PROLONGED TUMOR EXPOSURE TO TOPOISOMERASE I INHIBITOR

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LANGDURIGE BLOOTSTELLING VAN TUMOREN AAN TOPOISOMERASE-I-REMMERS

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Verdriet is voor de achterblijvers, de reiziger heeft er geen tijd voor

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CHAPTER 1

INTRODUCTION TO THE THESIS

Topoisomerase I -3' is a nuclear enzyme abundantly present in all eukaryotic cells. Human topoisomerase I-3' is a monomeric 100kDa polypeptide encoded by a single copy gene located on chromosome 20q12-13.2 [1,2]. Topoisomerase I becomes covalently bound to the 3'phosphate at the DNA (cleavable complex). The cleavable complex results in single strand DNA breaks. Topoisomerase I inhibitors stabilize the cleavable complex and in cells in S-phase cytotoxic effects are seen as a result of irreversible double strand DNA breaks.

Camptothecin, a plant alkaloid extract from the tree Camptotheca acuminata, was shown to be active against L1210 murine leukemia [3]. Because of unpredicatable toxicities including myelotoxicity, diarrhea and hemorrhagic cystitis in phase I-II studies with watersoluble sodium-camptothecin, further clinical development was precluded [4-8]. Years later the antitumor effect of camptothecin appeared to be related to specific topoisomerase I inhibition by the drug [9,10]. This finding resulted in the development of a new class of antitumor drugs (topoisomerase I inhibitors), and consequently various camptothecin-derivatives were developed [11]. One of the earliest semi-synthetic camptothecin-derivatives developed was topotecan (SKF 104864, 9-dimethyl-aminomethyl-10-hydroxycamptothecin, Hycamtin®) which demonstrated broad spectrum antitumor activity in preclinical studies [12,13]. Phase I-II studies with topotecan administered intravenously once daily for 5 days every 3 weeks showed brief and non-cumulative neutropenia being the dose-limiting toxicity. With topotecan i.v. once daily for 5 days important activity was observed against small-cell lung cancer and ovarian cancer [14-33]. 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 prolonged exposure resulted in less toxicity [34-44].

These preclinical findings were the stimulus for phase I-II studies with low dose continuous infusion of topoisomerase I inhibitors in cancer patients [45-47]. Because oral or intragastric administration of topoisomerase I inhibitors appeared to be effective in animal models studies on oral administration in humans became of interest [43,48-50].

This thesis includes clinical and pharmacologic studies. First with topotecan, focussing on prolonged administration, either by continuous infusion or by oral administration. The latter became possible in view of an oral bioavailability of topotecan in humans of 32-44% [51,52].

Pharmacokinetic-pharmacodynamic analysis is being presented for all studies with oral administration. Finally a promising new water-soluble topoisomerase I inhibitor GI147211 (7-(methylpiperazinomethylene)-10,11-ethylenedioxy-20-S-camptothecin) [53,54] was studied in a phase I study with a daily x 5 i.v. administration q 21 days, and aiming to develop a useful oral formulation also in a phase I oral bioavailability study.

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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 concentrations. 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 indeed prolonged exposure may 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 topologic problems encountered in DNA replication and DNA transcription. Topoisomerases may also be involved in recombinational processes and chromatin assembly, however their roles in these processes are less well defined [4].

Already in the 1970's camptothecin (CPT), an extract from the chinese tree Camptotheca acuminata, showed antitumor activity against several tumors. In phase I and II studies, however, unpredictable severe toxicities occurred which led to the discontinuation of further development [5-9]. In the late 1980's 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 entirely be 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, because 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 following removal of CPT from cultured cells probably due to the dissociation of topoisomerase I cleavable complexes from transcription units. Thus camptothecin demonstrated inhibition of DNA and RNA synthesis with fragmentation of nuclear DNA but upon removal of the drug nucleic acid synthesis inhibition and DNA fragmentation were reversible, and only at higher dose and longer exposure times did 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 protooncogenes, 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 were significantly higher as compared to quiescent cells. However topoisomerase I protein increased much less, which may be due to a shorter half life of the protein in proliferating cells as compared to resting cells [36-37]. The catalytic activity of topoisomerase I also depends on the phosporylation 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 nevertheless were resistant to topoisomerase I inhibitors which could 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 point-mutations 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 however are more resistant to the positively charged camptothecine 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 topo-isomerase 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 were 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 then 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 as compared to 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 waterinsoluble 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 as compared to 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 when compared to 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% and 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 coloncarcinoma [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 dose and schedule dependent. 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 70's 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 occured in several patients who recieved multiple doses. Cumulative leuco- and thrombocytopenia were also dose-limiting with the daily x5 schedule, that resulted 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 gastro-intestinal 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 myelosuppression, 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, 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 origin. Conversion of 9NC is less in hematopoetic cells. Cellular conversion of the lactone form of 9NC to 9AC is maximal at 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 occured 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 nontumorigenic breast cancer cells (MDA-MB-134) and tumorigenic cells (MDA-MB-231) were exposed to 9NC. The nontumorigenic cells accumulated in G_2/M without significant changes in S-fraction. Removal of 9NC from the cultures of nontumorigenic 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 a dose i.m. 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./day x3 q 21 days at dose levels of 0.75 and 1 mg/kg/day resulted in tumor regression, but with ultimately 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 nontumorigenic (DUN) ovarian cancer cells in vitro to a concentration of 1 ng/ml of 9NC resulted in accumulation of nontumorigenic 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 effacicious 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 than higher concentrations for short periods in inducing apoptosis. 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 crucial for optimal antitumor responses. Prolonged or intermittent administration of a lower dose of these drugs is most efficacious.

Clinical studies with prolonged or continuous exposure

72-hour infusion

Phase I studies of 9-amino-camptothecin in adult patients with solid tumors have initially been performed 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 comparable but the MTD in children exceeded that in adults [73].

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.	Refer. Year.
9-Amino-CPT i.v.	5-59 $\mu g/m^2/h$ 72h q 14d	48	0.9-10.6 nM	35 $\mu g/m^2/h$	Neutro.	Dahut 1996.
	47-74 $\mu g/m^2$ 72h q 14d + G-CSF			47 $\mu g/m^2/h$	Neutro. Thrombo.	
9-Amino-CPT i.v.	-- 72h	19	--	--	Neutro.	Rubin 1994.
9-Amino-CPT i.v.	36-62$\mu g/m^2/h$ 72h q 14d	18	2.23 ng/ml.	not (yet) reached	myelosupp.	Langevin 1996.
9-Amino-CPT i.v.	6.2-9.4 $\mu g/m^2/h$ 21d q 28d	19	--	> 9.4 $\mu g/m^2$ /h	not yet reached.	Hochster 1996.
9-Amino-CPT i.v.	17-25$\mu g/m^2/h$ 120h/wkx3 q 4wk	20	2.9 ± 1.6 (17 $\mu g/m^2/h$)	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

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 ug/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 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 that 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 TPT every 4 days (q4dx4 schedule). The maximum tolerated dose 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 regrowth 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 maximum tolerated dose 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 q 21 d 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, coloncarcinoma, head and neck cancer, renal cell carcinoma, cervix- and prostate carcinoma, appear much less sensitive to this regimen [90-99]. In these phase II studies CTC grade III-IV neutropenia (32-81%) is reported as major toxicity. Thrombocytopenia CTC grade III-IV is infrequent. Anemia greater than CTC grade II was reported in 27-60%.

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, and every 21 days; a 120 hour infusion every 3-4 weeks; a 21 day continuous infusion administered every 28 days. (Table 2).

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 -/72h q 2wk	12 7	-- --	2 mg/m^2/72h 2.6mg/m^2/72h	Neutro. Neutro.	Sabiers, 1993
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 0.68-1.6/day 72h q 3wk	14 32	5.5 ng/ml 2.0 ng/ml	0.68 mg/m^2/day 1.6 mg/m^2/day	Thrombo. Neutro.	Burris, 1994
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.

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 bloodtransfusions 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 hematologic 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]. Bloodtransfusions 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-hematologic 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 pediatric 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 q 28 days. In the second schedule oral TPT was given 5 days on, 2 days off for 15 total doses. In the 21 day schedule oral bioavailability was 46% $\pm$ 22% at 0.8 mg/m^2 and 34% $\pm$ 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 coloncarcinoma, chemo-responsive rhabdomyosarcoma, and sublines of rhabdomyosarcoma with in vivo resistance to vincristine, melphalan, and topotecan, as well as with 3 pediatric brain tumors [79,110]. As a single i.v. administration at the maximum tolerated dose (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 coloncarcinoma 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 to more toxic short term schedules.

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	Refer. Year.
CPT-11 i.v.	125-225 every other wk	20	--	$\geq$ 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.

Clinical studies with prolonged or continuous administration

In a phase I study with CPT-11 given as a 5 days 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 2 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].

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 for the time being 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-methyl piperazinomethylene)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-hematologic 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].

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

Drug	Dose Schedule mg/m^2	No. Pts	Cp-ss	MTD	DLT	Refer. 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.

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 $\geq$ 2 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).

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 manyfold and one of the conclusions from all of the above 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 necessity of having to perform too many clinical studies on scheduling. It is also recommendable to perform the clinical phase I and II studies with inclusion of pharmacokinetic/pharmacodynamic relationship studies. A nice 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 necessary studies could easily be limited. Also such studies would answer 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 still remains 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

PHASE II AND PHARMACOLOGIC STUDY OF TOPOTECAN ADMINISTERED AS A 21-DAYS CONTINUOUS INFUSION TO PATIENTS WITH COLORECTAL CANCER

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SUMMARY

Purpose: Topotecan is a specific inhibitor of topoisomerase I. Preclinical data have indicated that topoisomerase I inhibitors demonstrate more efficacy and have a greater therapeutic index with prolonged continuous exposure. The feasibility of this concept in humans using a 21-day continuous infusion of topotecan has been reported. We conducted a phase II study of this 21-day continuous topotecan administration schedule in patients with locally advanced, unresectable or metastatic colorectal cancer.

Patients and Methods: Topotecan, initially applied at a dose of 0.6 mg/m^2/day, was administered as a continuous infusion via an ambulatory pump for 21 days repeated every 4 weeks. The starting dose was reduced to 0.5 mg/m^2/day because in 5 of the first 11 patients the second course had to be delayed due to prolonged myelosuppression. Forty-two patients entered the study; one patient was ineligible and was excluded from further analyses.

Results: The overall response rate was 10%, with 1 complete and 3 partial responses. The median response duration was 7 months (range 4-11). With this schedule, the major toxicity was prolonged cumulative myelosuppression including a marked inhibition of erythropoiesis. A total of 250 units of erythrocyte transfusions were needed to keep the hemoglobine level above 6.0 mmol/l. Other side effects were mild, including alopecia (47%), periodic nausea (40%)/vomiting (22%) and fatigue (16%). Pharmacokinetic evaluation showed a mean steady-state plasma concentration (Css) of topotecan of 0.62 ng/ml (range 0.33-1.1) with a significant relationship between the Css of topotecan and CTC-grade of leukocytopenia.

Conclusion: Topotecan administered as a 21-day continuous infusion exerts minor activity as single-agent therapy in patients with metastatic colorectal cancer.

INTRODUCTION

Colorectal cancer is one of the most common malignancies in Western

countries. The primary treatment is surgical resection, but approximately half of the patients will eventually die of metastatic disease. Over the last 20 years 5-fluorouracil (5-FU) has remained the mainstay of systemic treatment in patients with metastatic colorectal cancer, with reported response rates ranging from 5-20%, responses usually being of short duration. Biomodulation with folinic acid, interferons, methotrexate (MTX) or phosphonacetyl-L-aspartate (PALA) induces higher response rates, but complete responses are rare and the impact on survival is marginal [1-3].

Thus, the poor prognosis for patients with metastatic colorectal cancer and the lack of essential progress in this common disorder results in researchers striving new drugs for their activity in this disease.

Topotecan, (S)-9-dimethylaminomethyl-10-hydroxycamptothecin is a water-soluble analogue of camptothecin [4,5], a plant alkaloid from the deciduous tree *Camptotheca acuminata*. Topotecan exerts its cytotoxic activity during the S-phase of the cell cycle through specific inhibition of the enzyme topoisomerase I. Topoisomerase I relaxes the supercoiled DNA by forming a covalent adduct with DNA, known as the cleavable complex, resulting in transient single-strand breaks through which the intact strand is allowed to pass. DNA relaxation occurs from swiveling at this nick and so plays an important role in DNA replication and RNA transcription. The enzyme breaks are then resealed by topoisomerase I (religation). Binding of topoisomerase I inhibitors to the cleavable complex permits uncoiling, but prevents religation in presence of the drug. Cytotoxicity occurs by interaction of the advancing replication fork with the drug-trapped cleavable complex, resulting in fork breakage and eventually cell death [6,7].

It has been shown that colon cancer cells contain a fivefold higher level of topoisomerase I than adjacent normal tissue [8] and preclinical data have indicated that topoisomerase I inhibitors demonstrate greater efficacy and have a greater therapeutic index with prolonged continuous exposure [9,10]. Hochster et al. [11] recently reported a phase I study of prolonged exposure to the drug using a 21-day continuous infusion repeated every 28 days. In pretreated patients, the infusion was tolerated at a dose of 0.53 mg/m^2/day. The dose-limiting toxicity was myelo-suppression, with thrombocytopenia somewhat more profound compared to

neutropenia. Responses were reported in tumor types that are usually considered resistant to chemotherapy.

In view of these data, we conducted a phase II study of topotecan administered as a 21-day continuous infusion repeated every 28 days in patients with metastatic colorectal cancer.

PATIENTS AND METHODS

Patients

Patients at least 18 years of age with histologically confirmed, locally advanced, unresectable, or metastatic colorectal cancer, who had not received any previous chemotherapy for metastatic disease, were enrolled in the study. All patients had at least one bidimensional lesion and were suitable and willing to have a permanent mediport device. A World Health Organization (WHO) performance status ≤ 2 and a life expectancy of ≥ 3 months was required. In patients who had received adjuvant chemotherapy, the treatment-free interval had to be a minimum of 12 months. Prior radiotherapy was allowed if the interval was ≥ 4 weeks. Eligibility further required adequate bone marrow function (white blood cells (WBC) $\geq 3.5 \times 10^9$/l, granulocytes $\geq 1.5 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l); creatinine ≤ 135 μmol/l; serum bilirubin ≤ 30 μmol/l, serum ASAT/ALAT $\leq$ 2x the upper limit of normal, in presence of liver metastasis $\leq$ 5x the upper limit. Informed consent was obtained according to institutional rules before entry into the study.

Treatment schedule

Topotecan, initially applied at a dose of 0.6 mg/m^2/day, was administered as a 21-day continuous infusion repeated every 28 days. Topotecan (Hycamtin®) was supplied in vials as a light yellow, lyophilized cake. Each lyophilized vial contained 5 mg of the free base. The lyophilized formulation was reconstituted with 2 ml of bacteriostatic water for injection, preserved with benzyl alcohol. The appropriate volume of the reconstituted solution was transferred to the cassette. Final dilution

to a total volume of 50 ml was made at a concentration such that the total daily dose was contained in every 6 ml of solution. The cassette was inserted in a CADD-PLUS ambulatory infusion pump (Pharmacia-Deltec Inc., St. Paul, USA) adjusted at a flow rate of 6 ml/24 hours and connected to the mediport device. Stability data for the topotecan solution have been generated for at least 8 days under the conditions used in the study. The cassette and batteries were changed every week.

Dose modifications

The next treatment cycle was administered if the granulocytes were $\geq$ 1.0 x 10^9/l and platelets $\geq$ 100 x 10^9/l. The dosage of topotecan was reduced by 0.1 mg/m^2/day if myelosuppression persisted beyond day 28 or if the previous cycle of topotecan was prematurely stopped because of grade 4 myelosuppression. If toxicity was $\leq$ grade 2, the dose of topotecan was increased by 0.1 mg/m^2/day.

Pretreatment and follow-up study scheme

A history, physical examination and assignment of toxicity, according to the Common Cytotoxicity Criteria (CTC) were performed every week [12]. Complete blood cell counts (CBC) including differential, were performed twice weekly. Blood chemistries including electrolytes, renal and liver functions, total protein, albumin and glucose were obtained weekly. Urinalysis was performed before every cycle. An ECG was performed before treatment and prior to the second cycle.

Evaluation

Responses were evaluated according to the World Health Organisation (WHO) criteria [13]. Response duration was defined as the interval between the initiation of treatment to the first sign of progression. Measurement of the involved lesions by computed tomographic scan, plain chest X-ray or ultrasonography was performed every 2 cycles. CEA was not measured routinely and was not taken as a surrogate marker for response, since only objective remission was considered to be of relevance.

Pharmacokinetics

Heparinized blood samples (2.8 ml) were collected during cycle 1 on days 2, 8 and 15 and during cycle 2 on day 8 to determine the steady-state plasma concentration (Css) of topotecan and the ring-opened product hydroxy acid. After collection, the blood samples were centrifuged immediately for 5 minutes at 3,500 rpm; 250 μl of the plasma was immediately transferred to a polypropylene tube containing 750 μl of cold methanol (-20 ^{0}C) and mixed on a whirl mixer for 15 seconds. Subsequently, the sample was immediately stored at -80 ^{0}C until analysis. Topotecan and the hydroxy acid were analyzed simultaneously with a reverse-phase HPLC system and fluorescence detection as described by *Loos et al.* [14], with a lower limit of quantification (LLQ) of 0.1 ng/ml for both compounds. The Spearman rank correlation coefficient was calculated between the Css and toxicity parameters.

RESULTS

A total of 42 patients were entered in the study. One patient was considered ineligible because there was no histologic confirmation of colorectal cancer. This patient was excluded from all analyses. Another patient was not evaluable because of the development of an intra-abdominal abscess and colocutaneous fistula in the first week after initiation of therapy, which led to discontinuation of treatment. Patient characteristics are summarized in Table 1.

Toxicity

Although a 21-day continuous infusion is rather inconvenient for patients, treatment was well tolerated. In 4 patients treatment was complicated by thrombosis of the subclavian vein at the site where the mediport device was inserted. Two patients experienced a local infection of the mediport device; with an early start of antibiotics the device was preserved.

Forty patients were assessable for toxicity and response and received a total of 142 courses. The median number of courses given per patient was 3 (range 1-14$^+$).

Table 1. Patient characteristics

	No. of patients
No. patients entered	42
No. patients	
evaluable	41
ineligible	1
Sex	
male	20
female	21
Age	
median	57
range	38-69
Performance status	
0	14
1	25
2	2
Prior treatment	
prior surgery	27
prior surgery and prior radiotherapy	3
prior surgery and adjuvant chemotherapy	3
no prior treatment	8
Metastatic sites	
lung	11
liver	26
lymph nodes	11
miscellaneous	12

The starting dose was reduced from 0.6 mg/m^2/day to 0.5 mg/m^2/day after the second course had to be delayed in 5 of the first 11 patients because of prolonged myelosuppression. The main toxicity was myelosuppression, with

neutropenia grade 3-4 in 20% of the courses with the median nadir ANC observed on day 25 (range 8-25); Thrombocytopenia was mild, being grade 3-4 in only 8% of the courses, and the platelet nadir also occurred on day 25 (Table 2). Despite the mild myelosuppression treatment had to be delayed in 26% of the courses due to prolonged myelosuppression. Following treatment delays, the protocol mandated a dose reduction in the subsequent course. Related to this, the mean dose-intensity decreased from 2.65 to 1.43 mg/m^2/week (Figure 1), illustrating that the myelosuppression was cumulative. In addition, a marked inhibition of the erythropoeisis was observed. The hospital policy is to correct the hemoglobin as soon as it drops below 6.0 mmol/l. A total of 250 units of erythrocyte transfusions were needed to keep the hemoglobin (Hb) > 6.0 mmol/l. The percentage of patients requiring transfusions for this reason varied from 58% in course 1 to 90% in course 6.

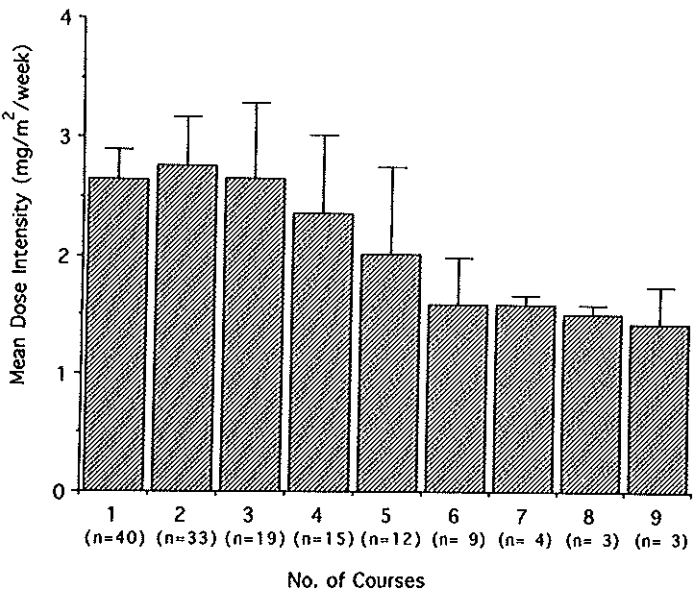

Figure 1. Graph shows the gradually decreasing mean dose-intensity ± SD (mg/m^2/wk) per treatment course

Non-hematologic side effects were alopecia (47%) being grade 1 in more than two thirds of the patients, mild periodic (often only in the first week of treatment) nausea (40%) and vomiting (22%), asthenia/fatigue (16%) (Table 2). The latter was partially associated with anemia as symptoms subjectively reduced after transfusion.

Table 2. Drug related toxicity per course (n = 142)

	CTC grade				grade 3-4 %
	1	2	3	4	
leucopenia	20	41	22	2	17
neutropenia	26	28	17	11	20
thrombocytopenia	19	24	8	4	8
nausea	52	5	-	-	0
vomiting	25	6	-	-	0
fatigue	20	3	-	-	0
alopecia (per pt.)	13	6	-	-	-

Pharmacokinetics

The Css levels of topotecan could be determined in 37 patients and varied widely, with a mean of 0.62 $\pm$ 0.17 ng/ml and a range of 0.33-1.1 ng/ml (Table 3). A low but statistically significant correlation was found between Css and CTC grade of leucocytopenia ($R=0.54$, $p=0.001$). There was no correlation between Css and the absolute dose of topotecan.

Response

Four patients achieved a response (10%; 95% confidence limits 3-23%), including 1 complete response lasting for 8 months and 3 partial responses with a duration (as measured from the initiation of treatment) of respectively, 4, 7 and 11 months. One of these 4 patients started at the dose level off 0.6 mg/m^2/day. The obtained drug plasma levels of the responding patients where in the same range as

those of other patients treated. Seventeen patients had stable disease (43%) at evaluation whereas 19 patients had progression (47%). The median time to progression for the total study population was 3 months.

Table 3. Pharmacokinetics of Topotecan and Topotecan plus hydroxy-acid

Pharmacokinetic parameter	Topotecan	Topotecan + HA
C_{ss} (ng/mL)*		
mean (SD)	0.62 (0.17)	1.94 (0.47)
range	0.33 - 1.1	1.23 - 3.02
CL (l/min)		
mean (SD)	1.14 (0.33)	0.35 (0.08)
range	0.63 - 2.04	0.24 - 0.54

* n = 37

HA, hydroxy-acid; C_{ss}, steady-state plasma concentration; CL, clearance

DISCUSSION

This phase II study in patients with locally, unresectable or metastatic colorectal cancer was initiated based on previous data of Hochster et al. [11] who reported favourable responses using a 21-days continuous infusion of topotecan via an ambulatory pump repeated every 28 days, pursuing the prolonged exposure that *in vitro* has shown to possess more cytotoxic activity and may have a greater therapeutic index [9,10]. In the heavily pretreated patient population of that phase I study topotecan was tolerated with a recommended dose of 0.53 mg/m^2/day, the dose-limiting toxicity was myelosuppression with thrombocytopenia somewhat exceeding leukopenia. Seven objective responses were observed including tumor

types usually considered chemotherapy resistant. Although no responses were seen in patients with colorectal cancer this was imputed to the fact that those patients were mainly entered at the lower dose levels (personal communication).

The starting dose of the presently reported study was 0.6 mg/m^2/day as our patients hardly received prior chemotherapy, but the starting dose had to be reduced to 0.5 mg/m^2/day after in 5 of first 11 patients the second course had to be delayed. Overall, the magnitude of myelosuppression was less pronounced, presumably because patients had not received prior chemotherapy, but the recovery from myelosuppression was delayed and therefore treatment had to be postponed in 26% of the courses. In addition, we demonstrated that the myelosuppression was cumulative, with a mean dose intensity gradually decreasing from 2.65 to 1.43 mg/m^2/week. The low correlation coefficient of 0.54 between Css and the CTC-grade of leukocytopenia illustrates that the pharmacokinetic variability is not a major determinant of this toxicity. Furthermore, we observed a marked inhibition of erythropoiesis. *In vitro* data have shown that topoisomerase inhibitors added to bone marrow cultures impair the formation of early BFU-E derived colonies, late CFU-E derived colonies and mixed hemotopoietic (CFU-GEMM derived) colonies in a time and concentration dependent fashion [15]. Topoisomerase inhibitors also impair the maturation of erythroblasts by inhibition of the hemoglobinization [15].

In *in vivo* studies topotecan had shown activity in the mouse coloncarcinomas 38 and 51, and in the human coloncarcinoma xenografts HT-29 and SW-48 [5,16]. The initial phase II studies with topotecan have been performed using a daily times five intravenous drug administration by 30 minute infusion. Using this schedule the *in vivo* activity could not be confirmed in the human situation, with an obtained response rate of 0-7% in a total of 76 patients [17,18]. However further *in vitro* studies had shown that topoisomerase I inhibitors (including topotecan) demonstrate a greater efficacy with prolonged continuous exposure [9,10]. Such an exposure can be achieved clinically by continuous infusion for instances for 21 days, the feasibility of which had been shown by Hochster et al [11]. Despite this, and the known data, that colon cancer cells contain a fivefold higher level op topoisomerase I than adjacent normal tissue [8], the efficacy of the 21-days infusion in patients with colorectal cancer was minor with an overall response rate of 10%

(1 CR and 3 PRs) with a median duration of 7 months (range 4-11).

Clinical studies with irinotecan (CPT-11), another topoisomerase I inhibitor reveal more promising results in patients with metastatic colorectal cancer. Several are published administering CPT-11 at a dose of 125 mg/m^2 every week x4, followed by a 2 weeks rest, showing a response rate in chemonaive patients of 27-32% and in 5-FU-pretreated patients of 22-25% [19-22], but severe (grade 3-4) diarrhea, despite the early and frequent use of loperamide, remains an important side effect. In view of these results, one might speculate why two different topotecan regimens did not show activity in colorectal cancer. With a daily times five regimen relatively high peak plasma levels of topotecan were achieved, and this regimen does show activity in other diseases. The intrapatient variation in achieved area under the curve is very limited, but the interpatient variation is large [23]. In colorectal cancer however only minor activity was noted [17,18]. The completely different approach reported here, using a 21-day continuous infusion did also not result in activity, possibly because the achieved plasma levels were lower than concentrations required for *in vitro* cell kill. Differences in drug-target interactions in colorectal cancer cells may be another, but unproven, reason for the difference in clinical activity between irinotecan and topotecan, while there also may be many others.

Despite the rather disappointing results on efficacy in this study, the concept of continuous exposure should not be refuted. A 21 day exposure may show to be efficacious in more sensitive tumor types, and for relatively resistant tumor types such as colorectal cancer a better balance between the necessity of achieving higher plasma concentrations without coinciding excessive toxicity may be obtained by shorter infusion durations. In addition, in view of the reported 32% bioavailability of oral topotecan [24] and the patient convenience related to oral dosing, prolonged topotecan administration by oral application should further be studied.

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CHAPTER 4

PHASE I AND PHARMACOLOGIC STUDY OF ORAL TOPOTECAN ADMINISTERED TWICE DAILY FOR 21-DAYS TO ADULT PATIENTS WITH SOLID TUMORS

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SUMMARY

Purpose: Topotecan is a specific inhibitor of topoisomerase I. Recently bioavailability of an oral formulation of approximately 30% with limited variability was reported. We conducted a phase I and pharmacokinetic study of the oral formulation of topotecan to characterize the maximum-tolerated dose (MTD), toxicities, pharmacokinetics and antitumor effects in patients with refractory malignancies.

