CLINICAL AND PHARMACOLOGICAL STUDIES ON THE TOPOISOMERASE I INHIBITOR TOPOTECAN

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Clinical and pharmacological studies on the topoisomerase I inhibitor topotecan

Klinisch en farmacologisch onderzoek met de topoisomerase I remmer topotecan.

PROEFSCHRIFT

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"Alleen wat niet ophoudt pijn te doen, blijft in de herinnering"

INTRODUCTION

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During the 1950's the National Cancer Institute (NCI) started a screening program of natural products. Among the thousands of plants analyzed, camptothecin, a plant alkaloid extract from the Camptotheca acuminata, an oriental tree which is cultivated throughout Asia, was found to be active against L1210 murine leukemia (1). In the early 1970's camptothecin underwent clinical testing. Although antitumor activity was observed in patients with colorectal cancer, melanoma and non-small-cell lung cancer further clinical development was precluded because of severe and unpredictable toxicities including, myelosuppression, diarrhea and hemorrhagic cystitis (2-5). In the mid 1980's several developments renewed the interest in camptothecin. Firstly, Hsiang et al. (6,7) identified topoisomerase I as the specific intracellular target of camptothecin. Secondly, overexpressed topoisomerase I level was found in advanced stages of human colon adenocarcinoma and other malignancies but not in normal tissue (8,9). This resulted in the development of several semisynthetic camptothecin analogues with more predictable toxicity profiles and consistent antitumor activity. One of these analogues is topotecan (SKF 104864, NSC 609699, (s)-9-dimethylaminomethyl-10-hydroxycamptothecin, Hycamtin®) which in preclinical studies demonstrated broad spectrum antitumor activity (10,11). Phase I studies revealed that topotecan was very well tolerated with generally a brief and non-cumulative neutropenia being the dose-limiting toxicity on all schedules (12,13). Major responses were observed in carcinomas of the ovary, lung (both small and non-small), oesophagus and colon. Phase II studies were initiated with a daily x5 schedule based on the fact that most objective responses in phase I studies were observed with this schedule.

This thesis includes clinical and pharmacological studies on topotecan which was focused towards the efficacy of the daily x5 schedule in patients with colorectal and ovarian cancer and towards the concept of prolonged exposure to the drug.

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NOVEL TOPOISOMERASE I INHIBITORS, TOPOTECAN AND IRINOTECAN

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Cancer treatment Reviews 20: 73-96; 1994

INTRODUCTION

Camptothecin is a plant alkaloid extract from the Camptotheca acuminata tree, which is cultivated throughout Asia (1). In the early 1970's camptothecin demonstrated objective antineoplastic activity against gastrointestinal adenocarcinoma, melanoma, non-small cell lung cancer and acute myelocytic leukemia (2,3). However, further clinical development of camptothecin was hampered because of unpredictable toxicity, notably severe myelosuppression, hemorrhagic cystitis and severe diarrhea (3-5). At the same point in time it was shown that camptothecin is a strong inhibitor of the DNA and RNA synthesis (6-9), while more recent studies revealed that camptothecin only induced single strand DNA-breaks in the presence of topoisomerase I, thus identifying this enzyme as a major target for the drug (10).

Topoisomerases are essential nuclear enzymes that function to resolve topological problems in DNA. Two major topoisomerases, type I, (which produces single strand breaks), and II, (which produces double strand breaks), have been identified in all eukaryotic cells. Topoisomerase I is a monomeric 100 kDa polypeptide encoded by a single copy gene located on chromosome 20q12-13.2 (11-13). Like all topoisomerases, topoisomerase I relaxes torsionally strained (supercoiled) duplex DNA so that replication and transcription can proceed. This is accomplished by forming a covalent adduct between topoisomerase I and DNA, termed the cleavable complex. This catalytic intermediate induces a single strand nick in the phosphodiester backbone of DNA, allowing the intact strand to pass through the nick (figure 1). DNA relaxation results from swivelling at this nick and so plays an important role in DNA replication and RNA transcription. The enzyme-bridged breaks are then resealed by topoisomerase I (relegation).

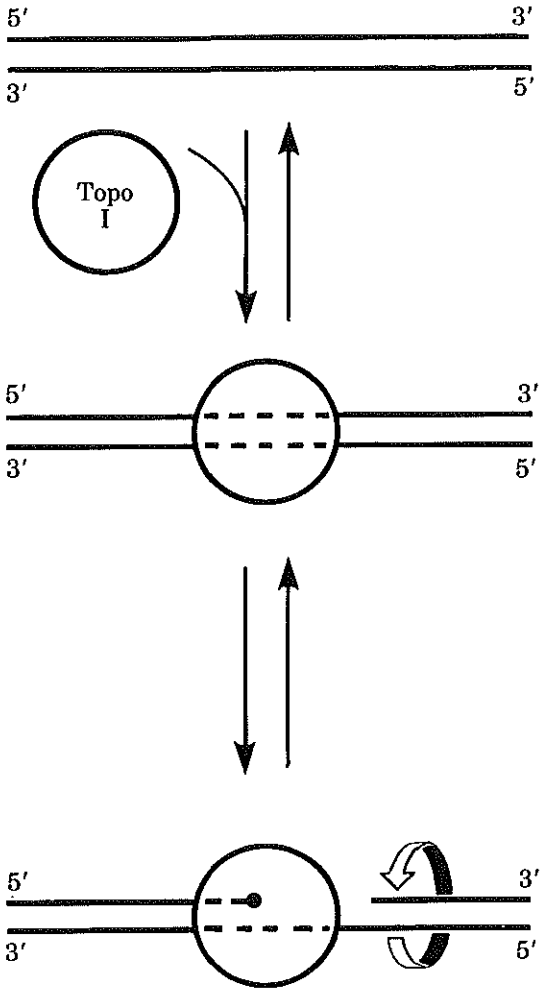


Figure 1:

Topoisomerase I becomes covalent bound to DNA, creating a DNA protein crosslink and a nick in the phosphodiester backbone. DNA relaxation results from swivelling at this nick.

Camptothecin inhibits topoisomerase I by stabilizing cleavable complexes resulting in single strand DNA breaks that cannot be relegated in the presence of the drug (14-19). Inhibition of RNA synthesis in cells is consistent with the enrichment of topoisomerase I on active transcription units (20-22) and the observation that topoisomerase I cleavable complexes occur preferentially within expressed genes (23-25). The reversibility of RNA synthesis inhibition that rapidly occurs following removal of camptothecin from cultured cells is probably due to the dissociation of topoisomerase I cleavable complexes from transcription units (25). Immunological studies have demonstrated that topoisomerase I is concentrated *in vivo* in areas of active transcription by RNA polymerases (20,22). This may indicate that the camptothecin altered cleavable complexes block elongation by impeding the progression of RNA polymerase molecules along the transcription unit.

DNA synthesis inhibition, although reversible following camptothecin exposure of short duration progressively becomes irreversible with increasing exposure duration (26). A specific mechanism for DNA synthesis inhibition has been suggested from an analysis of viral replication that accumulates in camptothecin treated cells (27-29). The analysis of these SV 40 replication intermediates has shown that strand breaks occur preferentially at replication forks (28). In presence of camptothecin, DNA synthesis is arrested, presumably due to collision between the replication fork and the drugtrapped cleavable complex resulting in formation of double strand DNA breaks (figure 2) (31). Recent studies have provided strong experimental evidence supporting this hypothesis. Halting the replication forks with aphidicolin, an inhibitor of DNA polymerase α and β, was shown to protect S-phase L1210 cells from camptothecin cytotoxicity without affecting cellular levels of cleavable complexes (32).

The cytotoxicity of camptothecin mainly occurs in the S-phase (9,33,34). Cells in S-phase are as much as 1000 fold more sensitive to the cytotoxic effects of

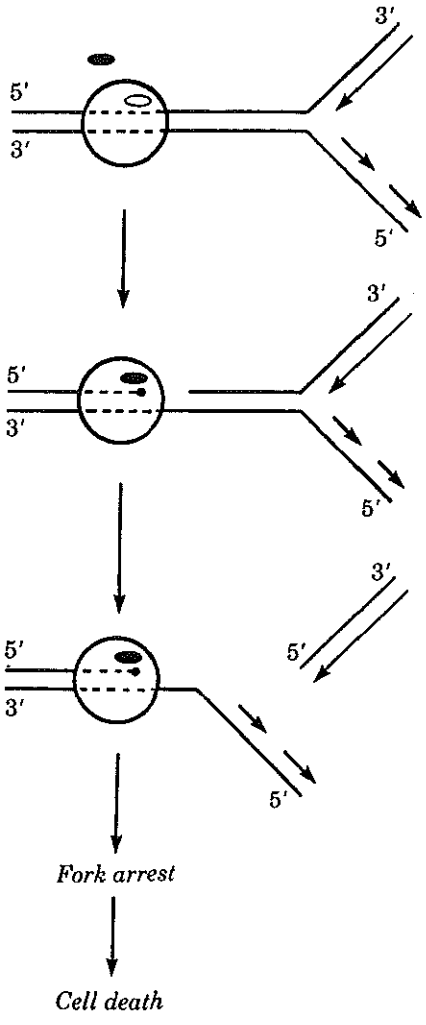


Figure 2:

Camptothecin and its binding site are represented. DNA synthesis is arrested by interaction of the drugtrapped cleavable complex with the advancing replication fork resulting in a fork breakage which is responsible for cell death.

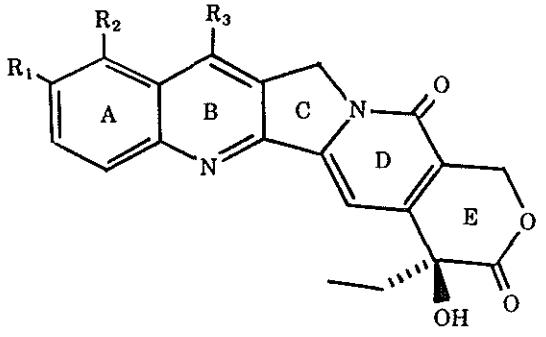
camptothecin than cells in the G1-or G2-phase at time of brief exposure to the drug (33,35). This also suggests that cytotoxicity occurs from an interaction of cleavable complexes with the replication forks. There is also evidence that the regulation of topoisomerase I is altered in neoplastic cells. For example colon cancer cells contain a fivefold higher level of topoisomerase I than normal adjacent mucosal cells (36). So with inhibition of topoisomerase I some degree of selectivity might be expected. Besides, topoisomerase I is present in relatively high levels in both proliferating and quiescent cells suggesting that its function may be independent of cellular growth rate and thus topoisomerase I inhibitors may be active in slowly as well in rapidly proliferating tumors (37,38).

With the increasing knowledge of function and inhibition of topoisomerase I a renewed interest in camptothecin occurred leading to the development of semisynthetic analogues with more predictable toxicity profiles and consistent antitumor activity. The two main analogues that have underwent clinical testing are topotecan and CPT-11 and these drugs will be discussed in more detail.

TOPOTECAN

Clinical pharmacology:

Topotecan (SKF 104864), [(S)-9-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride] is a semisynthetic analog of camptothecin with improved water solubility (figure 3). It incorporates a stable basic side chain at the 9-position of the A-ring of 10-hydroxycamptothecin, which provides water solubility at acid pH. Like camptothecin, topotecan undergoes a pH dependent reversible hydrolysis of the E-ring lactone, yielding an α, β dihydroxycarboxylic acid (figure 4). Only the lactone form of the drug, which is the predominant form of the agent in an acid environment is biochemically and pharmacologically active (39,40). For example at a pH level of 6.0 the closed lactone form accounts for more than 80% of the total compound. With high performance liquid chromatographic



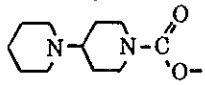
	R_1	R_2	R_3
CPT	H	H	H
TPT	OH	$(CH_3)_2NHCH_2$	H
CPT-11		H	C_2H_5
SN-38	OH	H	C_2H_5

Figure 3:

Structure of camptothecin, topotecan, CPT-11 and SN-38.

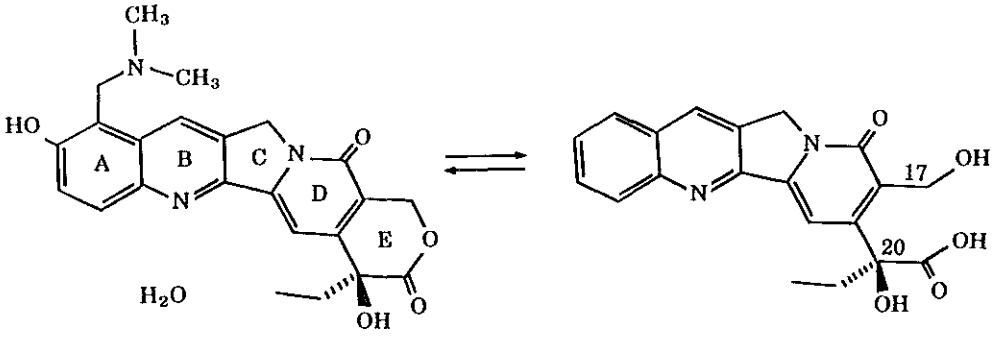


Figure 4:

Structure of topotecan lactone and the hydroxy acid form.

(HPLC) assay according to Beynen et al. (41) it is possible to differentiate between the closed lactone and the open hydroxy acid form.
Studies to examine the equilibrium of the lactone and the hydroxy acid form revealed that in physiologic saline the lactone is converted to the hydroxy acid in a substantial amount, while the relatively low pH of dextrose 5% solutions (pH 4.5) slows down this process. Hydrolysis of the lactone to the hydroxy acid form in dextrose 5% solutions occurs slowly, with a halflife of 30 minutes with a maximal conversion of approximately 10% (42,43). The difference in the pH of the diluents for topotecan, and the duration between dilution and the drug administration is therefore obviously critical. Formulations that ensure consistent delivery of the lactone form need to be explored (42).

In vivo topotecan is also rapidly converted to the open hydroxy acid form. At the end of a short infusion 50% of the total drug is present as the hydroxy acid form (43,44,45). Clinical pharmacology studies have attempted to measure both forms of the drug. Table 1 summarizes the reported results of the pharmacokinetics of topotecan.
Both the closed lactone form as well as the open hydroxy acid form of the agent are eliminated in a biexponential manner. The mean clearance of the lactone from the plasma is 27.0 liters/h/m^2 (range 10.8-132 L/h/m^2), with a mean elimination half life time of 3.0 h. (range 1.2-4.9 h). Renal elimination appears to be a major route of excretion for topotecan with approximately 45% (range 39-80%) of the total drug excreted in the urine over 24 hours (42-53).

Several phase I studies have examined a possible relation between toxic effects of topotecan and pharmacokinetics of the drug. A linear relationship has been described between the grade of leucopenia and the peak plasma concentration or the area under the concentration-time curve (AUC) of the lactone form of topotecan after a single 30 minute infusion (44). In patients treated with topotecan at a daily 30 minute infusion during 5 days the relationship between the dose and the magnitude of topotecan

TABLE 1: PHARMACOKINETICS OF TOPOTECAN

Schedule	Ref.	t ½ (h)	Peak lactone conc. ng/ml (dose mg/m^2 per day)	Clearance (L/h/m^2)	Vd ss (L/m^2)	24 h urine % dose
30 min. every 3 weeks	45	3.4	130 - 581 (2.5 - 22.5)	25.7	76.4	40
30 min. daily x 5 every 3 weeks	43	3.0	20.6 - 50.8 (0.5 - 2.5)	132	25.6	39
24 hr. infusion weekly*	46	4.9	7.0 (1.5)	10.8	61	-
30 min. daily x 5 every 3 weeks.	47	1.7	-	124	186	80
24 hr. infusion every 3 weeks**	48	2.9	6.4 - 30.7*** (2.0 - 7.5)	28.3	-	-
30 min. daily x 5 every 3 weeks	49	2.7	36.9-79.8***	31.8	86.1	20
30 min. one time	44	3.4	117 - 674 (2.5 - 22.5)	25.7	76.4	40
30 min. daily x 5 every 3 weeks	42	1.2	21-80*** (0.5 - 2.5)	73.2	26	30
72 hr. continuous infusion**	50	3.0	1.7 - 3.6 (0.75 - 1.9)	20.9	21.6	41.6

* Total topotecan
** Pediatric study
*** nM

induced neutropenia is characterized by a sigmoidal E_{max} model (42,43). This relation suggests that although the hydroxy acid form of topotecan is significantly less cytotoxic than the lactone form it may still contribute to the myelosuppressive effects of topotecan.

Preclinical studies:

Topotecan has impressive antitumor activity against a variety of tumors in murine models. It is curative against intravenously implanted P 388 leukemia and intravenously as well as subcutaneously implanted Lewis lung carcinoma, and has a high degree of activity against subcutaneously implanted solid tumors including, chemorefractory tumors such as mouse colon adenocarcinomas 38 and 51, mammary 16/c tumor and human coloncarcinoma xenograft HT-29 (40,54). Topotecan has been compared with camptothecin and 9-amino-camptothecin (a camptothecin analogue with an amino group at the 9 position, insoluble in aqueous solutions) in intravenously or intraperitoneally implanted L1210 leukemia, intraperitoneally implanted P388 leukemia, a camptothecin resistant P388 subline, s.c Lewis lung carcinoma, s.c B16 melanoma and s.c colon carcinoma 51. In addition, topotecan and 9-amino- topotecan were compared in nude mice bearing subcutaneously implanted xenografts of human colon tumors HT-29 and SW-48. Topotecan had superior activity in Lewis lung carcinoma and B16 melanoma and was the only compound of the three that induced regressions in the lung tumor model. Topotecan and 9-amino-camptothecin had similar activity in the leukemias and were more effective than camptothecin; all three were ineffective against in the P388 camptothecin resistant subline. Comparable extensive tumor growth delays were produced by all three agents in the murine colon tumor 51 model. Topotecan, like 9-amino-camptothecin, produced regressions and tumor growth delays in the human colon tumor xenografts; however, the duration of regression was superior for 9-amino-camptothecin (55). The preclinical toxicology studies of topotecan revealed that toxicity was dose related, reversible and limited to fast proliferating tissues such as bone marrow and gastro-intestinal epithelium.

From the murine models no single optimal dosing schedule could be defined for phase I clinical testing. Activity is retained when topotecan is administered intravenously, intraperitoneally, subcutaneously or orally.

Recent data from preclinical studies suggest that daily administration for a prolonged time is associated with an increased antitumor effect (36,56,57). A study comparing different dosing schedules of topotecan in *in vitro* tumor colony formation from freshly explanted human tumors showed that the activity of topotecan was more effective in the continuous exposure than in the short (1-hour) exposure experiment (57).

Phase I studies:

Table 2 summarizes the single agent phase I studies of topotecan (43,44,47-49,51-53,58-63). In these studies many different schedules have been tested; a single 30 minute infusion every 3 weeks, a 30 minute daily infusion during 5 days every 3 weeks, a 24 hour infusion weekly or every 3 weeks, and a 72 or 120 hour infusion repeated every week or at 3 weeks intervals. In addition, recently topotecan was administered as a continuous infusion during 21 days. In all studies except one involving leukemic patients the dose limiting toxicity was neutropenia either alone or in conjunction with thrombocytopenia. The neutropenia usually occurred between day 8 and 10, was generally brief and rarely associated with fever or treatment delays. At subsequent courses there was no evidence of cumulative toxicity. The maximum tolerated dose (MTD) was very schedule dependent. The highest amount of topotecan could be delivered as a short 30 minute infusion every 3 weeks (22.5 mg/m^2). Intermittent or prolonged infusion led to a relative increase in toxicity except the study using a 21-day continuous infusion. In this study the dose limiting toxicity occurred at a dose level of 0.7 mg/m^2/day. The total course dose in this study is much higher compared to the other studies using schedules with prolonged infusion times.

TABLE 2: PHASE I STUDIES OF TOPOTECAN

Schedule	Ref.	MTD* mg/m²/d (phase II)	Total course dose mg/m²	Dose limiting toxicity (DLT)
30 min. infusion every 3 weeks	44,45	22.5	22.5	Neutropenia
30 min. infusion daily x 5 every 3 weeks	43	1.5 - 2	7.5 - 10	Neutropenia
30 min. infusion daily x 5 every 3 weeks	47	1.5	7.5	Neutropenia
30 min. infusion daily x 5 every 3 weeks	49	1.5 (1.5)	7.5	Neutropenia
24 hr. infusion every 3 weeks**	48,52	5.5	5.5	Neutropenia/thrombocytopenia
24 hr. infusion every 3 weeks	53	PT 4 UT 5	PT 4 UT 5	Neutropenia/thrombocytopenia
24 hr. infusion every 3 weeks	58	8.4	8.4	Neutropenia/thrombocytopenia
24 hr. infusion weekly	46	1.75 (1.5)	1.5	Neutropenia
120 hr. infusion every 3 weeks	51,59	0.68	3.4	Neutropenia
72 hr. infusion every 3 weeks	59	1.6	4.8	Neutropenia
72 hr. infusion every 3 weeks**	60	1.0	3.0	Neutropenia
weekly 72 hr. infusion	61	2.0 (2.0)****	6.0	Myelotoxicity
120 hr. infusion every 3 weeks***	62	2.0	10.0	Mucositis
3 weeks continuous infusion	63	0.7	14.7	Not reached

* Maximum tolerated dose (recommended phase II dose), ** Pediatric study, *** Leukemic patients, **** mg/m²/72 hr, PT: pretreated, UT: untreated

There were only minimal differences in the magnitude of neutropenia in heavily versus minimally pretreated patients. Only one study (46) recommended different doses for untreated and pretreated patients in phase II studies. Mild anemia occurs but is not a clinically significant problem. Non-hematological toxic effects include nausea (12%), vomiting (10%), alopecia (11%), diarrhea (11%), fever (4%), fatigue (3%), and skin rash (2%). Rarely, mild elevation in liver function tests occurs. Hemorrhagic cystitis, one of the dose limiting toxicities of the parent drug camptothecin, was not reported.

