Prolonging the exposure to anti-cancer agents

Otto Soepenberg

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Prolonging the exposure to anti-cancer agents

Verlenging van de expositieduur van antikankermiddelen

Proefschrift

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Dosis solum facit venenum [Paracelsus]

Aan mijn ouders

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Introduction to the thesis

Introduction to the thesis

Approximately half a century ago, thousands of natural products were chemically screened in a program initiated by the National Cancer Institute USA to evaluate naturally-occuring substances for antitumor activity. This program led to the discovery by Wall and coworkers of camptothecin - isolated from the stem wood of the Chinese tree *Camptotheca acuminata* -, and paclitaxel - isolated from the Western yew *Taxus Brevifolia* - as potential cytotoxic agents [1-3]. The development of both classes of agents had in common that early phase I/II clinical trials showed unpredictable severe adverse events [4-7], which limited the initial enthusiasm and led to the suspension of research of these antineoplastic agents during the next two decades. After the recognition of the unique mechanisms of cytotoxic action of both camptothecin and paclitaxel, renewed interest in the further development of these cytotoxic agents was heralded [8-10].

Camptothecin belongs to the class of topoisomerase I inhibitors, which results in the inhibition of the nuclear enzyme DNA topoisomerase I and are active in the S-phase of the cell cycle. DNA topoisomerases are essential enzymes for cell growth and proliferation. The enzyme makes a transient single-strand break in the supercoiled DNA duplex resulting in relaxation of supercoiled DNA. Camptothecin stabilizes the covalent cleavable DNA-topoisomerase I complex, which causes arrest of the replication fork, resulting in inhibition of DNA synthesis and finally cell death [9-11].

The initial failure encountered in the clinical development of the first generation camptothecins was partly related to the poor aqueous solubility of the agent, which necessitated novel alkaline pharmaceutical formulations for intraveneous administration. Drug-research efforts led to the identification and development of new semi-synthetic derivatives with improved water solubility while maintaining the drug's unique mechanism of cytotoxic action. One of the second generation camptothecin analogs is irinotecan, which is at present registered for use in colorectal cancer, whilst other third generation analogs (*e.g.*, exatecan, diflomotecan) are currently in clinical development. A variety of different strategies is being used to modulate the systemic delivery of the camptothecin analogs, frequently in order to increase antitumor activity and/or reduce experienced side effects. An overview of pharmaceutical modulation of formulation vehicles as well as modulation of routes of administration and considerations on infusion duration of these analogs is discussed in **Chapter 1**.

Related to their S-phase specificity, the cytotoxicity of topoisomerase I inhibitors in preclinical models increases with duration of exposure. Long-term exposures

to low concentrations are more effective than short-term exposures to high concentrations [12]. Although, this concept established greater tumor efficacy compared with bolus administration in experimental studies, the optimal treatment and dosing regimens, such as continuous intravenous infusion, long-term oral delivery, or polymerized drug formulations have to be more crystallized in further clinical investigations.

Oral administration of irinotecan is an approach for continuous drug administration facilitating sustained exposure of the drug. The results of a phase I study of oral irinotecan, formulated as semi-solid matrix (SSM) capsules, are presented in **Chapter 2.** In addition, the results of a phase I study of the oral combination of irinotecan and capecitabine are described in **Chapter 3.**

An alternative approach for continuous drug administration is to make use of a macromolecular drug-delivery system, which can comprise an active topoisomerase I inhibitor linked to a polyalcohol polymer. Macromolecular carriers have been shown to alter drug pharmacokinetics at the whole organism and cellular level and facilitate controlled drug-release at the tumor site. This concept is based on the principle that macromolecular carriers will accumulate and be retained preferentially in tumor tissue by a phenomenon, termed *enhanced permeability and retention* (EPR) effect introduced by Hiroshi Maeda in 1986, which is thought to be due to tumor vasculature displaying a discontinuous endothelium, that allows macromolecular extravasation into tumor tissues, and also the lack of effective lymphatic drainage in tumors, which prevents efficient clearance of such accumulated macromolecules [13-16]. To study the biodistribution of DE-310, a novel macromolecular prodrug of the topoisomerase I inhibitor exatecan (DX-8951f), and its two major enzymatic products in human matrices (*i.e.*, erythrocytes and saliva), we developed and validated high liquid chromatographic assays as described in **Chapter 4.** In **Chapter 5** we reported the results of a phase I and pharmacokinetic study of DE-310. In addition, pharmacokinetic analysis was performed in normal skin and superficial tumor tissue.

The taxane diterpenoid, paclitaxel, belongs to the class of anti-microtubulin agents, which acts by promoting and stabilizing tubulin polymers against depolymerisation resulting in inhibition of the dynamic process of reorganization of microtubuli and ultimately cell death [17]. Due to its poor solubility in aqueous solution, paclitaxel is formulated in a mixture of Cremophor EL (polyoxyethylated Castor oil 35) and ethanol. Cremophor EL contributes to the nonlinear pharmacokinetic behavior of paclitaxel and to severe side effects, including hypersensitivity reactions and pheripheral neurotoxicity [18-20]. To overcome

these problems associated with the current formulation of paclitaxel, a variety of strategies are being explored to optimize the chemotherapeutic treatment with this agent [21]. One of the strategies is delivery of the drug by encapsulation in liposomes [22]. Liposomes are also macromolecular carriers, which are able to leak in tumor tissues by the proposed EPR effect [23,24]. Another issue for paclitaxel is to optimize its dose and schedule. Preclinical data have suggested that duration of exposure is an important factor in the cytotoxic activity of this drug [25]. This has been preliminarily confirmed by the activity of prolonged (96-hours) infusions of paclitaxel in patients with metastatic breast cancer having previously failed taxane therapy [26,27], and by data comparing 3-hour *versus* 24-hour infusion of high dose paclitaxel in breast cancer patients [28]. Another method to achieve extended cumulative exposure is frequent, repetitive dosing, such as by a weekly schedule [29-31]. We performed a phase I study of liposome encapsulated paclitaxel (LEP) administered six-times weekly in a period of eight weeks as reported in **Chapter 6**.

In summary, this thesis includes clinical and pharmacological studies, aiming at prolongation of the exposure to anti-cancer agents belonging to the class of topoisomerase I inhibitors, either by oral administration (*i.e.*, oral irinotecan, formulated as semi-solid matrix (SSM) capsules, and the oral combination of irinotecan and capecitabine), or by administration as macromolecular drug-delivery system (*i.e.*, DE-310), as well as an anti-microtubulin agent (taxane) by liposomal encapsulation (*i.e.*, LEP).

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Modulation of camptothecin analogs in the treatment of cancer: A review

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Abstract

The topoisomerase I inhibitors reviewed in this paper are all semisynthetic analogs of camptothecin. Modulation of this intranuclear enzyme translates clinically in antitumor activity against a broad spectrum of tumors and is therefore subject of numerous investigations. We present preclinical and clinical data on camptothecin analogs that are already being used in clinical practice [*i.e.*, topotecan and irinotecan (CPT-11)] or are currently in clinical development (*e.g.*, 9-aminocamptothecin, 9-nitrocamptotecin, lurtotecan, DX 8951f and BN 80915), as well as drugs that are still only developed in a preclinical setting (*e.g.* silatecans, polymer-bound derivates). A variety of different strategies is being used to modulate the systemic delivery of this class of agents, frequently in order to increase antitumor activity and/or reduce experienced side effects. Three principal approaches are being discussed, including (i) pharmaceutical modulation of formulation vehicles, structural alterations and the search for more water soluble prodrugs, (ii) modulation of routes of administration and considerations on infusion duration, and (iii) both pharmacodynamic and pharmacokinetic biomodulation.

Introduction

Camptothecin (CPT), a plant alkaloid isolated from *Camptotheca acuminata*, was first identified in the late 1950's [1]. Due to severe and unpredictable toxic side effects in early clinical studies, including myelosuppression, severe diarrhea and hemorrhagic cystitis, the clinical development of CPT was halted in the 1970's [2-5]. In the early 1980's several important events occurred that resulted in renewed interest in this agent; (i) the molecular target of CPT, *viz.* the nuclear enzyme topoisomerase I, was identified. This topoisomerase I was described as an enzyme involved in transient scission and relegation of DNA during the replication and transcription phases. Binding of CPT to the topoisomerase I-DNA complex (cleavable complex) and interference with the relegation step of this process was recognized as the primary mechanism of action of CPT, finally leading to a double stranded DNA break and, ultimately, cell death [6-8]. Subsequent investigations indicated overexpression of this topoisomerase I enzyme in various types of solid tumors, including ovarian and colon cancer [9,10]. (ii) At the same time, it was shown that the failures encountered in the clinical development of CPT were related, at least partially, to the drug's poor water solubility, which necessitated

pharmaceutical formulation in alkaline solutions for i.v. administration. This not only led to chemical modification of the original structure into an entity lacking antitumor activity, but also induced profound alterations in the toxicological behavior of the agent [11].

These two key findings then boosted drug-research efforts aimed at identifying and developing new [(semi-)synthetic] analogs with improved aqueous solubility while maintaining CPT's unique mechanism of action. Some of these agents are currently in clinical development, whilst irinotecan (CPT-11) and topotecan are now registered for use in colorectal cancer [12], and ovarian and lung cancer [13], respectively. Both topotecan and CPT-11 underwent extensive clinical evaluation in phase II and III trials and data suggested that both drugs are also active in various other tumor types in addition to the indications mentioned [14,15].

Unlike most other CPT analogs, CPT-11 is a prodrug with very little inherent antitumor activity. To form the active metabolite SN-38, which is 100- to 1000-fold more active than the parent compound [16], CPT-11 is hydrolyzed by a carboxylesterase [17]. SN-38 in its turn can be metabolized further by UDP glucuronosyltransferase 1A1 to form an inactive β-glucuronide derivative (SN-38-G) [18]. In *in vitro* studies, topoisomerase I inhibitors showed more pronounced antitumor efficacy with protracted exposure to low concentrations. In animal models, low-dose prolonged exposure also resulted in less toxicity [19-23]. It should be noted that for several reasons, including species differences in drug disposition and tolerability as well as intrinsic differences in tumor sensitivity, *in vitro* and animal models have been shown to be poor predictors of clinical efficacy and toxicity. Nonetheless, most clinical studies have focussed on low-dose exposure to topoisomerase I inhibitors in cancer patients [24-29]. Meanwhile, numerous researchers are unravelling the clinical pharmacodynamics and pharmacokinetics of the different analogs. This has lead to an explosion in publications on this subject. The present review is focussed on chemical and pharmacologic aspects of CPT analog development, with special emphasis on the choice of routes of delivery and on intrinsic differences in toxicity profiles of the various analogs and possible ways to modulate these either pharmacodynamically or -kinetically.

Chemical properties

Structure-activity relationships

Most of the currently known CPT analogs share a basic five-ring structure with a chiral center located at C20 in the terminal E-ring. Extensive studies on the

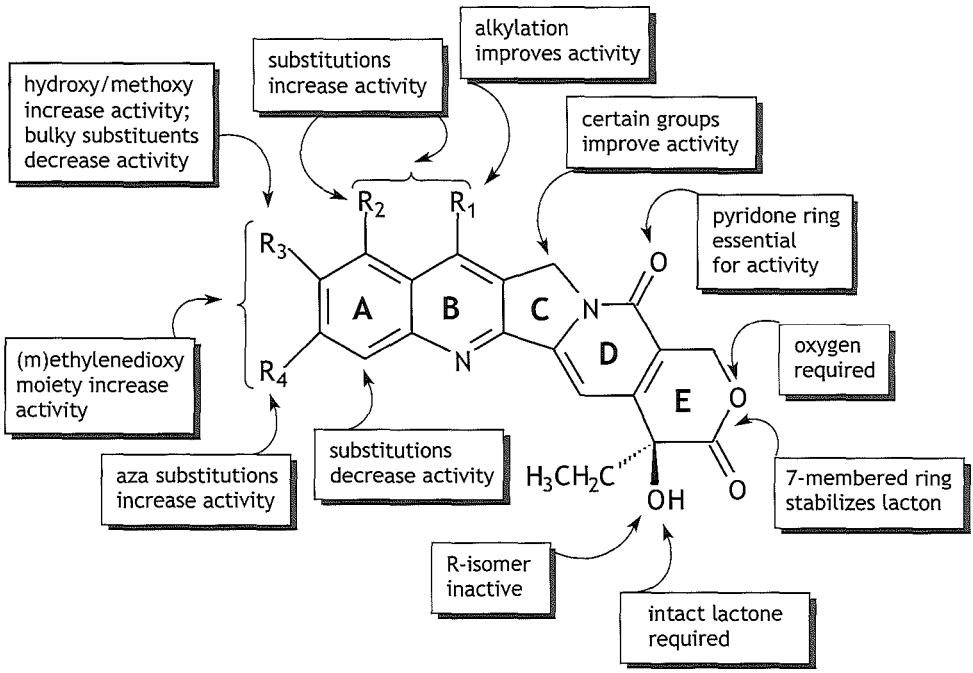


Figure 1. Structure-activity relationships of camptothecins.

synthesis of CPT analogs and the development of structure-activity relationships have been carried out over recent years and some important general relationships have emerged [30]. While these relationships will clearly be refined in the years to come, current knowledge is potentially adequate for the design of improved analogs of CPT. This current knowledge is here summarized in Figure 1 rather than being described exhaustively. Structure-activity studies have also shown a close correlation between the ability to inhibit topoisomerase I and overall cytotoxic potency [31]. For the purpose of this review, a number of regions in the CPT structure is particularly relevant:

(i) It has been shown that the topoisomerase I inhibitory activity of these agents is stereospecific, with the naturally occurring (*S*)-isomer being many-fold more potent that the (*R*)-isomer [7,9].

(ii) In general, substitutions at C7, C9 and C10 tend to increase topoisomerase I inhibition and sometimes increase water solubility, whereas substitutions at C12 decrease antitumor activity [16].

(iii) Similarly, the formation of certain additional ring structures, *e.g.* between C7 and C9 or C10 and C11 increases activity [32-35].

(iv) One of the principal chemical features of this class of agents is the presence of a lactone functionality in the E-ring, which is not only essential for antitumor activity, but it also confers a degree of instability to these agents in aqueous solutions [36]. All known camptothecins can undergo a pH-dependent reversible interconversion between this lactone form and a ring-opened carboxylate (or hydroxy acid) form (Figure 2), of which only the lactone form is able to diffuse across cell membranes, and exert the characteristic topoisomerase I inhibitory activity. At neutral or physiologic pH, the equilibrium between the two species favors the carboxylate form for all the camptothecins. As outlined, an understanding of this hydrolysis reaction helps to explain several observations in the early development of these agents. Because CPT was administered as the more water-soluble sodium salt, patients were exposed to high concentrations of the relatively inactive carboxylate species, whereas large amounts of drug were excreted in urine, where the low pH favored closure of the lactone ring with resulting hemorrhagic cystitis [2,4]. The equilibrium between the lactone moiety ring and the carboxylate form of the camptothecins is not solely dependent on the pH, but also on the presence of specific binding proteins in the biological matrix, most notably human serum albumin (HSA). Following establishment of equilibration at 37°C in phosphate buffered saline (PBS), equal amounts of the various CPT analogs are present in the pharmacologically active lactone form, with values of 17, 19, 15, 13 and 15% for CPT, 9-aminocamptothecin (9-AC), topotecan, CPT-11 and SN-38, respectively [37]. Addition of 40 mg/mL HSA shifts the equilibrium for CPT and 9-AC towards the carboxylate form, with approximately 1% present in the lactone form at equilibrium [37,38]. In contrast to HSA, addition of murine serum albumin to 9-AC leads to approximately 35% existing in the lactone form at equilibrium [38]. As opposed to CPT and 9-AC, HSA actually stabilizes the lactone moiety of CPT-11 and SN-38, with values of 30 and 39%, respectively, present in the lactone form at equilibrium, while almost no effect was seen for topotecan [37]. It has been proposed that the differences in the percentiles present in the lactone form at equilibrium is related to sterical considerations of the various substituents at the R_1 and R_2 positions (Figure 2A). For some of the more recently developed CPT analogs, the substituents cause sterical hindrance and prevent binding of the carboxylate forms to HSA, and so drive the equilibrium towards the lactone species.

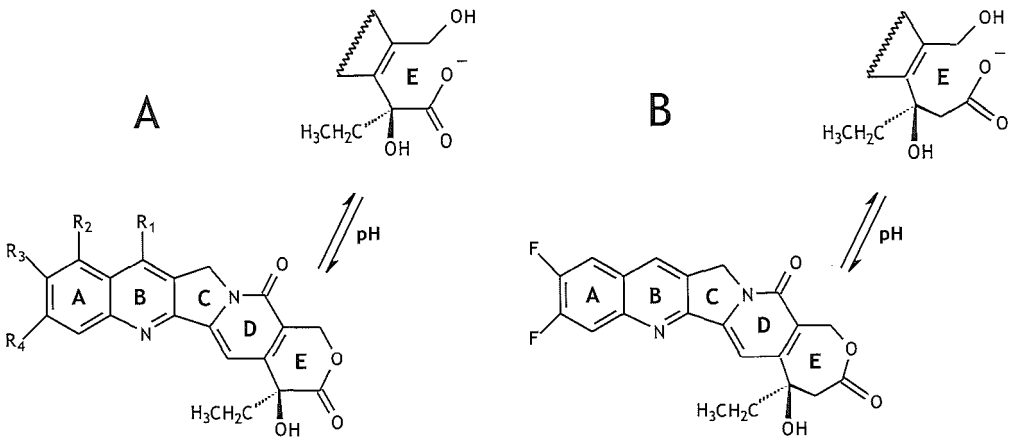


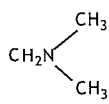
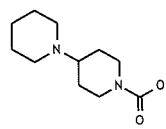
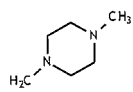
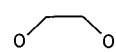
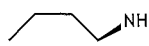
FIGURE A	R_1	R_2	R_3	R_4
CPT	H	H	H	H
9NC	H	NO_2	H	H
9AC	H	NH_2	H	H
TPT	H		H	H
CPT-11	CH_2CH_3	H		H
SN-38	CH_2CH_3	H	OH	H
LRT		H		
DX-8951			CH_3	F

Figure 2A and 2B. Chemical structures of camptothecin analogs

Conventional drug formulations

The inherent instability of the lactone form and the inactivity of the carboxylate form have posed a specific challenge to the development of a suitable dosage form of CPT analogs. Since the lactone form is strongly favored by an acidic pH, as outlined above, the currently registered agents topotecan and CPT-11 have both been formulated in buffered dosage forms. Topotecan is available as a powder containing topotecan hydrochloride and tartaric acid, yielding an aqueous solution for infusion of pH 2.5-3.5 after reconstitution. This solution is stable for at least 12 hours at room temperature, whereas the dry powder is stable for at least 2 years at room temperature. The pharmaceutical dosage form of CPT-11 is similarly based on formulation of the hydrochloride form of the drug in an aqueous solution, containing a lactic acid-sodium hydroxide buffer system of pH 3.5-4.5. Current shelf-life studies have shown that the injection concentrate is stable for at least 3 years at room temperature when protected from light.

Pharmaceutical modulation

Alternative formulations

In recent years, a variety of alternative pharmaceutical formulations have been or are currently being evaluated. Important properties of these alternatives will be that they allow drug doses to be delivered at levels (at least) similar to those achieved with the conventional formulations, and that the drugs should be stable for several hours in order to be handled in a clinical setting. Despite the tremendous efforts invested so far, only very few of the alternatives have fulfilled the requirements to justify clinical testing. The rationale for re-formulation of CPT or its analogs has been either to stabilize the lactone moieties or to induce sustained release combined with specific tumor targeting of these agents. An example of the first approach has been the formulation of CPT, CPT-11 or 10-hydroxy-CPT in microspheres composed of poly-D,L-lactic acid or poly(D,L-lactic-co-glycolic acid) [39-42]. The influence of various encapsulation procedures on the release of these agents has been extensively examined and has shown stabilization of the lactone form due to an acidic microclimate of the microspheres combined with enhanced pharmacokinetic characteristics in animal models [43]. Although from a theoretical perspective the modulation of CPT in this prolonged release system could be attractive by reducing local toxicity and improving therapeutic efficacy, no phase I studies have been performed so far.

Alternatively, substantial progress has been made recently toward liposomal formulation of a number of important CPT analogs. Liposomes are microparticulate carriers that consist of one or more lipid bilayer membranes enclosing an internal aqueous phase. The most common constituents are synthetic or naturally occurring phospholipids and cholesterol. Although several reports address the considerations in choosing the specific liposome constituents and their physical properties (*e.g.* for CPT [21], topotecan [44,45] and CPT-11 [46,47]), relatively little information has been presented on toxicity profiles and antitumor activity. A recent study reported, however, that liposomal topotecan, encapsulated in sphingomyelin/cholesterol liposomes using an ionophore-generated proton gradient, was eliminated from the plasma much more slowly than the free drug, resulting in a 400-fold increase in systemic exposure [48]. The liposomal preparation also protected topotecan from lactonolysis and increased antitumor activity in both murine and human tumor models. Likewise, data have been generated demonstrating that unilamellar liposomal formulations of lurtotecan (NX 211 [49] and SPI-355 [50]) have significant therapeutic advantage over free drug and that the increased activity is consistent with increased systemic exposure and enhanced tumor-specific delivery of the drug. Based on these exciting data, several phase I clinical trials have been initiated with NX 211 given to cancer patients in either weekly or 3-weekly regimens, and preliminary findings corroborate the preclinical pharmacological profile of this agent [51].
CPT and a number of analogs, notably topotecan, have also been formulated in solid-lipid nanoparticles [composed of stearic acid, soybean lecithin and polyoxyethylene-polypropylene copolymer (Poloxamer 188)] [52] and dimethyl-ß-cyclodextrin to stabilize the lactone form [53]. Although these systems are promising sustained release and drug-targeting systems in various preclinical models, their clinical merit has not yet been evaluated.

Synthetic derivatives

9-Aminocamptothecin

9-AC is a semisynthetic CPT derivative which showed outstanding preclinical activity against a wide spectrum of tumor types, including those of breast, colon, lung and prostate and melanoma [54]. In clinical trials, the drug has been very extensively studied using two different formulations based on the use of dimethylacetamide/polyethylene glycol 400 or a colloidal dispersion preparation, which enhances solubility and stability. Clinical phase I investigations have been

conducted using a variety of i.v. administration schedules, including a 30-min infusion given daily for 5 days every 3 weeks, and more prolonged infusion schedules using 24-hour, 72-hour, 96-hour and 7-day or 21-day continuous dosing repeated every 4 weeks [55-61]. All of the studies report neutropenia as the dose-limiting toxicity, while thrombocytopenia is also frequent and sometimes severe. Gastrointestinal toxicity is the second most reported, though not dose limiting. Other toxicities are considered mild to moderate. Numerous multi-institutional phase II studies have been conducted in several disease types, and overall, 9-AC shows only very modest single-agent activity and its further evaluation does not seem indicated [59, 62-64]. It has been suggested that the lack of clinically relevant antitumor efficacy relates to substantial inactivation of the agent due to the unfavorable lactone/carboxylate ratio in patients [38].

Homocamptothecins

In the search for more stabile CPT analogs, the synthetic preparation of derivatives bearing a 7-membered E-ring, the so-called homocamptothecins has been described (Figuur 2) [65]. The lactone ring is stabilized by modification of the naturally occurring 6-membered α-hydroxylactone ring into a 7-membered β-hydroxylactone ring by insertion of a methylene spacer between the alcohol and the carboxyl moiety. The lead compound in this series, *i.e.* homocamptothecin (hCPT), has been shown to be more stable than CPT, remains a highly potent inhibitor of both cell growth with superior topoisomerase I inhibitory activity as compared to CPT [66-68] and, most interestingly, changes the sequence-specificity of the drug-induced DNA cleavage by topoisomerase I [69]. Indeed, in contrast to CPT which shows a rapid hydrolysis of the lactone moiety until a pH- and protein-dependent equilibrium has been reached, hCPT displays a slow and irreversible hydrolytic lactone ring opening [70]. After a 3 h incubation of CPT and hCPT in human whole blood at 37°C, the fraction present in the lactone form was 6% in the case of CPT and 80% in the case of hCPT. This remarkable difference is not only due to the slower ring opening of hCPT, but also to a higher affinity of hCPT for red blood cells [71]. Based upon this promising feature of hCPT, a series of derivatives of this agent was developed that combine enhanced plasma stability and potent topoisomerase I-mediated cytotoxicity. Various fluorinated analogs were subsequently found to have potent cytotoxic activity against several cell lines, including those overexpressing a functionally active P-glycoprotein [72]. Figure 2 (B) shows the chemical structure of BN 80915, one of the most promising fluorinated hCPT analogs, which recently has entered clinical phase I testing.

Silatecans

Using a cascade radical annulation route to the CPT family, a novel series of CPT analogs, *viz.* 7-silylcamptothecins or silatecans, have been synthesized that exhibited potent inhibition of topoisomerase I, dramatically improved blood stability and sufficient lipophilicity to favor blood-brain barrier transit [73]. Preliminary evaluation in preclinical mouse models indicate that silatecans may hold significant promise for the treatment of high-grade gliomas and provide a rationale for proceeding with further (pre)clinical evaluation of their efficacy and safety versus commercially available CPT derivatives, including topotecan and CPT-11.

Hexacyclic camptothecins

Two representative agents of the hexacyclic CPT analogs are currently under investigation. The first of these, lurtotecan (also known as GI147211 or GG211), is a water-soluble, totally synthetic derivative with a dioxalane moiety between C10 and C11 [74]. This agent has been evaluated clinically in various phase I and phase II trials using a 30-min i.v. infusion given daily for 5 consecutive days or as a 72-hour continuous i.v. infusion [75-77]. The dose-limiting toxicity in both schedules was myelosuppression, including severe neutropenia and thrombocytopenia. Nonhematological toxicities were various and only mild to moderate. Because the oral bioavailability was highly variable and as low as 10% [78], alternative ways of drug administration are currently being developed, including a new liposomal formulation (NX 211; see above).

The second agent, DX-8951f or exatecan mesylate, is a new water-soluble, CPT analog with an amino group at C1 and a fluorine at C5 [79]. DX-8951f showed superior and a broader spectrum of antitumor activity *in vitro* and *in vivo* in comparison with the other CPT analogs tested [80,81]. Recently, the results of a phase I evaluation of DX-8951f have become available, with the drug administered as a 30-min i.v. infusion given daily for 5 days every 3 weeks [82,83]. Brief, noncumulative neutropenia was the most common toxicity, and was seen consistently at doses greater than 0.5 mg/m^2/day. Other nonhematologic toxicities were mild to moderate in severity. Various other schedules, including a once every 3 weeks regimen with 30-min i.v. infusions [84,85] and one based on continuous i.v. infusions of 5 to 21 days are presently under investigation [86]. The 30-min infusion regimen with daily administration for 5 consecutive days is now being tested in clinical phase II trials in various disease types, including non-small cell lung cancer [87], pancreatic cancer [88], ovarian cancer [89], and colorectal cancer [90].

Prodrugs

Because of the poor aqueous solubility of some CPT analogs, some major efforts have been put into the design and synthesis of more water-soluble prodrugs that could be more readily formulated than the parent drug. Many of the synthesized compounds have shown only marginal improvements in solubility or are too unstable to allow administration in a clinical setting. The instability of prodrug forms of, for example, CPT is particularly problematic, since the product of degradation (generally the parent drug) is insoluble and precipitates in aqueous solutions. Other synthetic approaches have produced fairly stable prodrugs, but the rate of active drug liberation proceeds at a too slow and variable rate. To date, two approaches in prodrug design have yielded agents that have progressed to clinical evaluation, *viz.* the 9-nitro derivative of CPT and polymer-coupled derivatives of CPT and DX-8951f.

C9-Substituted derivatives

One of the most extensively studied agents of this class is 9-nitrocamptothecin (9-NC), which acts as a partial prodrug of 9-AC [91,92]. 9-NC has a nitro radical in the C9-position and is highly insoluble in water, and was initially identified as a precursor in the semi-synthetic production of 9-AC. Since nearly all human cells are able to convert 9-NC to 9-AC, including tumor cells, it has been proven difficult to identify whether 9-NC-mediated antitumor activity is directly associated with the parent drug alone or with 9-AC alone or the combination of both [93]. Preliminary evidence generated in clinical phase II trials suggests that 9-NC may be potentially be useful in the treatment of advanced pancreatic cancer and refractory ovarian cancer using a daily times 4 or 5 per week schedule with the drug given orally [94]. An extensive clinical phase II program is currently being conducted to test the efficacy of this agent against various malignant diseases [95,96].

Polymer-bound derivatives

One of the possible ways to modulate anticancer agents is the use of its attachment to macromolecules because these high molecular weight prodrug carriers can lead to reductions in systemic toxicity, longer retention time within the body, alterations in biological distributions and possible improvements in therapeutic efficacy [97,98]. Its use is dependent on the concept of the enhanced permeability and retention (EPR) effect in solid tumors [99]. This EPR effect is based on four general characteristics of tumor tissues in comparison with normal tissues: (i) hypervascularity, (ii) hyperpermeability, (iii) defective vascular

architecture, and (iv) less efficacious drainage due to hypoplastic or minimally effective lymphatic system [99]. The pharmacokinetic characteristics of CPT were the starting point to modulate its structure in this way. As indicated, CPT is highly insoluble in water, and by converting the C20-OH moiety into an ester coupled to an amino acid spacer to allow better solubilization in aqueous environments, several macromolecular prodrugs of CPT have been generated in recent years [100]. Two of these, MAG-CPT and PEG-CPT, have progressed to clinical evaluation [97,100]. The former consists of CPT covalently bound to a soluble polymer [N-(2-hydroxypropyl)-methacrylamide (HPMA)] through a glycyl-aminohexanoyl-glycyl spacer, whereas the latter is a macromolecule derived through conjugation of chemically modified polyethyleneglycol with CPT at the C20-OH position. A variety of preclinical studies with MAG-CPT and PEG-CPT, have shown stabilization and sustained release of CPT, and also prolonged drug retention within experimental tumors [97,101]. Recently, the preliminary results of clinical phase I studies of PEG-CPT [102] and MAG-CPT (also referred to as PNU 166148) [103] have been reported, and indicate substantially prolonged exposure times to the active species as compared to administration schedules of the free drugs.
In an effort to prolong exposure times of DX-8951f to tumor tissues that might increase cytotoxic properties and antitumor efficacy, a prodrug has recently been generated by linking the agent to a biodegradable carrier via a peptide spacer (DE-310). Clinical evaluation of this prodrug to examine this hypothesis is currently being conducted.

