11 and its structurally related analogues is thought to be related to inhibition of the intranuclear enzyme topoisomerase-I, thereby indirectly impeding DNA-replication and RNA-transcription [4].

CPT-11 and its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecine; Fig. 1) are both subject to a rapid, reversible, pH-dependent hydrolysis of a lactone ring moiety in the molecule, generating an open-ring carboxylate [5].



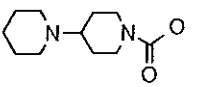
compound	R_1	R_2
irinotecan (I)		CH_2CH_3
SN-38 (II)	OH	CH_2CH_3
camptothecin (III)	H	H

Fig 1. Chemical structures of the lactone **(A)** and carboxylate **(B)** forms of CPT-11, its active metabolite SN-38 and the related compound camptothecine.

At neutral or physiologic pH, e.g. in blood, the equilibrium between the two drug species favors the pharmacologically less active carboxylate for most camptothecines [6]. However, the relative importance of the lactone-carboxylate interconversion of CPT-11 and SN-38 with respect to pharmaco-dynamic outcome is still not completely understood. Furthermore, the clinical pharmacoki-netic behavior of CPT-11 is associated with a substantial degree of interindividual variability. Thus, careful measurement of both drug forms is clearly warranted in order to fully characterize the clinical pharmacology of these agents.

The major dose-limiting toxicities encountered with single agent CPT-11 therapy were neutropenia and diarrhea.[7] Relationships for CPT-11 and SN-38 between clinical pharmacokinetics and pharmacodynamic outcome are extremely complex and not thoroughly discerned [reviewed in Ref. (7)]. Based on the knowledge of

camptothecine pharmacology, CPT-11 induced myelosuppression probably occurs due to the inhibition of topoisomerase-I by SN-38 lactone in bone marrow cells. In contrast, acute CPT-11 mediated diarrhea is most likely caused by the anticholinesterase activity of the parent compound. Supporting this theory are reports that the area under the plasma concentra-tion-time curve (AUC) of SN-38 correlated with myelosuppression and the AUC of CPT-11 correlated with diarrhea [8,9] However, some studies have not found such associations, indicating that additional studies applying selective analytical methods are essential to help clarify these contrasting results. Such studies will likely involve large numbers of patients and generally require the collection of upto 17 timed blood samples after i.v. drug administration [10]. Thus, the objective of the current study was to develop a model for prediction of the AUCs of CPT-11 and SN-38 from a limited blood sampling schedule in patients with advanced solid cancer receiving the drug in combination with cisplatin.

MATERIALS AND METHODS

Patients and Treatment. The pharmacokinetic models were developed and validated in 48 patients with proven malignant solid tumors, participating in a phase I and pharmacokinetic study of combined chemotherapy with CPT-11 and cisplatin [9]. Detailed clinical and toxicological profiles will be reported separately. Inclusion criteria included the following: (*a*) no more than one prior combination chemotherapy regimen or two single agent regimens; (*b*) off previous anticancer therapy for at least 4 weeks (6 weeks if nitrosoureas, mitomycin or radiotherapy); (*c*) no prior treatment with topoisomerase-I inhibitors or platinum derivatives; (*d*) age between 18 and 70 years; (*e*) WHO performance status ≤2; (*f*) life expectancy greater than 12 weeks; (*g*) adequate bone-marrow, liver and renal functions and symptomatic peripheral neurotoxicity graded 1 or less (according to NCI common toxicity criteria). Written informed consent was obtained from all patients prior to treatment in accordance with the guidelines of the Institutional Review Board.

CPT-11 was provided by Rhône-Poulenc Rorer (Antony, France) as an aqueous formulation containing *d*-sorbitol, lactic acid and sodium hydroxide with a final pH-value of 3.5. The drug was administered at dose levels ranging from 175 to 300 mg/m^2 as a 90-min i.v. infusion. Cisplatin was given as a 3-h i.v. infusion directly after the end of the CPT-11 infusion.

Pharmacokinetic Analysis. For CPT-11 and SN-38 pharmacokinetic analysis heparinized blood samples were drawn from an indwelling cannula at 0.5, 1.5, 1.67, 1.83, 2.0, 2.5, 3.5, 4.5, 5.0, 5.5, 6.5, 8.0, 12.0, 25.5, 32.0, 49.5 and 56.0 h after the start of the infusion. The plasma fraction was obtained by centrifugation and analyzed for CPT-11 and SN-28 using a validated reversed-phase high-performance liquid chromatography system (HPLC) with fluorescence detection [11]. The lower limits of quantitation were 0.5 ng/mL for the lactone forms (1-mL samples) and 2.0 ng/mL for the total forms (0.25-mL samples), respectively. The percentage deviation from the nominal value and the between-run and within-run precision were always less than 15.0%.

CPT-11 and SN-38 (lactone and total) concentration-time data of all patients were fitted to a tri-exponential equation, using Siphar v4.0 (SIMED, Créteil, France), based on discriminating tests described elsewhere [12]. All compartmental analyses were obtained by inverse square weighting of the observed concentration. The terminal drug disposition half-life [$t_{1/2}(\gamma)$] and the AUC from time zero to infinity were determined on the basis of the best fitted curves, whereas the peak plasma concentration (C_{max}) was determined graphically from semi-logarithmic concentration-time plots.

Model Development and Validation. Limited sampling models were constructed on a training data set that contained 24 patients, randomly assigned from each separate dose level to avoid bias in the predictive values of one set to another. The models were constructed by assuming that concentration(s) at a fixed time could predict the AUC of each of the compounds of interest simultaneously. Simple linear correlations were initially determined between the concentrations at each time point (independent variables) and the corresponding AUC (dependent variable) by a univariate linear-regression analysis, to find the optimal single-sample time point for each substance measured. Next, forward stepwise multivariate-regression analyses was undertaken to develop the best linear equation describing the association between concentrations at more than one time point and AUC, to increase the precision of the method. The optimal model was eventually identified on the basis of Pearson's correlation coefficient (r), and root mean square residual values as determined from the regression [13].

The pharmacokinetic data from the remaining 24 patients was used to validate the applicability of the constructed models. This was achieved by comparing actual AUCs from the tri-exponential computer fit with estimated AUCs using the best single or multiple time-point models developed from the training set. The predictive

performance of the developed models was evaluated using calculations of bias (or percentage mean predictive error; %MPE) and precision (or percentage root mean square error; %RMSE). Due to missing concentrations in this data set at the relevant time points, 3 patients were excluded from validation. Pearson's correlation coefficient was used to rank the concordance between measured and predicted AUCs. Differences in patient demographics and pharmacokinetics between training and validation set patients were evaluated by using Student's *t*-test. All statistical calculations were performed with the Number Cruncher Statistical System software v5.X (Dr. J.L. Hintze, Kaysville, Utah, USA, 1992).

RESULTS

Pharmacokinetic studies were completed in 48 patients with various types of solid tumors, treated with a 90-min i.v. infusion of CPT-11, directly followed by a 3-h infusion of cisplatin. The total group of patients was composed of 31 males and 17 females, with a mean age 53 years and a median Eastern Cooperative Oncology Group (ECOG) perfor-mance status of 0 [Table 1]. Patients were randomly divided in two groups, a training set and a validation set, both containing 24 patients. No significant differences were observed between both groups in patient characteristics [Table 1].

Table 1. Patient demographics

Characteristic	Training set	Validation set
No. of patients	24	24
Age (years/range)	53 (42-69)	52 (36-68)
Sex (male/female	16 : 8	15 : 9
ECOG performance status (0/1/2)	17 : 7 : 0	14 : 9 : 1
Primary tumor site		
colorectal	12	9
lung	3	3
pancreas	2	0
mesothelioma	2	0
tonsil	2	1
unknown	2	4
other	1	7

[a] Results expressed as mean value (range).