As neutropenia is the dose limiting toxicity, the feasibility of further dose escalating in combination with hematopoietic growth factors has been evaluated in two studies, with conflicting results. Murphy et al. (64) started granulocyte colony-stimulating factor (G-CSF) 5 μg/kg subcutaneously once daily from day 6 until full recovery, in a pretreated group of patients who were treated with topotecan 1.5 mg/m^2/d for 5 consecutive days as a 30 minute infusion. There was no effect on the day of nadir of the neutrophils, but the median duration of the neutropenia was only 2 days (range 0-5). However, the dose of topotecan could not be escalated as thrombocytopenia emerged as the dose limiting factor. In contrast Rowinsky et al. (65) showed that the same approach resulted in a higher neutrophil nadir without coinciding worsening of the platelet nadir, allowing further dose escalations. An interesting observation in the latter study was that concurrent administration of G-CSF with topotecan resulted in a more severe neutropenia and thrombocytopenia. This might reflect the G-CSF induced increase in the fraction of precursors transversing the S-phase concurrent with a S-phase selective cytotoxic agent.

In a recent study the effect of administration of prechemotherapy hematopoietic growth factors was evaluated (66). Hematopoietic growth factors can reduce the duration of neutropenia following chemotherapy but have no effect on the depth of the neutrophil nadir. All patients received topotecan at a dose of 1.5 mg/m^2 daily for 5 days followed by granulocyte-macrophage colony-stimulating factor (GM-CSF) 250 μg/m^2 sub-

cutaneously once daily for 7 days. Patients were randomized between no or prechemotherapy GM-CSF, at a dose of 250 $\mu g/m^2$ twice daily subcutaneously for 5 days before the topotecan infusions. A substantial decrease in the incidence of grade 4 neutropenia was observed, 27% in the prechemotherapy GM-CSF group versus 77% in the group not receiving prechemotherapy GM-CSF. Whether prechemotherapy hematopoietic growth factor administration allows further dose escalation with topotecan needs to be studied.
During phase I trials responses with topotecan have been observed in non-small cell lung cancer (1 complete response (CR), 4 partial responses (PR), epithelial ovarian cancer (1 PR), small-cell lung cancer (1 PR), oesophageal cancer (1 PR), colorectal cancer (1 PR), pancreatic cancer (1 PR) and acute leukemia (3 CR and 2 PR). In addition, minor responses have been observed in renal cell cancer, squamous cell carcinoma of the skin, non-small lung cancer and ovarian carcinomas.

The most convincing evidence of antitumor activity occurred with the daily x5 schedule and the 21-days continuous infusion schedule. These phase I observations in combination with the observation, that in preclinical models intermittent and prolonged infusion times are more effective, have prompted initiation of phase II trials utilizing topotecan in a dosing schedule of 1.5 $mg/m^2/d$ administered as 30 minute infusions for 5 consecutive days being repeated every 3 weeks. In addition phase II studies with the 21-days continuous infusion schedule are planned.

Phase II studies:
Phase II trials are ongoing with the daily x5 schedule in many different tumor types. The preliminary results of four published studies are summarized in table 3 (67-70). From these studies it can be concluded that with this dosing schedule topotecan is not active in renal cell carcinoma and hormone refractory prostate carcinoma. Topotecan has definite activity in ovarian cancer refractory to cisplatin and/or carboplatin therapy and exerts some antitumor activity in colorectal cancer. Further studies have to be awaited.

TABLE 3: PHASE II STUDIES WITH TOPOTECAN

Tumor type (Reference)	Schedule	Prior chemotherapy	No. patients	Response	Toxicity
Prostate (67)	1.5 mg/m^2 d x 5 every 3 weeks	PT	28	2 x PR 8 x SD	Neutropenia ≥ grade 3: 27/84 cycles (32.1%) Dose reduction 9/28 pts (32.1%) Treatment delay 22/84 cycles (26.2%)
Renal cell (68)	1.5 mg/m^2/d x 5 every 4 weeks	UT	15	2 x MR 7 x SD	Neutropenia ≥ grade 3: 12/15 (80%)
Epithelial ovary (69)	1.5 mg/m^2/d x 5 every 3 weeks	PT	28	4 x PR 7 x SD	Treatment delay 57%
Colorectal (70)	1.5 mg/m^2/d x 5 every 3 weeks	UT	16	1 x PR	Leucopenia (97%), being grade 3-4 in 50%, thrombocytopenia (47%)

PT : pretreated, UT : untreated, PR : partial response, SD : stable disease, MR : minor response

IRINOTECAN (CPT-11)

Clinical pharmacology:

Like topotecan, CPT-11 (7-ethyl-10 [4-(piperidino)-1-piperidino] carboxyloxy-camptothecin) is a semisynthetic analogue of camptothecin with improved watersolubility (figure 3).

In vivo, CPT-11 is converted to its active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38), which *in vitro* has a 100-fold or more antitumor activity than CPT-11 (figure 3) (71,72). Incubating CPT-11 with mouse serum or with homogenates of liver or intestinal mucosa before adding tumor cells results in an enhanced growth inhibitory activity which is coincided with the formation of SN-38 (73). Thus CPT-11 acts as a prodrug, mainly expressing antitumor activity after enzymatic conversion to its active form. It is speculated that the enzyme responsible for converting CPT-11 to SN-38 is carboxylesterase (74). A study comparing the metabolism of CPT-11 resistant non-small cell lung carcinoma cells (PC-7/CPT) to the CPT-11 sensitive parent cells (PC-7/S) showed that there was no difference in the intracellular accumulation of CPT-11, but the intracellular level of SN-38 was decreased in the CPT-11 resistant cells. The enzymatic carboxylesterase activity of these cells was decreased to one third compared as to the activity in the parent cells also suggesting that carboxylesterase is responsible for the conversion of CPT-11 in SN-38 (75).

In mice the plasma CPT-11 concentration decreases biexponentially after intravenous administration of CPT-11, with a biological half life 0.8 to 1.1 hour.

The area under the plasma concentration-time curve shows dose dependency. The peak plasma concentrations of SN-38 are approximately 100-fold lower than the peak plasma concentrations of CPT-11. The SN-38 concentration decreases for the first 30 minutes after administration and is then maintained stable for a few hours followed by the log-linear terminal phase with a half life of approximately 2 hours.

This elimination is independent of the dose. Maintenance of the plasma concentration of SN-38 is presumed to be caused by two mechanisms. One is that the concentration is the sum of the linear formation of SN-38 from CPT-11 in the blood and the liver, the other is that SN-38 elimination from the plasma is saturated or inhibited by coexisting CPT-11 (73,76).

Although peak plasma SN-38 concentrations achieved after direct intravenous administration of SN-38 to mice are higher compared to peak plasma SN-38 concentration after CPT-11 administration, the effective cytotoxic plasma concentration were sustained for a longer period after conversion from the CPT-11. The antitumor activity of CPT-11 and SN-38 are time dependent rather than dose dependent, except at extremely high concentrations (35,77).

The clinical pharmacology studies of CPT-11 have only mainly been reported in a preliminary form. Table 4 summarizes the pharmacokinetic parameters noted CPT-11 and SN-38 (78-85). Interpretating these pharmacokinetic parameters is difficult, not only caused by the diversity of schedules and/or infusion times. First, like topotecan and camptothecin, both CPT-11 and SN-38 undergo a pH-dependent hydrolysis from active lactone forms to hydroxy acid forms. In the earlier trials (82,83) the available HPLC assays only allowed measuring of the total concentration of CPT-11 and SN-38. In humans a rapid hydrolysis of both CPT-11 and SN-38 is observed, with equilibria established within 30 to 60 minutes after administration. At equilibrium 33-55% of the total CPT-11 and SN-38 are present as lactones, with stable proportions thereafter (80). Second, in all these studies there was a interindividual variability in the pharmacokinetics of CPT-11, implicating large interindividual differences in the metabolism and/or elimination. Third, the available data seem conflicting. In the earlier studies the pharmacokinetics of CPT-11 appeared to be non-linear (82,83), whereas more recent studies suggest a linear relation (78,79,80,84,85).

TABLE 4: PHARMACOKINETICS OF CPT-11

Schedule	Ref.	t½ (h) (SN-38)	Peak plasma concentration CPT-11 (μg/ml)	Peak plasma concentration SN-38 (μg/ml)	AUC CPT-11 (μg.h/ml)	AUC SN-38 (μg.h/ml)	Clearance (l/h/m^2)	VDss (l/m^2)
90 min. infusion 100 mg/m^2 weekly x 4	78 79	7.9 (15.8)	1.37	30.35	2.79	0.21	14.7	-
90 min. infusion every 3 weeks	80	3	-	-	-	-	18.6	33
90 min. infusion 100 mg/m^2 weekly x 4	81	-	1.35	26.20	6.41	0.23	-	-
90 min. infusion 100 mg/m^2 weekly	82	1.5 (3.0)	1.42	18.0	6.37	0.2	17.6	-
120 hr. continuous infusion (30 mg/m^2 per day)	83	37.7 (23.9)	0.15	10.5	20.5	0.96	55.0	-
30 min. infusion every 3 weeks	84 85	17	-	-	-	-	15.0	150

Peak plasma concentrations of CPT-11 occur directly after the infusion whereas peak plasma concentrations of SN-38 are observed at variable times. Like in murine models, the peak SN-38 concentrations and the AUC do not correlate with CPT-11 dose. There are no data in humans, about elimination of CPT-11. In rats, within 24-hours 33-58% of unchanged CPT-11 given intravenously is excreted in the bile and the urine. The metabolite SN-38 is mainly excreted into the bile (73,76).

Until now, there is no clear correlation between toxic effects and pharmacokinetic parameters. If more pharmacokinetic data become available pharmacodynamic parameters of both CPT-11 and its active metabolite SN-38 might become better defined.

Preclinical studies:
CPT-11 demonstrated significant antitumor activity against a broad spectrum of experimental tumors. Among the most sensitive tumors were S180, Meth-A fibrosarcoma, Lewis lung carcinoma, Ehrlich carcinoma, MH 134 hepatoma, mammary carcinoma of C3H/HeN mice, L1210 and P388 leukemia. Probable cures of these tumors were observed frequently. Almost all mice bearing S180 and Lewis lung carcinomas were cured by CPT-11 (86). CPT-11 was also highly effective against early and advanced stage pancreatic ductal adenocarcinoma 03, with 5/5 complete regressions and 3/5 longterm survivors (87,88). Activity was further observed in B16 melanoma and colon carcinomas 38 and 51. Comparing the activity of CPT-11 against L1210 leukemia to camptothecin and other analogues revealed that CPT-11 was the most effective, giving the highest maximum increased life span (ILS) over a broad dose range. Moreover comparing the antitumor activity of CPT-11 against intraperitoneally implanted L1210 leukemia to adriamycin showed that CPT-11 was superior to adriamycin in maximum ILS (300% versus 129%), the number of cured mice (5/6 versus 1/6) and therapeutic ratio (86). Administration of CPT-11, intravenously or orally was significantly effective against human tumor xenografts transplanted in nude mice. CPT-11 showed antitumor activity against coloncarcinoma Co-4, mammary carcinoma MX-1, gastric adeno-

carcinoma ST-15 and SC-16, and squamous cell lung carcinoma QG-56 (89). CPT-11 showed activity against human tumor cell lines, resistant to vincristine, doxorubicin, colchicine and vinblastine which is related to the total different mechanism of action of CPT-11 as compared to these drugs (90).

Like topotecan no single optimal dosing schedule could be defined for phase I testing, but in a study in human cell lines it is suggested that more prolonged exposure to CPT-11 has a better cytotoxic effect (91).

Phase I studies:
The first phase I study was conducted in 1987 in Japan (92). Subsequent studies were performed in Japan, France and the United States. Table 5 summarizes the recently published data of phase I studies of CPT-11 (78-80,82,83,93-97). In these studies a wide range of schedules have been used; a single 30 minute infusion weekly or every 3 weeks, a 90 minute infusion weekly or every 3 weeks, a 30 minute infusion daily for 3 days every 3 weeks, and a 5 days continuous infusion every 3 weeks.

The dose limiting toxicities in all these studies were mainly neutropenia and diarrhea. The neutropenia is, like for topotecan, dose related, generally brief and non-cumulative. Infrequent mild to moderate thrombocytopenia or anemia are also reported. Two types of diarrhea are observed. The first is an early onset type which begins during or immediately after the infusion of CPT-11. This diarrhea is often coincided by abdominal cramping and facial flushing, suggesting that it is mediated by vaso-active substances. *In vitro* it has been demonstrated that CPT-11 inhibits acetylcholine esterase activity (74). These acute gastrointestinal side effects are fairly well controlled by the use of standard dose of anticholinergic drugs such as scopolamine butylbromide or atropine sulphate.

TABLE 5: PHASE I STUDIES OF CPT-11

Schedule	Ref.	MTD mg/m^2/d (*)	Dose limiting toxicity	Other adverse events
5 days continuous infusion	83	30 (30)	Diarrhea	Nausea, vomiting, leucopenia, anemia, hepatic dysfunction
30 min. weekly infusion	93	145 (115)	Diarrhea, leucopenia	Vomiting, fatigue, alopecia, stomatitis
90 min. weekly infusion	82	100 (100)	Neutropenia, diarrhea	Nausea, vomiting, thrombocytopenia, alopecia, hepatic and renal dysfunction, pneumonitis
90 min. weekly infusion	78 79	> 100	-	Neutropenia, diarrhea, nausea, vomiting, anorexia, alopecia
30 min. infusion x 3 every 3 weeks	94 95	115	Neutropenia	Diarrhea, nausea, vomiting, alopecia, fatigue
30 min. infusion every 3 weeks	96 97	400	-	Leucopenia, neutropenia, anemia, thrombocytopenia, vomiting, diarrhea, alopecia, malaise, hyperperistalsis
90 min. infusion every 3 weeks	80	240 (240)	Neutropenia, vomiting, nausea	Thrombocytopenia, alopecia, anorexia, malaise, abdominal cramps, flushing, diarrhea
30 min. infusion every 3 weeks	98	≥ 600	-	Neutropenia, anemia, thrombocytopenia, nausea, vomiting, diarrhea, alopecia, transient elevation transaminases

(*) Recommended phase II dose

With prophylactic use of 5HT-3 antagonist-ondansetron in combination with the H1-histamine antagonist diphenhydramine, the acute adverse gastrointestinal toxicities could be prevented. Furthermore it permitted further dose escalation of CPT-11 (80). The second type of diarrhea is a delayed type occurring after several days. It can present as a cholera-like secretory diarrhea, leading to severe dehydration, requiring intravenous rehydration. Milder forms of this delayed type of diarrhea respond to the standard anti-diarrheal drugs such as loperamide. Other toxicities include malaise, alopecia, rash, mild elevations of liver function tests, nausea and vomiting. There has been no evidence of hemorrhagic cystitis.

During the phase I studies responses with CPT-11 have been observed in different tumor types, including non-small cell lung cancer (82), colon carcinoma (96,97,98), cervix carcinoma (93,98), mesothelioma (94,95) and breast carcinoma (94,95). Minor responses have been noted in oesophageal carcinoma (93), renal cell carcinoma (93), ovarian carcinoma (93), hepatoma (78,79), colon carcinoma (78,79,94-97), pancreatic carcinoma (94,95) and head and neck cancer (96,97).

In Japan, the recommended scheme for phase II studies is a weekly CPT-11 infusion of 100 mg/m^2. This is based on the apparent augmentation of the dose intensity with this weekly scheme compared to a single infusion every four weeks, which resulted in phase I studies to a dose intensity of 50 mg/m^2 per week. Instead, in Europe phase II studies are started with a single infusion of 350 mg/m^2 of CPT-11 repeated every three weeks, because in phase I studies performed in Europe this dosing schedule resulted in an increased dose intensity.

Phase II studies:

Phase II studies with CPT-11 have been performed in Japan. In the USA and in Europe many phase II studies are ongoing. Table 6 and 7 summarize the results of the phase II studies of CPT-11 in solid tumors and in hematological malignancies. These early results suggest that CPT-11 is active against a broad spectrum of human tumors.

TABLE 6: PHASE II STUDIES OF CPT-11 IN SOLID TUMORS

Tumor type	Ref.	Prior therapy*	Schedule mg/m^2	No. of evaluable patients	Number of responses CR	PR	Response rate (%)
Lung, non-small cell	99	UT	90 min. infusion, 100 mg/m^2 weekly	72	0	23	32
Lung, small cell	100	PT/UT	90 min. infusion, 100 mg/m^2 weekly	27/8	2/0	7/4	33/50
Gastric	101	PT/UT	100 mg/m^2 weekly or 150 mg/m^2 every two weeks	45/15	0	9/5	23
Pancreas	102	PT/UT	100 mg/m^2 weekly or 150 mg/m^2 every two weeks	35	0	4	11,4
Colorectal	103	PT/UT	90 min. infusion 100 mg/m^2 weekly or 150 mg/m^2 every two weeks	51/12	0	13/4	27
Epithelial ovary	104	PT	100 mg/m^2 weekly or 150 mg/m^2 every two weeks or 200 mg/m^2 every 3-4 weeks	14	1	2	21
Cervix	105	UT	100 mg/m^2 weekly or 150 mg/m^2 every two weeks	55	5	8	24

* UT : untreated, PT : pretreated

TABLE 7: PHASE II STUDIES OF CPT IN HAEMATOLOGICAL MALIGNANCIES

Tumor type	Ref.	Prior therapy*	Schedule mg/m^2	No. of evaluable patients	Number of response CR	PR	Response rate (%)
Non Hodgkin lymphoma	106	PT	200 mg/m^2 every 3-4 weeks	6	0	0	0
			40 mg/m^2 daily x 5 every 3-4 weeks	16	2	3	31
			40 mg/m^2 daily x 3, weekly	8	0	1	33
			20 mg/m^2 twice daily x 7 every 3-4 weeks	2	8	0	0
Non Hodgkin lymphoma	107	PT	40 mg/m^2 daily x 3, weekly	47	8	15	49
Hodgkin lymphoma	107	PT	40 mg/m^2 daily x 3, weekly	4	0	0	0
Acute leukemia	106	PT	200 mg/m^2 every 3-4 weeks	4	0	0	0
			40 mg/m^2 daily x 5 every 3-4 weeks	9	0	0	0
			40 mg/m^2 daily x 3, weekly	1	0	0	0
			20 mg/m^2 twice daily x 7 every 3-4 weeks	12	1	2	25

* UT : untreated
PT : pretreated

For the studies in solid tumors CPT-11 was mostly administered once weekly or once every two weeks. Activity was seen both in untreated patients as well as patients previously treated with chemotherapy and/or radiotherapy.

In a study with patients with untreated non-small cell lung cancer 23 partial responses were reported among the 72 evaluable patients, with a median duration of response of 15 weeks (99). CPT-11 seems to be more active than other currently available single agents. CPT-11 also showed clear activity against gastric carcinoma (23%), colorectal carcinoma (27%), small cell lung cancer (33%-50%), cervical carcinoma (24%) and epithelial ovarian carcinoma (21%), but the overall median duration of response was short, varying from 50 to 68 days. The response rate of 11.4 % in pancreatic carcinoma with this dosing schedule seems to be of no clinical relevance (102).

In hematological malignancies activity of CPT-11 was mainly reported in schedules using daily infusions for a prolonged time (106,107). It would be of interest to study the feasibility of these intensive dosing schedules in solid tumors.
Side effects in phase II studies are similar compared to the toxicities noted in phase I studies, with the exception of pulmonary toxicity that was more frequent in phase II studies. This serious complication seems to be exclusive to patients with lung cancer. It was only noticed once in a phase I study (82). In a study with previously untreated non-small cell lung carcinomas this complication was observed in 6 patients (8%). Dyspnea on exertion developed in five patients and fever in four. Five of them showed a diffuse reticular nodular pattern on a chest X-ray and in one patient abnormalities highly suggestive of interstitial infiltrates were seen on a CT scan. In three patients eosinophilia was found concomitant with pulmonary toxicity. The interval from beginning of the administration of CPT-11 to the onset of the pulmonary symptoms ranged from 42 to 175 days, with a median of 61 days. A median total dose of CPT-11 in these patients was 750 mg/m^2, with a range of 400 to 1000 mg/m^2. In one patient the toxicity resolved spontaneously within one week.

The other patients were treated with corticosteroids, which seems to be beneficial. One patient died of respiratory failure (99). In another study involving relapsed or refractory small cell lung cancer the complication was seen in two patients (13%). Transbronchial biopsy showed interstitial edema associated with fibroblastic proliferation, lymphoid cell infiltration and with the formation of hyaline-like membranes in several air spaces caused by a fibrinous exsudate. One patient responded to corticosteroids, in the other the pneumonitis caused progressive respiratory failure and death (108).