Patients and methods: Patients were treated with oral topotecan given twice daily for 21 days, cycles repeated every 28 days. In subsequent cohorts the dose was escalated from 0.15 to 0.6 mg/m^2 twice daily. Pharmacokinetics were performed on day 1 and 8 of the first course using a validated high-performance liquid chromatographic assay and noncompartmental pharmacokinetic methods.

Results: Thirty-one patients entered the study, one patient was not assessable for toxicity and response as therapy was prematurely interrupted on request of the patient who had not experienced toxicity. Thirty patients received a total of 59 courses. The dose-limiting toxicity (DLT) was reached at a dose of 0.6 mg/m^2 twice daily and consisted of diarrhea, starting subacutely at a median onset on day 15 (range 12-20) and resolving after a median of 8 days (range 7-16). Other toxicities were mild, including leucocytopenia, thrombocytopenia, nausea and vomiting. The MTD was 0.5 mg/m^2 twice daily. No responses were observed. Pharmacokinetics showed a substantial variation of the area under the plasma concentration-time curve at time point "t" (AUC(t)) of topotecan and ring-opened product hydroxy-acid. A significant correlation was observed between the percentage of decrease in WBC versus the AUC(t) of topotecan (R=0.75) which was modeled by a sigmoidal maximal effect concentration (E_{max}) function.

Conclusion: The DLT in this phase I study for chronic oral topotecan for 21 days was diarrhea. The recommended dose for phase II studies is 0.5 mg/m^2 twice daily.

INTRODUCTION

Topotecan, (s)-9-dimethylaminomethyl-10-hydroxycamptothecin, is a water-soluble semisynthetic analogue of camptothecin (CPT) [1]. Like camptothecin, topotecan is a specific inhibitor of topoisomerase I. Topoisomerase I is a nuclear enzyme that resolves topological problems of the supercoiled DNA. This is achieved by forming a covalent adduct between topoisomerase I and the DNA, termed the cleavable complex. This catalytic intermediate creates single-strand breaks, allowing the DNA molecule to rotate around the intact DNA strand at the cleavage site leading to a relaxation of the DNA molecule. In this way, replication, transcription, and other DNA functions can proceed. These enzyme-bridged breaks are then resealed by topoisomerase I. Topoisomerase I inhibitors interfere with the breakage-reunion process by stabilizing the cleavable complexes and thereby preventing the resealing of single breaks in the presence of the drug. Cytotoxicity is specific to the S-phase of the cell, cycle because the double-strand breaks that occur during this phase are more difficult to repair in the absence of the drug. So, in the absence of DNA replication or in case of short exposure, topoisomerase I inhibitors produce little or no cytotoxicity [2,3]. Moreover, preclinical studies using human colony-forming units *in vitro*, have indicated that prolonged exposure demonstrates more efficacy and has a greater therapeutic index [4,5].

The feasibility of this concept of prolonged exposure in humans was tested by Hochster et al. [6] in a phase I study that used a 21-day continuous infusion repeated every 28 days. Responses were seen in tumor types that are usually considered chemotherapy-resistant. In pretreated patients, the infusion was tolerated at a dose of 0.53 mg/m^2/day. For patients with no prior treatment, the tolerated dose was 0.7 mg/m^2/day. The dose-limiting toxicity (DLT) was myelosuppression, with thrombocytopenia being somewhat more profound compared with neutropenia.
Recently reported results showed a 32-44% bioavailability of the intravenous (i.v.) formulation of topotecan administered orally [7,8], and thus oral administration might be a more simple and convenient method for prolonged drug administration. In view of the relatively short half life of topotecan and in order to mimic as appropriate as possible the continuous infusion schedule, the oral formulation was

initially tested at a twice-daily administration. We performed a phase I study on oral topotecan given twice daily for 21 days repeated every 28 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: age between 18-75 years; Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2; estimated life expectancy $\geq$ 12 weeks; no previous anticancer therapy for $\geq$ 4 weeks (6 weeks for nitrosoureas or mitomycin C), adequate hematopoietic (WBC $\geq$ $4x10^9$/l and platelets $\geq$ $100x10^9$/l), hepatic (bilirubin within normal limits, AST, ALT and/or alkaline phosphatase $\leq$ 2x normal), and renal (serum creatinine $\leq$ 132.6 μmol/l) function. Specific exclusion criteria included active peptic ulcer or any gastrointestinal condition that could alter absorption or motility, and patients taking H_2-antagonists or proton pump inhibitors. All patients gave written informed consent before entry in the study.

Treatment and dose escalation

Based on the data of Hochster et al. [6] who used topotecan as a 21-day continuous infusion with a maximum-tolerated dose (MTD) of 0.53 mg/m^2/day and given a bioavailability of 32% of oral administration of topotecan [7], the starting dose for the 21-day oral administration was set at 0.15 mg/m^2 twice daily. Courses were to be repeated every 28 days as tolerated. Dose escalations were based on the prior dose level toxicity. For example, if no toxicity was seen in the prior dose, 100% dose escalation was allowed. However, if toxicity was seen, a dose escalation of 25-50% (which was determined by the worst significant toxicity) was prescribed. At least four patients were entered at each dose level. The MTD was defined as one dose level below the dose that induced DLTs, which were defined as common toxicity criteria (CTC) grade 4 hematologic toxicity and/or nonhematologic toxicity $\geq$ CTC grade 3 in more than two of six patients. If

neutropenia grade 4, thrombocytopenia ≥ grade 3, and/or non-hematologic toxicity ≥ grade 3 occurred during treatment days, topotecan administration was stopped immediately. Intrapatient dose escalation was not performed.

Topotecan was supplied as capsules containing topotecan hydrochloride, equivalent to either 0.2 mg or 0.3 mg of the anhydrous free base. Capsules had to be stored at a temperature between 2-8°C. Capsules were taken with an interval of 12 hours with a glass of water at least 10 minutes before non-standardized meals. Patients were treated on a outpatient basis.

Treatment assessment

Before 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, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, urea, uric acid, bilirubin, AST, ALT, alkaline phosphatase, total protein and albumin, 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 chemistries. CBC were determined twice weekly. Tumor measurements were performed after every two courses and evaluated according to the World Health Organization (WHO) criteria for response. Patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis, whole-blood samples (2.8 ml) in heparinized tubes were collected from an indwelling IV canula before dosing, and 15, 30, and 45 minutes, and 1, 1.5, 2.5, 3.5, 4.5, 8.5, and 12 hours after administration of the morning dose of the drug on days 1 and 8 of the first course. The samples were immediately prepared and analyzed according to the method described by Loos et al [9]. The lower limit of quantitation (LLQ) was 0.1 ng/ml for topotecan lactone as well as for the hydroxyacid.

The within-run precision of the LLQ samples of topotecan lactone was 4.4% and of the hydroxyacid 9.7% The accuracy was 93.2% and 106.6%, respectively,

and the between-run precision rates were 7.1% and 5.5%, respectively.

Area under the plasma concentration-time curves (AUC) of topotecan lactone and hydroxyacid were calculated by noncompartmental analysis (linear-logarithmic trapezoidal method). The AUC(t) was calculated up to the last measured time point "t", because in most cases the extrapolated part was ≥20% of the total AUC. The terminal half-life was calculated as ln2/k, where k is the elimination rate constant (h^{-1}); k was calculated using the decline of the logarithmic-transformed concentrations versus time in the terminal phase of the curve (an automatic feature of the Siphar package (SIMED, Creteil, France) when using the lin-log trapezoidal method). The Auc(t) was fitted to observe the percentage decrease in WBC calculated as (WBC nadir/WBC baseline) x 100, and absolute neutrophil count (ANC) calculated as (ANC nadir/ANC baseline) x 100, using the sigmoidal maximal effect concentration (E_{max}) model [10]. For all calculations, the Siphar software package release 4.0 was used. For statistical analysis, linear regression analysis was used to evaluate relationships between dose and dose/m^2 and AUC(t) and Pearson correlation coefficients were calculated. Spearman rank-correlation coefficients were calculated between AUC(t) and the CTC grade leucocytopenia and thrombocytopenia and diarrhea. For statistical analysis, Statgraphics was used (Manngistics, Rockville, MD, USA). The Wilcoxon signed-rank test was used to compare the AUC(t) values of topotecan lactone on days 1 and 8.

RESULTS

A total of 31 patients entered the study. Patients characteristics are listed in Table 1. All patients were eligible, but one patient with colorectal cancer was considered not assessable for toxicity and response because upon request of the patient he was taken off protocol on day 10 of the first course without any notable toxicity at that time. Therefore, 30 patients were assessable for toxicity and response. The majority of patients were either asymptomatic or only mildly symptomatic (ECOG performance status 0-1). All patients, except one, had received prior therapy.

Table 1. Patient characteristics

	No. of patients
No. patients entered	31
No. patients evaluable	30
Age	
median	55
range	33-73
Sex	
female	18
male	13
Performance status	
median	1
range	0-2
Tumor types	
colorectal	12
oropharynx	4
ovarian	3
lung (non-small cell)	2
melanoma	2
breast	2
carcinoma unknown primary	2
soft tissue sarcoma	1
gastric	1
kidney	1
cervix	1
Prior treatment	
radiotherapy	2
chemotherapy	14
radio- and chemotherapy	14
no prior therapy	1

The most common tumor type was colorectal cancer. The total number of assessable courses was 59. The median number of courses per patient was 2 (1-10). Dose levels studied were 0.15, 0.3, 0.4, 0.5 and 0.6 mg/m^2 twice daily, resulting in total daily doses of 0.3, 0.6, 0.8, 1.0 and 1.2 mg/m^2, respectively.

Hematologic toxicity

Overall, the hematologic toxicities were relatively mild (Table 2), with leucocytopenia and thrombocytopenia mainly occurring (if at all) during the third and fourth week of the course and being short-lasting.

Table 2. Hematologic toxicity (worst per patient)

Dose (mg/m^2/bi.d.)	No. pts	Leucocytes				Granulocytes				Platelets			
		CTC grade											
		1	2	3	4	1	2	3	4	1	2	3	4
0.15	4	0	1	0	0	0	0	0	1	0	0	0	0
0.3	8	1	1	1	0	0	0	1	0	0	0	1	0
0.4	8	0	1	0	0	1	0	0	0	0	0	0	0
0.5	8	0	2	2	2	2	2	0	2	0	0	0	2
0.6	3	0	1	1	0	0	2	0	0	2	0	0	0

The next treatment course had to be postponed due to prolonged myelosuppression in 2 courses in patients who experienced grade 3-4 leucopenia. Grade 3-4 leucopenia was observed in 6 of 59 courses (10.2%), it was complicated by neutropenic fever in 1 patient. In 3 courses (5%) grade 3-4 thrombocytopenia was noted, 2 in conjunction with leucocytopenia.

Non-hematologic toxicity

Diarrhea was the DLT of topotecan at a dose of 0.6 mg/m^2 twice daily in this schedule (Table 3).

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

Dose (mg/m^2/b.i.d)	No. pts	Nausea				Vomiting				Diarrhea			
		CTC grade											
		1	2	3	4	1	2	3	4	1	2	3	4
0.15	4	2	0	0	0	3	0	0	0	0	0	1	0
0.3	8	4	0	0	0	1	0	0	0	3	0	0	0
0.4	8	4	0	0	0	0	0	0	0	1	1	0	1
0.5	8	3	4	0	0	2	0	0	0	4	1	0	2
0.6	3	1	1	0	0	1	0	0	0	0	0	0	3

The onset of severe diarrhea was sudden with a median day of onset of day 15 (range 12-20). Diarrhea lasted for a median duration of 8 days (range 7-16 days) and resulted in dehydration requiring hospitalization for parenteral fluid and electrolyte therapy in all 3 patients at the 0.6 mg/m^2 twice daily dose level. One patient showed a different pattern of diarrhea. Two days after topotecan therapy was initiated, the patient developed diarrhea which gradually became worse. On day 17 the treatment was stopped according to protocol and the diarrhea resolved in 7 days. According to the protocol, topotecan administration was stopped after the development of severe diarrhea. Vigorous administration of loperamide every 2 hours for treatment of diarrhea was ineffective in reducing its severity in all patients with this side effect. Stool cultures and examination for fecal leucocytes were negative in these patients. Endoscopy was performed in two patients, revealing a mild non-specific colitis.

Prophylactic antiemetics were not routinely used in the study. Mild intermittent nausea and vomiting (grade 1-2) was observed in 32% and 11.8%, respectively, of the given courses and could be circumvented with standard antiemetics. Alopecia occurred in 2 patients. No other toxicities were seen. There was no stomatitis, hypotension, or liver or renal toxicity.

Pharmacokinetics and dynamics

Pharmacokinetics were performed in 14 patients and were well distributed over all dose levels. A representative plasma concentration-time curve of topotecan and the hydroxyacid on days 1 and 8 is shown in Figure 1.

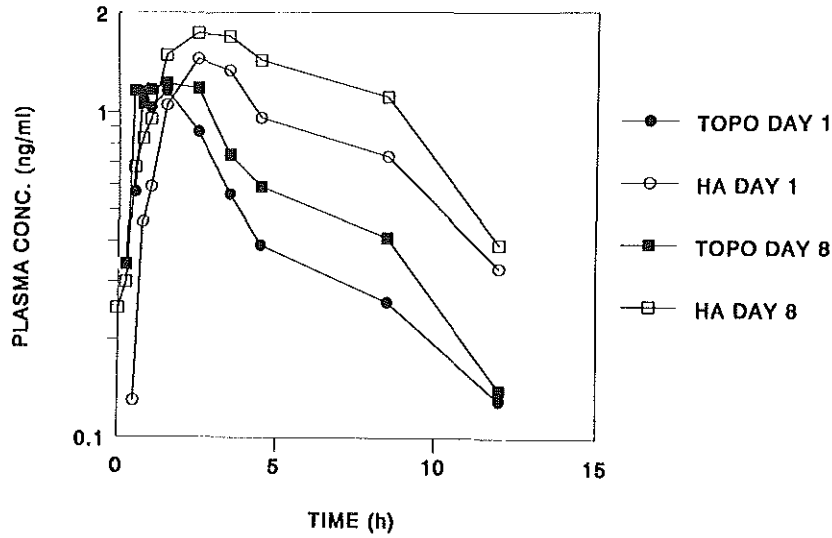

Figure 1. Representative AUCs of topotecan and hydroxyacid on days 1 and 8.

After oral administration, the percent of AUC which had to be extrapolated was more than 20% in most patients. Therefore, the AUC(t) was calculated, where "t" denotes the latest measured point. The pharmacokinetic data are listed in Table 4. In 6 patients, the percentage extrapolated was >20% for topotecan lactone and in 7 for hydroxy-acid.

The value of t for each patient was 12 hours and very constant (after drug intake). This cannot be a source of variability. The half-life (which was >3 hours in 5 of 14 patients) leads to measurable drug levels at the start of the second administration.

Table 4. Pharmacokinetics

		Topotecan Day 1		Hydroxy-acid Day 1		Topotecan Day 8		Hydroxy-acid Day 8	
Dose level mg/m^2	No. of Patients	AUC(t) ng.hr/ml	t1/2 h	AUC(t) ng.hr/ml	t1/2 h	AUC(t) ng.hr/ml	t1/2 h	AUC(t) ng.hr/ml	t1/2 h
0.15	2	1.18 ± 0.52	2.29 ± 1.31	2.90 ± 3.02	3.52 ± 1.16	2.06 ± 1.05	3.53 ± 1.36	5.11 ± 3.37	4.39 ± 2.18
0.30	3	4.30 ± 2.54	2.70 ± 1.23	6.88 ± 4.86	4.38 ± 1.97	5.53 ± 3.69	2.65 ± 1.05	9.03 ± 6.65	3.23 ± 0.60
0.40	2	3.16 ± 2.05	2.02 ± 0.06	4.00 ± 2.44	2.67 ± 0.06	5.96 ± 1.29	2.71 ± 0.11	7.98 ± 1.90	2.53 ± 0.13
0.50	4	3.92 ± 2.19	1.70 ± 0.62	7.58 ± 2.89	3.73 ± 0.70	5.46 ± 1.89	2.88 ± 0.67	10.77 ± 3.74	4.19 ± 1.69
0.60	3	6.13 ± 2.09	3.09 ± 1.47	9.28 ± 3.31	3.21 ± 0.08	8.86 ± 1.63	3.89 ± 1.30	9.41 ± 0.63	2.71 ± 1.65
All	14	3.98 ± 2.35	2.35 ± 1.05	6.61 ± 3.68	3.61 ± 1.04	5.79 ± 2.82	3.06 ± 0.91	8.93 ± 4.00	3.52 ± 1.40
% CV	-	59.1	44.7	55.6	28.9	46.8	29.6	44.6	39.9

%CV = coefficient of variation

This resulted in a small but detectable accumulation of the drug and in a higher AUC(t) on day 8; t values on days 1 and 8 were the same.

The AUC(t) of topotecan and hydroxyacid showed substantial variation. High correlation was found between the ratio of the AUC(t) of topotecan and the AUC(t) of hydroxyacid on days 1 and 8 (R=0.96). The AUC(t) values of topotecan and hydroxyacid were consistently higher on day 8 compared with day 1 (Figure 2) (p=0.001). The mean AUC(t) ± SD of topotecan on day 8 was 5.79 ± 2.83 ng.hr/ml and was 26% higher than the AUC(t) on day 1, which was 3.98 ± 2.35 ng.hr/ml (p=0.001). The interpatient variability (%CV) values in the AUC(t) values of topotecan on days 1 and 8 were 59.1% and 46.8%, respectively. The correlation between dose and AUC(t) of topotecan was relatively low (R=0.44).

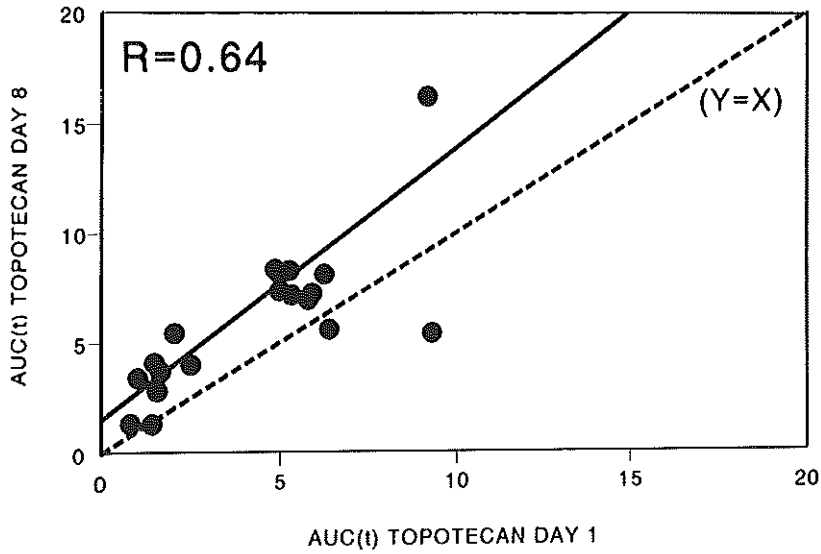

Figure 2. Relationship between the AUC(t) of topotecan on day 1 and day 8 of course 1.

There was no significant relationship between the dose of topotecan and the occurrence of leucocytopenia or thrombocytopenia (R=0.41, p=0.15). There was

a significant correlation between the AUC(t) of topotecan and percentage of decrease in WBC count (R=0.75, p=0.003). The latter data could be fitted best using a sigmoidal E_{max} model (Figure 3). The AUC at 50% of the maximal effect (AUC_{50}) in that model was 3.9 ng.h/ml and the slope factor γ was 2.6. There also was a significant correlation between AUC(t) and the CTC grade of ANC (R=0.69, p=0.02) and of thrombocytopenia (R=0.59, p=0.04).

Finally, while there was a trend towards a significant relationship between the CTC grade of diarrhea and the administered dose (R=0.51, p=0.08), there was no correlation between AUC(t) and the CTC grade of diarrhea. However, only 8 of 17 patients with diarrhea had pharmacokinetic studies performed, which limits the power of this observation.

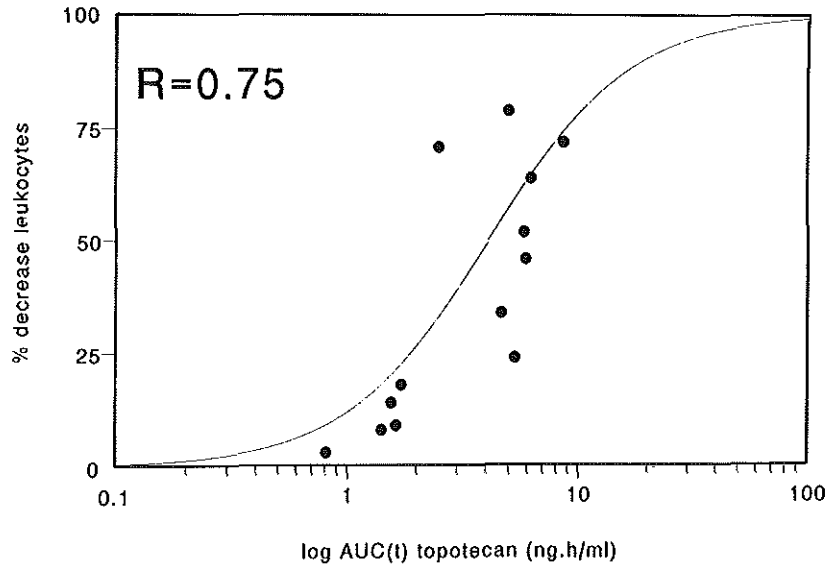

Figure 3. Percentage decrease in WBC count versus AUC(t) of topotecan during course 1. Curve is fit to a sigmoidal E_{max} model.

Responses

Objective responses have not been observed. Disease stabilization of 11

months was noted in one patient with colorectal cancer at the dose of 0.3 mg/m^2 twice daily.

DISCUSSION

The topoisomerase I inhibitors continue to move rapidly through clinical development, with their unique mechanism of action and their activity against a variety of malignancies. Topotecan has been extensively studied using different i.v. dosing schedules and has shown short-lasting noncumulative neutropenia and/or thrombocytopenia as the DLT [11,12].

The present study on prolonged drug administration was based on the promising results of preclinical studies on prolonged exposure and the oral bioavailability of topotecan in humans. *In vitro* experiments with topotecan using short-term 1-hour exposure at concentrations of 1.0 and 10 μg/ml showed responses in 10% and 25% of patients, respectively. In contrast, using continuous exposure at concentrations of 0.1 and 1.0 μg/ml, responses were seen in 34% and 76%, respectively [5]. Prolonged exposure experiments with topotecan against xenografts derived from adult and childhood solid tumors also showed significant activity against several human cancers (rhabdomyosarcoma and colon cancer) without any toxicity, and indicated that its efficacy may be schedule-dependent [4]. The oral bioavailability using the i.v. formulation given orally was determined at 30% $\pm$ 7.7% in a study using topotecan at a dose of 1.5 mg/m^2, with moderate interpatient variation (21%-45%) [7]. In another study, the bioavailability was determined to be 44% using the MTD of the oral (14 mg/m^2) and i.v. (17.5 mg/m^2) routes of administration [8]. In contrast to the intravenous route, the DLT for the twice-daily oral administration for 21 days was diarrhea at the dose of 0.6 mg/m^2 twice daily. Mostly, diarrhea started in the third week of drug administration. It was always self-limiting after a median duration of 8 days (range 7-16). At the time the diarrhea occurred, vigorous administration of loperamide was ineffective in reducing this toxicity. Treatment consisted of supportive care with the administration of fluids and electrolytes. Diagnostic evaluation (i.e. stool cultures and biopsies) in several of these patients failed to find a pathogenetic

mechanism for this diarrhea. The pattern and severity of this diarrhea resembles the severe diarrhea seen in patients treated with irinotecan, another topoisomerase I inhibitor, particularly when the latter is used in intermittent dosing schedules employing higher single doses. With irinotecan grade 3-4 delayed diarrhea is observed in approximately 20% of patients [11-16]. Irinotecan is converted by endogenous carboxylesterase to its active metabolite SN-38. As diarrhea has never been reported as a major side effect with the i.v. route of topotecan administration, it is speculated that the cause of diarrhea with chronic oral administration may be a local effect of topotecan on the intestinal mucosa.

Nausea and vomiting were intermittent and mild, and never exceeded grade 2 toxicity, similar to the experience obtained with long-term continuous infusion of topotecan [11,17].

Hematological toxicity was relatively mild. Grade 3-4 hematologic toxicity was observed in 10.2% of the courses and consisted of leucocytopenia which in 2 of 6 courses occurred in conjunction with thrombocytopenia grade 3-4. In comparison, the DLT in the phase I study using a 21-day continuous infusion was hematologic, with thrombocytopenia being somewhat more profound than leucocytopenia [6]. In addition, myelotoxicity was the single relevant side effect in a phase II study that used long-term continuous infusion of topotecan at a dose of 0.5 mg/m^2/day in patients with untreated metastatic colorectal cancer. Moreover, that study revealed that the myelosuppression was prolonged, cumulative and coincided with a marked inhibition of the erythropoiesis [17].

Pharmacokinetic data from the present study showed a substantial interpatient variability in the AUC(t) and the AUC(t) on day 8 was consistently higher, which indicates some (presumably clinically less relevant) accumulation of the drug.

Nevertheless, in this study, a significant correlation was found between the systemic exposure and the percentage decrease in the leucocytes (Figure 3).
Topoisomerase I inhibitors are highly S-phase-specific, and, *in vitro*, cytotoxicity is a function of exposure time to the drug above some critical concentration [4,5,18]. Recently, Hochster et al. [19] showed that with continuous infusion of topotecan in humans, progressive depletion of topoisomerase I levels is observed until the end of the second week. Pursuing such a less protracted exposure time in using the oral

administration might enable the use of higher doses per day and could result in higher concentrations. Moreover, this way, the troublesome diarrhea may possibly be circumvented, as in the present study it mainly started in the third week of treatment.

In conclusion, in this phase I study with oral administration of topotecan given twice daily for 21 days, the MTD was reached at a daily dose of 0.5 mg/m^2, with diarrhea being the DLT. The concept of prolonged administration via oral administration remains attractive, but from the experience in the present study, it is possible that a shortened exposure time might result in better tolerance with a better chance to see antitumor activity. Based on these data, studies are planned to test 5 and 10 days of oral administration.

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CHAPTER 5

TEN DAYS ONCE DAILY AND TWICE DAILY DOSING OF ORAL TOPOTECAN: A PHASE I AND PHARMACOLOGY STUDY IN ADULT PATIENTS WITH SOLID TUMORS

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SUMMARY

Background and Purpose: Prolonged exposure to topotecan (TPT) in in vitro experiments and in vivo studies in animals yielded the highest antitumour efficacy. An oral bioavailability of TPT of 32-44% enables convenient prolonged administration. Because of unpredictable diarrhea in the third week of the b.i.d. 21 day schedule of orally administered TPT and the finding of optimal downregulation of topoisomerase I level after 10-14 days in mononuclear peripheral blood cells, a shorter period of administration (10 days) was chosen for phase I and pharmacologic studies of oral administration of TPT.

Patients and Methods: Adult patients with malignant solid tumors refractory to standard forms of chemotherapy were entered. Two dose schedules were studied: once daily (o.d.) and twice daily (b.i.d.) x10 administration every 3 weeks. TPT o.d. x10 was studied at dose levels 1.0, 1.4, 1.6 mg/m^2/day, and dose levels were 0.5, 0.6, 0.7, 0.8 mg/m^2 with the b.i.d. x10 schedule. Pharmacokinetics were performed on day 1 and 8 of the first course using a validated high performance liquid chromatographic assay and noncompartmental pharmacokinetic methods.