Routes of delivery

Intravenous dosing and considerations of infusion duration

I.v. administration of CPT analogs is currently the most commonly used route of delivery. The advantages of this route are many, including the fact that the total amount and duration of the administered drug can be controlled. As mentioned earlier, this has the advantage of controlled prolonged delivery of the drug. In this regard, most of the studies have been done with topotecan [5], and several of the most promising regimens have been selected to enter phase II testing, *viz.* a daily-times-five every 3 weeks 30-min schedule [104,105], weekly or 3-weekly 24-hour infusion [106,107] and a 21-day continuous low-dose infusion administered every 4 weeks [13,25,27, 29,108]. Besides proposed schedule dependency on antitumor activity, toxicity also appears to vary considerably. Overall, the dose-limiting toxicity is myelosuppression, consisting primarily of neutropenia, whereas in

some continuous infusion schedules thrombocytopenia was more pronounced [25,104]. Anemia and nonhematological side effects, including nausea, vomiting, diarrhea, fatigue, asthenia, alopecia and mucositis are common but usually mild, and do not seem to be schedule dependent. Most of the studies showed that prolonged exposure to i.v. administered topotecan is feasible. Randomized comparison of the daily-times-five schedule every 3 weeks and 24-h infusion once a week for 4 weeks repeated every 6 weeks in patients with ovarian cancer suggests that the daily-times-five topotecan regimen was significantly superior with respect to response rate [109,110]. Randomized studies between the 5-day schedule and the 21-day continuous infusion schedule are not yet available [111]. Regarding the administration of CPT-11, different schedules are currently being used in Europe (350 mg/m^2 as a 90-min infusion once every 3 weeks) [112], the USA (125 mg/m^2 given weekly as a 90-min infusion for 4 or 6 weeks) [113] and Japan (100 mg/m^2 given weekly as a 30-min infusion) [114]. In all these dosing schedules, the total amount of CPT-11 that can be tolerated in any time period is similar [115]. Schedules with protracted infusions that have been investigated vary between 96-hours weekly to 14-days continuous infusion [26,116,117]. The maximum-tolerated dose in these studies (10-30 mg/m^2/day) is much lower then for the short-duration schedules. Surprisingly, the AUC of the active metabolite SN-38 reaches comparable levels as reported for the short duration regimens, which is not completely understood. One possible explanation would be that the enhanced metabolism of CPT-11 relates to saturation of carboxylesterase-mediated conversion of CPT-11 with (high-dose) short infusion schedules. As for toxicity, myelosuppression and diarrhea are the dose-limiting events in all tested regimens. Like with topotecan, protracted low-dose schedules give more rise to thrombocytopenia [116], while in shorter schedules neutropenia is more prominent. The influence of infusion duration of antitumor activity has not yet been evaluated in a randomized setting.

Oral dosing

As indicated, the high specificity in the mechanism of action of CPT analogs for the S-phase in the cell cycle has led to the recognition that the compounds may require prolonged exposure to maximize the fractional cell kill. In this regard, the availability of clinically useful oral formulations of currently available CPT analogs would provide increased convenience for the administration of chronic dosing regimens and the opportunity for cost-effective outpatient therapy [94]. Since most of the CPT analogs have relatively short terminal disposition half-lives, the use of protracted oral dosing is not necessarily the same as continuous

i.v. infusion, although if the postulated concept of time over threshold concentration is a valid indication of both toxicity and efficacy, oral dosing can mimic continuous infusion regimens. Formal oral bioavailability studies have been conducted for several agents, and have yielded bioavailability values for topotecan of 30 to 44% [118-120], for 9-AC of ~50% depending on the formulation applied [121,122], and for lurtotecan of 11% [78]. Clinical data are not yet available for CPT-11, although murine data show an oral bioavailability of between 10 and 20%, depending on the dose administered [123].

The development toward suitable oral regimens for CPT analogs has to date been most extensively studied for topotecan using daily or bi-daily administration of 5-day [124], 10-day [125], or 21-day schedules [126]. A variety of clinical studies have shown that with an increase in prolonged topotecan administration by this route, a shift occurs in dose-limiting toxicity from hematological toxicity (mainly granulocytopenia) toward severe gastrointestinal side effects, most notably diarrhea [127]. These investigations further indicated that the schedule applied, rather than the applied systemic exposure per course seemed to be related to the type of experienced toxicity [127]. Based on these considerations, the daily-times-five schedule has been recommended for future studies. The need for further clinical development of the oral topotecan formulation became even more important in view of recent findings that the oral formulation has similar efficacy in the treatment of advanced ovarian and small-cell lung cancers as compared to the i.v. formulation, while less hematological toxicity was observed [128,129].

Based on theoretical considerations, including the fact that carboxylesterases are highly expressed in human liver and the gastrointestinal tract that could result in presystemic metabolism to SN-38 [130], it appears particularly attractive to deliver CPT-11 by the oral route. Indeed, the preliminary findings of substantially increased SN-38 to CPT-11 concentration ratios with oral CPT-11 administration as compared to i.v. administration seems to sustain this notion [131,132]. In addition, oral drug administration was associated with increased persistence of circulating levels of the lactone form of SN-38, which might be an additional advantage with potential pharmacodynamic importance [133]. The clinical utility of oral CPT-11 administration is currently under further investigation.

Local drug administration

Hepatic arterial dosing

The narrow therapeutic window of systemic administration of CPT and its analogs has prompted a search toward local drug administration, with the rationale to obtain selectively higher activity against locally confined tumors and/or lower systemic toxicity without loss of antitumor activity. The pharmacokinetic behavior of CPT-11 was recently compared during 5-day hepatic arterial and i.v. infusion in a group of cancer patients [134]. These findings indicated that arterial drug administration leads to significantly higher conversion of CPT-11 into the active metabolite as compared to the i.v. administration, although the clinical relevance of this observation is, as yet, unknown [135]. In recent years, various agents have also been used for arterial embolization in an attempt to encapsulate the concomitantly administered chemotherapeutic agent and, thereby, further enhance the local drug concentration. This concept has been tested with CPT-11 administered with hepatic arterial chemoembolization to patients with primary and metastatic hepatic malignancies [134]. Further studies are clearly required to confirm efficacy of this treatment and should aim at measuring systemic and tumor tissue concentrations.

Pulmonary delivery

Liposomal aerosol formulations of CPT and 9-NC have recently been developed for nasal inhalation treatment of experimental lung tumors xenografted in nude mice. It was found that this preparation was strikingly effective in the treatment of these xenografts growing subcutaneously over the thorax at doses much lower than those traditionally used in preclinical models administered by other routes [136]. Interestingly, 9-NC aerosol therapy was also effective against established melanoma and osteosarcoma lung metastases [137]. Concurrent pharmacokinetic studies showed that this type of treatment results in a prompt pulmonary distribution at substantial levels that could not be achieved with conventional routes of delivery, including oral and intramuscular [138,139]. Overall, these data suggest that local delivery of CPT analogs to the respiratory tract by liposome aerosol treatment might offer advantages over existing methods in the treatment of some diseases.

Intraperitoneal dosing

Intraperitoneal (i.p.) administration has been used as a strategy for increasing total drug delivery to ovarian cancers confined to the peritoneal cavity.

The pharmacokinetic behavior of topotecan suggests that a substantial pharmacokinetic advantage might be obtained following i.p. injection. Indeed, recent clinical evidence suggests very slow peritoneal clearance of topotecan and high peritoneal:plasma concentration ratios of >10 after i.p. drug administration [140]. I.p. administration of CPT-11 has also been studied recently in animal models and showed some potential advantages over the i.v. route. It appeared that the therapy was more efficient with an increase in life span and was less toxic as compared to the i.v. route in mice bearing C26 colon tumors. In addition, substantially elevated AUCs of CPT-11 and SN-38 were found in the peritoneum, although plasma levels were comparable to i.v. dosing [140]. The clinical implications of these observations have not yet been evaluated.

Biomodulation

Pharmacodynamic alterations

The use of biomodulators to increase the therapeutic index of chemotherapeutic treatment has made a significant impact on certain diseases [141]. A number of cancer chemotherapy biomodulators has been approved for clinical use in humans and these agents can modulate anticancer drugs through either pharmacodynamic or -kinetic modulations. Among extensively studied biomodulating agents are the class of hematopoietic growth factors to decrease chemotherapy-induced neutropenia and anemia. Initial attempts to increase topotecan dose intensity with the use of granulocyte colony-stimulating factor (G-CSF) failed, since thrombocytopenia and fatigue rapidly emerged as dose-limiting effects with the daily-times-five schedule [142,143]. On the other hand, G-CSF administered after 5 daily infusions of topotecan permitted a 2.3-fold dose escalation above the maximum-tolerated dose [144,145], although it was concluded that the substantial toxicity, inconvenience and costs associated with this high-dose topotecan/G-CSF regimen does not warrant further development. Similar disappointing results have been obtained with topotecan or 9-AC administered by prolonged continuous infusions [146,147]. The addition of G-CSF with CPT-11 administration has also been advocated by some investigators [148,149], evidence for increased dose intensity or clearly improved chemotherapy based on G-CSF support is still lacking. Therefore, the use of G-CSF outside clinical trials to support chemotherapeutic treatment is not recommended.

The principal nonhematologic toxicity for all topoisomerase I inhibitors is gastrointestinal. Nausea and vomiting are frequent [56,75,104,112], but with the

introduction of selective serotonine antagonist of the $5HT_3$ -receptor, this side effect is adequately manageable. Diarrhea is also frequent and mild to moderate in severity with i.v. administration schedules. It appears to be unrelated to the schedule used [56,75,104,112], except in case of CPT-11, where with the oral administration diarrhea becomes the prime dose-limiting toxicity especially when using the prolonged schedules [24,122, 125,126,150].

In the treatment with CPT-11 two types of diarrhea can be distinguished, *viz.* an early- and a late-onset form. The early-onset diarrhea is part of a cholinergic-like syndrome and manifests in sweating, salivation, abdominal cramping and diarrhea [151]. Interestingly, this cholinergic syndrome has not been described for other CPT analogs. It has been argued that the unique structural features of CPT-11, including a bipiperidino group that shows similarity with a known stimulant of nicotine receptors of autonomic ganglia, dimethylphenylpiperazinium iodide, is responsible for this phenomenon [152]. More recently, it has been found that the mechanism behind the transient cholinergic reaction observed clinically is more likely mediated through a rapid reversible inhibition of acetylcholinesterase by the lactone form of CPT-11 [153]. Clinical evidence indicates that this side effect can be adequately treated in the acute phase as well as prophylactically with the use of i.v. atropine.

CPT-11-induced delayed type diarrhea has been reported to be severe (NCI-CTC grade 3-4) in 11-23% of the patients [113,154], but even the less severe diarrhea still might influence continuation of therapy. Moreover, diarrhea related to drug-induced colon-mucosal damage, as observed in both rodents [155] and humans [156], can cause severe and potentially lethal illness especially during concomitant occurrence of neutropenia. Once delayed-type diarrhea has occurred, a high-dose regimen loperamide renders the diarrhea manageable [157].

Prophylactic treatment of this frequently observed side effect has been investigated in numerous studies. Potential modulation of delayed-type diarrhea has been examined clinically with several agents, including an enkephalinase inhibitor [158] and glutamine [159], and in animal models with a lipopeptide [160] and interleukin-15 [161], with different results. A recent preliminary report on co-treatment with thalidomide claims a good protection against diarrhea, but we still have to await pharmacokinetic results to weigh this study on its proper value [162]. To explain the mechanism by which CPT-11-mediated delayed-type diarrhea is triggered, many pharmacokinetic analyses in humans have been performed in order to predict the incidence of this diarrhea, with conflicting results. Some studies described a correlation between late-onset diarrhea and the systemic glucuronidation rates of SN-38 (*i.e.*, the biliary index) [163]. Recently,

it was suggested from animal models, that β-glucuronidase activity from the microflora in the large intestines may play a major role in the development of CPT-11-induced diarrhea, by mediating the hydrolysis of biliary secreted SN-38G, thereby causing local accumulation of SN-38, which subsequently causes damage to the intestinal epithelium [164]. This observation has led to experiments in which antibiotic treatment inhibited the β-glucuronidase activity from the intestinal microflora, thereby decreasing the luminal SN-38 concentration and subsequently reducing intestinal damage and ameliorating diarrhea [155]. A recent study in humans showed that antibiotic treatment with neomycin did not alter SN-38 pharmacokinetics in plasma, and gave protection to recurrent diarrhea in over 85% of the patients experiencing diarrhea in the first course (unpublished data, DK and AS). The expected mechanism of blocking bacterial β-glucuronidase activity causing a subsequent rise in SN-38/SN-38G concentration ratio has recently been confirmed. It would be even more attractive to use an agent that specifically inhibits the microbial β-glucuronidase activity. Hange-shasin-to (also referred to as TJ14), a herbal medicine that contains the β-glucuronidase inhibitor baicalin, has recently been described to be a potent inhibitor of delayed-type diarrhea caused by CPT-11 in a rat model [165] as well as in humans [166]. Unfortunately there is no information yet on possible changes in the systemic disposition of CPT-11 and its metabolites which are likely to occur due to inhibition of plasma β-glucuronidase activity by this agent, information of vital importance in view of antitumor activity.

It has also been speculated based on *in vitro* studies, that raising pH-values in the intestines by intestinal alkalization might decrease reabsorption of biliary secreted SN-38 after i.v. CPT-11 administration and, as a result, lowers intestinal side effects [167]. Again, demonstration of unaltered pharmacokinetics of SN-38 in the presence of intestinal alkalization is of crucial importance. Thus, although this approach might show reduced CPT-11-mediated intestinal toxicity, this may be a phyrric victory if a simultaneously altered metabolic clearance (by way of a decreased enterohepatic recirculation of SN-38) results in reduced antitumor activity.

Pharmacokinetic alterations

Intestinal metabolic systems and drug efflux pumps located in the intestinal mucosa represent a major limitation in the bioavailability of orally delivered drugs [168]. Several enzymes located in the enterocyte, like the cytochrome P450-3A4 isoenzyme (CYP3A4), are involved in the presystemic metabolism of many anticancer agents, including etoposide and paclitaxel, thereby limiting the oral

absorption of these drugs. Since CYP3A4 is involved in the metabolism of CPT-11, it has been proposed that the bioavailability of this agent might be substantially enhanced by pharmacokinetic modulation of enteric CYP3A4 activity, *e.g.* by concomitant administration of ketoconazole, erythromycin or quinidine [169]. Similarly, P-glycoprotein and the Breast Cancer Resistance Protein (BCRP), which are abundantly present in the gastrointestinal tract, have been shown recently to limit the intestinal absorption of various agents, including topotecan [170]. Combined inhibition of intestinal P-glycoprotein and BCRP by the investigational agent GF120918 was shown recently to increase the systemic exposure to topotecan in both animals and patients with the bioavailability increasing from a mere 30-44% to >90%, suggesting that modulation of these transporters simultaneously could be considered in the development of substrate anticancer agents given by the oral route [171].

As indicated, several preclinical studies have identified CYP3A4 to form two pharmacologically inactive oxidation products known as APC and NPC, as one of the principal enzymes involved in CPT-11. In addition, it was shown that ketoconazole, a synthetic imidazole-type broad-spectrum antifungal agent as well as a potent inhibitor of CYP3A4, inhibited APC and NPC formation by 98 and 99%, respectively, at tested concentrations as low as 1 μM [172,173]. Previous investigations indicated that with standard oral doses of ketoconazole (200 to 400 mg/day), peak plasma concentrations are in the range of 4 to 20 μM, suggesting that concomitant treatment of ketoconazole is likely to substantially alter the disposition of CPT-11 administered i.v. to cancer patients. Indeed, a recent pilot pharmacokinetic study in a cancer patient receiving CPT-11 with or without ketoconazole indicated a substantial pharmacokinetic interaction between the two drugs at the level of drug metabolism, and indicated that these agents can not be administered together without dose adjustments (unpublished data, AS and JV). If confirmed in a larger group of patients, the concomitant administration of ketoconazole might enable CPT-11 dose reductions without affecting systemic exposure to the active metabolite, SN-38, and potentially eliminates interpatient variability in CPT-11 pharmacokinetics that arise as a result of (genetically-defined) patient differences in CYP3A4 expression levels [174].

There are many other potential approaches to improve the therapeutic index of CPT-11 through pharmacokinetic biomodulation, including modulation with inhibitors of biliary secretion processes mediated by P-glycoprotein and/or cMOAT (*e.g.*, cyclosporin A) and with inducers of UDP glucuronosyltransferase isoforms involved in SN-38 glucuronidation (*e.g.*, phenobarbital) [175,176]. A clinical trial to evaluate the pharmacological and toxicological implications of

a combination regimen of CPT-11, cyclosporin A and phenobarbital is currently in progress, and preliminary findings indicate substantial antitumor activity with this combination without the occurrence of significant diarrhea despite a very low systemic exposure to SN-38 [177]. This finding is supportive of the conjecture that activation of CPT-11 by intratumoral carboxylesterases might be even more important than circulating SN-38 concentrations [178,179].

Conclusions and future perspectives

Camptothecins are among the most promising new anticancer drugs that have been developed in recent years. Topotecan and CPT-11 are now registered for the treatment of ovarian and colon cancer, respectively. The unique mechanism of action on topoisomerase I and activity against a broad spectrum of other malignancies are an ongoing stimulus for further clinical development. With the growing knowledge on pharmacodynamics and pharmacokinetics of the different campotothecin analogs, the poor water solubility and pH-dependent reversible interconvention between the active lactone and inactive carboxylate form, as well as increase in activity or stability by substitutions to specific sites on the molecule, much effort has and will be done to increase antitumor activity of this group of drugs. Meanwhile pharmacological modulation, particularly of CPT-11, can be of interest to reduce toxicity and to influence metabolic pathways. Although much effort is being put into development of new analogs, the question to answer remains if drugs under development will ultimately lead to the theoretically expected higher activity and/or reduced toxicity. Last but not least, optimization of schedules, routes of administration and combination therapies will lead to numerous new studies in different tumor types. It is to be expected that in the future many new formulations and/or combinations will be developed. In contrast to other previously registered anticancer drugs, the pharmacological knowledge on how camptothecin analogs behave in humans will lead to a more logical and quicker development of these agents.

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Phase I, pharmacokinetic, food effect, and pharmacogenetic study of oral irinotecan given as semi-solid matrix capsules in patients with solid tumors

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Submitted

Abstract

Purpose: To characterize the maximum-tolerated dose (MTD), recommended dose, dose-limiting toxicities (DLTs), pharmacokinetic profile, and food effect of orally administered irinotecan formulated as new semi-solid matrix capsules.
Patients and Methods: Irinotecan was administered orally in fasted condition once daily for 5 consecutive days repeated every 3 weeks. Patients were randomly assigned to take the drug along with a high-fat, high-calorie breakfast for the administration at day 1 of the first or second cycle. Dosages tested were 70 and 80 mg/m^2/day.
Results: Twenty-five patients received 101 cycles of therapy (median 2 cycles, range 1 - 15). During the first cycle grade 3 delayed diarrhea and grade 3 fever were the DLTs at the dosage 80 mg/m^2/day in three out of five patients. Hematological and nonhematological toxicities were mild to moderate. Exposure to the active metabolite SN-38 was relatively high compared to intravenous infusion, but no relevant accumulation was observed. Food had no significant effect on irinotecan pharmacokinetics. One confirmed partial remission and ten disease stabilizations were observed in previously treated patients. No association was found between the UGT1A1*28 genotype and the risk of severe irinotecan-induced toxicity.
Conclusion: For oral irinotecan, a dose of 70 mg/m^2/day for 5 consecutive days every 3 weeks is recommended for further studies. Delayed diarrhea was the main DLT as to that observed with intravenously administered irinotecan. This study confirms that oral administration of irinotecan is feasible and may have favorable pharmacokinetic characteristics.

Introduction

In clinical studies, irinotecan has demonstrated single-agent activity against a spectrum of solid tumors [1], in particular colorectal cancer [2-9]. In two phase III studies, the combination of irinotecan with both bolus or infusional 5-fluorouracil plus folinic acid regimens for the first-line treatment of advanced and metastatic colorectal cancer was significantly superior over the corresponding control regimen in terms of response rate, time to progression and overall survival [10,11]. As a result, irinotecan has been approved for clinical use in advanced colorectal cancer, both as first-line therapy in combination with 5-fluorouracil and as salvage treatment in refractory disease [1].

Irinotecan requires bioactivation to form its biologically active metabolite SN-38 [12], that subsequently is detoxified to SN-38 glucuronide (SN-38G) by the polymorphic enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) [13-15]. In addition, irinotecan is metabolized by cytochrome P450 isoenzymes CYP3A4 and CYP3A5 to form the metabolites APC and NPC [16,17]. Furthermore, the elimination pathways of irinotecan and SN-38 are partially mediated by membrane-localized, energy-dependent outward drug pumps, belonging to the superfamily of ATP-binding cassette (ABC) transporters, like MDR1 P-glycoprotein (ABCB1) [18,19].

The highest dose intensity of irinotecan is achieved with short-time infusion once every 2 or 3 weeks [20,21], and the lowest dose intensity is achieved with protracted continuous schedules [22,23]. However, the antitumor activity of irinotecan in preclinical studies is dose- and schedule-dependent, with protracted low-dose administration being more effective and less toxic than short duration high-dose schedules [24,25]. This is consistent with the S-phase specificity of topoisomerase I inhibitors. The cytotoxicity of these drugs increases with the duration of exposure as a consequence of the longer drug exposure allowing a greater number of cells to enter into, and be targeted in, the S-phase of their cycle [26,27]. Therefore, prolonged plasma concentrations following continuous oral delivery would ensure therapeutic efficacy and facilitate sustained exposure of these cytotoxic agents. Furthermore, the high concentrations of tissue carboxylesterases in the liver and gastrointestinal tract may promote presystemic conversion of irinotecan to SN-38 [28]. Finally, approximately 90% of cancer patients expressed a preference for oral compared with intravenous administration of chemotherapy, predominantly due to the convenience of administration outside a clinical setting of current concerns or previous problems with intravenous access, provided that the efficacy of the drug is not compromised [29].

We performed this phase I study to evaluate the oral administration of irinotecan formulated as new SSM capsules in patients with refractory solid tumors. The objectives of this trial were (*a*) to determine the maximum-tolerated dose (MTD) and dose-limiting toxicities (DLTs) of irinotecan when administered once daily for 5 consecutive days every 3 weeks, (*b*) to characterize the pharmacokinetics of irinotecan and its metabolite SN-38, (*c*) to correlate the observed irinotecan-associated toxicity with genetic polymorphisms in genes involved in the pharmacokinetics of irinotecan, (*d*) to analyse the effect of food on the bioavailability, and (*e*) to evaluate preliminary antitumor activity.

Patients and methods

Eligibility Criteria

Patients with a histologically confirmed diagnosis of a malignant solid tumor refractory to conventional chemotherapy or for whom no effective therapy existed were eligible. Other eligibility criteria were similar to those described previously [30]. Specific exclusion criteria included prior treatment with irinotecan, concomitant treatment with CYP3A4 inhibitors or inducers (wash-out period of at least 7 days since last intake), symptomatic brain metastases or leptomeningeal involvement, active inflammatory bowel disease, bowel (sub-)obstruction, chronic diarrhea, known chronic malabsorption or total colectomy or other major abdominal surgery that might result in substantial alteration in transit or absorption of oral medication. The institutional Ethical Boards approved the study protocol, and all patients gave written informed consent.

Treatment and Dose-escalation

Irinotecan was supplied as semi-solid matrix capsules, containing 5, 20 or 50 mg of the active drug substance, and these were stored at room temperature. The capsules also contained lecithin and lauroyl macrogolglycerides as inactive ingredients and a yellowish waxy mass. The drug was supplied by Aventis Pharma (Antony, France) in 30 mL bottles, containing 20 capsules of the 50 mg dosage form and 40 capsules of the 5 mg and 20 mg dosage form. Capsules were taken once a day in the morning for five consecutive days with about 180 mL of water after an overnight fasting for at least four hours prior to the daily oral dose and one hour following dosing, except for the first dose of the 5-day treatment as described below. Compliance with the scheduled treatment was assessed at the end of each cycle by counting the used and returned capsules of irinotecan.

Prophylactic anti-emetics (either metoclopramide or a serotonin 5-hydroxytryptamine-3 receptor antagonist) were allowed one hour before irinotecan dosing and up to two additional times daily if necessary during all cycles of treatment, except for the first dose of the 5-day treatment of the first two cycles.

For irinotecan induced delayed type diarrhea, high-dose loperamide therapy was administered orally consisting of a starting dose of 4 mg at the first episode of diarrhea followed by 2 mg every 2 hours for at least 12 hours. The patient was allowed to stop loperamide only after a 12-hour diarrhea free interval. If the diarrhea persisted for more than 48 hours despite the recommended loperamide treatment, a seven days prophylactic oral antibiotic therapy (ciprofloxacine 500 mg b.i.d.) was added in subsequent cycles.

The effect of food on the pharmacokinetics of irinotecan and metabolite SN-38 was assessed on the first day of the first two cycles, for which patients were randomly assigned to take the study drug in the fed state after a FDA-standardized high-fat, high-calorie breakfast, containing approximately 20% of proteins, 60% of lipids, and 20% of carbohydrate (approximating 1000 kcalories) [31], and then in the fasted state or to the inverse sequence. In the fed condition for the first dose of the 5-day treatment of either cycle 1 or 2, capsules were taken within 5 minutes after completion of the breakfast, which was to be ingested within 30 minutes.

The starting dose of irinotecan, 70 mg/m^2 given once daily for 5 consecutive days, was based on a previous phase I study with a different formulation involving powder-filled capsules of irinotecan [32]. Preclinical data indicated that the new formulation exhibited a similar absolute bioavailability of irinotecan. Hence, the starting dose was 10 mg/m^2/day below the MTD (80 mg/m^2/day) of the previous study. Further dose-escalations were based on the prior dose level toxicity. If no significant toxicity was observed at the previous dose level, the dose was escalated to the next higher dose level with 10 mg/m^2/day increments. A treatment cycle was defined as the 5 consecutive days of irinotecan administration plus the necessary time for the patient to recover from any toxicities. Cycles were to be repeated every 21 days. A minimum of 3 patients was to be treated at each dose level, with a minimum 1-week interval between the entry of the first patient and the entry of the subsequent 2 patients at any given dose level. Before escalation to the next dose levels all 3 patients had to have received at least one treatment cycle. If one of three patients experienced DLT, three additional patients were entered at that dose level. The MTD was defined as one dose level below the dose that induced DLTs in 2 out of 6 patients during the first cycle. DLT was defined as National Cancer Institute Common Toxicity Criteria (NTC-CTC) version 2.0 grade 4 neutropenia lasting for ≥ 5 days, neutropenic fever (defined as grade 4 neutropenia with fever ≥ 38.5°C), neutropenic infection (defined as grade 3 - 4 neutropenia with ≥ grade 3 infection or documented infection), thrombocytopenia less than 25 x 10^9/L, ≥ grade 3 diarrhea, despite maximal loperamide support, ≥ grade 2 nausea or vomiting, failing maximal oral antiemetic therapy, or vomiting leading to discontinuation of the study drug intake ≥ 3 days, other ≥ grade 3 nonhematological toxicities, and treatment delay due to toxicities attributed to the study drug for more than 2 weeks [33]. Intrapatient dose-escalation was not allowed. The treatment was resumed when the neutrophil count had recovered to ≥ 1.5 × 10^9/L, the platelet count to ≥ 100 × 10^9/L, diarrhea was grade 0, and any other treatment-related

toxicities were ≤ grade 1. Once the MTD was confirmed at least ten additional patients were to be enrolled at this dose level to ensure that this dose was feasible for phase II/III studies.

Treatment Assessment

Prior to therapy, a complete medical history was taken and a physical examination and clinical chemistry evaluation was performed. Weekly evaluations included history, physical examination, toxicity assessment. Complete blood cell counts were obtained twice weekly throughout cycle 1 and weekly thereafter, serum biochemistry was determined on day 8 and 15 of cycle 1 and weekly thereafter until recovery, at every subsequent cycle it was determined once every 3 weeks. Response evaluation was performed after every two cycles and assessed according to RECIST [34]. Patients were treated for at least two cycles of therapy unless disease progression or unacceptable toxicity was encountered.

Pharmacologic Analysis

For pharmacokinetic analysis, blood samples were taken immediately prior to drug administration, and at 0.5, 1.0, 1.5, 2, 3, 4, 6, 10, 18 and 24 hours after administration on days 1 and 5 at cycle 1, and on day 1 at cycle 2. Urine was collected prior to drug administration, and at time intervals: 0 - 10 hours and 10 - 24 hours after administration on days 1 and 5 at cycle 1 and day 1 at cycle 2. The concentrations of irinotecan and SN-38 in plasma and urine were quantified by a validated assay based on liquid chromatography with fluorescence detection. The lower limits of quantitation were 1 ng/mL in plasma for both compounds and 100 ng/mL and 25 ng/mL in urine for irinotecan and SN-38, respectively, using 50-µL aliquots.

Pharmacokinetic parameters were calculated by standard non-compartmental methods using WinNonlin software version 3.3 (Pharsight, Mountain View, CA, USA), using standard equations. Non-predicted accumulation was calculated as the ratio of AUC-over-one-dosing- interval (24 h) on day 5 over AUC-extrapolated-to-infinity on day 1.

Pharmacogenetic Data Analysis

Genomic DNA was extracted from 200 µL plasma using a total nucleic acid extraction kit on a MagnaPure LC (Roche, Mannheim, Germany). Variations in the ABCB1 (nucleotide 3435 C>T) [35], CYP3A4 (CYP3A4*3, CYP3A4*17, and CYP3A4*18), CYP3A5 (CYP3A5*3) genes were analyzed by polymerase chain reaction (PCR)-restriction fragment length polymorphism as previously

reported [36,37]. For UGT1A1*28, a 35 cycle PCR (1 min 94°C, 1 min 60°C, and 1 min 72°C) was performed using primers 5'-FAM-AAG TGA ACT CCC TGC TAC CT-3' and 5'-AAA GTG AAC TCC CTG CTA CC-3'. The number of TA-repeats in the 253 bp PCR product was determined using capillary electrophoresis on an ABI 310 (Applied BioSystems, Foster City, USA) [14,15]. Genetic polymorphisms were correlated with pharmacokinetic parameters obtained in fasted condition cycles in 23 patients.