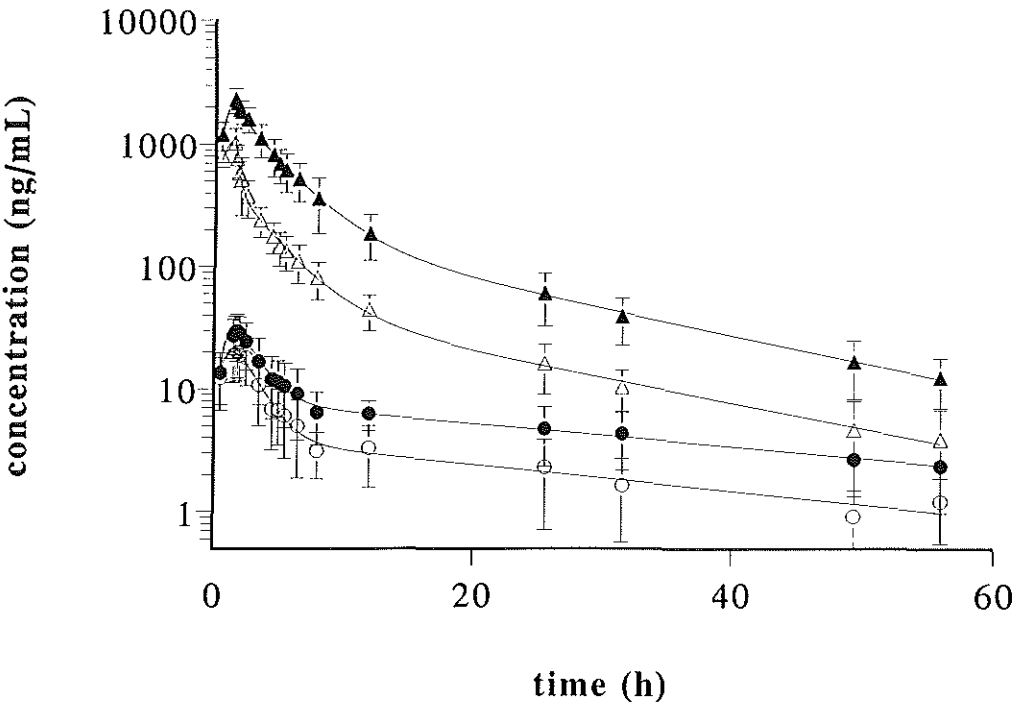


Fig 2. Plasma concentration-time curves of CPT-11 (triangles) lactone and total and of SN-38 (circles) lactone and total in 17 patients given CPT-11 at 200 mg/m^2 as a 90-min i.v. infusion. The open symbols represent the lactone forms and the closed symbols represent the total forms of CPT-11 and SN-38. All pharmacokinetic curves were fitted to a tri-exponential equation, using Siphar v4.0 (SIMED, Créteil, France), assuming a three-compartment model for distribution and elimination of the compounds. Data are presented as mean values (symbols) ± SD (error bars).

The mean plasma concentration-time curves for the lactone and total forms of CPT-11 and SN-38 in all patients treated at a dose level of 200 mg/m^2 of CPT-11 are shown in Fig. 2. During the drug infusion, the plasma concentrations of the lactone and total forms of CPT-11 and SN-38 increased steadily, after which a decrease was observed with a decay best described by a tri-exponential equation. Relatively large amounts of CPT-11 and SN-38 were continually present in the lactone forms of these substances, as described for these compounds previously [14].

Table 2. Summary of the plasma pharmacokinetic parameters of CPT-11 total and lactone after a 90-min i.v. infusion of CPT-11 in the training (A) and validation (B) sets. Data represent mean values ± SD

Dose level (mg/m^2)	Set	*n*	$AUC_{0-\infty}$ total (μg.h/mL)	$AUC_{0-\infty}$ lactone (μg.h/mL)	C_{max} total (μg/mL)	C_{max} lactone (μg/mL)	$t_{1/2}(\gamma)$ total (h)	$t_{1/2}(\gamma)$ lactone (h)
175	A	1	6.53	2.03	1.37	0.60	12.14	12.80
	B	2	7.77, 15.9	2.36, 4.78	2.39, 3.16	1.07, 1.80	8.48, 11.1	5.43, 7.94
200	A	9	11.5±3.75	3.79±0.98	2.49±0.35	1.04±0.22	14.2±7.80	17.3±10.4
	B	7	12.8±3.01	3.66±0.78	2.51±0.41	1.12±0.22	13.6±5.82	12.2±5.65
230	A	3	13.1±2.65	4.32±1.43	2.98±0.72	1.50±0.68	9.21±2.20	13.4±3.72
	B	4	17.7±2.05	5.02±1.11	3.32±0.30	1.39±0.29	11.5±2.22	9.62±2.61
260	A	6	13.5±4.65	4.01±1.07	2.61±0.65	1.31±0.30	12.6±5.86	12.8±4.21
	B	6	13.6±5.88	4.86±2.19	2.70±0.72	1.38±0.43	11.4±1.81	11.2±2.41
300	A	5	22.3±13.3*	6.20±2.96	4.04±1.45*	1.71±0.59	11.5±2.97*	12.9±3.67
	B	5	21.2±5.92	7.27±2.61	4.17±1.22	1.97±0.59	12.5±1.72	12.0±2.64

All parameters were obtained from a nonlinear three-compartment computer-fitted model with $1/(concentration)^2$ weighting. Results are shown as mean ± SD. Abbreviations: *n*, number of data sets; AUC, area under the plasma-concentration time curve; C_{max}, maximum plasma concentration; $t_{1/2}(\gamma)$, terminal elimination half-life. * $n = 4$.

Table 3. Summary of the plasma pharmacokinetic parameters of SN-38 total and lactone after a 90-min i.v. infusion of CPT-11 in the training (A) and validation (B) sets. Data represent mean values ± SD

Dose level lactone (mg/m^2)	Set	*n*	$AUC_{0-\infty}$ total (μg.h/mL)	$AUC_{0-\infty}$ lactone (μg.h/mL)	C_{max} total (ng/mL)	C_{max} lactone (ng/mL)	$t_{1/2}(\gamma)$ total (h)	$_{1/2}(\gamma)$ (h)
175	A	1	0.13	0.07	11.43	6.52	7.72	6.76
	B	2	0.29, 0.35	0.16, 0.17	44.6, 47.4	27.5, 27.7	16.87, 10.3	13.9, 13.2
200	A	9	0.43±0.20	0.21±0.08	36.7±10.9	25.1±7.33	30.4±15.1	24.8±7.61
	B	7	0.30±0.08	0.16±0.05	29.2±7.25	20.8±7.35	20.3±7.64	19.3±5.09
230	A	3	0.39±0.16	0.16±0.04	27.4±3.50	18.4±5.43	42.0±35.2	18.3±7.99
	B	4	0.79±0.46	0.27±0.08	57.9±25.8	42.8±26.5	29.9±14.5	23.4±6.83
260	A	6	0.34±0.18	0.22±0.07	40.3±15.8	25.7±11.3	23.1±10.6	29.8±15.5
	B	6	0.51±0.22	0.31±0.13	44.4±14.5	31.5±8.00	26.9±10.9	23.0±5.80
300	A	5	0.43±0.25	0.33±0.21	47.9±27.6	36.7±27.2	29.9±18.0	39.9±19.3
	B	5	0.51±0.22	0.35±0.14	51.2±18.5	38.4±12.5	29.8±14.6	30.8±11.7

All parameters were obtained from a nonlinear three-compartment computer-fitted model with $1/(concentration)^2$ weighting. Results are shown as mean ± SD. Abbreviations: *n*, number of data sets; AUC, area under the plasma-concentration time curve; C_{max}, maximum plasma concentration; $t_{1/2}(\gamma)$, terminal elimination half-life.

The terminal elimination phases of CPT-11 lactone and total were more rapid than those of the SN-38 forms, which resulted in prolonged biological half-lives for the active metabolite relative to CPT-11. A summary of the main pharmacokinetic parameters, including AUC, C_{max} and $t_{1/2}(\gamma)$ of CPT-11 and SN-38 between the two patient groups is presented in Tables 2 and 3, respectively. There were no significant differences in any pharmacokinetic parameter between the two groups as shown by an unpaired two-sided Student's *t*-test [Tables 2 and 3]. Interpatient variability in the concentrations of CPT-11 and SN-38 in the training and validation sets at each of the 17 sample-time points was large (coefficient of variation ranged from 64% (0.5 h) to 96% (1.67 h). The interpatient variability in corresponding AUCs of CPT-11 and SN-38 was slightly dependent on the dose level and ranged from 46% to 64%.

Table 4. Univariate correlation of CPT-11 and SN-38 (lactone and total) concentrations at each sample-time point with the corresponding AUC in the training-data set

Time point	CPT-11 lactone		CPT-11 total		SN-38 lactone		SN-38 total	
(h)	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
0.5	23	0.633	23	0.611	17	0.362	24	0.097
1.5	22	0.607	22	0.790	22	0.478	23	0.332
1.67	21	0.422	23	0.841	19	0.823	23	0.523
1.83	22	0.676	23	0.923	22	0.505	24	0.611
2.0	24	0.473	23	0.929	24	0.691	24	0.651
2.5	23	0.766	23	0.942	22	0.477	24	0.686
3.5	22	0.883	23	0.954	20	0.690	24	0.637
4.5	23	0.857	23	0.959	24	0.686	24	0.712
5.0	23	0.886	23	0.968	23	0.742	24	0.636
5.5	23	0.920	23	0.965	24	0.598	23	0.743
6.5	23	0.873	22	0.950	22	0.486	23	0.661
8.0	21	0.938	21	0.946	20	0.621	22	0.586
12.0	21	0.619	21	0.873	20	0.419	20	0.621
25.5	23	0.842	23	0.898	23	0.426	15	0.480
32.0	20	0.756	22	0.894	19	0.477	12	0.528
49.5	23	0.481	22	0.723	20	0.378	8	0.223
56.0	23	0.396	23	0.712	18	0.377	8	0.126

Abbreviations: *n*, number of data sets at that specific time point; *r*, Pearson's correlation coefficient.