Results: 19 patients were entered in the o.d. x10 schedule, with a total of 48 courses given. Dose limiting toxicity (DLT) was reached at 1.6 mg/m^2/day and consisted of CTC-grade IV thrombocytopenia and CTC-grade III diarrhea. The maximum tolerated dose (MTD) was 1.4 mg/m^2/day. In the b.i.d. x10 administration of TPT a total of 64 courses were studied in 20 patients. DLT was reached at a dose of 0.8 mg/m^2 b.i.d. and consisted of CTC-grade IV myelosuppression and CTC-grade IV diarrhea. MTD was 0.7 mg/m^2 b.i.d.. Non hematologic toxicities with both schedules included mild nausea and vomiting, fatigue and anorexia. Pharmacokinetics revealed a substantial variation of the AUC of TPT lactone in both schedules. Significant correlations were observed between the myelotoxicity parameters and the AUC(t) day 1 of TPT lactone o.d.and b.i.d..

Conclusions: DLT of daily x10 administration of oral topotecan every 3 weeks consisted of a combination of myelosuppression and diarrhea for both schedules studied.

The recommended dose for phase II studies is 1.4 mg/m^2/day x10 for the once daily administration and 0.7 mg/m^2 b.i.d. x10 for the twice daily dose schedule.

INTRODUCTION

Topotecan, 9-dimethylaminomethyl-10-hydroxycamptothecin, is a water soluble semi-synthetic analogue of camptothecin, and a specific topoisomerase I inhibitor [1-8]. Topotecan was extensively studied at a daily x 5 i.v. schedule every 3 weeks. These studies showed brief myelosuppression as most important side effect and promising antitumor effects were reported in patients with small cell lung cancer and in pretreated patients with ovarian cancer [9-19]. Recently the drug was registered in Europe and the USA for the latter indication. Cytotoxicity of topoisomerase I inhibitors is more specific to the S-phase of the cell cycle and preclinical in vitro and in vivo studies indicate that prolonged exposure to low dose topoisomerase I inhibitors yields higher antitumour efficacy [20-25].

The clinical feasibility of using prolonged exposure to topotecan was initially reported by Hochster et al. in a phase I study using a 21-day continuous infusion every 28 days [26]. Myelosuppression was the dose limiting toxicity and antitumor effects were seen. Recent studies in humans reported a 32-44% bioavailability of topotecan when given orally [27,28]. We have previously reported a phase I study with oral topotecan given twice daily for 21 days in a 28 days cycle. Side effects were mainly diarrhea occurring during the last week of drug administration [29].

Topoisomerase I down-regulation in peripheral blood mono-nuclear cells was optimal after 2 weeks continuous infusion of topotecan [30]. In view of the latter observation it was considered of interest to also study shorter schedules of oral administration of topotecan than the 21 day schedule. In the present phase I study we investigated once and twice daily oral topotecan given for 10 days every 3 weeks.

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: age $\geq$ 18 years; WHO performance score $\leq$ 2; an estimated life expectancy of $\geq$ 12 weeks; no anticancer therapy in the preceding 4 weeks (6 weeks for nitrosoureas or mitomycin C); adequate hematopoetic (WBC $\geq$ $4x10^9$/l and platelets $\geq$ $100x10^9$/l), hepatic (bilirubin within normal limits, AST, ALT and/or alkaline phosphatase $\leq$ 2x upper limit of normal), and renal function (serum creatinine $\leq$ 133 μmol/l (2.0 mg/dL)). Specific exclusion criteria included: active peptic ulcer or any gastrointestinal condition which could alter absorption or motility or patients taking H_2-antagonists or proton pump inhibitors. All patients gave written informed consent.

Treatment and dose escalation

Based on the data of Creemers et al. [29] with a MTD of 0.5 mg/m^2 b.i.d. for oral topotecan in a 21 days schedule, the starting dose for the twice daily x10 day administration was set at 0.5 mg/m^2 b.i.d. and 1.0 mg/m^2/day for o.d. administration. Courses were to be repeated every 21 days as tolerated.
Topotecan was supplied as capsules containing topotecan-HCL, equivalent to either 0.25 mg, 0.50 mg or 1.0 mg of the anhydrous free base. Capsules had to be stored between 2-8 degrees Celsius. Capsules were taken with a glass of water in the morning on an empty stomach with a 2 hours period of fasting. With twice daily administration of topotecan the second dose was taken with an interval of 12 hours with a glass of water at least 10 minutes before meals preferably on an empty stomach. Patients were treated as outpatients. Dose escalations were based on the prior dose level toxicity.

The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose limiting toxicities (DLT), which were defined as CTC grade IV hematologic toxicity and/or $\geq$ CTC grade III non-hematologic toxicity during the first course in more than 2/6 patients. If neutropenia grade IV,

thrombocytopenia ≥ grade III and/or nonhematologic toxicity ≥ grade III occurred during treatment days, topotecan administration was stopped immediately. Intrapatient dose escalation was not allowed.

Treatment assessment

Prior to therapy, a complete medical history was taken and a physical examination was performed. A complete blood count (CBC) including WBC differential, and serum chemistry including sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, urea, uric acid, bilirubin, AST, ALT, alkaline phosphatase, total protein and albumin was performed, as were urinalysis, ECG and chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the CTC criteria [31] and serum chemistries. CBC was determined twice weekly.
Tumor measurements were performed after every two courses and evaluated according to the WHO criteria for response [32]. Patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis, whole blood samples (2.8 ml) in heparinized tubes were collected from an indwelling i.v. canula, prior to dosing, 15, 30, 45, minutes and 1, 1.5, 2.5, 3.5, 4.5, 8.5 and 12 hours after administration of the drug on day 1 and 8 of the first course. For the b.i.d. schedule this was done after the morning dose. The samples were immediately prepared and analyzed according to the method previously described [33]. The lower limit of quantitation (LLQ) was 0.1 ng/ml for topotecan lactone as well as for the hydroxy-acid. Area under the plasma concentration-time curves (AUC) of topotecan lactone and hydroxy-acid were calculated by noncompartmental analysis (linear-logarithmic trapezoideal method). The AUC(t) was calculated up to the last measured time point "t", because in half of the cases with b.i.d. administration the extrapolated part was ≥ 20% of the total AUC. The terminal half-life was calculated as ln2/k, where k is the elimination rate constant (h^{-1}). The AUC(t) was fitted to the observed percentage decrease in WBC using the sigmoidal E_{max} model [34]. For all calculations the

Siphar software package release 4.0 (Siphar SIMED, Cedex, Creteil, France) was used. For statistical analysis, linear regression analysis was employed to evaluate relationships between dose and dose/m^2 and AUC(t), and Pearson correlation coefficients were calculated.

Spearman rank correlation coefficients were calculated between AUC(t) and the percentage of decrease of leucocytes, granulocyts and platelets.

Table 1. Patient characteristics.

	Drug administration	
	O.D.	**B.I.D.**
Number patients entered	19	20
Number patients evaluable	19	18
Age		
median	53	55
range	19-85	41-69
Sex		
female	8	7
male	11	13
WHO performance		
0	12	12
I	7	7
II	0	1
Tumour types		
colorectal	4	11
ovarian	4	3
SCLC	3	1
NSCLC	2	1
miscellaneous	6	4
Prior treatment		
chemotherapy	11	14
radiotherapy	1	1
both	5	1
immunotherapy	0	1
none	2	3

RESULTS

A total of 39 patients entered on study (Table 1). All patients were eligible and 37 were fully evaluable for toxicity and response. Two patients with b.i.d. administration withdrew from the study during the first course, one patient after 3 days because of nausea and the second patient after 9 days because of abdominal cramping and weakness.

Most patients had prior chemotherapy. Dose levels studied were 0.5, 0.6, 0.7, 0.8 mg/m^2 b.i.d., and 1.0, 1.4, 1.6 mg/m^2/day o.d.. The total number of evaluable courses was 48 for o.d. and 64 for b.i.d. administration. The median number of courses per patient was 2 (range: 1-17).

Hematologic toxicity

O.D.schedule: Overall, haematologic toxicity was mild. CTC-grade III-IV leucopenia and granulocytopenia was observed in 3 (6.2%) and 2 (4.2%) out of 48 courses respectively (Table 2), with a day of onset for both on day 12, 14 and 19 and a duration of 8, 10 and 12 days. One patient had neutropenic fever lasting for 2 days.

Treatment delay was necessary in 2 patients because of slow recovery from grade II and grade III leucopenia, respectively.

One patient treated at 1.6 mg/m^2/day had CTC grade IV thrombocytopenia as DLT. In 4 courses (8.3%) CTC-grade III-IV thrombocytopenia was noted, twice in conjunction with leucopenia. The onset of thrombocytopenia CTC-grade III-IV was on day 12 in 3 patients and day 14 in 1 patient. Duration of thrombocytopenia was 8 and 12 days in the 2 patients with complete hematologic follow up. Five platelet transfusions were given in 2 (10.5%) patients. Anemia CTC-grade $\geq$ II occurred in 15 of 48 courses (31.2%), twenty three units of red blood cells were given to 7 (36.8%) patients to keep their hemoglobin $\geq$ 6.0 mMol/l.

B.I.D. schedule: CTC-grade III-IV leucocytopenia and granulocytopenia were both observed in 3 (4.7%) out of 64 courses (Table 2).

Table 2. Hematologic toxicity in 10 day administration of oral Topotecan. (CTC grades: worst per course)

Dose level mg/m^2	Number patients	Number courses	Leucocytes III	Leucocytes IV	Granulocytes III	Granulocytes IV	Platelets III	Platelets IV
1.0 o.d.	7 (3)*	17 (9)*	0	0	0	0	1	0
1.4 o.d.	9 (1)*	18 (1)*	0	2	0	2	0	2
1.6 o.d.	7	13	1	0	0	0	0	1
Total	**19**	**48**						
0.5 b.i.d.	3	26	0	0	0	0	0	0
0.6 b.i.d.	7	21	0	0	0	0	0	0
0.7 b.i.d.	6	15	0	1	0	1	0	1
0.8 b.i.d.	2	2	0	2	0	2	1	0
Total	**18**	**64**						

*: number between brackets is the number of patients also studied at this dose level but previously treated at a higher dose level.
o.d. = once daily administration. bid= twice daily administration.

The day of onset of leucopenia and granulocytopenia III-IV were similar and was day 8, 10 and 14, respectively. Duration of granulocytopenia was 8 and 14 days in the two patients with complete follow up. Granulocytopenia was complicated by fever in 3 patients, all were treated with empiric broad spectrum antibiotics. Both patients treated at DLT (0.8 mg/m^2 b.i.d.) had granulopenic fever, one patient died from pneumonia with sepsis. The third patient with granulopenic fever was treated at 0.7 mg/m^2 b.i.d..

Thrombocytopenia CTC-grade III-IV was noted in 2 courses (3.3%), with nadirs on day 12 and 14, both events occurring in conjunction with granulocytopenia requiring platelet transfusion (5 units in total).

Treatment never had to be delayed due to slow recovery from myelosuppression.

Anemia CTC-grade $\geq$ II occurred in 11 (17.2%) courses and 24 units of red blood cell transfusions were required in 7 (38.8%) patients.

Non-hematologic toxicity

O.D. schedule: Diarrhea was dose limiting toxicity in patients treated at 1.6 mg/m^2/day o.d. Two patients developed CTC grade III diarrhea in the first course with day of onset day 8 and day 9 and a duration of 5 and 3 days, respectively. Another patient at this dose level had CTC grade IV thrombocytopenia as DLT. Further dose escalation was not considered possible.

Diarrhea CTC-grade $\geq$ II was observed in a total of in 7 patients, with a median day of onset on day 9 (range 7-13) and a median duration of 4 days (range 1-7). Two patients treated at 1.4 mg/m^2/day had CTC grade III diarrhea One patient had diarrhea in the first course and was hospitalized because of concomittant nausea and vomiting. The second patient treated at 1.4 mg/m^2/ day developed CTC grade III diarrhea in the second course (1 day) and in the third course (4 days). Diarrhea did respond poorly to administration of loperamide. Other non-hematologic toxicities were mild. Mild nausea and vomiting could easily be circumvented with standard dopamine antagonists. CTC-grade III nausea and CTC-grade III vomiting each only occurred in 1 (2.1%) course.

Table 3. Non-hematologic toxicity in 10 day administration of oral topotecan. (CTC grades; worst per course).

Dose level mg/m^2	Number patients	Number courses	Nausea			Vomiting				Diarrhea				Fatigue		Anorexia		Abd. discomfort
			I	II	III	I	II	III	IV	I	II	III	IV	I	II	I	II	
1.0 o.d.	7 (3)*	17 (9)*	8	2	0	5	1	0	0	8	0	0	0	8	6	8	1	2
1.4 o.d.	9 (1)*	18 (1)*	2	2	1	1	2	1	0	4	5	3	0	3	2	5	0	5
1.6 o.d.	7	13	5	1	0	3	1	0	0	3	1	2	0	3	2	1	1	1
Total	**19**	**48**																
0.5 bid	3	26	6	0	0	3	1	0	0	3	0	0	8	8	0	0	0	3
0.6 bid	7	21	9	1	1	4	0	0	2	5	2	1	1	14	1	2	0	4
0.7 bid	6	15	8	0	1	3	1	0	0	2	3	1	1	7	1	3	0	5
0.8 bid	2	2	1	0	1	2	0	0	0	0	0	0	2	0	0	1	0	0
Total	**16**	**64**																

*: number between brackets is the number of patients also studied at this dose level but previously treated at a higher dose level. o.d.: once daily administration. bid: twice daily administration.

Table 4. Pharmacokinetics in 10 day administration of oral administration of oral topotecan. (median ± (standard deviation)).

Dose level mg/m²	Topotecan lactone day 1				Topotecan hydroxy-acid day 1				Topotecan lactone day 8				Topotecan hydroxy-acid day 8			
	AUC(t) ng.h/ml		t½ (h)		AUC(t) (ng.h/ml)		t½ (h)		AUC(t) (ng.h/ml)		t½ (h)		AUC(t) (ng.h/ml)		t½ (h)	
1.0 o.d. n=3	6.87	(1.61)	3.13	(0.10)	16.79	(2.97)	4.25	(0.44)	9.46	(3.70)	4.63	(2.39)	18.09	(6.35)	4.21	(1.18)
1.4 o.d. n=3	13.17	(2.38)	3.46	(0.14)	28.15	(6.73)	3.91	(0.48)	21.02	(7.56)	2.34	(0.74)	32.92	(5.63)	3.71	(0.64)
1.6 o.d. n=4	14.58	(5.68)	3.37	(11.64)	21.19	(10.00)	3.36	(1.04)	12.88	(3.82)	2.93	(0.74)	19.82	(6.74)	3.33	(0.57)
0.5 b.i.d. n=1	2.62		1.20		5.74		3.22		5.55		6.91		9.27		2.93	
0.6 b.i.d. n=4	5.13	(2.48)	2.09	(2.07)	8.38	(4.34)	3.22	(0.47)	7.64	(3.06)	2.03	(1.00)	15.04	(7.76)	2.81	(0.81)
0.7 b.i.d. n=4	5.64	(2.39)	3.55	(0.79)	10.57	(6.18)	5.48	(1.02)	7.34	(3.50)	3.24	(0.77)	12.85	(10.00)	3.18	(1.16)
0.8 b.i.d. n=1	16.73		3.17		31.56		-		-		-		-		-	

AUC(t) = area under the plasmaconcentration curve; OD = once daily administration ;BID = twice daily administration.
t½ = half life hours (h)
( ) = standard deviation
n = number of patients per dose level

Anorexia and fatigue was reported in 16 (33%) and 24 (50%) courses respectively (Table 3). Fatigue was mainly experienced during topotecan intake and subsided after a few days. It occurred in 6 (32%) patients. One patient withdrew from the study after 7 courses because of progressive fatigue and malaise. Other non-hematologic toxicities reported were: abdominal discomfort (16.7%), headache (4.2%) of courses, and alopecia CTC-grade I in 4 patients (21%). B.I.D. schedule: Diarrhea CTC grade IV in combination with CTC grade IV granulocytopenia occurred in both patients treated at 0.8 mg/m^2 b.i.d. Thus DLT also consisted of a combination of diarrhea and granulocytopenia. Diarrhea CTC-grade $\geq$ II occurred in a total of 9 patients, was always self limiting, had a median day of onset on day 10 (range 6-17) and had a median duration of 3 days (range 1-12).

Other non-hematologic toxicities were usually mild. Nausea and vomiting, CTC grade I-II, were observed in 25 (39.1%) and 14 courses (21.9 %.), respectively (Table 3). CTC-grade III nausea and CTC grade IV vomiting occurred in 3 (4.7%) and 2 (3.1%) courses. Other toxicities reported were: abdominal discomfort (18.7%), and headache (7.8%). Two patients developed alopecia CTC-grade I and 1 patient grade II. Skin rash, hematuria, cardiac, pulmonary, renal, hepatic or neurologic toxicity were not observed in both dose schedules studied.

Pharmacokinetics and dynamics

Pharmacokinetics were performed in 10 patients in each schedule of administration (Table 4). The AUC(t) of topotecan lactone was consistently higher on day 8 as compared to day 1, but for the once daily administration this did not reach statistical significance, while for the b.i.d. x10 administration it was significant ($p < 0.05$) (Table 4). The mean AUC(t) on day 8 of topotecan lactone once daily x10 was 14.0 $\pm$ 7.1 ng.h/ml and was 21.0% higher than the AUC(t) day 1 which was 11.6 $\pm$ 4.7 ng.h/ml. In the b.i.d.x10 schedule the mean AUC(t) day 8 was 8.0 $\pm$ 3.0 ng.h/ml which was 37.0% higher than the AUC(t) day 1 of 5.9 $\pm$ 4.3 ng.h/ml.

The interpatient variability (%CV) in AUC(t) of topotecan lactone on day 1 was 40.1% and 73.4 % for once daily and twice daily x10 administration respectively. (Figure 1). For both schedules of administration no significant

correlation was found between the dose (mg), dose level (mg/m2) and AUC(t) day 1 of topotecan lactone.

For both 10 days schedules the relation between AUC(t) day 1 versus percentage decrease of leucocytes was sigmoidal. The correlation coefficients of the AUC(t) day 1 of topotecan lactone and the percentage of decrease of leucocytes were R=0.61 (p=0.06) for o.d. x10 and R= 0.69 (p=0.03) for b.i.d. administration. The correlation between the AUC(t) day 1 of topotecan lactone and the percentage decrease of granulocytes was R=0.64 (p=0.08) for o.d. and R=0.-70 (p=0.03) for b.i.d. A significant correlation between the AUC(t) of topotecan lactone and the percentage decrease in platelets was observed in both dose schedules with R=0.83 (p=0.008) for o.d. x10 and R= 0.78 (p=0.03) for b.i.d. x10, respectively.

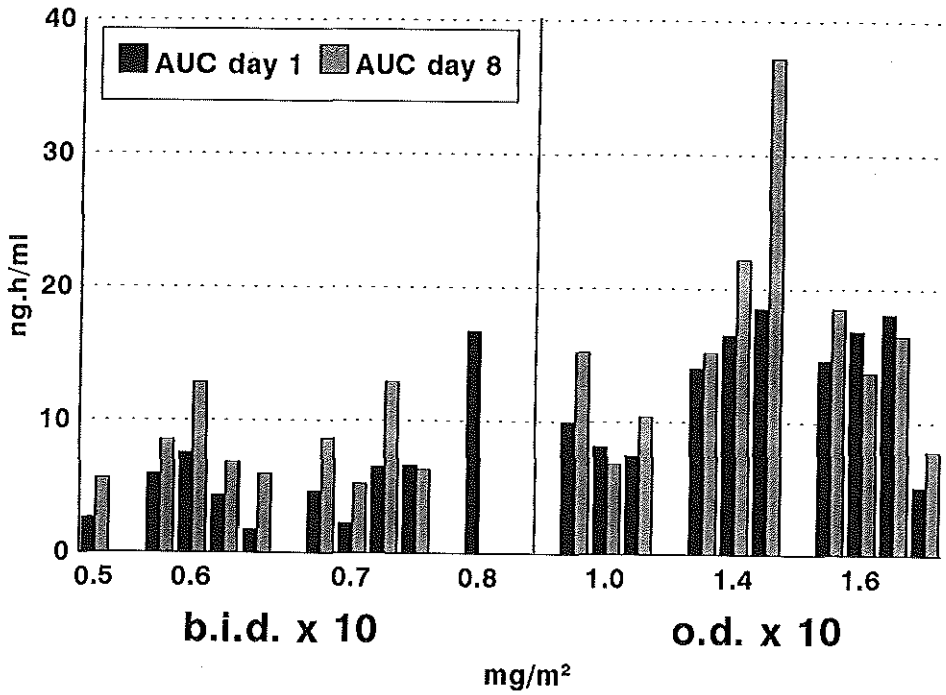

Figure 1. Interpatient variation of AUC day 1 versus AUC day 8 of Topotecan lactone

Responses

No complete or partial responses were noted. At the b.i.d x10 dose schedule, 1 patient with ovarian carcinoma had stable disease and was treated for 17 courses (51 weeks) at the dose level of 0.5 mg/m^2, and 2 patients with colorectal cancer had stable disease for 18 and 24 weeks. With the once daily administration, 1 patient with SCLC and 1 patient with carcinoma of unknown primary origin had stable disease for 18 and 21 weeks, respectively.

DISCUSSION

Topoisomerase I inhibitors have shown activity against a variety of malignancies and their clinical developement is rapidly progressing. Topotecan has extensively been studied using different schedules of intravenous dosing, revealing shortlasting non-cumulative neutro-penia and/or thrombocytopenia as the dose-limiting toxicity [35,36]. In vitro experiments and in vivo studies with human xenografts revealed a better anti-tumour effect with prolonged exposure to topoisomerase I inhibitors [20-25]; this dose scheduling of topoisomerase I inhibitors might be of importance in humans as well.

In a phase I study 21 days of continuous infusion (c.i.v.) of topotecan was well tolerated and some antitumor effects were seen [26]. The tolerability was confirmed in a recent phase II study in colorectal cancer although unfortunally the response rate was disappointing [37]. Continuous infusion is incovenient for patients and sometimes leads to complications of the central venous catheters [25,37]. Oral topotecan with a bioavailability of 32-44% and a moderate intra-patient variation may be more convenient for prolonged administration in patients. In a previous study with oral topotecan for 21-days out of every 28 days, diarrhea apppeared to be dose limiting with relatively mild hematologic toxicities [29]. Diarrhea occurred mainly after day 14. Because of this and in view of the fact that topoisomerase I down-regulation appeared to be optimal after 10-14 days [26], we studied the presently reported 10 day dosing schedules.

DLT consisted of diarrhea and myelosuppression and occurred at 1.6 mg/m^2 o.d.and at 0.8 mg/m^2 b.i.d. In the previously reported 21 days of b.i.d. oral topotecan diarrhea was the only DLT. Through all studies, diarrhea was always self limiting.

Diarrhea is a well known side effect of camptothecin and its derivatives, but the types of diarrhea appear to differ. CPT-11 adminstered intravenously induced delayed onset diarrhea with a median day of onset on day 5 and had a median duration of 5 days and was severe in 12 % of courses requiring hospitalisation [38]. This diarrhea can be treated by vigorous administration of loperamide to prevent hospitalisation. CPT-11 also induced acute onset early diarrhea on day 1 that can be treated by atropine [38]. Oral 20-S-camptothecin for 21 days q 28 days and oral 9-nitro-camptothecin for 5 days a week induced severe diarrhea in 40% and 33% of patients, respectively [39,40]. Diarrhea from these drugs apparently could be controlled easier. With 21 days c.i.v. topotecan diarrhea occurred in 13.6.% of patients [26]. 20-S-camptothecin and CPT-11 were shown to induce intestinal mucosal destruction in animal models [39,41]. The CPT-11 delayed onset diarrhea is related to the biliary excretion of the glucuronated SN-38 metabolite. The biliary index of SN-38 predicts the risk on delayed onset diarrhea of CPT-11 [42]. Thus local intestinal effects of camptothecin and its derivatives are responsible for the diarrhea. In our study on 21 days of oral topotecan b.i.d. diarrhea occurred in 23 % of patients with a median day of onset on day 15 and a duration of 8 days (range: 7-16) while vigorous administration of loperamide had no effect on the diarrhea [29]. With 10 days of oral topotecan diarrhea seems to last shorter with a median duration of 4 days (range 1-12). Although diarrhea from oral topotecan may be due to local efects to the intestinal mucosa, the exact mechanism of diarrhea is yet unknown. These findings suggest that the duration of diarrhea is related to the duration of oral topotecan administration.

Myelosuppression, in particular neutropenia, is a well known toxicity with the daily x 5 intravenous administration of topotecan with the nadir of granulocytes occurring between day 8 and 15 days, and myelosuppression reported to be brief (3-10 days) [9-12]. With 21 days c.i.v. administration of topotecan, WBC nadir occurred on day 18 (range:12-28) and platelet nadir on day 22 (range:11-28) [26].

In the present studies granulocytopenia nadirs occurred at day 12-19 (o.d.) and day 8-14 (b.i.d.), while cumulative myelotoxicity was not observed. Latter findings are consistent with previous reports of daily x5 administration of topotecan. In contrast cumulative myelotoxicity requiring dose reductions were reported for 21 days c.i.v. [26,37].

Anemia is a well documented side effect of i.v. topotecan [9,12-15,26,37]. In our present studies with the two 10 day schedules of oral topotecan anemia $\geq$ CTC-grade II occurred in 31.2% and 17.2% of courses, respectively. Marked inhibition of erythropoesis appears to occur in all intravenous and oral schedules of topotecan administration. Apart from diarrhea and similar to the experience with i.v. topotecan, other non-hematologic toxicities were mainly mild.

Pharmacokinetic analysis of topotecan o.d. x10 showed that at MTD level AUC(t) was twice as high as the AUC(t) of topotecan b.i.d. x10. Mean half lifes were similar and are in concordance with the half lifes of intravenous daily x5 administration. As in previous studies with topotecan a significant correlation of the AUC(t) day 1 topotecan and percentage decrease of leucocytes was found for both 10 days dose schedules, and a sigmoidal relationship (data not shown) could be established.

In conclusion: orally administered topotecan for 10 days every 21 days is feasible with DLT consisting of a combination of myelosuppression and diarrhea. The recommended phase II doses are 0.7 mg/m^2 b.i.d. and 1.4 mg/m^2 o.d..

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CHAPTER 6

FIVE DAYS OF ORAL TOPOTECAN (HYCAMTIN®), A PHASE I AND PHARMACOLOGIC STUDY IN ADULT PATIENTS WITH SOLID TUMORS

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ABSTRACT

Background and Purpose: Topotecan is a specific inhibitor of topoisomerase I. An oral formulation of topotecan is available with a bioavailability of 32-44% in humans.

A phase I and pharmacologic study of the oral formulation of topotecan administered daily for 5 days every 21 days was performed as a next step in defining the optimal oral dosing, moving from 21 days to 10 days (o.d. and b.i.d.) to 5 days in adult patients with solid tumors to determine the MTD.

Patients and Methods: Adult patients with a WHO performance status ≤ 2, adequate hematologic, hepatic and renal functions, with malignant solid tumors refractory to standard forms were entered. Pharmacokinetics were performed on day 1 and 4 of the first course using a validated high performance liquid chromatographic assay.

Results: 29 patients entered the study, all patients were evaluable for toxicity and response. Doses studied in 29 patients were 1.2, 1.8, 2.3, 2.7 mg/m^2/day and a fixed dose of 4 mg/day without surface area adjustment. A total of 109 courses were given. Dose limiting toxicity (DLT) was reached at a dose of 2.7 mg/m^2/day and consisted of CTC grade IV granulocytopenia. The maximum tolerated dose (MTD) was 2.3 mg/m^2/day. The regimen was well tolerated. Non hematologic toxicities were mild including fatigue, anorexia, nausea, vomiting and diarrhea.

A significant correlation was observed between the percentage decrease in WBC versus the AUC(t) of topotecan lactone ($R=0.76$ $p< 0.01$) which was modelled by a sigmoidal E_{max} function. The correlation coefficient between the absolute topotecan dose administered and AUC(t) was $R=0.52$ ($p=0.04$). Pharmacokinetics of the fixed dose of 4 mg/day were comparable to the 2.3 mg/m^2/day dose.

Conclusions: Dose limiting toxicity in this phase I study of daily x5 oral topotecan every 21 days was granulocytopenia. The recommended dose for phase II studies is 2.3 mg/m^2/day or alternatively, a fixed dose of 4 mg/day.