Statistical Analysis

Pharmacokinetic parameters from the various treatment groups were compared statistically using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA). To compare the pharmacokinetic parameters with the genetic polymorphisms a Kruskal-Wallis test (SPSS version 10.1, Paris, France) was used or a non-parametrical trend analysis (Stata version 7.0; Stata Corp., College Station, TX, USA) [15]. All tests results with a $P < .05$ were regarded as statistically significant.

Results

Patient Characteristics

A total of 25 patients (10 male and 15 female), with a median age of 53 years (range, 31 - 76 years) was enrolled into the study (Table 1). All patients were eligible, treated and evaluable for toxicity and DLT. All patients, except four, had received prior chemotherapy and/or radiotherapy. A total of 101 cycles of treatment were given. Dose levels studied were 70 mg/m^2/day, and 80 mg/m^2/day daily-times-five every 3 weeks. Twenty-one patients were assessable for response. Three patients, who were not assessable for response, withdrew from the study before the first scheduled tumor reassessment, and one patient had a response, which was not properly assessed. A total of 16 patients were evaluable for the food effect, whereas nine patients were not evaluable for the food effect because of vomiting within one hour after the meal.

Dose-Limiting Toxicity

At the starting dose of 70 mg/m^2/day 3 patients were treated. Because no DLT was observed, the next dose level of 80 mg/m^2/day was explored. After determination of a DLT consisting of grade 3 delayed diarrhea in one out of three patients, this cohort was expanded (Table 2). One additional patient at this dose level experienced grade 3 fever, regarded as a DLT because no infection

was documented and other causes were not found. Subsequently, the dose of 70 mg/m^2/day was further explored with 3 additional patients. None of these three patients experienced DLT. Therefore, the recommended dose for phase II trials was set at 70 mg/m^2/day for 5 consecutive days every 3 weeks, and fourteen additional patients were enrolled at this dose to fully assess feasibility and the food effect. One of these patients experienced DLT (*i.e.*, grade 3 fatigue and grade 3 anorexia).

Hematologic Toxicity and Nonhematologic Toxicity

Hematologic and nonhematologic toxicities were mild to moderate (Table 3 and 4, respectively). The majority of patients (68%) developed grade 1 or 2 anemia. The nonhematologic toxicities consisted mainly of nausea, vomiting, diarrhea, stomatitis, anorexia and asthenia. Most (76%) patients experienced grade 1 to 2 nausea, and 13 out of 25 patients (52%) experienced grade 1 to 2 vomiting at both dose levels. Prophylactic anti-emetics either metoclopramide or serotonine antagonists were used to manage nausea and vomiting. Since almost every patient experienced vomiting during the feasibility step (dose level 70 mg/m^2/day), it was recommended to use oral 5-HT3-blockers from the first intake of oral irinotecan capsules. Grade 3 delayed diarrhea was observed in five (20%) patients at the 70 and 80 mg/m^2/day dosages, although was not considered DLTs in three patients due to suboptimal supportive treatment with loperamide. Grade 1 to 2 diarrhea was noted in all other patients and was easily manageable with loperamide support and if necessary with use of ciproxin. Grade 1 and 2 anorexia was noted in seven (28%) patients, mostly at dose level 70 mg/m^2/day. A grade 3 anorexia was observed in one patient at dose level 80 mg/m^2/day at subsequent cycle and was coincidenced by inadequate anti-emetic treatment of severe nausea and vomiting. A grade 1 to 2 asthenia was observed in eleven (44%) patients at both dose levels. Four patients experienced mild alopecia (grade 1) and one patient moderate alopecia (grade 2). Finally, a mild cholinergic syndrome was observed in two patients (one in cycle 1 and the other in cycle 2). No patient received prophylatic atropine for this adverse event.

Table 1. Patient characteristics

Characteristic	No. of Patients
No. of Patients	
Total	25
Assessment	
For Dose-Limiting Toxicity	25
For Food Effect	16
For Efficacy	21
No. of cycles/patient	
Median	2
Range	1 - 15
Gender, male : female	10 : 15
Age, years	
Median	53
Range	31 - 76
WHO performance status	
0	7
1	17
2	1
Previous therapy	
Chemotherapy only	21
≤ 2 prior regimens	13
≥ 3 prior regimens	8
Radiotherapy only	1
Both	8
None	4
Tumor types	
Lung	3
Melanoma	5
Gastro-intestinal tract, including:	6
Esophageal, duodenal, colorectal	1 each
Gastric	3
Gynaecologic	2
Unknown primary tumor	2
Genitourinary	1
Miscellaneous	6

Abbreviations: no., number; WHO, World Health Organization.

Table 2. Dose-escalation scheme and dose-limiting toxicity [DLT]

	No. of Patients				Patients with DLT		
Dose level [mg/m²/day]	No. of pts.	With one dose reduced	With one cycle delayed	No. of cycles	First cycle	All cycles	DLT Events at 1st cycle
70	20	2[a]	2	89	1/20	3/20	grade 3 asthenia and grade 3 anorexia [N=1]
80	5	2[b]	2	12	2/5	2/5	grade 3 diarrhea [N=1]; and grade 3 fever [N=1]
TOTAL	25	4	4	101			

Note: [a] dose reduction from 70 mg/m²/day to 60 mg/m²/day; [b] dose reduction from 80 mg/m²/day to 70 mg/m²/day.
Abbreviation: no., number; pts., patients.

Table 3. Hematologic toxicity [worst grade per patient]

			Anemia			Leukocytopenia				Neutropenia				Thrombocytopenia		
			Grades													
Dose level [mg/m²/d]	No. of pts.	No. of cycles	1	2	3-4	1	2	3	4	1	2	3	4	1	2	3-4
70	20	89	7	6	0	1	2	0	0	1	0	1[a]	0	0	0	0
80	5	12	0	2	0	1	1	0	0	0	2	0	0	0	1	0

Note: [a] not considered a DLT at subsequent cycle according to protocol definitions.
Abbreviation: d, day; no., number; pts., patients.

Table 4. Nonhematologic toxicity [worst grade per patient]

			Diarrhea			Nausea			Vomiting			Stomatitis			Anorexia			Asthenia		
			Grades																	
Dose level [mg/m²/d]	No. of pts.	No. of cycles	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
70	20	89	11	7	2[a,b]	12	5[c]	0	7	3[c]	0	1	0	0	4	2	1[a]	5	4	2[d]
80	5	12	1	1	3[a,e]	1	1[c]	3[c]	1	2[c]	2[c]	0	1	0	1	0	1[f]	1	3	0

Note: [a] considered a DLT at first cycle in 1 pt., [b] not considered a DLT at first cycle in 1 pt. due to insufficient supportive treatment with loperamide, [c] not considered a DLT at first or subsequent cycles due to insufficient supportive treatment with anti-emetics, [d] considered DLTs at first and subsequent cycle, [e] not considered DLTs at first and subsequent cycle in 2 pts. due to insufficient supportive treatment with loperamide, [f] not considered a DLT at subsequent cycle.
Abbreviation: d, day; no., number; pts., patients.

Table 5. Mean ± SD [CV%] pharmacokinetic parameters of irinotecan and SN-38

		Irinotecan			SN-38		
	No. of pts.	Cycle 1		Cycle 2	Cycle 1		Cycle 2
Parameters		Day 1	Day 5	Day 1	Day 1	Day 5	Day 1
		Dose: 70 mg/m²/day					
C_{max} [ng/mL]	20[a]	81.3 ± 38.3 [47]	136 ± 88 [65]	101 ± 86 [86]	10.7 ± 6.0 [56]	10.0 ± 6.2 [62]	9.3 ± 4.9 [53]
Median T_{max} [h] [range]		3 [1-6]	2 [0.5-6]	3 [1-6]	3 [1-6]	1.5 [1.5-3]	3 [1.5-4]
$AUC_{(0-24h)}$ [ng·h/mL]		601 ± 241 [40]	1029 ± 814 [79]	689 ± 493 [72]	69.8 ± 52.0 [75]	77.8 ± 53.5 [69]	66.3 ± 49.3 [74]
AUC [ng·h/mL]		735 ± 400 [54]	1181 ± 940 [80]	758 ± 542 [71]	89.6[c] ± 55.9 [62]	81.1[c] ± 50.5 [62]	93.9 ± 68.1 [73]
$T_{½}$ [h]		8.1 ± 4.0 [50]	8.3 ± 2.1 [25]	6.8 ± 1.7 [25]	10.6[c] ± 13.3 [125]	16.8[c] ± 21.6 [128]	11.6 ± 11.8 [102]
Metabolic ratio		—	—	—	0.14 ± 0.08 [57]	0.08 ± 0.04 [46]	0.20 ± 0.22 [113]
		Dose: 80 mg/m²/day					
C_{max} [ng/mL]	5[b]	86 ± 48.0 [56]	116 ± 61 [53]	78.0 ± 79.3 [102]	6.3 ± 3.4 [53]	6.2 ± 2.2 [36]	5.8 ± 3.7 [64]
Median T_{max} [h] [range]		3 [0.5-6]	2 [2-4]	4 [3-6]	3 [2-3]	3 [1.5-6]	3 [2-4]
$AUC_{(0-24h)}$ [ng·h/mL]		656 ± 223 [34]	864 ± 562 [65]	718 ± 560 [78]	46.2 ± 29.2 [63]	49.8 ± 31.4 [63]	41.2 ± 38.1 [92]
AUC [ng·h/mL]		794 ± 335 [42]	1003 ± 652 [65]	792 ± 600 [76]	88.7 ± 78.9 [89]	54.4[d] ± 25.5 [47]	70.6[d] ± 31.1 [44]
$T_{½}$ [h]		8.7 ± 3.6 [41]	9.0 ± 1.4 [16]	6.9 ± 0.7 [10]	18.2 ± 20.2 [111]	8.9[d] ± 8.7 [97]	5.2[d] ± 1.8 [34]
Metabolic ratio		—	—	—	0.11 ± 0.09 [80]	0.08 ± 0.08 [101]	0.07 ± 0.01 [17]

Note: AUC_{0-24h} corresponding to AUC_{0-t} on day 1 at cycle 1 and cycle 2; [a] PK parameters were calculated in 16 pts. out of 20 pts. on day 1 at cycle 2; [b] PK parameters were calculated in 3 pts. out of 5 pts. on day 1 at cycle 2; [c] $T_{½}$ and AUC were calculated in 19 pts.; [d] $T_{½}$ and AUC were calculated in 4 pts; metabolic ratio is defined as the AUC ratio of SN-38-to-irinotecan.

Abbreviations: SD, standard deviation; CV, coefficient of variation; no., number; pts., patients; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life of terminal phase.

Table 6. Effect of food on irinotecan pharmacokinetics: AUC ratio for oral administration after a high-fat, high-calorie breakfast compared to fasting state

Parameter		Irinotecan	SN-38
C_{max}	Mean [%]	132	77
	90% CI [%]	94 - 187	60 - 98
	p-value	0.172	0.075
AUC	Mean [%]	113	117
	90% CI [%]	86 - 148	88 - 155
	p-value	0.443	0.360

Note: six out of twenty-five patients were not evaluable for the food effect due to vomiting on day 1 at cycle 1 and two additional patients were not evaluable for SN-38.
Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; CI, confidence interval.

Pharmacokinetics

After oral administration, irinotecan and SN-38 achieved peak plasma concentrations within 2 to 4 hours (Table 5). Typical concentration-time profiles for SN-38 are shown in Figure 1. The mean AUC accumulation-ratio on day 5 *versus* day 1 for irinotecan was 117% (90% confidence interval (CI), 86-160%) (P = .40), suggesting no accumulation of the parent drug. For SN-38, this ratio was 85% (90% CI, 61-119%) (P = .43). For the entire population, the mean AUC ratio of SN-38-to-irinotecan was approximately 13%. This metabolic ratio was dose-independent, and substantially higher than the ratio of about 3% measured after intravenous administration [38]. This indicates extensive presystemic biotransformation of irinotecan (*i.e.*, in the gastrointestinal tract and/or during first-pass extraction). As expected, the 24-hour urinary excretion of both irinotecan and SN-38 was low, and accounted for less than 3% and 1% of the dose, respectively. The AUC-ratio for fed-to-fasting was approximately 1.13 for irinotecan and 1.17 for SN-38 (both not statistically significant; Table 6), indicating no change in absorption of irinotecan after a high-fat meal.

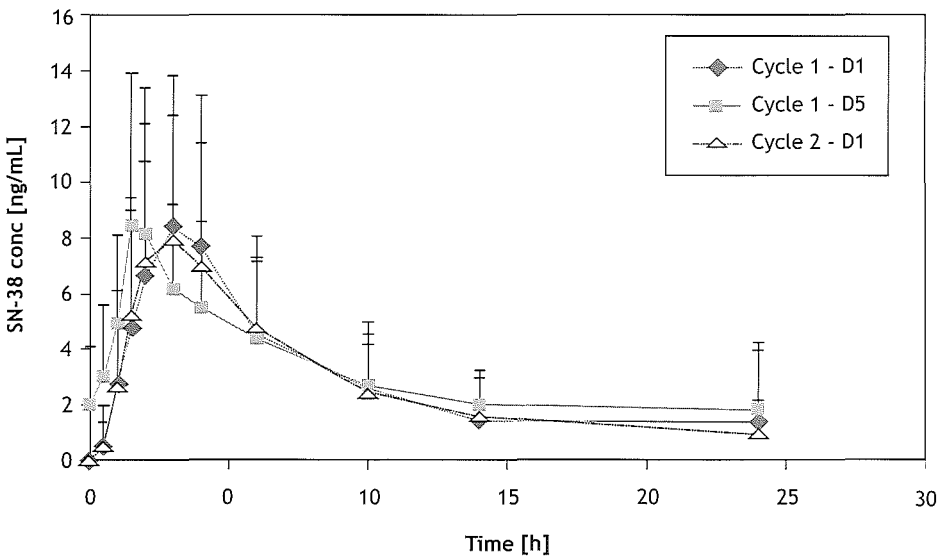

Figure 1. Mean [+ SD] plasma concentration-time curves of SN-38 on day 1 [D1] and day 5 [D5] at cycle 1 [N = 20] and on day 1 [D1] at cycle 2 [N = 16] after oral administration of irinotecan at dose level 70 mg/m^2/day

Table 7. Genotype and allele frequencies in genes studied

Polymorphism	Description	No. of samples	Genotype frequencies			
			wild-type	heterozygous	homozygous variant	other
UGT1A1*28[a]	promoter	23	13	8	1	1
CYP3A4*3	M445T	9	8	1	0	
CYP3A4*17	F189S	17	17	0	0	
CYP3A4*18	L293P	20	20	0	0	
CYP3A5*3	splice variant	22	0	1	21	
ABCB1 3435 C>T	E1143E	22	2	8	12	

Note: [a] TA_6/TA_6 is defined as wild-type, TA_6/TA_7 as heterozygous, TA_7/TA_7 as homozygous variant, and TA_5/TA_6 as other.
Abbreviation: no., number.

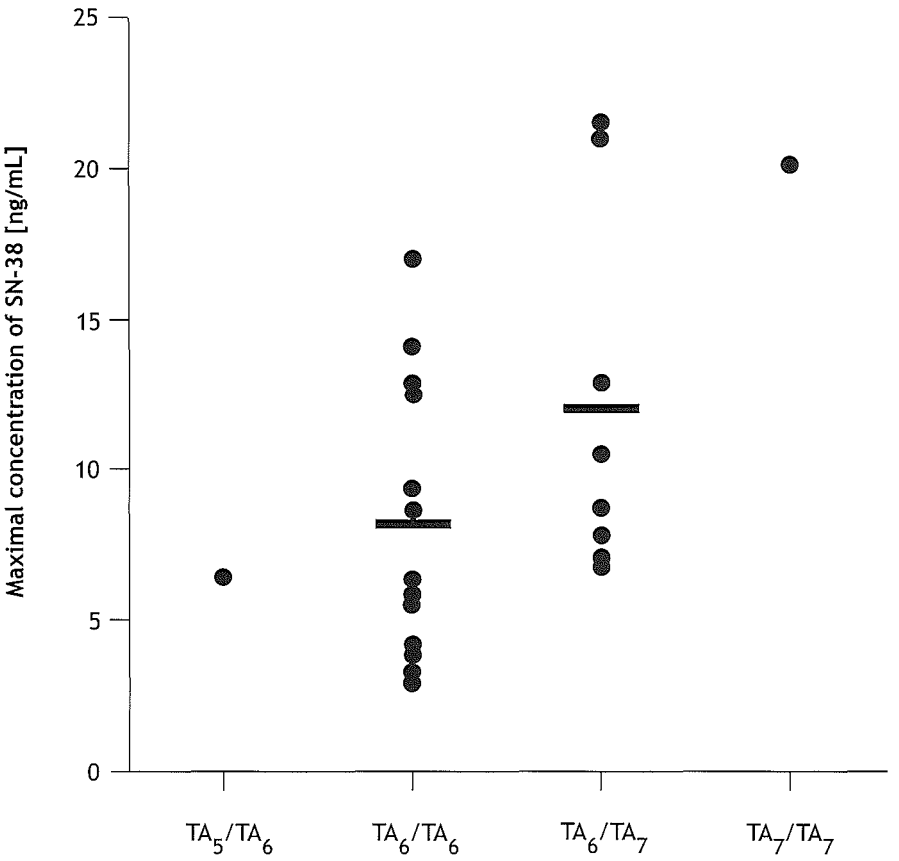

Figure 2. UGT1A1*28 polymorphism [N = 23] related to maximal concentration of SN-38 [ng/mL]

Pharmacogenetics

Five SNPs and one dinucleotide repeat were analyzed in four genes of putative relevance for the irinotecan absorption and disposition (Table 7). One patient had an extra TA repeat in both alleles ($(TA)_7TAA$) of the UGT1A1 gene promoter (UGT1A1*28), whereas another patient had one TA repeat less in one of both alleles (TA_5/TA_6). The genotype frequency for TA_6/TA_6 (N = 13) and TA_6/TA_7 (N = 8) were comparable with previously reported estimates in European Caucasians [39]. Although the dose-normalized peak concentration of SN-38 in the fasted condition was significantly affected by UGT1A1*28 genotype (P = .026) (Figure 2), this was not associated with increased toxicity (*i.e.*, severe diarrhea and/or neutropenia) in patients carrying the variant allele (data not shown). No statistically significant associations with SN-38 pharmacokinetic parameters were

observed in a total of 23 patients with variant alleles in CYP3A5*3 and ABCB1 3435 C>T (P > .23). The AUC_{0-24h} of irinotecan of the CYP3A4*1/*3 heterozygous individual was comparable with the AUC_{0-24h} of irinotecan of CYP3A4*3 wild-type carriers, 379 *versus* 582.92 (SD ± 302.06) ng·h/mL, respectively. No CYP3A4*17 (*viz.* associated with low CYP3A4 activity) nor CYP3A4*18 (*viz.* associated with high CYP3A4 activity) individuals were identified.

Efficacy

A 69-year-old male with metastatic colorectal cancer achieved a confirmed partial response lasting 4 months. A total of ten patients had disease stabilization for 6 (N = 5), 12 (N = 2), 18 (N = 2), and 24 weeks (N = 1). Ten patients had progressive disease after 2 cycles of chemotherapy and one patient had early progressive disease.

Discussion

The present phase I study indicates that oral irinotecan, formulated as a semi-solid matrix capsules, administered daily for 5 consecutive days every 3 weeks, is feasible and safe. The principal DLT of this oral regimen was nonhematologic and consisted of delayed diarrhea and fever observed at dose level 80 mg/m^2/day. The pattern of the delayed diarrhea is similar to that associated with intravenous administration of irinotecan and can be relieved by support of loperamide [40]. It has been suggested that the delayed diarrhea after irinotecan administration results from the direct effect of SN-38 on the intestinal mucosa [40]. At a daily dose of 80 mg/m^2/day, two of five patients experienced grade 3 diarrhea and grade 3 fever, which, according to the predefined criteria for the MTD, precluded further dose-escalation. Hematologic toxicity was mild to moderate and did not result in DLT in the first cycle. The recommended dose is 70 mg/m^2/day for 5 consecutive days every 3 weeks, and this dose level was tested for the feasibility and food effect. Nonhematologic toxicities attributed to oral irinotecan treatment, including vomiting, stomatitis, anorexia, asthenia, alopecia and symptoms associated with mild cholinergic syndrome, were similar to historical experience with intravenous irinotecan [1].

Substantial interpatient variability in pharmacokinetics of irinotecan and SN-38 was observed in our study, which is in agreement with other phase I studies of oral irinotecan [30, 32,41-44], and can be linked to the complex pharmacology of the drug. After absorption of oral irinotecan, both the parent drug and SN-38 achieved

peak plasma concentrations within 2 to 4 hours of administration of the drug. There was no statistically significant accumulation of SN-38 or irinotecan, and there was no statistically significant influence of food on the pharmacokinetics of irinotecan and SN-38.

Furthermore, we found that the metabolic-ratio, defined as the AUC of SN-38-to-irinotecan was higher with oral administration than with intravenous administration of irinotecan (13% *versus* 3%) [38], suggesting extensive presystemic metabolism of irinotecan. This is consistent with the high expression levels of irinotecan-converting carboxylesterases in the gastrointestinal tract and liver [45]. Presystemic metabolism of irinotecan was also observed in the other phase I studies with irinotecan administered either as a solution with CranGrape juice [30], as powder-filled capsules [32,41-43], or as semi-solid matrix capsules [44]. The results of the six phase I studies of orally administered irinotecan are summarized in Table 8.

In contrast with our study, the preliminary results of another phase I study of oral irinotecan formulated as semi-solid matrix capsules revealed an MTD of 60 mg/m^2/day daily-times-five every 3 weeks [44]. A mean (± SD) bioavailability of orally administered irinotecan of 25% ± 23% was found. Furthermore, the AUC of the active metabolite SN-38 following oral administration of irinotecan was 50% of the value from an equivalent intravenous dose, implicating presystemic metabolism of irinotecan as well [44]. In addition, over a 5-day dosing-interval orally administered irinotecan produced substantially less systemic exposure to parent drug compared to intravenous treatment on the weekly-times-4 every 6 weeks schedule, while maintaining comparable exposure to SN-38, suggesting that the oral route could be associated with less irinotecan-related toxicity [44].

In a phase I study of irinotecan given as 5-days continuous infusion in 36 patients, the recommended dose was 30 mg/m^2/day, with diarrhea as dose-limiting toxicity at dose 40 mg/m^2/day [46]. Large variations in clearance and half-life of irinotecan at the different dose levels (range 5 - 40 mg/m^2/day) were documented [46], and in this study the calculated mean metabolic ratio was only 3% to 7%. In another phase I study of irinotecan administered as a continuous low-dose infusion for 14 days, the recommended dose was 10 mg/m^2/day times-14 every 3 weeks [22]. Diarrhea was a cumulative toxicity if doses were repeated at doses above 10 mg/m^2/day or for more than 17 days [22]. The dose intensity of this schedule was approximately 40% of the dose intensity obtained with 90-min intravenous infusion of irinotecan (350 mg/m^2 once every 3 weeks). The mean metabolic ratio was 16% and was constant over dose range tested. In comparison with the short infusion of irinotecan, it was demonstrated that prolonged exposure to

Table 8. Phase I studies with orally administered irinotecan

Oral substance	Schedule [Dose range]	No. of pts.	DLT events	RD	Reference
Solution of IV irinotecan mixed in CranGrape juice	Once daily x 5 q3w [20 - 100 mg/m^2/day]	28	Grade 4 diarrhea	Pts. < 65 yrs: 66 mg/m^2/d x 5 q3w Pts. ≥ 65 yrs: 50 mg/m^2/d x 5 q3w	[30]
Powder-filled capsules	Once daily x 5 q3w [30-90 mg/m^2/day]	46	Neutropenic infection Grade 3 diarrhea Grade 4 vomiting	80 mg/m^2/day x 5 q3w	[32]
Powder-filled capsules	Once daily x 5 q3w [30-60 mg/m^2/day]	19	Grade 3 nausea, vomiting Grade 3 diarrhea Febrile neutropenia	50 mg/m^2/day x 5 q3w	[41]
Powder-filled capsules	Once daily x 14 q3w [7.5-40 mg/m^2/day]	34	Grade 4 diarrhea Grade 3 vomiting	30 mg/m^2/day x 14 q3w	[42]
Powder-filled capsules	Once daily x 14 q3w [7.5-40 mg/m^2/day]	19	Grade 3-4 vomiting Grade 3 diarrhea	30 mg/m^2/day x 14 q3w	[43]
Semi-Solid Matrix capsules	Once daily x 5 q3w [50 - 70 mg/m^2/day]	43	Grade 3 - 4 diarrhea Grade 4 neutropenia	60 mg/m^2/day x 5 q3w	[44]

Abbreviations: no., number; pts., patients; DLT, dose-limiting toxicity; RD, recommended dose; IV, intravenous; q, every; w, weeks.

low-doses of irinotecan resulted in more efficient conversion of irinotecan in SN-38 [22]. Furthermore, the study showed that there was no saturation of the carboxylesterase or UGT enzyme systems during 14- to 21-days of infusion of irinotecan at the doses tested [22], in contrast with *in vivo* experiments, which showed nonlinear pharmacokinetics of irinotecan as a result of decreased metabolic clearance reflected by carboxylesterase saturation [47,48].

As mentioned earlier, the cytotoxicity of topoisomerase I inhibitors is more displayed by exposure time-dependent rather than concentration-dependent [24]. Schedule dependency as a result of the cell cycle specificity of the topoisomerase I inhibitors is more dependent on pharmacodynamics rather than pharmacokinetics [22]. The present study revealed that at the recommended dose the cumulative AUC of irinotecan is 69% of that after continuous low-dose (10 mg/m^2/day) infusion of irinotecan for 14 days [22], and about 25% of that after 350 mg/m^2 every three weeks [20], or 145 mg/m^2 weekly for 4 weeks every 6 weeks [49]. However, for SN-38 the mean cumulative AUC was about 70% of both the slow infusion and single high-dose infusion schedules and 50% of that in the weekly regimen. It therefore appears that both slow infusion and oral administration result in more efficient conversion of irinotecan into SN-38, which is reflected in the higher metabolic ratios observed with these schedules.

In our study, no correlation was noted between irinotecan-associated toxicity and the UGT1A1*28 genotype, in contrast to previous observations [15,50]. The limited number of patients in this study may obscure such relationships. Nevertheless, there was a statistically significant trend (P = .026) showing that less dinucleotide repeats in the promoter correlate to a reduced peak concentrations of SN-38, and therefore to a higher level of activity of UGT in accordance with a previous study [51]. Furthermore, no statistically significance between the genetic polymorphisms of CYP3A5*3 and ABCB1 3435 C>T and SN-38 pharmacokinetic parameters (P > .23) were found, in accordance with the results of a previous study on intravenous irinotecan metabolism and genetic polymorphisms [38].

This study confirms that oral administration of irinotecan, formulated as SSM capsules, is safe and feasible and may have improved pharmacokinetic characteristics with no statistical significant effect of food on drug absorption. The oral formulation can achieve sustained drug exposure in the absence of the disadvantages of intravenous delivery and thus with greater convenience for patients. A phase II study of this oral formulation of irinotecan in patients with metastatic breast cancer is scheduled.

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Phase I and pharmacokinetic study of oral irinotecan given once daily for 5 days every 3 weeks in combination with capecitabine in patients with solid tumors

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Submitted

Abstract

Purpose: To assess the maximum-tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetics, and preliminary antitumor activity of oral irinotecan given in combination with capecitabine to patients with advanced, refractory solid tumors.

Patients and Methods: Patients were treated from day 1 with irinotecan capsules given once daily for 5 consecutive days (dose, 50 to 60 mg/m^2/day) concomitantly with capecitabine given twice daily for 14 consecutive days (dose, 800 - 1000 mg/m^2); cycles were repeated every 21 days.

Results: Twenty-eight patients were enrolled into the study and received 155 cycles of therapy (median 5 cycles, range 1 - 18). At dose level irinotecan 60 mg/m^2/day and capecitabine 2 × 800 mg/m^2/day, grade 3 delayed diarrhea in combination with grade 2 nausea, despite maximal anti-emetic support, and with grade 3 anorexia and colitis were the first cycle DLTs in two out of six patients, respectively. At the recommended doses (irinotecan 50 mg/m^2/day; capecitabine 2 × 1000 mg/m^2/day), side effects were mostly mild to moderate and uniformly reversible. Pharmacokinetic analysis showed no interaction between oral irinotecan and capecitabine. Confirmed partial responses were observed in two patients with gallbladder carcinoma and in one patient with melanoma. Disease stabilization was noted in sixteen patients.

Conclusion: The recommended phase II doses for oral irinotecan and capecitabine are 50 mg/m^2/day for 5 consecutive days, and 2 × 1000 mg/m^2/day for 14 consecutive days repeated every 3 weeks, respectively. This study shows that the combination of oral irinotecan and capecitabine is feasible, well tolerated and convenient for the outpatient setting, and warrants further evaluation.

Introduction

Irinotecan is a semisynthetic derivative of the natural alkaloid camptothecin, and is a potent inhibitor of the enzyme topoisomerase-I [1,2]. It is registered for use in a broad range of tumor types and a variety of irinotecan including combination regimens continue to be investigated [3,7].

Capecitabine is an oral fluoropyrimidine carbamate, which is preferentially converted into 5-fluorouracil (5-FU) in tumors through a cascade of three

enzymes, carboxylesterase, cytidine deaminase, and thimidine phosphorylase (dThdPase) [8]. After oral intake of capecitabine, the parent drug passes mainly unchanged from the gastrointestinal tract and is metabolized in the liver by carboxylesterases to 5'-deoxy-5-fluorocytidine (5'-DFCR), then by cytidine deaminase to 5'-deoxy-5-fluorouridine (5'-DFUR) in the liver and tumor tissues and finally by dThdPase to 5-FU in tumors. This minimizes the exposure of healthy tissues to systemic active 5-FU.