CPT-11 and SN-38 (both lactone and total) concentrations at each sample-time point were correlated with their AUC by using univariate-regression analysis for the training-data set [Table 4]. The correlation coefficient ranged from 0.396 to 0.938 for CPT-11 lactone, from 0.611 to 0.968 for CPT-11 total, from 0.362 to 0.823 for SN-38 lactone and from 0.097 to 0.743 for SN-38 total [Table 4].

The best correlation was found at the sample-time point of 5.50 h, taken all substances into account simultaneously. Following this univariate-regression, multivariate-regression with a restriction to models with no more than two additional time points was evaluated. In the trivariate models, sample-time point combinations with the highest correlation and precision (lowest %RMSE), were found at different sample-time points for CPT-11 total, CPT-11 lactone, SN-38 total and SN-38 lactone (data not shown). Assuming the lactone forms being most predictive for toxicities of the given drug, the best sample-time points for the combination of CPT-11 lactone and SN-38 lactone were used for further model development.

Table 5. Limited-sampling models for the prediction of the AUCs of CPT-11 lactone and total and of SN-38 lactone and total

Models:

A $AUC_{CPT\text{-}11\ lactone}$ (µg.h/mL) = $1.11*C_{0.5} + 0.0531*C_{1.67} + 16.53*C_{5.5} + 0.439$

B $AUC_{CPT\text{-}11\ total}$ (µg.h/mL) = $1.84*C_{0.5} + 1.19*C_{1.67} + 11.5*C_{5.5} + 0.215$

C $AUC_{SN\text{-}38\ lactone}$ (µg.h/mL) = $2.46*C_{0.5} + 2.36*C_{1.67} + 9.66*C_{5.5} + 0.0521$

D $AUC_{SN\text{-}38\ total}$ (µg.h/mL) = $-6.27*C_{0.5} + 3.72*C_{1.67} + 20.2*C_{5.5} + 0.121$

Model	Training set			Validation set		
	r	%MPE	%RMSE	r	%MPE	%RMSE
A	0.953	0.34	7.88	0.936	0.36	11.3
B	0.982	0.22	6.83	0.966	0.10	3.31
C	0.903	0.52	7.94	0.443	1.06	31.7
D	0.764	0.85	21.1	0.869	1.08	29.8

Abbreviations: *r*, Pearson's correlation coefficient; %MPE, percentage mean predictive error; %RMSE, percentage root mean-squared predictive error; AUC, area under the plasma-concentration time curve; $C_{0.5}$, $C_{1.67}$ and $C_{5.5}$ are the plasma concentrations in µg/mL at 0.5, 1.67 and 5.5 h after start of infusion.

The most predictive sample-time points were found at 0.5, 1.67 and 5.5 h after the start of infusion. In the training-data set, the models of all substances demonstrated little bias, with values for the %MPE ranging from 0.22 to 0.85 [Table 5]. In this set, the correlation coefficient of SN-38 total was estimated at 0.764 with a %RMSE of 21.1%, suggesting less correlation and lower accuracy than found for the other compounds (correlation coefficients ranging from 0.903 for SN-38 lactone to 0.982 for CPT-11 total and %RMSE ranging from 6.83 for CPT-11 total to 7.94 for SN-38 lactone).

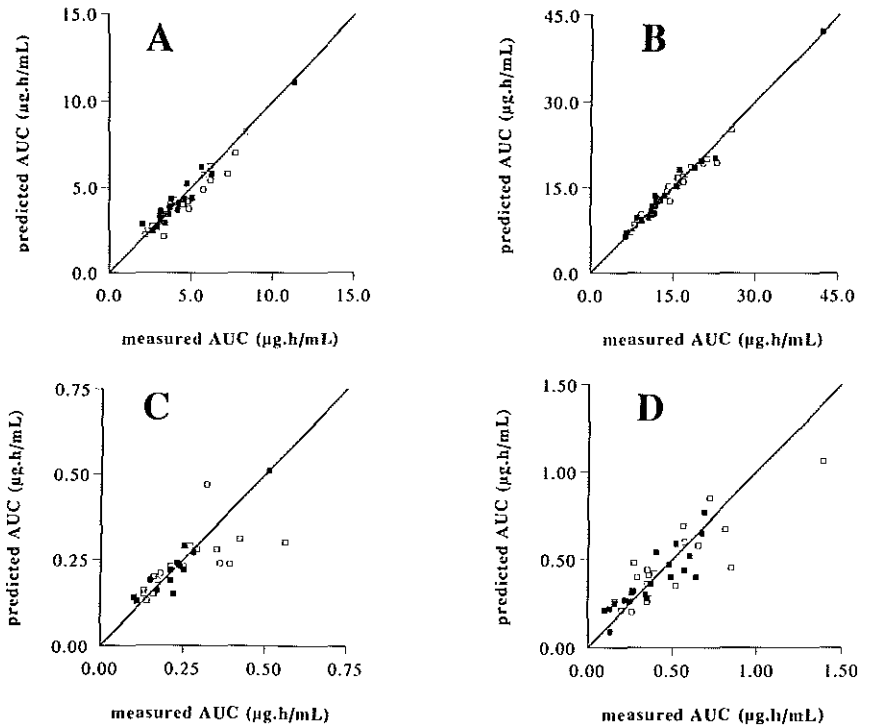


Fig 3. Observed correlations between the measured AUC using a nonlinear three-compartment computer-fitted model with 1/(concentration)^2 weighting and the predicted AUC from the limited sampling models (Table 5) for CPT-11 lactone (**A**), CPT-11 total (**B**), SN-38 lactone (**C**) and SN-38 total (**D**). In all models plasma samples were taken at sample-time points of 0.5, 1.67 and 5.5 h after the start of infusion. Closed symbols represent data from the training set and open symbols data from the validation set. Pearson's correlation coefficients in the training and validation sets were 0.953 and 0.936 for CPT-11 lactone, 0.982 and 0.966 for CPT-11 total, 0.903 and 0.443 for SN-38 lactone, and 0.764 and 0.869 for SN-38 total, respectively. The solid lines represent the lines of identity.

Next, we used the validation-data set, containing the remaining 24 patients to evaluate the predictive performance of the developed models. For CPT-11 lactone and total, correlation coefficients were 0.936 and 0.966 respectively, with the %MPE (0.36 and 0.10 respectively) and the %RMSE (11.30 and 3.31 respectively) were low, indicating minor bias and excellent precision [Table 5; Fig. 3]. For SN-38 total, similar acceptable data could be obtained using the same model. However, in the validation data-set SN-38 lactone data showed poor results for the correlation coefficient, %MPE and %RMSE, due to a poor correlation between AUC and concentrations at the higher dose levels of CPT-11 (230 to 300 mg/m^2). Hence, the limited-sampling model was not suitable for prediction of SN-38 lactone AUCs in patients treated at dose levels of CPT-11 higher than 230 mg/m^2.

We also performed an additional analysis to evaluate the use of CPT-11 and SN-38 total concentrations for prediction of the AUCs of the respective lactone drug forms. In general, the best models demonstrated deteriorated correlations and poor accuracy, with values for the %RMSE of upto 42% (data not shown).

DISCUSSION

In recent years, various statistical models have been developed for antineoplastic agents to predict pharmacokinetic parameters from a limited blood sample schedule [15]. Previous studies have indicated that such strategies are also feasible for the estimation of CPT-11 and SN-38 pharmacokinetics with the drug given by i.v. infusion, although no differentia-tion has been made sofar between the lactone and carboxylate forms of the compounds [16-19]. In view of the discrepant data published on relationships between drug levels and the observed toxicity, further investigations of CPT-11 kinetics including separate quantitation of the lactone and the total forms of CPT-11 and SN-38 are clearly needed. Previously described limited-sampling models differed considerably in administered dose and infusion duration and are only valid for CPT-11 given as a single agent. In our current study, the follow-up period for sample collection after infusion was much longer as compared to the other studies, resulting in more reliable pharmacokinetic data. Moreover, our limited-sampling models are the first applicable for CPT-11 given in combination with another drug, in this case cisplatin.

To achieve high predictive values for the developed models, i.e. high correlation coefficients and precision with low bias [12], at least three sample-time points were required in all models. Although inclusion of additional samples might have upgraded

the predictive performance of the models, fewer samples are more cost-effective and convenient for the patients. In addition, sampling over shorter periods enables pharmacokinetic-pharmacodynamic studies during day-time treatment in an outpatient setting, even in multi-institutional clinical trials.