COMBINATION THERAPY

In view of the single agent activity of topoisomerase I inhibitors, recently studies are started focusing on combination strategies. *In vitro* and/or *in vivo* the topoisomerase I inhibitors are combined with other cytostatic agents, radiotherapy and hyperthermia.

The combination of topoisomerase I inhibitors with cisplatin has been reported to be additive (55,109-111). This combination might be very promising as the drugs have non-overlapping toxicities, different mechanisms of action, and topoisomerase I inhibitors potentially can interfere with the repair from cisplatin induced DNA damage, a major mechanism of cisplatin resistance. In a phase I study, escalating doses of CPT-11 were administered as a 90 minute infusion on day 1, 8, and 15 every four weeks with a fixed dose of cisplatin of 80 mg/m^2 on day 1 (112). At the dose level of 70 mg/m^2 of CPT-11, diarrhea and neutropenia were the dose limiting toxicities. There were 14 partial responses among the 26 evaluable patients (a response rate of 54%). Recommended dose for phase II studies is CPT-11 60 mg/m^2 on day 1,8,15 and cisplatin 80 mg/m^2 on day 1, cycles to be repeated every four weeks.
Based on the assumption of the existence of a dose-response relationship a subsequent study was performed where an attempt was made to further escalate the dose of CPT-11 by adding G-CSF. CPT-11 was given on day 1, 8, and 15, with a starting

dose of 60 mg/m^2, repeated every four weeks, again combined with cisplatin at a dose of 80 mg/m^2 on day 1, plus G-CSF at a dose of 2 μg/kg administered once daily from day 4-21 except on the days of CPT-11 was given. The use of G-CSF allowed safe administration of 80 mg/m^2 of CPT-11 combined with 80 mg/m^2 of cisplatin, a 33.3% dose intensification, without the occurrence of dose limiting diarrhea or leucopenia. Eight partial responses out of the 19 evaluable patients were noted (response rate of 42%). In these two studies there was a clear linear relationship between the Cmax of SN-38 and the severity of diarrhea (113).

Topotecan has also been used in conjunction with cisplatin. In one study the dose limiting neutropenia occurred at the dose level of 75 mg/m^2 of cisplatin given on day 1 and topotecan at a dose of 1.0 mg/m^2/day for 5 consecutive days. It was concluded that the combination of topotecan with cisplatin is more toxic than either drug given alone (114). In the other study patient accrual only recently started at the dose level of 1.0 mg/m^2/day of topotecan (115).

While the concomitant combination of topoisomerase I and II inhibitors appears antagonistic (111,117,118), the sequentially combination of the two types of drugs appears additive or synergistic (55,116-118). The synergism might be related to the upregulation of cellular topoisomerase II levels which have found to increase 24-48 hours after exposure to topoisomerase I inhibitors (119). Based on these data two phase I studies have been performed with the combination of topoisomerase I and II inhibitors. The first was a Japanese study including patients with advanced lung cancer and these patients were treated with escalating doses of CPT-11, administered on day 1, 8, and 15 in combination with fixed doses of 80 mg/m^2 etoposide on day 1, 2, and 3 plus G-CSF 2 μg/kg given subcutaneously once daily from day 4-21, except on the days of CPT-11 administration. This treatment was repeated every four weeks (120). The dose limiting toxicity was reached at a dose level of 80 mg/m^2 of CPT-11 and consisted of diarrhea and neutropenia. Seven partial responses (37%) and one complete response (5%) were noted among the 19 assessable patients.

The overall response was 58% for the small cell lung cancer patients and 15% for the non-small cell lung cancer patients. The recommended CPT-11 dose for phase II studies is 70 mg/m^2. Escalating doses of topotecan given as a 3 days continuous infusion, with a starting dose of 0.17 mg/m^2/day, was tested in combination with etoposide with a fixed dose of 100 mg/m^2, given as a 2 hours infusion, on day 7, 8 and 9. The hematological toxicities were more severe than expected, especially in pretreated patients. These toxicities might reflect a synergistic effect of the combination, but this hypothesis has to be studied in the future with previously untreated patients with detailed pharmacokinetic monitoring (119).

From *in vitro* studies synergistic activity has also been observed when CPT-11 was combined with cytosine arabinoside or mitomycin C. Additive effects were observed for its combination with amsacrine, bleomycin, vincristine and 5-fluorouracil (111). These results might be used for the design of future in clinical trials.

The primary cytotoxic effect of ionizing radiation is DNA damage. Normally, most forms of DNA damage can be reversed by means of a number of repair pathways. In a variety of preclinical models camptothecin, topotecan and CPT-11 have been shown to enhance the lethality of ionizing radiation (121-124). It is suggested that this enhancement of cytotoxicty is caused by the inhibition of the DNA repair by the topoisomerase I inhibitors.

Hyperthermia is an other local treatment that has been explored in combination with topoisomerase I inhibitors. Hyperthermic treatment of the tumor bed enhances the cytotoxicity of topoisomerase I inhibitors in FSa11C murine fibrosarcoma cells (125). Both radiotherapy as well as hyperthermia in combination with the camptothecin analogues have not been applied in the clinical setting.

MECHANISMS OF RESISTANCE TO TOPOISOMERASE I INHIBITORS

Resistance to the cytotoxic effects of topoisomerase I inhibitors has been induced by selecting cells for their ability to survive continuous exposure of increasing concentrations of drug. These drug resistant cell lines provide powerful tools for understanding the mechanisms how cellular sensitivity to these agents is controlled, their target sites and the mechanisms of acquirement of drug resistance (126,127).

Different mechanisms appear to be involved in drug resistance. First, reduced levels of topoisomerase I have found to occur frequently in tumor cells (128). Mutations that alter in the affinity of the drugs to topoisomerase I represent another recognized mechanism of resistance. A human cancer cell line selected under continuous exposure to camptothecin was shown to contain topoisomerase I having a single amino acid change which related camptothecin resistance (129). In a CPT-11 resistant human lung cancer cell line (PC-7/CPT) resistance is caused by pleiotropic changes; decreased levels of topoisomerase I, low affinity of the drug to topoisomerase I and low affinity of carboxylesterase which catalyses CPT-11 to its active metabolite SN-38 (72).

Lengthening of the cell cycle may also be a mechanism of resistance in certain tumors that contain a large fraction of non-S-phase cells (130). Overexpression of the MDR-1 gene is a major general cause of drug resistance. The topoisomerase I inhibitors camptothecin and CPT-11 are unaffected with increase in P-170 membrane glycoprotein expression (90). In contrast, topotecan, a positively charged camptothecin analogue, shows a decreased accumulation and cytotoxicity in cells overexpressing MDR-1 (131,132). This effect of P-170 glycoprotein pump mechanism on the uptake and cytotoxicity of topotecan is small compared to the effect in the uptake and cytotoxicity of doxorubicin or etoposide in the same cell lines. Whether this mechanism of resistance to topotecan is of any clinical relevance needs to be investigated.

CONCLUSIONS

Topoisomerase I inhibitors have a novel mechanism of action and they seem to have activity against a broad spectrum of tumors, including to tumors which are resistant to the currently available chemotherapeutic drugs. Further preclinical and clinical studies are in progress to define the role of topoisomerase I inhibitors in cancer therapy.

Many questions remain unanswered. For example, the optimal dosing schedule is unknown. From preclinical models it seems that prolonged exposure is superior. In a recently published clinical study it has been reported that a continuous infusion of topotecan for 21-days has an excellent tolerance with evident antitumor activity. So far most phase II studies and topotecan have been performed with dosing schedules using short infusion times for 5 days.

As topoisomerase I inhibitors are unlikely to be curative as a single agent, the combination of these drugs with other chemotherapeutic agents, radiotherapy and biological response modifiers need to be studied.

In addition, testing of new camptothecin analogues and oral formulations are important issues for evaluating the role of topoisomerase I inhibitors in cancer therapy.

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TOPOTECAN IN COLORECTAL CANCER: A PHASE II STUDY OF THE EORTC EARLY CLINICAL TRIALS GROUP

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SUMMARY

Purpose: This phase II study with the topoisomerase I inhibitor topotecan was performed to determine its clinical activity and toxicity in patients with metastatic or locally irresectable colorectal cancer.

Patients and methods: Topotecan 1.5 mg/m^2/day was administered intravenously by 30 minute infusion for 5 days repeated every 3 weeks. Fifty-nine patients entered the study. Two were considered to be ineligible, 57 patients were evaluable for response and toxicity.

Results: A partial response was obtained in 4 of 57 evaluable patients (7%). The median duration of the response was 11 months (range 9.3 to 12.2). This topotecan regimen was very well tolerated. A total of 290 courses were given, with a median of 4 courses per patient (range, 1 to 18). The major toxicity was leuco- and neutropenia (91%), being grade 3-4 in 48% and 79% of courses respectively, but with only 2 infectious complications. Other side effects were alopecia (77%), being grade 1 in 46%, nausea (35%), vomiting (10%), and maculo-papular rash (6%).

Conclusion: Topotecan administered as a daily times five regimen only has minor activity as a single agent therapy in colorectal cancer.

INTRODUCTION

5-Fluorouracil (5-FU) is still the mainstay of systemic treatment for patients with metastatic colorectal cancer, with reported response rates averaging 5-20 %, usually of short duration. Biomodulation of 5-FU induces higher response rates, but the impact on survival has been minimal (1,2). Thus, new active antitumor agents are needed to improve the prognosis of these patients.

Topotecan, (S)-9-dimethylaminomethyl-10-hydroxycamptothecinhydrochloride, is a semisynthetic watersoluble analogue of the parent drug camptothecin. Topotecan is a specific inhibitor of the enzyme topoisomerase I (3,4). Its detailed mechanism of action has extensively been reported elsewhere (5).

Topotecan was shown to have antitumor activity against a variety of tumors in preclinical testing such as P 388 leukemia, Lewis lung carcinoma, mouse colon carcinoma 38 and 51 and human colon carcinoma xenograft HT-29. In all phase I studies reversible non-cumulative neutropenia either alone or in conjunction with mild thrombocytopenia was the dose-limiting toxicity (5). The daily x 5 schedule was selected for phase II studies. We performed a phase II study in metastatic colorectal cancer.

PATIENTS AND METHODS

Patients

Patients with metastatic or irresectable locally advanced colorectal cancer were included. Eligibility further required; (1) measurable disease; (2) age $\leq$ 75 years; (3) WHO performance status $\leq$ 2; (4) adequate bone marrow function (white blood cells (WBC) $\geq$ 3.5 x 10^9/l, granulocytes $\geq$ 1.5 x 10^9/l and platelets $\geq$ 100 x 10^9/l); (5) serum creatinine $\leq$ 140 μmol/l; (6) serum bilirubin $\leq$ 25 μmol/l, serum SGOT/PT $\leq$ 2x the upper limit of normal, in instances of liver metastasis $\leq$ 5x the upper limit; (7) no prior chemotherapy for advanced disease (an interval of at least twelve months was required since prior adjuvant chemotherapy); (8) informed consent.

Treatment schedule

Topotecan 1.5 mg/m^2/day was administered as a 30 minute infusion for 5 consecutive days every 3 weeks. Topotecan 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 sterile water for injection prior to dilution with 5% dextrose injection. In instances of bone marrow suppression retreatment was postponed until full hematological recovery. If a delay in the treatment cycle was more than 2 weeks, the daily infusion dose was decreased by 0.25 mg/m^2/day. The infusion dose was reduced by 0.5 mg/m^2/day if granulocytes were $\leq 0.5 \times 10^9$/l and/or platelets $\leq 50 \times 10^9$/l lasting for more than 14 days; or if grade 3-4 non-hematological toxicity occurred (with the exception grade 3 nausea and vomiting without prophylactic anti-emetics). A modification of 0.25 mg/m^2/day was made if granulocytes $\leq 0.5 \times 10^9$/l and/or platelets $\leq 50 \times 10^9$/l associated with infection/ bleeding or lasting between 7-14 days; or if grade 2 non-hematological toxicity occurred. The minimum allowed infusion dose was 1.0 mg/m2/day.

Evaluation

Responses were evaluated according to the World Health Organisation (WHO) criteria (6). Response duration was defined from the initiation of treatment. The involved lesions were measured every two cycles. White blood cells (WBC) and platelets were determined biweekly during the first 2 cycles thereafter weekly. Blood chemistry tests including renal and liver functions and urinalysis were performed weekly. Toxicity was assessed every week according to the CTC criteria (7).

RESULTS

A total of 59 patients were entered in the study. Two patients were considered ineligible: one because there were no measurable lesions, the other because of prior chemotherapy for metastatic disease. These patients were excluded from all analyses. Patient characteristics are summarized in table 1.

Table 1. Patient characteristics

	No. of patients	
No. patients entered	59	
No. patients eligible	57	
Median age (range)	60	(34-74)
Sex		
male	30	(53%)
female	27	(47%)
Performance status		
0	36	(63%)
1	19	(33%)
2	2	(4%)
Prior therapy		
surgery	52	
radiotherapy	9	
adjuvant chemotherapy	2	

The daily x 5 schedule was very well tolerated. There were 290 courses evaluable for toxicity. The median number of courses given per patient was 4 (range, 1-18). In 2 patients the administered dose had to be reduced: in one because of a sepsis during grade 4 neutropenia and in the other because of a grade 4 thrombocytopenia lasting for more than 7 days. Toxicities are listed in table 2. The major adverse reaction was myelosuppression. Leuco- and neutropenia occurred in more than 90 % of the courses, being grade 3-4 in respectively 48% and 79%. Despite the severe leuco-neutropenia only 2 infectious complications were observed. Concurrent anemia and thrombocytopenia occurred frequently but were mild and of no clinical significance, with the exception of one grade 4 thrombocytopenia. There was no evidence of cumulative myelosuppression.

Table 2. Drug related toxicities per course (n = 290)

	CTC				total	%
	1	2	3	4		
Leucopenia	25	113	106	21	265	91
Neutropenia	20	33	117	90	263	91
Anemia	119	119	6		244	84
Thrombocytopenia	113	7	4	1	125	43
Nausea	90	11	1		102	35
Vomiting	21	8			29	10
Asthenia, fatigue	69	16	1		86	29
Alopecia (per pt.)	26	18			44	77

The non-hematologic toxicity was usually mild. Nausea and vomiting mainly occurred on the infusions days and could usually be easily circumvented with prophylactic use of standard anti-emetics, sometimes in combination with steroids.
Four patients (7%; 95% CI 2-17%) achieved a PR, with a median duration of 11 months (range 9.3 to 12.2), 32 patients had SD (56%) at evaluation whereas the remaining 21 patients had PD.

DISCUSSION

Despite the recent progress in cancer chemotherapy, metastatic colorectal cancer remains a frustrating issue in clinical medical oncology. The effect of systemic combination chemotherapy on the survival remains unclear. In addition, the cost-effectiveness of such therapy is debatable. Therefore it is essential to test the activity of new drugs, in an effort to improve the dismal outlook for patients with metastatic colorectal cancer. In the early 1970's camptothecin (CPT) demonstrated antitumor activity against gastrointestinal adenocarcinoma. Further clinical development was hampered because of unpredictable toxicity, notable severe myelosuppression, hemorrhagic cystitis and diarrhea. Topotecan is a new watersoluble semi-synthetic analogue of CPT.
The present study shows that topotecan at this dose and schedule has only limited activity as a single agent therapy in colorectal cancer with a response rate of 7% with a median duration of 11 months. The number of patients with stable disease is noteworthy. The regimen was very well tolerated, with bone marrow suppression being the main toxicity. Although leuco- and neutropenia were very prominent, occurring in more than 90 % of the courses being grade 3-4 in about 50 %, infectious complications were seldom seen, probably because of the brief period of deep leucopenia.
At this moment, irinotecan (CPT-11), another camptothecin analog shows more promising results in patients with metastatic colorectal cancer. Recently, several studies were published in patients with colorectal cancer administrating CPT-11 at the dose of 125 mg/m2 every week x4, followed by a 2 week rest, showing a response

rate in un- and 5-FU-pretreated patients of 27-32% and 22-25% respectively (8-11). Topoisomerase II inhibitors exhibit marked schedule dependency. This may also be the case for topoisomerase I inhibitors such as topotecan. Preclinical data have indicated that topoisomerase I inhibitors demonstrate greater efficacy and have a greater therapeutic index with prolonged continuous exposure(12,13). The feasibility of the concept of prolonged exposure in humans using a 21-days continuous topotecan infusion repeated every 28 days was recently reported by Hochster et al. (14). In these pretreated patients the infusion was tolerated at a dose of 0.53 mg/m^2/day, with dose-intensity exceeding other schedules of topotecan, except the bolus injection every 3 weeks. The dose limiting toxicity was also hematologic, with thrombocytopenia somewhat more profound than the to leucopenia. In view of the low numbers of responses we report with the daily-times-5 schedule in colorectal cancer, a study using prolonged infusion times seems warranted in this tumor type.
In addition, recently reported results reveal a 32% bioavailability of oral topotecan, and thus oral administration may appear to be a more simple and convenient method for prolonged exposure and combination therapy with other cytotoxic agents (15). Phase I studies testing the feasibility of prolonged oral administration are ongoing.

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TOPOTECAN, AN ACTIVE DRUG IN THE SECOND-LINE TREATMENT OF EPITHELIAL OVARIAN CANCER: RESULTS OF A LARGE EUROPEAN PHASE II STUDY

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SUMMARY

Purpose: Topotecan is an inhibitor of topoisomerase I that has shown preclinical activity against different tumor types. Based on the results of a phase II study of topotecan as salvage therapy in ovarian cancer we conducted a large multicenter phase II study with topotecan as second-line treatment in ovarian cancer in patients who failed one platinum based chemotherapeutic regimen, to further evaluate and detail the efficacy and toxicity in this group of patients.

Patients and Methods: Topotecan 1.5 mg/m^2/day was administered intravenously by 30 minute infusion for 5 days repeated every 3 weeks. As the platinum-free interval is known to influence the response in subsequent treatment patients were stratified in subgroups, i.e. in platinum refractory, platinum resistant and platinum sensitive patients.

Results: One-hundred eleven patients entered the study. Nineteen patients were considered to be ineligible, 92 patients were evaluable for response. A total of 552 courses were given, with a median of 4 per patient (range 1-17). The major toxicities were leuco- and neutropenia, being grade 3-4 in 54.2% and 69.1% of the courses respectively, but with only 4.3% of courses with grade 4 neutropenia and fever or infectious complications. Prophylactic G-CSF was given in 20.5% of the courses to maintain dose intensity. Other relatively frequent side effects were alopecia (82%) and mild nausea (36.4%) and vomiting (17.5%). The overall response rate was 16.3%, with 1 complete response and 14 partial responses. If expressed according to strata platinum refractory, platinum resistant and platinum sensitive the response rates were 5.9%, 17.8% and 26.7%, respectively. The median duration from time of documented response was 21.7 weeks (range 4.6-41.9).

Conclusion: Topotecan in a daily x 5 schedule is an effective regimen as second-line treatment in ovarian cancer, with a favourable toxicity profile. Further investigations of topotecan in ovarian cancer including first line use and combination with other active agents are indicated.

INTRODUCTION

Ovarian cancer is the most common cause of death among women with gynecologic malignancies. Its incidence is the highest in Europe and North America (1). Most ovarian tumors in the Western world are of the common epithelial type, accounting for nearly 90% of all ovarian malignancies. Many patients present with advanced stage disease. The mainstay of therapy for advanced stage ovarian cancer involves adequate surgical staging and cytoreduction followed by platinum-based chemotherapy, with initial response rates of 70-80% including a high portion of complete responses. However, the majority of patients eventually relapse and ultimately die of chemoresistant disease (2).

Response rates to salvage treatment are modest and the duration of response is usually relatively short. Recently taxanes were identified to have second-line activity in ovarian cancer (3-11), but further studies on the optimal schedule of paclitaxel and circumvention of the side effects of docetaxel are necessary to properly define their potential in second-line treatment. Even then, it is clear that further new agents have to be identified for the salvage setting to hopefully improve the dismal outlook for patients with epithelial ovarian cancer.

Topotecan,[(S)-9-dimethylaminomethyl-10-hydroxycamptothecinhydrochloride],is a semisynthetic watersoluble analogue of the parent drug camptothecin. Topotecan is a specific inhibitor of the enzyme topoisomerase I, a cellular enzyme that is involved in maintaining the topographic structure of DNA. Topoisomerase I covalently binds to double-stranded DNA forming the cleavable complex which enables uncoiling of the DNA helix during replication and transcription resulting in transient single-strand breaks and relieves torsional strain along the fork of replicating DNA. Topoisomerase I inhibitors form a ternary complex with the covalently bound DNA-topoisomerase I enzyme and interfere with the DNA breakage and resealing process. It permits uncoiling of the double-stranded DNA but it prevents the resealing. The advancing replication fork then collides with the cleavable complex, resulting in fork breakage and eventually cell death (12-13). In preclinical testing topotecan has shown to have antitumor activity

against a variety of tumors including human ovarian xenografts (13-14). In phase I studies clinical antitumor activity was most clear in the daily x5 schedule with non-cumulative neutropenia as the dose limiting toxicity.