In randomized phase III studies capecitabine has demonstrated essentially equivalent survival with decreased toxicity compared to intravenous 5-FU regimens [9], and currently irinotecan administered intraveneously is being investigated in combination with capecitabine [6, 7, 10]. The development of oral irinotecan raises the possibility of using this agent with capecitabine in an all-oral regimen, possibly increasing the convenience of therapy and therefore potentially improving patient's quality of life.

Against this background, we performed a phase I study to evaluate the combination treatment of irinotecan capsules and capecitabine given concomitantly once daily for 5 consecutive days and twice daily for 14 consecutive days every 3 weeks, respectively, in patients with refractory solid tumors. The objectives of this trial were (*a*) to determine the maximum-tolerated dose (MTD) and dose-limiting toxicities (DLTs) of this combination, (*b*) to characterize the pharmacokinetics of irinotecan, its metabolite SN-38, capecitabine, and its major metabolites, to assess a potential interaction between the compounds, and (*c*) to document any evidence of antitumor activity.

Patients and methods

Eligibility Criteria

Patients with a histologically confirmed diagnosis of a malignant solid tumor refractory to conventional chemotherapy or for whom no effective therapy existed were eligible. Other eligibility criteria included the following: age ≥ 18 years; World Health Organization performance status 0 - 2; estimated life expectancy of ≥ 12 weeks; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas or mitomycin-C); and adequate hematopoietic (absolute neutrophil count (ANC) ≥ 2,000/μL, platelet count ≥ 100 × 10^9/L and hemoglobin ≥ 10.0 g/dL (or 6.2 mmol/L)), hepatic (serum total bilirubin within the normal institutional ranges, and serum aspartate transaminase (ASAT), alanine transaminase (ALAT), alkaline phosphatase ≤ 3.0 times the institutional

upper normal limit (UNL) (≤ 5.0 times UNL in case of liver metastases), and renal (serum creatinine concentration ≤ 1.5 mg/dL (≤ 135 μmol/L)) function. Specific exclusion criteria included prior treatment with irinotecan, capecitabine or 5-FU (except as a bolus), concomitant treatment with cytochrome P450 3A inhibitors or inducers (wash-out period of at least 7 days since last intake), symptomatic brain metastases or leptomeningeal involvement, active inflammatory bowel disease, bowel (sub)obstruction, chronic diarrhea, known chronic malabsorption or total colectomy or other major abdominal surgery that might result in substantial alteration in transit or absorption of oral medication. The institutional Ethical Boards approved the study protocol. All patients gave written informed consent before study entry.

Treatment and Dose-escalation

Irinotecan was provided as semi-solid matrix capsules, containing 5, 20 or 50 mg of the active drug substance, and stored at room temperature. The capsules contained also lecithin and lauroyl macrogolglycerides as inactive ingredients and a yellowish waxy mass. The drug was supplied by Aventis Pharma (Antony, France) in 30 mL bottles, containing 20 capsules of the 50 mg dosage form and 40 capsules of the 5 mg and 20 mg dosage form.

Capecitabine was provided as film-coated, oblong-shaped tablets containing 150 and 500 mg of active substance. The tablets were already packed for the study in high density polyethene bottles, whereby the 150 mg tablet was packed in bottles containing 28 tablets and the 500 mg tablets in bottles containing 56 tablets. Capecitabine was provided by Hoffmann-La Roche (Basel, Switzerland).

Irinotecan and capecitabine were concomitantly administered orally during the first 5 days of each cycle, and afterwards treatment with capecitabine continued for 9 additional consecutive days. Irinotecan capsules were taken once daily in the morning, at the same time of the day, with about 180 mL of water after an overnight fasting for at least four hours prior to the daily oral dose and one hour following dosing.

Capecitabine tablets were taken twice daily at the end of a meal (*i.e.*, breakfast and dinner), in the morning two hours after intake of irinotecan capsules and approximately 10 to 12 hours later, for 14 consecutive days with a glass of water. Cycles were repeated every 3 weeks. Compliance with the scheduled treatment was assessed at the end of each cycle by counting the used and returned capsules and tablets. With the exception of days 5, 6, 14, and 15 of the first cycle, in which patients were hospitalized for pharmacokinetic sampling, patients were treated on an outpatient basis.

Prophylactic anti-emetics (either metoclopramide or a serotonin receptor antagonist) were allowed one hour before irinotecan dosing and up to two additional times daily if necessary during all cycles of treatment.
For irinotecan induced delayed-type diarrhea, high-dose loperamide therapy was administered orally consisting of a starting dose of 4 mg at the first episode of diarrhea followed by 2 mg every 2 hours for at least 12 hours. The patient was allowed to stop loperamide only after a 12-hour diarrhea free interval. If the diarrhea persisted for more than 48 hours despite the recommended loperamide treatment, a seven days prophylactic oral antibiotic therapy (ciprofloxacine 500 mg b.i.d.) was added during subsequent cycles.
In case of severe acute cholinergic symptoms, patients were treated curatively and as secondary prevention with either orally or subcutaneously administered atropine.
The starting doses for irinotecan and capecitabine were 50 mg/m^2/day given once daily and 800 mg/m^2 given twice daily. The starting dose of irinotecan was based on results of a previous phase I study with single agent oral irinotecan and was 20 mg/m^2/day lower than the single agent MTD [11].
Further dose-escalations were based on the prior dose level toxicity. If no DLT was observed during the first cycle at a given dose level, then the dose was escalated to the next higher dose level with 10 mg/m^2/day increments for irinotecan. After the determination of the MTD for oral irinotecan with 2 $\times$ 800 mg/m^2/day of capecitabine, the dose of capecitabine was increased to 1000 mg/m^2 given twice daily for 14 consecutive days and the dose of irinotecan tapered to the next previous dose in combination with capecitabine 800 mg/m^2 twice daily.
At least three patients were entered at each dose level. If one of three patients experienced DLT at the first cycle, three additional patients were entered at that dose level. The MTD was defined as one dose level below the dose that induced DLTs in 2 out of 6 patients during the first cycle, DLTs were defined as National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC) grade 3 neutropenia before day 7, or grade 4 neutropenia during the treatment cycle, or grade 4 neutropenia lasting $\geq$ 5 days, neutropenic fever (defined as grade 4 neutropenia with fever $\geq$ 38.5°C), neutropenic infection (defined as grade 3 - 4 neutropenia with $\geq$ grade 3 infection or documented infection), thrombocytopenia less than 25 $\times$ 10^9/L, $\geq$ grade 3 diarrhea, despite maximal intensive loperamide support, $\geq$ grade 2 nausea or vomiting, despite maximal oral antiemetic therapy, or vomiting leading to discontinuation of the study drugs intake $\geq$ 3 days, other $\geq$ grade 3 nonhematological toxicities (*e.g.*, grade 3 stomatitis, grade 3 palmar-plantar erythrodysesthesia (hand-foot syndrome)),

and treatment delay due to toxicities attributed to the study drugs for more than 2 weeks (12). Intrapatient dose-escalation was not allowed. The treatment was resumed when the neutrophil count had recovered to $\geq 1.5 \times 10^9$/L, the platelet count to $\geq 100 \times 10^9$/L, diarrhea was absent, and any other treatment-related toxicities were $\leq$ grade 1.

Treatment Assessment

Before initiating therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell (CBC) count, including hemoglobin, white cells with differential count, platelets, and serum biochemistry was performed, as were ECG, and chest X-ray. Weekly evaluations included history, physical examination, CBC, and toxicity assessment according to the NCI-CTC. CBC was done twice weekly throughout cycle 1 and weekly thereafter, serum biochemistry were done on days 8 and 15 of cycle 1 and weekly thereafter until recovery, and every 3 weeks at every subsequent cycle. Tumor evaluation was performed after every two cycles and response assessed according to RECIST [13]. Patients were treated for at least two cycles of therapy unless disease progression or unacceptable toxicity was encountered.

Sample Collection and Drug Analysis

For pharmacokinetic analysis, a total of 34 blood samples (total volume of ~170 mL) (corresponding to 11 samples for irinotecan and 23 samples for capecitabine) were obtained from an indwelling intravenous canula and collected into heparin-coated tubes for irinotecan and into EDTA-containing tubes for capecitabine, respectively, on days 5 (irinotecan and capecitabine) and 14 (capecitabine only) of the first cycle. The samples were taken immediately prior to intake of irinotecan and at 0.5, 1, 1.5, 2, 2.25, 2.50, 3, 4, 5, 6, 7, 8, 10, 12, 18 and 24 hours after administration on day 5 at cycle 1. Samples were also taken immediately prior to intake of capecitabine, and at 0.25, 0.50, 1, 2, 3, 4, 5, 6, 8 and 10 hours after the morning intake of capectabine on day 14 of cycle 1. After sampling, all blood specimens were immediately put in an ice-water bath (4°C) until centrifugation at 2000 rpm for 15 minutes at 4°C. Plasma samples were stored frozen at a temperature below -20°C, until analysis.

Concentrations of irinotecan and its active metabolite SN-38 were quantified as total drug (*i.e.*, the total of lactone and carboxylate forms) with a validated high-performance liquid chromatographic (HPLC) assay with fluorescence detection following solid-phase extraction of 50-µL samples. The lower limit of quantitation was 1 ng/mL. The accuracy of the assay was defined as the present difference

between the nominal and the mean measured concentrations of quality controls, ranged from -2.1% to 5.4% for irinotecan, and from -0.11 to 1.2% for SN-38 in plasma over the analysis period. The precision of the assay, established by the coefficients of variation (CVs) of the quality controls, was lower than 6.5% for both compounds. Concentrations of capecitabine and its metabolites 5'-DFCR, 5'-DFUR, 5-FU, and α-fluoro-β-alanine (FBAL) were determined by HPLC with tandem mass-spectrometric detection. The lower limits of quantitation were 50 ng/mL for 5'-DFUR, 11.3 ng/mL for FBAL, 10 ng/mL for capecitabine and 5'-DFCR, and 2 ng/mL for 5-FU). The accuracy of the assay ranged from -4.3 to 3.3% for 5-FU, and from -5.8 to 4.0% for FBAL. The precision of the assay was lower than 8%, 13%, 6.2%, 11%, and 16% for capecitabine, 5'-DFCR, 5'-DFUR, 5-FU, and FBAL, respectively.

Pharmacokinetic Data Analysis

Pharmacokinetic parameters were calculated by standard non-compartmental methods using WinNonlin software version 3.3 (Pharsight Corp., Mountain View, CA, USA). The following parameters were determined for irinotecan, capecitabine, and their metabolites in the dosing interval (τ): peak concentration (C_{max}), time to C_{max} (T_{max}), area under the curve (AUC), half-life of the terminal phase ($T_{½}$), and the metabolic ratio of AUCs of SN-38 and irinotecan.

Statistical Analysis

The effect of capecitabine on irinotecan pharmacokinetics was assessed by comparing C_{max} and AUC on day 5 of this study (concomitant combination step) with day 5 of monotherapy with oral irinotecan (single agent step; [11]). The comparison was made with 25 patients treated at 70 mg/m²/day or 80 mg/m²/day for 5 days with the single agent. The statistical analysis was carried out on dose-normalized C_{max} and $AUC_{(0\text{-}24h)}$ of irinotecan and SN-38 and after log-transformation. The Proc-Mixed procedure of SAS software version 8.2 (SAS Institute Inc., Cary, NC, USA) was used with treatment (concomitant intake of irinotecan and capecitabine *versus* oral irinotecan monotherapy) taken as fixed effect. For comparison of the metabolic ratios, Student's *t*-test was used. To assess a possible influence of irinotecan and SN-38 on the pharmacokinetics of capecitabine, a Proc-Mixed procedure was applied on log-transformed C_{max} and $AUC_{(0\text{-}10h)}$ of capecitabine and its metabolites at day 5 (concomitant intake of irinotecan with capecitabine) *versus* day 14 (intake of capecitabine only) taken as fixed effect and patient as random effect. Of note, 10 hours corresponds to the time between the first and second daily intake of capecitabine. All tests results with a $P < .05$ were considered statistically significant.

Results

Patient Characteristics and Treatment administration

Twenty-eight patients (14 male and 14 female), with a median age of 54 years, received 155 cycles of treatment at three dose levels. The median number of cycles administered per patient was five (range, 1 - 18). All patients were eligible, but one was not evaluable for DLT. This patient received only 4 days of treatment due to very rapid progressive melanoma. All but five patients had received prior chemotherapy and/or radiotherapy. A total of 26 patients was assessable for response. The two patients not assessable for response withdrew from the study due to adverse events before the first scheduled tumor reassessment and not proper tumor assessment, respectively. Patient characteristics are listed in Table 1. Dose levels studied were irinotecan 50 mg/m^2/day, and 60 mg/m^2/day daily-times-five every 3 weeks, and capecitabine 800 and 1000 mg/m^2 twice daily-times-fourteen every 3 weeks.

Dose-limiting toxicity

Grade 3 colitis associated with grade 3 anorexia and grade 3 delayed diarrhea with grade 2 nausea, despite maximal anti-emetic support with a serotonin antagonist, were the DLTs in the first cycle at the second dose level irinotecan 60 mg/m^2/day and capecitabine 800 mg/m^2 twice daily in two out of six patients (Table 2). Three additional patients were entered at the next lower dose level, *i.e.* the study starting dose level (irinotecan 50 mg/m^2/day and capecitabine 2 $\times$ 800 mg/m^2/day). No DLTs were observed in the first cycle in these patients. Subsequently, the dose level irinotecan 50 mg/m^2/day and capecitabine 1000 mg/m^2 twice daily was explored. No DLTs were observed in the first 6 patients at this dose level. Since capecitabine 1000 mg/m^2 twice daily is the most commonly used combination dose, it was unlikely that a higher dose in combination with irinotecan would be feasible, therefore the dose of capecitabine was not further escalated. Hence, the recommended dose for phase II trials of the oral irinotecan and capecitabine combination was set at 50 mg/m^2/day for 5 consecutive days (*i.e.*, total dose of 250 mg/m^2) and 1000 mg/m^2 twice daily for 14 consecutive days every 3 weeks, respectively. Thirteen additional patients were treated at this dose level to confirm its feasibility. Ultimately, two out of 16 patients experienced DLTs in the first cycle, *viz.* grade 3 delayed diarrhea, grade 2 nausea despite maximal anti-emetic supportive treatment, and grade 3 fatigue, thereby confirming the recommended dose level.

Table 1. Patient characteristics

Characteristic	No. of Patients
No. of Patients	
Total	28
Assessment	
For Dose-Limiting Toxicity	27
For Efficacy	26
No. of cycles/patient	
Median	5
Range	1 - 18
Gender, male : female	14 : 14
Age, years	
Median	54
Range	20 - 74
WHO performance status	
0	5
1	20
2	3
Previous therapy	
Chemotherapy	
≤ 2 prior regimens	16
≥ 3 prior regimens	5
Radiotherapy only	0
Both	8
None	5
Tumor types	
Gastro-intestinal tract, including:	11
Esophageal	3
Gastric	4
Gallbladder	3
Cholangiocarcinoma	1
Melanoma	9
Renal cell	2
Miscellaneous	6

Abbreviations: no., number; WHO, World Health Organization.

Table 2. Dose-escalation scheme and dose-limiting toxicity [DLT]

Dose level [mg/m²/day]		No. of Patients				Patients with DLT		
		No. of pts.	With one dose reduced	With one cycle delayed	No. of cycles	First cycle	All cycles	DLT Events at 1st cycle
Irinotecan	Capecitabine							
50	2 × 800	6	1[a]	2	33	0/6	2/6	
60	2 × 800	6	0	2	29	2/6	2/6	grade 3 diarrhea and grade 2 nausea [b] [N=1]; grade 3 diarrhea, grade 3 anorexia and grade 3 colitis [N=1]
50	2 × 1000	16[c]	2[d]	7	93	2/15	5/15	grade 3 diarrhea and grade 2 nausea [b] [N=1]; grade 3 asthenia [N=1]
TOTAL		28	3	11	155			

Note: [a] 25% dose-reduction of capecitabine due to grade 3 hand-food syndrome at 9th cycle, [b] grade 2 nausea despite maximal anti-emetic support, [c] one patient was not assessable for DLT, [d] 25% dose-reduction of capecitabine due to above-mentioned dose-limiting toxicities.
Abbreviations: no., number; pts., patients.

Hematologic Toxicity

Worst grade hematologic toxicities per patient are listed in Table 3. Overall, hematologic toxicity was mild to moderate across all dose levels with no grade 4 observed. Although grade 3 leukocytopenia and grade 3 neutropenia were observed in four patients at doses of irinotecan 50 mg/m^2/day and capecitabine 2 $\times$ 1000 mg/m^2/day, this was not considered a DLT because the adverse event occurred after the first cycle and the grade 3 myelosuppression occurred after day 7 of the cycle. Grade 3 anemia was seen in two patients at the feasibility dose level irinotecan 50 mg/m^2/day and capecitabine 2 $\times$ 1000 mg/m^2/day, considered DLTs at subsequent cycles.

Nonhematologic Toxicity

Other nonhematologic toxicities were mild to moderate with no grade 4 toxicity observed are summarized in Table 4. Overall, gastrointestinal toxicity (*i.e.*, diarrhea, nausea and vomiting) was the most common adverse event. Most (82%) patients experienced grade 1 to 2 nausea, and 17 out of 28 patients (61%) experienced grade 1 to 2 vomiting. Prophylactic anti-emetics, either metoclopramide or serotonine antagonists, were used to manage nausea and vomiting. Grade 3 delayed diarrhea was observed in 5 (18%) patients across all dose levels, including one patient at the first dose level (irinotecan 50 mg/m^2/day and capecitabine 800 mg/m^2 twice daily) considered a DLT at subsequent cycle, and two patients at the second dose level (irinotecan 60 mg/m^2/day and capecitabine 800 mg/m^2 twice daily) not considered DLTs because of inappropriate supportive treatment with loperamide. Grade 1 and 2 diarrhea was noted in 19 (70%) patients, and was manageable with supportive treatment with loperamide. Grade 2 anorexia was observed in 9 (32%) patients and one patient had grade 3 anorexia considered a DLT at the second dose level. In addition, grade 1 and 2 asthenia was seen in nine (32%) patients and one patient experienced dose-limiting (grade 3) asthenia in the first cycle at dose level irinotecan 50 mg/m^2/day and capecitabine 2 $\times$ 1000 mg/m^2/day after 5 days of the treatment. Mild alopecia (grade 1) was seen in eight patients and moderate alopecia (grade 2) was encountered in four patients. Across all dose levels mild to moderate abdominal pain was noted in 8 (29%) and 2 (7%) patients, respectively. Hand-foot syndrome (palmar-plantar erythrodysesthesia), a well-known adverse event of capecitabine, was seen in six patients (21% grade 1 to 2) all at the recommended dose of capecitabine (1000 mg/m^2 twice daily). Grade 3 hand-foot syndrome was observed in one patient at cycle 9 at the first dose level, considered a DLT at subsequent cycle. Mild to moderate conjunctivitis due

Table 3. Hematologic toxicity [worst grade per patient]

Dose level [mg/m²/day]				Anemia			Leukocytopenia				Neutropenia				Thrombocytopenia		
Irinotecan	Capecitabine	No. of pts.	No. of cycles	Grades													
				1	2	3	1	2	3	4	1	2	3	4	1	2	3-4
50	2 × 800	6	33	1	4	0	2	2	0	0	2	1	0	0	1	0	0
60	2 × 800	6	29	2	3	0	3	1	0	0	2	0	0	0	0	0	0
50	2 × 1000	16	93	5	5	3[a,b]	2	4	3[c]	0	0	4	3[c]	0	1	1	0

Note: [a] considered a DLT at first cycle in 1 pt., [b] considered a DLT at subsequent cycles in 2 pts., [c] not considered a DLT according to protocol definitions because adverse events occurred after day 7 of the cycle.
Abbreviations: no., number; pts., patients

Table 4. Nonhematologic toxicity [worst grade per patient]

Dose level [mg/m²/day]		No. of pts.	No. of cycles	Diarrhea			Nausea			Vomiting			Stomatitis			Anorexia			HFS			Asthenia		
				Grades																				
Irinotecan	Capecitabine			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
50	2 × 800	6	33	3	1	1[a]	4	1[a]	0	3	0	0	1	0	0	1	2	0	0	0	1[a]	1	1	0
60	2 × 800	6	29	3	1	2[b]	3	2[b,c]	0	5	0	0	0	1	0	1	2	1[c]	0	0	0	0	1	0
50	2 × 1000	16	93	6	5	2[c]	2	11[b]	0	6	3[b]	3[b]	0	0	0	3	5	0	5	1	0	3	3	1[c]

Note: [a] considered a DLT at subsequent cycles, [b] not considered a DLT due to inappropriate supportive treatment according to the protocol definitions, [c] considered a DLT at 1st cycle.
Abbreviation: HFS, hand-foot syndrome; no., number; pts., patients.

to capecitabine treatment was seen in two patients. Finally, mild and moderate increase of liver transaminases was noted in six (21%) and two (7%) patients, respectively, whereas mild hyperbilirubinemia was observed in 2 patients at the recommended dose level. All toxicities were reversible. In 39% of patients (N = 11), cycles were delayed by one week or more. A total of three patients (11%) required at least one dose reduction of irinotecan or capecitabine.

Pharmacokinetics

Blood samples were obtained from 26 patients, and complete pharmacokinetic profiles were available in 25 patients. The mean (± SD) irinotecan and SN-38 pharmacokinetic parameters are summarized in Table 5. Oral irinotecan was rapidly absorbed and metabolized with peak plasma concentrations of irinotecan and SN-38 within 2 to 2.5 hours. Interpatient variability was generally high with coefficients of variation of 36-48% for the AUC of irinotecan, and 34-84% for the AUC of SN-38. The SN-38-to-irinotecan AUC metabolic ratio was approximately 13.2%, which is similar to previous observations with single agent oral irinotecan formulated as semi-solid matrix capsules [11]. Pharmacokinetic parameters of capecitabine and its metabolites were also similar to previous findings (Table 6) [14]. Statistical analysis revealed that the pharmacokinetics of irinotecan and SN-38 were not altered by the coadministration of capecitabine, although there was a trend towards a higher metabolic ratio (Table 7), and showed that the pharmacokinetics of capecitabine and its metabolites were similar on day 5 and day 14, suggesting the absence of a pharmacokinetic interaction with irinotecan (Table 8).

Efficacy

Twenty-six patients were assessable for therapeutic activity. Three confirmed partial responses were documented, two in patients with metastatic gallbladder carcinoma lasting for ≥ 24 weeks, and one in a patient with metastatic melanoma lasting 42 weeks. A total of 16 patients demonstrated disease stabilization for 6 (N = 5), 12 (N = 8), 18 (N = 2), 24 (N=2), and 30 weeks (N = 1). One patient was not evaluable for response because he went off study after the first cycle due to DLT, and no tumor reassessment was done at that time.

Table 5. Mean ± SD [CV%] pharmacokinetic parameters of irinotecan and SN-38 on day 5 of the first cycle

	No. of pts.	Irinotecan	SN-38
Parameter		**Dose: 50 mg/m²/day**	
C_{max} [ng/mL]	19	65.6 ± 37.1 [57]	6.98 ± 5.49 [79]
Median T_{max} [h]		2.0	2.0
$AUC_{(0\text{-}24h)}$ [ng·h/mL]		529 ± 254 [48]	68.9 ± 50.9 [74]
$AUC_{(0\text{-}t)}$ [ng·h/mL]		529 ± 254 [48]	67.4 ± 52.0 [77]
$T_{½}$ [h]		8.15 ± 1.59 [20]	
Metabolic ratio			0.131 ± 0.083 [63]
		Dose: 60 mg/m²/day	
C_{max} [ng/mL]	6	81.5 ± 30.3 [37]	8.98 ± 5.64 [63]
Median T_{max} [h]		2.0	2.0
$AUC_{(0\text{-}24h)}$ [ng·h/mL]		642 ± 231 [36]	91.7 ± 76.9 [84]
$T_{½}$ [h]		8.94 ± 1.21 [13.5]	
Metabolic ratio			0.133 ± 0.066 [50]

Note: Metabolic ratio is defined as the AUC ratio of SN-38-to-irinotecan.
Abbreviations: SD, standard deviation; CV, coefficient of variation; no, number; pts., patients; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; AUC, area under the plasma concentration-time curve; $T_{½}$, half-life of terminal phase.

Table 6. Mean ± SD [CV%] pharmacokinetic parameters of capecitabine, 5'-DFCR, 5'-DFUR, 5-FU, and FBAL during the first cycle

		Capecitabine		5'-DFCR		5'-DFUR		5-FU		FBAL	
	No.of pts.	Day									
Parameter		5	14	5	14	5	14	5	14	5	14
		Dose: 2 × 800 mg/m²/day									
C_{max} [ng/mL]	12	3227 ± 2757 [85]	2402 ± 999 [42]	4513 ± 1316 [29]	3548 ± 1165 [33]	5421 ± 2703 [50]	4401 ± 1328 [30]	312 ± 194 [62]	257 ± 164 [64]	3378 ± 984 [26]	3568 ± 851 [24]
T_{max} [h]	12	1.00	0.50	1.21	2.00	1.71	2.00	1.21	2.00	2.50	3.00
$AUC_{(0-t)}$ [ng·h/mL]	12	4128 ± 2409 [58]	3967 ± 1542 [39]	8856 ± 3126 [35]	7880 ± 2067 [26]	9664 ± 3063 [32]	8929 ± 1982 [22]	554 ± 286 [52]	488 ± 222 [46]	19028 ± 6674 [35]	17921 ± 4786 [27]
$AUC_{(0-10h)}$ [ng·h/mL]	12	4145 ± 2402 [58]	3974 ± 1542 [39]	8885 ± 3128 [35]	7924 ± 2088 [26]	9758 ± 3081 [32]	9009 ± 1988 [22]	558 ± 284 [51]	491 ± 223 [45]	19028 ± 6674 [35]	17921 ± 4786 [27]
$T_{½}$ [h]	10	—	0.59 ± 0.43 [72]	—	0.82[a] ± 0.24 [29]	—	0.75 ± 0.31 [41]	—	0.79 ± 0.48 [61]	—	2.74 ± 0.48 [18]
		Dose: 2 × 1000 mg/m²/day									
C_{max} [ng/mL]	13	6003 ± 5827 [97]	5651 ± 5360 [95]	5156 ± 3359 [65]	4578 ± 2090 [46]	4942 ± 2435 [49]	4906 ± 2928 [60]	210 ± 123 [58]	211 ± 130 [61]	4283 ± 1187 [28]	4419 ± 1101 [25]
T_{max} [h]	13	2.00	1.00	2.00	1.00	2.00	2.00	2.00	2.00	3.00	3.00
$AUC_{(0-t)}$ [ng·h/mL]	13	7915 ± 5020 [63]	6797 ± 3904 [57]	11025 ± 3888 [35]	10214 ± 3672 [36]	10224 ± 3299 [32]	10426 ± 3524 [34]	417 ± 151 [36]	430 ± 161 [37]	22894 ± 9344 [41]	22307 ± 8719 [39]
$AUC_{(0-10h)}$ [ng·h/mL]	13	7929 ± 5021 [63]	6810 ± 3904 [57]	11034 ± 3893 [35]	10299 ± 3663 [36]	10347 ± 3321 [32]	10519 ± 3533 [34]	422 ± 153 [36]	434 ± 161 [37]	22894 ± 9344 [41]	22307 ± 8719 [39]
$T_{½}$ [h]	11	—	0.98 ± 0.61 [63]	—	0.99[b] ± 0.17 [17]	—	0.81[c] ± 0.10 [12]	—	1.04[c] ± 0.57 [55]	—	2.80 ± 0.57 [20]

Note: $AUC_{(0-\tau)} = AUC_{(0-10h)}$; [a] calculated on data from 11 pts.; [b] calculated on data from 13 pts.; [c] calculated on data from 10 pts.
Abbreviations: 5'-DFCR, 5-deoxy-5-fluorocytidine; 5'-DFUR, 5-deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; FBAL, α-fluoro-β-alanine; no., number; pts., patients; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life of terminal phase.

Table 7. Statistical analysis of the effect of capecitabine co-administration on the pharmacokinetics of irinotecan and SN-38

Compound	Parameters	p-value	Estimate [%] [90% CI]
Irinotecan	C_{max} $AUC_{(0-24h)}$	0.2137 0.5120	79.6 [58.7-107.9] 88.9 [66.0-119.8]
SN-38	C_{max} $AUC_{(0-24h)}$	0.8610 0.4145	103.2 [76.2-139.9] 119.3 [83-170.7]
–	Metabolic ratio	0.1127	133.0 [98.9-178.7]

Note: C_{max} and AUC on day 5 of the combination treatment in 25 patients were compared using SAS Proc-mixed procedure with those on day 5 of single agent oral irinotecan in 25 patients [11]. Metabolic ratio defined as the AUC-ratio of SN-38-to-irinotecan.
Abbreviations: CI, confidence interval; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration.

Table 8. Statistical analysis of the effect of irinotecan co-administration on the pharmacokinetics of capecitabine and its metabolites

Compound	Parameters	p-value	Estimate [%] [90% CI]
Capecitabine	C_{max}	0.56	108.6 [85.6-137.6]
	$AUC_{(0-10h)}$	0.53	104.9 [92.3-119.3]
5'-DFCR	C_{max}	0.16	114.1 [97.7-133.3]
	$AUC_{(0-10h)}$	0.30	108.0 [95.4-122.4]
	Metabolic ratio	0.88	102.9 [75.4-140.5]
5'-DFUR	C_{max}	0.57	106.7 [87.8-129.7]
	$AUC_{(0-10h)}$	0.74	101.6 [93.6-110.3]
	Metabolic ratio	0.83	96.8 [76.0-123.4]
5-FU	C_{max}	0.62	107.1 [84.5-135.7]
	$AUC_{(0-10h)}$	0.60	103.5 [92.7-115.6]
	Metabolic ratio	0.93	98.6 [74.6-130.5]
FBAL	C_{max}	0.91	100.5 [93.0-108.6]
	$AUC_{(0-10h)}$	0.38	103.2 [97.2-109.6]
	Metabolic ratio	0.93	98.3 [72.8-132.8]

Note: Pharmacokinetic parameters on day 5 (test), *i.e.*, after five days of concomitant treatment, were compared to day 14 (reference), *i.e.*, nine days after stop of irinotecan administration. Metabolic ratio is defined as AUC-ratio of the metabolite-to-capecitabine.