The pharmacokinetic behavior of SN-38, the principal (active) metabolite of CPT-11, is markedly different from that of the parent drug. The objective of our approach was to accurately predict the AUCs of CPT-11 and SN-38 simultaneously in both lactone and total drug forms, from only three sample-time points. The best compromise for the concurrent determination of the AUCs was found at the sample-time points at 0.5, 1.67 and 5.5 hours after the start of infusion. In selecting these sample-time points, clinical constraints also were taken into consideration. For example, the samples have to be taken as early as possible after infusion, in view of the potential future usage of CPT-11 in clinical practice with drug-level monitoring for adaptive controlled dosing. In addition, a late sample-time point is not clinically convenient as it makes outpatient treatment difficult or even impossible. For all four limited-sampling models developed, the first sample-time point (at 0.5 h) is critical, for it is part of the ascending part of the concentration-time curves. The second point (at 1.67 h) lies just after the end-of-infusion time-point, and is indicative for near-maximum plasma concentrations of CPT-11 and SN-38. The third sample point (at 5.5 h) is also important for it is part of the descending part of the concentration-time curves. Theoretically limited-sampling strategies employing other sample-time points which are also predictive, could have been constructed, but would probably lack the above mentioned advantages.

The CPT-11 total AUC is an important pharmacologic parameter, essential for the calculation of the total body clearance and for the calculation of individual metabolic ratios. The active lactone forms, especially that of SN-38, are important as they are assumed to be the real cytotoxic species and responsible for the toxic effects of CPT-11 therapy [8]. The proposed models for estimation of the AUCs of CPT-11 lactone and total and SN-38 total were shown to be valid, with excellent predictive utility in a large group of patients given CPT-11 at different dose levels in combination with cisplatin. In case of SN-38 lactone, however, the AUC estimates were slightly biased and less predictable especially at dose levels of >230 mg/m^2. In combination therapy studies, for instance with cisplatin, these high dose levels of CPT-11 may be less relevant for clinical practice, and therefore this model can still be considered useful in a normal clinical setting. Other studies confirmed the variable and less predictive

behavior of SN-38 in limited-sampling model development, probably due to the complex pharmacokinetics of this metabolite [20,22].

In future clinical studies, our limited-sampling models will enable prediction of the systemic exposure to CPT-11 and SN-38. Studies to examine the relationships between CPT-11 and SN-38 pharmacokinetics and pharmacodynamics could be explored conven-iently using our model and sampling strategy. In our continued investigations, we will be examining these relationships in a future clinical phase II trial with combined CPT-11 and cisplatin chemotherapy.

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Summary and Conclusions

This thesis includes phase I and pharmacological studies on topoisomerase I inhibitors administered orally and/or in combination with cisplatin.

Topoisomerase I inhibitors are of great clinical interest because of their unique mode of action, their important anti-tumor activity as single agents in a broad spectrum of tumor types and the high expression of the enzyme in various human tumor types. **Chapter 2** gives an overview of the preclinical data on the potential relevance of prolonged drug administration of the topoisomerase I inhibitors 20-S-camptothecine, 9-nitro-camptothecine, 9-amino-camptothecine, topotecan, irinotecan and GI147211 for anti-tumor efficacy. In studies on human xenografts, prolonged administration of topoisomerase I inhibitors at lower concentrations seemed to be more effective in inducing tumor regression than higher concentrations for short periods. However, in preclinical studies no true consistency in the use of schedules and models exists. Early clinical studies have shown the feasibility of protracted drug administration. The optimal dose and schedule of the various agents remain to be elucidated.

As stated, preclinical studies indicate that prolonged systemic exposure to 9-aminocamptothecine (9-AC) might enhance anti-tumor activity. An oral formulation would provide a convenient method for prolonged drug administration. In **chapter 3** the oral availability of 9-AC polyethylene glycol 1000 capsules is described. Twelve patients were randomized to receive either 1.5 mg/m^2 9-AC p.o. on day 1 and 1.0 mg/m^2 9-AC i.v. on day 8 or *vice versa*. Serial plasma samples were collected and analyzed for 9-AC by high performance liquid chromatography. At equilibrium, the plasma concentration of the lactone form of 9-AC accounted for <10% of the total drug concentration. The overall bioavailability of 9-AC was 48.6±17.6%, indicating significant systemic exposure to the drug. Oral delivery was not associated with an increased interpatient variability in systemic exposure compared to the i.v. administration of 9-AC.

In **chapter 4** a phase I and pharmacological study is presented of oral capsulated [PEG-1000] 9-AC administered once daily for 7-14 days every 3 weeks to patients with solid tumors. Twenty-seven patients were eligible for toxicity and response. The dose-limiting toxicity was reached at a dose of 1.1 mg/m^2/day x 14 and consisted of myelosuppression and diarrhea starting median on day 12 (range 1-23), which was self-limiting after a median of 3 days (range 1-23). Other toxicities were mild and

consisted of nausea and vomiting, mucositis, fatigue and alopecia. The maximum tolerated dose and recommended dose for phase II studies is 0.84 mg/m²/day x 14.

As reported in **Chapter 5** we subsequently developed and validated limited-sampling strategies for prediction of the area under the plasma-concentration time curves (AUCs) of the lactone and total (i.e. lactone plus carboxylate) forms of 9-amino-20(*S*)-camptothecin (9-AC). In the study described in chapter 4, complete pharmacokinetic curves were obtained from 32 patients who received the drug orally in a clinical phase I setting at dose levels ranging from 0.25 to 1.10 mg/m^2. The concentrations of the lactone and carboxylate forms of 9-AC in plasma were measured by HPLC. Using data from 20 randomly-selected patients, forward-stepwise multivariate-regression analysis was employed to generate modeling strategies incorporating data from 1, 2 or 3 plasma samples. The simultaneous optimal prediction of both 9-AC lactone and 9-AC total AUCs was obtained with sample-time points at 0.33, 3.0 and 11.0 h after drug dosing. Validation of the models on an independent data set, comprising data of the remaining 12 patients demonstrated that 9-AC lactone and 9-AC total AUCs could be predicted sufficiently unbiased and precise using one and two time points: {AUC (ng.h/mL) = $7.103*C_3$ + 4.333} for 9-AC lactone and {AUC (ng.h/mL) = $9.612*C_3 + 13.77*C_{11} - 44.11$} for 9-AC total, where C_3 and C_{11} represent the 9-AC plasma concentrations in ng/mL at 3 h and 11 h after drug dosing. Application of the proposed models will be valuable in the determination of 9-AC population pharmacokinetics and permits treatment optimization for patients on the basis of individual pharmacokinetic characteristics.

In **chapter 6** the pharmacokinetics and pharmacodynamics of 9-AC are described after repeated oral dosing. Serial plasma and saliva samples were obtained on days 1 and 6 or 8 of the first treatment cycle in the32 patients, who received oral [PEG-1000] 9-AC at doses ranging from 0.25 to 1.5 mg/m²/day (see chapter 4), and were analyzed for the lactone and carboxylate forms of 9-AC by high-performance liquid chromatography. 9-AC demonstrated linear and dose-independent pharmacokinetics, with extremely small intrapatient kinetic variability (coefficient of variation <10%). However, interpatient variability in plasma pharmacokinetics was large (coefficient of variation >90%). The relative extent of lactone to carboxylate interconversion was large, and predictable from individual pretreatment serum albumin values. The 9-AC concentration ratio in plasma and saliva was strongly patient dependent and highly variable, suggesting that saliva is an unreliable matrix for kinetic modeling. To

determine the impact of a pleural effusion on the pharmacokinetics of 9-AC, plasma and pleural fluid samples were obtained for drug analysis in a single patient with a malignant pleural effusion. The mean pleura versus plasma concentration ratio of 9-AC lactone was 4.95±2.32%, indicating that pleural effusion does not constitute a major pharmacokinetic compartment for this drug. The area under the plasma concentration-time curve of 9-AC lactone was significantly correlated with the hematological toxicity. In view of the substantial interpatient variation in AUC, the related toxicity and the availability of a limited sampling model, a pharmacokinetic guided study design is recommended in future clinical trials.

Chapter 7 summarizes the development of combination therapies involving irinotecan and topotecan. Considering their mechanism of action, topoisomerase I inhibitors may interfere with processes involved in DNA repair. This renders them attractive for combination therapy with DNA-damaging agents. Interactions of topoisomerase I with other anticancer agents have been studied *in vitro* and *in vivo*. The interaction varied with the cell lines examined. However, synergism was observed in combination with topoisomerase II inhibitors, platinum-derivatives, alkylating agents and anti-metabolites in several human cancer cell lines and tumor xenografts. For several combinations a sequence dependent cytotoxic effect was noted, with synergy increasing when the topoisomerase I inhibitor was preceded by a platinum-derivative or was followed by a topoisomerase II inhibitor. Where observed, synergism might at least partly be explained by interference of the topoisomerase I inhibitor in the repair of CDDP-induced DNA interstrand cross-links and up-regulation of topoisomerase II α levels.