INTRODUCTION

Topotecan, 9-dimethylaminomethyl-10-hydroxycamptothecin, is a water soluble semi-synthetic analogue of camptothecin [1]. Like camptothecin, topotecan is a specific inhibitor of topoisomerase I. Topoisomerase I is a nuclear enzyme that resolves topological problems of torsionally strained (supercoiled) DNA. This is achieved by forming a covalent adduct between topoisomerase I and DNA, termed the cleavable complex. This catalytic intermediate creates single strand breaks, allowing the DNA molecule to rotate around the intact DNA strand at the cleavage site leading to a relaxation of the DNA molecule and in this way replication, transcription and other DNA functions can proceed. These enzyme-bridged breaks are then resealed by topoisomerase I [2-6].

Topoisomerase I inhibitors interfere with the breakage-reunion process by stabilizing the cleavable complexes thereby preventing the resealing of single strand DNA breaks in the presence of the drug. Cytotoxicity is more specific to the S-phase of the cell cycle in which double strand breaks occur due prolonged stabilisation of the cleavable complexes [7,8].

Phase I studies with single i.v. bolus daily for 5 days every 3 to 4 weeks, show a maximum tolerated dose of 1.5 mg/m^2/day. with myelosuppression, in particular neutropenia, as the dose limiting toxicity [9-12]. Non hematologic toxicities were usually mild and reversible. Phase II studies with this regimen given every 21 days showed promising results in patients with small cell lung cancer and in pretreated patients with ovarian cancer [13-19]. Recently the drug was registered for the latter indication. Other solid tumors appear much less sensitive to this regimen [20-29]. Daily intravenous administration of topotecan is inconvenient to patients, and oral administration would be a more convenient method of drug adminstration.

Recent studies in humans using oral administration of the i.v. formulation revealed a 32-44% bioavailability of oral topotecan [30,31]. No relationship was found between bioavailability of topotecan and age, gender, performance score and presence of liver metastasis [31]. We present a phase I study of oral topotecan once daily x5 every 3 weeks, a dose schedule comparable to the daily x5 intravenous

administration.

PATIENTS AND METHODS

Patient selection

Patients with a histologically confirmed diagnosis of malignant solid tumor refractory to standard forms of therapy were eligible. Other eligibility criteria included: age $\geq$ 18 years; Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2; an estimated life expectancy of $\geq$ 12 weeks; no previous anticancer therapy $\geq$ 4 weeks (6 weeks for nitroso-ureas or mitomycine C); adequate hematopoetic (WBC $\geq$ $4x10^9$/l and platelets $\geq$ $100x10^9$/l), hepatic (bilirubin within normal limits, AST ALT and/or alkaline phosphatase $\leq$ 2x normal), and renal function (serum creatinine $\leq$ 133 μmol/l (2.0 mg/dl)). Specific exclusion criteria included: active peptic ulcer or any gastrointestinal condition which could alter absorption or motility; patients taking H_2-antagonists or proton pump inhibitors. All patients gave written informed consent before entry in the study.

Treatment and dose escalation

Based on our previous data [32] using topotecan for 10 days once daily and given the human bioavailability data [30,31] the starting dose for the 5 day oral administration was set at 1.2 mg/m^2/day once daily. Courses were to be repeated every 21 days as tolerated. Dose escalations were based on the toxicity seen at the prior dose level. If no toxicity was seen in the prior dose, $\leq$ 100% dose escalation was allowed. However, if toxicity was seen, a dose escalation of 25-50% was prescribed. Three patients were to be entered at the lowest dose level, 6 or more patients were to be accrued on the higher dose levels.

The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose limiting toxicities (DLT), which were defined as CTC grade IV hematologic toxicity and/or non-hematologic toxicity $\geq$ CTC grade III during the first course in more than 2/6 patients. If neutropenia grade IV, throm-

bocytopenia ≥ grade III and/or nonhematologic toxicity ≥ grade III occurred during treatment days, topotecan administration was stopped immediately. Intrapatient dose escalation was not allowed.

For a patient with an average body surface area of 1.75 m^2 an absolute dose of 4 mg/day could be calculated at the achieved MTD dose level. Six patients with different body surface areas were to be studied, including pharmacokinetics, at this fixed dose to see whether dosing on a mg/m^2/day basis offered any advantage over the easier fixed dose.

Topotecan was supplied as capsules containing topotecan HCL, equivalent to either 0.25, 0.50 mg or 1.00 mg of the anhydrous free base. Capsules had to be stored between 2-8 degrees Celsius. Capsules were taken with a glass of water in the morning on an empty stomach with a period of 2 hours fasting thereafter. Patients were treated as outpatients.

Treatment assessment

Prior to therapy, complete medical history was taken and physical examination was performed. A complete blood count (CBC) including WBC differential, and serum biochemistry involving sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, creatinine, urea, uric acid, bilirubin, AST, ALT, alkaline phosphatase, total protein and albumin were performed, as were urinalysis, ECG and chest X-ray. Weekly evaluations included history, physical examination, serum chemistry and toxicity assessment according to the CTC criteria [33]. CBC was determined twice weekly. Tumor measurements were performed after every two courses and evaluated according to the WHO criteria for response [34]. Patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis, whole blood samples (2.8 ml) in heparinized tubes were collected from an indwelling i.v. canula, prior to dosing, 15, 30, 45, minutes and 1, 1.5, 2.5, 3.5, 4.5, 8.5 and 12 hours after administration of the drug on day 1 and 4 of the first course. The samples were processed and analyzed

according to the method previously described [35]. The lower limit of quantitation (LLQ) was 0.1 ng/ml for topotecan lactone as well as for the ring-opened hydroxy-acid. Area under the plasma concentration-time curves $(AUC)_{o\text{-}infinity}$ of topotecan lactone and hydroxy-acid were calculated by noncompartmental analysis (linear-logarithmic trapezoideal method). The terminal half-life was calculated as ln2/k, where k is the elimination rate constant (h^{-1}). The AUC was fitted to the observed percentage decrease in WBC using the sigmoidal E_{max} model [36]. For all calculations the Siphar software package release 4.0 (Siphar SIMED, Cedex, Creteil, France) was used. For statistical analysis, linear regression analysis was employed to evaluate relationships between dose and dose/m^2 and AUC, and Pearson correlation coefficients were calculated. Spearman rank correlation coefficients were calculated between AUC and the percentage of decrease of leucocytes, granulocytes and platelets.

RESULTS

A total of 29 patients entered on the study. Patient characteristics are given in Table 1. All patients were eligible and evaluable for toxicity and response. The total number of evaluable courses was 109. The median number of courses given per patient was 2 (range: 1-14).
Dose levels studied were 1.2, 1.8, 2.3, 2.7 mg/m^2/day and an additional dose level of 4 mg/day fixed dose.

Hematologic toxicity
CTC grade III-IV leucopenia and granulocytopenia were observed in 13 (11.9%) and 23 (21.1%) out of 109 courses, respectively (Table 2). Myelosuppression was dose limiting at 2.7 mg/m^2/day with both granulocytopenia and thrombocytopenia. CTC grade III-IV granulocytopenia occurred in all 7 patients at the dose level of 2.7 mg/m^2/day, 3 patients had concommitant CTC grade III-IV thrombocytopenia. Granulocytopenia CTC-grade IV was complicated in 1 patient by neutropenic fever lasting for 2 days.

Table 1. Patient characteristics

No. patients entered	29
No. patients evaluable	29
Age (years)	
Median	53
Range	27-72
Sex	
Female	16
Male	13
WHO Performance Status	
Median	0
Range	0-1
Prior treatment	
Chemotherapy	19
Radio- and chemotherapy	3
No prior therapy	7
Tumor types	
Colorectal	10
Ovarian	3
Hepatocellular	2
Breast	2
NSCLC	2
SCLC	2
Miscellaneous	8

CTC grade III-IV myelosuppression occurred in 4 out of 15 courses in 4 out of 8 patients treated with 2.3 mg/m^2/day, two of these patients were heavily pretreated with three prior chemotherapy regimens. The median day of onset of leucopenia CTC grade III-IV was day 12 (range: 9-15) with a median duration of

5.5 days (range: 3-10). CTC grade III-IV granulocytopenia occurred at a median of 11 days (range: 8-15) and its median duration was 6.5 days (range: 2-12).
In 8 courses (7.3%) CTC grade III-IV thrombocytopenia was observed, 7 times in conjunction with CTC grade III-IV granulocytopenia. Thrombocytopenia CTC grade III-IV occurred on day 14 (range: 10-15) and its median duration was 7 days (range: 2-12 days). In view of these side effects 2.3 mg/m^2/day topotecan for 5 days was considered to be the maximum tolerated dose.

Treatment delay due to prolonged myelosuppression occurred in 8 patients. One patient treated at the 4 mg/day dose level had treatment delays of 1 week in 3 out of 10 courses because of CTC grade II granulocytopenia on day 21. In 7 patients treatment delay of 1 week occurred after the first course because of slow recovery from CTC grade III-IV granulocytopenia. Anemia $\geq$ CTC grade II occurred in 37 (33.9%) of 109 courses in 17 patients (58.6%). A total of 54 units of packed cells were given to 14 patients over 27 (24.8%) courses. Platelet transfusions were given in 4 (3.7%) courses to 4 (13.8%) patients.

Table 2. Hematologic toxicities (CTC grades; worst per course)

Dose level	No patients	No courses	Leucocytes		Granulocytes		Platelets	
mg/m^2			III	IV	III	IV	III	IV
1.2	4(1)*	19	0	0	0	0	0	0
1.8	10(5)*	31	2	0	4	1	2	1
2.3	8	15	2	3	1	3	0	2
2.7	7	10	5	1	4	4	1	2
4 mg flat	6	34	0	0	6	0	0	0
Total	**29**	**109**						

*: number between brackets is the number of patients also studied at this dose level but previously treated at a higher dose level.

Table 3. Non-hematologic toxicities (worst per course) (CTC grade)

Dose level	No. courses	Nausea			Vomiting				Diarrhea				Fatigue		Anorexia		Abd.dis comfort
		I	II	III	I	II	III	IV	I	II	III	IV	I	II	I	II	
1.2	19	3	0	1	4	0	0	1	1	0	0	0	4	0	1	1	0
1.8	31	17	0	1	6	0	1	0	2	1	0	0	5	2	1	0	0
2.3	15	6	1	2	5	1	0	2**	1	2	0	0	6	0	3	1	4
2.7	10	6	1	1	5	2	0	0	3	1	0	1**	3	3	4	0	3
4 mg flat	34	23	0	0	10	2	0	0	2	3	0	0	7	0	1	0	3
Total	**109**																

**: In both patients relationship to TPT possible

In the patients treated with the 4 mg/day fixed dose no clinically relevant myelosuppression was seen, 6 courses with uncomplicated CTC grade III granulocytopenia occurred. In 1 patient treatment delays for one week occurred related to persistent CTC grade II granulocytopenia on day 21 in 3 out of 10 treatment cycles.

Non-hematologic toxicity

Non-hematologic toxicity was mild. The most frequent of these side effects are listed in Table 3. Nausea CTC grade III and vomiting CTC grade III-IV were observed in 5 (4.6%) and 4 (3.7%) of all courses respectively. Mild nausea and vomiting could be circumvented with standard antiemetics (peripheral dopamine antagonists).

Mild anorexia and mild fatigue were reported in 8 (27.6%) and 14 (48.3%) patients, moderate anorexia and fatigue occurred in 2 (6.9%) and 3 (10.3%) patients respectively.

Fatigue was experienced during topotecan intake or in the first week there after and subsided within a few days. In 1 patient (dose level 2.7 mg/m^2/day) fatigue was reason for 1 week delay of the next course. Alopecia CTC grade II occurred in 4 patients at the higher dose levels. Diarrhea $\geq$ CTC grade II was seen in 6 patients (20.7%) at all dose levels except 1.2 mg/m^2/day. Five patients had CTC grade II diarrhea which was self limiting and lasted 1-6 days. One patient who had disease progression of a peritoneal carcinomatosis of colon cancer developed CTC-grade IV diarrhea. Other mild non-hematologic toxicities reported were: upper abdominal discomfort (10 courses), headache during topotecan intake (2 courses) and CTC grade I stomatitis (2 courses).

At the 4 mg/day fixed dose level studied, 5 patients experienced only mild nausea, vomiting and mild fatigue, 1 patient CTC grade II diarrhea, and 1 patient CTC grade II alopecia. Skin rash, hematuria, liver or renal toxicity were not observed with topotecan administration.

Table 4. Pharmacokinetics of oral topotecan once daily x5 (median ± (S.D.)).

Dose level mg/m² n=No pts	Dose mg	Topotecan lactone day 1		Hydroxy acid day 1		Topotecan lactone day 4		Hydroxy-acid day 4	
		AUC ng.h/ml	t 1/2 h	AUC ng.h/ml	t 1/2 h	AUC ng.h/ml	t 1/2 h	AUC ng.h/ml	t 1/2 h
1.20 (n=3)	2.25 (0.14)	16.65 (4.58)	4.22 (1.17)	34.83 (6.28)	3.99 (2.43)	15.46 (4.31)	2.17 (1.57)	27.17 (11.20)	3.65 (0.63)
1.80 (n=3)	3.00 (0.38)	19.39 (3.57)	2.07 (1.19)	37.75 (8.07)	2.62 (1.23)	21.92 (2.99)	2.91 (0.12)	46.05 (11.17)	3.35 (0.19)
2.30 (n=6)	3.95 (0.25)	20.93 (7.15)	3.80 (1.09)	35.51 (11.04)	3.23 (0.64)	28.70 (10.87)	3.58 (1.36)	39.46 (20.29)	4.23 (1.27)
2.70 (n=4)	5.20 (0.45)	37.01 (13.90)	2.73 (0.60)	62.36 (26.70)	2.70 (1.11)	47.44 (11.90)	4.03 (0.46)	79.81 (41.31)	4.31 (0.34)
4 mg flat (n=6)	4.00	23.80 (8.81)	3.24 (2.78)	46.60 (14.95)	4.00 (0.50)	25.19 (6.65)	4.51 (2.37)	43.33 (10.20)	3.87 (0.88)

SD = standard deviation; N = No of patients studied at dose level; t1/2 = half life in hours;
AUC = Total area under the curve

Pharmacokinetics and dynamics

Pharmacokinetics were performed in 22 patients (Table 4). The AUC of topotecan lactone and hydroxy-acid showed substantial variation. The mean AUC of topotecan lactone for all patients on day 4 (27.7 ± 11.8 ng.h/ml) is similar to the AUC on day 1 (23.4 ± 9.7 ng.h/ml) (N.S.). The interpatient variation (%CV) in AUC of topotecan lactone on day 1 was 41.5% and the intrapatient variation was 18.5%. There was a low but significant correlation between doselevel and AUC of topotecan lactone day 1 (R=0.59 p=0.02). The correlation coefficient was R=0.52 (p=0.04) between the absolute dose of topotecan and AUC of topotecan lactone.

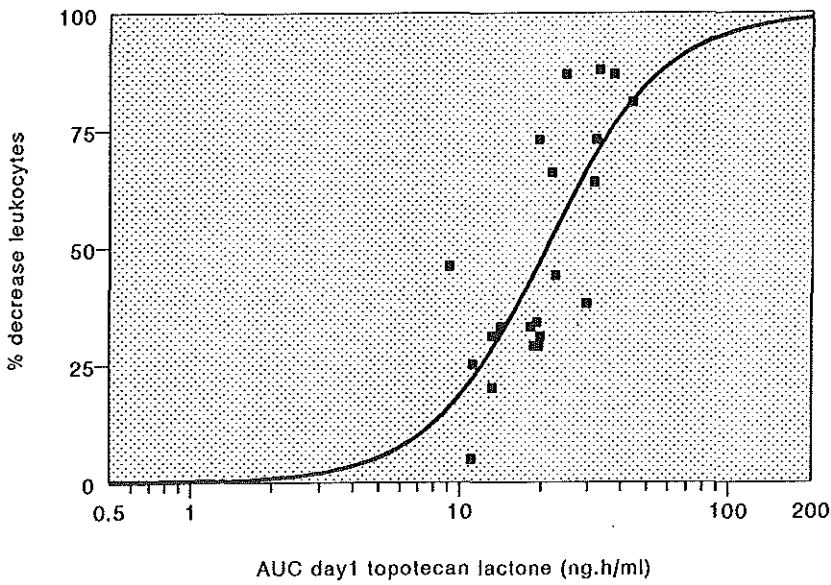

Figure 1. Sigmoidal relationship AUC topotecan and percentage decrease leucocytes

The correlation between the AUC of topotecan lactone and the percentage of decrease of granulocytes and platelets was significant with R=0.85 (p<0.01) and R=0.66 (P<0.01), respectively. A sigmoidal relationship (gamma of the slope: 3.06) was found between the AUC day 1 and percentage of decrease of leucocytes (Figure 1).

Finally the fixed dose of 4 mg/day topotecan for 5 days was studied in 3 female and 3 male patients of different height and weight and thus body surface area. Pharmacokinetic analysis of the 4 mg/day dose level revealed similar AUC and $T_{1/2}$ as compared to the dose of 2.3 mg/m^2/day (N.S.) (Table 4). At the 4 mg/day dose level the mean AUC of topotecan lactone was 22.8 $\pm$ 8.8 ng.h/ml and 23.0 $\pm$ 7.1 at the 2.3 mg/m^2/day dose level.

Responses

A minor response (40% tumor reduction) was seen in 1 patient with liver metastasis of gallbladder cancer lasting for 30 weeks. Stable disease was noted in 5 patients for a median duration of 30 weeks (range: 18 to 52 weeks).

DISCUSSION

Intravenous administration of topotecan daily for 5 days every 3 weeks has shortlasting non-cumulative neutropenia and/or thrombocytopenia as dose limiting toxicity [9-12]. The daily x5 intravenous administration of topotecan has shown antitumor activity in a variety of malignancies such as ovarian cancer and SCLC, and was recently registered in Europe and USA for the second line treatment of patients with ovarian cancer [13-19].

A bioavailability of 32-44% of oral topotecan with moderate intrapatient variation creates the opportunity to study different dose schedules for oral administration.

In the present study topotecan was adminstered daily for 5 days every 3 weeks similar to the most frequently studied and recently registered schedule of i.v. administration [13-29].

DLT with oral topotecan daily x5 every 3 weeks was myelosuppression which is comparable to the i.v daily x5 schedule. The nadirs of leucopenia and granulocytopenia were at day 12 and 11 and lasted 5.5 and 6.5 days respectively. The day of onset of granulocytopenia is in concordance with previous studies of i.v. topotecan daily x5 in which the nadir of granulocytes was reported between

day 8 and 15. The median duration of granulocytopenia was 6.5 days (range: 2-12 days) with orally administered topotecan, the duration was reported 3-5 days in the studies with intravenous administration [9-12].

DLT was reached at 2.7 mg/m^2/day and MTD was determined as 2.3 mg/m^2/day. At 2.3 mg/m^2/day ultimately 8 patients were included, in 4 patients CTC grade III-IV uncomplicated neutropenia occurred. However, 2 of these 4 patients were heavily pretreated with three prior chemotherapy regimens. We considered the 2.3 mg/m^2/day dose level as MTD.

Assuming an average body surface area of 1.75 m^2 an average fixed dose of 4 mg/day was calculated at the MTD of 2.3 mg/m^2/day. Although this flat dose was only studied for pharmacokinetic purposes, it is remarkable that myelotoxicity of topotecan seemed limited to uncomplicated granulocytopenia grade III. However at this dose level 2 out of 6 patients studied had been pretreated with two prior chemotherapy regimens in contrast to 4 out of 8 patients treated at the 2.3 mg/m^2/day dose level who were pretreated with $\geq$ 2 chemotherapy regimens. In other words patient selection might explain the difference in toxicity, as discussed later, pharmacokinetics were not dissimilar.

Anemia $\geq$ CTC grade II occurred in 17 patients (58.6%) in 24.8% of courses. Anemia is a well documented toxicity occurring in 11-37% of phase I-II studies with i.v. administration of TPT [13-15,19,22]. Similar to daily x5 i.v. topotecan, non-hematologic toxicities with the oral formulation were mild and consisted mostly of nausea and vomiting which could be easily controlled with conventional antiemetics. Other non-hematologic toxicities consisted of fatigue, anorexia, upper abdominal discomfort. In contrast to the i.v. daily x5 administration were diarrhoea if occuring is always CTC grade $\leq$ 1, diarrhoea $\geq$ CTC grade II occurred in 20.7% of patients with oral administration of topotecan. Diarrhoea was mild in the majority of cases and always self limiting.

In a phase I study with oral administration of topotecan twice daily for 21 days every 28 days DLT consisted of diarrhea with a median day of onset on day 15 and a median duration of 8 days (range 7-16) [37]. Prolonged daily oral administration appears to have more intestinal side effects with more vigorous diarrhea of longer duration. Diarrhea $\geq$ CTC grade II has been reported as severe

toxicity in patients treated with oral administration of 20-S-camptothecin (40%) and 9-nitro-camptothecin (38%) [38,39]. In patients treated with intravenous CPT-11 and 20-S-camptothecin diarrhea had been reported as severe toxicity [40-42]. In view of the difference between results from oral and i.v. topotecan, one possibility is that diarrhea of oral topotecan is due to local effects at the intestinal mucosa. However the exact mechanism of the cause diarrhea is unknown, but oral exposure of topotecan of limited duration seems feasible and should certainly be considered.

Pharmacokinetic analysis were performed at all dose levels showing a low but significant correlation between the dose levels and the area under the plasma curve of the lactone form of topotecan. Pharmacokinetics were studied at a fixed dose of 4 mg/day in order to see if dosing on a mg/m^2 basis offered any advantage over the easier fixed dose. Pharmacokinetics showed no differences between patients treated with 4 mg/day and patients treated at the 2.3 mg/m^2/day dose level. AUC and $t_{1/2}$ were similar. Non-hematologic toxicities observed were comparable. Differences in myelotoxicity with the two dosages compared could be explained from differences in levels of pretreatment of patients as stated previously. Significant sigmoidal relationships were established between AUC of topotecan lactone and the percentage of decrease of leucocytes, granulocytes and platelets. Compared to the daily x 5 i.v. administration, oral topotecan resulted in a lower systemic exposure of the drug.

Since the side effects are nevertheless similar, an appropriate explanation for the difference is lacking. However since oral topotecan showed significant myelotoxocity and data on a dose-response relationship are lacking from studies with the i.v. formulation of topotecan, systemic exposure from oral administration may still be sufficient to induce anti-tumor effects. Six patients in our study with stable disease were treated for 6 to 14 courses with topotecan, no cumulative hematologic or non-hematologic toxicity occurred.

In conclusion: orally administered TPT given daily for 5 days every 3 weeks has myelosuppression as DLT. The recommended dose for phase II studies is 2.3 mg/m^2/day, or alternatively a fixed dose of 4 mg/day. Further studies are needed to fully explore the potential of oral topotecan as an antitumor agent.

Studies comparing daily x5 i.v to daily x5 oral as well as a phase I study of oral topotecan combined with i.v. cisplatinum are ongoing.

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CHAPTER 7

A COMPARISON OF CLINICAL PHARMACODYNAMICS OF DIFFERENT ADMINISTRATION SCHEDULES OF ORAL TOPOTECAN (HYCAMTIN®).

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SUMMARY

Background and Purpose: Prolonged exposure to topotecan in in vitro and in vivo experiments yielded the highest antitumor efficacy. An oral formulation of topotecan with a bioavailability of 32-44% in humans enables convenient prolonged administration. Pharmacokinetic/pharmacodynamic relationships from 4 phase I studies with different schedules of administration of oral topotecan in adult patients with solid tumors were compared.

Patients and Methods: 99 patients with malignant solid tumors refractory to standard forms of chemotherapy were entered on 4 phase I studies. Topotecan was administered: once daily (o.d.) x5 q 21 days (29 pts), o.d. x10 q 21 days (19 pts), twice daily (b.i.d.) x10 q 21 days (20 pts) and b.i.d. x21 q 28 days (31 pts). - Pharmacokinetic analysis was performed in 55 patients using a validated high performance liquid chromatographic assay and non-compartmental pharmacokinetic methods.

Results: A total of 109 (o.d.x5), 48 (o.d.x10), 64 (b.i.d.x10) and 59 (b.i.d.x21) courses were given. Dose limiting toxicity (DLT) consisted of granulocytopenia (o.d.x5), a combination of myelosuppression and diarrhea in both 10 day schedules, and diarrhea only in the 21 day schedule. Pharmacokinetics revealed a substantial variation of the AUC of topotecan lactone in all dose schedules with a mean intrapatient variation of 25.4 $\pm$ 31.0% (o.d. x5), 34.5 $\pm$ 25.0% (o.d. x10), 96.5 $\pm$ 70.1% (b.i.d. x10) and 59.5 $\pm$ 51.0% (b.i.d. x21). Significant correlations were observed between myelotoxicity parameters and AUC(t) day 1 and AUC(t) per course of topotecan lactone. In all studies similar sigmoidal relationships could be established between AUC(t) per course and the percentage decrease of WBC. At MTD dose level, no significant difference in AUC(t) per course was found: AUC(t) per course was 107.4 $\pm$ 33.7 ng.h/ml (o.d.x5), 145.3 $\pm$ 23.8 ng.h/ml (o.d.x10), 100.0 $\pm$ 41.5 ng.h/ml (b.i.d.x 10), and 164.9 $\pm$ 92.2 ng.h/ml (b.i.d.x21), respectively.

Conclusions: For oral topotecan, schedule rather than AUC(t) per course appeared to be related to the type of toxicity. Prolonged oral administration results in intestinal side effects as DLT, short term administration results in

granulocytopenia.
Based upon this pharmacokinetic study no schedule preference could be expressed. Based upon patient convenience the once daily x5 administration could be favoured.

INTRODUCTION

Topotecan, 9-dimethylaminomethyl-10-hydroxycamptothecin, is a water soluble semisynthetic analogue of camptothecin [1]. Like camptothecin, topotecan is a specific inhibitor of topoisomerase I. Topotecan administered daily by 30 minutes infusion on 5 subsequent days every 3 weeks results in brief myelosuppression as the most important side effect [2-5]. Antitumor activity was reported in patients with small cell lung cancer [6,7] and in pretreated patients with ovarian cancer [8-12]. Recently topotecan was registered in Europe and the USA for the latter indication. Cytotoxicity of topoisomerase I inhibitors is more specific to the S-phase of the cell cycle in which double strand breaks occur [13-19].
Preclinical in vitro and in vivo studies indicate that prolonged exposure to low dose topoisomerase I inhibitors is most efficacious [20-25]. The feasibility of the concept of prolonged exposure to topotecan in humans was initially reported by Hochster et al in a phase I study using a 21-days continuous infusion [26]. Myelosuppression was the dose limiting toxicity and antitumor activity was seen. Continuous infusion though is relatively patient inconvenient. Recent studies in humans reported a 32-44% bioavailability of the intravenous formulation of topotecan when given orally [27,28]. Oral administration would be a more simple and perhaps a more convenient method to achieve prolonged exposure.
We performed 4 phase I and pharmacologic studies with different schedules of oral administration of topotecan in adult patients. The present analysis was performed to see if from a pharmacokinetic/pharmacodynamic point of view there was a preference for a particular schedule to be taken forward for further development.

Chapter 7

PATIENTS AND METHODS

Patient selection

Patients with a histologically confirmed diagnosis of malignant solid tumor refractory to standard forms of therapy were eligible. Eligibility criteria included: age $\geq$ 18 years; WHO performance status $\leq$ 2; an estimated life expectancy of $\geq$ 12 weeks; no previous anticancer therapy $\geq$ 4 weeks (6 weeks for nitroso-ureas or mitomycin C); adequate hematopoetic (WBC $\geq$ $4x10^9$/l and platelets $\geq$ $100x10^9$ /l), hepatic (bilirubin within normal limits, AST,ALT and/or alkaline phosphatase $\leq$ 2x normal), and renal function (serum creatinine $\leq$ 133 μmol/l (2.0 mg/dl). Specific exclusion criteria included: active peptic ulcer or any gastro-intestinal condition which could alter absorption or motility; patients taking H_2-antagonists or proton pump inhibitors. All patients gave written informed consent.