Abbreviations: 5'-DFCR, 5-deoxy-5-fluorocytidine; 5'-DFUR, 5-deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; FBAL, α-fluoro-β-alanine; CI, confidence interval; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration.

Discussion

The present phase I trial indicates that the oral combination of irinotecan, formulated as novel semi-solid matrix capsules, and capecitabine administered concomitantly daily for 5 and 14 consecutive days, respectively, every 3 weeks in patients with advanced solid tumor, is clinically safe, feasible and has demonstrated antitumor activity. Irinotecan and capecitabine have different mechanisms of action, and have synergistic or additive cytotoxicity in experimental models. In addition, both agents have only partially overlapping toxicities.

Delayed diarrhea accompanied by nausea, anorexia and colitis were the principal DLTs of this combination regimen. Diarrhea was easily manageable by the use of loperamide. Hematological toxicity was mild to moderate and did not result in DLT in the first cycle. Nonhematologic toxicities attributed to the oral combination of irinotecan and capecitabine included nausea, vomiting, stomatitis, fatigue, alopecia, and hand-foot-syndrome. The recommended dose of this oral combination for further phase II study evaluation is irinotecan 50 mg/m^2/day given daily for 5 days and capecitabine 1000 mg/m^2 given twice daily for 14 days, repeated every 3 weeks.

Currently, the combination of irinotecan and capecitabine is tested in different schedules in phase I and phase II studies in advanced colorectal cancer, although only preliminary data are yet available [15-19]. In the majority of these studies intravenous irinotecan is used, while two studies apply flat-dose capecitabine [20, 21] and a third uses oral irinotecan for 14 consecutive days every 3 weeks in combination with capecitabine [22]. Two randomized phase II studies compared weekly to 3-weekly administration of irinotecan with capecitabine [6, 17]. Higher incidences of diarrhea and life-threatening toxicity on the weekly regimen have led to the conclusion that the 3-weekly administration is preferable. Efficacy and safety of the 3-weekly regimen were interesting and have led to the design of phase III trials which are currently performed.

Preclinical studies showed that the therapeutic efficacy of the combination of intravenous irinotecan and intravenous 5-FU is dose- and sequence-dependent [23-26]. The sequence of irinotecan preceding 5-FU by an interval of 24 h appeared superior. Furthermore, the toxicity associated with irinotecan could be eliminated by reducing the dose of irinotecan to at least 50% of its MTD while keeping dose of 5-FU at 50 - 75% of its MTD, without loss of the antitumor activity [26]. Increasing the dose of 5-FU did not contribute to additional therapeutic activity, but only resulted in increased toxicity. In this combination, doses of 5-FU are critical for therapeutic efficacy, whereas doses of irinotecan play a modulatory function in

sensitizing tumor cells for the subsequent 5-FU administration after 24 h [26]. *In vitro* studies in HCT-8 colon carcinoma cell lines showed that the combination of SN-38 and 5-FU resulted in increased deoxythymidine triphosphate levels and inhibition of deoxyuridine monophosphate (dUMP) synthesis [26]. This depletion of dUMP enhanced the 5-FU associated inhibition of thymidylate synthese, which explained the enhanced cytotoxicity of 5-FU after irinotecan administration [26].

The pharmacokinetic results of oral irinotecan and SN-38 in the present study are in agreement with results of a previously published phase I study of single agent oral irinotecan [11], showing rapid absorption and metabolism of the drug with peak plasma concentrations of irinotecan and SN-38 within 2 to 3 hours after intake, high interpatient variability and the same dose-independent SN-38-to-irinotecan AUC ratio. This ratio is substantially higher than that measured after oral administration of irinotecan than after intravenous administration of irinotecan [27, 28], suggesting significant presystemic biotransformation of irinotecan into SN-38 within the gastrointestinal tract and/or the liver. This is consistent with the high expression levels of irinotecan-metabolizing carboxylesterases in intestinal cells [29]. These enzymes are also involved in capecitabine biotransformation, and a pharmacokinetic drug-drug interaction was thus theoretically possible. However, the pharmacokinetic analysis of the present study revealed no significant interaction between oral irinotecan and capecitabine, although there was a trend towards a higher metabolic ratio SN-38/irinotecan with concomitant administration. The pharmacokinetics of capecitabine and its metabolites either at day 5 or at day 14 were not modified by the 5-day coadministration of irinotecan.

In conclusion, the oral combination of irinotecan with prolonged administration of capecitabine appears to be feasible. The antitumor activity observed in patients with various drug-refractory malignancies, including gallbladder carcinoma and melanoma, is encouraging and provides further impetus for the development of this combination in phase II trials. In addition, the convenience of oral administration of cytotoxic drugs brings new alternatives to current intravenous therapy.

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Liquid chromatographic assays for DE-310, a novel camptothecin analog, and two major enzymatic products in human matrices

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Abstract

Assays were developed for determination of DE-310, a carboxymethyldextran polyalcohol conjugate of the topoisomerase I inhibitor DX-8951 (exatecan) and two enzymatic products (*i.e.*, glycyl-DX-8951 and unconjugated DX-8951) in human whole blood, erythrocytes and saliva. Sample pretreatment involved a single protein-precipitation step, followed by a thermolysin-mediated deconjugation for the parent molecule. Separation of the compounds was achieved on an Inertsil ODS-80A column (150 × 4.6 mm i.d; 5 µm PS), using isocratic elution. The column effluent was monitored at excitation and emission wavelengths of 375 and 445 nm, respectively. Validation results indicated that the methods are accurate and precise at lower limits of quantitation of 0.5 - 6.9 ng/mL. The methods was used to study the blood distribution and salivary concentrations in patients receiving DE-310.

1. Introduction

DE-310 comprises the active moiety DX-8951 (exatecan; [1*S*,9*S*]-1-amino-9-ethyl-5-fluoro-1,2,3,9,12,15-hexahydro-9-hydroxy-4-methyl-10*H*,13*H*-benzo[*de*]pyranol)-[3',4':6,7]-indolizino[1,2-*b*]quinoline-10,13-dione), a hexacyclic camptothecin derivative, linked to a biodegradable macromolecular carboxymethyldextran polyalcohol drug-delivery system via a glycyl-glycyl-phenylalanyl-glycyl-peptidyl spacer (Figure 1) [1]. The biodegradable polymer portion of DE-310 is designed to provide preferential tumoural uptake and sustained release of the active moiety within the tumour, as a result of an enhanced permeability and retention effect [2-4]. After entering the tumour area, DE-310 is taken up by tumour cells through endocytosis. The breakdown of the carrier is mediated by lysosomal enzymes, *i.e.* cathepsins, which results in two enzymatic products, *viz.* glycyl-DX-8951 (G-DX-8951) and unconjugated DX-8951 (DX-8951).

Currently, DE-310 is being tested in clinical trials administered as a 3 h infusion given once every 4 or 6 weeks [5,6]. Preliminary pharmacokinetic analysis showed that the apparent plasma half-lifes of conjugated DX-8951, glycyl-DX-8951 (G-DX-8951) and unconjugated DX-8951 (DX-8951) in humans, were in the order of 10 - 13 days [5]. This pharmacokinetic behavior is very distinct from that of other known camptothecin analogs [7]. To allow more detailed studies on the

distribution of DE-310 and to elucidate the basis for its unique pharmacokinetic behavior, information of dynamic changes of free drug *in vivo* in different body compartments is essential.

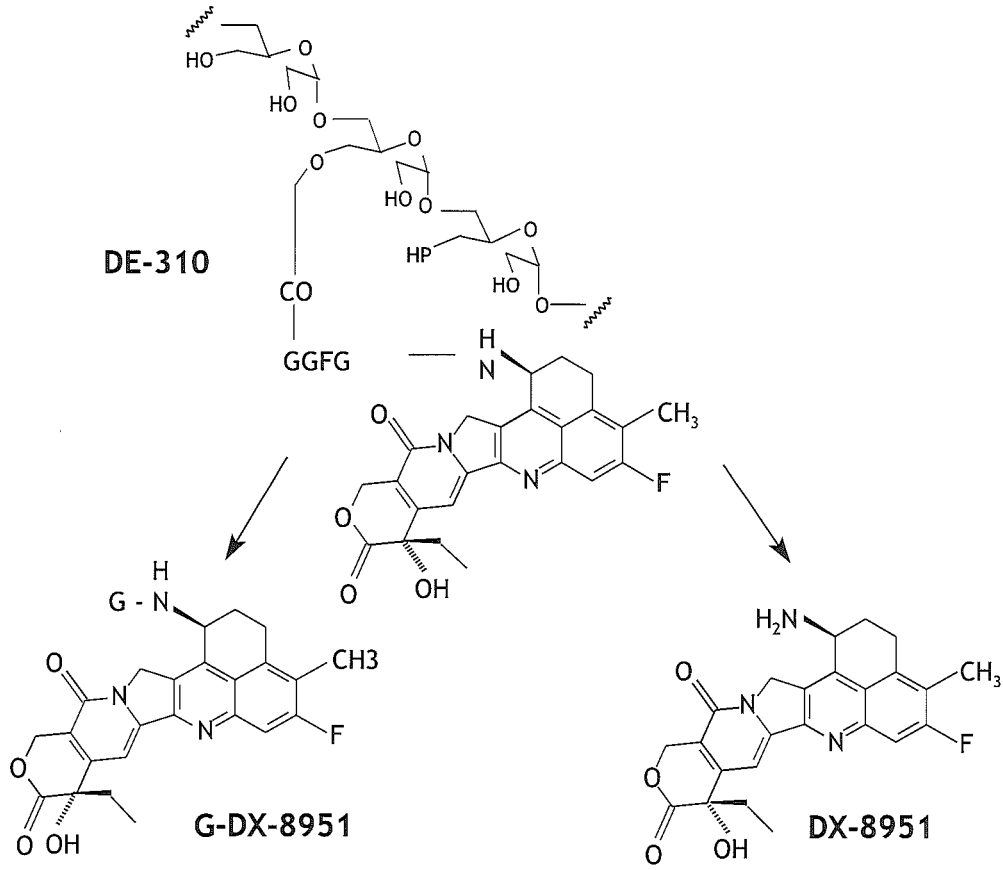

Figure 1. The chemical structures of DE-310 [M.W. 3.4 × 10^5], DX-8951 [M.W. 435.45], and G-DX-8951

Previously, several physiologically based approaches have been put forward for the determination of the non-protein-bound fraction of drugs in dynamically functioning biological systems, including analysis of saliva [8], cerebrospinal fluid [9], and red blood cell partitioning [10]. Here, we report on the development and validation of HPLC-based analytical methods for the determination of conjugated DX-8951, DX-8951 and G-DX-8951 in human whole blood, erythrocytes and saliva.

2. Experimental

2.1. *Chemicals and reagents*

Reference standards of DE-310 (batch: P-IW114B; purity, 100%), DX-8951f (batch: 654-654-98-003; purity, 99.9%), which refers to the monomethanesulfonate dihydrate salt form of DX-8951, G-DX-8951 trifluoroacetate (batch: AR068103; purity, 98.8%), and the internal standard DW-8579 (batch: AR141301; purity, 98.2%) were supplied by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Acetonitrile was obtained from Biosolve (Valkenswaard, the Netherlands), Tris-hydrochloride buffer and thermolysin from Sigma (St. Louis, MO, USA), and zinc sulfate and calcium chloride from J.T. Baker (Deventer, the Netherlands). All other chemicals and HPLC solvents were of highest grade available commercially. Milli-Q-UF (Millipore, Bedford, MA, USA) water was used throughout. Blank human plasma, whole blood and saliva were obtained from healthy volunteers via the Central Laboratory of the Blood Transfusion Service (Amsterdam, the Netherlands). Unwashed erythrocytes were isolated from whole blood samples using MESED instruments (Kelmis, Belgium), as described previously [11].

2.2. *Sample preparation*

2.2.1. *DX-8951 and G-DX-8951 in whole blood, erythrocytes and saliva*

Aliquots of 100 µL blood were transferred to 4.5 mL glass tubes, followed by the addition of 900 µL of human plasma, 500 µL of acetonitrile, 100 µL of internal standard DW-8579 (100 ng/mL in acetonitrile-water (1:1, (v/v)), and 100 µL of zinc sulfate [70% (w/v) in water]). After vigorous vortex-mixing for 5 min on a multi-tube vortex mixer (Dade S8215-1X, Miami, FL, USA), samples were centrifuged using a Hettig Universal 30RF centrifuge (Tüttlingen, Germany) for 5 min at 4000 × *g*. The supernatant was transferred to a clean 4.5 mL glass tube and partially evaporated under a gentle stream of nitrogen at 60°C for exactly 30 min. An aliquot of 200 µL of the residue was transferred to a low-volume insert of glass, and 100 µL was injected into the HPLC system.

Prior to extraction of erythrocyte samples, 100 µL volumes of erythrocytes were diluted with 150 µL of human plasma, followed by vortex-mixing on an MS2 minishaker (IKA Works, Inc., Wilmington, NL, USA). This procedure was performed to achieve a uniform assay in order to interpolate the chromatographic data on a single standard measure line in the matrix (plasma). Aliquots of 100 µL of plasma-diluted erythrocytes were further prepared as blood (see above).

Sample pretreatment for DX-8951 and G-DX-8951 in saliva was identical to that described for whole blood, using saliva aliquots of 250 µL diluted with 750 µL

of human plasma. This procedure was performed to achieve a uniform assay in order to interpolate the chromatographic data on a single standard measure line in the matrix (plasma). At the end of the evaporation period, 250 µL of the residue was transferred into a low volume insert of glass, and 200 µL were subjected to chromatography.

2.2.2. Conjugated DX-8951 in whole blood, erythrocytes and saliva

Aliquots of 100 µL of blood, plasma-diluted erythrocytes, or saliva were transferred to 1.5 mL polypropylene microtubes (Eppendorf, Hamburg, Germany), followed by the addition of 100 µL of plasma and 600 µL of methanol. Following vigorous mixing for 1 min on the multi-tube mixer, samples were centrifuged at ambient temperature for 5 min at 10,000 × *g*. Next, 600 µL of the upper layer were transferred to a 4.5 mL glass tube and then evaporated to dryness under nitrogen at 60°C. The residue was reconstituted in 100 µL of water, 200 µL Tris-hydrochloride buffer (0.1 M; pH 8.5), and 100 µL of a thermolysin solution (2.0 mg/mL in 0.1 M calcium chloride). This mixture was incubated for 1 h at 50°C in a water bath to release phenylalanylglycyl-DX-8951 (FG-DX-8951) from conjugated DX-8951. The reaction was stopped by adding 500 µL of a mixture of 0.5 M hydrochloric acid-water (1:1, (v/v)). The resulting solution was centrifuged for 5 min at 4000 × *g*, and 100 µL of the supernatant were diluted with 100 µL of phosphate-buffered saline (pH 7.2), from which 100 µL were injected into the HPLC system.

2.3. Equipment and chromatographic conditions

Chromatographic analyses were performed using a constaMetric 3200 pump (LDC Analytical, Riviera Beach, FL, USA), a 717plus autosampler (Waters, Milford, MA, USA) and a Jasco FP-920 fluorescence detector (Hachioji City, Japan). The analytical column used was packed with Inertsil ODS-80A material (150 × 4.6 mm i.d; 5-µm PS) from GL Science (Tokyo, Japan). The column temperature was maintained at 60°C by using a Spark Mistral column heater (Meppel, the Netherlands). The fluorescence detector operated at excitation and emission wavelengths of 375 and 445 nm, respectively, with the emission bandwidth set at 18 nm.

The mobile phase for determination of DX-8951 and G-DX-8951 consisted of a 0.1 M sodium acetate (pH 5.0, acetic acid)-tetrahydrofuran mixture (15:2, (v/v)), whereas that for conjugated DX-8951 was composed of 0.1 M sodium acetate (pH 5.0, acetic acid)-methanol (10:12, (v/v)). The mobile phases were prepared daily, filtered and degassed before use, and delivered isocratically at a flow rate of 1.0 mL/min.

2.4. Calibration

Separate standard stock solutions of DX-8951, G-DX-8951 and DW-8579 were prepared in triplicate at 1.0 mg/mL in dimethylsulfoxide and were stored at -80°C. The amount of compounds in the triplicate solutions was measured by injection aliquots of 50 µL of 2500-fold diluted stock solutions in acetonitrile-water (1:1, (v/v)), followed by a 50-fold dilution in the mobile phase (all in triplicate) and injection into the HPLC system. The mean value of the individual stock solutions was within 2.0 - 4.6% of each other. Stock solutions of DE-310 were prepared at 1.0 mg/mL (equivalent to 69.0 µg/ml of DX-8951) in water, and were stored at -80°C. The amount of compound in the triplicate solutions was measured by incubation of aliquots of DE-310 with thermolysin for 1 h in a water bath at 50°C, followed by injection of 10 µL aliquots after stopping the enzyme reaction.

Acquisition and integration of HPLC data was performed with Chrom-Card data analysis system (Fisons, Milan, Italy), running on an ICW chromatographic workstation. Six-point calibration curves were prepared daily in a blank matrix by serial dilution from the standard stock solution. For DX-8951 and G-DX-8951, weighted $1/x^2$ linear-regression analysis of the peak-height ratio of the compounds and the internal standard *versus* the concentration were used for the calculation of the calibration curves. For conjugated DX-8951, weighted $1/x^2$ linear-regression analysis of the peak height *versus* the concentration was used.

2.5. Method validation

The selectivity of the methods was assessed using a set of five independent blank (drug-free) human blood and saliva samples that were handled as described above. The presence of potentially interfering endogenous compounds with retention times similar to that of DX-8951, G-DX-8951, conjugated DX-8951, and/or the internal standard was investigated by visual inspection of the chromatograms.

The precision and accuracy of the analytical procedures was evaluated by repeat analysis of quality control (QC) samples, prepared in batch at various concentrations in each matrix and stored at -80°C, on separate occasions. QC samples of unwashed erythrocytes were prepared by incubation of 30 mL of whole blood for a period of 30 min at 37°C with DX-8951 and G-DX-8951 (each at a final concentration of 50 ng/mL). Subsequently, 1.5 mL aliquots of whole blood were transferred to 20 MESED instruments for isolation of unwashed erythrocytes. The remaining blood was used for the determination of DX-8951 and G-DX-8951 concentrations, and for hematocrit measurement.

The between-run precision (BRP) was evaluated prospectively as the percentage relative standard deviation obtained by one-way analysis of variance (ANOVA) using the run day as classification variable, calculating between-groups mean square (*B*), the within-groups mean square (*W*), and the grand mean (*M*) of the observed concentrations across run days. BRP was defined as:

$$BRP = 100 \times [(B - W) / n]^{0.5} / M$$

The within-run precision (WRP) was estimated in a similar manner using the one-way ANOVA as:

$$WRP = 100 \times [(W)^{0.5} / M]$$

In case where *W* is greater than *B*, the resulting variance is negative, implying that no significant additional variation was observed on performing the assay in different runs. The average accuracy of the determinations were calculated as percentage deviation from nominal values (%DEV) and was defined as:

$$\%DEV = 100 \times (M / \text{nominal concentration})$$

For determination of the lowest standard concentration with a definite level of certainty, *i.e.* the lower limit of quantitation (LLQ), blank samples were spiked with each of the analytes and subjected to repeat analysis as described for the QC samples. Stability of the analytes in the tested matrices was evaluated following (1) three freeze-thaw cycles of 15 min each, (2) at 37°C for 24 h, and (3) after extraction at room temperature (autosampler stability). The extraction efficiency was established by comparing peak areas of samples prepared in plasma with those for non-processed samples prepared in mobile phase. All statistical analyses were performed using the software package Number Cruncher Statistical System v5.0 (Dr. J.L. Hintze, Kaysville, UT, USA; 1992).

2.6. Patient samples

The patients studied participated in a clinical phase I and pharmacokinetic study of DE-310 in various nonhematological malignancies [5]. The DE-310 doses of 6.0 or 7.5 mg/m^2 were delivered as a 3 h intravenous infusion, with treatment cycles repeated every 6 weeks. The protocol was approved by the Institutional Review Board of Erasmus University Medical Center (Rotterdam, the Netherlands) and written informed consent was obtained prior to treatment.

Blood samples were collected in sodium heparinized coated tubes before and at 1, 2, 4, 6, 8, 24, 48, 72, 168, 240, 336, 504, 672 and 840 h after the end of the DE-310 infusion. After sampling, the blood specimens were immediately put in an ice-water bath (4°C) until centrifuged at 2000 rpm for 15 min at 4°C. Plasma was transferred into plastic specimen storage vials and the red blood cell fraction into Falcon vials, and both were stored frozen at -20°C until analysis. Saliva samples were collected in plastic specimen storage vials before and at 3, 7, 27, 51, 72, 168, 240, 336, 504, 672, 840 h after the end of the DE-310 infusion and stored frozen at -20°C until analysis as described above.

3. Results and discussion

3.1. Chromatography and detection

Because of the pH-dependent instability of the α-hydroxy-δ-lactone moiety in the core structure of most camptothecin analogs [12], including DX-8951, resulting in the ring-opened carboxylate form at high pH and the ring-closed lactone form at low pH, we decided to focus only on measurement of total concentrations in the present study, since disruption of the carboxymethyldextran polyalcohol side chain while maintaining the physiologic lactone-to-carboxylate ratio may not be feasible. In addition, for most camptothecin analogs it has been shown that total drug monitoring serves as an appropriate surrogate of the lactone forms [13]. The choice of the internal standard DW-8579, which lacks the C4-methyl substituent as compared to DX-8951 (Figure 1), and the optimal fluorescence wavelength couple of the analytes (365/445 nm) was based on earlier work described for determination of DX-8951 in human plasma [14] and mouse plasma [15]. Furthermore, no other metabolites were expected around the retention time of the internal standard [14].

The choice of the Inertsil ODS-80A analytical column was based on previous experience during the development of an assay for total drug forms (*i.e.*, lactone plus carboxylate) of the related agent topotecan in human whole blood and unwashed erythrocytes [16], and resulted in optimal selectivity factors and resolution. The composition and pH of the mobile phase was selected in order to optimize separation factors and peak shapes of the analytes. To ensure sufficient selectivity in our assays, tetrahydrofuran was added to the mobile phase as an organic modifier.

In order to allow for convenient and inexpensive sample-pretreatment procedure of the biological samples, we opted for a solvent extraction, rather than taking

recourse to solid-phase cartridges [13]. This was achieved by the use of a protein-precipitation step in the presence of aqueous zinc sulfate [17]. This sample handling was also chosen for its optimal elimination of endogenous interferences, while maintaining a high extraction efficiency for the analytes in all matrices.

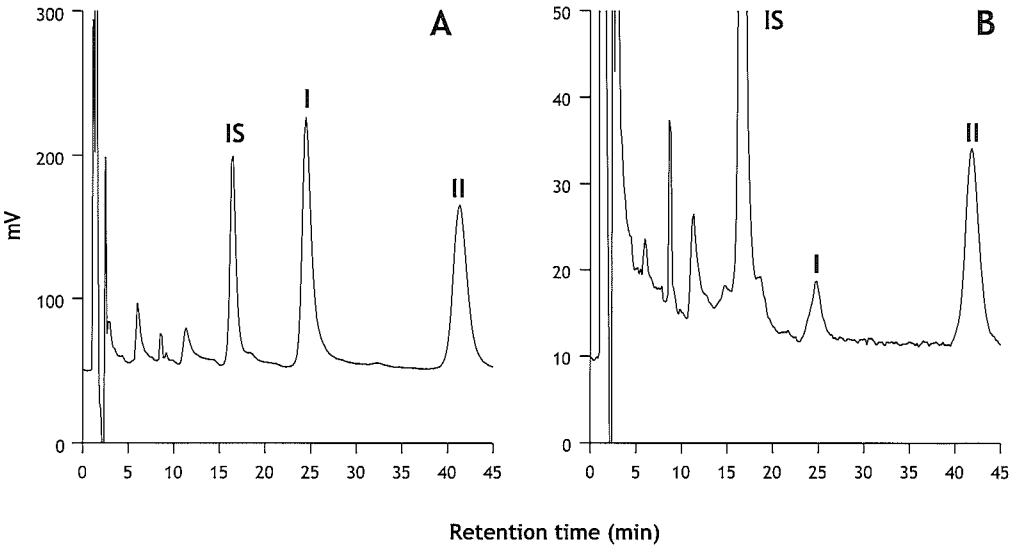

Figure 2A and 2B. HPLC chromatograms of internal standard [IS] DW-8579, [I] DX-8951 and [II] G-DX-8951 in [A] whole blood and [B] erythrocytes

3.2. Method validation

3.2.1 Whole blood and erythrocytes

Chromatograms of blank and spiked human whole blood and erythrocyte samples are shown in Figure 2A and 2B, respectively. The selectivity for the analytes is shown by the sharp and symmetrical resolution of the peaks, with no significant interfering peak for all compounds in drug-free specimens, obtained from five different individuals. The retention times of the internal standard DW-8579, unconjugated DX-8951 and G-DX-8951 were 16, 24, and 42 min, respectively, with an overall chromatographic run time of 45 min. This long run time was chosen to obtain optimal chromatographic resolution and to eluate potential interfering substances previous to injection of a new sample. In addition, there was no need for high-throughput assay because a limited number of saliva and erythrocyte samples per patient were expected. The calibration curves of assay of DX-8951 and G-DX-8951 in human blood were found to be linear in the range of 5.0 - 200 ng/mL when applying a weight factor of $1/x^2$, with correlation coefficients of at least 0.995 and 0.997 for DX-8951 and G-DX-8951, respectively.

Table 1. Validation characteristics of DX-8951 and G-DX-8951 in human blood

Spiked concentration [ng/mL]	Observed concentration [ng/mL]		Precision [%]		Number of replicate observations
	Whole blood	Erythrocytes	Within-run	Between-run	
DX-8951					
50	–	7.26	7.8	4.6	5
50	50.6	–	1.3	0.65	2
G-DX-8951					
50	–	35.1	2.6	5.3	5
50	47.6	–	0.64	3.9	2

Table 2. Stability data of DX-8951 and G-DX-8951 in human blood

Spiked concentration [ng/mL]	Freeze-thaw [three cycles]		24 h, 37°C		Number of replicate observations
	Observed concentration [ng/mL]	Difference [%]	Observed concentration [ng/mL]	Difference [%]	
DX-8951					
20.3	20.6	1.8	19.1	-5.8	3
74.1	72.0	-2.8	72.8	-1.7	3
158.8	155.1	-2.3	152.2	-4.2	3
G-DX-8951					
17.7	18.4	4.0	16.5	-7.0	3
65.1	64.7	-0.7	64.8	-0.6	3
144.2	138.8	-3.8	136.6	-6.0	3

QC samples in whole blood spiked with DX-8951 and G-DX-8951 (50 ng/mL, final concentration) were assayed in duplicate in four days, whereas QC samples in erythrocytes were assayed in quintuplicate in four days. Validation data of the analytical runs in terms of accuracy and precision indicated that all of samples were within the acceptable range (Table 1). The mean overall extraction recoveries were 118.0 and 101.0% for DX-8951 and G-DX-8951, respectively. Stability data for DX-8951 and G-DX-8951 in human blood indicated that both compounds were stable following three freeze-thaw cycles as well as after incubation at 37°C for 24 h (Table 2).

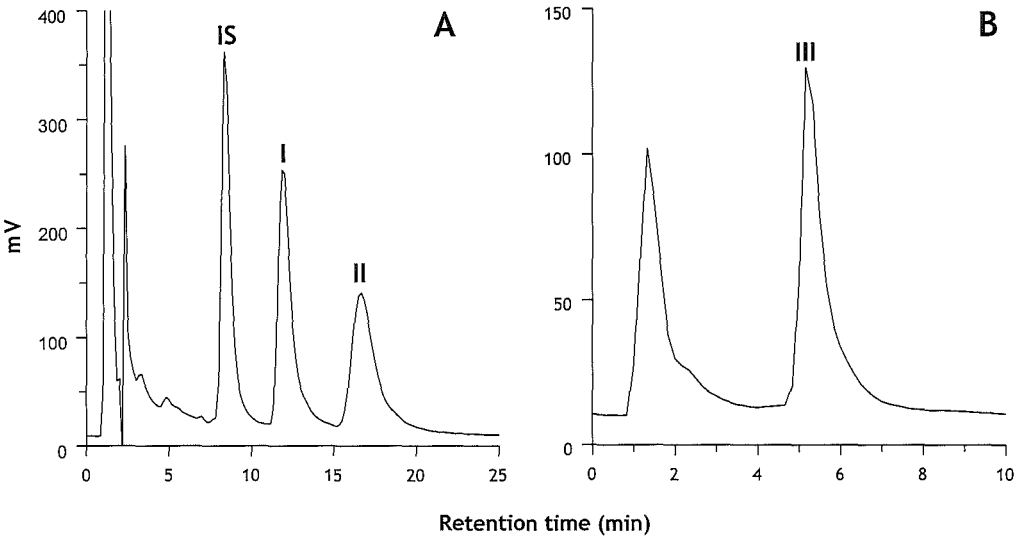

Figure 3A and 3B. HPLC chromatograms of [A] internal standard [IS] DW-8579, [I] DX-8951, [II] G-DX-8951; and [B] [III] FG-DX-8951 in human saliva

Following incubation of human blood spiked with DE-310 at a concentration of 5000 ng/mL (*i.e.*, equivalent to DX-8951 at a concentration of 345.0 ng/mL) at 37°C for 30 min, no conjugated DX-8951 could be detected in the erythrocytes following their isolation in the MESED instruments. For this reason, no further validation characteristics could be made for conjugated DX-8951 in erythrocytes (data not shown). This is consistent with the high molecular weight of the conjugated analyte, precluding (extensive) red blood cell partitioning.

Table 3. Validation characteristics of DX-8951 and G-DX-8951 in human saliva

Concentration [ng/mL]		Precision [%]		Accuracy [%]
Spiked	Observed	Within-run	Between-run	
DX-8951				
0.5 [LLQ[a]]	0.51	6.1	3.0	101.6
2.5	2.43	2.7	3.1	97.2
7.5	7.19	2.1	3.6	95.8
15.0	14.4	2.3	2.6	95.9
G-DX-8951				
0.5 [LLQ]	0.48	11.5	[b]	95.4
2.5	2.25	3.7	[b]	90.1
7.5	6.67	2.8	3.8	90.2
15.0	13.8	2.4	3.6	91.7

Abbreviation: [a] LLQ: lower limit of quantitation.
Note: [b] No additional variation was observed on performing the assay in different runs.