Various phase I and phase II studies have investigated combinations of topoisomerase I inhibitors and other cytotoxic agents. Irinotecan can be combined with antimetabolites and taxanes at relatively high doses. The feasibility of combining irinotecan with platinum-derivatives seems to depend on the schedule used. Combinations of topoisomerase I and II inhibitors yielded much toxicity, especially myelotoxicity, mucositis and diarrhea. Even after addition of G-CSF the gain in dose intensity was limited.

Combination therapy with topotecan required even more dose reductions as compared to the single agent dose. The reason for this particular difference between irinotecan and topotecan is not yet known.

Also in the clinical setting, the sequence of drug administration influenced the severity of the side effects. This might in part be explained by pharmacokinetic

interactions, by up-regulation of topoisomerase IIα, by a change in cell cycle distribution of the tumor and blood progenitor cells and/or by the interaction between topoisomerase I inhibitor and DNA-adducts. Clearly, all future trials should be paralleled by pharmacokinetics in order to evaluate possible pharmacological interactions and sequence dependent effects of a combination therapy, especially for topoisomerase I inhibitors with a short half-life of elimination. Further investigations are also needed to determine whether the more toxic sequences are also the more cytotoxic ones.

The combination of irinotecan and cisplatin had promising anti-tumor activity in patients with non-small cell and small cell lung cancer, gastric cancer and ovarian cancer.

In order to reduce the side effects and maximize the dose intensity of the combination therapy, we recommended that other schedules of administration of the drugs should be evaluated, such as administration once every three weeks of both CDDP and irinotecan, the reversed sequence of administration of topotecan and CDDP and schedules with different time intervals between drug administrations.

In **chapter 8** a phase I and pharmacological study is described, in which the feasibility of the combination of oral topotecan and cisplatin, the pharmacokinetic interaction, and sequence dependent effects are assessed as recommended in chapter 7.

Topotecan was administered orally daily for five days in escalating doses and cisplatin was given at a fixed dose of 75 mg/m² as a 3-hour infusion either before topotecan on day 1 (CT) or after topotecan on day 5 (TC) once every 3 weeks. Patients were treated in a randomized cross-over design. The CT sequence induced significantly worse myelosuppression than the alternate sequence, and resulted in MTD at a topotecan dose of 1.25 mg/m²/dx5. In the reversed sequence (TC), further dose escalation was possible resulting in DLT consisting of a combination of myelosuppression and diarrhea at a topotecan dose of 2.3 mg/m²/dx5. Pharmacokinetics of topotecan and cisplatin were linear over the dose range studied. No sequence dependent effects were observed in the pharmacokinetic parameters of topotecan. In addition, topotecan did not influence the protein binding of cisplatin and the platinum-DNA adduct formation in peripheral leukocytes in either sequence.

The recommended dose for phase II studies in selected patients is oral topotecan 1.25 mg/m²/day x 5 preceded by cisplatin 75 mg/m² day 1 once every 3 weeks, or topotecan 2.0 mg/m²/day followed by 75 mg/m² of cisplatin on day 5. The antitumor

efficacy of both administration schedules should be evaluated in a randomized phase II study.

Chapter 9. As reviewed in chapter 7,until now, phase I studies on the combination of irinotecan and cisplatin focused on fractionated dose schedules. Here, the results are presented of a phase I study of irinotecan given as a 90-minutes infusion followed by cisplatin as a 3-hour infusion on day 1, cycles repeated once every 3 weeks in patients with solid tumors. After determination of the maximum tolerated doses, the sequence of drug administration was reversed. Fifty-two patients, who had received no more than one prior combination chemotherapy regimen or two single-agent regimens entered on study and 194 courses were administered. Dose limiting toxicity (DLT) was a combination of neutropenic fever, diarrhea and fatigue at a dose level combining irinotecan 300 mg/m^2 with cisplatin 80 mg/m^2. Dose reduction of cisplatin to 60 mg/m^2 also resulted in DLT. Neutropenia grade 3 and 4 was common ranging from 42% of the cycles at the first dose level to 90% at the higher dose levels, with a median duration of the nadir of 7 days. Compared to the median time to neutrophil nadir of 8 days (range, 5-28) after treatment with single agent irinotecan at a dose of 350 mg/m^2 every three weeks, the nadir in our study, which usually occurred around day 18 (range 7-23), was delayed. In regard of the high percentage of grade 3 and 4 neutropenia observed, the incidence of neutropenic fever (5%) was strikingly low. The delayed neutrophil nadir in our study reduced the period of overlap of neutropenia and diarrhea, which seems to predestine patients to the development of neutropenic fever and might have reduced the risk of patients for the development of neutropenic fever.
The sequence of drug administration had no apparent influence on severity and frequency of the observed side effects.
The effect of additional G-CSF on the observed hematological toxicity was studied at the dose level combining irinotecan 200 mg/m^2 and cisplatin 80 mg/m^2 . The use of G-CSF resulted in a reduction of the grade and the duration of the myelosuppression, enabling optimal administration of both agents without treatment delay. The timing of G-CSF administration had no apparent influence on the reduction of the hematological toxicity.
The pharmacokinetics of irinotecan and SN-38 were linear and dose-independent over the dose range studied. Tumor responses included three complete responses and seven partial responses.

The recommended doses for phase II studies are irinotecan 260 mg/m² and cisplatin 80 mg/m² in highly selected patients in a good physical condition. In other patients, doses of irinotecan 200 mg/m² combined with cisplatin 80 mg/m² should be considered. Addition of G-CSF at this dose level substantially reduces the hematological side effects.

Chapter 10 describes the influence of the administration sequence of irinotecan and cisplatin in a 3-weekly schedule on the toxicity and pharmacokinetic parameters evaluated in 11 patients with solid tumors. Each patient was randomized to one of two treatment groups that determined the order of drug administration. In group A, irinotecan 200 mg/m² was administered as a 90-min i.v. infusion on day 1, immediately followed by cisplatin 80 mg/m² as a 3-h i.v. infusion in the first course and the reverse sequence in the second course. Patients in group B received the two treatment cycles in the reversed order as compared to group A. Quantitative determination of irinotecan and its four principal metabolites (SN-38, SN-38 glucuronide, APC and NPC) was performed using liquid chromatography. Non-protein bound and total cisplatin levels in plasma were determined by flameless atomic absorption spectrometry.
No significant differences in any toxicity were observed between the treatment schedules. Pharmacokinetic parameters of the lactone and total drug forms of irinotecan were similar to single agent data, and not significantly different between study courses. The metabolic disposition of irinotecan was also sequence independent. In addition, irinotecan had no influence on the protein binding of cisplatin and the platinum DNA-adduct formation in peripheral leukocytes in either sequence. These data indicate that the toxicity of the combination of irinotecan and cisplatin is schedule independent and that there is no pharmacokinetic interaction.

In **chapter 11** the pharmacokinetic, metabolic and pharmacodynamic profiles were reported of irinotecan and cisplatin administered once every 3 weeks in the dose-escalating study described in chapter 9. Fifty-two cancer patients were treated with irinotecan administered as a 90-minutes infusion at doses ranging from 175-300 mg/m² followed by cisplatin as a 3-hour i.v. infusion at doses ranging from 60-80 mg/m². After reaching maximum tolerated dose (MTD), the sequence of drug administration was reversed. For pharmacokinetic analysis serial plasma samples were obtained on day 1-3 of the first cycle. Quantitative determination of irinotecan, SN-38, its β-glucuronide derivative (SN-38G), 7-ethyl-10-[4-*N*-(5-aminopentanoic

acid)-1-piperidino]-carbonyloxycamptothecine (APC) and 7-ethyl-10-[4-*N*-(1-piperidino)-1-amino]-carbonyloxycamptothecine (NPC) were performed using a reversed-phase high-performance liquid chromatography assay. Cisplatin concentrations and platinum DNA-adduct levels in leukocytes were determined by flameless atomic absorption spectrometry.
Irinotecan and cisplatin demonstrated linear and dose-independent pharmacokinetics comparable with single agent data. SN-38G constituted the major plasma metabolite of irinotecan, whereas NPC was only a very minor metabolite in plasma, possibly indicating a rapid conversion of NPC to SN-38. The terminal elimination phases of SN-38 and SN-38G were similar, and relatively delayed compared to the elimination of irinotecan. Maximal DNA adduct formation did not significantly differ from single agent data. The percentage decrease in WBC was significantly related to the AUCs of the lactone form of irinotecan (P=0.0245) and SN-38 (P=0.0123). The severity of diarrhea was not significantly related to the AUCs of irinotecan and SN-38, nor to the systemic glucuronidation rate of SN-38 (i.e. the biliary index).
There was no apparent pharmacokinetic interaction between irinotecan and cisplatin in this study. Reversal of the administration sequence of the drugs did not seem to have any influence on the pharmacokinetic data. The incidence and severity of delayed type diarrhea was not related to any of the studied parameters.