Treatment and dose escalation

Oral administration of topotecan was studied in 4 phase I studies: twice daily for 21 days every 28 days, once or twice daily for 10 days every 21 days, and once daily for 5 days every 21 days. The 21 day administration was studied based on the 21 days continuous intravenous administration [26,29,30]. In view of the relatively short half-life of topotecan the twice daily dosing was given. Dose levels studied were 0.15, 0.3, 0.4, 0.5 and 0.6 mg/m^2 twice daily, resulting in total daily doses of 0.3, 0.6, 0.8, 1.0 and 1.2 mg/m^2, respectively. The 10 day schedules were studied because of severe diarrhea occurring in the third week of the 21 day administration of oral topotecan, and the finding that topoisomerase I down-regulation was optimal after 10-14 days with continuous infusion of topotecan [26,30,31]. Dose levels studied with the 10 days administration were 0.5, 0.6, 0.7, 0.8 mg/m^2 b.i.d., and 1.0, 1.4, 1.6 mg/m^2/day o.d. The reduction from 2 to 1 administration per day was intended to reduce gastrointestinal toxicities. A daily x5 q 21 days administration was based on the daily x5 i.v. administration, with dose levels being 1.2, 1.8, 2.3 and 2.7 mg/m^2/day.

Dose escalations were based on the toxicity seen at the prior dose level. If no toxicity was seen in the prior dose, $\leq$100% dose escalation was allowed.

However, if toxicity was seen, a dose escalation of 25-50% was prescribed. The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose limiting toxicities (DLT), which were defined as CTC grade IV hematologic toxicity and/or non-hematologic toxicity ≥ CTC grade III during the first course in more than 2/6 patients. Intrapatient dose escalation was not allowed. Topotecan was supplied as capsules which were taken with a glass of water on an empty stomach with a 2 hours period of fasting. With twice daily administration an interval of 12 hours was chosen and for the second daily dose capsules were taken with a glass of water at least 10 minutes before meals, preferably on an empty stomach. Patients were treated as outpatients.

Treatment assessment

Prior to therapy and weekly during therapy, evaluations were performed including history, physical examination, toxicity assessment according to the CTC criteria and serum chemistries [32]. CBC were determined twice weekly. Tumor measurements were performed after every two courses and evaluated according to the WHO criteria for response [33]. Patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis, whole blood samples (2.8 ml) in heparinized tubes were collected during the first course, prior to dosing, 15, 30, 45 minutes and 1, 1.5, 2.5, 3.5, 4.5, 8.5 and 12 hours after administration of the drug on day 1 and on day 4 (o.d. x5), or day 8 (x10 and x21 schedules). For the twice daily dosing schedules pharmacokinetic samples were taken following the morning dose. The samples were immediately processed and analyzed according to a method previously described [34].

Area under the plasma concentration-time curves (AUC) of topotecan lactone and hydroxy-acid were calculated by noncompartmental analysis (linear-logarithmic trapezoidal method). Because >20% extrapolation was needed to calculate the total AUC of topotecan lactone in most cases of the b.i.d. x21 and b.i.d x10 administration, pharmacokinetic-pharmacodynamic analysis was carried out with

AUC(t) in all studies. AUC(t) was calculated up to the last measured time point "t". In all patients samples were obtained up to 12 hours after drug intake. The terminal half-life was calculated as ln2/k, where k is the elimination rate constant (h^{-1}). The AUC(t) day 1 and AUC (t) per course were fitted to the observed percentage decrease in WBC using the sigmoidal E_{max} model [32]. The AUC(t) per course was calculated by multiplying the AUC(t) day 1 with the number of doses per course. For all calculations the Siphar software package release 4.0 (Siphar SIMED, Cedex, Creteil, France) was used. Spearman rank correlation coefficients were calculated between AUC(t) day 1 and AUC(t) per course and the percentage of decrease of WBC, granulocytes and platelets.

Two way analysis of variance was used to compare AUC(t) per course for the different schedules at MTD dose level. Analysis of variance was used for analysis on difference in myelotoxicity, diarrhea, C_{max} day 1, and intrapatient variation. Intrapatient variation was calculated as follows:

$$\frac{AUC\ day\ 1\ -\ AUC\ day\ 4/8}{AUC\ day\ 1}\ x\ 100\%$$

Cytotoxicity of topoisomerase I inhibitors is cell cycle specific. Duration of exposure seems important for antitumour effects. In vitro experiments with continuous exposure of topotecan were performed with a minimum concentration of 100 ng/ml [23], and steady state plasma concentrations were 0.62 and 4.4 ng/ml in studies in humans with 21 day continuous infusion [26,29]. An arbitrary threshold plasma concentration of >1 ng/ml was chosen to study the differences in duration of exposure in the schedules used. Duration of time of topotecan >1 ng/ml per course was calculated from the duration measured on day 1 multiplied with the number of doses per course.

RESULTS

A total of 99 eligible patients were entered into the studies of whom 96 were

evaluable for toxicity. Pharmacokinetic analysis could be performed in 55 patients. The patient characteristics are given in Table 1. The median WHO performance status of patients was: 0 (range 0-2). The majority of patients recieved prior chemotherapy.

Table 1. Patient characteristics oral topotecan

	Once Daily x5		Once Daily x10		Twice Daily x10		Twice Daily x21	
Number pts								
entered:	29		19		20		31	
evaluable:	29		19		18		30	
Age								
median (range)	53	(27-72)	53	(19-85)	55	(41-69)	55	(33-73)
WHO performance								
median (range)	0	(0-I)	0	(0-I)	0	(0-I)	0	(0-II)
Tumor types	10		4		11		12	
colorectal	3		4		3		3	
ovarian	2		2		2		2	
NSCLC	2		3		1		0	
SCLC	2		0		0		2	
breast	2		1		0		0	
Hepatocellular	8		5		3		12	
miscellaneous								
Prior therapy	19		11		14		14	
chemotherapy	0		1		1		2	
radiotherapy	3		5		1		14	
both	7		2		3		1	
none	0		0		1		0	
immunotherapy								
N° of courses	10		48		64		59	
median (range)	9	(1-14)	2	(1-7)	2	(1-17)	2	(1-10)
	2							

pts = patients; No = number; (N)SCLC = (Non) Small Cell Lung Cancer.

Table 2. Toxicities in patients treated with oral topotecan (CTC grades. Worst per course.)

Schedule	Dose levels*	No pts	No courses	Leucocytes		Granulocytes		Platelets		Nausea	Vomiting		Diarrhea	
				III	IV	III	IV	III	IV	III	III	IV	III	IV
Once daily	All	29	109	9	4	15	8	3	5	5	1	3	0	1
x5	MTD	8	15	2	3	1	3	0	2	2	0	2**	0	0
Once daily	All	19	48	1	2	0	2	1	3	1	1	0	5	0
x10	MTD	9 (1)[1]	18 (1)[1]	0	2	0	2	0	2	1	1	0	3	0
Twice daily	All	18	64	0	3	0	3	1	1	3	0	2	2	3
x10	MTD	6	15	0	1	0	1	0	1	1	0	0	1	1
Twice daily	All	30	59	4	2	1	2	1	2	0	0	0	1	6
x21	MTD	8	?	2	2	0	2	0	2	0	0	0	0	2

* All = pts studied at all dose levels
MTD = pts studied at MTD dose level

** in both patients relationship to topotecan possible

[1] number between brackets is the number of patients also studied at this dose level but previously treated at a higher dose level

Hematologic toxicity

Occurrence of CTC-grade III-IV leucocytopenia and granulocytopenia with the various schedules is listed in Table 2. They were observed in 11.9% and 21.1%, of courses at the daily x5 administration, 6.2% and 4.2% of courses of o.d. x10, both 4.6% of courses with b.i.d. x10, and 10.2% and 5.1% of courses for b.i.d. x21, respectively.

Granulocytopenia was significantly ($p < 0.001$) more frequent in the daily x5 administration as compared to the other schedules, for leucocytopenia this was not different.

At MTD, granulocytopenia was more frequent with the daily x5 administration, were the median duration was 6.5 days (range: 2-12 days), while it was never complicated by fever. At MTD, granulocytopenia was relatively mild in the b.i.d. x21 and o.d. x10 schedules. Granulocytopenia was complicated by fever in one patient treated at MTD with the b.i.d. x10 administration.

CTC-grade III-IV thrombocytopenia was noted in 8 (7.3%), 4 (8.3%), 2 (3.1%) and 3 (5.1%) courses of the o.d.x5, o.d.x10, b.i.d.x10 and b.i.d.x21 administration (N.S.), most often in conjunction with CTC grade III-IV leucocytopenia.

Non-hematologic toxicity

Diarrhea CTC-grade III-IV was seen in 1.0% of courses o.d.x5, 10.5% of courses o.d.x10, 7.8% of courses at b.i.d.x 10, and 11.9% of courses with b.i.d.x21 ($p=0.03$). Other non-hematologic toxicities were infrequent and usually mild with all schedules studied (Table 2).

Diarrhea was the only DLT at 0.6 mg/m^2 b.i.d for the 21 day administration. DLT consisted of a combination of myelosuppression and diarrhea at 0.8 mg/m^2 b.i.d. x10 and 1.6 mg/m^2 o.d. x10. Granulocytopenia was DLT at 2.7 mg/m^2 o.d. x5. At MTD, no CTC-grade III-IV diarrhea occurred with the daily x5 administration. CTC-grade IV diarrhea was seen in 2/8 patients treated at MTD with the 21 day schedule. For the different schedules of administration MTDs were 0.5 mg/m^2 b.i.d. x21, 0.7 mg/m^2 b.i.d. x10, 1.4 mg/m^2/day x10 and 2.3 mg/m^2/day x5.

Table 3. Pharmacokinetics after oral administration of topotecan in patients treated at MTD (Median (range); SD = standard deviation)

Schedule	No pts at MTD (MTD, mg/m²/day)	AUC(t) Topotecan day 1 (ng.h/ml)	AUC(t) Topotecan per course	AUC(t) Topotecan per week	C_{max} Topotecan day 1 (ng/ml)
o.d. x5	n = 6	19.6	97.9	32.6	8.4
q 3 weeks	(2.3)	(13.1-33.0)	(65.7-165.0)	(21.9-55.0)	(4.6-11.1)
		SD: 6.7	SD: 33.7	SD: 11.2	SD: 2.2
o.d. x10	n = 3	13.2	131.7	43.9	3.3
q 3 weeks	(1.4)	(13.1-17.3)	(131.4-172.8)	(43.8-57.6)	(3.0-6.8)
		SD: 2.4	SD: 23.8	SD: 7.9	SD: 3.3
b.i.d. x10	n = 4	5.6	111.1	37.2	1.3
q 3 weeks	(1.4)	(2.2-6.6)	(44.20-132.8)	(14.7-44.3)	(0.7-2.4)
		SD: 2.1	SD: 41.5	SD: 13.8	SD: 0.7
b.i.d. x21	n = 4	4.1	172.6	43.1	1.6
q 4 weeks	(1.0)	(1.6-5.9)	(68.5-246.1)	(17.1-61.5)	(0.9-2.4)
		SD: 2.2	SD: 92.2	SD: 23.1	SD: 0.6

Pharmacokinetics and dynamics

The AUC(t) of topotecan lactone was consistently higher on day 4 (o.d. x5) and day 8 (10 and 21 day schedules) compared to day 1. Significant correlations were found between AUC(t) day 1 and day 4/8 with R=0.81 (p=0.001) (o.d. x5), R=0.76 (p=0.01) (o.d.x10), R=0.74 (p=0.02) (b.i.d. x10) and R=0.95 (p=0.001) (b.i.d. x21). In the b.i.d. x10 schedule AUC(t) day 8 was significantly higher compared to day 1 ($p < 0.05$). Thus, limited cumulation of topotecan occurred in this schedule. Bearing this in mind, the mean intrapatient variation of AUC(t) topotecan lactone was 25.4% ± 31.0% (o.d.x5)(n=22), 34.5% ± 25.0% (o.d.x10) (n=10), 96.5% ± 70.1% (b.i.d.x10) (n=10) and 59.5 ± 51.0% (b.i.d. x21) (n=13), respectively. Intrapatient variation appeared lower in the o.d. dose schedules because of a more limited increase of AUC(t) topotecan lactone as compared to the b.i.d. schedules. Interpatient variation (% CV) was 43.1% (o.d.x5), 40.1% (o.d. x10), 73.4% (b.i.d.x10) and 59.1% (b.i.d. x21).

C_{max} was correlated to percentage of decrease of granulocytes (R=0.55; p=0.02) for o.d. x5, and (R=0.72; p=0.02) for b.i.d. x21. C_{max} did not correlate with other myelotoxicity parameters. T_{max} and $t_{1/2}$ were of similar magnitude for all 4 dose schedules (N.S.)

The AUC per course at MTD was not significantly different between the 4 schedules of oral administration (Table 3). The resulting mean AUC per course at MTD was 107.4 ± 33.7 ng.h/ml for o.d. x5, 145.3 ± 23.8 ng.h/ml for o.d. x10, 100.0 ± 41.5 ng.h/ml for b.i.d. x10 and 164.9 ± 92.2 ng.h/ml for b.i.d. x21 (N.S.). The mean AUC per week at MTD dose level, a measure for dose intensity, was 35.8 ± 11.2 ng.h/ml (o.d. x5), 48.4 ± 7.9 ng.h/ml (o.d. x10), 33.3 ± 13.8 ng.h/ml (b.i.d. x10) and 41.2 ± 23.1 ng.h/ml (b.i.d. x21), respectively. (Table 3) (N.S.).

Calculating AUC per course at MTD from AUC(t) day 4/8 resulted in an AUC per course of 124.8 ± 50.2 ng.h/ml (o.d. x5), 217.2 ± 75.6 ng.h/ml (o.d. x10), 164.1 ± 70.0 ng.h/ml (b.i.d. x10) and 229.4 ± 79.5 ng.h/ml (b.i.d. x21), respectively (N.S.).

Duration of time of topotecan lactone >1 ng/ml per course was lowest in the o.d x5 administration with a mean duration of 20.1 ± 7.9 h/course. Duration of

topotecan >1 ng/ml per course was 26.5 ± 13.6 h (o.d.x10), 47.9 ± 49.2 h (b.i.d.x10) and 44.6 ± 12.2 h (b.i.d. x21), respectively. Duration of time of topotecan lactone >1 ng/ml per course was significantly lower (p=0.006) for the 5 day o.d. schedule compared to the 10 and 21 b.i.d. schedules.

The correlation between topotecan lactone >1 ng/ml per course with the percentage of decrease of leucocytes was low for o.d. x5 (R=0.44 p=0.04), but higher in the 10 day schedules with R=0.74 p=0.02 (o.d. x10) and R=0.99 p=0.0001 (b.i.d. x10).

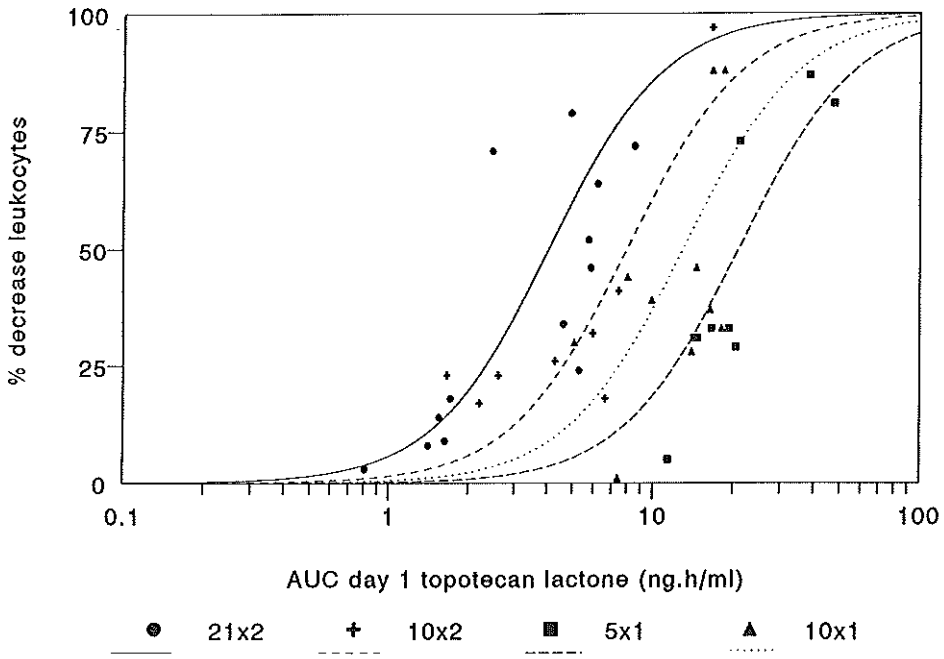

Figure 1. Sigmoidal relationship between AUC day 1 and percentage decrease of leucocytes (all oral studies).

The correlation for the 21 day schedule could not be calculated reliably. The correlation between the AUC(t) day 1 of topotecan and the percentage of decrease of leucocytes is significant in 3 schedules of administration with correlation

coefficients of R=0.76 (p= 0.001) (o.d. x5), R= 0.69 (p=0.03) (b.i.d. x10) and R=0.66 (p=0.03) for b.i.d.x21 administration. The correlation between AUC(t) day 1 topotecan and percentage decrease of leucocytes showed a same trend for the o.d. x10 administration (R=0.61 p=0.06). The relationship between the AUC(t) day 1 of topotecan lactone and the percentage of decrease of leucocytes could be fitted best using a sigmoidal E_{max} model (Figure 1).

A significant correlation between the AUC(t) of topotecan lactone and the percentage decrease of platelets was observed in the 10 day dose schedules with R= 0.78 (p=0.03) b.i.d. and R= 0.83 (p=0.01) o.d. and in the 5 day schedule (R=0.60 p=0.004). Thus, significant correlations with myelotoxicity parameters are found with all schedules.

When plotting AUC day 1 and day4/8 per course against the percentage decrease of leucocytes all the sigmoidal curves showed a similar pattern (Figure 2 a+b).

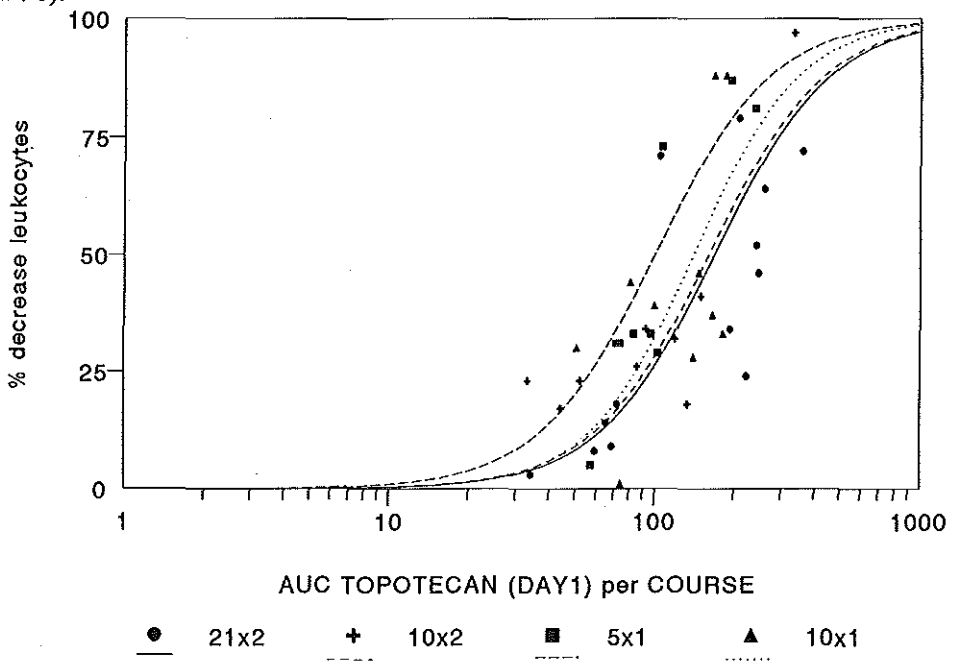

Figure 2a. Sigmoidal relationship between AUC (day 1) per course and percentage decrease of leucocytes (all oral studies).

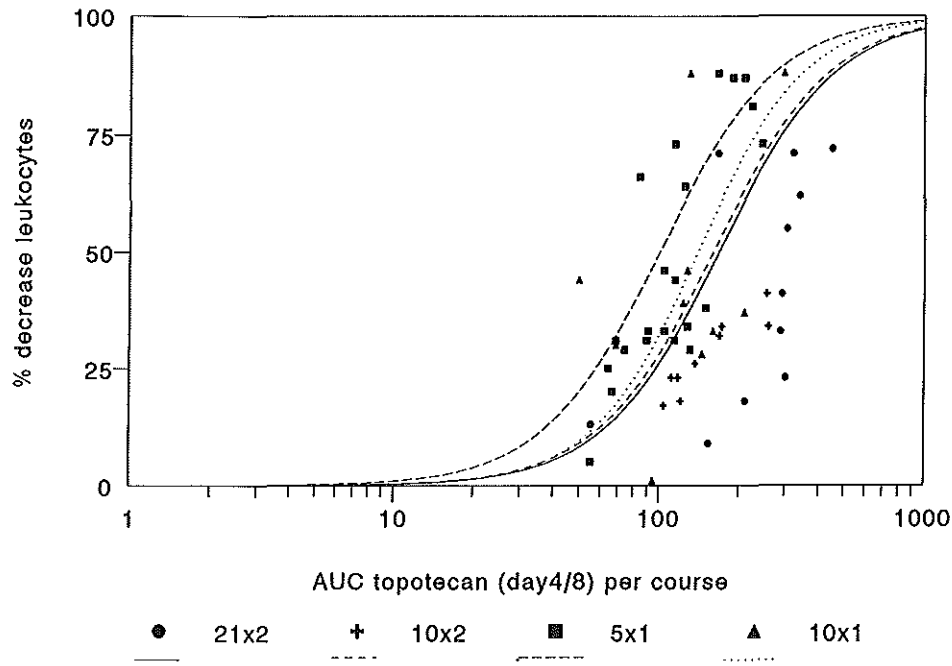

Figure 2b. Sigmoidal relationship between AUC (day 4/8) per course and percentage decrease of leucocytes (all oral studies).

DISCUSSION

In vitro experiments and in vivo studies with human xenografts revealed that prolonged exposure rather than concentration was relevant to achieve antitumor activity with topoisomerase I inhibitors [20-25]. Based upon these studies scheduling of administration of topoisomerase I inhibitors in clinical studies appeared to be important.

In a phase I study 21 days of continuous topotecan infusion was well tolerated and antitumor effects were seen [26]. Continuous infusion is inconvenient for patients and sometimes leads to complications of the central venous catheters [26,29].

Oral administration of topotecan might perhaps be more convenient in patients and was considered worthwhile testing in view of a bioavailability of 32-44% of the i.v. formulation when given orally [27,28].

Both for oral and intravenous topotecan administered on 5 consecutive days every 3 weeks myelosuppression was dose limiting. No clinically important diarrhea was seen in the daily x5 administration. In contrast, for the twice daily x21 administration of oral topotecan uncontrollable diarrhea was the single dose limiting side effect, while it was myelotoxicity in studies on 21 days continuous infusion. The latter studies did not report severe diarrhea. Diarrhea is a well known side effect of camptothecin and its derivatives, but the types of diarrhea appear to differ.

CPT-11 administered intravenously causes acute onset diarrhea on day 1 responding to atropine and delayed onset diarrhea starting around day 5 and controllable by vigorous administration of loperamide [36]. CPT-11 delayed onset diarrhea is related to the biliary excretion of the glucuronated SN-38 metabolite and the biliary index of SN-38 predicts the risk of delayed onset diarrhea of CPT-11. Oral administration of camptothecin for 21 days q 28 days and 9-nitro-camptothecin for 5 days a week resulted in severe diarrhea in 40% and 33% of patients, respectively [37,38]. Camptothecin and CPT-11 induce intestinal mucosal destruction in studies with animal models [37,39]. Thus local intestinal effects of camptothecin and its derivatives appear responsible for diarrhea. Diarrhea induced by oral topotecan was always self-limiting but did not respond to loperamide administration.

Taking into account the bioavailability of topotecan DLT in the 5 day oral schedule was approximately 30% lower as compared to the i.v. administration. For the 21 days schedules it was approximately 70% lower. These data might suggest local intestinal exposure as an inducing factor for the observed diarrhea. The exact mechanism of topotecan induced diarrhea is unknown. DLT consisted of a combination of both myelotoxicity and diarrhea in the studies with 10 day administration of oral topotecan. Thus with oral administration of topotecan, the toxicity profile appeared to change gradually from granulocytopenia to diarrhea when administration was prolonged.

Neutropenia is the major side effect of daily x 5 intravenous topotecan, with the nadir of granulocytes being reported between day 8 and 15 (2-5). The continuous i.v. administration of topotecan for 21 days q 28 days showed a granulocyte nadir on day 18 (range:12-28) [26]. Granulocytes nadirs of the daily x5 administration of oral or i.v. topotecan were similar, as were those of myelotoxicity of the o.d. x10 (day 12 and 16) and b.i.d. x10 (day 8-14) oral schedules. In none of the schedules of oral administration of topotecan was myelotoxicity cumulative. These findings are consistent with previous reports on daily x5 administration of topotecan. Neutropenia had a median duration of 6.5 days (range: 2-12 days) and was uncomplicated in the daily x5 administration of oral topotecan. In contrast cumulative myelotoxicity requiring dose reductions was seen in schedules with 21 days continuous infusion [26,29].

With the 21 days oral administration plasma concentrations of topotecan lactone >1 ng/ml never lasted for more than 3 hours per administration. In contrast, 20 (91%) out of the 22 patients analyzed in the daily x5 study had a plasma concentration of topotecan lactone >1 ng/ml lasting for more than 3 hours, as did 5 patients (50%) on o.d. x10 and 1 patient (10%) with b.i.d. administration. The duration of topotecan lactone plasma-concentration >1 ng/ml per course however was highest with the 21 day schedule, and lowest for o.d.x5. Since granulocytopenia was significantly more frequent in the o.d. x5 administration, myelotoxicity appears to be related to plasma concentration per administration rather than duration of exposure to >1 ng/ml topotecan per course.

Compared to oral administration o.d. x5, AUC(t) of topotecan lactone is substantially higher with i.v. administration, and neutropenia more pronounced [40-44]. Furthermore, mild myelotoxicity was the major side effect of 21 days continuous infusion of topotecan with achieved mean steady state topotecan lactone plasma-concentrations varying from 0.62 ± 0.17 [29] and 4.4 ± 0.99 ng/ml [26]. Together with the finding of mild myelotoxicity in the b.i.d. x21 oral administration, with a low mean C_{max} of 1.40 ± 0.74, myelotoxicity might be related to topotecan plasma level rather than time of duration of exposure to the drug. Systemic exposure from low dose prolonged administration of camptothecin and its derivatives 9-amino-campthotecin and 9-nitro-camptothecin showed more

efficacy in tumor reduction in studies with human xenografts [21,22] and these schedules were tolerated better than the intravenous schedules with higher doses. Apperently myelotoxicity can be circumvented by prolonged administration of low dose topoisomerase I inhibitors.

Pharmacokinetic-pharmacodynamic analysis of oral topotecan showed that half lifes are similar for all schedules and in concordance with those of i.v. administration.

Interpatient and (especially) intrapatient variation appeared to be most limited with o.d. x5 oral administration. As in previous studies with i.v. topotecan a significant correlation of the AUC(t) day 1 topotecan and percentage decrease of leucocytes was found with all schedules. When AUC(t) per course is plotted against the percentage of decrease of leucocytes similar sigmoidal curves are found. At MTD, AUC per course and AUC per week, were similar for all oral schedules. Thus, AUC per week, as a measure of dose intensity, was not significantly different in the 4 schedules studied.

Oral administration of topotecan, especially in the o.d x5 day schedule, is safe, with uncomplicated granulocytopenia as the main side effect, limited intrapatient variation and similar dose intensity as compared to the other schedules of oral administration. The 10 day and especially the 21 day administrations can result in unpredictable and sometimes clinically severe uncontrollable diarrhea. For the reasons mentioned above, and because a 5 day schedule is more convenient to patients, the once daily x5 oral administration of topotecan is preferred for future studies.

Further studies of orally administered topotecan either as a single agent or in combinations with other chemotherapeutics are ongoing.