3.2.2. Saliva

No chromatographic interference was noted from endogenous compounds in blank human saliva specimens (Figure 3A). The calibration curves for DX-8951 and G-DX-8951 in saliva were linear in the range of 0.5 - 20.0 ng/mL, with correlation coefficients of at least 0.990. The retention times for DW-8579, DX-8951 and G-DX-8951 were 9, 12 and 17 min, respectively, with an overall chromatographic run time of 45 min. A representative chromatogram of a QC sample containing DX-8951 and G-DX-8951 spiked at a concentration of 7.5 ng/mL is also shown in Figure 3. The LLQ samples were assayed in quintuplicate on four separate occasions, and indicated that 95 and 80% of the analyzed samples containing DX-8951 and G-DX-8951, respectively, were within the acceptable range for accuracy of 80 - 120%. Likewise, QC samples were analyzed in quintuplicate on four consecutive days and accuracy and precision data were within acceptable limits (Table 3). Stability data of DX-8951 and G-DX-8951 in human saliva after three freeze-thaw cycles (-80°C) and 24 h at 37°C are given in Table 4. These data suggest a minor loss of material due to instability, necessitating rapid freezing of clinical samples after blood collection.

The calibration curves of the assay for conjugated DX-8951 in human saliva were linear in the range of 100.0 - 5000 ng/mL (equivalent to 6.9 - 345 ng/mL of DX-8951), with correlation coefficients of at least 0.996. The retention time of conjugated DX-8951 was 5.2 min, and the total run time was set at 10 min. A representative chromatogram of a QC sample containing DE-310 spiked at a concentration of 200.0 ng/mL (13.0 ng/mL of DX-8951) is shown in Figure 3B. All of the analyzed LLQ and QC samples fell within the range of accuracy of 80 - 120%. Because of the fact that 100% of the analyzed LLQ samples and QC samples were within the acceptable range of accuracy, only three analytical runs were performed (Table 5). Stability experiments showed that conjugated DX-8951 was stable during the chromatographic run when using plastic inserts and at 4°C in the dark, but not after three freeze-thaw cycles (-80°C) and upon storage for 24 h at 37°C (Table 4).

Table 4. Stability data of DX-8951, G-DX-8951 and DE-310 in human saliva

Spiked concentration [ng/mL]	Freeze-thaw [three cycles]		24 h, 37°C		Number of replicate observations
	Observed concentration [ng/mL]	Difference [%]	Observed concentration [ng/mL]	Difference [%]	
DX-8951					
2.5	2.2	-12.0	2.1	-16.0	2
7.1	6.4	-9.8	5.9	-16.9	2
14.3	12.7	-11.2	11.8	-17.5	2
G-DX-8951					
2.5	2.0	-20.0	1.7	-32.0	2
7.4	5.8	-21.6	5.0	-32.4	2
15.4	12.4	-19.5	10.4	-32.5	2
DE-310 [DX-8951 eq.]					
11.86	12.06	1.7	9.1	-23.2	3
129.8	129.5	-0.3	106.7	-17.8	3
303.3	291.5	-3.9	236.2	-22.1	3

Table 5. Validation characteristics of DE-310 in human saliva

Concentration [ng/mL]		Precision [%]		Accuracy [%]
Spiked	Observed	Within-run	Between-run	
100.0 [6.0 ng eq. DX-8951]	98.4	7.5	2.2	98.3
200.0 [13.8 ng eq. DX-8951]	204.2	3.8	4.2	102.0
1000.0 [69.0 ng eq. DX-8951]	940.0	3.4	3.1	94.6
2000.0 [138 ng eq. DX-8951]	1879.6	3.8	5.1	93.6

3.3. *Pharmacokinetics*

The suitability of the developed methods for the clinical use was demonstrated by the determination of DE-310 and its enzymatic products in human erythrocytes and saliva obtained from eight patients with different advanced solid tumours, treated with DE-310 at dose levels 6.0 or 7.5 mg/m^2 once every 6 weeks. In all analyzed samples of erythrocytes and saliva, the concentration of DE-310, G-DX-8951, and DX-8951 were below lower limit of quantitation (< 0.5 ng/mL). This would fit with the hypothesis of preferential drug uptake in tumour tissue, but not in other tissues.

In conclusion, we have developed and validated HPLC methods with fluorescence detection for DE-310 and two major metabolites in human whole blood, erythrocytes, and saliva. The methods were shown to meet the current requirements as to the validation of bioanalytical methods, providing good accuracy and precision. The results obtained from the analysis of patient samples revealed that DE-310, G-DX-8951, and DX-8951 are neither distributed to erythrocytes, nor excreted in saliva, which makes the biodistribution of this topoisomerase I inhibitor substantially different from that of topotecan [11], or irinotecan and its metabolite, SN-38 [18].

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Phase I and pharmacokinetic study of DE-310, a macromolecular prodrug of the topoisomerase-I-inhibitor exatecan (DX-8951f), administered once every 2 or 6 weeks in patients with advanced solid tumors

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Submitted

Abstract

Purpose: To assess the maximum-tolerated dose (MTD), toxicity and pharmacokinetics of DE-310, a macromolecular prodrug of the topoisomerase I inhibitor exatecan (DX-8951f), in patients with advanced solid tumors.

Patients and Methods: Patients received DE-310 as a 3-h infusion once every 2 weeks (dose, 1.0 to 2.0 mg/m^2) or once every 6 weeks (dose, 6.0 to 9.0 mg/m^2). Conjugated DX-8951 (the carrier linked molecule), and the metabolites DX-8951 and glycyl-DX-8951 were assayed in various matrices up to 35 days post first and second dose.

Results: Twenty-seven patients were enrolled into the study and received a total of 86 administrations. Grade 4 neutropenia and grade 3 thrombocytopenia, and grade 3 hepatotoxicity with veno-occlusive disease (VOD), were dose-limiting toxicities. Other hematological and nonhematological toxicities were mild to moderate and reversible. The apparent half-life of conjugated DX-8951, glycyl-DX-8951, and DX-8951 was 13 days. The area under the curve ratio for conjugated DX-8951-to-DX-8951 was 600. No drug concentration was detectable in erythrocytes, skin and saliva, although low levels of glycyl-DX-8951 and DX-8951 were detectable in tumor biopsies. One patient with metastatic adenocarcinoma of unknown primary achieved a histologically proven complete remission. One confirmed partial remission was observed in a patient with metastatic pancreatic cancer and disease stabilization was noted in 14 additional patients.

Conclusion: The recommended phase II dose of DE-310 is 7.5 mg/m^2 given once every 6 weeks. The active moiety DX-8951 is released slowly from DE-310 and over an extended period, achieving the desired prolonged exposure to this topoisomerase I inhibitor.

Introduction

DE-310 is a novel macromolecular drug-delivery system for the topoisomerase I inhibitor DX-8951f (exatecan mesylate), in which each molecule is linked via a glycyl-glycyl-phenylalanyl-glycyl-peptidyl spacer to a biodegradable carboxymethyldextran polyalcohol polymer. This carrier part of DE-310 is intended to provide passive accumulation in tumor tissue and sustained

release of the active moiety DX-8951 within the tumor, which occurs as result of enzymatic cleavage of the peptidyl spacer by cathepsin B and cathepsin L, thereby enhancing its activity and reducing its systemic toxicity [1]. The main chain is acid-labile, suggesting that it is biodegradable and can be depolymerized after endocytosis in a lysosomal acidic-environment (Figure 1) [1].

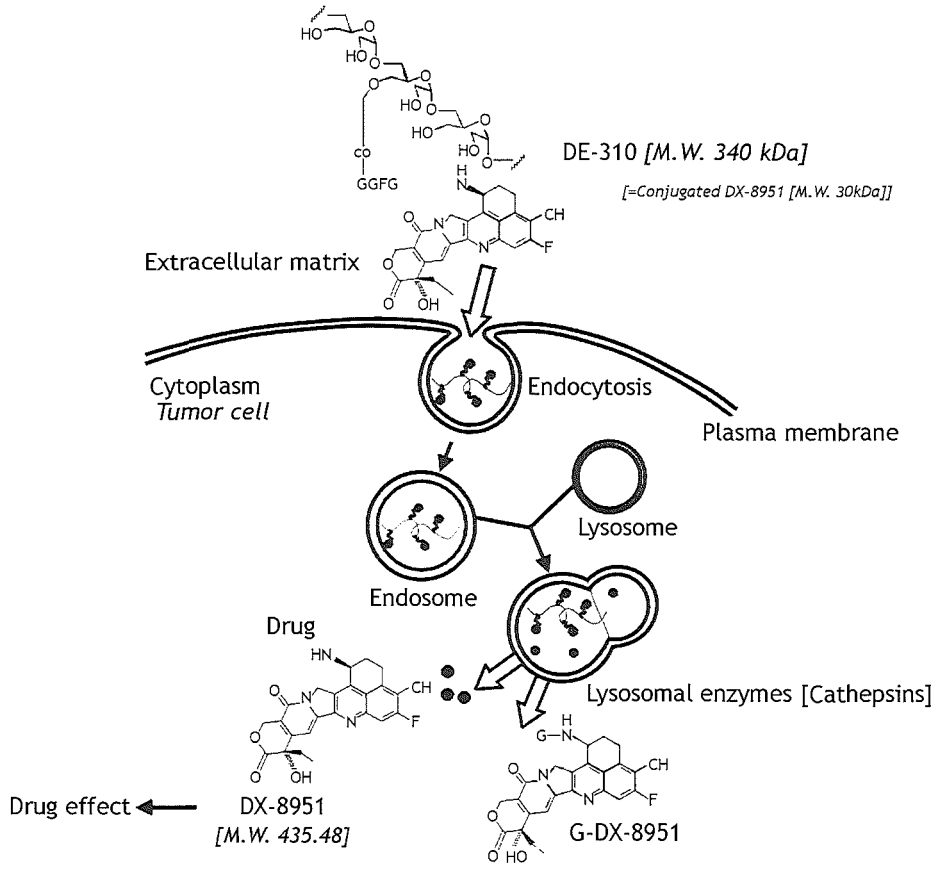

Figure 1. Schematic representation of intracellular drug release by cleavage of the peptidyl linker by lysosomal enzymes. After internalization of the macromolecular carrier DE-310 [MW 3.4×10^5] by endocytosis, the macromolecule is transferred to the lysosomal compartment, where it is exposed to lysosomal enzymes [*i.e.*, cathepsin B and cathepsin L]. Both glycyl-DX-8951 and free DX-8951 [MW 435.48] are liberated intracellularly. The biodegradable polymer is metabolized and afterwards excreted renally.

The cytotoxicity of topoisomerase I inhibitors in animal models increases with duration of exposure, and long-term exposures to low concentrations are more effective than short-term exposures to high concentrations [2]. The concept behind the development of DE-310 is that the macromolecular carrier will accumulate and be retained preferentially in tumor tissue by an enhanced permeability and retention (EPR) effect [3-5].

Preclinical studies have revealed that the macromolecular carrier is stable in plasma and is resistant to clearance by the reticuloendothelial system. DE-310 showed antitumor activity in various tumor models by single and repeated administration [1], and antitumor activity was noted at lower doses than with DX-8951. The two DE-310 breakdown products, DX-8951 and glycyl-DX-8951, were found to exert antitumor activity *in vivo*, whereas the CYP3A4 and CYP1A2-mediated DX-8951 metabolites referred to as UM-1 and UM-2 were less potent [6, 7, 9].

Previous phase I clinical studies with various administration schedules of DX-8951f have shown reversible, noncumulative, and dose-dependent neutropenia and sometimes thrombocytopenia [10-15]. Other side effects included mild to moderate gastrointestinal toxicity (nausea, vomiting, stomatitis, diarrhea), fatigue, asthenia and alopecia [10-15]. Reversible, noncumulative, neutropenia was the dose-limiting toxicity (DLT) in all schedules [10-14], as well as transient and reversible liver dysfunction [11, 13], or stomatitis in advanced leukemia [15].

The objectives of this phase I study were (*a*) to determine the maximum-tolerated dose (MTD) and dose-limiting toxicities (DLTs) of DE-310 when administered intravenously once every 2 or 6 weeks, (*b*) to characterize the pharmacokinetics of DE-310 (as conjugated DX-8951) and its metabolites DX-8951 and glycyl-DX-8951, and (*c*) to evaluate preliminary antitumor activity.

Patients and methods

Eligibility Criteria

Patients with a histologically confirmed diagnosis of a malignant solid tumor refractory to conventional chemotherapy or for whom no effective therapy existed were eligible. Other eligibility criteria included the following: age ≥ 18 years; Eastern Cooperative Oncology Group performance status 0 - 2; estimated life expectancy of ≥ 8 weeks; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas, mitomycin-C or carboplatin); and adequate hematopoietic (absolute white blood cell count (WBC) > 3,000/μL, absolute

neutrophil count (ANC) > 1,500/μL, platelet count > 100 × 10^9/L and hemoglobin > 8.5 g/dL (or 5.2 mmol/L)), hepatic (serum total bilirubin < 1.5 mg/dL (25.6 μmol/L), serum aspartate transaminase (ASAT), and alanine transaminase (ALAT) < 2.5 times the institutional upper normal limit (UNL) (< 5.0 times UNL in case of liver metastases)), and renal (serum creatinine concentration < 1.5 mg/dL or if raised between 1.5 - 2.0 mg/dL, then a creatinine clearance of > 60 mL/min) function. Specific exclusion criteria included symptomatic brain metastases, active or uncontrolled infection, known allergy to camptothecin derivatives, concomitant treatment with CYP 3A4 inhibitors or inducers (wash-out period of at least 7 days since last intake), or diarrhea (more than 2 - 3 stools/day above normal frequency during the past 4 weeks). The institutional Ethical Board approved the study protocol. All patients gave written informed consent before study entry.

Treatment and Dose-escalation

DE-310 for injection (37.9 mg of conjugated DX-8951 (*i.e.*, equivalent to 2.5 mg of DX-8951) and 165 mg maltose-monohydrate per 5 mL vial) was provided as a lyophilized powder, and was stored in a refrigerator at 2 - 8 °C and protected from light in a closed package. The drug was supplied by Daichii Pharmaceutical Co., Ltd. (Tokyo, Japan). DE-310 was reconstituted with 5 mL of 0.9% sodium chloride to obtain a concentration of 0.5 mg DX-8951 equivalent/mL solution and then filtered. The appropriate volume of stock solution needed to yield the required dose was diluted in a polyvinylchloride infusion bag with 0.9% sodium chloride to 250 mL. This dilution was stable for at least 24 hours when protected from light. The diluted drug product was administered protected from light over a period of 3 hours, using a programmed peristaltic pump system. With the exception of the first and second administration of the first and second cycle of the q2w schedule and the first and second cycle of the q6w schedule, respectively, in which patients were hospitalized for pharmacokinetic blood sampling, patients were treated on an outpatient basis. Prophylactic therapy was prohibited for the first administration, but could be given from the second administration if treatment was delayed (> 24 h) or when constant nausea and vomiting occurred. Prophylactic growth factors were not allowed.

The starting dose of DE-310 was 1.0 mg/m^2, and each infusion was followed by a two weeks recovery period for every 28 days of treatment (q2w schedule), and was based on 1/6 of the highest non-severe toxic dose found in the dog. In the q2w schedule, cycles were repeated every 28 days. After protocol amendment to a six-weekly schedule, based upon available information from a parallel phase I

study using an every four-weeks schedule [16], the dose of DE-310 was escalated to 6.0 mg/m^2, followed by a six-weeks recovery period (q6w schedule). Further dose-escalations were based on the prior dose level toxicity.

At least three patients were entered at each dose level. If one of three patients experienced DLT, three additional patients were to be entered at that dose level. The MTD was defined as one dose level below the dose that induced DLTs in at least 2 out of 6 patients, defined as the National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC) grade 4 neutropenia lasting for ≥ 5 days, neutropenic fever (defined as grade 4 neutropenia with fever ≥ 38.5°C), thrombocytopenia less than 25×10^9/L, any other clinically significant grade 4 hematological toxicity, grade 3 or 4 vomiting despite maximum supportive care, any other grade 3 or 4 nonhematological toxicity, and for the q2w schedule inability to start the second administration of the first cycle or to start a second cycle after a 1-week delay because of unresolved toxicity [17]. Unresolved toxicity was defined as WBC < 3.0×10^9/L, ANC < 1.5×10^9/L, platelet count < 100×10^9/L, and all associated nonhematological toxicities (excluding alopecia) not yet recovered to grade 0 to 1. Intrapatient dose-escalation was not allowed. The treatment was resumed when the neutrophil count had recovered to ≥ 1.5×10^9/L, the platelet count to ≥ 100×10^9/L, and any other treatment-related toxicities were ≤ grade 1.

Treatment Assessment

Before initiating therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell count (CBC), and serum biochemistry were performed, as were ECG, urinalysis, and chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the NCI-CTC, serum biochemistry and urinalysis. CBCs were determined twice weekly throughout every cycle. Tumor evaluation was performed after every two cycles in the q2w schedule, and after every cycle in the q6w schedule, respectively, according to RECIST [18].

Sample Collection and Drug Analysis

For pharmacokinetic analysis, a total of 40 blood samples (~6 mL each) over a period of 8 weeks for the q2w schedule, and over a period of 12 weeks for the q6w schedule, respectively, was obtained from an indwelling intravenous canula and collected into a heparin-containing tube. In the q2w schedule, samples were taken immediately prior to drug administration of DE-310, at the end of infusion, and at 1, 2, 4, 6, 8, 24, 48, 96, and 144 hours post-end of infusion. In

the q6w schedule, samples were taken immediately prior to drug administration of DE-310, and at 1, 2, 4, 6, 8, 24, 48, 72, 168, 240, 336, 504, 672 and 840 hours post-end of infusion. Blood specimens were immediately put in an ice-water bath (4°C) until centrifugation at 2000 rpm for 15 minutes at 4°C. Plasma was transferred into plastic specimen storage vials and stored frozen at -20°C until analysis. Also, urine was collected for pharmacokinetic analysis over a 24-hour period on days 1 - 8 in the first 2 cycles of the q2w schedule and over a 24-hour period on days 1 - 2, and over a 12-hour period on days 3, 7, 14, 21, 28, 35, and 42 in the first two cycles of the q6w schedule. Urine samples were stored frozen at -20°C until analysis.

The plasma concentrations of conjugated DX-8951, glycyl-DX-8951 and DX-8951 were quantified by a validated liquid chromatographic method (MDS Pharma Services, Montreal, Canada). Concentrations of conjugated DX-8951 were determined using a thermolysin enzymatic reaction and fluorescence detection. Samples of saliva and erythrocytes, normal skin, and superficial tumor tissue, if possible, were obtained from patients at the dose levels 6.0 and 7.5 mg/m^2. Saliva was obtained during the first cycle prior to drug administration, at the end of infusion, 4 hours post-end of infusion, and once a day on days 2, 3, 4, 8, 11, 15, 22, 29, and 36. Samples of erythrocytes were collected at the time points indicated for plasma. The concentrations of conjugated DX-8951, glycyl-DX-8951 and DX-8951 in saliva, erythrocytes and ascites were quantified by a validated method as described previously [19]. Normal skin and tumor tissue was obtained on day 8 or 9 of the first cycle and analyzed as described for plasma (Shin-Nappon Biomedical Laboratory Ltd., Kainan, Japan).

Pharmacokinetic and Pharmacodynamic Data Analysis

Pharmacokinetic parameters were calculated by standard non-compartmental methods using WinNonlin software version 3.3 (Pharsight, Mountain View, CA, USA), and included time to peak concentration (T_{max}), peak concentration (C_{max}), terminal half-life ($T_{½}$), and area under the curve (AUC). Urine excretion was calculated in percentage of the dose administered. Pharmacokinetic parameters are provided as mean values with the percent coefficient of variation, and the level of significance (P) for statistical tests was set at 0.05.

Results

Patients and Treatment

A total of twenty-seven patients (16 males and 11 females) with a median age of 57 years was enrolled into the study (Table 1). All patients were eligible and assessable for toxicity. A total of 61 cycles (*i.e.*, 86 administrations) of treatment was given at dose-levels of 1.0 mg/m^2 (q2w), 2.0 mg/m^2 (q2w), 6.0 mg/m^2 (q6w), 7.5 mg/m^2 (q6w), and 9.0 mg/m^2 (q6w). Twenty-five patients were assessable for response; one patient died due to massive pulmonary embolism before scheduled tumor reassessment, and another patient withdrew because of rapid disease progression and was replaced (*i.e.*, dose-level 6.0 mg/m^2).

Dose-Limiting Toxicity

At the starting dose of 1.0 mg/m^2, the cohort was expanded to 6 patients because of the initial classification of DLT due to prolonged grade 1 thrombocytopenia in one patient, delaying the second administration of DE-310 of the first cycle. In retrospect, this mild thrombocytopenia was related to occult gastrointestinal bleeding from cirrhosis induced esophageal varices. No further DLTs were observed with this schedule. Since pharmacokinetics revealed a drug terminal half-life exceeding the 2 weeks administration interval, the protocol was amended to a 6 weeks interval between administrations.

The initial 3 patients at the q6w schedule were treated at a dose of 6.0 mg/m^2 and experienced no DLTs. Subsequently, a dose of 9.0 mg/m^2 was explored, where two patients developed DLT. A female patient with metastatic pancreatic cancer had grade 4 leukocytopenia, and grade 4 neutropenia with fever on day 8, as well as grade 3 thrombocytopenia, grade 2 anemia, and grade 3 hepatotoxicity (elevated transaminases). All side effects were reversible. The second and third cycles were given at a dose of 6.0 mg/m^2, and were uneventful. A second female patient with metastatic adenocarcinoma of unknown primary (ACUP) had an uncomplicated first cycle, but on day 11 of her second cycle she developed grade 3 thrombocytopenia, grade 3 neutropenia, grade 2 anemia, grade 3 hepatotoxicity (*i.e.*, hyperbilirubinemia and elevated transaminases). An ultrasound guided liver biopsy revealed veno-occlusive liver disease (VOD). Hyperbilirubinemia persisted for 7 weeks and subsequently recovered but no further DE-310 was given. In view of the DLTs observed at 9.0 mg/m^2, the cohort of 6.0 mg/m^2 was further expanded with 3 additional patients, none of whome experienced DLTs.

Table 1. Patient characteristics	
Characteristic	**No. of Patients**
No. of Patients	
Total	27
Assessment	
For Dose-Limiting Toxicity	27
For Efficacy	25
Gender, male : female	16 : 11
Age, years	
Median	57
Range	31 - 78
ECOG performance status	
0	7
1	19
2	1
Previous therapy	
Chemotherapy only	19
Median no. of regimens	2
Range	1 - 4
Radiotherapy	2
Both	3
None	3
Tumor types*	
Gastro-intestinal tract, including:	12
Colorectal cancer	6
Esophageal cancer	3
Pancreatic cancer	3
Melanoma	5
Lung cancer (NSCLC)	3
Soft tissue sarcoma	3
Unknown primary adenocarcinoma	2
Miscellaneous	4

Note: *Two patients had double tumors.
Abbreviations: no., number; ECOG, Eastern Cooperative Oncology Group; NSCLC, non small cell lung cancer.

Subsequently, an intermediate dose of 7.5 mg/m^2 was explored. At this dose level, one out of six patients developed DLT consisting of grade 3 hepatotoxicity (*i.e.*, hyperbilirubinemia and elevated transaminases) occurring on day 15 of the second cycle. An ultrasound guided liver biopsy again revealed VOD, which was fully reversible within 15 weeks. The recommended dose for phase II trials of DE-310 was set at 7.5 mg/m^2 once every 6 weeks.

Hematologic and Nonhematologic Toxicity

A summary of the worst grade hematologic toxicities per patient is provided in Table 2. In addition to the dose-limiting toxicities, the other observed nonhematologic toxicities were only mild to moderate and consisted of nausea, vomiting, stomatitis, anorexia, asthenia, and alopecia (Table 3). Fifty-one percent of patients experienced short lasting nausea, and 37% experienced incidental vomiting. Prophylactic anti-emetics were not given. Reversible increase of the liver transaminases was encountered in 12 out of 27 patients (44%) across all dose levels, except 1.0 mg/m^2 (Table 4).

Pharmacokinetics

Pharmacokinetic analysis was performed in all patients. Across the various dose levels, the apparent terminal half-lives of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were 13, 12, and 13 days, respectively (Table 5A and 5B). Consequently, plasma concentrations of all compounds conjugated were sustained for several weeks. This parallel decline suggests that the terminal phase seen with the DE-310 metabolites (*i.e.*, glycyl-DX-8951 and DX-8951) is not due to real elimination-processes but due to their much slower formation from conjugated DX-8951. At the recommended dose of 7.5 mg/m^2, substantial interindividual variability was observed (coefficient of variation, up to 92%). The AUC ratio of conjugated DX-8951-to-DX-8951 was approximately 600, a similar value seen as predicted by preclinical studies [1]. The AUCs of conjugated DX-8951, glycyl-DX-8951, and DX-8951 appeared to increase in near proportion with an increase in dose.

Urinary excretion was very slow, and total drug recovery over the entire sampling period amounted to less than 1%. In line with the small distribution volume of conjugated DX-8951, concentrations of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were not detectable in erythrocytes and saliva, further pointing to limited distribution outside the plasma compartment. In tumor biopsies of four patients, concentrations of both glycyl-DX-8951 and DX-8951 were detectable at low levels, whereas in normal skin biopsies of nine patients concentrations of DX-8951 remained below the detection limit (Table 6).

Table 2. Hematological toxicity [worst grade per patient]

				Anemia		Leukocytopenia				Neutropenia				Thrombocytopenia		
				Grades												
Dose [mg/m^2]	No. of pts.	No. of cycles	No. of adm.	1	2	1	2	3	4	1	2	3	4	1	2	3
1.0 [q2w]	6	13	24	0	1	0	0	0	0	0	0	0	0	2	0	0
2.0 [q2w]	4	16	30	0	2	0	0	0	0	0	0	0	0	0	0	0
6.0 [q6w]	7	17	17	1	2	2	0	0	0	2	0	0	0	1	0	0
7.5 [q6w]	6	10	10	2	3	0	1	0	0	0	0	1	0	0	1	1
9.0 [q6w]	4	5	5	0	2	2	1	0	1[a]	2	0	1	1[a]	0	0	2[a]

Note: [a] considered DLTs in 2 patients at dose level 9.0 mg/m^2 [q6w] in the first and second cycle, respectively.
Abbreviations: no., number, pts., patients, adm., administrations; q, every; w, weeks.

Table 3. Nonhematological toxicity [worst grade per patient]

				Nausea		Vomiting		Stomatitis		Anorexia		Fatigue		Alopecia	
				Grades											
Dose [mg/m^2]	No. of pts.	No. of cycles	No. of adm.	1	2	1	2	1	2	1	2	1	2	1	2
1.0 [q2w]	6	13	24	1	0	1	0	0	0	0	0	2	1	0	0
2.0 [q2w]	4	16	30	2	1	2	1	0	0	0	0	1	2	1	0
6.0 [q6w]	7	17	17	5	0	0	3	2	1	3	1	5	2	1	0
7.5 [q6w]	6	10	10	2	1	0	1	1	0	3	0	6	0	1	0
9.0 [q6w]	4	5	5	2	0	2	0	0	0	1	0	2	1	1	0

Abbreviations: no., number, pts., patients, adm., administrations; q, every; w, weeks.

Table 4. Hepatotoxicity [worst grade per patient]

				ALAT			ASAT			Total bilirubin		
				Grades								
Dose [mg/m^2]	No. of pts.	No. of cycles	No. of adm.	1	2	3	1	2	3	1	2	3
1.0 [q2w]	6	13	24	0	0	0	0	0	0	0	0	0
2.0 [q2w]	4	16	30	0	1	0	1	1	0	1	0	0
6.0 [q6w]	7	17	17	2	0	0	4	0	0	0	0	0
7.5 [q6w]	6	10	10	0	2	1[a]	0	1	2[a]	1	1	1[a]
9.0 [q6w]	4	5	5	1	0	2[b]	1	1	2[b]	0	0	1[a]

Note: [a] considered a DLT in 1 patient at dose level 7.5 mg/m^2 [q6w], and in 1 patient at dose level 9.0 mg/m^2 [q6w] in the second cycle; [b] considered a DLT in 1 patient at dose level 9.0 mg/m^2 [q6w] in the first cycle.
Abbreviations: ALAT, alanine transaminase; ASAT, aspartate transaminase; no., number, pts., patients, adm., administrations; q, every; w, weeks.

Table 5A. Mean [CV%] pharmacokinetic parameters of conjugated DX-8951, glycyl-DX-8951, and DX-8951 of 1st administration at 1st course in q2w schedule and at 1st course in q6w schedule

Dose [mg/m²]		Conjugated DX-8951					Glycyl-DX-8951					DX-8951				
		AUC_{0-t} [ng·h/mL]	AUC_{0-336h} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{½}$ [h]	AUC_{0-t} [ng·h/mL]	AUC_{0-336h} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{½}$ [h]	AUC_{0-t} [ng·h/mL]	AUC_{0-336h} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{½}$ [h]
q2w																
1.0 [N=6]	mean	38402.0[a]	n/a	460.8	5.3	n/a	238.5	n/a	1.54	132.0	n/a	158.6	n/a	0.87	135.0	n/a
	CV%	67.7		53.8	47.6		67.8		71.7	51.9		72.5		71.7	44.6	
2.0 [N=4]	mean	61104.0[b]	n/a	582.6[b]	4.0[b]	n/a	515.8[c]	n/a	2.23[b]	99.0[a]	n/a	366.6[c]	n/a	1.6	87.0	n/a
	CV%	n/a		n/a	n/a		130.2		123.7	39.6		111.7		100.7	52.8	
q6w																
6.0 [N=7]	mean	661924.4	498855[d]	3344.7	5.0	243.4	1731.2	929.2[d]	6.71	251.0	348.4	960.4	642.8[d]	3.2	150.4	260.8
	CV%	45.5	36.0	17.5	38.3	40.8	55.5	60.0	93.3	78.9	47.0	39.2	34.7	56.6	50.8	21.1
7.5 [N=6]	mean	1124472.3	793120.2	5706.3	4.7	338.8	3907.2	2492.4	11.6	123.0	330.2	1781.1	1168.6	5.2	151.0	302.0
	CV%	19.4	20.1	18.9	50.1	18.3	90.1	92.6	93.7	42.7	78.5	78.9	83.6	84.5	68.5	54.0
9.0 [N=4]	mean	902743.5	629527.3	4218.9	5.5	309.5	3178.7	2299.6	12.6	183.0	186.3	2062.8	1479.0	6.9	135.0	169.5
	CV%	60.3	56.5	33.8	54.5	37.3	71.5	89.9	118.0	71.4	16.2	53.2	56.3	66.8	65.7	18.4

Note: AUC_{0-t}: AUC calculated from time 0 to the last non-zero concentration; AUC_{0-336h}: AUC calculated from time 0 to the concentration at time 336h post end of infusion, where possible. Since the distribution phase of glycyl-DX-8951 and DX-8951f from conjugated DX-8951 and the glycyl-DX-8951 and DX-8951 elimination phase could be simultaneously occurring over the dosing interval, a true terminal elimination phase cannot be accurately characterized using non-compartimental methods. An apparent elimination phase half-life [$T_{½}$ app.] is therefore reported here; [a] calculated on data from 4 pts.; [b] calculated on data from 1 pt.; [c] calculated on data from 3 pts.; [d] calculated on data from 6 pts.