An initial phase II study using this schedule in 28 patients with ovarian cancer, of whom 80% were considered platinum-refractory, suggested activity for topotecan in this disease (15). The median number of prior chemotherapy regimens in these patients was 2 (range 1-4). The use of G-CSF to maintain dose intensity was prohibited in this study.

The interval between prior platinum therapy and relapse is known to influence the response to subsequent therapy (16-18). This way, relapses can be discriminated in three groups: platinum-refractory disease, platinum-resistant relapse and platinum-sensitive relapse.

To properly define the efficacy of topotecan in a daily x5 schedule as a second-line treatment, in patients who failed one platinum based chemotherapeutic regimen, we performed a large phase II study taking these strata into account.

PATIENTS AND METHODS

Patients

Patients with a histological diagnosis of epithelial ovarian cancer, FIGO stage III or IV at the time of entry into the study, who had failed first line therapy with one regimen containing cisplatin or carboplatin were eligible for the study. The population was defined by the following strata: 1. *Platinum-refractory patients:* Patients with progressive or stable disease while on initial platinum-based chemotherapy. 2. *Platinum-resistant patients:* Patients who responded and subsequently relapsed within 6 months after discontinuation of initial platinum-based chemotherapy. 3) *Platinum-sensitive patients:* Patients who responded but relapsed after more than 6 months after discontinuation of initial platinum-based chemotherapy. Eligibility further included; measurable disease must be $\geq$ 2 cm (in case of tumor measurement solely relying on physical examination, verification was to be performed by a second physician) ; age $\geq$ 18 years; WHO

performance status ≤ 2; adequate bone marrow function (white blood cells (WBC) ≥ 3.5 x 10^9/l, granulocytes ≥ 1.5 x 10^9/l and platelets ≥ 100 x 10^9/l); serum creatinine ≤ 132.6 μmol/l; serum bilirubin ≤ 34.2 μmol/l; serum SGOT(AST)/SGPT(ALT) and alkaline phosphatase ≤ 2x the upper limit of normal, in case of liver metastasis ≤ 5x the upper limit; at least 4 weeks since last surgery, radiotherapy (indicator lesions must not be in the field of prior radiation) or chemotherapy; no active concomitant malignant disease, with the exception of adequately treated cone-biopsied in-situ carcinoma of the cervix and basal cell or squamous cell carcinoma of the skin; no prior treatment with taxanes; informed consent was obtained according to the local or national rules from all patients before entry of the study.

Treatment schedule

Topotecan 1.5 mg/m^2/day was administered as a 30 minute infusion for 5 consecutive days every 3 weeks. 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 sterile water for injection prior to dilution with 5% dextrose solution. The appropriate volume of the reconstituted solution was then further diluted in 50 ml 5% dextrose.

Follow-up study scheme

Complete blood count (CBC), blood chemistries including renal and liver functions, total protein, albumin and glucose were performed weekly. Urinalysis and determination of the CA-125 were performed before every cycle. An ECG was performed before treatment and prior to the second cycle.

Dose modifications

The next treatment cycle was administered if granulocytes were ≥ 1.0 x 10^9/l and platelets ≥ 100 x 10^9/l. Prophylactic administration of G-CSF (Filgrastim®) 5 μg/kg subcutaneously injected once daily for 10 days beginning on day 6 of the treatment cycle could be started if the patient experienced grade 4 neutropenia and fever or

grade 4 neutropenia lasting ≥ 14 days or grade 3 neutropenia requiring a delay in the next cycle. A dose reduction of 0.25 mg/m^2/day was performed if, with the addition of G-CSF, a grade 4 neutropenia was complicated by fever/infection or lasted ≥ 14 days, or if a grade 3 neutropenia lasted beyond day 21 of the treatment cycle. A dose reduction of 0.25 mg/m^2/day was also performed if a grade 3-4 thrombocytopenia was associated with bleeding or if thrombocytopenia grade 3-4 lasted ≥ 14 days or if a grade 3-4 non-hematologic toxicity was seen. The single infusion dose was increased by 0.25 mg/m^2/day if during the previous cycle there was no toxicity greater than grade 2. The minimum allowed infusion dose was 1.0 mg/m^2/day and the maximum allowed infusion dose was 2.0 mg/m^2/day.

Evaluation

Responses were evaluated according to the World Health Organisation (WHO) criteria (19). All reported responses were confirmed by independent extramural review. The duration of stable disease (SD), partial response (PR) and complete response (CR) was defined as the interval between the initial documented response to the first sign of progression. Measurement of the involved lesions by computed tomographic scan, plain chest X-ray, and ultrasonography was performed every 2 cycles. Toxicity was assessed before every cycle according to the Common Toxicity Criteria (CTC)(20).

RESULTS

A total of 111 patients were entered into this phase II study. Nineteen patients were considered to be ineligible. Reasons for ineligibility were; more than one prior platinum based therapy (7), indicator lesions within the field of prior radiotherapy (5), no measurable disease (4), concomitant malignancy (1), surgery within 28 days before start treatment (1) and performance status > 2 (1). In order not to underestimate toxicity and overestimate efficacy an intention to treat analysis was performed including all entered patients. Ninety-two patients were formally evaluable for response.

Patient characteristics are summarized in table 1. These patients were stratified in 3 subgroups, i.e. platinum-refractory (34 patients), platinum-resistant (28 patients) and platinum-sensitive relapses (30 patients).

Table 1. Patient characteristics

	No. of patients	
No. patients entered	111	
No. patients eligible	92	
Mean age in years (range)	57.6	(25-76)
Performance status (WHO)		
0	48	
1	39	
2	5	
Tumor size, cm		
< 5	32	
≥ 5	60	
Response to first-line therapy		
complete response	33	
partial response	25	
stable disease	16	
progressive disease	18	
Classification of relapse		
platinum refractory	34	
platinum resistant	28	
platinum sensitive	30	

The majority of patients had bulky disease (tumor > 5 cm in diameter) and in addition to first-line platinum based chemotherapy 5 patients were pretreated with radiotherapy and 1 patient was previously treated with immunotherapy.

For toxicity an intention to treat analysis was performed, including all patients. A total of 552 courses of topotecan were given, with a median number of 4 (range 1-17) per patient. The major side effect was myelosuppression. Leuco- and neutropenia grade 3-4 occurred in 54.2 % and 69.1 %, respectively (table 2). The median nadir of neutropenia was 0.6 x 10^9/l (range 0.0-13.6) and was seen on day 11 (range 2-29). The median duration of neutropenia was 7 days. Myelosuppression was not cumulative. Despite the severe neutropenia, fever ≥ grade 2 was observed in only 4,3% of the courses with infectious fever in 3.1% of cycles with grade 4 neutropenia, including one fatal neutropenic fever that occurred shortly after completion of the first treatment cycle. Treatment delays occurred in 25.4% of courses, delays lasting ≤ 2 days in 10%, 3-7 days in 11.8% and ≥ 7 days in 3.6% of courses. Hematological toxicity accountered for 14.5% of all treatment delays. G-CSF (Filgrastim®) was given in 20.5% of the courses, in 16.1% it was given prophylactically, in 4.3% of courses it was given as treatment. Dose reductions were very infrequent, only 6.8% of all courses were given at reduced doses, merely due to hematologic toxicity. On the contrary, 7.2% of the courses the dose of topotecan could be escalated (table 3).If expressed as mean or median dose intensity (mg/m^2/week) the targeted dose intensity of 2.50 mg/m^2/week was achieved in 94.4% and 97.6% of the courses respectively (table 4). Thrombocytopenia was relatively mild, grade 3-4 thrombocytopenia was observed in 13.8% of the courses, with prophylactic platelet transfusions in 2.4%. Concurrent anemia, although easily manageable, was frequent; erythrocyte transfusions were needed in 20.7% of the courses (table 2).

Non-hematological toxicities were mild and consisted of nausea, vomiting, stomatitis, fatigue and asthenia. Alopecia was seen in 82% of the patients being partial in 24.3% and total in 57.5% (table 5).

Table 2. Hematological toxicity per course (N = 552)

	CTC grade				grade 3 - 4 %
	1	2	3	4	
Leucopenia	56	123	245	52	54.2
Neutropenia	26	68	156	218	69.1
Thrombocyto-penia	237	68	44	32	13.8
Anemia	154	312	56	5	11.1

Table 3. Number of patients and courses per dose (mg/m^2/day)

Dose (mg/m^2/day)	No. of patients	No. of courses	Course median	Course range
1.00	4	5	1	1-2
1.25	14	33	1	1-8
1.50	111	474	3	1-17
1.75	11	28	2	1-5
2.00	5	12	1	1-5
Overall	111	552	4	1-17

Table 4. Dose intensity (mg/m^2/week) per course

Course	n	Mean	Median
1	111	2.31	2.50
2	104	2.40	2.50
3	79	2.48	2.50
4	69	2.38	2.50
5	53	2.45	2.50
6	44	2.47	2.50
7	27	2.47	2.50
8	23	2.43	2.50
9	11	2.46	2.50
10	10	2.30	2.50
11	5	2.50	2.50
12	4	2.38	2.50
13	3	2.50	2.50
14	3	2.50	2.50
15	3	2.50	2.50
16	2	2.45	2.45
17	1	2.50	2.50
Overall	111	2.36	2.44

Table 5. Non-hematological toxicity per course (N = 552)

	CTC				total	%
	1	2	3	4		
Nausea	152	40	9		201	36.4
Vomiting	60	30	7		97	17.5
Fatique	61	19			80	14.4
Stomatitis	44	26	2	1	73	13.2
Asthenia	36	19	2	1	58	10.5

Table 6. Response in relation to the interval of relapse after prior platinum based chemotherapy

Relapse	Refractory	Resistant	Sensitive
No. pts	34	28	30
CR	0	0	1
PR	2	5	7
SD	9	5	5
total (%)	2 (5.9%)	5 (17.8%)	8 (26.7%)

Response data were analyzed in eligible patients. All responses were confirmed by independent review. None of the responses were assessed by physical examination. The overall response rate was 16.3% (95% confidence interval (CI) 9.4-23.5%) with 1 complete and 14 partial responses. If expressed according to the defined strata platinum-refractory, platinum-resistant and platinum-sensitive the response rates were 5.9%, 17.8% and 26.7%, respectively (table 6). In addition, 19 patients (20.7%) had stable disease. The median time to response was 10.4 weeks. The median response duration (measured from the time of first documented response) for the responders was 21.7 weeks (range 4.6-41.9). The median time to progression for all patients was 11.9 weeks. If the data were analyzed according to the intention-to-treat principle the overall response rate was 16 responses of 111 patients (14.4%; CI 7.9-20.9%).

DISCUSSION

Topotecan at this dose and schedule is active in ovarian cancer. The regimen was very well tolerated. Although neutropenia was very prominent, being grade 3-4 in 69,1% of the courses with a median nadir of 0.6 $x10^9$/l (range 0.0-13.6), infectious complications (3.1%) and treatment delays due to neutropenia (14.5%), with subsequently prophylactic administration of G-CSF (Filgrastim®) occurred only in a minority of the courses. Dose reduction were rare and in addition, the targeted dose intensity was achieved in practically all courses. Presumably this reflects the brief duration of leuco- and neutropenia. Thrombocytopenia was much less common, and even grade 3-4 thrombocytopenia was never complicated by bleeding.
Non-hematological toxicities were mild. Alopecia occurred in almost all patients, nausea and vomiting were easily circumvented with the use of standard anti-emetics, sometimes in combination with steroids.
The present study showed that topotecan at this dose and schedule resulted in a response rate of 16.3% with a median duration of 21.7 weeks. In addition, 20.7% of patients had at least temporary stabilization of disease. This study that included 92 patients, largely confirms the results of Kuldelka et al. (15) who obtained a response

rate of 14% with a median duration of 8.7 months, in a patient population of 28 patients. In addition, these results fit with the recently published preliminary data of Armstrong et al. (21) with the daily x5 topotecan schedule as salvage therapy for ovarian cancer. They reported 1 complete and 3 partial responses in the first 16 evaluable patients (25%), in a study for which accrual continues.

If expressed according to the defined strata, platinum-refractory, platinum-resistant and platinum-sensitive, the observed response rates were respectively, 5.9%, 17.8% and 26.7%. So in truly platinum-refractory disease topotecan shows only modest activity.

Many new drugs have been tested as salvage therapy in ovarian cancer in clinical trials; of these, especially the taxanes paclitaxel (Taxol) and docetaxel (Taxotere) seem promising (22). The obtained response rate with paclitaxel varies from 11.9-29% (3-8,23), and for docetaxel from 25-41% (9-11,24). These results can not easily be compared with those of the present study since in many reports on taxanes the definitions of, and/or detailed information on the different strata is missing and for paclitaxel many different doses and infusion durations were applied. The European-Canadian study (8) indicated that the efficacy of paclitaxel is more dose- than schedule dependent. Furthermore, prolonged infusions revealed more severe neutropenia, and as a consequence this resulted in more dose reductions. The latter study also included an independent response review and at the registered dose of 175 mg/m^2 given over 3 hours the response rate to paclitaxel was 15%, which is similar to the results presently reported for topotecan. In addition, increasingly paclitaxel is being used in the first line treatment of ovarian cancer which further stresses the need to develop new drugs with activity in second line such as topotecan.

As compared to taxanes, and with the exception of shortlasting neutropenia, topotecan lacks, many side effects. Toxicities of taxanes include, alopecia, nausea/vomiting, mucositis, arthralgia/myalgia and anaphylactoid hypersensivity reactions. In addition paclitaxel induces peripheral neuropathy, and less frequently, cardiac dysrhythmias (3-8,23,25), while docetaxel induces skin toxicity, onycholysis and fluid retention, the latter two being related to the cumulative dose (9-11,23,25).

Although comparison of topotecan versus taxanes cannot be easily performed with the currently available data it is suggested that the efficacy might be in the same range and therefore a randomized study has been initiated to compare topotecan versus paclitaxel as second-line therapy in ovarian cancer.

In an attempt to further improve the current results of topotecan in ovarian cancer other dosing schedules warrant exploration. Preclinical data have indicated that topoisomerase I inhibitors demonstrate greater efficacy and have a greater therapeutic index with prolonged continuous exposure (26,27). The feasibility of this concept of prolonged exposure in humans using a 21-days continuous infusion repeated every 28 days is recently reported (28). The dose intensity exceeded most other schedules of topotecan. Phase II studies with this schedule in different tumor types, including ovarian cancer, are ongoing. In addition, recently reported results reveal a bioavailability of oral topotecan of 32% with limited variation, and thus oral administration may appear to be a more simple and more convenient method to achieve prolonged exposure (29).

Combination therapy with cisplatin, the current most effective agent in ovarian cancer is of great interest because of the non-overlapping toxicities, the different mechanisms of action and the fact that topotecan potentially can interfere with repair from cisplatin induced DNA damage, a major mechanism of cisplatin resistance (30,31). The first dose-finding studies of this combination were recently be published (32,33), and other studies are on the way.

In summary, topotecan at a dose of 1.5 mg/m^2/day for 5 days repeated every 21 days is an efficacious drug in ovarian cancer, with only relatively minor and readily managed, non-cumulative side effects. Future directions should include exploring alternative regimens and combinations with other agents active in ovarian cancer.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF TOPOTECAN ADMINISTERED AT A DAILY TIMES FIVE SCHEDULE IN PHASE II CLINICAL TRIALS USING A LIMITED SAMPLING PROCEDURE

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SUMMARY

Topotecan is a novel semisynthetic derivative of the anticancer agent camptothecin and inhibits the intranuclear enzyme topoisomerase I. The lactone structure of topotecan, which is in equilibrium with the inactive ring-opened hydroxy acid is essential for this activity. We performed a pharmacokinetic study, as part of phase II clinical trials in patients with various types of solid tumours with topotecan 1.5 mg/m^2/day administered by a 30 minutes infusion for 5 consecutive days which was repeated every 3 weeks. Previously validated limited sampling models, using concentration measurements in samples obtained 2 hours after infusion, were used to calculate the area under the plasma concentration-time curves (AUCs) of both chemical forms. Samples were obtained from a total of 36 patients over 136 treatment days. The mean AUC of the closed ring form (AUC_{closed}) was 8.74 μM·min/day (range 2.3- 16.3), and the mean AUC of the ring-opened form (AUC_{open}) was 11.5 μM·min/day (range 3.2- 46.0) (interpatient variability: 34 to 61%). In each patient, the AUC-values achieved on the first day of administration were similar to, and thus predictive for, those achieved during the following days, with a day-to-day variation of 7.39% for the AUC_{closed}, and 12.6% for the AUC_{open}. There was no drug accumulation during the five consecutive treatment days of each cycle. However, despite the large interpatient pharmacokinetic variability, the importance of regular drug monitoring in this schedule can be questioned, as the pharmacodynamic variability was relatively small.

INTRODUCTION

Topotecan ([S]-9-dimethylaminomethyl-10-hydroxy-camptothecin, SK&F104864, NSC 609699, Hycamtin®), is a semisynthetic derivative of camptothecin, an anticancer drug derived from the Asian tree Camptotheca acuminata. Due to camptothecin's serious and unpredictable gastrointestinal, urothelial and myelosuppressive toxicities, clinical evaluation had to be discontinued in the early 1970s (12,13). Compared with camptothecin, topotecan as the hydrochloride salt is better water soluble, has a reduced protein binding and shows promising pre-clinical and clinical efficacy with a strongly reduced toxicity profile (7,9,15,25). The dose limiting toxicity of topotecan in phase I trials is reversible myelotoxicity, particularly granulocytopenia (15,16,18,24,25). The cytotoxicity of topotecan and other camptothecin analogues is ascribed to inhibition of the intranuclear enzyme topoisomerase I (1,8,19). The lactone structure, which is in equilibrium with the open-ring hydroxy acid (SK&F 105992) at constant pH is essential for this activity (figure 1) (7,26).

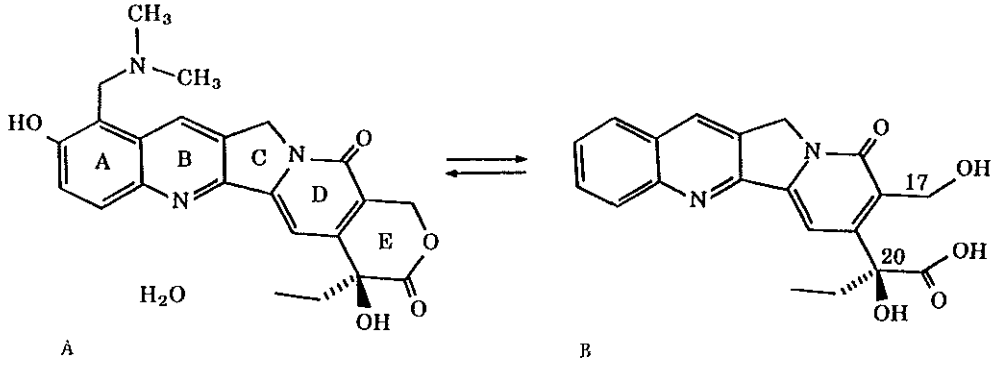

Figure 1:
Chemical structures of topotecan (A) and its lactone ring-opened hydroxy acid (B). Both forms are in equilibrium at constant pH.

The closed lactone ring predominates at acidic pH, but the reverse reaction of the parent into the open ring form is favoured at physiological pH (2,4,21). The hydrolysis of the parent drug into the inactive form may thus have important pharmacokinetic and pharmacodynamic implications.

The results of phase I clinical trials have suggested that pharmacokinetic parameters are well correlated with pharmacodynamic outcome (4,5,20,23,25). Especially the area under the plasma concentration-time curve (AUC) of topotecan was pre-eminently related to the degree of neutropenia and leucopenia (4,5,20,23,25). This relationship might be of value for the optimization of topotecan therapy since, by measuring the AUC, patients at high risk for developing serious toxicity might be identified at an early stage. Furthermore, the dose could in theory be adjusted upwards or downwards during a daily times five treatment to achieve the AUC associated with an optimal clinical outcome. However, since the topotecan dose in the phase I clinical trials was linearly related to the AUC (4,23,25), the escalation of the dose might have, consequently, defined predominantly the relationships between the AUCs and the toxicities.

In order to find out whether this is true, we felt it important to further investigate the relationships between the AUCs and the toxicities in different subjects treated with a fixed topotecan dose. The Pharmacology And Molecular Mechanisms (PAMM) group and Early Clinical Trials Group (ECTG) of the EORTC (European Organization for Research and Treatment of Cancer) have recently taken the initiative to coordinate and stimulate pharmacokinetic studies as part of phase II clinical trials (14). The larger and more homogenous phase II patient population allows more insight into the variability in pharmacokinetics and their relationships with pharmacodynamics, such as toxicity and tumour response, as compared to phase I studies. We previously developed and validated a limited sampling model for topotecan (22) that can be used to get the necessary pharmacokinetic data with a minimum of samples taken from the patients. This limited sampling strategy was used for the present study in patients with ovarian, colorectal or small cell lung cancer (SCLC). Pharmacokinetic monitoring is also performed in other ongoing phase II studies coordinated by the EORTC-PAMM and

ECTG groups with the investigational anticancer agents irinotecan, docetaxel, EO9, and rhizoxin (14).