In **chapter 12** the development of a new simple reversed-phase high-performance liquid chromatographic method for the determination of irinotecan and three metabolites in human plasma, urine and feces homogenate is described. The metabolites of interest were 7-ethyl-10-hydroxycamptothecin (SN-38), its β-glucuronide derivative (SN-38G) and 7-ethyl-10-[4-N-(5-aminopentanoicacid)-1-piperidino]carbonyloxycamptothecin (APC). Sample pretreatment from the various biological matrices involved a rapid protein precipitation with simultaneous solvent extraction of 250-µl aliquots of sample with 500 µl of methanol-5% (w/v) aqueous perchloric acid (1:1, v/v). Separation of the compounds was achieved on an analytical column packed with Hypersil ODS material (100x4.6 mm I.D., 5 µm), and isocratic elution with a mixture of methanol-0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). The column effluent was monitored at excitation and emission wavelenghts of 355 and 515 nm, respectively. Results from a 4-day validation study indicated that this single-run determination allows for simple, simultaneous and rapid quantitation and identification of all analytes with excellent reliability.

The assay was used a.o. in the study reported in **chapter 12** to determine the metabolic fate and disposition of irinotecan. Ten patients were treated with irinotecan 200 mg/m² as a 90-min i.v. infusion, followed by a 3-h i.v. infusion of cisplatin (60-80 mg/m²). Plasma, urine, and feces were collected for 56 h and analyzed by a specific reversed-phase high-performance liquid chromatographic assay for the parent drug and all four metabolites positively identified to date: SN-38; SN-38G; 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecine (APC) and 7-ethyl-10-[4-*N*-(1-piperidino)-1-amino]-carbonyloxycamptothecine (NPC).
A three-exponential decline was observed in plasma for all compounds, with a clear predominance of irinotecan [25.6±5.71 μM.h (irinotecan) versus 15.8±3.51 μM.h (total metabolites)]. Total urinary excretion was 28.1±10.6% of the dose, with unchanged irinotecan and SN-38G as the main excretion products. Whereas renal clearance of SN-38 was only a minor route of drug elimination, fecal concentration of this compound was unexpectedly high (on average, 2.45% of the dose), suggestive of intestinal hydrolysis of SN-38G by bacterial β-glucuronidase. Irinotecan and the other metabolites could also be identified from fecal extracts, with a very minor contribution overall of the cytochrome P-450-mediated compound APC and NPC. Surprisingly, fecal excretion accounted for only 24.4±13.3% of the dose, leading to a total excretion of ~52%. These data indicate that half of the dose in urine and feces may constitute some further unknown non-extractable or non-fluorescent metabolites.

Chapter 14. The clinical pharmacokinetics of irinotecan (CPT-11) are associated with substantial interpatient variability. The degree to which this variability in CPT-11 exposure impacts upon the response and toxicity of the drug has not yet been properly determined. In general, the area under the plasma concentration-time curve (AUC) is an appropriate indicator of exposure, but requires collection of upto 17 timed blood samples. This presents difficulties if large-scale population samplings are required. A limited sampling strategy was developed to estimate the AUCs of the lactone and total (i.e. lactone *plus* carboxylate) forms of CPT-11 and its active metabolite SN-38 from a limited number of blood samples in patients co-treated with cisplatin. Using data from 24 patients, univariate and multivariate regression analyses were employed to generate the models. The best predictive models for simultaneous estimation of CPT-11 and SN-38 AUCs were obtained with three time points at 0.5 h, 1.67 h and 5.50 h after start of the 90-min i.v. infusion of CPT-11. The models were tested separately in another group of 24 patients receiving the same

combination treatment. This validation set demonstrated that CPT-11 and SN-38 AUCs after standard dose administration could be predicted sufficiently unbiased and precise with three timed samples to warrant clinical application.

Final conclusions and future perspectives. Topoisomerase I inhibitors are an important addition to the presently available classes of drugs. Based on pre-clinical data the concept of prolonged drug exposure with topoisomerase I inhibitors remains attractive, especially in view of the availability of oral formulations of topotecan, 9-nitro-camptothecine and 9-aminocamptothecine respectively, all with an adequate bioavailability.

Both the pre-clinical and clinical data underscore the importance of pharmacokinetic analysis in all phase I studies involving combination therapies to evaluate the existence of pharmacological interactions between the drugs administered and sequence dependent effects. For topotecan further investigations are also needed to determine whether the sequence causing more side-effects, is also the more potent one. Since it is less desirable to address this issue in phase III studies, it would be worthwhile to have these studies preceded by randomised phase II studies in order to exclude a major impact of drug-sequences on antitumor activity.

The knowledge of the metabolism and disposition of irinotecan, provides a basis for the modulation of the gastrointestinal toxicity, especially the late diarrhea. A clinical trial to evaluate the effects of pretreatment with the antibiotic neomycin before the administration of irinotecan is in progress at our institute.

Samenvatting en Conclusies

In dit proefschrift worden de resultaten besproken van onderzoekingen verricht met topoisomerase I remmers, oraal toegediend en/of in combinatie met cisplatin.

Topoisomerase I remmers vormen een nieuwe groep cytostatica met een uniek werkingsmechanisme. Antitumor activiteit van deze middelen is aangetoond bij verschillende soorten tumoren. In **hoofdstuk 2** wordt een overzicht gegeven van de in vitro en vivo onderzoeken, die het belang bestuderen van de duur van blootstelling aan topoisomerase I remmers, met name 20-S-camptothecine, 9-nitro-camptothecine, 9-amino-camptothecine, topotecan, irinotecan en GI47211 voor een optimale werking.
De studies tonen een verhoogde antitumor activiteit, indien de topoisomerase I remmers langdurig in lage concentraties worden toegediend in vergelijking met kortdurende, hoge concentraties. Ook de wijze van toediening van de topoisomerase I remmers was van invloed op de antitumor activiteit. Vroeg klinische studies tonen aan dat het mogelijk is om bij de mens topoisomerase I remmers gedurende langere tijd toe te dienen, met acceptabele bijwerkingen. Het is echter nog niet mogelijk om aan te geven of deze manier van toediening ook resulteert in een betere antitumor activiteit bij de mens.

Zoals gezegd suggereren preklinische studies het potentiële belang van langdurige toediening van onder andere 9-aminocamptothecine (9-AC). Een orale toedieningsvorm zou de mogelijkheid van langdurige toediening aanzienlijk vereenvoudigen voor de patiënt. In **hoofdstuk 3** wordt de biologische beschikbaarheid van oraal toegediende 9-AC PEG 1000 capsules beschreven. Twaalf patiënten werden gerandomiseerd. De helft van de patiënten kreeg 1,5 mg/m^2 9-AC oraal toegediend op dag 1, gevolgd door 1,0 mg/m^2 9-AC intraveneus op dag 8. Bij de andere helft van de patiënten werd de volgorde van toediening omgekeerd. In afgenomen bloedmonsters werd de concentratie 9-AC bepaald met behulp van HPLC. Na het bereiken van een evenwicht, bedroeg de concentratie 9-AC lacton in het bloed minder dan 10% van de totale concentratie van 9-AC. De biologische beschikbaarheid van 9-AC was 48,6±17,6%. Deze resultaten tonen aan, dat na orale toediening van 9-AC een adequate bloedspiegel wordt bereikt. Orale toediening leidt niet tot een toename van de interpatiënt variatie van de bloedspiegel in vergelijking met de intraveneuze toediening van 9-AC.

Hoofdstuk 4 bespreekt de resultaten van een fase I studie waarin [PEG-1000] 9-AC eenmaal per dag oraal werd toegediend gedurende 7 tot 14 dagen per 3 weken aan patiënten met solide tumoren. De dosis beperkende bijwerkingen werden bereikt bij een dosis van 1,1 mg/m²/dag gedurende 14 dagen en bestonden uit myelosuppressie en diarree, welke mediaan optrad op dag 12 (spreiding: 1-23) en een mediane duur had van 3 dagen (spreiding 1-23). Andere bijwerkingen bestonden uit misselijkheid en braken, mucositis, vermoeidheid, en haarverlies. De geadviseerde dosis voor fase II onderzoek is 0,84 mg/m²/dag gedurende 14 dagen per 3 weken.