Abbreviations: CV, coefficient of variation; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life of terminal phase; q, every; w, weeks; n/a, not available; pts., patients.

Table 5B. Mean [CV%] pharmacokinetic parameters of conjugated DX-8951, glycyl-DX-8951, and DX-8951 of 2nd administration at 1st course in q2w schedule and at 2nd course in q6w schedule

Dose [mg/m²]		Conjugated DX-8951					Glycyl-DX-8951					DX-8951				
		AUC_{0-t} [ng·h/mL]	AUC_{0-336h} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{½}$ [h]	AUC_{0-t} [ng·h/mL]	AUC_{0-336h} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{½}$ [h]	AUC_{0-t} [ng·h/mL]	AUC_{0-336h} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{½}$ [h]
q2w																
1.0 [N=4]	mean	31256.5	n/a	324.9	3.8	n/a	321.7	n/a	3.8	36.5	n/a	321.8	n/a	4.9	51.0	n/a
	CV%	114.8		72.9	25.5		111.5		134.4	116.3		66.7		98.5	66.6	
2.0 [N=3]	mean	221846.0	n/a	1230.3	3.5	n/a	834.5[a]	n/a	4.0[a]	292[a]	n/a	631.0[a]	n/a	3.1[a]	326[a]	n/a
	CV%	75.1		42.8	16.5		93.9		112.7	143.1		86.2		115.7	119.9	
q6w																
6.0 [N=5]	mean	750514.6	519098.0	3541.4	6.0	295.8	2989.8	1540.0	7.5	247.8	226.2	1202.7	727.3	2.9	180.6	210.2
	CV%	32.6	25.6	22.0	47.1	19.7	100.4	72.4	98.4	64.8	36.7	68.5	54.1	54.8	38.3	25.9
7.5 [N=3]	mean	1180980.0	813841.0	5081.0	6.3	418.3	13080.3	6324.2	33.4	179.0	152.7	4032.8	2484.3	10.5	163.0	210.7
	CV%	42.7	39.0	13.2	63.8	38.2	102.8	98.0	110.9	87.2	7.7	66.7	61.8	56.2	51.7	4.8
9.0 [N=2][b]	mean	131375.1	920467.3	4868.5	15.0	267.0	13893[c]	3814.9[c]	36.5[c]	339[c]	616[c]	7039.3[c]	2811.5[c]	19.8[c]	339[c]	181[c]
	CV%	71.7	73.8	55.7	113.1	4.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Note: AUC_{0-t}: AUC calculated from time 0 to the last non-zero concentration; AUC_{0-336h}: AUC calculated from time 0 to the concentration at time 336h post end of infusion, where possible. Since the distribution phase of glycyl-DX-8951 and DX-8951f from conjugated DX-8951 and the glycyl-DX-8951 and DX-8951 elimination phase could be simultaneously occurring over the dosing interval, a true terminal elimination phase cannot be accurately characterized using non-compartimental methods. An apparent elimination phase half-life [$T_{½}$ app.] is therefore reported here; [a] calculated on data from 3 pts.; [b] dose reduction from 9.0 to 6.0 mg/m² [q6w] for 1 pt. in course 2; [c] calculated on data from 1 pt.
Abbreviations: CV, coefficient of variation; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life of terminal phase; q, every; w, weeks; n/a, not available; pts., patients.

Table 6. Concentrations [ng/g] of DE-310 [as conjugated DX-8951], G-DX-8951 and DX-8951 in normal skin tissues [Normal] and tumor biopsies [Tumor]

Patient [Tumor type]	Dose [mg/m²] [q6w]	Time of biopsy at first cycle [day]	Normal *vs* Tumor	Biopsy of Tumor type	Weight of sample [mg]	Conjugated DX-8951	G-DX-8951	DX-8951
19 [Sarcoma]	6.0	8	Normal		5.8	BLQ	BLQ	BLQ
20 [Colorectal]	6.0	8	Normal		9.8	BLQ	62.731	BLQ
21 [Melanoma]	6.0	9	Normal		32.7	BLQ	24.524	BLQ
			Tumor	Melanoma	153.5	166.159	134.457	8.037
22 [Melanoma]	7.5	9	Normal		18.9	BLQ	53.448	BLQ
			Tumor	Melanoma	2294.1	303.557	155.276	4.188
23 [Melanoma]	7.5	8	Normal		15.9	BLQ	59.173	BLQ
			Tumor	Melanoma	2223.0	222.019	119.103	4.157
24 [NSCLC]	7.5	8	Normal		9.3	BLQ	10.870	BLQ
25 [ACUP]	7.5	9	Normal		9.2	BLQ	5.617	BLQ
26 [Sarcoma]	7.5	8	Normal		27.2	BLQ	8.419	BLQ
		9	Tumor	Sarcoma	414.2	160.690	54.202	2.159
27 [Melanoma]	7.5	8	Normal		29.1	BLQ	10.199	6.613

Abbreviations: G-DX-8951, glycyl-DX-8951; vs, versus; q, every; w, weeks; BLQ, below limit of quantitation; NSCLC, non small cell lung cancer; ACUP, adenocarcinoma unknown primary.

Table 7. Concentrations [ng/mL] of DE-310 [as conjugated DX-8951], G-DX-8951, and DX-8951 in plasma and ascites in one patient with metastatic pancreatic cancer [dose 6.0 mg/m² [q6w]]

		Plasma			Ascites		
Cycle	Day	Conjugated DX-8951	G-DX-8951	DX-8951	Conjugated DX-8951	G-DX-8951	DX-8951
1	1	2987.9[b]	2.333[c]	0.545[c]	n.d.	n.d.	n.d.
	15	424.3	1.974	0.448	1401.97	3.66	0.61
	29	119.3	0.610	0.149	382.18	0.46	0.11
	36	BLQ	0.352	0.084	232.82	BLQ	BLQ
	39	n.d.	n.d.	n.d.	197.51	BLQ	BLQ
2	1	4067.1[a]	2.643[c]	0.785[c]	172.49	BLQ	BLQ
	4	1759.9	7.912	1.578	3164.66	6.74	0.67
	18	n.d.	n.d.	n.d.	572.32	1.64	0.21
	22	305.9	0.948	0.181	466.62	1.10	0.18
	30	BLQ	0.321	0.073	103.1	BLQ	BLQ

Note: maximum concentration at [a]2, [b]4, and [c]24 hrs. post end of infusion.
Abbreviations: G-DX-8951, glycyl-DX-8951; q, every; w, weeks; n.d., not determined; BLQ, below limit of quantitation (for ascites < 0.5 ng/mL).

During the first and second cycle at 6.0 mg/m^2 the concentrations were also assessed in ascites of a patient with metastastic pancreatic cancer (Table 7).

Efficacy

A histologically proven complete remission, confirmed by surgery and still persisting at a follow up duration of more than 1 year, was documented in a 56-year old female with lymph node metastases of ACUP, who experienced DLT at dose level 9.0 mg/m^2 and only received 2 cycles.

A partial response lasting 3 months was achieved in a 46-year old female with liver metastases from pancreatic cancer initially treated at 9.0 mg/m^2 during the first cycle, and treated at 6.0 mg/m^2 during the second and third cycle. While according to actual measurements, another partial remission of liver metastases was seen in a 58-year old female with metastatic ovarian cancer, treated at 2.0 mg/m^2 for 7 cycles, we downstaged this response to stable disease since the liver metastases were small and difficult to measure. Progression occurred in this patient after 8 months. A total of 14 patients demonstrated disease stabilization for 6 weeks (wks) (N=1; fibrous histiocytoma), 8 wks (N=1; colorectal cancer (CRC)), 10 wks (N=1; CRC), 12 wks (N=5; pancreatic cancer (N=2), CRC, ACUP and melanoma (each N=1), 16 wks (N=1; urothelial cancer), 18 wks (N=2; NSCLC and leiomyosarcoma uteri), 24 wks (N=2; CRC and alveolar soft part sarcoma), and 32 wks (N=1; ovarian cancer).

Discussion

We performed this study to assess the safety, tolerability, MTD, and pharmacokinetics of DE-310, a macromolecular prodrug of the topoisomerase I inhibitor exatecan (DX-8951f), administered as a 3-hour intravenous infusion once every 2 or 6 weeks.

The present study showed that prolonged concentrations of DX-8951 were achieved by only single short infusion of DE-310 given every 6 weeks, which confirmed the pharmacokinetic proof of principle yielding the extended exposure to the topoisomerase I inhibiting active moiety.

Because of the long half-life, which approximated a 2 weeks administration interval and largely exceeded the half-life expected based on preclinical data, further exploration of the q2w schedule was considered illogical in view of the potential for drug accumulation, and therefore the study protocol was amended to a 6 weeks schedule. The pharmacokinetics showed near dose proportionality of conjugated DX-8951, glycyl-DX-8951, and DX-8951.

Reversible grade 4 myelosuppression and grade 3 hepatotoxicity with VOD were the principal DLTs and were observed at dose levels 9.0 mg/m^2 (2 out of 4 patients) and 7.5 mg/m^2 (1 out of 6 patients), respectively. It is of interest to note that the AUCs of conjugated DX-8951, glycyl-DX-8951 and DX-8951 were 10-fold higher in the second cycle of the two patients with resulting VOD compared to the other patients at the same dose levels. The reason for this large interpatient variation in pharmacokinetics remains to be elucidated. The recommended dose for phase II studies is 7.5 mg/m^2 once every 6 weeks. At this dose and schedule, circulating concentration of the active moiety DX-8951 after a single infusion of DE-310 were similar to those achieved at the MTD of a 21-day continuous infusion of DX-8951f at 0.15 mg/m^2/day (Figure 2) [20].

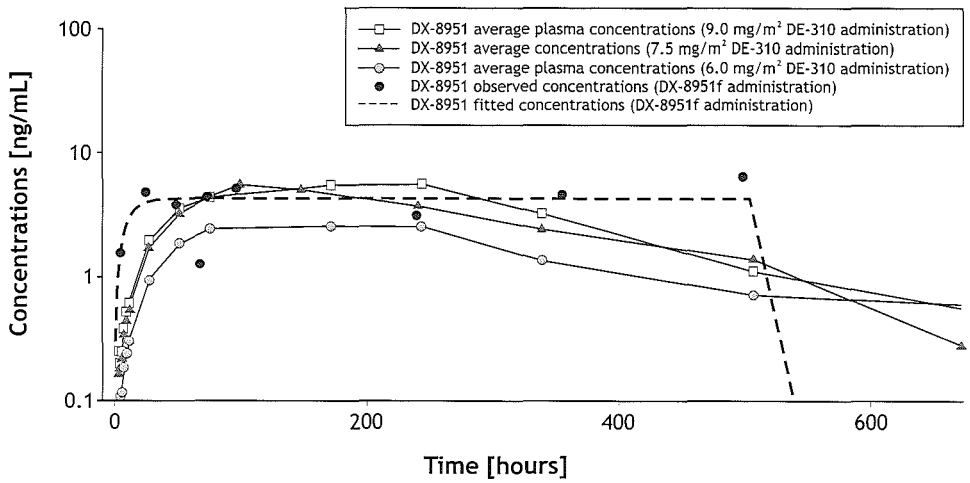

Figure 2. Comparative exposure of 21-day continuous infusion of 0.15 mg/m^2/day of DX-8951f [exatecan] [20] *versus* single 3-hour infusions of increasing doses of DE-310

Other nonhematalogic toxicities attributed to the treatment of DE-310 included mild to moderate nausea, vomiting, stomatitis, anorexia, asthenia, and alopecia, a toxicity profile similar to that of single agent exatecan mesylate (DX-8951f) [10-14].

The observed myelosuppression was an expected side effect, known to be a class-effect of topoisomerase I inhibitors. The nadirs of ANC and platelets were reached at 22 days (range, 18 - 29 for ANC, and 11 - 36 for platelets) post infusion.

Myelosuppression was always fully reversible after a median of 14 days (range, 3 - 23) post nadir. In view of the presence of circulating active drug moiety at the time of nadir, and the conceptual likelihood that colony-stimulating growth factors could thus actually increase severity and duration of myelosuppression by stimulating stem cells while exposed to cytotoxics, the administration of agents like G-CSF was specifically avoided.

The other DLT was hepatotoxicity consisting of reversible increases in liver transaminases and bilirubin, and in 2 patients correlated with presence of hepatic VOD. Hepatic VOD results from toxic injuries to the hepatic sinusoids (zone 3 of the liver acinus), followed by a series of biologic processess that lead to circulatory compromise of centrilobular hepatocytes, fibrosis, and obstruction of liver blood flow [21, 22]. Histology showed diffuse damage in the centrilobular zone of the liver, in combination with sinusoidal fibrosis, necrosis of pericentral hepatocytes, narrowing and fibrosis of central veins. Although drug-induced VOD is most frequently observed following stem cell transplantations with high-dose busulfan and cyclofosfamide, it can occur after various regimens of chemotherapy at conventional doses (*e.g.*, actinomycin D, dacarbazine, cytosine arabinoside, mitramycin, and 6-thioguanine), combination chemotherapy, or (total body) irradiation. It has been suggested that drugs or toxins can induce depletion of glutathione leading to cell death and yielding increased activity of matrix metalloproteinases, resulting in degradation of the extracellular matrix and loss of sinusoidal endothelial cells from the space of Disse [21, 22].

Hepatotoxicity was also reported in a phase I study of N-(2-hydroxypropyl)-methacrylamide (HPMA) copolymer conjugates of doxorubicine (PK1) [23], but not for MAG-camptothecin (MAG-CPT) [24], and the phase I study of pegylated-camptothecin (PEG-CPT) [25]. It therefore remains unclear if the carrier is responsible for this phenomenon, or whether both carrier and active drug are responsible. While VOD is a severe side effect, the fact that even the most severe cases of hepatotoxicity in our trial showed full reversibility indicates that with proper monitoring the drug DE-310 can be safely used at the recommended dose of 7.5 mg/m^2 every 6 weeks.

Concentrations of conjugated DX-8951, glycyl-DX-8951, and DX-8951 were determined in ascites samples in a patient with advanced pancreatic disease treated at dose level 6.0 mg/m^2. These data indicated significant exposure of the peritoneal cavity to conjugated DX-8951, glycyl-DX-8951, and DX-8951 at concentrations exceeding those in simultaneously obtained plasma samples.

The results of studies assessing DE-310, glycyl-DX-8951 and DX-8951 in normal skin, erythrocytes, and saliva would seem to fit with the long circulation times combined with the formation rate-limited elimination and the hypothesis of preferential drug uptake by tumor tissue and not in other tissues. However the

sample size and available data are too limited to draw definitive conclusions. Notwithstanding, it can be concluded that the biodistribution of this polymerized agent is substantially different from that of the registered topoisomerase I inhibitors topotecan [26], and irinotecan and its metabolite SN-38 [27].

In summary, by employing a polyalcohol carrier with cleavable linking peptide as a drug-delivery system, the pharmacokinetics profile of the topoisomerase I inhibitor DX-8951f seems to be improved, providing slow release of the active moiety over a very extended period. This together with the presence of discernable drug levels in tumor and the below limits of quantitation drug levels in normal tissues provides supportive evidence to the validity of the concept of using macromolecular carriers to enhance the potential efficacy and diminish the toxicity of topoisomerase I inhibitors and warrants further evaluation in phase II studies.

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Real-time pharmacokinetics guiding clinical decisions: Phase I study of a weekly schedule of liposome encapsulated paclitaxel in patients with solid tumours.

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Abstract

The purpose of this weekly schedule phase I study of liposome encapsulated paclitaxel (LEP) was to define the maximum-tolerated dose (MTD), the recommended dose (RD), the dose-limiting toxicities (DLTs), the pharmacokinetic profiles, and to evaluate preliminary antitumour effects in patients with refractory solid malignancies. LEP was administered as an intravenous infusion over 45 minutes once every week for 6 out of 8 weeks. Fourteen patients were treated at doses ranging from 90 to 150 mg/m^2/week. In one patient DLT was observed at dose level 150 mg/m^2/week, who received less than 70% of the intended cumulative dose. No cumulative toxicities were observed. Stabilization of disease for 8 weeks was documented in two patients. The whole blood clearance of total paclitaxel was similar for LEP (15.3 ± 8.98 L/h/m^2) and Taxol® (17.5 ± 3.43 L/h/m^2), and the extraliposomal-to-total-drug ratio increased rapidly to unity at later sampling time points. The trial was discontinued upon completion of enrolment of the 150 mg/m^2/week cohort because an assessment of the pharmacokinetics and clinical data suggested that LEP was unlikely to have any advantages over Taxol®. It is concluded that this formulation of LEP is unlikely to provide improvements over taxanes currently in clinical use.

1. Introduction

Paclitaxel, a complex diterpenoid natural product derived from the bark of the Western yew tree, *Taxus brevifolia*, belongs to the class of anti-microtubule agents and is active in a broad variety of human malignancies, including breast, ovarian and non-small cell lung cancer. Due to the agent's poor solubility in aqueous solutions, paclitaxel is formulated for clinical use in a mixture of Cremophor EL and ethanol (Taxol®). Previous work has indicated that Cremophor EL contributes to the nonlinear pharmacokinetic behavior of paclitaxel and to severe hypersensitivity reactions in humans observed after the administration of Taxol®[1, 2]. The incidence of these severe hypersensitivity reactions is approximately 41% despite the use of pre-medication with corticosteroids and antihistamines (see: http://www.taxol.com). It has been proposed that the hypersensitivity reaction to Taxol® is caused by a Cremophor EL-mediated activation of the complement system [3]. The clinical formulation of Taxol® has also been associated with other side effects, including peripheral neurotoxicity [4].To overcome the problems associated with the current

formulation of paclitaxel, several chemical, pharmaceutical, and/or biological strategies are being explored to optimize chemotherapeutic treatment with paclitaxel [5]. One of the strategies is delivery of the drug by the use of liposomes (size ranging from 10 nm to 20 μm) consisting of an aqueous core surrounded by one or more membranes consisting of naturally or synthetic phospholipids arranged in a bilayer configuration [6, 7]. These spherical vesicles can encapsulate various therapeutic agents, including anticancer agents [8]. One rationale for encapsulating cytotoxic drugs in liposomes is based on the hypothesis that macromolecular (liposomal) carrier leakage will occur in tumour tissue due to its enhanced permeability and retention (EPR) effect [9,10]. This EPR effect is caused by discontinuation of the endothelium of tumour blood vessels, as a result of structural and functional anomalies, and the co-existing lack of a fully functional system of lymphatic drainage [11]. The interplay between these characteristics of tumour tissue can result in the extravasation and retention of liposomes within the tumour interstitium, with the potential for providing more active drug to tumour with less exposure to normal tissue.

Since liposome encapsulation is suitable for the intravenous delivery of poorly water soluble compounds, paclitaxel has also been proposed for administration in liposomes [8]. A liposome formulation (without Cremophor EL) could have considerable potential given the substantial liabilities associated with Cremophor EL. Several liposome-based formulations of paclitaxel have been tested *in vivo* for antitumour activity in various models [12-15], and for various liposome formulations the maximum-tolerated dose (MTD) was 2- to 7-fold greater than for Taxol® [12].

The encapsulation of cytotoxic agents into liposomes (*e.g.*, anthracyclines) has been shown to substantially modulate the pharmacokinetic behavior of these drugs [1]. This approach may enhance the efficacy of anticancer drugs and reduce their systemic toxicity through a lower exposure to the drug of normal tissues. The aim of this study was to define the MTD, RD, DLTs, pharmacokinetic profiles, and evaluate preliminary antitumour effects of a weekly schedule of LEP in patients with refractory solid malignancies.

2. Patients and methods

2.1 Patient selection

Patients with a histologically confirmed diagnosis of a malignant solid tumour refractory to conventional chemotherapy or for whom no effective therapy

existed were eligible. Other eligibility criteria included the following: age ≥ 18 years; Eastern Cooperative Oncology Group performance status ≤ 1; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosourea or mitomycin-C); and adequate haematopoietic (absolute neutrophil count (ANC) ≥ 1,500/μL, platelet count ≥ 100 × 10^9/L and hemoglobin ≥ 10.0 g/dL (or 6.2 mmol/L), hepatic (serum total bilirubin ≤ 1.5 mg/dL, and serum aspartate transaminase (ASAT), alanine transaminase (ALAT) ≤ 2.5 times the institutional upper normal limit (UNL) (≤ 5.0 times UNL in case of liver metastases), and renal function (serum creatinine concentration ≤ 1.5 mg/dL). Prior surgery or radiation therapy (irradiation field encompassing < 25% of bone marrow) was acceptable as long as it had been completed at least 4 weeks before study registration. Specific exclusion criteria included known hypersensitivity to Cremophor EL and/or paclitaxel-containing regimens, known brain metastases, spinal cord compression, and/or carcinomatous meningitis. The study protocol was approved by the institutional Ethical Board, and all patients gave written informed consent before study entry.

2.2 Treatment and dose-escalation

LEP was provided in vials containing 25 mg of paclitaxel per vial, and was supplied by the Pharmacia (Nerviano, Italy) as a freeze-dried product. The vials also contained cardiolipin, egg phosphatidyl choline, cholesterol, d-α-tocopheryl acid succinate (vitamin E), and mannitol as inactive ingredients. The addition of mannitol as a cryoprotectant to this liposomal formulation of paclitaxel ensured that sonication before the administration of the drug was not required. The vials were stored at 5°C in the dark, and were kept at room temperature for at least 2 hours before reconstitution. After that, 25 mL of 0.9% sodium chloride injection per 25 mg of paclitaxel were added to the LEP vials. The solution was injected in the middle of the lyophilized cake using a 50 mL sterile and pyrogen free syringe. The vials were gently shaken for 2 to 3 minutes. The reconstituted product was a sterile dispersion, and in-line filters were not used for administration. The content of the reconstituted vials was transferred to an infusion bag using a syringe, and the infusion bag was gently turned for 30 seconds before infusion.

LEP was given as a 45-minute infusion, preceded by pre-medication consisting of 20 mg dexamethasone, 2 mg clemastine, and 50 mg ranitidine, each administered intravenously 30 minutes before the initiation of LEP infusion. Prophylactic anti-emetics were not given. The treatment was administered every 7 days for 6 out of 8 weeks, unless the patient did not recover adequately from treatment-

related adverse events of prior infusions. A period of 8 weeks was defined as one cycle. The starting dose of LEP was 90 mg/m^2/week. This dose and schedule was selected on the basis of both clinical and pharmacokinetic data regarding weekly paclitaxel and from a phase I study of a sonicated preparation of LEP given as a single agent once every 3 weeks (unpublished data, Pharmacia). Subsequent dose levels scheduled were: 120 mg/m^2/week (33% dose increment), 150 mg/m^2/week (25%), and 180 mg/m^2/week (20%).

At least three patients were entered at each dose level. The MTD was defined as one dose level below the dose that induced DLTs during the first cycle in ≥ 2 out of 6 patients. DLTs were defined using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 and included: grade 4 neutropenia > 7 days, grade 4 haematological toxicity of any duration (except for grade 4 neutropenia), febrile neutropenia, non-haematological toxicities ≥ grade 3, severe hypersensitivity reaction suggestive of an anaphylactic reaction, and receiving less than 70% of the intended cumulative dose of LEP [17]. If grade 2 neutropenia and/or grade 2 thrombocytopenia occurred during treatment, the dose of LEP was decreased by 50% for the subsequent administration. In case of grade ≥ 3 neutropenia and/or thrombocytopenia, treatment with LEP was omitted for that week and then decreased by 50% for subsequent administrations when the neutrophil count had recovered to ≥ 1500/μL and the platelet count to ≥ 75 × 10^9/L. Intrapatient dose-escalation was not allowed.

2.3 Treatment assessment

Before initiating therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell (CBC) count, including haematology tests (hemoglobin, WBC with differential count, and platelets), and serum biochemistry, (sodium, potassium, calcium, magnesium, chloride, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, ASAT, ALAT, and gamma-glutamyltransferase) were performed, as were urinalysis (pH and albumin), ECG, and chest X-ray. Weekly evaluations included physical examination, toxicity assessment according to the NCI-CTC criteria, and haematology tests. Urinalysis was performed at week 1, 3 and 5 before administration of LEP. Tumour evaluation was performed after every cycle of 8 weeks and response was assessed according to RECIST [18]. Patients were discontinued from treatment in the case of disease progression, unacceptable toxicity, or patient request.

2.4 Sample collection and drug analysis

The pharmacokinetic of LEP was evaluated by following the time concentration profile of paclitaxel, measured both as total paclitaxel in blood (liposome-associated plus non-liposome-associated) and extraliposomal paclitaxel in plasma (non-liposome-associated, bound and unbound to plasma proteins). The pharmacokinetics of extraliposomal and total paclitaxel after LEP administration were evaluated in all patients enrolled into the study during the first cycle of treatment. A total of 33 blood samples (about 7 mL) were drawn from each patient during the first and last week of the first cycle of treatment at pre-dose, end of infusion and at 5, 15, 30 min and 1, 2, 4 h, any time between 8 and 16 h, 24, 48, 72 and 168 h (this latter only at the sixth week) post-infusion. In addition, blood samples were also collected at pre-dose and end of infusion at the second, third, fourth, and fifth week of treatment. Blood samples were collected in precooled (ice-water, 4°C) vials containing lithium heparin as anticoagulant. An aliquot of blood (2 mL) was frozen at -20°C and used for the analysis of total paclitaxel; the remaining amount of blood was centrifuged (1200 x *g* for 15 min at 4°C) and the harvested plasma was frozen at -20°C and used for the analysis of extraliposomal paclitaxel.

Concentrations of total paclitaxel (*i.e.*, the sum of liposome-associated and non-liposome-associated paclitaxel) and extraliposomal paclitaxel (*i.e.*, the non-liposome-associated, protein-bound and unbound) were determined in blood and plasma, respectively, with validated methods based on liquid chromatography with tandem mass-spectrometric detection (MS/MS). For the quantitation of total paclitaxel, Triton X-100 (5%, (v/v)) was added to whole blood and an aliquot (100 µL) of blood was extracted using tert-butyl methyl ether (MTBE). To determine extraliposomal concentrations of paclitaxel in human plasma (250 µL), liposomes were separated from plasma proteins using a solution containing dodecantungstophosphoric acid and magnesium chloride; an aliquot of supernatant (100 µL) was extracted with MTBE. For both methods, paclitaxel was separated using a Zorbax C18 column and eluted under gradient conditions with a mobile phase containing acetonitrile and 2 mM ammonium acetate buffer (pH 5). MS/MS detection was conducted with a PE-Sciex API 3000 mass spectrometer using a turbo ionspray source and multiple reaction monitoring in a positive ion mode. The lower limits of quantitation were 5 ng/mL and 1 ng/mL for total paclitaxel in blood and extraliposomal in plasma, respectively.

Analysis of the protein-unbound fraction of the extraliposomal paclitaxel concentrations was attempted but could not be determined separately due to interference in the equilibrium dialysis method [19].

2.5 *Pharmacokinetic analysis*

Pharmacokinetic parameters were calculated by standard non-compartmental methods using WinNonlin version 3.1 (Pharsight, Mountain View, CA, USA). The area under the plasma concentration-time curve (AUC) was calculated up to the last detectable concentration ($C_{(t,z)}$), using the linear trapezoidal rule. Other parameters, including volume of distribution at steady state (V_{ss}) and clearance (CL) were estimated using standard equations. The AUC was extrapolated to infinity by addition of $C_{(t,z)}/k$, where k is the terminal rate constant, which was estimated from a log-linear regression analysis of the terminal disposition phase. The half-life of the terminal phase was calculated as ln(2)/k.

3. Results

A total of 14 patients was entered into the study and received at least one dose of LEP. All patients were assessable for toxicity and response. Patient characteristics are listed in Table 1. Four patients did not complete the first cycle; one patients with adenocarcinoma of the duodenum, developed tumour related haematemesis and three patients had disease progression. The total number of assessable cycles was 16. The median number of cycles per patient was 1 (range, 1 - 2).

3.1 *Dose-limiting toxicity*

At 150 mg/m^2/week, one patient experienced DLT by receiving less than 70% of the intended cumulative dose of LEP. The patient received the first two doses as planned. The third and fourth infusions were omitted because of grade 3 and grade 4 neutropenia, respectively, and the patient received no subsequent therapy because of disease progression. In view of this single DLT, the cohort was expanded without observing further DLTs. Dose-escalation to 180 mg/m^2/week did not take place because of discontinuation of the study (see below). For this reason, the MTD and RD were not determined.

3.2 *Haematological toxicity*

Haematological toxicities per patient over the entire cycle of treatment are summarized in Table 2. One patient experienced grade 3 neutropenia at the 120 mg/m^2/week dose level and one patient at the 150 mg/m^2/week dose level experienced grade 3 and grade 4 neutropenia. At the 150 mg/m^2/week dose level, haematological side effects generally lasted less than 7 days. Mild to moderate anemia and thrombocytopenia was documented at all dose levels tested.