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CHAPTER 8

PHASE I AND PHARMACOLOGICAL STUDY OF THE NEW TOPOISOMERASE I INHIBITOR GI147211, USING A DAILY X 5 INTRAVENOUS ADMINISTRATION

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SUMMARY

Purpose: Topoisomerase I inhibitors are interesting anti-cancer agents with a novel mechanism of action. We performed a phase I study with intravenous GI147211, a new semisynthetic camptothecin analogue, using a daily x5 schedule administered every 3 weeks, to evaluate the side effects and pharmacokinetics of the agent.

Patients and methods: Patients with a histologically confirmed diagnosis of a solid tumor refractory to standard forms of therapy were eligible for the study. GI147211 was given as a 30 minute intravenous infusion daily for 5 consecutive days, repeated every 3 weeks. In subsequent patient cohorts the dose was escalated from 0.3 to 1.5 mg/m^2/day. Pharmacokinetics analysis was performed on days 1 and 4 of the first course using a validated high performance liquid chromatographic assay and non-compartmental methods.

Results: A total of 19 patients were entered into the study, one patient was not evaluable for toxicity because only one drug administration was given. Eighteen patients received a total of 67 courses through four dose levels. The dose-limiting toxicities were neutropenia and thrombocytopenia at the dose of 1.5 mg/m^2/day. Nadirs occurred on day 15 and day 15, respectively. Other toxicities were mild and infrequent and included nausea/vomiting, headache and alopecia. The maximal tolerated dose was 1.2 mg/m^2/day. One partial response was observed in a patient with colorectal cancer. The total plasma clearance was 999 $\pm$ 184 ml/min (range 640-1329). The volume of distribution was 190 $\pm$ 46 l/m^2 and the terminal half-life was 3.7 $\pm$1.2 h. The AUC increased linearly with the administered dose. A steep and significant sigmoid relationship was established between the AUC and the % decrease of ANC.

Conclusions: GI147211 is a new topoisomerase I inhibitor that induced dose-limiting neutropenia and thrombocytopenia in this phase I study. The recommended dose for phase II studies with this schedule is 1.2 mg/m^2 x5 every 3 weeks.

INTRODUCTION

GI147211, (7-(methylpiperazinomethylene)-10,11-ethylenedioxy-20(S) camptothecin dihydrochloride, is a water-soluble semisynthetic analogue of camptothecin (CPT). Early clinical trials with CPT in the late 1960s showed hints of activity of this plant alkaloid in a variety of solid tumors [1-3]. Its further development was stopped because of unpredictable and severe myelosuppression, gastrointestinal toxicity and hemorrhagic cystitis.

In the late 1980s two discoveries brought a renewal of interest in CPT; firstly, topoisomerase I was identified as the single cellular target of CPT [4,5], and, secondly, an overexpression of topoisomerase I was found in various tumor cell lines but not in normal tissues [6-8]. Topoisomerase I is a nuclear enzyme that resolves topological problems of the torsionally strained (supercoiled) DNA [9]. This is achieved by forming a covalent adduct between topoisomerase I and the DNA, termed the cleavable complex. This catalytic intermediate creates single-strand DNA breaks, allowing the DNA molecule to rotate around the intact DNA strand at the cleavage site, leading to a relaxation of the DNA molecule and in this way replica-tion, transcription and other DNA functions can proceed. These enzyme-bridged breaks are then resealed by topoisomerase I. CPT stabilises the cleavable complexes, thereby preventing resealing of single-strand DNA breaks in the presence of the drug [10-12]. Cytotoxicity is specific to the S-phase of the cell cycle because the double-strand breaks that occur during this phase are more difficult to repair in the presence of the drug [13].

Recently, several semisynthetic CPT analogues [14,15] have been developed, aiming at reduced toxicity and sustained or improved activity.

One of these analogues, GI147211, demonstrated significant cytotoxicity against several xenografts of human cancers including HT-29 and SW-48 colon, PC-3 prostate, MX-1 breast, H460 lung, SKOV3 ovarian and KB epidermoid carcinomas [16-18]. The relative effect on tumor growth was dose-schedule dependent with a greater reduction in tumor volume achieved by prolonged dosing. LD_{10} in mice was 75 mg/m^2 (20 mg/kg) given as a single bolus injection. Animal toxicology studies by the intravenous route showed that myelosuppression was the

main toxicity and was dose-limiting.
We performed a phase I and pharmacologic study with intravenous GI147211 on a daily x5 regimen, repeated every 3 weeks, in patients with solid tumors.

PATIENTS AND METHODS

Patient selection

Patients with a histologically confirmed diagnosis of a solid tumor refractory to standard forms of therapy were eligible for this study. Other eligibility criteria included: (1) age $\geq$ 18 years; (2) an Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2; (3) a predicted life expectancy of at least 3 months; (4) no previous anti-cancer therapy for at least 3 weeks (6 weeks for previous nitrosoureas or mitomycin C); (5) adequate hematopoietic (WBC $\geq$ $3x10^9/l$, ANC $\geq$ $1.5x10^9/l$ and platelets $100x10^9/l$), hepatic (bilirubin within normal limits, AST, ALT and/or alkaline phosphatase $\leq$ 2.0x normal), and renal (serum creatinine $\leq$ 130 μmol/l) functions. All patients gave written informed consent.

Treatment and dose escalation

In the published phase I studies [19,20] using topotecan on a daily x5 schedule, the starting dose was 1/30th of the murine LD_{10} level because of the significant interspecies differences in toxicity. By rough estimate the murine LD_{10} for GI147211 was equivalent to topotecan. However, the *in vitro* and *in vivo* pharmacology suggested that topotecan is 2.2-fold less potent than GI 147211 [15-16]. Therefore it was felt that a safe starting dose for GI147211 should be less than 1/30 of the mouse LD_{10}; 0.3 mg/m^2/day given as a 30 minute infusion for 5 consecutive days was selected. Courses were to be repeated every 3 weeks as tolerated. Dose escalations were based on the prior dose level toxicity. For example if no toxicity was seen in the prior dose, $\leq$ 100% dose escalation was allowed. However, if toxicity was seen, a dose escalation of 25-50%, which was determined by the worst significant toxicity, was prescribed. At least three patients were entered at each

dose level. The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose-limiting toxicities (DLTs), which were defined as at least one of the following: (1) ANC $\leq$ 0.5x10^9/l or platelets $\leq$ 50x10^9/l for more than 5 days; (2) ANC $\leq$ 0.5 x10^9/l with fever requiring parenteral antibiotics, and/or non-hematologic toxicity $\geq$ CTC grade 3 in more than one-third of GI147211 naive patients (at least two of a maximum of six patients). Intrapatient dose escalation was not performed.

GI147211 was supplied by Glaxo as a clear solution in vials of 2.0 ml. The vials contained a mixture of 0.5 mg GI147211 and 100 mg dextrose. The pH was adjusted to 3.5 with sodium hydroxide or hydrochloric acid. GI147211 was diluted in D5W. The infusion bag (GI147211 + D5W) contained 100 ml exactly.

During the first course patients were hospitalised, all other courses were given at the outpatient clinic.

Treatment assessment

Before therapy medical history was taken and complete physical examination, complete blood cell (CBC) count, serum chemistries including sodium, potassium, chloride, bicarbonate, calcium, phosphorus, creatinine, urea, uric acid, glucose, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT, were performed, as were urinalysis, coagulation parameters (APTT, PT), ECG and chest X-ray. Weekly evaluations between the courses included history, physical examination, toxicity assessment according to the CTC criteria [21] and serum chemistries. CBC and urinalysis were determined twice weekly. Tumor measurements were performed after every two courses and evaluated according to the WHO criteria for response [22]; patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis whole blood samples (7 ml) in heparinised tubes were collected from an indwelling i.v. canula, placed in the arm contralateral to that receiving the drug, before infusion and at 15, 25, 45 minutes and 1, 1.5, 2, 4, 6, 8, 10, 12 hours after the initiation of the infusion on days 1 and 4 of the first course. Urine was collected within a two hour interval prior to the dosing and over

the intervals; 0-4, 4-8, 8-12 and 12-24 hours. Both blood and urine samples were analysed for the lactone form using a validated chromatographic assay, according to a method published by Stafford CG et al. [23]. The AUC was calculated using the trapezoidal method with extrapolation of the curve to infinity on day 1 and extrapolated to 24 hrs. on day 4. The terminal half-life was calculated as $\ln 2/\lambda$ where λ is the elimination rate constant, the total plasma clearance (Cl) as dose/AUC and the apparent volume of distribution at steady state (Vdss) as

$$\frac{Dose \cdot AUMC}{AUC^2} - \frac{Dose\ (0.5)}{2\ AUC}$$

Sigma Plot for Windows (release 2.0, Jandel scientific) and PCNONLIN (release 4.0, SCI software) were used for pharmacological data analysis. The sigmoid E_{max} model (Hill equation) was used to explore relationships between pharmacokinetic parameters and % decrease ANC and % decrease thrombocytes. The Gauss-Newton algorithm was used without weighing factor. The concentration of the drug in the infusion bags was also quantitated by high-performance liquid chromatography (HPLC).

RESULTS

A total of 19 patients entered the study. Patient characteristics are given in Table 1. All patients were eligible but 1 patient with NSCLC was considered not evaluable for toxicity and response because the patient was taken off study after the first drug administration, because of development of broncho-oesophageal fistula. In total 18 patients were evaluable for toxicity and response. The total number of evaluable courses was 67. The median number of courses per patient was 4 (range 4-10). Dose levels studied were 0.3, 0.6, 1.2 and 1.5 mg/m^2/day.

Table 1. Patient characteristics

No. of patients	19	
Sex (male/female)	10/9	
Median age (range)	59	(34-74)
Median performance score (ECOG)		
0	11	
1	8	
2	0	
Prior therapy		
chemotherapy	6	
radiotherapy	1	
both	7	
none	5	
Tumor types		
NSCLC	2	
colorectal cancer	9	
sarcoma	3	
unknown primary	1	
pancreastic cancer	1	
breast cancer	1	
mesothelioma	1	
oropharyngeal cancer	1	

Hematologic toxicity

Neutropenia and thrombocytopenia were the dose-limiting toxicities of GI 147211 on this schedule (Table 2). No myelotoxicity was observed at the first two dose levels. At the third dose level (1.2 mg/m^2/day), grade 3-4 neutropenia and thrombocytopenia were seen in 7/15 and 6/15 of the courses respectively. The median ANC nadir at this dose level was 1.29 x 10^9/l (range 0.08-5.6), for platelets it was 74 x 10^9/l (range 33-379).

Table 2. Hematological toxicity

Dose (mg/m^2 day)	No. Pts.	No.Eval. Courses	Leucocytes				Neutrophils CTC grades				Platelets			
			1	2	3	4	1	2	3	4	1	2	3	4
0.3	3	10	0	0	0	0	0	0	0	0	0	0	0	0
0.6	3	18	8	0	0	0	4	1	0	0	1	0	0	0
0.9	3	9	3	1	1	0	0	4	1	0	4	2	1	0
1.2	6	15	1	1	4	2	2	2	1	6	0	1	6	0
1.5	6	15	0	9	3	3	2	0	2	11	2	6	4	3

Table 3. Neutropenia and thrombocytopenia at the two highest dose levels

	Median day of occurrence grade 3-4 thrombocytopenia	Day nadir	Median no. days from grade 3-4 to recovery ≥ grade 2 thrombocytopenia	median day of occurrence grade 3-4 neutropenia	Day nadir	median no. days from grade 3-4 to recovery ≥ grade 2 neutropenia
1.2 mg/m^2/day	15 (15-15)	15 (10-19)	7 (2-8)	15 (10-19)	22 (13-22)	10 (1-10)
	N = 6			N = 7		
1.5 mg/m^2/day	15 (10-15)	15 (13-15)	8 (5-12)	10 (5-25)	15 (13-22)	11 (4-25)
	N = 7			N = 13		

The median duration of severe myelosuppression, expressed as the number of days between the first occurrence of grade 3-4 toxicity and recovery to ≤ grade 2 toxicity, was 10 days for neutropenia and 7 days for thrombocytopenia (Table 3). In three of six patients first receiving 1.2 mg/m²/day, the dose was subsequently reduced to 0.9 mg/m²/day; in two of the patients because of slow recovery from myelosuppression (with retreatment being permitted on day 28 and day 35 respectively); and in one patient because of febrile neutropenia with sepsis during the first course. At 1.5 mg/m²/day the dose-limiting toxicity was reached (Figure 1).

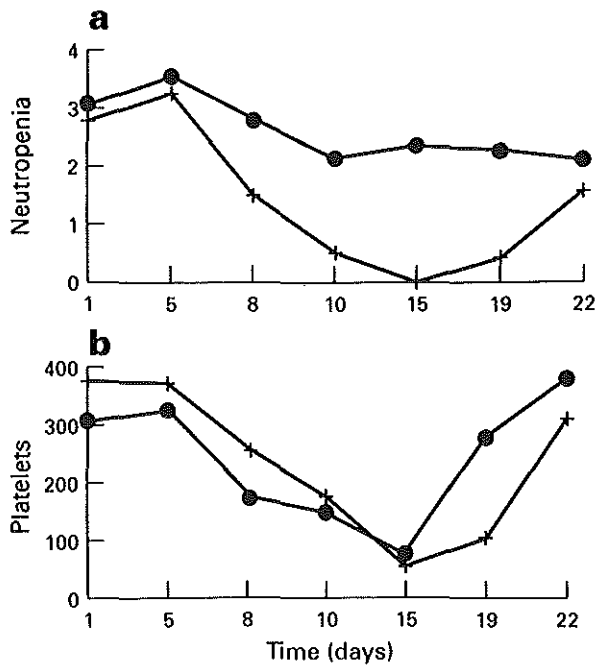

Figure 1. Comparison of the median ANC (a) and median platelet (b) counts of all given courses at the dose of 1.2 and 1.5 mg/m²/day

Grade 3-4 neutropenia was noted in 13/15 courses (86%). The median ANC nadir was 0.09 x10^9/l (range 0.01-1.7). The median number of days to recover from grade 3-4 neutropenia was 11 days (range 4-25). Three infectious complications were observed, two of them were life-threatening septic complications caused by gram-negative bacteria. In addition, thrombocytopenia grade 3-4 was seen in 7/15 courses (46%). At this dose of 1.5 mg/m^2/day the median platelet nadir was 62 x10^9/l (range 10-116).

The recovery of grade 3-4 thrombocytopenia occurred in 8 days (range 5-12). Thrombocytopenia was complicated by gastrointestinal bleeding in two patients. Anaemia occurred frequently but was never severe. Regularly, subsequent courses had to be postponed, due to delayed recovery of myelosuppression. At the dose of 1.2 mg/m2/day a treatment delay of 1 week was required in 4 courses and a delay of 2 weeks was required in 2 courses. At 1.5 mg/m^2/day a treatment delay of 1 week was necessary 3 courses and a delay of 2 weeks in 2 courses.

Cumulative myelotoxicity was not observed. The pattern of myelosuppression during the first course predicted the pattern in all subsequent courses (Figure 2).

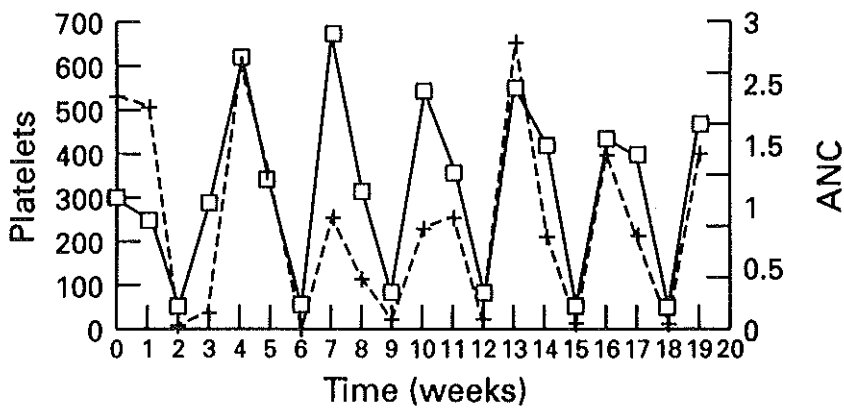

Figure 2. Graph showing course of ANC and platelets during subsequent cycles with GI147211 of patient 16 at the dose of 1.5 mg/m^2/day

Table 4. Pharmacokinetic data after i.v. administration of GI147211 on day 1 of the first course

Patient number	Dose (mg/m^2)	Day	AUC_{inf} (ng/ml/h)	C_{max} (ng/ml)	t_{max} (h)	t½ (h)	MRT (h)	Clearance ($ml/min/m^2$)	V_{dss} ($/m^2$)
1	0.3	1	4.93	3.63	0.42	2.96	2.89	1014	161
2	0.3	1	4.04	4.27	0.42	1.99	1.66	1238	104
3	0.3	1	4.77	3.73	0.42	2.63	3.33	1048	194
		Mean	**4.58**	**3.88**	**0.42**	**2.52**	**2.63**	**1100**	**153**
4	0.6	1	10.10	9.16	0.42	3.35	4.01	990	223
5	0.6	1	15.63	9.41	0.42	2.81	3.74	640	134
6	0.6	1	14.00	11.46	0.42	2.80	3.52	714	140
		Mean	**13.25**	**10.01**	**0.42**	**2.98**	**3.76**	**781**	**166**
7	1.2	1	17.57	17.33	0.42	3.62	3.01	1138	189
8	1.2	1	24.95	11.97	0.42	4.04	5.18	802	237
9	1.2	1	16.70	15.99	0.42	2.54	2.94	1198	193
10	1.2	1	15.05	12.83	0.42	2.40	3.04	1329	223
11	1.2	1	19.86	14.11	0.25	5.10	3.98	1007	225
12	1.2	1	19.42	17.65	0.42	2.97	2.66	1030	149
		Mean	**18.93**	**14.98**	**0.39**	**3.45**	**3.47**	**1084**	**203**
13	1.5	1	24.66	15.08	0.25	4.98	5.11	1014	296
14	1.5	1	-	27.47	0.42	-	-	-	-
15	1.5	1	24.39	19.21	0.42	4.62	3.57	1025	204
16	1.5	1	23.32	19.53	0.25	4.37	3.32	1072	197
17	1.5	1	22.64	18.91	0.25	4.61	3.65	1104	225
18	1.5	1	28.37	15.90	0.75	4.42	3.78	881	186
		Mean	**24.68**	**19.35**	**0.39**	**4.60**	**3.89**	**1019**	**222**

AUC_{inf}: AUC after extrapolation to infinity; C_{max}: maximal plasma concentration; t_{max}: time to maximal plasma concentration; t½: elimination half-life; clearance: total plasma clearance; V_{dss}: apparant volume of distribution at steady state; MRT: mean residence time

Table 5. Pharmacokinetic data after i.v. administration of GI147211 on day 4 of the first course

Patient number	Dose (mg/m^2)	Day	AUC_{inf} (ng/ml/h)	C_{max} (ng/ml)	t_{max} (h)	t½ (h)	MRT (h)	Clearance ($ml/min/m^2$)	V_{dss} ($/m^2$
1	0.3	4	6.19	4.73	0.25	4.82	4.62	808	207
2	0.3	4	6.79	4.30	0.25	2.34	4.73	736	199
3	0.3	4	7.46	5.11	0.42	3.81	5.30	670	199
		Mean	**6.81**	**4.71**	**0.31**	**3.65**	**4.88**	**738**	**202**
4	0.6	4	11.21	7.60	0.42	3.42	4.89	892	246
5	0.6	4	15.10	8.13	0.42	3.78	5.38	662	203
6	0.6	4	15.94	9.29	0.25	4.15	6.35	627	224
		Mean	**14.08**	**8.34**	**0.36**	**3.78**	**5.54**	**727**	**224**
7	1.2	4	20.59	18.66	0.25	3.02	3.06	971	163
8	1.2	4	37.83	20.03	0.42	4.07	5.62	529	167
9	1.2	4	21.10	16.21	0.42	2.70	3.47	948	182
10	1.2	4	14.84	9.62	0.42	2.20	3.96	1348	299
11	1.2	4	26.62	12.74	0.42	6.99	7.06	802	307
12	1.2	4	16.96	11.63	0.42	4.99	4.44	1179	291
		Mean	**22.99**	**14.82**	**0.39**	**3.99**	**4.60**	**963**	**235**
13	1.5	4	26.92	20.02	0.42	4.95	5.06	929	261
14	1.5	4	29.51	23.81	0.42	3.44	2.56	847	117
15	1.5	4	30.85	25.41	0.25	4.35	3.04	810	144
16	1.5	4	34.51	32.19	0.42	5.62	4.47	724	178
17	1.5	4	24.85	21.18	0.25	5.82	5.75	1006	319
18	1.5	4	52.70	28.25	0.42	4.43	4.74	474	126
		Mean	**33.22**	**25.14**	**0.36**	**4.47**	**4.27**	**798**	**191**

AUC_{inf}: AUC after extrapolation to infinity; C_{max}: maximal plasma concentration; t_{max}: time to maximal plasma concentration; t½: elimination half-life; clearance: total plasma clearance; V_{dss}: apparant volume of distribution at steady state; MRT: mean residence time

Non-hematologic toxicity

Overall, non-haematologic toxicities were relatively mild. Nausea and vomiting were not dose-related and occurred in respectively 30 (44%) and 15 (22%) of the given courses respectively and were never worse than grade 1-2. These symptoms were only present during the period of drug administrations and could easily be circumvented by the prophylactic use of standard anti-emetics.

Alopecia was dose related, alopecia grade 1 was observed in 3 patients at the highest two dose levels. Mild headache was not dose-dependent and occurred in 8 courses (12%). Prophylactic use of analgetics such as paracetamol during the days of drug administration prevented this symptom. No other toxicities were seen. There was no diarrhea, mucositis, liver or renal toxicity.

Table 6. Pharmacokinetic data after i.v. administration of GI147211 on days 1 and 4 of the first course

Patient number	Day	fe	Clr (ml/min)	Clr	Day	fe	Clr (ml/min)	Clr (ml/min/m^2)
1	1	0.114	232	116	4	0.109	175	88
2	1	0.081	184	101	4	0.095	128	70
3	1	0.212	333	222	4	0.428	430	287
4	1	0.115	231	115	4	-	-	-
5	1	0.156	161	101	4	0.181	192	120
6	1	0.149	194	107	4	0.197	225	123
7	1	0.106	201	122	4	0.122	196	119
8	1	0.101	151	84	4	0.219	212	118
9	1	0.088	203	107	4	0.133	241	127
10	1	0.085	226	113	4	0.128	344	172
11	1	0.116	208	119	4	0.239	314	179
12	1	0.347	663	358	4	0.210	459	248
13	1	-	-	-	4	-	-	-
14	1	0.191	-	-	4	-	-	-
15	1	0.135	280	150	4	-	-	-
16	1	-	-	-	4	0.177	218	128
17	1	-	-	-	4	0.090	182	91
18	1	-	-	-	4	0.125	101	59
	Mean	0.143	251	140		0.0175	244	138
	s.d.	0.070	133	74		0.087	106	64
	CV (%)	49	53	53		50	43	47

fe: fraction of the drug excreted unchanged; Clr: renal clearance

Pharmacokinetics and kinetic-dynamic relationships

Complete plasma sampling was obtained from all 18 patients on days 1 and 4 during the first course.

The pharmacokinetic data are summarized in Tables 4, 5 and 6. The AUC was linearly related to the dose (Figure 3). Total plasma clearance, determined on day 1, was 1014 ± 177.0 ml/min/m_2 (mean ± SD), V_{dss} was 193 ± 45.9 l/m^2 (mean ± SD), $t_{½}$ was 3.54 ± 0.99 (mean ± SD) and Mean Residence Time (MRT) was 3.53 ± 0.93 h (mean ± SD).

Significant sigmoid relationships were observed between the AUC on day 4 of treatment and the % decrease ANC (Figure 4). No significant influence of pretreatment on these relationships were observed (Figure 5).

The fraction of the drug excreted unchanged in the urine (fe) on day 1 was 0.14 ± 0.07 and the renal clearance was 140 ± 74 ml/min/m^2 (Table 4).

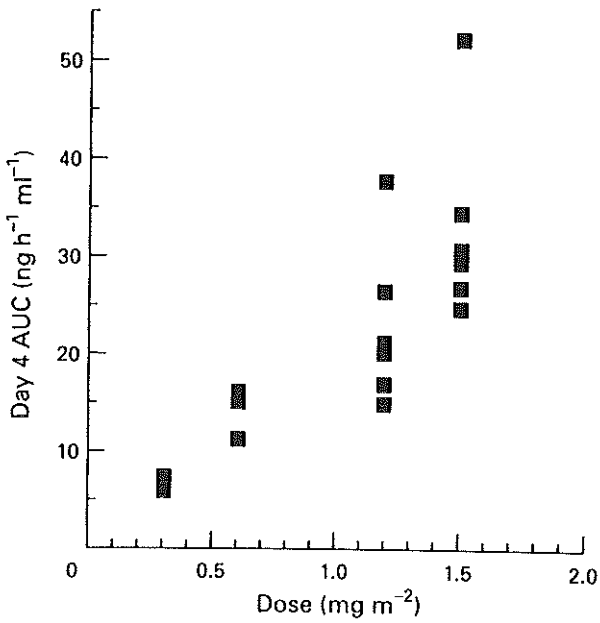

Figure 3. Relationship between the dose/m^2 and the area under the curve (AUC) of GI 147211 determined on day 4 of course one.

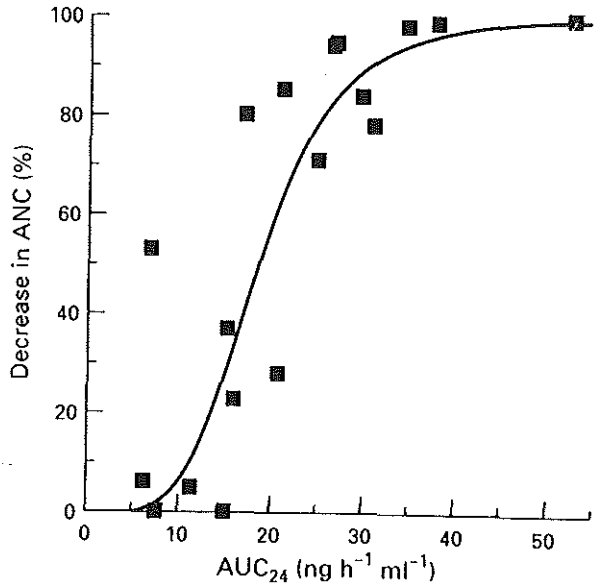

Figure 4. Relationship between the AUC of GI 147211 determined on day 4 of the first course and the % decrease in AUC. The sigmoid E_{max} model was applied.

Responses

One partial response was seen in a patient with metastatic colorectal cancer. His tumor had previously been shown to be resistant to 5-FU/folinic acid. The remission achieved by GI147221 lasted 6 weeks. A minor response was observed after 2 courses in a patient with liver metastasis of a leiomyosarcoma of the stomach, previously progressive after 2 courses of doxorubicin/ifosfamide. The patient refused further treatment because the second course was complicated by a non-drug-related upper gastrointestinal bleeding. Stable disease was seen in 10 patients.

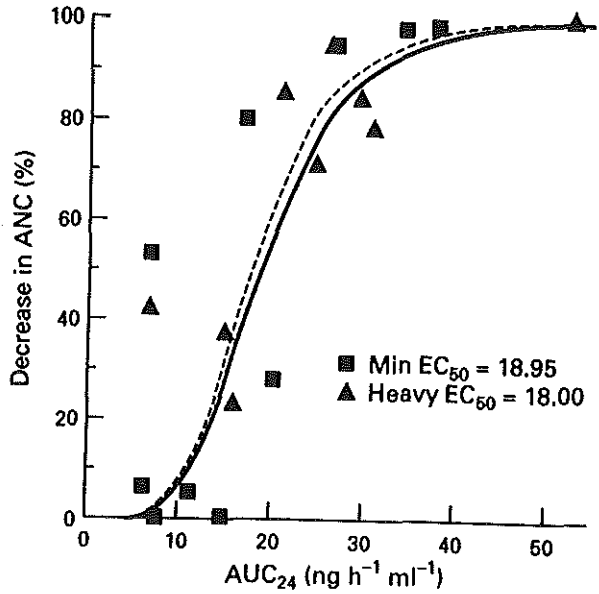

Figure 5. Relationship between the AUC of GI 147211 determined of day 4 of the first course and the % decrease in AUC, in minimally and heavily pretreated patients.