Hoofdstuk 5 beschrijft de ontwikkeling van een limited sampling model om de expositie aan 9-AC te voorspellen na orale toediening van 9-AC. Bij patiënten, die behandeld werden in een fase I studie, werd de concentratie van 9-AC in het bloed bepaald op verschillende tijdstippen. Met behulp van gegevens van 20 patiënten en een multivariate-regressie analyse werd een model opgesteld om met behulp van 1, 2 of 3 plasma monsters de AUC van 9-AC lacton en totaal 9-AC in het plasma te voorspellen. Dit model werd gevalideerd in de overige 12 patiënten. Met 1 bepaling van de plasmaspiegel 3 uur na toediening van 9-AC, kan de AUC van 9-AC lacton nauwkeurig worden voorspeld. Dit limited sampling model maakt het mogelijk om de farmacokinetiek van 9-AC in grotere groepen van patiënten te bepalen. Daarnaast kan op basis van individuele farmacokinetiek gegevens de dosis 9-AC, welke aan elke patiënt afzonderlijk wordt toegediend, worden geoptimaliseerd.

In **hoofdstuk 6** wordt de farmacokinetisch-farmacodynamische analyse beschreven van de meerdaagse orale toediening van 9-AC bij patiënten met solide tumoren. Bij 32 patiënten werden bloed- en speekselmonsters afgenomen op de eerste en 6de of 8ste dag tijdens de eerste behandeling ter analyse van de lacton en carboxylaat vormen van 9-AC. De toegediende dosis 9-AC varieerde van 0,25 tot 1,5 mg/m²/dag. De farmacokinetiek van 9-AC was lineair en onafhankelijk van de toegediende dosis met een zeer geringe intrapatiënt variatie (variatiecoëffecient <10%). De interpatiënt variatie was echter erg groot (variatiecoëfficient >90%). De lacton vorm van 9-AC werd bijna volledig omgezet naar de carboxylaat vorm. De ratio van de plasma en speekselconcentraties van 9-AC wisselde sterk tussen de patiënten, zodat het bepalen van medicijn-concentraties in speekselmonsters geen goed alternatief bleek. Om na te gaan of pleuravocht een invloed heeft op de plasma kinetiek van 9-AC, werden bij een patiënt met een maligne pleuritis zowel pleuravocht als

bloedmonsters afgenomen voor het bepalen van de kinetiek van 9-AC. De gemiddelde verhouding van de pleura concentratie van 9-AC ten opzichte van de plasma concentratie bedroeg 4,95±2,32%. Dit betekent dat er geen stapeling van 9-AC in pleuravocht optreedt en dat het bestaan van pleuravocht geen invloed heeft op de plasma kinetiek van 9-AC. Farmacodynamisch onderzoek liet een significante correlatie zien van de $AUC_{(t)}$ van de lacton vorm van 9-AC met de procentuele daling van de leukocyten en de trombocyten. Gezien de grote interpatiënt variatie in de kinetiek van 9-AC, de relatie van de kinetiek met het optreden van toxiciteit en de mogelijkheid om met slechts 1 of 2 bloedmonsters de $AUC_{(t)}$ van 9-AC nauwkeurig te bepalen, lijkt het aanbevelenswaardig om bij verdere studies met 9-AC gebruik te maken van een farmacokinetisch-farmacodynamisch model voor dosis-individualisatie.

Hoofdstuk 7 geeft een literatuur-overzicht van de ontwikkeling van combinatietherapiëen met irinotecan en topotecan. Gezien het werkingsmechanisme van topoisomerase I remmers, lijkt het aannemelijk dat topoisomerase I remmers kunnen intervenieren in het herstel van aangerichte DNA schade. Deze eigenschap maakt een combinatie met cytostatica, die DNA schade aanrichten, aantrekkelijk. Interacties tussen topoisomerase I remmers en andere cytostatica zijn zowel in in vitro als in vivo onderzocht. Synergie werd gezien bij combinaties van topoisomerase I remmers met topoisomerase II remmers, alkylerende cytostatica, antimetabolieten en platinaverbindingen. Voor sommige combinaties bleek de volgorde van toedienning van de middelen bepalend te zijn voor de interactie: indien de topoisomerase I remmer werd vooraf gegaan door een platinaverbinding of werd gevolgd door een topoisomearse II remmer, nam het synergisme toe. Deels kan dit verklaard worden door een reactieve stijging van het topoisomerase IIα bij remming van topoisomerase I en een vertraagd herstel van de dwarsverbindingen tussen twee DNA-ketens, welke veroorzaakt worden door platinaverbindingen.
Vervolgens werden in klinische studies verschillende combinatietherapieen bestudeerd. Irinotecan kan in relatief hoge dosis gecombineerd worden met taxanen en antimetabolieten. In combinatie met platinaverbindingen lijkt het gebruikte schema van invloed op de dosisintensiteit die bereikt kan worden. Topoisomerase I en II remmers zijn moeilijker te combineren ten gevolge van de bijwerkingen. Over het algemeen lijkt topotecan moeilijker te combineren met andere cytostatica dan irinotecan.

Ook in klinische studies werd de invloed van de volgorde van toediening van de chemotherapie op de bijwerkingen gezien. Naast interacties op cellulair niveau zoals beschreven in de preklinische studies, kan ook een farmacokinetische interactie hierbij een rol spelen. Op grond hiervan zouden studies van topoisomerase I remmers in combinatie met andere soorten cytostatica dan ook gecombineerd moeten worden met farmacokinetisch onderzoek om een farmacologische interactie en de invloed van het toedieningsschema hierop te beoordelen. Tevens moet nader onderzocht worden of een toedieningsschema met meer bijwerkingen ook gepaard gaat met een betere anti-tumor activiteit.

In **hoofdstuk 8** wordt een fase I studie beschreven, waarin opklimmende doseringen oraal topotecan in een 5-daags schema gecombineerd werden met cisplatin 75 mg/m² intraveneus toegediend eenmaal per 3 weken. Om het effect van de volgorde van toediening van beide middelen op de bijwerkingen en de farmacokinetiek te onderzoeken, werd cisplatin afwisselend op de 1ste (volgorde CT) of 5de (TC) dag van de topotecan toediening gegeven. Toediening van de chemotherapie in volgorde CT leidde tot meer beenmergonderdrukking, dan de omgekeerde volgorde. Hierdoor kon een hogere dosis topotecan gecombineerd worden met cisplatin, indien dit laatste op de 5de dag werd toegediend. Dit verschil in bijwerkingen kon niet verklaard worden door een verschil in de kinetiek van topotecan of cisplatin. Evenmin werd de vorming van platinum-DNA adducten in witte bloedcellen beïnvloed door de volgorde van toediening. De geadviseerde dosis voor fase II onderzoek is oraal topotecan 1,25 mg/m²/dag gedurende 5 dagen gecombineerd met cisplatin 75 mg/m² op dag 1, of topotecan 2,0 mg/m²/dag gedurende 5 dagen in combinatie met cisplatin op dag 5. Een eventueel verschil in effectiviteit van deze 2 schemata dient in een gerandomiseerde fase II studie verder te worden onderzocht.

Voor irinotecan concentreerde het fase I onderzoek zich tot nu toe op het combineren van cisplatin met irinotecan in een wekelijks schema. Hoofdstuk 9 beschrijft een fase I studie waarin irinotecan intraveneus werd toegediend in 90 minuten, gevolgd door een infuus met cisplatin gedurende 3 uur, in ee 3-wekelijkse cyclus. Na het bepalen van de maximaal tolerabele dosis, werd de volgorde van toediening van beide middelen veranderd. In totaal participeerden 52 patiënten aan de studie. De dosis beperkende bijwerkingen bestonden uit een combinatie van neutropene koorts, diarree en vermoeidheid bij een dosis irinotecan van 300 mg/m² gecombineerd met cisplatin 80 mg/m². Op alle dosisniveau's werd in een hoog

percentage van de patiënten neutropenia graad 3 en 4 gezien. Het aantal patiënten dat neutropene koorts ontwikkelde was echter beperkt. Een mogelijke verklaring hiervoor is de verschuiving van de nadir van de leukocyten van dag 8 bij een behandeling met irinotecan alleen, naar dag 18 bij de combinatie behandeling. Hierdoor werd de periode, waarin patiënten zowel klachten van diarree als daling van de leukocyten ondervonden verkort. Met name in deze periode zijn patiënten gevoelig voor het ontwikkelen van neutropene koorts. Een verkorting van deze periode kan het risico op het ontwikkelen van neutropene koorts mogelijk hebben verminderd.
De volgorde van toediening van irinotecan en cisplatin was niet van invloed op het optreden of de ernst van de bijwerkingen.
De beschermende invloed van toediening van G-CSF op de duur van de leukocytopenie werd bestudeerd bij 6 patiënten, die behandeld werden met irinotecan 200 mg/m^2 en cisplatin 80 mg/m^2. Het gebruik van G-CSF reduceerde zowel de ernst als de duur van de neutropenie, waardoor minder uitstel in de toediening van de chemotherapie optrad.
De geadviseerde dosis voor fase II studies is irinotecan 260 mg/m^2 gecombineerd met cisplatin 80 mg/m^2. Deze dosis is echter alleen haalbaar voor patiënten,die niet eerder zijn behandeld met chemotherapie en een goede lichamelijke conditie hebben. In alle andere situaties dient de dosis irinotecan gereduceerd te worden tot 200 mg/m^2 en dient de toevoeging van G-CSF te worden overwogen.