PATIENTS AND METHODS

Patient population

The patients participated in three phase II clinical trials in which topotecan was administered daily at a dose of 1.5 mg/m^2 for five consecutive days every three weeks. These phase II trials have recently been completed, and the detailed clinical results will be published elsewhere. Eligible were patients with ovarian cancer failing treatment with a platinum-based chemotherapeutic regimen, non-chemotherapy pretreated patients with colorectal cancer, or patients with small cell lung cancer (SCLC) failing or relapsing after first-line chemotherapy. All patients had an adequate bone marrow function (White Blood Cells (WBC) $\geq 3.5 \times 10^9$/l, absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l), normal serum bilirubin ($\leq 26\ \mu$M) and serum creatinine ($\leq 140\ \mu$M), no prior history of haemorrhagic cystitis, WHO performance status ≤ 2, life expectancy of ≥ 12 weeks and age 18- 75 years. All patients gave informed consent.

Treatment plan

Topotecan (SmithKline Beecham, King of Prussia, USA) was supplied as a freeze-dried product, with topotecan as the hydrochloride salt (5 mg as free base) and 100 mg mannitol as bulking agent (i.e. topotecan AA formulation). These vials were reconstituted before infusion with 2 ml of Water for Injection. The appropriate daily dosage of the drug was diluted in 50 ml of normal saline (NaCl 0.9%) and was administered intravenously by a syringe pump during 30 minutes. To assess the haematological and non-haematological toxicities, patients were evaluated each course by clinical history, physical examination, and serum chemistry. Haematology screen was performed twice weekly. Response was assessed every 2 courses.

Pharmacokinetic studies

Blood samples (5 ml each) were taken by a venepuncture from a cubital vein, in the arm contralateral to the arm receiving topotecan. The blood samples were collected at one single time point: exactly at 2.5 hours after the start of the 30 minutes infusion. An error in sample timing of maximal 5 minutes was allowed. Each sample was collected in a heparinized tube at one and, when possible, at more days of drug administration. Plasma was obtained by immediate centrifugation (5 minutes; 1500·g) of the samples, which was followed by protein precipitation with cold methanol (-30°C); 1.0 ml plasma was added to 4.0 ml of methanol. Thereafter, the mixture was vortex-mixed and centrifuged (5 minutes; 1500·g), and the clear supernatant was transferred to a polypropylene tube and stored (-30°C) until analysis. The closed lactone ring and the lactone ring-opened form of topotecan were determined by a validated high performance liquid chromatographic (HPLC) method using fluorescence detection (2). The AUCs were calculated using the following validated limited sampling models (22), for the closed ring form:

$$AUC_{closed}\ (\mu M \cdot min) = 499(min) \cdot C_{2h}(\mu M) + 0.85(\mu M \cdot min \cdot m^2/mg) \cdot dose(mg/m^2),$$

and for the ring-opened form,

$$AUC_{open}\ (\mu M \cdot min) = 551(min) \cdot C_{2h}(\mu M) - 0.011(\mu M \cdot min \cdot m^2/mg) \cdot dose\ (mg/m^2),$$

where C_{2h} is the plasma concentration of the closed or the ring-opened form, respectively, at 2 hours after the end of the 30 minutes infusion. The AUC of the lactone plus the ring-opened form (AUC_{total}) was calculated by: $AUC_{closed} + AUC_{open}$. The intrapatient (day-to-day) and interpatient variation in pharmacokinetics were calculated by analysis of variance (ANOVA).

The pharmacodynamics, especially the myelosuppression, were explored using plots

of percentage decrease (%decr) in ANC, %decr in WBC and %decr in platelet count versus the AUC_{closed}/day, the AUC_{open}/day, the AUC_{total}/day, and the total dose (mg). The percentage decrease (%decr) is defined as:

$$\%decr = \frac{\text{Pretreatment value - value of the nadir}}{\text{Pretreatment value}\cdot 100\%}$$

The data were fit to the respective sigmoidal maximum effect ($sigE_{max}$) models derived from the phase I clinical trial (23). Mathematically, these models are defined as:

$$\%decr = \frac{100\cdot(P)^H}{(P_{50})^H + (P)^H}$$

where P is the value of the pharmacokinetic parameter or the dose, P_{50} the P that results in 50% decrease, and H is the Hill constant, which determines the shape of the curve. These values were for the %decr in ANC versus the dose(mg): H = 2.27, P_{50} = 1.02; AUC_{closed}: H = 1.55, P_{50} = 1.55; AUC_{open}: H = 1.09, P_{50} = 1.97; and AUC_{total}: H = 1.30, P_{50} = 4.96 (23). The performance of these models for the phase II pharmacokinetic data was evaluated by using the relative Root Mean Square Error (%RMSE)-value (17). The %RMSE is a measure of precision and is defined as:

$$RMSE\% = \left[N^{-1}\cdot\sum_{i=1}^{N}(pe_i)^2\right]^{1/2}\cdot 100\%$$

where N is e.g. the number of P-pairs (i.e. true with predicted values), and pe is the prediction error [$\ln(P_{true\ value})$-$\ln(P_{predicted})$]. The smaller the %RMSE, the better the relation is described by the model. To investigate the determinators of inter- and intraindividual kinetic variability, we correlated continuously-scaled patients characteristics with the pharmacokinetics by linear regression analysis. These characteristics were age, weight, height, creatinine clearance, and the number of topotecan courses received. The investigated pharmacokinetic parameters were the AUC_{closed}/day, the AUC_{open}/day, their ratio (AUC_{closed}/AUC_{open}), and the AUC_{total}/day. These values were calculated

per day to account for the missing values during the five administration days. This is justified since the intrapatient variability was very small (< 15%). Discontinuously scaled patient characteristics, such as gender (male versus female), WHO-performance status (0 versus 1 or 2), pretreatment status (prior chemotherapy or not), were also tested (Student's t-test) for differences in the values of the kinetic parameters. In addition, we investigated whether the kinetic parameters and/or patient characteristics were related to the toxicities by linear regression analysis and the Student's t-test, respectively. The computer program NCSS® (Number Cruncher Statistical System, Kaysville, Utah, USA, 1992) and Quattro Pro® (Borland International, Scotts Valley, CA, USA, 1992) were used for all calculations.

RESULTS

A total of 36 patients entered in this pharmacokinetic trial of which 18 patients (8 female, 10 male) had colorectal cancer, 14 patients had ovarian cancer, and 4 patients (all female) had SCLC. All patients with ovarian cancer were pretreated with platinum-containing chemotherapy, all patients with SCLC were relapsing after or failing first-line chemotherapy involving a variety of drugs, whereas the colorectal cancer patients had not received prior chemotherapy. The median age was 59 (range 48-75) years, the median performance status was 1 (range 0-2), and the median creatinine clearance was 80 (range 50- 112) ml/min.

Toxicity

In all 36 patients the major toxicity was granulocytopenia, with a mean %decr in ANC of 85% (48%- 99%) (being WHO-grade IV in 38/61 courses). Thrombocytopenia was much less frequent and less severe, with a mean %decr in platelet count of 68% (range 45%- 99%) (being WHO-grade 3 in 5/61 courses and grade 4 in 2/61 courses). Mild anaemia grade 1-2 occurred regularly, with a mean %decr in the haemoglobin (Hb) of 16% (range 4%-34%). The nadirs of both granulocytopenia and thrombocytopenia were between day 8 and 15, and were of short duration (3-5

days). Non-haematologic toxicities were grade II or less and included nausea, vomiting, diarrhoea, alopecia, fatigue, and stomatitis.

Responses

All patients were evaluable for response. Most patients (10/18) with colorectal cancer were progressive under topotecan treatment. However, one patient had a partial remission, and seven patients remained stable for at least 2 months. Of the ovarian cancer patients 1 achieved a complete remission, 2 a partial remission, 7 were stable, and 4 progressive. Two patients with SCLC remained also stable, while the two others progressed.

Feasibility

It appeared that the requirements for the sample acquisition were generally very demanding for the clinical staff; the sample had to be timely withdrawn at exactly 2 hours after the end of infusion, which had to be followed by immediate centrifugation and careful volumetric pipettation into cold and volatile methanol, whereafter centrifugation was required again, followed by refrigeration and finally shipment to our laboratory. In addition, it appeared that patients treated on an outpatient-basis were frequently not willing to wait for the blood sampling at 2 hours after infusion. For these reasons, despite the fact that over 200 patients entered the full phase II programmes in over 30 institutions, pharmacokinetic samples could only be obtained from two institutes in the Netherlands over 136 treatment days, in 61 courses.

Pharmacokinetics

Intrapatient data

The AUC-values for the first day of administration were similar to and thus predictive for those achieved in the following days in the same patient (n = 19 patients), with a day-to-day variation of 9.7% for the AUC_{total}, 12.6% for the AUC_{open}, and 7.39% for the AUC_{closed}. Thus, no accumulation of topotecan occurred during the consecutive

days of administration. Furthermore, the intrapatient variability between courses was also small (n = 10 patients, 39 courses), with a course-to-course variation ranging between 9.4% to 10.7%.

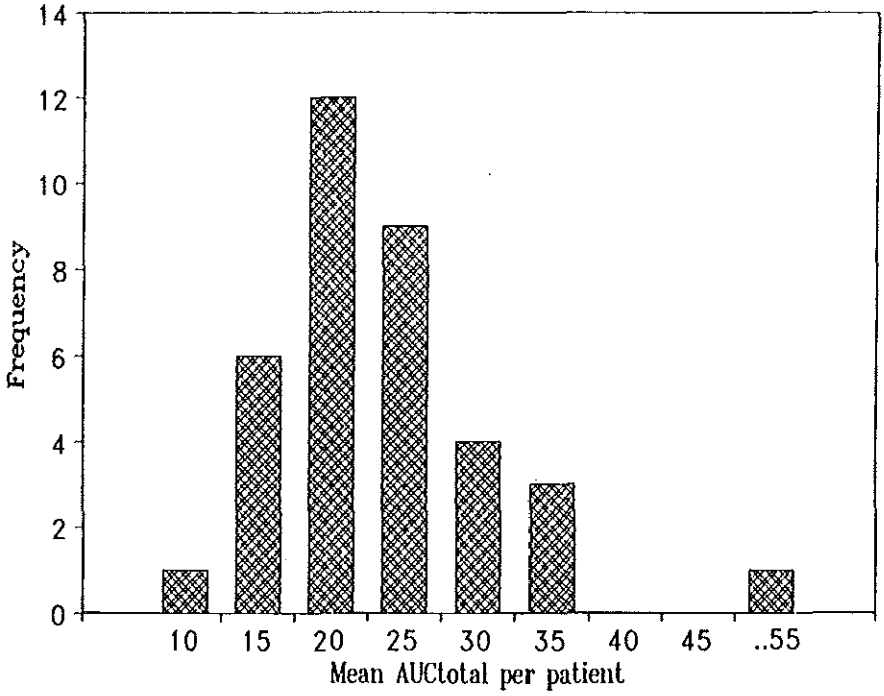

Figure 2:
Frequency plot of the mean AUC_{total} per patient.

Interpatient data

However, a wide interpatient (n = 36 patients) variability in the pharmacokinetics of topotecan was found, being 39.3% for the AUC_{total} (Figure 2), 60.9% for the AUC_{open}, and 34.4% for the AUC_{closed}. The mean AUC of topotecan was 8.71 μM·min/day (range 2.3-15.8), and the mean AUC of the ring-opened form was 11.49 μM·min/day

(range 3.2-46.0). The ratios of the AUC_{open} and AUC_{closed} ranged between 0.15 and 1.96, with a mean ratio of 0.91. The value of this ratio was patient-dependent (inter-patient variation 67.2%), with a day-to-day (intrapatient) variation of 10.1%. The patients with ovarian cancer appeared to have higher AUC_{closed} and AUC_{total}-levels than patients with colorectal cancer ($0.00001 < p < 0.006$). Patients with SCLC had lower AUC-levels than patients with colorectal cancer, but this was not statistically significant ($0.06 < p < 0.35$; $n = 4$). Other parameters, such as age, weight, height, gender, creatinine clearance, performance status, or the number of topotecan courses received were not significantly related with the kinetic parameters (Table 1).

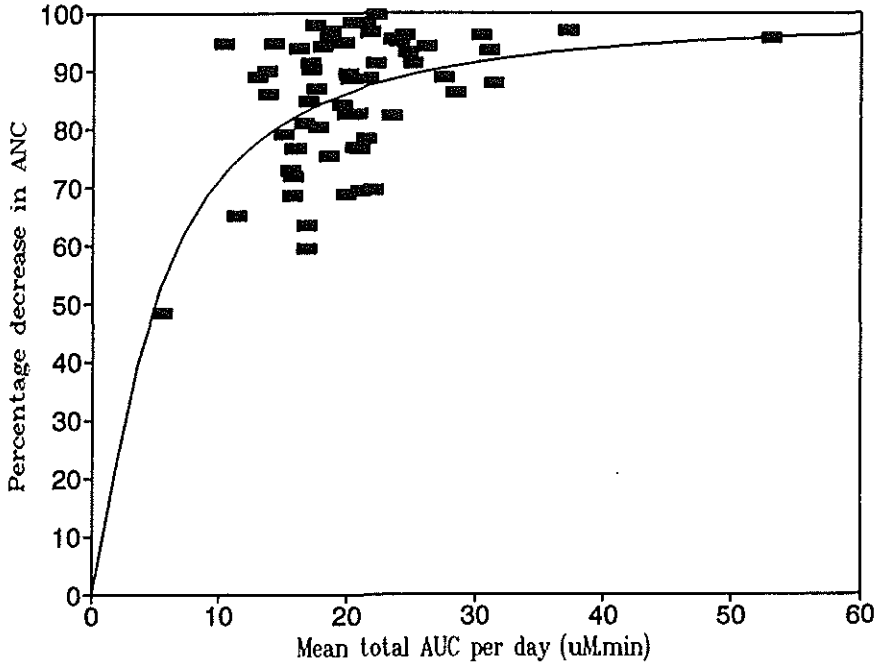

Figure 3:

Relation between the mean total AUC per day (closed form + ring-opened form) and the percentage decrease in ANC. The solid line represents the fit of the data to the $sigE_{max}$ model as derived from the preceding phase I trial:

$$\%decr = \frac{100 \cdot (AUC)^{1.30}}{(4.96)^{1.30} + (AUC)^{1.30}}$$

Table 1 Relationships between patient characteristics and pharmacokinetic parameters

Patient Characteristic	AUC_{closed}		AUC_{open}		AUC_{total}		AUC_{closed}/AUC_{open}	
	r	p-value	r	p-value	r	p-value	r	p-value
Patients with ovarian cancer								
Age	0.21	0.45	0.19	0.06	0.03	0.04	0.40	0.06
Creatinine clearance	0.03	0.01	0.02	0.07	0.01	0.55	0.04	0.92
Height	0.16	0.26	0.28	0.07	0.27	0.07	0.16	0.41
Number of courses received	0.17	0.45	0.13	0.51	0.01	0.35	0.33	0.35
Weight	0.18	0.68	0.07	0.04	0.03	0.05	0.14	0.30
Performance Status	-	0.14	-	0.43	-	0.20	-	0.42
Progression vs no progression	-	0.02	-	0.008	-	0.006	-	0.50
Patients with colorectal cancer								
Age	0.15	0.88	0.24	0.32	0.21	0.37	0.44	0.18
Creatinine clearance	0.02	0.99	0.16	0.65	0.15	0.67	0.17	0.33
Height	0.10	0.21	0.15	0.73	0.16	0.62	0.03	0.98
Number of courses received	0.08	0.01	0.05	0.01	0.06	0.01	0.08	0.01
Weight	0.17	0.91	0.07	0.67	0.09	0.71	0.06	0.70
Gender	-	0.64	-	0.17		0.56		0.16
Performance Status	-	0.87	-	0.47	-	0.48	-	0.78
Progression vs no progression	-	0.48	-	0.47	-	0.41	-	0.99

Abbreviations: AUC: area under the concentration-time curve of the lactone from (closed), the ring-opened form (open), and the sum of both (total), respectively; r: correlation coefficient; -: not applicable (Student's t-test only); vs: versus

Pharmacokinetic-Pharmacodynamic relationships

The mean AUC_{closed}/day, AUC_{open}/day, the AUC_{total}/day, and the total dose/day (mg) were plotted against the %decr in WBC, the %decr in ANC and the %decr in platelets. The sigmoidal E_{max} models previously derived from the phase I clinical trial (23), were used to describe the data and evaluated for their precision. For example, plots of the toxicity versus the dose (mg) could not adequately be described by the previously defined sigmoidal E_{max} model (RMSE% > 35%). This is in contrast to plots of the toxicity versus the AUCs of the ring-opened form, the closed ring form, and the sum of both, which were well described by their respective sigmoidal E_{max} models. Especially plots of the %decr in ANC, which was the most prominent side-effect, versus the AUCs fitted adequately to the previously defined sigmoidal models, with RMSE%-values ranging from 12.0% to 13.5% (Figure 3). These values were not significantly different from the RMSE%-values found in the phase I study ($p > 0.05$). For the %decr in platelets and %decr in WBC, the RMSE%-values ranged from 18.3% to 19.1% and from 25.1 to 25.6%, respectively. Besides the AUC-values, other predictive parameters for the %decr in ANC were the number of topotecan courses received ($r = 0.42$, $p = 0.001$), and the performance status ($p = 0.022$). There were no relationships between the grade of the non-haematological toxicities and the investigated patient characteristics or pharmacokinetic parameters. The number of responses was to small to allow a statistically meaningful analysis of relationships between response and the AUCs or other parameters.

DISCUSSION

The toxicities of topotecan in this 1.5 mg/m²/day daily times 5 schedule are similar to those reported in the phase I (4,23,24) and II (10,11) clinical trials using this schedule. Myelosuppression, especially granulocytopenia was most prominent, with 62% of the pharmacokinetically monitored courses resulting in a grade 4 neutropenia. Also the pharmacokinetics and their relationships with the pharmacodynamics were similar, despite the use of a fixed dosage in this study. The plots of the %decr in

ANC versus the AUCs could adequately be described by the sigmoidal E_{max} models that were originally fitted to the data reported for the preceding phase I clinical trial (Figure 3) (23). In that study, we predicted from the fitted models that a fixed dose of 1.5 mg/m²/day administered in phase II studies would result in an average %decr in ANC of 87%, which is in good agreement with the %decr of 85% found in the present study.

The closed ring structure is assumed to be essential for cytotoxic activity (6,7). Since the ratios of the AUC_{closed} and AUC_{open} varied between 0.15 and 1.96, and were patient-dependent, patients with equal AUC_{total}-values have been exposed to different concentrations of the lactone form. However, either the $AUCs_{closed}$, $AUCs_{open}$, or $AUCs_{total}$ were equally related to the haematological toxicity, which is also in agreement with the phase I clinical trial (23). Thus, it may seem that the fraction of topotecan present in plasma as the active lactone form is not that important for the toxicity. This may be explained by the reversibility of the conversion of the closed ring form into the ring-opened form; the open ring might spontaneously close to form the lactone form at the target under the right conditions. This hypothesis is supported by the fact that the parent compound camptothecin originally was administered as the sodium-salt of the ring-opened carboxylate form, and was also toxic and active (3,12,13). However, it is still advisable to administer topotecan dissolved in an acidic infusion fluid to yield the closed ring form. The closed ring form has the largest volume of distribution (4,23), indicating that this form is better distributed over the body, which may be important for higher tumour penetration. Unfortunately, because of limited numbers in the present study a statistically meaningful relationship between the AUCs and response was not present. It is, however, interesting to note that progressive patients with ovarian cancer had higher AUC-values compared to patients who remained stable or the few that responded to therapy. If this could be confirmed in larger numbers of patients, it suggests that the contribution of the drug exposure (AUC) to the tumor response is minimal at the fixed phase II dosage.

Then it is likely that other, tumor-dependent factors, such as type and amount of topoisomerase I levels, the number of cells in S-phase and the expression of P-glycoprotein (19), are more important for antitumor effect.

The variability in the ratio of the AUC_{closed} and AUC_{open} that was found in the present study may be explained by the differences in the pH of the administered infusion fluid. Measurement of the pH in the NaCl 0.9% containing infusion fluid of several patients yielded pH-values between 3.5 and 6.1, indicating that different fractions of the drug present as lactone form were administered. Similar results were reported by Grochow et al.(4) for topotecan added to 5% dextrose infusions. For future studies with topotecan, however, it is expected that the variability in the AUC_{closed} and AUC_{open} will be much less, since the AA formulation as used in the present study has now been replaced by the AC/AF formulation containing tartaric acid to increase the stability of the lactone form.

Measurements of the pH of these infusions indicated that 100% of the drug is present in the lactone form during infusion (pH < 3.5), which we confirmed by HPLC analysis.

In general, therapeutic drug monitoring is beneficial for toxic drugs that display a high pharmacokinetic variability and a high pharmacodynamic variability, in combination with a good relationship between the two. For topotecan, the difference in AUCs varied up to 10-fold between patients, but resulted in only a small interindividual difference in toxicity. This can be explained by the use of the maximum tolerable dose of topotecan in this phase II study producing AUCs generally being more than 15 μM·min, which is associated with maximum toxicity (%decr in ANC around 85%) and therefore small variability. As the pharmacodynamic variability was relatively small, it seems questionable whether regular drug monitoring of topotecan in this schedule will be valuable to account for differences in toxicity or response.