DISCUSSION

The characterisation of the inhibition of topoisomerase I as the mechanism of action of CPT has resulted in the development of several semisynthetic CPT analogues, of which some are under extensive clinical investigation. This is the first report of a clinical phase I study with GI147211.

The dose-limiting toxicity of GI147211 administered as a 30 minute i.v infusion for 5 consecutive days in patients with solid tumors was neutropenia in conjunction with thrombocytopenia. The dose of 1.2 mg/m^2/day was considered the maximally tolerated dose. At this dose the onset of neutropenia grade 3-4 occurred between days 10 and 19, with a median ANC nadir of 1.29 x10^9/l (range 0.08-

5.6). The median day of the platelet nadir and the first day of grade 3-4 toxicity was day 15. The median number of days to recovery was 7 (range 2-8). The recovery of neutropenia was even more prolonged, it lasted 10 days (range 1-10). Due to this prolonged myelosuppression subsequent courses had to be postponed in 6/15 courses at this dose level of 1.2 mg/m^2/day. At the dose of 1.5 mg/m^2/day the dose-limiting toxicity was reached. At this dose the grade 3-4 neutropenia already was noticed on day 10 (range 5-25) and lasted for 11 days (4-25) with a median ANC nadir of 0.09 x10^9/l (range 0.01-1.7). This deep and prolonged recovery from neutropenia was complicated by 3 septic episodes, and resulted in treatment delay in 5 of the 15 courses given at this dose level. Allthough the depth, the time of occurrence and recovery from thrombocytopenia at this dose level was equal to the dose of 1.2 mg/m^2/day, at this dose the thrombocytopenia was complicated in 2 courses by gastrointestinal bleeding.

As only one patient was heavily pretreated (10 coures of anthracyclin-containing chemotherapy) at the higher dose levels, these treatment delays were not related to prior myelosuppressive therapies. There were no indications of cumulative myelosuppression, the pattern of myelosuppression in subsequent courses was equal (see Figure 2). The use of hematopoeitic growth factors might be useful in preventing infections, but will be of limited value in further dose escalation of GI147211, since dose-limiting thrombocytopenia occurred in conjunction with neutropenia.

The pharmacokinetics reveal moderate interpatient variability. The pharmacokinetic data, obtained days 1 and 4 of course one, demonstrate limited intrapatient variability. There was a linear relationship between the AUC and the administered dose. A steep sigmoid relationship was observed between the AUC on day 4 and % decrease ANC, indicating that the AUC is predictive for the myelosuppression.

In this study one short-lasting partial response was noted in a patient with metastatic colorectal cancer. This fits with the observations of activity of GI147211 in preclinical models against colorectal cancer. In addition, it is of interest that we observed a minor response in a patient with leiomyosarcoma. In a recently reported phase II study in metastatic soft tissue sarcoma the topoisomerase I inhibitor topotecan only showed responses in patients with leiomyosarcoma [24].

In phase I studies with daily x5 of topotecan the dose-limiting toxicity was also myelosuppression, predominantly severe neutropenia of brief duration not necessitating treatment delays [19,20]. Thrombocytopenia mainly occurred in prolonged continuous regimens and the myelosuppresion was not cumulative [25]. As the daily x5 schedule appeared to be most active in early phase I studies many different phase II studies were iniated with this scheme. Although a randomised comparison is obviously lacking, GI147211 seems to induce more prolonged myelosuppression than topotecan. Presumably related to this, in contrast to topotecan, GI147211 administration necessitated relatively frequent delays of retreatment. These human data therefore confirm preclinical studies in bone marrow cultures where GI147211 was found to be more myelotoxic than topotecan. In preclinical studies this increased myelotoxicity of GI147211 seems to coincide with more anti-tumor activity [18].

The other topoisomerase-I inhibitor in a well-advanced stage of clinical development, Irinotecan (CPT-11), induces neutropenia in addition to diarrhea. Diarrhea was not observed at all for GI147211. Unlike GI147211, which is the active compound, CPT-11 is a prodrug. CPT-11 has to be converted to the active metabolite SN-38. It has been hypothesised that the conversion in the intestinal mucosa might be responsible for the diarrhea. The fact that such a conversion is not required for GI147211 may result in relatively low intestinal mucosal drug levels as compared to SN-38, and thereby less mucosal damage.

Preclinical data have indicated that topoisomerase I inhibitors, like topoisomerase II inhibitors, demonstrate more efficacy with prolonged continuous exposure [26, 6, 27]. Therefore, future development of GI147211 will be focussed on prolonged infu-sions, and the apparent bioavailability of oral administration will be determined.

The recommended dose for phase II studies with a daily x5 intravenous schedule is 1.2 mg/m^2/day repeated every 3 weeks. Phase II studies in various tumor types have recently been initiated.

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CHAPTER 9

THE BIOAVAILABILITY OF ORAL GI147211 (GG211), A NEW TOPOISOMERASE I INHIBITOR

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SUMMARY

Purpose: Topoisomerase I inhibitors are new compounds of interest for cancer chemotherapy. We performed a study with GI147211, a new semisynthetic camptothecin analogue, to determine the absolute bioavailability of the drug given orally.

Patients and Methods: Patients with a histologically confirmed diagnosis of a solid tumor refractory to standard forms of therapy were eligible for the study. GI147211 was given orally on day 1 and as a 30 minute infusion daily on day 2-5. The treatment course was repeated every three weeks. In subsequent patient cohorts the dose of the oral formulation was escalated from 1.5 mg/m^2 to 6.0 mg/m^2 the dose for i.v. administration was fixed at 1.2 mg/m^2. Plasma pharmacokinetics were performed on day 1 and 2 of the first course and on day 1 of the second course using a validated high performance liquid chromatographic assay.

Results: 19 patients were entered on study, one patient was not evaluable because the treatment course was stopped prematurely. Eighteen patients recieved a total of 47 treatment courses. The absolute bioavailability of GI147211 averaged 11.3% $\pm$ 5.2%. Drug appeared quickly in plasma with a median T_{max} at 0.5 hours. Fasting or fed state had no significant influence on the bioavailability of GI147211. The terminal half-life after administration of oral GI147211 was 6.85 $\pm$ 3.13 h, comparable to the half-life after intravenous administration. The major toxicities were neutropenia and thrombocytopenia. Nadirs for neutropenia and thrombocyopenia occurred on day 8 and day 15 respectively. Other toxicities predominantly consisted of mild and infrequent nausea and vomiting, and fatigue.

Conclusions: The oral administration of the drug is well tolerated. Oral administration of topoisomerase I inhibitor GI147211 results in a low bioavailability with relatively wide interpatient variation. The intravenous route of administration is advised for further development of this promising topoisomerase-I inhibitor.

INTRODUCTION

GI147211, (7-(methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-

camptothecin dihydrochloride, is a water soluble semisynthetic analogue of camptothecin (CPT). Early clinical trials with CPT in the late 1960s showed activity of this plant alkaloid in a variety of solid tumors. Its further development was stopped because of unpredictable and severe myelosuppression, gastrointestinal toxicity and hemorrhagic cystitis [1-3].

The interest in CPT was renewed in the 1980s, because topoisomerase I was identified as the single cellular target of CPT [4-5], and an overexpression of topoisomerase I was found in various tumor cell lines but not in normal tissues [6-7]. Topoisomerase I is a nuclear enzyme that resolves topological problems of the torsionally strained (supercoiled) DNA by forming a covalent adduct between topoisomerase I and the DNA, termed the cleavable complex. This catalytic intermediate creates single strand DNA breaks, allowing the DNA molecule to rotate around the intact DNA strand at the cleavage site leading to a relaxation of the DNA molecule and in this way replication, transcription and other DNA functions can proceed. These enzyme-bridged breaks are then resealed by topoisomerase I [8-11].

The sensitivity of malignant cells to topoisomerase I inhibitors has been correlated positively with topoisomerase I activity [6,12-17]. It has been documented that camptothecin (CPT) interferes with the breakage-reunion process of topoisomerase I by stabilizing the enzyme-DNA cleavable complexes [18]. Formation of these complexes results in various effects, including inhibition of DNA replication, termination of RNA transcription at sites of complex formation, induction of expression of early-response genes, induction of differentiation and ultimately internucleosomal DNA fragmentation, a characteristic of programmed cell death or apoptosis [19-24].

Recently several semisynthetic CPT analogues [25-27] have been developed, aiming at reduced toxicity and sustained or improved activity. One of these analogues, GI147211, demonstrated significant cytotoxicity against several xenografts of human cancers including HT-29 and SW-48 colon, PC-3 prostate, MX-1 breast, H460 lung, SKOV3 ovarian and KB epidermoid carcinomas [28-29].

The relative effect on tumor growth was dose-schedule dependent with a greater reduction in tumor volume achieved by prolonged dosing. Animal toxicolo-

gy studies by intravenous route showed that myelosuppression was the main toxicity and was dose-limiting.

Previously we reported myelosuppression as main toxicity of GI147211 administered intravenously to adult patients with solid tumors on a daily x5 schedule every 3 weeks [30]. Here we present a bioavailability study in patients with solid tumors using oral administration of GI147211 on day 1 followed by i.v. infusion on day 2-5, courses repeated every 3 weeks.

PATIENTS AND METHODS

Patient selection

Patients with a histologically confirmed diagnosis of a solid tumor refractory to standard forms of therapy were eligible to this study. Other eligibility criteria included: (1) age $\geq$ 18 years; (2) an Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2; (3) an estimated life expectancy of at least 3 months; (4) no previous anti-cancer therapy for at least 4 weeks (3 months for previous nitrosoureas or mitomycin C); (5) adequate hematopoetic (WBC $\geq$ $4x10^9/l$, ANC $\geq$ $1.5x10^9/l$, platelets $120x10^9/l$, and Hgb >6.0 mMol/l), hepatic (bilirubin within normal limits; AST, ALT $\leq$ 2.0x normal), and renal (serum creatinine $\leq$ 140 μmol/l) functions; (6) no known brain and/or leptomeningeal disease, no symptomatic peripheral neuropathy. All patients gave written informed consent. Patients with prior gastric of upper gastrointestinal surgery were excluded.

Treatment and dose escalation

Patients were to be treated with GI147211 on a daily times-5 schedule every three weeks. The first two courses patients recieved GI147211 orally on day 1. GI147211 was given by infusion on day 2-5 of the first two courses, and for 5 days in subsequent courses.

The anticipated oral bioavailability of GI147211 was around 15%. Thus compared to an intravenous bioavailability of 100% a higher oral dose would produce much less systemic exposure. To provide a safe administration of the drug

the starting dose was set at 1.5 mg/m^2. Dose escalations of the oral administration were based on the prior dose level toxicity and pharmacokinetic profile. If no toxicity was seen in the prior dose, $\leq$ 100% dose escalation of the oral dose was allowed. However, if toxicity was seen, a maximum dose escalation of 33- 66% was allowed, determined by the worst significant toxicity.

At least three patients were entered at each dose level. At the highest oral dose, bioavailability of oral GI147211 was studied in half of the patients after an overnight fast during the first course and in a fed state during the second course. The i.v. dose of GI147211 was fixed at 1.2 mg/m^2/day according to the recommended dose for phase II studies [30]. Intrapatient dose escalation was not performed. The maximum tolerated dose (MTD) was defined as one dose level below the dose that induced dose-limiting toxicities (DLT), which were defined as at least one of the following: (1) ANC $\leq$ 0.5x10^9/l or platelets $\leq$ 50x10^9/l for more than 5 days; (2) ANC $\leq$ 0.5 x10^9/l with fever requiring parenteral antibiotics, and/or nonhematologic toxicity $\geq$ CTC grade 3 in more than one third GI147211 naive patients (at least two of a maximum of six patients).

GI147211 was supplied by Glaxo Inc. as a clear solution in vials of 2.0 ml. The vials contained a mixture of 0.5 mg GI147211 and 100 mg dextrose. The pH was adjusted to 3.5. GI147211 was diluted in 5% dextrose. GI147211 for oral intake was mixed with 50 ml 5% dextrose in a plastic dosing container, and consumed within one minute after which an additional 50 ml of 5% dextrose was used. The infusion bag (GI147211 + 5% dextrose) exactly contained 100 ml and was administred as a 30 minute infusion on days 2-5.

Treatment assessment

Prior to therapy medical history was taken and complete physical examination, complete blood cell (CBC) count, serum chemistries including sodium, potassium, chloride, bicarbonate, calcium, phosphorus, creatinine, urea, uric acid, glucose, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT, were performed, as were urinalysis, coagulation parameters (APTT, PT), ECG and chest X-ray. Weekly evaluations between the courses included history, physical examination, hematology and serum chemistries and toxicity assessment according to the

CTC criteria [31]. Tumor measurements were performed after every two courses and evaluated according to the WHO criteria for response [32]; patients were taken off protocol in case of disease progression.

Pharmacokinetics

For pharmacokinetic analysis whole blood samples (7 ml) were collected in heparinized tubes from an indwelling i.v. canula, placed in the arm contralateral to that receiving the drug, prior to dosing and at 15, 30, 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours post-dosing on day 1 and 2 of the first course. Blood samples for the second course were only obtained during day 1. Plasma was harvested from blood. Blood samples were analyzed for the lactone and total GI147211 using a validated chromatographic assay, according to the method published by Stafford et al. [33]. The area under the plasma concentration-time curve [AUC] was calculated by non-compartmental analysis using the trapezoidal method with extrapolation of the curve to infinity on day 1. The absolute bioavailability was calculated as the ratio of the AUC after oral and intravenous dosing.

$$F = x \frac{AUC\ oral}{AUC\ iv} x \frac{Dose\ iv}{Dose\ oral} x\ 100\%$$

The intrapatient variability of the absolute oral bioavailability was calculated according

$$\frac{F_1 - F_2}{F_1} x\ 100\%$$

F_1 is absolute bioavailability during the first course,

F_2 is bioavailability during the second course.

The terminal half-life was calculated as $\ln 2/\lambda$ where λ is the elimination rate constant.

The effect of feeding on oral bioavailability was tested with a standard meal (breakfast) in 8 patients at the highest oral dose level.

Statistical methods

The paired t-test and Wilcoxon signed-rank test were used for statistical analysis on T_{max}., $t_{1/2}$ and AUC.

Table 1. Patient characteristics

No. of patients	19	
Sex (male/female)	7/12	
Median age (range)	55	(21-67)
Median performance score (ECOG)		
0	16	
1	3	
2	0	
Prior therapy		
chemotherapy	9	
radiotherapy	0	
both	5	
none	5	
Tumor types		
ovarian cancer	4	
colorectal cancer	8	
sarcoma	4	
unknown primary	1	
breast cancer	1	
NSCLC	1	

RESULTS

A total of 19 patients entered the study. Patient characteristics are given in Table 1. One patient requested to be taken off study after three days of drug administration. In another patient tumor response could not be evaluated since only one course was given because of toxicity. The total number of evaluable courses was 47.

In total 17 patients were evaluable for response. The median number of courses per patient was 2 (range 1-6). Seven patients received 3 or more treatment

courses. Dose levels studied for the oral dosing of GI147211 were 1.5 mg/m^2, 3.0 mg/m^2, 6.0 mg/m^2. In order to study the influence of a fed versus fasting state on pharmacokinetics of orally administered GI147211 a number of 8 additional patients were recruited at the highest dose level.

Hematologic toxicity

Neutropenia and thrombocytopenia were major side effects observed. Myelotoxicity was not observed at the first two dose levels. At the third oral dose level (6.0 mg/m^2) CTC-grade III-IV granulocytopenia and thrombocytopenia were seen in respectively 5/30 and 4/30 of the courses. The ANC nadir at this dose level was 0.09 x10^9/l in the one case with CTC-grade IV and 0.73-0.98 x10^9/l in the 2 cases with CTC grade III granulocytopenia. Three patients developed CTC-grade III-IV thrombocytopenia with a median platelet count of 25x10^9/l (range 4-40x10^9/l). The median duration of severe myelosuppression, expressed as the number of days between the first occurrence and recovery to CTC-grade II toxicity was 7 days (range 7-19 days) for granulocytopenia and 8 days for thrombocytopenia (range: 3-16 days).

CTC-grade I-II anemia occurred regularly, erythrocyte transfusions were given in 24/47 courses. Mild leucopenia CTC-grade I-II occurred in 17 (36.2%) of 47 courses. Treatment delay because of slow recovery of mild leucopenia occurred in 6 patients, 5 patients had a delay of 1 week, 1 patient had 2 weeks delay. Treatment delay occurred in 5 patients on dose level 6.0 mg/m^2 and in 1 patient at dose level 3.0 mg/m^2.

Non-hematologic toxicity

Overall, non-hematologic toxicities were relatively mild (Table 2). Nausea and vomiting occurred in respectively 24 (51.6%) and 9 (19.1%) of 47 courses and were CTC-grade I. Vomiting CTC-grade II was present in 2 (4.2%) cycles. Nausea and vomiting after oral administration was not different compared to intravenous dosing of the drug. Nausea and vomiting were only present during the period of drug administration and could easily be circumvented by the prophylactic use of standard antiemetics.

Patients (34%) frequently complained of mild fatigue. Abdominal discomfort, mostly cramping, occurred in 6 (13%) courses. Alopecia grade I was observed in 3 patients (17%). Mild headache was not dose-dependent and occurred in 2 courses (4.2%), reversible CTC grade I peripheral neuropathy was reported in 1 patient (2.1%). Mild stomatitis occurred in 2 patients, and 1 patient developed mild diarrhea grade I.

Renal and liver toxicity were not reported. Neutropenic sepsis in 1 patient was the main serious adverse event during administration of GI147211.

Table 2. Drug related non-hematologic toxicity per course. (n=47). (All toxicities CTC-grade I).

	Dose level			
	1.5 mg/m^2	3.0 mg/m^2	6.0 mg/m^2	TOTAL
Nausea*	4	4	16	24
Vomiting**	2	2	7	11
Fatigue***	4	2	10	16
Diarrhea	0	0	1	1
Stomatitis	0	1	1	2
Abd.dys.	0	3	3	6

*: No difference between oral and intravenous administration.
**: 2 courses vomiting CTC-grade II.
***: 4 courses fatigue CTC-grade II.
Abd.dys.: Abdominal dyscomfort.

Pharmacokinetics

At the dose level of 6.0 mg/m^2 plasma concentration-time curves of oral lactone and total GI147211 could be measured up to 12 hours after administration in 68% of courses.

Table 3. Pharmacokinetic data of 19 patients after oral dosing (day 1) and after intravenous administration (day 2-5) of GI147211. Bioavailability of Lactone and Total GI147211.

Patient	IV Dose (mg/m^2)	IV AUC (ng.h/ml)	IV T½ (h)	Oral Dose (mg/m^2)	Oral C_{max} (h)	Oral T_{max} (h)	Oral AUC Lactone (ng.h/ml)	Oral AUC Total (ng.h/ml)	Oral T½ (h)	Absolute bioavailability	
										Lactone (%)	Total (%)
1	1.2	22.29	6.2	1.5	1.01	0.25	2.25	3.12	2.5	8.1	6.7
2	1.2	26.37	6.0	1.5	2.15	0.25	7.47	11.16	4.0	22.7	12.5
3	1.2	56.04	14.3	3	2.06	1.5	16.28	70.82	12.2	11.6	22.4
4	1.2	28.17	6.2	3	1.73	0.5	6.93	12.81	7.7	9.8	11.3
5	1.2	68.73	5.6	6	17.45	2	83.40	157.68	4.2	24.3	17.2
6	1.2	23.51	6.7	3	1.82	0.25	4.07	15.21	9.5	6.9	8.9
				3 (C2)	1.51	0.75	5.25		2.0	8.9	
7	1.2	33.80	7.0	6	3.45	0.75	16.56	30.60	4.5	9.8	9.8
				6 (C2)	5.26	0.5	16.05		7.8	9.5	
8	1.2	31.28	5.2	3	2.43	0.25	9.84	24.05	5.1	12.6	15.7
				3 (C2)	4.25	0.25	16.72		11.7	21.4	
9	1.2	32.71	9.5	6	4.79	0.5	24.00	36.73	4.3	14.7	11.1
				6 (fed)	6.44	0.5	31.78		8.7	19.4	
10	1.2	40.11	10.9	6	1.54	1	10.80	39.35	6.1	5.4	8.2
				6 (C2)	2.85	0.75	17.84		7.2	8.9	
11	1.2	34.40	10.5	6	9.04	0.25	29.32	55.59	10.4	17.0	17.0
				6 (fed)	5.74	0.75	24.80		6.0	14.4	
12	1.2	24.41	8.9	6	1.63	0.25	10.39	27.76	9.7	8.5	9.5
				6 (fed)	2	1	7.14		8.2	5.8	
13	1.2	30.29	6.8	6	1.75	0.5	8.12	13.89	7.8	5.4	5.1
14	1.2	27.12	4.5	6	4.40	0.5	15.07	28.78	6.2	11.1	12.0
				6 (fed)	2.09	0.75	14.26		11.1	10.5	
15	1.2	40.73	18.6	6	3.45	0.5	18.82	30.71	13.0	9.2	9.4
				6 (fed)	2.45	1	9.68		11.9	4.8	
16	1.2	30.03	14.6	6	6.08	0.5	22.88	40.52	6.7	15.2	15.1
17	1.2	20.75	7.2	6	2.45	0.25	10.70	40.50	4.3	10.3	14.0
				6 (fed)	3.07	1	12.37		4.1	11.9	
18	1.2	15.03	1.9	6 (fed)	0.65	0.25	3.62	14.69	3.4	4.8	6.0
				6	0.77	0.5	6.78		5.0	9.0	
19	1.2	19.11	4.9	6	1.77	1	7.53		3.7	7.9	
				6 (fed)	3.38	0.5	9.07		3.5	9.5	
Mean		31.84	8.18		3.53	0.63	15.48	36.33	6.85	11.3	11.8
Std Dev		12.82	4.09		3.21	0.40	14.66	34.63	3.13	5.2	4.5
% CV		40	50		91	64	95	95	46	46	38

C2: second course. C_{max}: Maximum concentration. AUC: area under the curve. T_{max}: Time to maximum concentration. Total=Lactone plus acid concentrations. FED: after breakfast.

Table 4. Pharmacokinetic parameters of GI147211 Lactone in 13 patients recieving 6.0 mg/m^2 oral solution doses in fasted state and fed state. 8 patients analyzed in fasted and fed state.

Patient	IV Dose (mg/m^2)	IV AUC (ng.h/ml)	IV T½ (h)	Oral Dose		Oral Tmax		Oral AUC		Oral T½		Absolute bioavalability	
				Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted (%)	Feeding. (%)
5	1.2	68.73	5.6	6	-	2	-	83.40	-	4.2	-	24.3	
7	1.2	33.80	7.0	6	-	0.75	-	16.56	-	4.5	-	9.8	
9	1.2	32.71	9.5	6	fed	0.5	0.5	24.00	31.78	4.3	8.7	14.7	19.4
10	1.2	40.11	10.9	6	-	1	-	10.80	-	6.1	-	5.4	
11	1.2	34.40	10.5	6	fed	0.25	0.75	29.32	24.80	10.4	6.0	17.0	14.4
12	1.2	24.41	8.9	6	fed	0.25	0.75	10.39	24.80	9.7	6.0	8.5	14.4
13	1.2	30.29	6.8	6	-	0.5	-	8.12	-	7.8	-	5.4	
14	1.2	27.12	4.5	6	fed	0.5	0.75	15.07	14.26	6.2	11.1	11.1	10.5
15	1.2	40.73	18.6	6	fed	0.5	1	18.82	9.68	13.0	11.9	9.2	4.8
16	1.2	30.03	14.6	6	-	0.5	-	22.88	-	6.7	-	15.2	
17	1.2	20.75	7.2	6	fed	0.25	1	10.70	12.37	4.3	4.1	10.3	11.9
18	1.2	15.03	1.9	6	fed	0.5	0.25	6.78	3.62	5.0	3.4	9.0	4.8
19	1.2	19.11	4.9	6	fed	1	0.5	7.53	9.07	3.7	3.5	7.9	9.5
Mean		32.09	8.53			0.65	0.69	20.34	16.30	6.61	6.84	11.4	11.2
Std Dev		13.45	4.45			0.47	0.26	20.21	9.71	2.87	3.36	5.2	5.0
% CV		42	52			72	38	99	60	43	49	46	44

Fasted: fasted state. Fed: after breakfast. AUC: Area under the curve.
C_{max}: Maximum concentration. T_{max}: Time to maximum concentration

Plasma concentrations could be measured at 24 hours in 21% of courses of oral GI147211 administration.

T_{max} was $\leq$ 0,5 hours in 13 of 19 cases. Mean T_{max} after oral dosing was 0.63 $\pm$ 0.40 h (Table 3). At the dose level of 6.0 mg/m^2 T_{max} after oral administration was not significantly influenced by a fed or fasted state (p=0.17) (Table 4).

The mean maximal plasma concentration (C_{max}) at the 6.0 mg/m^2 dose level was 4.02 $\pm$ 3.57 ng/ml after oral and 21.76 mg/ml $\pm$ 6.37 ng/ml after intravenous dosing. The mean AUC of lactone GI147211 after oral dosing at the 6.0 mg/m^2 dose level was 20.3 $\pm$ 20.2 ng.h/ml and 32.1 $\pm$ 13.5 ng.h/ml after intravenous administration of 1.2 mg/m^2.

The absolute bioavailability of GI147211 lactone was 11.3% $\pm$ 5.2%. Absolute bioavailability ranged from 4.8 to 24.3%. Absolute bioavailability based on total GI147211 (lactone plus acid) was similar to the one observed with lactone alone. Absolute bioavailability from lactone is 11.3% $\pm$ 5.2% compared to an absolute bioavailability of 11.8% $\pm$ 4.5% for total GI147211 (Table 3). The ratio of lactone to total GI147211 after intravenous dosing was comparable with the ratio after oral administration. The median intrapatient variability of the absolute bioavailability was 31% (range 3-88%).

At the highest dose level of 6.0 mg/m^2 the influence of fasted or fed state in absorption of the drug was studied in 8 patients. The AUC after fasting was 15.3 $\pm$ 8.1 ng.h/ml and after a breakfast 16.3 $\pm$ 9.7 ng.h/ml (p= 0.36 N.S.).

After oral administration the terminal half-life of GI147211 lactone ranged from 2.0 to 13.0 hours (mean: 6.8 $\pm$ 3.1 h) and were of the same magnitude as after intravenous administration (mean:8.1 $\pm$ 4.1 h) (p=0.04).

Responses

Tumor responses were evaluable in 17 patients. In 2 patients tumor response could not be analysed because of early withdrawal. Best response to treatment was stable disease in 7 patients. Short lasting stable disease occurred in five patients with colon cancer, in one patient with adenocarcinoma of unknown primary, and one patient with sarcoma.

DISCUSSION

The characterization of the inhibition of topoisomerase I as the mechanism of action of CPT has resulted in the development of several semisynthetic CPT analogues, of which some are under extensive clinical investigation. This is the first clinical bioavailability study of orally administered GI147211.

In preclinical studies absolute bioavailability of GI14721 was 2-5% in mice and 16% in dogs. In the present study in humans the absolute bioavailability averaged 11.3% ± 5.2%. In comparison bioavailability studies of topotecan showed a variable systemic exposure of 32% and 44%, which is higher than the bioavailability of oral GI147211 [34-35]. The bioavailability after oral administration of GI147211 showed wide interpatient variability ranging from 4.8 to 24.3 %. Intrapatient variability however was more limited.

There was little difference in the ratio of lactone to total GI147211 between oral and intravenous dosing indicating that the acid metabolite is not formated during first pass.

T_{max} of oral GI147211 was 0.5 hours or less in 13 of 19 cases indicating rapid absorption which was not influenced by the presence of food. Oral dosing of GI147211 appeared to have comparable blood half-lives as the intravenous formulation indicating no prolonged absorption of the oral drug. In conclusion, the absolute bioavailability after administration of an oral solution of GI147211 was low and showed wide interpatient variability. Oral GI147211 bioavailability was not dose dependent, and was not affected by the presence of food. It was not possible in this study to determine the contributions of first-pass metabolism versus incomplete absorption to GI147211 bioavailability.