In **hoofdstuk 10** wordt de invloed van de volgorde van toediening bestudeerd voor de combinatie van irinotecan 200 mg/m^2 en cisplatin 80 mg/m^2, welke beide eenmaal per 3 weken werden toegediend aan patiënten met solide tumoren. Elf patiënten kregen ofwel tijdens hun eerste behandelingskuur irinotecan gevolgd door cisplatin en in de tweede kuur cisplatin gevolgd door irinotecan toegediend, ofwel de twee kuren in omgekeerde volgorde. Bij beide kuren werd farmacokinetisch onderzoek verricht, waarbij de concentraties van irinotecan, cisplatin en de metabolieten van irinotecan in het bloed werden bepaald.
De volgorde van toediening van irinotecan en cisplatin had geen significante invloed op de ernst of de duur van de bijwerkingen, welke optraden na de behandeling. Evenmin kon een significant verschil in de kinetiek van irinotecan en cisplatin worden vast gesteld, wat farmacokinetische interactie grotendeels uitsluit.

Hoofdstuk 11. In dit hoofdstuk wordt de farmacokinetische en farmacodynamische analyse beschreven van de combinatie therapie beschreven in hoofdstuk 9, bestaande uit irinotecan en cisplatin, waarin beide éénmaal per 3 weken worden toegediend. Bij tweeënvijftig patiënten met een solide tumor, die werden behandeld met irinotecan in een dosis variërend van 175 tot 300 mg/m², toegediend als een infuus van 90 minuten, gevolgd door een 3 uur durend infuus met cisplatin in dosis variërend van 60 tot 80 mg/m², werden op de eerste tot derde dag van de eerste behandeling bloedmonsters afgenomen voor bepaling van cisplatin, irinotecan en de bekende metabolieten van irinotecan : SN-38, SN-38G, 7-ethyl-10-[4-*N*-(5-aminopentanoic zuur)-1-piperidino]-carbonyloxycamptothecine (APC) en 7-ethyl-10-[4-*N*-(1-piperidino)-1-amino]-carbonyloxycamptothecine (NPC).
De kinetiek van irinotecan en cisplatin was lineair en niet afhankelijk van de toegediende dosis. SN-38G was de belangrijkste metaboliet van irinotecan in het plasma. Daarentegen kon NPC slechts in een kleine hoeveelheid worden aangetoond, mogelijk wijzend op een snelle omzetting van NPC in SN-38. De waarden van de maximale cisplatin DNA-adduct vorming waren vergelijkbaar met waarden, die gevonden worden na behandeling met cisplatin alleen. Voor de combinatie cisplatin en irinotecan in het gebruikte schema kon geen farmacokinetische interactie worden aangetoond. Farmacodynamisch onderzoek liet een significante correlatie zien van de AUC van de lacton vorm van irinotecan en SN-38 enerzijds en de procentuele daling van de leukocyten anderzijds. De ernst van de late diarree was niet gecorreleerd aan de kinetiek van irinotecan en zijn metabolieten.

Hoofdstuk 12 beschrijft de ontwikkeling van een vereenvoudigde en snelle methode voor de bepaling van irinotecan en zijn metabolieten in plasma, urine en feces.

Hoofdstuk 13. Bij 10 patiënten met een solide tumor, die behandeld werden met irinotecan 200 mg/m² gecombineerd met cisplatin, werd in het bloed, de urine en in de feces het metabolisme en de excretie van irinotecan en de metabolieten SN-38, SN-38G, 7-ethyl-10-[4-*N*-(5-aminopentanoic zuur)-1-piperidino]-carbonyloxycamptothecine (APC) en 7-ethyl-10-[4-*N*-(1-piperidino)-1-amino]-carbonyloxycamptothecine (NPC) bepaald. Van de toegediende dosis werd 28,1±10,6% in de urine terug gevonden, grotendeels bestaande uit irinotecan en SN-38G. SN-38 werd met name in de feces teruggevonden in een opvallend hoge concentratie (2,45% van de toegediende dosis). Deze bevinding steunt de hypothese

dat SN-38G in de darmen door β-glucuronidase afkomstig van de darmflora in SN-38 wordt omgezet. Het totale percentage van de toegediende dosis, dat in de feces kon worden terug gevonden bedroeg 24,4±13,3%. Dit betekent dat na analyse van de urine en feces slechts ~52% van de toegediende dosis irinotecan kon worden terug gevonden. Vermoedelijk vindt verdere excretie van irinotecan plaats via de urine en feces als een nog niet geïdentificeerde, niet-fluorescerende metaboliet.

De expositie aan irinotecan varieert aanzienlijk tussen patiënten onderling na behandeling met dezelfde dosis irinotecan. Het is op dit moment niet bekend in hoeverre het verschil in expositie verantwoordelijk is voor het verschil in effectiviteit en toxiciteit, welke gezien wordt na behandeling met irinotecan. De expositie, welke gemeten wordt als de AUC van irinotecan en SN-38, de actieve metaboliet van irinotecan, kan slechts bepaald worden na afname van vele bloed monsters. In **hoofdstuk 14** wordt de ontwikkeling van een limited sampling model beschreven, waarmee het mogelijk is om met 3 meetpunten, de AUC van zowel irinotecan als SN-38 nauwkeurig te voorspellen. Hiermee wordt toepassing van farmacokinetiek op grotere schaal mogelijk.

Conclusies en vooruitzichten. Topoisomerase I remmers vormen een belangrijke aanwinst voor ons arsenaal aan cytostatica. Op basis van zowel theoretische als preklinische gegevens, blijft het concept van langdurige toediening van topoisomerase I remmers aantrekkelijk, zeker nu verschillende topoisomerase I remmers in een orale toedieningsvorm te verkrijgen zijn.
Zowel uit preklinisch, als uit klinisch onderzoek zoals beschreven in dit proefschrift blijkt het belang van het verrichten van farmacokinetisch onderzoek tijdens fase I studies, waarin een nieuwe combinatietherapie wordt onderzocht. Dit met het doel om een eventuele farmacokinetische interactie tussen de middelen vast te stellen en de invloed van de volgorde van toediening op de kinetiek of bijwerkingen te bestuderen. Indien de bijwerkingen beïnvloed worden door de volgorde van toediening, is het van belang te beoordelen of meer bijwerkingen ook leiden tot een effectievere antikanker behandeling. Een gerandomiseerde fase II studie verdient de voorkeur om een eventueel groot verschil in effectiviteit op te sporen.
De verbeterde inzichten in het metabolisme van irinotecan geven ons de gelegenheid om naar mogelijkheden te zoeken om de bijwerkingen na behandeling met irinotecan, met name het optreden van de late diarree, te beinvloeden.

Momenteel wordt in ons ziekenhuis onderzocht of toediening van het antibioticum neomycine het optreden van de late diarree na de behandeling met irinotecan kan verminderen.

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CURRICULUM VITAE

Maja de Jonge werd op 13 juli 1962 geboren te Rotterdam. In 1980 behaalde zij het diploma Gymnasium β aan het Gymnasium Erasmianum te Rotterdam, waarna zij in hetzelfde jaar haar studie Geneeskunde begon aan de Erasmus Universiteit te Rotterdam. In juli 1987 behaalde zij haar arts examen.
Van oktober 1987 tot juli 1989 werkte zij als arts-assistent Cardiologie in het St. Clara Ziekenhuis te Rotterdam (R Wardeh, FMA Harms) gevolgd door 3 maanden op de afdeling Intensive Care (AF Grootendorst) in hetzelfde ziekenhuis. Daarna startte zij haar opleiding in de Inwendige Geneeskunde in het Universitair Ziekenhuis Antwerpen (Prof Dr L de Leeuw). In oktober 1994 begon zij haar vervolgopleiding in het aandachtsgebied Medische Oncologie in het Universitair Ziekenhuis Antwerpen (Prof Dr AT van Oosterom). In de periode oktober 1995 tot maart 1997 werd de opleiding voortgezet in de Dr Daniel den Hoed Kliniek (Prof Dr G Stoter). In deze periode kwam dit proefschrift tot stand.
Sindsdien is zij werkzaam als internist in de Dr Daniel den Hoed Kliniek.

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