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PHASE II AND PHARMACOLOGIC STUDY ON 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-days continuous infusion with topotecan has been reported. We conducted a phase II study with this 21-days continuous topotecan administration in patients with locally advanced, irresectable, or metastatic colorectal cancer.

Patients and Methods: Topotecan 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. Fourty-two patients entered the study, 1 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 duration of response was 7 months (range 4-11). With this schedule the major toxicity was prolonged cumulative myelosuppression with a striking inhibition of the erythropoiesis. Other side effects were mild, including alopecia (47%), periodic nausea (40%)/ vomiting (22%) and fatigue (16%). Pharmacokinetics revealed a mean Css of topotecan of 0.62 ng/ml (range 0.33-1.1) with a significant relationship between Css of topotecan and CTC-grade of leucopenia.

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

INTRODUCTION

Colorectal cancer is one of the most common malignancies in the 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) 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-aspertate (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 is a continuous impulse to test new drugs for their activity in this disease.

Topotecan, (S)-9-dimethylaminomethyl-10-hydroxycamptothecin is a watersoluble analogue of camptothecin (4,5), a plant alkaloid from the decidous 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 swivelling at this nick and so plays an important role in DNA replication and RNA transcription. The enzyme breaks are then resealed by topoisomerase I (relegation). Binding of topoisomerase I inhibitors to the cleavable complex permits uncoiling but prevents relegation 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 pursuing prolonged exposure to the drug using a 21-days 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 myelosuppression, 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 with topotecan administered as a 21-days 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, irresectable, 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 medi-port device. A WHO performance status of ≤ 2 and a life expectancy of $\geq$ 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 0.6 mg/m^2/day was administered as a 21-days 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 flowrate of 6 ml per 24-hours and connected to the medi-port device. Stability data for the topotecan solution have been generated for at least 8 days under the conditions used in the study. Cassette and batteries were changed every week.

Dose modifications

The next treatment cycle was administered if granulocytes were $\geq 1.0 \times 10^9$/l and platelets $\geq 100 \times 10^9$/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 grade 2 or lower, the dose of topotecan was increased by 0.1 mg/m^2/day.

Pretreatment and follow-up study scheme

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.

Pharmacokinetics

Heparinized blood samples (2.8 ml) were collected during cycle 1 on day 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 3500 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 the colorectal cancer. This patient was excluded from all analyses. Another patient was not evaluable because of the development of an intra-abdominal abscess and colo-cutaneous fistula in the first week after initiation of therapy, for which reason treatment was discontinued. Patient characteristics are summarized in table 1.

Although a 21-days 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 medi-port device was inserted. In 3 patients this was the first sign of progression. Two patients experienced a local infection of the medi-port device; with an early start of antibiotics the device was preserved.

Fourty 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).

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

	CTC grade				grade 3-4 %
	1	2	3	4	
leucopenia			22	2	17
neutropenia			17	11	20
thrombocytopenia			8	4	8
anemia	250 units of erythrocyte transfusions were needed to keep the hemoglobin > 6.0 mol/l				
nausea	52	5			40
vomiting	25	6			22
fatigue	20	3			16
alopecia (per pt.)	13	6			47

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 (mg/m^2/week) 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 erythropoiesis was observed. A total of 250 units of erythrocyte transfusions were needed to keep the hemoglobin (Hb) > 6.0 mmol/l.

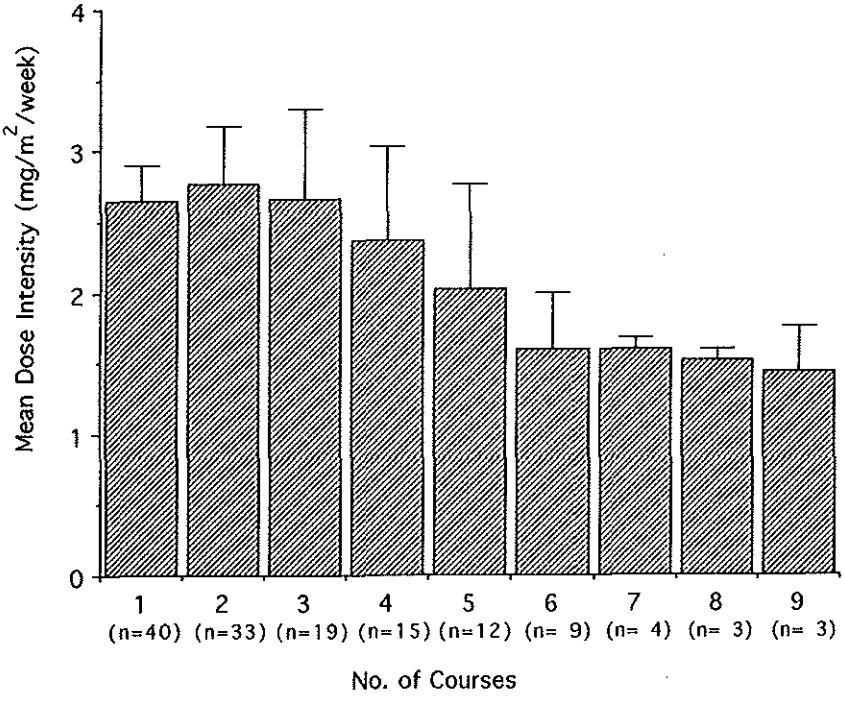

Figure 1:
Graph showing the gradually decreasing mean dose intensity ± SD (mg/m^2/week) per treatment course.

Non-hematologic side effects were alopecia (47%) being grade 1 in more than two-third of the patients, mild periodic (often only in first week of treatment) nausea (40%) and vomiting (22%), asthenia/fatigue (16%).

The latter was partially associated with anemia as symptoms subjectively reduced after transfusion.

Steady-state (Css) levels of topotecan could be determined in 37 patients and varied widely; 0.62 ± 0.17 ng/ml (range 0.33-1.1) (table 3). A low but statistically significant correlation was found between Css and CTC grade of leucopenia ($R = 0.54$, $p = 0.001$). There was no correlation between Css and the absolute dose of topotecan.

Four patients achieved a response (10%), including 1 complete response lasting for 8 months and 3 partial responses with a duration of respectively, 4, 7 and 11 months. Seventeen patients had stable disease (43%) at evaluation whereas 19 patients had progression (47%).

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, irresectable 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 at a dose of 0.53 mg/m^2/day, the dose-limiting toxicity was myelosuppression with thrombocytopenia somewhat exceeding leucopenia. 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. 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. Topoisomerase inhibitors also impair the maturation of erythroblasts by inhibition of the hemoglobinization (15). Contrasting preclinical data, the efficacy of the 21-days infusion in patients with colorectal cancer was modest with an overall response rate of 10% (1 CR and 3 PRs) with a median duration of 7 months (range 4-11).

The low correlation coefficient of 0.54 between Css and the CTC-grade of leucopenia illustrates that the pharmacokinetic variability is not a major determinant of this toxicity. Clinical studies with irinotecan (CPT-11), another topoisomerase I inhibitor reveal more promising results in patients with metastatic colorectal cancer. Several are published administrating 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% (16-19), but severe (grade 3-4) diarrhea, despite the early and frequent use of loperamide, remains an important side effect.
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 (20) and the patient convenience related to oral dosing, prolonged topotecan administration by oral application should further be studied.

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BIOAVAILABILITY AND PHARMACOKINETICS OF ORAL TOPOTECAN, A NEW TOPOISOMERASE I INHIBITOR

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SUMMARY

Background and purpose: Results of preclinical and clinical studies indicate enhanced antineoplastic activity of topotecan (SKF 104864-A) when administered on a chronic treatment. We determined the apparent bioavailability and pharmacokinetics of topotecan administered orally to 12 patients with solid tumors in a two-part crossover study.

Patients and methods: The oral dose of 1.5 mg/m^2 was administered as a drinking solution of 200 ml on day 1. The i.v. dose of 1.5 mg/m^2 was administered as a 30 minute continuous infusion on day 2. The bioavailability was calculated as the ratio of the oral and i.v. AUC calculated up to the last measured time point.

Results: The oral drinking solution was well tolerated. The bioavailability revealed moderate interpatient variation and was 30% ± 7.7% (range 21-45%). The time to maximal plasma concentration after oral administration (Tmax) was 0.78 hr (median; range 0.33-2.5). Total i.v. plasma clearance of topotecan was 824 ± 154 ml/min (range 535-1068 ml/min). The AUC ratio of topotecan and the lactone ring-opened hydrolysis product (hydroxy-acid) was of the same order after oral (0.34-1.13) and i.v. (0.47-0.98) administration.

Conclusions: The bioavailability of topotecan after oral administration illustrates significant systemic exposure to the drug, which may enable chronic oral treatment.

INTRODUCTION

Topotecan, (s)-9- dimethylaminomethyl-10-hydroxy-camptothecin is a semisynthetic watersoluble analogue of camptothecin (1). Like camptothecin, topotecan is a specific inhibitor of topoisomerase I (1). Topotecan incorporates a stable basic side chain at the 9-position of the A-ring, which provides water solubility at acid pH. Topotecan is converted to its lactone ring opened hydrolysis product (hydroxy-acid), which reversible pathway is strongly pH dependent. The biologic activity of the hydroxy-acid has not fully been elucidated (2). Antitumor activity has been demonstrated in preclinical models and in phase I and II studies (3-9). The results of preclinical and clinical studies indicate enhanced antineoplastic activity of topotecan when administered daily for prolonged periods of time (4,7,8,10-12). Also, preclinical studies with oral topotecan have shown efficacy against rhabdomyosarcoma and coloncarcinoma in mice (12). The unique mechanism of action and lack of cross resistance evidence with many well known currently available antitumor agents may provide therapeutic advantage in first line or second line chemotherapy. Furthermore, combination therapy of topotecan and other available antineoplastic agents may well be advantageous (13, 14). In view of the data suggesting the importance of prolonged exposure to topotecan for better antitumor activity and the impracticality to achieve this exposure by intravenous administration an oral formulation is being developed.
In the present study the bioavailability and pharmacokinetics of oral topotecan are explored in patients with solid tumors with the aim to provide a basis for further development of clinical studies utilizing chronic oral topotecan.

PATIENT SELECTION, MATERIALS AND METHODS

Patient selection and treatment schedule

All patients gave written informed consent according to local regulatory requirements. Eligibility for the clinical study required a pathologically confirmed small cell lung cancer, colon cancer or ovarian cancer refractory to standard therapy or for which

cancer type no therapy of proven benefit exists. The performance status had to be ≤ 2 on the WHO scale and life expectancy $\geq$ 3 months. The minimal age had to be 18 and maximal age 75 years. The hemoglobin had to be $\geq$ 6.0 mmol/l (9 g/dl), WBC $\geq 3.5 \times 10^9$/l, granulocytes $\geq 1.0 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l. The serum creatinine had to be $\leq$ 140 μmol/l (1.6 mg/dl), the serum bilirubin $\leq$ 26 μmol/l (1.5 mg/dl) and alkaline phosphatase and transaminases $\leq$ 2 times the upper normal limit or $\leq$ 5 times the upper limit of normal if liver metastases were present.
Topotecan was administered orally and intravenously at a dose of 1.5 mg/m^2. Topotecan as supplied for intravenous infusion was diluted for oral dosage. Each 5 mg vial of topotecan was reconstituted with 10 ml 5% dextrose injection solution to give a 0.5 mg/ml solution of the drug. The dose was placed in a beaker containing 100 ml of 5% dextrose injection solution. On day 1, patients were asked to drink the entire contents. Subsequently, an additional 100 ml of 5% dextrose injection solution were added to the beaker and drank by the patient. On day 2 topotecan was administered at the same dose as a continuous 30 minute intravenous infusion. The 5 mg vials of topotecan were reconstituted in 5% dextrose and the appropriate volume was then added to a volume of 50 ml of 5% dextrose. The drug was given at approximately the same time of day, between 8 am and 11 am. On both days of drug administration the patients were fasted from food for 4 hours prior to the time schedule for dosing. Fasting continued for a further 2 hours after drug administration on both days. Fluid intake was not restricted.

Blood sample collection

Serial heparinized blood samples (2.8 ml) were collected on both days of drug administration through an indwelling intravenous cannula, which was inserted in the arm contralateral to that which was used for the infusion. Blood samples were taken at 0, 10, 20, 30, 45 minutes and 1, 1.5, 2.5, 3.5, 4.5, 6.5 and 8.5 hours after start of drug administration. After collection, the blood samples were centrifuged immediately for 5 minutes at 3500 rpm. One ml of the plasma was immediately transferred to a polypropylene tube containing 4 ml of cold methanol

(-20°C) and mixed on a whirl mixer for 15 seconds. Subsequently, the mixture was centrifuged for 5 minutes at 3500 rpm and the clear supernatant was transferred to a clean polypropylene tube, closed tightly and stored immediately at -80 °C until analysis. Analysis took place within one month.

Chemicals

All chemicals were obtained from Baker (Deventer, The Netherlands) and were of analytical grade or higher.

Instruments

The HPLC apparatus consisted of a pumping device model 6000A (Waters Assoc., Milford, MA, USA), an automated sample injection device model SP 8880 (Spectra Physics, Santa Clara, CA, USA), Perkin Elmer LS40 fluorescence detector (excitation wavelength: 381 nm, emission wavelength: 527 nm; Perkin Elmer, Norwalk, CT, USA) and a model SP-4290 data analysis system (Spectra Physics). Separation was achieved with a LiChrosorb RP-18 (particle size: 5 μm) column (125x4 mm i.d., Merck, Darmstadt, Germany).

Assay of topotecan and hydroxy-acid

Topotecan and the hydroxy-acid were analyzed simultaneously with an automated reverse phase HPLC system and fluorescence detection as described (15), with modifications to lower the lower limit of quantitation (LLQ) (16). The LLQ was 0.1 ng/ml for topotecan and as well as for the hydroxy-acid. Data were accepted if the deviation from the nominal value of the individual calibration samples and quality controls was $<15\%$ (20% at the LLQ).

Pharmacokinetic analysis

Area under the plasma concentration-time curves (AUC) of topotecan and hydroxy-acid were calculated with compartmental and noncompartmental analysis using the Siphar software package release 4.0 (Siphar SIMED, Cedex, Creteil, France). The trapezoidal

method was used for the noncompartmental analysis. The AUC(t) was calculated up to the latest measured time point. The total AUC was calculated after extrapolation of the curve to infinity where appropriate using the terminal elimination rate constant k. The apparent bioavailability was calculated as the ratio of the AUC(t) after oral and i.v. administration. All concentration-time profiles were fitted using the extended least squares method (LSM) (17). The volume of distribution at steady state ($V_{d,ss}$) and total plasma clearance (CL = Dose/AUC) were calculated after i.v. administration of topotecan. The terminal half-life (t½) was calculated after oral and i.v. administration as ln2/k.

Visual inspection of the concentration-time curves, the objective function and the Akaike information criterion (18) were applied to chose the optimal model for quantitation of the AUC.

Statistical analysis

The Pearson correlation coefficient was calculated between the ratio of the AUC(t) of the hydroxy-acid and topotecan after oral and i.v administration and between the ratio of the AUC(t) of the hydroxy-acid after oral and i.v. administration and the bioavailability. Two-sided paired and unpaired t-test were applied to determine any significant difference between the half-lives of topotecan and hydroxy-acid after oral and i.v. administration. The influence of the presence of liver metastases, concomitant medication on bioavailability and clearance were evaluated with the Mann-Whitney U test. The relationship between age and bioavailability and clearance was evaluated with the Pearson correlation coefficient and the Wilcoxon rank sum test.

RESULTS

Patients and treatment

Twelve patients were included in the study (7 males and 5 females). The mean age was 62 ± 7.5 years (range 46-70). The median performance score was 1 (range 0-2). Nine patients had metastatic colon cancer, 2 had small cell lung cancer and

1 ovarian cancer. Nine patients had documented liver metastases. Five patients did not use concomitant medication, two used a benzodiazepine, one used slow release morphine and diclofenac, one disopyramide, one alizapride and diclofenac, one atenolol and one atenolol, furosemide, nifedipine, naproxen, budesonide by inhalation, acetylsalicylic acid and nitroglycerine. All patients had been entered in phase II studies applying topotecan in a daily times 5 schedule with cycles repeated every 3 weeks. Oral administration of topotecan was without exception the first day of one of the 5-day treatment cycles. Neither i.v. nor oral administration was associated with any significant acute side effect. The oral drinking solution was well tolerated.

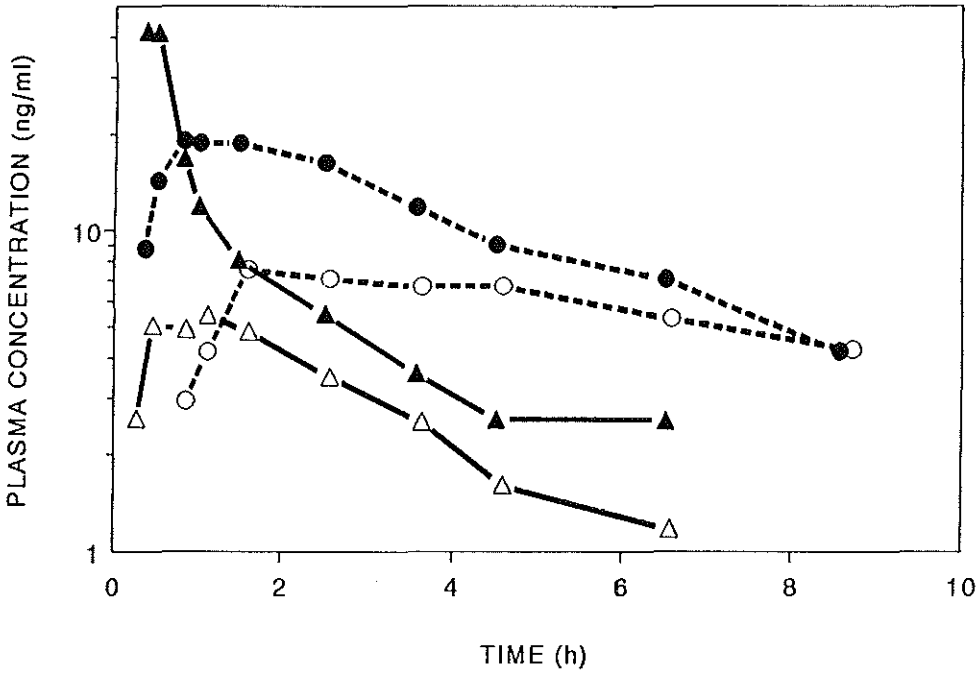

Figure 1.

Plasma concentration-time curve of topotecan and hydroxy-acid in a patient after oral and i.v. administration of 3.2 mg of topotecan.

▲ I.v. topotecan, ● i.v. hydroxy-acid, △ oral topotecan, ○ oral hydroxy-acid, closed lines represent topotecan and the dotted lines the hydroxy-acid.

Pharmacokinetic analysis

Representative plasma concentration-time curves of topotecan and hydroxy-acid after oral and i.v. administration are given in Fig. 1. Concentrations of topotecan lactone and hydroxy-acid assays were always undetectable on day two, prior to administration of the second dose. In only 6 out of 12 patients visual inspection of the obtained concentration-time profiles of topotecan after oral administration revealed a good fit of the measured plasma concentration-time points, applying extended LSM and one-, two-, or three compartments (the corresponding correlation coefficients [R] were > 0.97). All i.v. profiles of topotecan could be fitted well (R>0.97) applying LSM and the outlined models. In 10 patients the model using a 2-exponential decline of the concentration-time curve resulted in the highest correlation coefficent and lowest Akaike value. In 1 patient the model with a 3-exponential decline of the curve and in 1 other patient a 1-exponential decline resulted in the best fit. In most patients the curves of the hydroxy-acid could not be fitted properly using the extended LSM, assuming linear pharmacokinetics. The plasma concentration-time curves of the hydroxy-acid were higher than of topotecan in all patients. The pharmacokinetic data are summarized in tables 1 and 2.

After oral administration the percent extrapolated of the AUC in 8 patients was >20%, therefore, the bioavailability was calculated using the ratio of AUC(t) instead of the AUC extrapolated to infinity. The mean ± SD of the AUC(t) of topotecan after oral administration was 15.18 ± 5.51 ng.h/ml (range 9.38-25.37 ng.h/ml). The AUC(t) of topotecan after i.v. administration was 49.87 ± 10.87 ng.h/ml (range 32.50-73.68 ng.h/ml). The AUC of topotecan after i.v. administration was 61.02 ± 10.57 ng.h/ml (range 43.70-81.08 ng.h/ml). The bioavailability was 30% ± 7.7% (range 21%-45%). The coefficient of variation (%CV) was 25.4%. If the curves would have been extrapolated to infinity, then the bioavailability would have been calculated as 32% ± 11.5%.

The median of the Tmax was 0.78 hours and the range 0.33-2.5 hours.

Table 1.