At an oral dose of 6.0 mg/m^2/day GI147211 on day 1 followed by injection of the drug at the dose of 1.2 mg/m^2/day on days 2-5 the onset of neutropenia CTC-grade III-IV occurred between day 7 and 19, with a nadir count ranging from 0.09-0.98 x10^9/l. The day of the platelet nadir was 8 days ranging from day 3-16 and the value of CTC-grade III-IV thrombocytopenia ranged from 4-40x10^9/l. In contrast to the findings in our phase I study on intravenous GI147211 the current study shows CTC-grade III-IV myelotoxicity occurring in patients who have been

heavily pretreated [30].

In other patients mild leucopenia (CTC-grade I-II) with slow recovery frequently occurred and subsequent courses had to be postponed for 1 week in 10/30 courses at the dose level of 6.0 mg/m^2 irrespective of pretreatment of patients. Treatment courses with CTC-grade III-IV myelosuppression were all uneventful except for one patient with septicaemia.

In topotecan studies dose limiting toxicity also was non cumulative myelosuppression, predominantly a severe neutropenia of brief duration not necessitating treatment delays [36-37]. Thrombocytopenia and anaemia occurred mainly in regimens with prolonged intravenous topotecan administration [38-39].

A single oral administration of GI147211 did not result in diarrhea. No human data are available on effects on the intestinal mucosa with repeated oral GI147211.

Unlike GI147211, which is the active compound, CPT-11 is a prodrug. CPT-11 has to be converted to the active metabolite SN-38. It has been hypothesized that biliary excretion of SN-38 induces diarrhea due to a secretory and exudative mechanism. With oral 9-nitro-camptothecin administration 33% of patients developed CTC-grade $\geq$ II diarrhea [40].

Preclinical data have indicated that topoisomerase I inhibitors, like topoisomerase II inhibitors, demonstrate more efficacy with prolonged continuous exposure [41]. An oral administration would be most convenient for prolonged dosing. Because of the low absolute bioavailability of GI147211 and the wide range in the interpatient variation, resulting in a non predictable level of individual drug exposure, development of an oral formulation seems unattractive. The intravenous route is advised for further development of this active and promising new topoisomerase-I inhibitor.

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CHAPTER 10

SUMMARY EN NEDERLANDSE SAMENVATTING

SUMMARY

This thesis includes phase I and pharmacologic studies on topoisomerase I inhibitors focussing on prolonged administration of the drug.

Chapter 2 summarizes the available data on the potential relevance of prolonged exposure to topoisomerase I inhibitors, a new class of anticancer agents. Cytotoxicity of topoisomerase I inhibitors is S-phase specific, and in vitro and in vivo studies have suggested that for antitumor efficacy prolonged exposure might be more important than short-term exposure to high concentrations. Findings from preclinical studies and preliminary data on clinical studies are presented of 20-S-camptothecin, 9-nitro-camptothecin, 9-amino-camptothecin, topotecan, irinotecan and GI147211.

Intramuscular (twice weekly) or intragastric (two days followed by one day rest) administration of 20-S-camptothecin dispersed in intralipid 20% showed inhibitory effects in studies with human cancer xenografts. In humans 20-S-camptothecin in gelatin capsules administered orally once a day for 21 days q 28 days induced diarrhea as dose limiting toxicity.

Protracted intramuscular administration of 9-nitro-camptothecin and 9-amino-camptothecin twice a week was effective in nude mice bearing melanoma, breast cancer and ovarian cancer xenografts. Intragastric application of 9-amino-camptothecin on a 5 day/week schedue for 3-6 weeks induced complete remission in human xenografts of malignant melanoma and non-small cell lung cancer. In humans continuous intravenous 9-amino-camptothecin for 5 days every 3 out of 4 weeks, and continuous i.v. administration for 21 days every 28 days, result in higher dose intensities than the recommended phase II 72 hour infusion every 2 weeks. Oral 9-nitro-camptothecin for 5 days every week in adult patients revealed hematologic toxicity as dose limiting.

In vitro inhibitory effects were marked with long term incubation with low dose topotecan.

In studies on human xenografts with prolonged oral or intraperitoneal administration of topotecan, the inhibitory effects were as efficacious as similar

parental application schedules and more efficacious than high-dose short exposure schedules. Continuous infusion of topotecan has been studied in various schedules in man: a 24 hour infusion weekly q 3 weeks; 72 hour infusion weekly every 2, and every 3 weeks;120 hour infusion every 3-4 weeks, and 21 day continuous infusion q 28 days. All continuous infusion schedules showed leucocytopenia, frequently combined with thrombocytopenia, as dose limiting toxicity. Protracted preclinical scheduling of irinotecan showed greater antitumor activity than shorter intense courses. For clinical studies it is to early to suggest differences in antitumor effects between different dose schedules.
Thus the preclinical studies with the various topoisomerase I inhibitors all show that drug scheduling is more active while limited clinical studies have shown the feasibility of this approach.

Chapter 3 describes a phase II study performed with topotecan administered as a 21 day continuous infusion every 28 days at a dose of 0.5-0.6 mg/m^2/day in patients with locally advanced, irresectable or metastatic colorectal cancer. The overall response rate was 10% (1CR, 3PRs). The major side effect was prolonged and cumulative myelosuppression and a marked inhibition of the erythropoiesis.
Pharmacokinetics revealed a significant correlation (R=0.54) between the CTC grade of leucopenia and the steady state plasma concentration of topotecan. It is concluded that this schedule, which is inconvenient for patients, exerts only modest activity in patients with colorectal cancer. Because of the inconvenience of continuous infusion, the oral formulation known to have a bioavailability of 32-44% in man, was further used.

In **chapter 4** a phase I and pharmacologic study is presented of topotecan administered orally in capsules twice daily for 21 days every 28 days to adult patients with solid tumors.
Thirty patients were evaluable for toxicity and response. The dose limiting toxicity was reached at a dose of 0.6 mg/m^2 b.i.d. and consisted of diarrhea starting at a median on day 15 (range: 12-20), which was self-limiting after a median of 8 days (range: 7-16). Other toxicities were mild and included leuco- and

thrombocytopenia, nausea and vomiting.
The maximum tolerated dose and recommended dose for phase II studies is 0.5 mg/m^2 b.i.d.. Pharmacokinetics revealed substantial variation of the AUC(t) of topotecan. A significant correlation was seen between the percentage of decrease in WBC and the AUC(t) of topotecan lactone. At that time data became available from a study with continuous infusion of topotecan in humans that showed a progressive depletion of topoisomerase I levels in peripheral mononuclear blood cells with maximal effects reached after 2 weeks. In view of this, and the onset of diarrhea in our study on day 15 we postulated that the optimal concentration-time relationship of topotecan might be less than 3 weeks. For the oral administration a shorter schedule might enable higher doses per day resulting in higher plasma concentrations.

These findings were the basis for a phase I and pharmacologic study of 10 day administration of oral topotecan described in **chapter 5**. In view of the relatively short half life of topotecan two dose schedules were studied: a once daily (o.d) and a twice daily (b.i.d.) administration for 10 days every 21 days. Nineteen adult patients were studied on the o.d.x10 schedule with a total of 48 courses. Dose limiting toxicity was reached at 1.6 mg/m^2/day and consisted of thrombocytopenia in 1 patient and diarrhea in 2 patients. The maximum tolerated dose was 1.4 mg/m^2/day.
Twenty patients were studied on the b.i.d. x10 administration of topotecan with a total of 64 courses. Dose limiting toxicity was reached at 0.8 mg/m^2 b.i.d. and consisted of a combination of myelosuppression and diarrhea. The maximum tolerated dose was 0.7 mg/m^2 b.i.d. Other non-hematologic toxicities were mild and consisted of nausea, vomiting, diarrhea and fatigue.Pharmacokinetics revealed a substantial variation of the AUC(t) of topotecan lactone in both dose schedules. Significant correlations were observed between AUC(t) and myelotoxicity parameters. The relation between AUC(t) topotecan lactone and the percentage of decrease of leucocytes was modelled by a sigmoidal E_{max} function.

Chapter 6. A phase I and pharmacologic study was performed with orally

administered topotecan once daily for 5 days every 21 days similar to the i.v. administration for which the drug has been registered recently. Twenty-nine patients entered on study and 109 courses were administered. Dose limiting toxicity was reached at 2.7 mg/m^2/day and consisted of granulocytopenia. The day of onset and duration of granulocytopenia were comparable to the findings from the i.v. daily x5 administration. The maximum tolerated dose and recommended phase II dose was 2.3 mg/m^2/day. Non-hematologic toxicities were mild with nausea, vomiting, fatigue and anorexia.

The difference in pharmacokinetics were studied in patients with an administered dose calculated in milligram per square meter and patients treated with a flat dose level.

At the MTD dose of 2.3 mg/m^2/day an adult patient with an average body surface of 1.75 m^2 would recieve 4 mg topotecan as an absolute dose. Pharmacokinetics were studied in 6 patients with varying body surface areas treated with a flat dose of 4 mg/day, and these parameters were compared to the parameters of patients treated with 2.3 mg/m^2/day, and no differences were found. Dosing oral topotecan at a flat dose level of 4 mg/day is as accurate as dosing at milligram per square meter.

Pharmacodynamic analysis showed a significant correlation between total AUC of topotecan lactone and the percentage of decrease of leucocytes (R=0.79) and platelets (R=0.83), and a sigmoidal relationship was found with the decrease of WBC.

In **chapter 7** the pharmacokinetic-pharmacodynamic relationship was studied for all 4 different schedules of administration of oral topotecan. A total of 99 adult patients with malignant solid tumors had entered the above mentioned studies: o.d.x5 q 21 days, o.d. x10 q 21 days, b.i.d.x10 q 21 days, and b.i.d. x21 q 28 days. A total of 109 (o.d. x5), 48 (o.d. x10), 64 (b.i.d. x10) and 59 (b.i.d.x21) courses were given. Dose limiting toxicity differed with the schedules used with granulocytopenia with o.d. x5 administration, combined myelosuppression and diarrhea in both x10 schedules, and diarrhea with 21 day oral administration.

Intrapatient variation was lowest in the o.d. x5 administration.

In all studies significant correlations were observed between myelotoxicity parameters and AUC(t) day 1 and AUC(t) per course of topotecan lactone, and sigmoidal relationships between AUC(t) per course and the percentage of decrease of WBC were similar for all schedules. At the maximum tolerated dose no significant difference in AUC(t) lactone per course was found between the schedules: AUC(t) per course was 107.4 ± 33.7 (o.d.x5), 145.3 ±23.8 (o.d. x10), 100.0 ± 41.5 (b.i.d. x10) and 164.9 ± 92.2 (b.i.d. x21), respectively.
AUC(t) per week, as a measure of dose intensity, was also not significantly different for the 4 studies. While prolonged oral administration schedules in humans may result in intractable diarrhea the granulocytopenia resulting from o.d. x5 oral administration of topotecan is uncomplicated in almost all cases. If anti-tumor effects are related to dose intensity, no preference can be made for one of the schedules, since there was no difference in exposure to topotecan lactone in the 4 dose schedules. But because of uncomplicated toxicity and comparable dose intensity per course,the o.d. x5 administration of oral topotecan is preferred for patient convenience.

Chapter 8. A phase I and pharmacological study was performed with a new watersoluble topoisomerase I inhibitor GI147211, using a daily x 5 30 min. i.v. administration every 3 weeks. In subsequent cohorts the dose was escalated from 0.3 to 1.5 mg/m^2/day in a total of 18 adult patients with solid tumors. A total of 67 courses were given through 4 dose levels. The dose limiting toxicities were leucocytopenia and thrombocytopenia at the dose of 1.5 mg/m2/day. Other toxicities were mild and infrequent and included nausea/vomiting, headache and alopecia. The maximum tolerated dose was 1.2mg/m^2/day. The total plasma clearance was 999 ± 184 ml/min (range: 640-1329). The volume of distibution was 190 ± 46 l/m2 and the terminal half life was 3.7 ± 1.2 h. The AUC increased linearly with the administered dose. A steep and significant sigmoidal relationship was established between AUC and the percentage of decrease of ANC. The recommended dose for phase II studies with this schedule is 1.2 mg/m^2 x5 every 3 weeks.

Chapter 9. Again aiming at the potential of prolonged administration, subsequently the oral bioavailability of GI147211 was studied. Nineteen adult patients entered on study and GI147211 was given orally on day 1 and as 30 min infusion daily on day 2-5 q 3 weeks.

The dose of the oral formulation was escalated from 1.5 to 6.0 mg/m^2 in subsequent patient cohorts, the i.v. dose was fixed at the recommended phase II dose of 1.2 mg/m^2/day. Pharmacokinetics were performed on day 1 and 2.

Eighteen evaluable patients received 47 treatment courses. The absolute bioavailability of orally administered GI147211 was 11.3 $\pm$ 5.2%. The drug appeared quickly in plasma with a median T_{max} at 0.5 hours. Fasting or fed state of patients had no influence on the bioavailability of GI147211. The terminal half-life after administration of oral GI147211 was 6.85 $\pm$ 3.13 h, comparable to the half-life after intravenous administration. The major toxicities were neutropenia and thrombocytopenia. Other toxicities predominantly consisted of mild nausea and vomiting, and fatigue. In view of the relatively low bioavailability and relatively high variation, further development of this formulation was not recommended.

Future Perspectives. The topoisomerase I inhibitors are certainly active drugs. The issue of i.v. scheduling of topotecan has not yet fully been resolved. For the oral administration further studies should focus on the o.d. x5 schedule. We are presently performing a study with this schedule in combination with intravenous cisplatin. In view of the inappropriate bioavailability of GI 147211 and the side effects found by others using more prolonged infusion, the daily x5 i.v. schedule is to be preferred for further development.

SAMENVATTING

In dit proefschrift worden de resultaten besproken van onderzoekingen verricht met 2 topoisomerase I remmers, topotecan en GI147211.

In Hoofdstuk 2 worden de beschikbare literatuur gegevens samengevat betreffende in vitro en in vivo onderzoekingen die wijzen op het mogelijke belang van langdurig blootstelling aan topoisomerase I remmers voor het grootste rendement in tumorreductie.
De resultaten worden besproken voor die topoisomerase I remmers welke thans in klinische ontwikkeling zijn: 20-S-camptothecine, 9-nitro-camptothecine, 9-amino-camptothecine, topotecan, irinotecan en GI147211.
Intragastrische (dagelijks x2 met een dag rust) of intramusculaire toediening (tweemaal/week) van 20-S-camptothecine geformuleerd in intralipid 20% had bij proefdieren een remmende werking op de groei van implantaten van humaan tumorweefsel. Bij patienten bleek diarree de dosis beperkende bijwerking van 20-S-camptothecine bij eenmaal daagse inname gedurende 21 dagen.
Langdurige intramusculaire toediening van 9-nitro-camptothecine en 9-amino-camptothecine, bleek effectief bij geimplanteerde humane tumoren in muizen. Toediening van 9-amino-camptothecine via de maag gedurende 3-6 weken induceerde complete remissie van humane tumor transplantaten. Continue infusie van 9-amino-camptothecine gedurende 5 dagen en gedurende 21 dagen elke 4 weken, geeft een hogere dosis-intensiteit dan die van de aanbevolen fase II infusie-duur van 72 uur. Inname van 9-nitro-camptothecine eenmaal daags gedurende 5 dagen/week had bij patienten beenmergsuppressie als dosis beperkende bijwerking.
In vitro onderzoekingen met langdurige blootstelling aan lage concentraties topotecan laten een uitgesproken remming zien van de groei van tumorcellen. Langdurige orale of intraperitoneale toediening van relatief lage doseringen aan proef-dieren was duidelijk effectiever dan kortdurende expositie aan hogere doses.
De klinische onderzoekingen met verschillende duur van continue intraveneuze toediening van topotecan laten zien dat leucocytopenie de dosis beperkende bijwerking is. Tumor responsen in fase I onderzoeken werden met name gezien bij

21 daagse continu infusie van topotecan.
Hoewel in vitro en in vivo proeven wijzen op een grotere antitumor activiteit bij langdurige toediening van lage doseringen irinotecan, zijn de gegevens hierover bij de mens ontoereikend voor conclusies. Ook voor GI147211 was in preklinische modellen langdurige blootstelling het meest effectief. In klinische onderzoekingen met GI147211 continu intraveneus toegediend, is beenmergsuppressie de dosis limiterende bijwerking.

Hoofdstuk 3. Het effect van verlengde expositie bij patienten met een inoperabel of gemetastaseerd coloncarcinoom werd bestudeerd in een fase II onderzoek met 21 dagen continu infusie van topotecan. Topotecan werd toegediend in een dagelijkse dosis van 0,5-0,6 mg/m^2 en de belangrijkste bijwerking was langdurige en cumulatieve myelosuppressie, met een opvallende remming van de erythropoiese. Het bereikte respons percentage was slechts 10% (1CR, 3 PRs). Er bestond een significante correlatie (R= 0,54) tussen de "steady state" plasmaconcentratie en de mate van leucopenie. 21 daagse continu infusie heeft beperkte therapeutische activiteit bij colorectale tumoren en is belastend voor patienten. Aangezien bekend raakte dat de orale biologische beschikbaarheid van topotecan relatief hoog was, werd vervolgens orale toediening bij patienten onderzocht.

Hoofdstuk 4. De resultaten worden besproken van een fase I studie waarin topotecan tweemaal per dag oraal werd toegediend gedurende 21 dagen aan 30 patienten met solide tumoren. De dosis beperkende bijwerking werd bereikt bij een dosis van 0,6 mg/m^2 2dd en bestond uit diarree, welke mediaan optrad op dag 15 (spreiding: 12-20) en een mediane duur had van 8 dagen (spreiding: 7-16). Andere bijwerkingen waren leuco- en thrombocytopenie, misselijkheid en braken. De geadviseerde dosis voor fase II onderzoek is 0,5 mg/m^2 2dd. Er was een substantiele interpatient variatie in de AUC(t) van topotecan. Er werd een significante correlatie (R= 0,75) vastgesteld tussen de AUC(t) van topotecan en de procentuele daling van de leucocyten.

Hoofdstuk 5. Bij continue infusie van topotecan treedt daling op van topoisomerase

I spiegels in mononucleaire cellen welke optimaal is na 2 weken. De optimale concentratie-tijdsrelatie van topotecan lijkt derhalve korter dan 21 dagen, en gezien het optreden van diarrhee rond dag 15 in bovengenoemd schema hebben wij de orale toediening van topotecan vervolgens bestudeerd in twee 10 daagse schema's, waarbij het middel eenmaal daags of tweemaal daags werd ingenomen. Er werden 48 kuren gegeven met eenmaal daagse toediening van topotecan, en bij de dosis van 1,6 mg/m^2/dag bestond de dosis limiterende bijwerking uit een combinatie van thrombocytopenie en diarree. Myelumsuppressie en diarree waren ook de dosis beperkende bijwerkingen in het onderzoek met de tweemaal daags toediening, en deze traden op bij de dosis van 0,8 mg/m^2 2dd. De maximaal getolereerde en geadviseerde dosis voor fase II studies waren respectievelijk 1,4 mg/m^2/dag eenmmaal daags en 0,7 mg/m^2 tweemaal daags. Andere niet-hematologische bijwerkingen waren gering en bestonden met name uit misselijkheid, braken, diarree en moeheid.
De AUC(t) van topotecan toonde substantiele intrapatient variatie in beide doseringsschema's. Significante correlaties werden vastgesteld tussen AUC(t) topotecan en parameters van myelumsuppressie.

Hoofdstuk 6. Intravenueze toediening van topotecan is geregistreerd voor dagelijkse toediening gedurende 5 dagen elke 3 weken. Analoog aan dit schema werd een studie verricht met eenmaal daags oraal toegediend topotecan gedurende 5 dagen, bij 29 patienten waaraan in totaal 109 kuren werden toegediend. Bij de dosis van 2,7 mg/m^2/dag was granulocytopenie de dosis beperkende bijwerking. Het optreden en de duur van granulocytopenie was vergelijkbaar met die van de intraveneuze 5-daagse schema's. De geadviseerde fase II dosis is 2,3 mg/m^2/dag. De niet-hematologische toxiciteit was gering en bestond uit misselijkheid, braken, moeheid en anorexie. Farmacokinetisch onderzoek werd verricht bij patienten die een vaste dosis per dag (mg/dag) kregen, en deze gegevens werden vergeleken met patienten bij wie de dosis was berekend op basis van met milligram per vierkante meter (mg/m^2). Voor patienten met een gemiddeld lichaamsoppervlak van 1,75 m^2 kan een absolute dosis van 4 mg worden berekend bij de geadviseerde dosis van 2,3 mg/m^2/dag. De farmacokinetische parameters van de 6 patienten met

verschillend lichaamsoppervlak die met 4 mg/dag waren behandeld, bleken niet te verschillen van de patienten behandeld met 2,3 mg/m^2/dag. Farmacodynamisch onderzoek laat een significante correlatie zien van de AUC(t) dag 1 topotecan lacton met de procentuele daling van leucocyten (R=0,79) en thrombocyten (R= 0,83). Dagelijks orale toediening van topotecan gedurende 5 dagen bleek goed haalbaar met granulocytopenie als dosis beperkende bijwerking.

Hoofdstuk 7. In dit hoofdstuk wordt de farmacokinetisch-farmacodynamische analyse beschreven van de 4 bestudeerde schema's van toediening van oraal topotecan.

In totaal participeerden 99 patienten aan deze studies met de schema's: 1dd x5, 1dd x10, 2dd x10 en 2 dd x21. Het totaal aantal toegediende kuren bedroeg 109 (1dd x5), 48 (1dd x10), 64 (2dd x10) en 59 (2dd x21). Dosis beperkende bijwerkingen blijken af te hangen van het gehanteerde toedieningsschema: granulocytopenie bij 5 daagse toediening, een combinatie van diarree en beenmergsuppressie in beide 10 daagse schema's en diarrhee bij 21 daagse toediening. De intrapatient variatie bedroeg gemiddeld 47,9 $\pm$ 49,2% en de interpatient variatie 53.9% $\pm$ 15,4%. De intrapatient variatie was het geringst bij de 5 daagse toediening. In alle studies werden significante correlaties gevonden tussen de AUC(t) van topotecan en parameters voor myelotoxiciteit en kon een sigmoidale relatie worden vastgesteld tussen de AUC(t) per kuur en de procentuele daling van leucocyten. De AUC(t) per kuur was niet significant verschillend tussen de schema's bij de patienten die werden behandeld met de maximaal getolereerde dosis. Deze AUC(t) per kuur was: 107,4 $\pm$ 33,7 (1dd x5), 145,3 $\pm$ 23,8 (1dd x10), 100 $\pm$ 41,5 (2dd x10) en 164,9 $\pm$ 92,2 (2dd x21). De AUC(t) per week, als maat voor de dosis intensiteit, was niet significant veschillend tussen de 4 schema's.

Op grond van deze farmacokinetische bevindingen kan geen voorkeur worden uitgesproken voor een bepaald schema. Aangezien het 5 daagse schema slechts ongecompliceerde granulocytopenie als belangrijkste bijwerking heeft, en een vergelijkbare dosis intensiteit bereikt als de andere schema's, wordt gezien het relatieve gemak van dit schema voor patienten een voorkeur uitgesproken voor dit schema voor verdere studies.

Hoofdstuk 8. De nieuwe, wateroplosbare topoisomerase I remmer GI147211 werd bestudeerd in een eenmaal daagse intravenueze toediening gedurende 5 dagen elke 3 weken. Dosis escalatie vond plaats van 0,3 tot 1,5 mg/m^2/dag bij 18 patienten. De dosis beperkende bijwerking bestond uit leuco- en thrombocytopenie en werd bereikt bij een dosis van 1,5 mg/m^2/dag. Andere bijwerkingen waren weinig frequent en gering en bestonden uit misselijkheid, braken, hoofdpijn en haaruitval. De maximaal getolereerde dosis en geadviseerde dosis voor fase II studies was 1,2 mg/m^2/dag. De totale plasmaklaring was 999 $\pm$ 184 ml/min.(spreiding 640-1329). Het distributie volume was 190 $\pm$ 46 l/m2 en de terminale half waarde tijd was 3,7 $\pm$ 1,2 uur. Er werd een significante sigmoidale relatie vastgesteld tussen AUC en de procentuele daling van granulocyten.

Hoofdstuk 9. Aangezien ook van GI14721 gegevens bestonden dat langdurige expositie aan lage doseringen in modellen effectiever waren dan kortdurende exposities aan hoge doseringen werd vervolgense van GI147211 de biologische beschikbaarheid bepaald na orale toediening. Bij de eerste kuur werd GI147211 oraal toegediend op dag 1 en intraveneus op dag 2-5 dagelijks via een 30 min. durende infusie. Farmacologisch onderzoek werd zowel op dag 1 als op dag 2 verricht. Bij de volgende kuren werd GI147211 dagelijks x 5 i.v gegeven elke 3 weken. De orale dosis kon worden opgevoerd van 1,5 tot 6,0 mg/m^2.
Achtien patienten participeerden aan deze studie en kregen in totaal 47 kuren toegediend. De absolute biologische beschikbaarheid van oraal toegediend GI147211 was 11,3 $\pm$ 5,2%. Het middel werd snel opgenomen met een mediane T_{max} van 0,5 uur. De half waarde tijd van oraal GI147211 was met 6,85 $\pm$ 3,13 uur vergelijkbaar met de halfwaarde tijd na intraveneuze toediening. De biologische beschikbaarheid via orale toediening werd niet beinvloed door het feit of patienten nuchter waren of een maaltijd hadden gebruikt. De belangrijkste bijwerking in dit onderzoek was beenmergsuppressie overeen komend met de resultaten van de fase I studie met dit middel. Eenmalige orale toediening van oraal GI147211 resulteerde niet in het ontstaan van diarree. Gezien deze lage biologische beschikbaarheid en de relatief grote interpatient variatie werd geadviseerd de orale toediening van GI147211 niet verder te ontwikkelen.

Conclusies en vooruitzichten. De topoisomerase I remmers vormen een groep van actieve antikanker middelen. De verrichte onderzoeken geven nog geen eenduidig antwoord op de vraag wat het meest effectieve schema van toediening is. Wel is duidelijk dat voor de orale formulering verdere ontwikkeling van het 5-daagse schema kan worden aanbevolen. Momenteel bestuderen we dit schema in combinatie met intraveneus cisplatin. Gezien de beperkingen in de biologische beschikbaarheid van GI147211 en de door anderen gevonden bijwerkingen gerelateerd aan langdurige infusies, kan dit middel slechts verder ontwikkeld worden in intraveneuze 5-daagse schema's.

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CURRICULUM VITAE

Cornelis Johannes Hendricus Gerrits werd op 1 april 1957 geboren te Escharen. Na het behalen van het Atheneum diploma en een "parkeer-jaar" Scheikunde begon hij in 1978 aan de studie geneeskunde aan de Katholieke Universiteit van Nijmegen. Het arts examen werd in maart 1985 behaald.

Aansluitend startte hij met de opleiding in de inwendige geneeskunde aan het Canisius-Wilhelmina Ziekenhuis te Nijmegen (opleider: Dr. I.H. Go). In 1989 werd de opleiding voortgezet in het Radboud Ziekenhuis te Nijmegen (opleider: Prof.dr. A. van 't. Laar). Vanaf februari 1991 is hij geregistreerd als algemeen internist.

De opleiding in het aandachtsgebied Hematologie werd gestart in april 1991 in het Academisch Ziekenhuis "Dijkzigt" te Rotterdam. In de periode 1992 tot en met maart 1994 werd de opleiding voortgezet in de Dr. Daniel den Hoed Kliniek (opleider: Prof.dr. B. Löwenberg). Aansluitend startte hij met de opleiding in het aandachtsgebied Medische Oncologie in het zelfde instituut (opleider: Prof.dr. G. Stoter). In deze periode kwam dit proefschrift tot stand.

Sinds 14 april 1997 werkt hij als algemeen internist met hematologische en oncologische belangstelling in het Streekziekenhuis "Midden-Twente" te Hengelo.

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