TOPOTECAN									HYDROXY-ACID	
patient (no.)	dose (mg)	Tmax, 0 (hr)	Cmax, 0 (ng/ml)	Cmax, IV (ng/ml)	AUC(t), 0 (ng.hr/ml)	AUC(t), IV (ng.hr/ml)	AUC, IV (ng.hr/ml)	F (%)	AUC(t), 0 (ng.hr/ml)	AUC(t),IV (ng.hr/ml)
1	2.7	0.5	5.95	41.08	10.48	49.17	64.49	21	NE	59.86
2	2.8	1.03	5.93	35.92	9.38	32.50	43.70	30	12.20	37.08
3	3.2	0.33	5.16	43.86	10.85	42.37	56.34	25	15.10	60.72
4	3.0	1.13	5.45	41.81	18.81	49.37	58.15	38	47.96	90.79
5	2.7	0.52	5.71	33.40	11.25	37.96	46.64	30	NE	56.34
6	3.0	0.55	7.14	47.18	12.75	55.09	69.62	23	29.30	100.83
7	2.8	1.03	4.71	40.35	9.79	45.31	59.53	22	NE	65.07
8	2.8	2.5	5.08	41.41	21.98	48.57	52.04	45	63.99	103.25
9	3.1	0.5	6.48	41.21	12.46	55.00	66.00	23	19.22	57.70
10	2.6	1.5	6.67	42.11	25.37	73.68	81.08	34	51.47	121.14
11	2.8	1.0	5.38	25.35	21.56	62.31	70.14	35	19.01	63.77
12	2.7	0.5	6.93	19.57	17.00	46.61	64.55	36	24.46	62.48
MEAN SD			5.88 0.78	37.77 8.04	15.18 5.51	49.87 10.87	61.02 10.57	30 7.7	31.41 18.47	73.25 24.69

0 = oral, IV = intravenous, Dose = absolute dose (oral = i.v.), Tmax = time to maximal plasma concentration, Cmax = maximal plasma concentration, AUC(t) = area under the curve up to the latest measured time point, AUC = area under the curve extrapolated to infinity, NE = not evaluated

Table 2. Mean pharmacokinetic data of topotecan lactone

	oral	No. of patients	i.v.	No. of patients
Cl (ml/min)	-		824 ± 117 (535 -1068)	N = 12
Vd_{ss} (l)	-		128 ± 37.1 (86 - 231)	N = 12
t½β (h)	2.43 ± 1.15 (1.02 - 3.20)	N = 10	2.40 ± 0.38 (1.72 - 2.93)	N = 12

The half-life for the initial decline of the plasma concentration-time curve (t½,α) after i.v. administration was 0.186 ± 0.054 hours based on the data for the 10 patients which were fitted with a bi-exponential model.

The ratio of the AUC(t) of topotecan and the hydroxy-acid after oral administration was 0.63 ± 0.25 (n = 9) and after i.v. administration 0.72 ± 0.16. The correlation coefficient was 0.87 (p = 0.002). The ratio of the AUC(t) of the hydroxy-acid after oral and i.v. administration was 0.38 ± 0.12 (n = 9) which is of the same magnitude as the bioavailability of topotecan. The correlation coefficient between this ratio and the bioavailability was 0.84 (p = 0.004)

There was no significant relationship between age or gender and the bioavailability. In addition, there was no significant relationship between the presence of liver metastases and the magnitude of the bioavailability.

The Cmax of the hydroxy-acid after oral administration was 7.50 ± 2.57 ng/ml (range 4.66-12.32 ng/ml) and after i.v. administration 18.97 ± 2.44 ng/ml (range 15.35-24.12 ng/ml). The terminal t½ after oral administration was 2.82 ± 0.85 hours and after i.v. administration 3.22 ± 0.73 hours (not significantly different).

DISCUSSION

The present study is the first to provide data on the systemic exposure of topotecan after oral administration. Chronic administration of topotecan resulted in an enhanced antineoplastic activity (4,7,8,10-12). The concept of chronic administration is to some extent applied in a large number of phase II studies, which are currently in progress utilizing a daily times 5 intravenous infusion of 30 minutes or 21 day continuous infusion. Topotecan may show an even more pronounced antitumor response if the exposure duration is even further prolonged (7). It would increase the convenience for the patient substantially if the drug on a chronic treatment schedule could be taken orally.

The apparent bioavailability was determined in 12 patients with various types of solid tumors. The bioavailability ranged from 21 to 45%. The term apparent bioavailability has been used because topotecan undergoes a reversible, pH dependent conversion to the hydroxy-acid at physiologic pH and the standard equation for the calculation of bioavailability no longer applies. The correct equations contain terms for the AUC of topotecan, and hydroxy-acid after i.v. administration of the hydroxy-acid itself. The standard equation for bioavailability, as used in this study, is a function not only of dose and input rate, but of conversion clearance. The accuracy of the apparent bioavailability data will depend, to a large extent, on the magnitude of the conversion clearance, for which no *in vivo* data on topotecan are available. Even though at physiologic pH the formation of the hydroxy-acid is the predominant reaction, experiments on camptothecin in the rat have shown that the lactone can be formed following the i.v. administration of the hydroxy-acid (19). As camptothecin and topotecan have the same basic ring structure, it is highly likely that topotecan could also be formed following administration of the topotecan hydroxy-acid.

The bioavailability was determined after oral administration on day 1 and i.v. administration on day 2. This strategy was followed to deviate as little as possible from the phase II daily times 5 schedule which has documented therapeutic activity in several tumor types. The approach is justified because there is no carryover of

topotecan into the second treatment period. In addition, topotecan is not metabolised and therefore cannot induce its own elimination. Plasma samples were collected up to 8 hours after oral administration. This was slightly too short to extrapolate the AUC up to infinity. The sampling time was determined based on preclinical data on the oral administration in dogs and on the elimination pharmacokinetics after i.v. administration in man. The bioavailability after oral administration in dogs was 35.7 ± 16.3% (unpublished data), which is close to the result obtained in humans. The calculation of the bioavailability using the AUC calculated to infinity resulted in only a marginally higher value of 32% instead of 30%. Apparently, the applied ratio of AUC(t) gives a good estimation of the bioavailability.

The $t_{½,\beta}$ in the patient with the highest bioavailability of 45 % was 1.8 h after i.v. and 3.2 h after oral administration. This difference was relatively large in comparison to the data obtained in the other patients. This patient had a large tumor in the upper part of the abdomen. It cannot be excluded that this extensive tumor mass has influenced the rate of passage of topotecan through the gastrointestinal tract and thereby the magnitude of the absorption. The Tmax of this patient was 2.5 h, which is very delayed compared to the range of the other 11 patients (0.33-1.5 h). The %CV of the AUC(t) after oral administration was 36.3 % and after i.v. administration 21.8%. Hence oral administration increased the interpatient difference in systemic exposure markedly. It has to be elucidated in follow-up studies whether this variation has clinical implications. In addition, the intrapatient variability needs to be determined in future studies.

The %CV of the plasma clearance of topotecan after i.v. administration was 18.8%. The range of the data was of the same order as reported by Rowinsky *et al.* (4) and Verweij *et al.* (8).

No relationships were found between patient characteristics as age, gender, performance score and the bioavailability. In addition, the presence of liver metastases or concomitant drugs did not seem to be related to the magnitude of the bioavailability.

The ratio of topotecan and hydroxy-acid was of the same order after oral and i.v. administration. There would appear only two reasonable explanations for the observed

data: either topotecan is poorly absorbed from the gastrointestinal tract or that topotecan is well absorbed but that a large part of the dose is converted presystemically, in the gut, into the hydroxy-acid which is itself not absorbed to any appreciable extent. The good water solubility of topotecan coupled with the rapid absorption, would suggest that the second explanation can best describe the data obtained in this study. There is no evidence for the formation of other metabolites which may explain the observed data.

The bioavailability of topotecan after oral administration illustrates significant systemic exposure to the drug, which may enable chronic oral treatment.

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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

Background and 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 b.i.d. Pharmacokinetics were performed on day 1 and 8 of the first course using a validated high liquid performance chromatographic assay and noncompartmental pharmacokinetic methods.
Results: 31 patients entered the study, one patient was not evaluable for toxicity and response as therapy was prematurely interrupted on request of the patient without experiencing toxicity. 30 patients received a total of 59 courses. The dose limiting toxicity was reached at a dose of 0.6 mg/m^2 b.i.d. 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 leucopenia, thrombocytopenia, nausea and vomiting. The maximum-tolerated dose was 0.5 mg/m^2 b.i.d. No responses were observed. Pharmacokinetics revealed a substantial variation of the 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 modelled by a sigmoidal E_{max} function.
Conclusions: The dose-limiting toxicity 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 b.i.d.

INTRODUCTION

Topotecan, (s)-9-dimethylaminomethyl-10-hydroxycamptothecin, is a watersoluble 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 and 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 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 reported by Hochster et al. (6), in a phase I study using a 21-days 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 was myelosuppression with thrombocytopenia being somewhat more profound compared to neutropenia.

Recently reported results reveal a 32-44% bioavailability of oral topotecan (7,8), and thus oral administration might be a more simple and convenient method to achieve prolonged exposure. In view of the relatively short half life of topotecan and in order

to mimic as good as possible the continuous infusion schedule, the oral formulation was initially tested at a bidaily schedule. 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; an Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2; an estimated life expectancy of $\geq$ 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 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 the data of Hochster et al (6). using topotecan as a 21-days continuous infusion with a 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-days oral administration was set at 0.15 mg/m^2 given 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, $\leq$ 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 four 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 (DLT), which

were defined as CTC grade 4 hematologic toxicity and/or nonhematologic toxicity ≥ CTC grade 3 in more than 2/6 patients. If neutropenia grade 4, thrombocytopenia ≥ grade 3 and/or non-hematologic toxicity ≥ grade 3 occurred during treatment days, the topotecan administration was stopped immediately. Intrapatient dose escalation was not performed.

Topotecan was supplied as capsules containing topotecan HCL, 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 degrees celsius. Capsules were 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.

Treatment assessment

Prior to therapy, complete medical history was taken and a 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, 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 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 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. The samples were immediately prepared and analyzed according to the method as described by Loos et al. (9). The lower limit of quantitation

(LLQ) was 0.1 ng/ml for topotecan as well as for the hydroxy-acid. Area under the plasma concentration-time curves (AUC) of topotecan and hydroxy-acid 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}). The AUC(t) was fitted to observed percentage decrease in WBC and ANC using the sigmoidal E_{max} model (10). 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 CTC-grade leuco- and thrombocytopenia and diarrhea. For statistical analysis statgraphics was employed (Manngistics, MD, USA).

RESULTS

A total of 31 patients entered the study. Patient characteristics are given in table 1. All patients were eligible but one patient with colorectal cancer was considered not evaluable 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 evaluable for toxicity and response. The majority of patients were either asymptomatic or only mildly symptomatic (i.e ECOG performance status 0-1). All patients, except one, had received prior therapy. The most common tumor type was colorectal cancer. The total number of evaluable 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 b.i.d., resulting in total daily doses of 0.3, 0.6, 0.8, 1.0 and 1.2 mg/m^2 respectively.

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

Hematologic toxicity

Overall, the hematologic toxicities were relatively mild (table 2), with leucopenia and thrombocytopenia mainly occurring (if at all) during the third and fourth week of the course and being shortlasting. The next treatment course had to be postponed due to prolonged myelosuppression in 2 courses in patients experiencing grade 3-4 leucopenia. Grade 3-4 leucopenia was observed in 6 of the 59 courses (10.2%), in 1 patient being complicated by neutropenic fever. In 3 courses (5%) grade 3-4 thrombocytopenia was noted, 2 in conjunction with leucopenia.

Table 2: Hematologic toxicity (worst per patient)

Dose (mg/m^2/b.i.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

Non-hematologic toxicity

Diarrhea was the dose-limiting toxicity (DLT) of topotecan at a dose of 0.6 mg/m^2 - b.i.d. in this schedule (table 3). The onset of severe diarrhea was abruptly (figure 1) with a median day of onset being on day 15 (range 12-20).

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 diarrhea lasted for 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 b.i.d. dose level. One patient showed a different pattern of diarrhea. Two days after initiating topotecan therapy 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 developing severe diarrhea. Vigorous administration of loperamide for treatment of diarrhea was ineffective in reducing its severity. 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 anti-emetics were not routinely used in the study. Mild intermittent nausea and vomiting (grade 1-2) was observed in respectively 32% and 11.8%of the given courses and could be circumvented with standard anti-emetics.

Alopecia occurred in 2 patients. No other toxicities were seen. There was no stomatitis, hypotension, liver or renal toxicity.

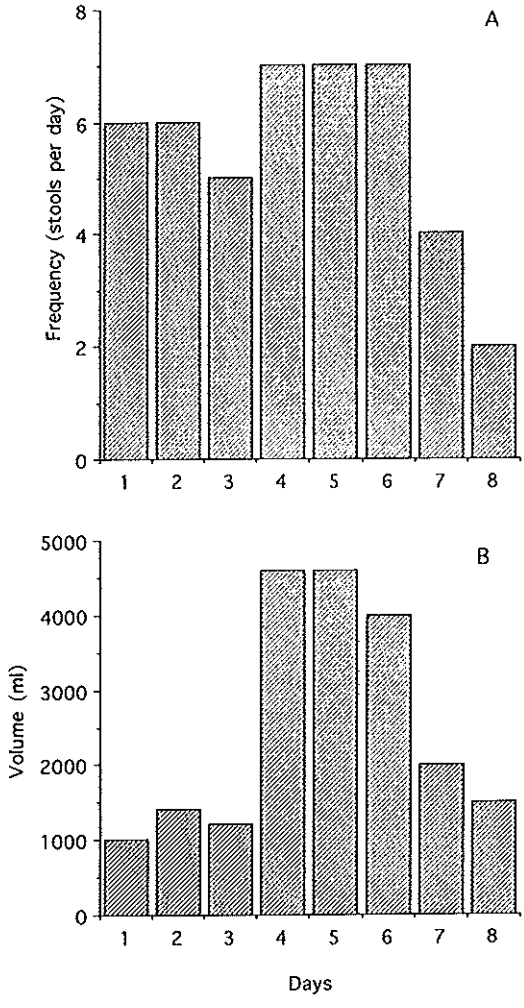

Figure 1:

Graph showing the severity of diarrhea in two different patients at the dose of 0.6 mg/m^2 b.i.d. At the top (patient A) the frequency and at the bottom (patient B) the volume is depicted in relation to the duration (days) of diarrhea.

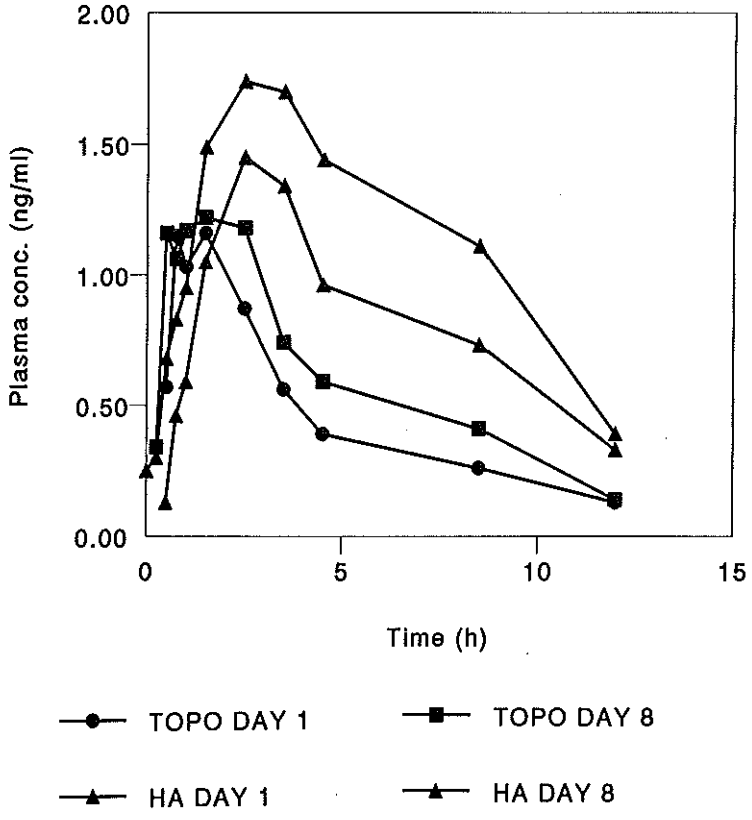

Figure 2:
Graph showing representative AUCs of topotecan and hydroxy-acid on day 1 and 8.

Pharmacokinetics and dynamics

Pharmacokinetics were performed in 14 patients, well distributed over all dose levels. A representative plasma-concentration-time curve of topotecan and the hydroxy-acid on day 1 and 8 is shown in figure 2. 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 summarized in table 4.

Table 4. Pharmacokinetics

		Topotecan Day 1		Hydroxy-acid Day 1		Topotecan Day 8		Hydroxy-acid Day 8	
Dose level mg/m^2	Dose mg	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	0.40	0.81	1.36	0.76	2.97	1.32	2.56	2.72	2.85
0.15	0.30	1.55	3.22	5.03	4.34	2.80	4.49	7.49	5.93
0.30	0.50	6.20	3.39	9.70	6.29	8.12	2.90	11.80	2.97
0.30	0.70	1.41	1.28	1.27	2.36	1.30	1.49	1.44	3.91
0.30	0.60	5.29	3.45	9.66	4.49	7.17	3.55	13.84	2.80
0.40	0.70	1.71	2.06	2.27	2.62	5.04	2.78	6.64	2.62
0.40	0.80	4.61	1.98	5.72	2.71	6.87	2.63	9.32	2.43
0.50	0.90	5.86	0.99	10.12	3.63	7.24	2.70	15.40	4.44
0.50	0.90	5.75	2.49	9.08	4.75	6.95	3.76	11.84	6.47
0.50	1.10	1.63	1.80	3.53	3.24	3.67	2.14	6.65	2.69
0.50	1.10	2.47	1.52	7.58	3.55	3.99	2.94	9.20	3.17
0.60	1.00	4.93	1.68	6.67	3.29	7.66	2.97	9.41	3.88
0.60	1.30	8.54	3.09	13.00	3.13	10.72	4.81	NQ	NQ
0.60	1.10	4.91	4.61	8.18	3.22	8.20	NQ	10.30	1.54
mean.	0.81	3.98	2.35	6.61	3.61	5.79	3.06	8.93	3.52
SD	0.30	2.35	1.05	3.68	1.04	2.82	0.91	4.00	1.40
%CV	36.3%	59.1%	44.7%	55.6%	28.9%	46.8%	29.6%	44.6%	39.9%

NQ = not quantificable, %CV = coefficient of variation

The AUC(t) of topotecan and hydroxy-acid showed substantial variation. High correlation was found between the ratio AUC(t) of topotecan and the AUC(t) of hydroxy-acid on day 1 and 8 (R = 0.96). The AUC(t) of topotecan and hydroxy-acid were consistently higher on day 8 compared to day 1 (figure 3). The mean AUC(t) ± SD of topotecan on day 8 was 5.79 ± 2.82 ng.hr/ml and was 26% higher than the AUC(t) on day 1 which was 3.98 ± 2.35 ng.hr/ml. The interpatient variability (%CV) in AUC(t) of topotecan on day 1 and 8 were 59.1% and 46.8%, respectively.

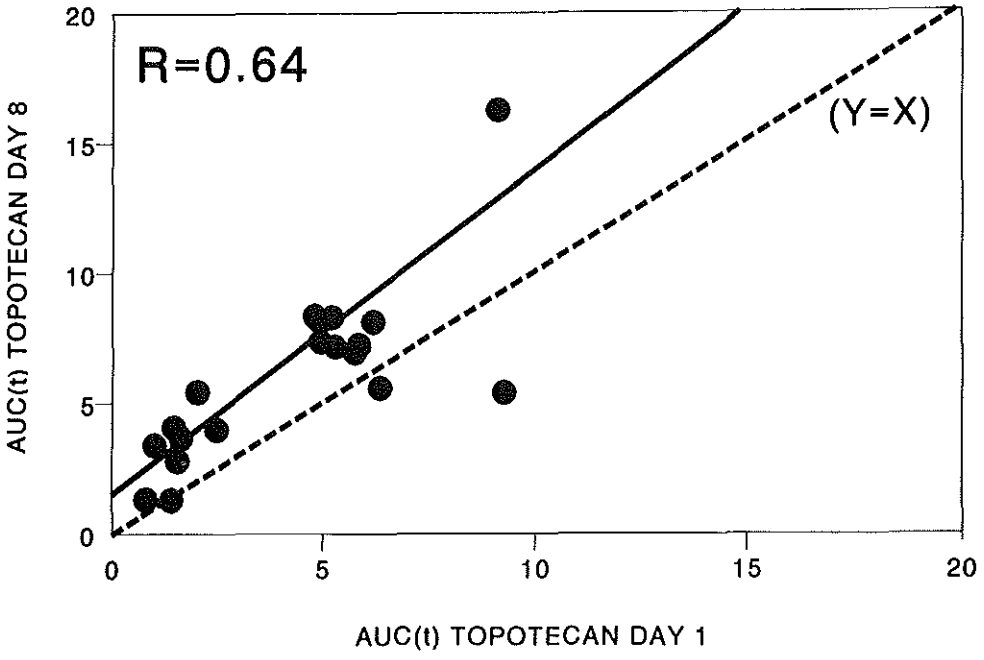

Figure 3:
Relationship between the AUC(t) of topotecan on day 1 and day 8 of course 1.

The correlation between dose and AUC(t) of topotecan was relatively low (R=0.44). There was no significant relationship between the dose of topotecan and the occurrence of leucopenia 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 ($R = 0.75$, $p = 0.003$). The latter data could be fitted best using a sigmoidal E_{max} model (figure 4). There also was a significant correlation between AUC(t) and the CTC-grade 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.

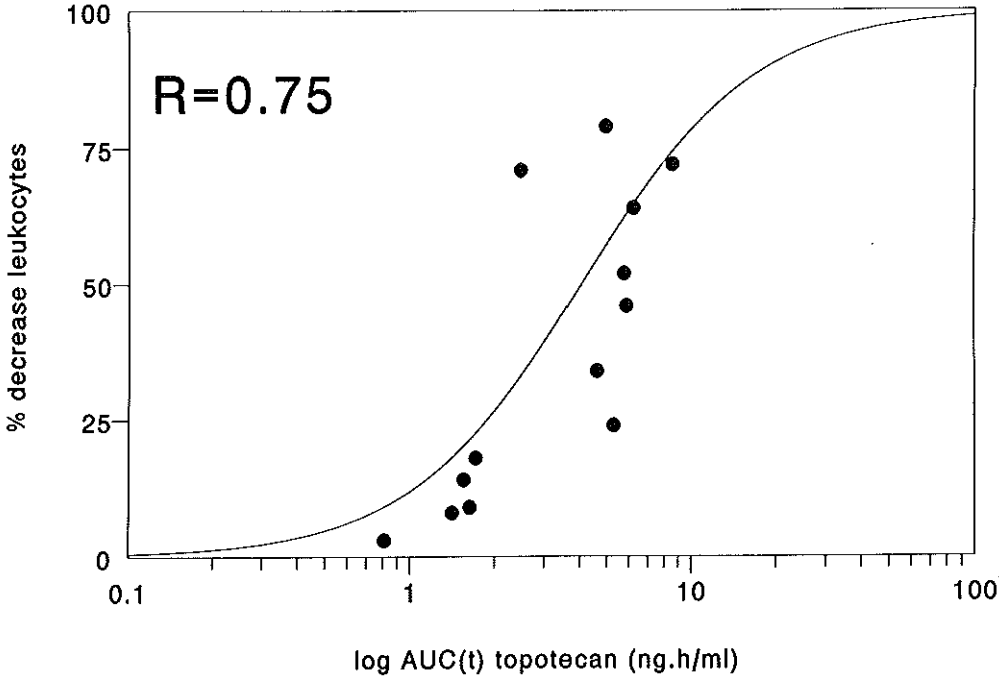

Figure 4:
The percentage decrease in WBC versus AUC(t) of topotecan during course 1. The curve is fit to a sigmoidal E_{max} model.

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 b.i.d.

DISCUSSION

The topoisomerase I inhibitors continue to rapidly move through clinical development with their unique mechanism of action and their activity against a variety of malignancies. Topotecan has been extensively studied utilizing different intravenous dosing schedules, revealing shortlasting non-cumulative neutropenia and/or thrombocytopenia as the dose-limiting toxicity (DLT) (11,12).

The present study was based on the promising results of prolonged exposure and the oral bioavailability of topotecan. *In vitro* experiments with topotecan using short-term 1-hour exposure at a concentration of 1.0 and 10 μg/ml showed responses in respectively 10% and 25%. 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 revealed significant activity against several human cancers (rhabdomyosarcoma and colon cancer) without any toxicity and its efficacy may be schedule dependent (4). The oral bioavailability was determined at 30% ± 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 utilizing the maximum tolerated dose (MTD) of the oral (14 mg/m^2) and intravenous (17.5 mg/m^2) routes of administration, revealing a bioavailability of 44% (8). In contrast to the intravenous routes, the dose-limiting toxicity (DLT) for the twice daily oral administration for 21-days was diarrhea at the dose of 0.6 mg/m^2 b.i.d. Mostly, the diarrhea started in the third week of drug administration. It was always self-limiting after a median duration of 8 days (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, 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 the patients (11-16). Irinotecan is converted by endogenous carboxylesterase to its active metabolite SN-38. The development of diarrhea might be associated with the amount of carboxylesterase activity in the intestinal mucosa, liver and plasma (17). As diarrhea has never been reported as a major side-effect with the intravenous route of topotecan administration it is speculated that the cause of diarrhea with chronic oral administration is a local effect of topotecan and/or the excipient on the intestinal mucosa. The CTC-grade of diarrhea appeared to be correlated with the administered dose.
Nausea and vomiting, were intermittent and mild, never exceeding grade 2 toxicity, resembling the gastrointestinal toxicities noted with longterm continuous infusion of topotecan (11,18).
Hematological toxicity was relatively mild, grade 3-4 hematological toxicity was observed in 10.2% of the courses and consisted of leucopenia which in 2/6 courses occurred in conjunction with thrombocytopenia grade 3-4. In comparison, the DLT in the phase I study using a 21-days continuous infusion was hematologic with thrombocytopenia being somewhat more profound than leucopenia (6). In addition, myelotoxicity was the single relevant side effect in a phase II study using 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 by a marked inhibition of the erythropoiesis (18).
Pharmacokinetic data from the present study revealed a substantial interpatient variability in the AUC(t) and the AUC(t) on day 8 was consistently higher, indicating some cumulation of the drug.
Nevertheless, in this study a significant correlation was found between the systemic exposure and the % decrease in the leucocytes (figure 4).
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 (4,5,20). Recently, Hochster et al. (19) have shown that with continuous infusion of topotecan in humans, progressive depletion of topoisomerase I levels is observed until the end

of the second week. Pursing such a less protracted exposure-time in using the oral administration might enable the use of higher doses per day resulting 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 exposure via oral administration remains attractive, but from the experience in the present study, it is possible that shortening the exposure time might result in better tolerance with a better chance to see antitumor activity. Based on that studies are planned to test 5 and 10 days of oral administration.

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SUMMARY

This thesis describes clinical and pharmacological studies on the topoisomerase I inhibitor topotecan, with a focus on the efficacy of the daily x5 intravenous schedule in patients with colorectal and ovarian cancer. In addition, the concept of prolonged exposure was studied.

In **chapter 1** an introduction is presented about the synthetic chemistry efforts, which have led to the development of the watersoluble analogue topotecan, which is a derivative of the parent drug camptothecin; an extract from the Asian tree Camptotheca acuminata.

Chapter 2 gives an overview of the two main topoisomerase I inhibitors under clinical investigation: topotecan and irinotecan (CPT-11). Both drugs, like camptothecin, undergo a pH dependent reversible hydrolysis of the active lactone (closed form) to the inactive hydroxy-acid (open form). The plasma clearance of topotecan is biexponential with a half-life of approximately 3 hours. The volume of distribution at steady state is large (25-190 l) and the major route of excretion is renal. The pharmacokinetics of irinotecan are complex since irinotecan is a prodrug that is rapidly converted *in vivo* to its active metabolite SN-38. The half-lives of both lactone forms of irinotecan and SN-38 are 7-8 hrs on average. Hepatic glucuronidation and biliary excretion is the major route of elimination for both compounds. In preclinical models, topotecan and irinotecan show impressive antitumor activity. In phase I studies, the dose-limiting toxicities were of brief duration, i.e. non-cumulative neutropenia with or without mild thrombocytopenia for topotecan, and for irinotecan neutropenia and diarrhea.
In the initial phase I studies of topotecan it was shown that a daily x 5 schedule had the most convincing evidence of antitumor activity. This had led us to clinical studies of the daily x5 schedule of topotecan.

In **chapter 3** the phase II study results are presented on the daily x 5 schedule in patients with metastatic or locally irresectable colorectal cancer. Partial responses were obtained in 4 (7%) of the 57 evaluable patients. The treatment was very well tolerated. The major side effect was neutropenia (91%), being grade 3-4 in 79% of the courses, without any neutropenic infection due to the short duration of neutropenia. It is concluded that topotecan in this schedule has minor activity as a single agent in colorectal cancer.

In **chapter 4** the phase II study results are detailed of the daily x5 schedule as second-line treatment in patients with epithelial ovarian cancer, who developed recurrent or progressive disease after one line of platinum combination therapy. Ninety-two patients were evaluable for response. The overall response rate was 16% (1 CR, 14 PRs). When calculated according to platinum refractoriness, platinum resistance and platinum sensitivity the response rates were 6%, 18% and 27%. The major toxicity was neutropenia grade 3-4 in 69%, with 4% neutropenic infections. It is concluded that topotecan in a daily x5 schedule is an active regimen with a favourable toxicity profile as second-line therapy in ovarian cancer.

In **chapter 5** pharmacokinetics and pharmacodynamics of topotecan in a daily x5 schedule in phase II studies are described using a limited sampling procedure, in which AUC of topotecan and hydroxy-acid were calculated by plasma concentration determination of only one blood sample taken 2 hours after the infusion. Samples were obtained of 36 patients in 136 treatment days. The mean AUC of topotecan was 8.74 μM.min/day and the mean AUC of hydroxy-acid was 11.5 μM.min/day, with a large interpatient variability. Intrapatient variability during treatment days was minor, which indicates that no drug accumulation occurs. The intrapatient variability between the courses was also small. The relationship between the AUC of topotecan and the percentage of decrease in the absolute neutrophil count in these phase II studies strongly resembled the relationship as observed in a previous phase I study. It is concluded that despite the large interpatient pharmacokinetic variability, the

usefulness of regular drug monitoring in this schedule is marginal because the pharmacodynamic variability was relatively small.

Chapter 6. In preclinical studies it has been shown that topoisomerase I inhibitors exhibit better efficacy and have a greater therapeutic index with prolonged continuous exposure to the drug. Here, the results are presented of a phase II study of topotecan administered as a 21-days continuous infusion at a dose of 0.5-0.6 mg/m^2/day to 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 including 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, and the high oral bioavailability reported in animals, the oral formulation of topotecan was studied in man.

In **chapter 7** the oral bioavailability of topotecan is described. The oral bioavailability of topotecan was calculated as the ratio between the AUC(t), in which "t" denotes the latest measured point of an oral dose of 1.5 mg/m^2 given as a drinking solution and the AUC(t) of an intravenous dose of 1.5 mg/m^2 given as a 30 minute infusion. The oral bioavailability was 30% ± 7.7%. It is concluded that the oral bioavailability of topotecan results in adequate systemic exposure with modest interpatient variability.

In **chapter 8** a phase I and pharmacological study is presented of topotecan administered in capsules twice daily for 21 days to 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 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. Significant correlation was seen between the percentage of decrease in WBC and the AUC(t) of topotecan. One study with continuous infusion of topotecan in humans has shown a progressive depletion of topoisomerase I levels until the end of the second week. Consequently, the optimal concentration-time relationship of topotecan might be less than 3 weeks. For the oral administration a shorter than 3 weeks exposure-time might enable higher doses per day resulting in higher plasma concentrations.

Final conclusions

Topoisomerase I inhibitors are a new class of drugs in medical oncology.

From the results of phase I studies with topotecan the daily x5 schedule was initially selected for phase II studies. We and others have shown that this dosing schedule is effective and has a favourable toxicity profile as second-line treatment in patients with ovarian cancer and small cell lung cancer.

Although this thesis does not contain efficacy data of prolonged treatment with topotecan, based on theoretical considerations and on preclinical data the concept of prolonged exposure with topotecan in the treatment of solid tumors remains attractive, especially as a chronic oral administration. Therefore, further studies including the subject of the optimal concentration-time relationship are warranted.

SAMENVATTING

Dit proefschrift bevat klinisch en farmacologisch onderzoek met de topoisomerase I remmer topotecan. De onderzoeken waren vooral gericht op de anti-tumor effecten van 5 dagelijkse intraveneuze toedieningen één maal per 3 weken bij patiënten met colorectale en ovariumcarcinomen. Tevens is het concept van verlengde expositieduur bestudeerd.

Hoofdstuk 1 is een inleiding over de ontwikkeling van de semisynthetische topoisomerase I remmer topotecan uit de moederstof camptothecine.

In **hoofdstuk 2** wordt een overzicht gegeven van de klinisch belangrijkste topoisomerase I remmers; topotecan en irinotecan (CPT-11). Evenals camptothecine ondergaan beide stoffen een pH afhankelijke reversibele hydrolyse van de actieve lacton (gesloten) vorm naar de inactieve hydroxy (open) vorm. De plasma klaring van topotecan is biëxponentieel met een halfwaarde tijd van circa 3 uur. Het verdelingsvolume is groot en de excretie vindt hoofdzakelijk plaats via de nieren. De farmacokinetiek van irinotecan is complex doordat irinotecan een prodrug is die *in vivo* snel wordt omgezet in de actieve metaboliet SN-38. De halfwaarde tijd van zowel irinotecan en SN-38 is langer dan die van topotecan. Biliaire excretie is voor zowel irinotecan als SN-38 de belangrijkste eliminatie route. In preklinische modellen vertonen zowel topotecan als irinotecan indrukwekkende anti-tumor activiteit. De dosis limiterende toxiciteit in fase I studies met topotecan was kortdurende, niet cumulatieve neutropenie soms gepaard gaand met milde trombocytopenie. Voor irinotecan was de dosis limiterende toxiciteit neutropenie en diarree. In de eerste fase I studies met topotecan werd de meest uitgesproken anti-tumor activiteit gezien met het schema waarbij op 5 achtereenvolgende dagen een kort infuus werd gegeven.

In **hoofdstuk 3** worden de resultaten beschreven van de fase II studie met het 5-daagse schema bij patiënten met een gemetastaseerd of inoperabel colorectaal carcinoom.

Er werden 4 (7%) partiële responsen gezien bij 57 evalueerbare patiënten. Topotecan werd zeer goed verdragen. De belangrijkste bijwerking was neutropenie (91%); CTC-graad 3-4 in 79% van de kuren. Door de korte duur van de neutropenie traden geen infectieuze complicaties op. Dit toedienings-schema van topotecan heeft slechts beperkte activiteit bij colorectale carcinomen.

In **hoofdstuk 4** worden de resultaten vermeld van de fase II studie met het 5-daagse schema als tweedelijns behandeling bij patiënten met een epitheliaal ovarium carcinoom, die progressie of een recidief hadden na eerstelijns behandeling met platina bevattende chemotherapie. Twee-en-negentig patiënten waren evalueerbaar voor respons. Het respons percentage was 16% (1 CR, 14 PRs). Wanneer de patiënten werden gegroepeerd als platina refractair, platina resistent en platina sensitief, waren de respectievelijke respons percentages 6%, 18% en 27%. De belangrijkste bijwerking was neutropenie; CTC-graad 3-4 in 69% van de kuren. Vier procent van de kuren ging gepaard met infectieuze complicaties. Dit 5-daagse schema met topotecan is actief als tweede lijns behandeling van ovarium kanker.

In **hoofdstuk 5** worden de farmacokinetische en farmacodynamische resultaten uit fase II studies met 5-daagse topotecan schema bepaald volgens de "limited sampling methode" geanalyseerd. Bij deze methode worden de AUC van topotecan en hydroxy vorm berekend door bepaling van slechts één bloedmonster 2 uur na de infusie. Er werden monsters genomen van 36 patiënten op 136 behandeldagen. De gemiddelde AUC van topotecan was 8,74 μM.min/dag en de gemiddelde AUC van de hydroxy vorm was 11,5 μM.min/dag, met een grote interpatient variatie. De intrapatient variabiliteit was gering. Dit suggereert dat er geen cumulatie van topotecan is gedurende de 5 behandelingsdagen. Ook was de intrapatient variabiliteit tijdens de kuren gering. De relatie tussen de AUC van topotecan en de procentuele daling van de neutrofielen in deze fase II studies kwam goed overeen met de relatie in de fase I studies met het 5-daagse topotecan schema. Ondanks de grote farmacokinetische interpatient variabiliteit is de waarde van regelmatige farmacokinetische metingen beperkt is gezien

de geringe farmacodynamische variabiliteit.

Hoofdstuk 6. In preklinische modellen zijn er aanwijzingen dat topoisomerase I remmers effectiever zijn en een grotere therapeutische index hebben in geval van een verlengde continue expositie. Hier worden de resultaten weergegeven van de fase II studie met topotecan gedurende 21 dagen in een dagelijkse dosis van 0,5-0,6 mg/m^2 door middel van een continue infusie via een draagbare pomp. De patiënten hadden een inoperabel of gemetastaseerd colorectaal carcinoom. Het bereikte respons percentage was 10% (1 CR, 3 PRs). De belangrijkste bijwerking was langdurige en cumulatieve myelosuppressie, met een opvallende remming van de erythropoiëse. Er bestond een significante correlatie (R=0,54) tussen de mate van leucopenie en de "steady-state" plasma concentratie van topotecan. Dit schema heeft geringe therapeutische activiteit bij colorectal carcinomen en is belastend voor patiënten.
Bij orale toediening van topotecan aan proefdieren bleek de biologische beschikbaarheid groot te zijn. Daarom hebben wij de orale toediening van topotecan ook bij patiënten bestudeerd.

In **hoofdstuk 7** wordt de biologische beschikbaarheid van oraal toegediend topotecan beschreven. De biologische beschikbaarheid werd berekend als de ratio tussen de AUC(t), waar "t" staat voor het laatst gemeten tijdstip, van oraal topotecan (1,5 mg/m^2) toegediend als een drinkbare oplossing en de AUC(t) van een intraveneuze dosis (1,5 mg/m^2) toegediend in 30 minuten. De biologische beschikbaarheid was 30% ±7,7%. Deze resultaten tonen dat orale toediening van topotecan leidt tot adequate systemische expositie met beperkte interpatient variatie. Deze bevinding vormde het argument voor een studie met continue orale toediening.

In **hoofdstuk 8** wordt een fase I studie gepresenteerd waarin topotecan, in capsule vorm, tweemaal per dag gedurende 21 dagen aan patiënten met solide tumoren werd toegediend. Dertig patiënten waren evaluabel voor toxiciteit en respons. De dosis-limiterende toxiciteit werd bereikt bij een dosis van 0,6 mg/m^2 2dd en bestond uit diarree,

welke mediaan optrad op de dag 15 (spreiding 12-20) en mediaan 8 dagen later spontaan verdween (spreiding 7-16). Andere bijwerkingen waren leuco- en trombocytopenie, misselijkheid en braken. De maximaal tolereerbare dosis en geadviseerde dosis voor fase II studies is 0,5 mg/m^2 2dd. De AUC(t) van topotecan en de hydroxy vorm lieten een substantiële interpatient variatie zien. Er bestond een significante correlatie tussen de procentuele daling van de leucocyten en de AUC(t) van topotecan. In een studie met continue infusie van topotecan werd tot ongeveer twee weken een progressieve depletie van topoisomerase I spiegels geobserveerd. Daarom is de optimale concentratie-tijds relatie van topotecan mogelijk korter is dan 21 dagen. Voor orale toediening van topotecan kan een expositieduur korter dan 3 weken mogelijk resulteren in een hogere dagdosis en hogere plasma concentraties.

Topoisomerase I remmers zijn een nieuwe klasse cytostatica. Gezien de resultaten in fase I studies met het 5-daagse topotecan schema werd dit schema geselecteerd voor fase II studies. Wij en andere onderzoekers hebben aangetoond dat dit schema effectief is als tweedelijns therapie bij patiënten met ovarium carcinoom en kleincellig bronchus carcinoom, met relatief weinig bijwerkingen.
Ondanks dat in dit proefschrift geen effectiviteit is gezien met verlenging van expositieduur van topotecan, blijft op basis van zowel theoretische als preklinische gegevens het concept van langdurige expositie blijft attractief, zeker de chronische orale toediening. Daarom zijn verdere studies geïndiceerd om de optimale concentratie-tijds relatie van topotecan te bepalen.

DANKWOORD

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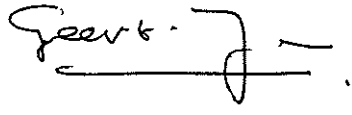

CURRICULUM VITAE

De auteur van dit proefschrift werd op 12 maart 1960 geboren te Eindhoven. Na het behalen van het Atheneum-B diploma aan het Hertog-Jan college te Valkenswaard werd in 1978 aangevangen met de studie geneeskunde aan de Universiteit van Amsterdam, alwaar in mei 1986 het artsexamen werd afgelegd. Na het behalen van het artsexamen was hij werkzaam als arts-assistent-niet-in-opleiding (AGNIO) op de afdeling interne geneeskunde in het St. Elisabeth ziekenhuis te Tilburg, waar in maart 1987 werd gestart met de opleiding tot internist (opleider: Dr. J.H.M. Lockefeer, vanaf september 1988 Dr. C. v.d. Heul). Vervolgens werd in december 1990, als onderdeel van de opleiding tot internist, een twee-jarige stage gevolgd op de afdeling hematologie van de Dr. Daniel den Hoed Kliniek (opleider: W. Sizoo/ Prof. dr. B. Löwenberg). In maart 1993 werd hij geregistreerd als internist. In de periode van januari 1993 tot januari 1995 was hij werkzaam als junior-internist op de afdeling interne oncologie van de Dr. Daniel den Hoed Kliniek, waar de studies werden verricht die resulteerden in dit proefschrift (hoofd: Prof. dr. G. Stoter). Sedert januari 1995 is hij geregistreerd voor het aandachtsgebied interne oncologie en werkzaam als internist in het streekziekenhuis Walcheren te Vlissingen.