Table 1. Patient characteristics

Characteristic	No. of Patients
No. of Patients	
Total	14
Assessment	
For Dose-Limiting Toxicity	13
For Efficacy	12
No. of cycles/patient	
Median	1
Range	1 - 2
Gender, male : female	8 : 6
Age, years	
Median	54
Range	30 - 66
ECOG performance status	
0	4
1	10
Previous therapy	
Chemotherapy only	11
Radiotherapy	3
Surgery	9
Tumour types	
Colorectal	3
Gastro-intestinal tract, including:	8
Esophageal	1
Gastric	2
Gallbladder	1
Pancreatic	2
Unknown primary tumour	2
Miscellaneous	3

Abbreviations: no., number; ECOG, Eastern Cooperative Oncology Group.

Table 2. Haematological toxicity [worst grade per patient]													
			Anemia		Leucocytopenia			Neutropenia			Thrombocytopenia		
			Grades										
Dose [mg/m²/week]	No. of pts.	No. of cycles	1-2	3-4	1-2	3	4	1-2	3	4	1-2	3	4
90	3	3	3	0	2	0	0	1	0	0	1	0	0
120	4	4	2	0	2	0	0	1	1	0	2	0	0
150	7	9	5	0	3	3	0	3	1	1	2	0	0

Abbreviations: no., number; pts., patients.

3.3 Non-haematological toxicity

Gastrointestinal toxicities of mild to moderate severity were observed at all dose levels tested, and consisted of nausea [grade 1 (N = 7), grade 2 (N = 1), grade 3 (N = 1)], diarrhea [grade 1 (N = 8), grade 2 (N = 1), grade 3 (N = 1)], and vomiting [grade 1 (N = 3)]. Mild hypersensitivity reactions, consisting mainly of a facial flush and shortness of breath, were documented in four patients at the 150 mg/m^2/week dose level. All of these reactions had a rapid onset within the first minutes after the start of the LEP infusion and promptly recovered after stopping of the infusion and intravenous administration of 2 mg clemastine and 100 mg hydrocortisone. After rechallenging, none of these patients experienced a repeat of the infusion reaction. In two patients, a transient grade 1 to 2 skin reaction was documented. Mild alopecia (grade 1) was observed in 1 patient and neurotoxicity was not observed, but particularly the latter should be interpreted with caution in view of the very small number of cycles evaluated per patient.

3.4 Pharmacokinetics

Pharmacokinetic analysis was performed on all the 14 patients enrolled in this study. The pharmacokinetic parameters for total and extraliposomal paclitaxel during the first and sixth week of the LEP treatment are summarized in Tables 3 and 4. After the administration of LEP at dose levels 90, 120, and 150 mg/m^2/ week, the levels of total and extraliposomal paclitaxel reached the maximum value near the end of infusion on both the first and sixth week of treatment. After the administration of 90, 120 and 150 mg/m^2/week, the mean ($\pm$ SD) peak concentration of extraliposomal paclitaxel was 190 $\pm$ 94 and 186 $\pm$ 70 ng/mL, 363 $\pm$ 241 and 224 ng/mL, and 424 $\pm$ 166 and 326 $\pm$ 120 ng/mL, after the first and sixth week, respectively. The corresponding values of total paclitaxel were 2787 $\pm$ 1262 and 3083 $\pm$ 807 ng/mL, 3918 $\pm$ 1325 ng/mL and 2020 ng/mL, and 5004 $\pm$ 2334 and 5032 $\pm$ 3527 ng/mL, after the first and sixth week, respectively, at the 90, 120 and 150 mg/m^2/week dose level. After the end of infusion, blood levels of total paclitaxel and plasma levels of extraliposomal paclitaxel declined polyexponentially with an apparent terminal half-life ranging between 77 and 195 h, and 80 and 144 h, respectively. Total paclitaxel exhibited a relatively slow clearance from whole blood (range, 9 to 26 L/h/m^2), with a steady-state volume of distribution ranging from 120 to 2189 L/m^2. There was large interpatient variation in both drug clearance and volume of distribution at a coefficient of variation of approximately 60%.

Table 3. Mean ± SD plasma pharmacokinetic parameters of total paclitaxel during the 1st week and 6th week of LEP treatment

		1st week						6th week			
Dose [mg/m²/week]	No. of patients	C_{max} [ng/mL]	$T_{½,z}$ [h]	$AUC_{0\text{-}t(last)}$ [ng·h/mL]	$AUC_{0\text{-}\infty}$ [ng·h/mL]	CL [L/h/m²]	V_{ss} [L/m²]	No. of patients	C_{max} [ng/mL]	$T_{½,z}$ [h]	$AUC_{0\text{-}t(last)}$ [ng·h/mL]
90	3	2787 ± 1262	135 ± 67	6444 ± 12	8035 ± 1111	11 ± 2	1004 ± 506	3	3083 ± 807	195[a] ± 61	12494 ± 5194
120	4	3918 ± 1325	77[a] ± 68	6566 ± 3231	7611 ± 2492	17 ± 5	618 ± 316	1	2020	112	5731
150	7	5004 ± 2334	118 ± 83	10609 ± 2852	12539 ± 3260	13 ± 6	1036 ± 824	5	5032 ± 3527	145[b] ± 125	13759 ± 5143

Note: [a]N = 2, [b]N = 3.

Abbreviations: SD, standard deviation; no., number; C_{max}, peak plasma concentration; $T_{1/2,z}$, half-life of the terminal disposition phase; $AUC_{0\text{-}t(last)}$, area under the plasma concentration-time curve up to the last time point with measurable levels; $AUC_{0\text{-}\infty}$, AUC extrapolated to infinity; CL, total clearance; V_{ss}, steady-state volume of distribution.

Table 4. Mean ± SD plasma pharmacokinetic parameters of extraliposomal [protein-bound] paclitaxel during the 1st week and 6th week of LEP treatment

		1st week				6th week			
Dose [mg/m²/week]	No. of patients	C_{max} [ng/mL]	$T_{½,z}$ [h]	$AUC_{0\text{-}t(last)}$ [ng·h/mL]	$AUC_{0\text{-}\infty}$ [ng·h/mL]	No. of patients	C_{max} [ng/mL]	$T_{½,z}$ [h]	$AUC_{0\text{-}t\ (last)}$ [ng·h/mL]
90	3	190 ± 94	80[a]	910 ± 171	N/A	3	186 ± 70	135 ± 67	4242 ± 2658
120	4	363 ± 241	138[b] ± 74	1357 ± 287	2119 ± 576	1	224[a]	N/A	2826
150	7	424 ± 166	126[b]± 32	2410 ± 704	3031 ± 839	5	326 ± 120	144[c] ± 48	3967 ± 1321

Note: [a]N = 1, [b]N = 3, [c]N = 2.

Abbreviations: SD, standard deviation; no., number; C_{max}, peak plasma concentration; $T_{1/2,z}$, half-life of the terminal disposition phase; $AUC_{0\text{-}t(last)}$, area under the plasma concentration-time curve up to the last time point with measurable levels; $AUC_{0\text{-}\infty}$, AUC extrapolated to infinity; N/A, not available.

After the first week of treatment, the levels of total and extraliposomal paclitaxel increased in direct proportion with the dose, suggesting linear pharmacokinetics. Furthermore, over the tested dose range, total blood clearance was dose-independent (P = 0.490, Kruskal-Wallis one-way ANOVA, corrected for ties), supporting the above observation of a linear kinetics for total paclitaxel. Pharmacokinetic information obtained during the sixth week indicated on average an one- to two-fold accumulation, which is in reasonable agreement with the half-life of the apparent terminal disposition phase.

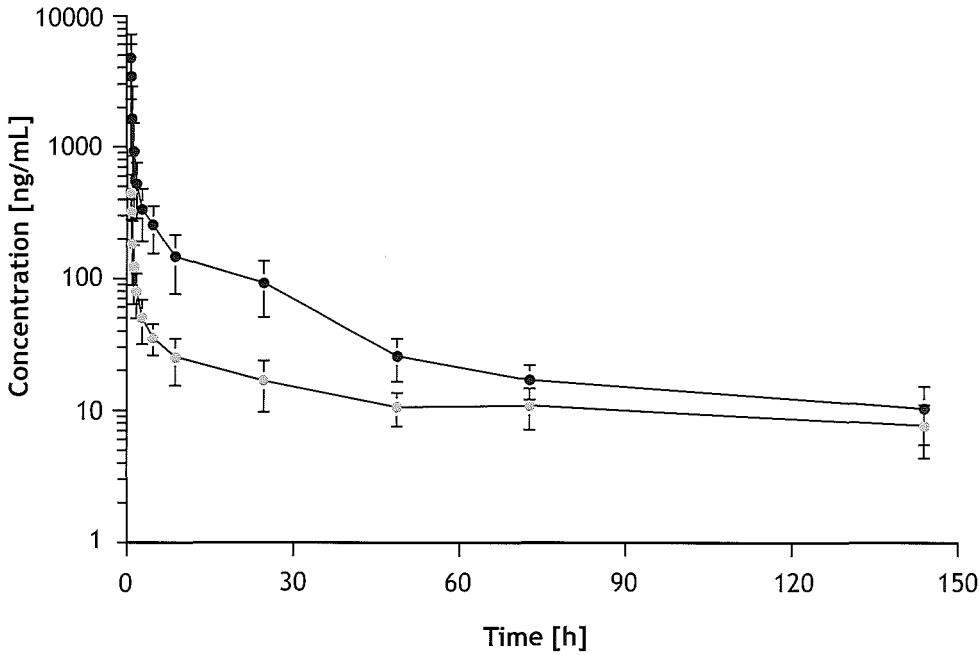

Figure 1. Plasma concentration time profiles of total paclitaxel in whole blood [closed symbols] and extraliposomal paclitaxel in plasma [open symbols] in patients receiving LEP at a dose of 150 mg/m^2/week. Data are presented as mean values [symbols] ± standard deviation [SD]

Assuming an equal distribution between plasma and blood for extraliposomal paclitaxel following administration of LEP [20], extraliposomal paclitaxel represented only a minor portion of the total paclitaxel [*i.e.*, the sum of liposome-associated and non-liposome-associated paclitaxel] in the systemic

circulation. The proportion of extraliposomal and total paclitaxel changed with time and among patients, ranging from 3 to 14% of the total paclitaxel at the first time point to about 23 to 100% at the final sampling time points. On average, considering the overall exposure, extraliposomal paclitaxel in plasma accounted for approximately 14 to 49% of the total paclitaxel exposure. The mean extraliposomal paclitaxel plasma concentrations and the total paclitaxel concentrations in blood *versus* time curves observed at the 150 mg/m^2/week dose level are displayed in Figure 1 and 2.

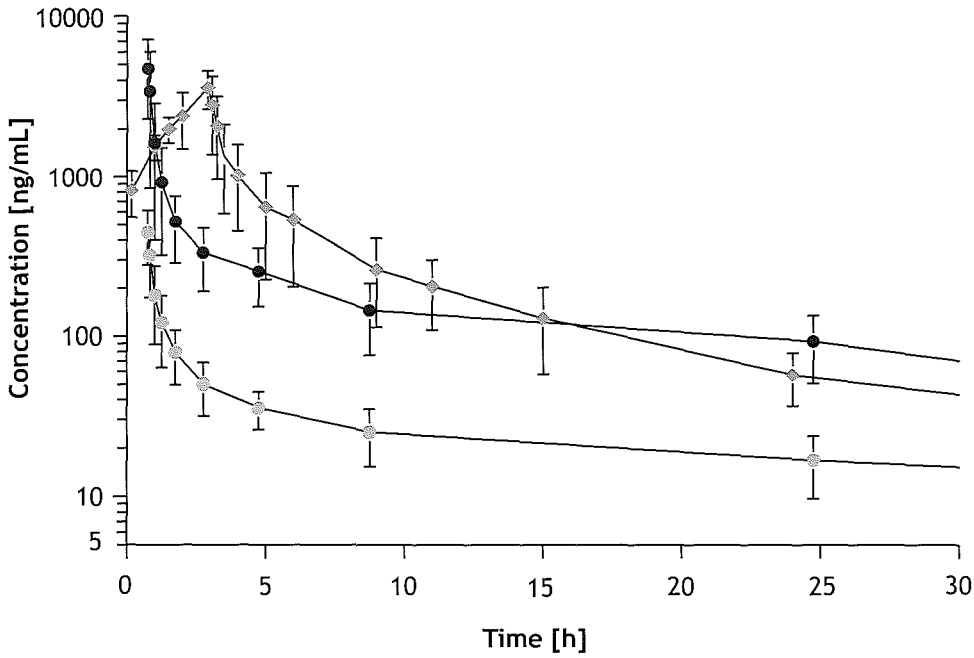

Figure 2. Plasma concentration time profiles of total paclitaxel in whole blood [closed symbols], extraliposomal paclitaxel in plasma [open symbols] in patients receiving LEP at a dose of 150 mg/m^2/week, and total paclitaxel in plasma [open lozenges] in 14 patients receiving Taxol® at a dose of 150 mg/m^2 [3-hour infusion]. Data are presented as mean values [symbols] ± standard deviation [SD]

3.5 Antitumour efficacy

At the 150 mg/m^2/week dose level, disease stabilisation for 8 weeks was documented in two patients with liver metastases of an adenocarcinoma of unknown primary and with pleural and peritoneal metastatic esophageal

carcinoma, respectively. Other patients had progressive disease after either the tumour assessment at 8 weeks, and 2 patients were discontinued because of early progression after the first and fourth administration of LEP, respectively.

4. Discussion

The current study was performed to explore the safety, feasibility, and pharmacokinetics of a new liposomal formulation of paclitaxel administrated as a 45-minute intravenous infusion once weekly for 6 out of 8 consecutive weeks. A previous formulation requiring a cumbersome sonication step just prior to infusion had been tested in a phase I trial using a single dose given every 21 days. The new, freeze-dried formulation has the same composition as the previously tested LEP formulation with the exception of the addition of mannitol as a cryoprotectant. The non-sonicated formulation was developed with the objectives of improving the overall profile of the drug. Real-time pharmacokinetics complimented clinical findings indicating that LEP was unlikely to offer significant advantages over taxanes currently employed in the clinic. With the new formulation, myelosuppression was seen at the 150 mg/m^2/week dose level, suggesting that a possible recommended phase II dose using a weekly schedule would be in the range of 150 - 180 mg/m^2/week. However, the current results do not allow any conclusions regarding long-term or cumulative toxicity, including the critical neurotoxicity.

To be effective as a carrier a liposome must be able to efficiently balance stability in the circulation with the ability to make the drug available at the tumour [8]. In order to achieve the optimum efficacy for a drug-delivery system, it is necessary to encapsulate the maximum possible quantity of a drug [7]. Comparison of the encapsulation efficiency of the drug in liposomes with the therapeutic dose indicates whether liposomes can be used as a suitable drug-delivery system [7]. The retention of the encapsulated drug is determined by the physicochemical characteristics of the drug itself and by the lipid composition and number of concentric membranes of the liposomal vesicle [21, 22]. Highly hydrophobic drugs, like paclitaxel, tend to associate mainly with the bilayer compartment of the liposome, resulting in lower entrapment stability due to faster redistribution of the drug to plasma components [9].

In comparison with small molecules, the volume of distribution of the drug encapsulated in liposomes is usually significantly reduced [9], and when a drug is stably encapsulated within the liposomal matrix it displays the pharmacokinetic

profile of the intact liposome rather than that of the encapsulated agent [11]. In general, this should achieve a significant increase in the AUC in the circulation and possibly in tumour tissue, and mimicking the effect of administering cytotoxic drugs as continuous intravenous infusion, without the inconvience of intravenous devices and toxicities associated with systemic drug exposure [11]. Likewise, the clearance of anticancer drugs encapsulated in liposomes is usually reduced, and the elimination half-life prolonged [23], as has been shown previously for anthracyclines [24], vincristine [25], and lurtotecan [26].

The pharmacokinetics of total paclitaxel when administered as LEP appeared to be dose-independent, providing further evidence of the previous supposition that the non-linearity of paclitaxel disposition following the administration of Taxol® is caused by its excipient Cremophor EL [1]. However, as compared to Taxol®, the interpatient variation in paclitaxel pharmacokinetic parameters following the administration of LEP was large (up to 60%). In contrast to that expected for a liposomal formulation, the clearance of total paclitaxel in whole blood following LEP (15.3 ± 8.98 L/h/m^2) was very similar to that reported after administration of Taxol® (17.5 ± 3.43 L/h/m^2) [27]. The observed values for volume of distribution at steady state of total paclitaxel in patients receiving LEP was very high (about 1000 L/m^2). Furthermore, in the systemic circulation, most paclitaxel was associated with the liposomes, since the extraliposomal paclitaxel AUC accounted only for 14 - 49% of the total paclitaxel AUC; however, the proportion of extraliposomal drug in plasma and total drug in whole blood increased with the time, reaching unity at the end of the sampling time period.

Previous work has shown that the plasma protein binding of the fraction of unbound paclitaxel in plasma in the absence of formulation excipients is approximately 85% in humans [1]. Assuming this value of plasma protein binding also for paclitaxel after LEP administration, at the 150 mg/m^2/week dose level, the predicted $AUC_{0\text{-}t(last)}$ is 362 ± 106 ng·h/mL, which is similar to 397 ± 69.7 ng·h/mL observed for Taxol® at the recommended weekly dose of 100 mg/m^2 [28]. This clearly suggests that at approximate equitoxic doses, exposure to the clinical relevant pharmacokinetic parameter is comparable for both formulations, and that LEP provides no pharmacologic advantages over Taxol®.

The release of complement fragments C3a and C5a can cause a hypersensitivity reaction, called complement activation-related pseudoallergy (CARPA), by release of anaphylatoxins and a cascade of cellular mediators of inflammation [3,29,30]. Activation of complement can rapidly induce a pallet of symptoms, including pain (*e.g.*, chest-pain, low back pain, headache), chills, choking, nausea, confusion, skin toxicity (*e.g.*, erythema, pruritus, urticaria), symptoms

of respiratory distress (*e.g.*, bronchospasm, dyspnea), and severe cardiac arrhythmias [3,29]. The frequency of CARPA due to intravenous infusion of conventional or pegylated liposomes ranged from 3 to 7% in several studies. Symptoms were observed within 5 to 10 min after the start of the first infusion. In most patients symptoms disappeared shortly after stopping of the infusion [3]. The rapid induction of this event seems to indicate that minimal amounts of liposomes can induce these side effects [30]. In the present study, hypersensitivity reactions were observed shortly after the intravenous infusion of the first LEP treatment in 3 patients. Rechallenge of the LEP infusion after treatment of corticosteroids and antihistamines was possible in all 3 patients without any new reaction or other complications. While the frequency of reactions was relatively low, LEP did not distinguish itself from currently used taxanes, since all patients received standard premedication with dexamethasone and antihistamines.

Collectively, the results of pharmacokinetic data supported the decision to terminate the study prior to reaching the primary objective of determining the MTD and RD and strengthened the importance of performing real-time pharmacokinetic analysis during a phase I study in order to bring as much as relevant information as possible to guide the decisions for further development.

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Summary and conclusiolns, Samenvatting en conclusies

This thesis includes phase I clinical and pharmacological studies on second and third generation topoisomerase I inhibitors, either administered orally, or intravenously as a macromolecular drug-delivery system, and on the anti-microtubulin agent, paclitaxel encapsulated in liposomes, to investigate the suitability of prolonged exposure of these anti-cancer agents and formulations. These studies were performed since in preclinical models, the cytotoxicity of both topoisomerase I inhibitors and paclitaxel increases with duration of exposure.

In ***Chapter 1*** we review the semi-synthetic camptothecin analogs currently registered or under clinical development as well as novel derivatives in preclinical development. In order to increase antitumor activity and to reduce toxicity, a variety of different strategies is being used to modulate the systemic delivery of these analogs. Three principal approaches are being discussed, (*a*) pharmaceutical modulation of formulation vehicles, structural alterations, and the search for more aqueous soluble prodrugs, (*b*) modulation of routes of administration and considerations on infusion duration, and (*c*) both pharmacodynamic and pharmacokinetic biomodulation.

Oral administration of irinotecan is a more patient convenient approach to mimic continuous drug-delivery facilitating prolonged exposure of the agent. In ***Chapter 2*** a phase I and pharmacokinetic, food effect, and pharmacogenetic study on oral irinotecan given as semi-solid matrix (SSM) capsules to patients with solid tumors is presented. The purpose of the study was to characterize the maximum-tolerated dose, recommended dose, toxicity and pharmacokinetic profile of orally administered irinotecan once daily for 5 consecutive days repeated every 3 weeks with the drug being taken in fasted condition. Patients were randomly assigned to take the drug along with high-fat and high-calorie breakfast for the first administration at day 1 of the first or second cycle. Twenty-five assessable patients received 101 cycles (median 2 cycles, range 1 - 15) at dose levels 70 and 80 mg/m^2/day. Grade 3 delayed diarrhea in combination with grade 3 fever were dose-limiting toxicities (DLTs) at 80 mg/m^2/day in three out of five patients. Exposure to the active metabolite SN-38 was relatively high compared to intravenous infusion, but no relevant accumulation was observed. Food had no significant effect on irinotecan pharmacokinetics. One confirmed partial remission and ten disease stabilizations were observed in previously pretreated patients. No association was found between UGT1A1*28 genotype and the risk of severe irinotecan-induced toxicity (*i.e.*, diarrhea and neutropenia).

The recommended dose is 70 mg/m^2/day for 5 consecutive days every 3 weeks. The study confirms that oral administration of irinotecan is feasible and may have improved pharmacokinetic characteristics as compared to intravenous administration of the drug.

In general, combination chemotherapy is more effective than single agent treatment. Irinotecan and capecitabine have different mechanisms of action, and have synergistic or additive cytotoxicity in experimental models. Furthermore, both agents have only partially overlapping toxicities. Against this background, we performed a phase I and pharmacokinetic study on oral irinotecan in combination with capecitabine in patients with solid tumors as described in ***Chapter 3***. Twenty-eight patients were enrolled into the study and received 155 cycles of therapy (median 5 cycles, range 1 - 18). They were treated with irinotecan SSM capsules given once daily for 5 consecutive days (dose, 50 - 60 mg/m^2/day) concomitantly with capecitabine given twice daily for 14 consecutive days (dose, 800 - 1000 mg/m^2), cycles repeated every 21 days. At dose level irinotecan 60 mg/m^2/day and capecitabine 2 x 800 mg/m^2/day, grade 3 diarrhea in combination with grade 2 nausea, despite maximal anti-emetic support, and with grade 3 anorexia and colitis were noted as DLTs in the first cycle in two out of six patients, respectively. At the recommended doses (irinotecan 50 mg/m^2/day; capecitabine 2 x 1000 mg/m^2/day), side effects were mostly mild to moderate and uniformly reversible. Pharmacokinetic analysis showed no interaction between both agents. Three confirmed partial responses and 16 disease stabilizations were observed. The study showed that the combination of oral irinotecan and capecitabine is a feasible, well tolerated all oral regimen, especially convenient for the outpatient setting and warrants further evaluation.

An alternative approach for continuous drug administration is using a macromolecular drug-delivery system, which can comprise an active topoisomerase I inhibitor linked to a polyalcohol polymer. Macromolecular carriers will accumulate and be retained preferentially in tumor tissue by a phenomenon, termed *enhanced permeability and retention* (EPR) effect, that is thought to be due to tumor vasculature displaying a discontinuous endothelium, which allows macromolecular extravasation into tumor tissue, and also the lack of effective lymphatic drainage in tumors, which prevents efficient clearance of such accumulated macromolecules. Macromolecular carriers have been shown to alter drug pharmacokinetics at the whole organism and cellular level and facilitate controlled drug release at the tumor site.

DE-310 is a novel macromolecular prodrug of the topoisomerase I inhibitor exatecan (DX-8951f). To study the biodistribution of this macromolecular carrier and its two major enzymatic products (*i.e.*, glycyl-DX-8951 and DX-8951), high liquid chromatographic assays in human whole blood, erythrocytes and saliva were developed and validated as described in ***Chapter 4***. Sample pretreatment involved a single protein-precipitation step, followed by a thermolysin-mediated deconjugation for the parent molecule. Separation of the compounds was achieved on an Inertsil ODS-80A column (150 × 4.6 mm i.d.; 5 µm PS), using isocratic elution. The column effluent was monitored at excitation and emission wavelengths of 375 and 445 nm, respectively. Validation results indicated that the methods are accurate and precise at lower limits of quantitation of 0.5 - 6.9 ng/mL. The suitability of the developed methods for the clinical use was demonstrated by the determination of DE-310 and its enzymatic products in human erythrocytes and saliva obtained from eight patients with different advanced solid tumors, treated with DE-310 at dose levels or 7.5 mg/m^2 (q6w). In all analyzed samples of erythrocytes and of saliva the concentration of DE-310, glycyl-DX-8951, and DX-8951 were below lower limit of quantitation (< 0.5 ng/mL). This would fit with the hypothesis of preferential drug uptake in tumor tissue, but not in other tissues. Therefore, the biodistribution of DE-310 is substantially different from other topoisomerase I inhibitors, such as irinotecan and topotecan.

In ***Chapter 5*** we reported a phase I and pharmacokinetic study of DE-310 administered as a 3 hours intravenous infusion once every 2 or 6 weeks in patients with advanced solid tumors. Twenty-seven patients were treated and received a total of 86 administrations (*i.e.*, 61 cycles). Initially, patients were treated once every 2 weeks in a 4-weekly schedule at doses ranging from 1.0 - 2.0 mg/m^2 (q2w). Because of the long half-life, which approximated the 2 weeks administration interval and largely exceeded the half-life expected based on preclinical data, further exploration of the q2w schedule was considered illogical in view of the potential risk for drug accumulation, and therefore the study protocol was amended to an intravenous administration once every 6 weeks at doses ranging from 6.0 - 9.0 mg/m^2 (q6w). Grade 4 myelosuppression and grade 3 hepatotoxicity with veno-occlusive disease (VOD) were the principal dose-limiting side effects. Other hematological and nonhematological toxicities were mild to moderate and reversible. Pharmacokinetics could performed in all patients. Across the various dose levels, the apparent terminal half-lives of conjugated DX-8951 (the carrier linked molecule), glycyl-DX-8951, and DX-8951

were 13, 12, and 13 days, respectively. Consequently, plasma concentrations of all compounds conjugated were sustained for several weeks. This parallel decline suggests that the terminal phase seen with the DE-310 metabolites (*i.e.*, glycyl-DX-8951 and DX-8951) is not due to real elimination-processess but due to their much slower formation from conjugated DX-8951. Substantial interindividual variability was observed (coefficient of variation, up to 92%) at dose level 7.5 mg/m^2. The AUC ratio of conjugated DX-8951-to-DX-8951 was approximately 600. The AUCs of conjugated DX-8951, glycyl-DX-8951, and DX-8951 appeared to increase in near proportion with an increase in dose. In tumor biopsies of four patients, concentrations of both glycyl-DX-8951 and DX-8951 were detectable at low levels, whereas in normal skin biopsies of nine patients concentrations of DX-8951 remained below the detection limit. During the first and second cycle at 6.0 mg/m^2 the concentrations of the three compounds were also assessed in ascites of a patient with metastastic pancreatic cancer. One patient with metastatic adenocarcinoma of unknown primary achieved a histologically proven complete remission. One confirmed partial remission was observed in a patient with metastatic pancreatic cancer and disease stabilization was noted in fourteen additional patients. The recommended phase II dose is 7.5 mg/m^2 given once every 6 weeks. The study showed that prolonged concentrations of DX-8951 were achieved by only single short infusion of DE-310 given every 6 weeks, which confirmed the pharmacokinetic proof of principle yielding the extended exposure to the topoisomerase I inhibiting active moiety.

Liposomes are macromolecular vehicles for drug delivery which are able to leak in tumor tissues according to the proposed EPR effect. We conducted a phase I and pharmacokinetic study of liposome encapsulated paclitaxel (LEP) as described in ***Chapter 6***. Fourteen patients were treated at doses ranging from 90 - 150 mg/m^2/week with LEP administered as an intravenous infusion over 45 minutes once every week for 6 out of 8 weeks. In one patient DLT was observed at dose-level 150 mg/m^2/week, who received less than 70% of the intended cumulative dose. No cumulative toxicities were observed. Stabilization of disease for 8 weeks was documented in two patients. The whole blood clearance of total paclitaxel was similar for LEP (15.3 ± 8.98 L/h/m^2) and Taxol® (17.5 ± 3.43 L/h/m^2), and the extraliposomal-to-total-drug ratio increased rapidly to unity at later sampling time points. The trial was discontinued upon completion of enrolment of the 150 mg/m^2/week cohort because an assessment of the pharmacokinetics and clinical data suggested that LEP was unlikely to have any advantages over Taxol®. It was concluded that this formulation of LEP is unlikely to provide improvements over taxanes currently in clinical use.

Final conclusions and Future perspectives

In conclusion, in preclinical models prolonged exposure to low concentrations of cytotoxic agents, especially suitable for topoisomerase I inhibitors and taxanes due to their unique mechanisms of action, shows more antitumor efficacy compared with bolus administration. However, the optimal treatment and dosing regimens, such as continuous intravenous infusion, long-term oral delivery, liposomal encapsulation, or polymerized drug formulations have to be more crystallized in further clinical investigations. So far, current insights, albeit only derived from phase II clinical trials, suggested that prolonged drug exposure may be at least as effective and less toxic than more conventional schedules of intermittant administration of higher doses given as short bolus intravenous infusion. Traditionally, continuous intravenous infusion of cytotoxic agents is suitable for this concept, but this way of administration poses several disadvantages (*e.g.*, catheter-related infections). For a variety of reasons oral regimens of anticancer drugs are more convenient for continuous drug administration and more preferred by patients, provided the efficacy of the drug is not compromised.

From a pharmacological point of view, the use of oral irinotecan has to overcome some disadvantages. These include maintenance absorption through the biochemical barrier of efflux pumps (*i.e.*, drug transporters), and modulation by cytochrome P-450 isoenzymes in the epithelium of the intestine, the first pass effect in the liver and the excretion in the bile, that all influence the relatively low, and highly variable, bioavailability of the drug. In addition, the narrow therapeutic index offers a potential risk of an accidental overdose and consequently unpredictable excessive toxicity, or suboptimal exposure and hence lower efficacy. But on the other hand, a higher metabolic ratio, defined as the AUC ratio of SN-38-to-irinotecan, was determined after oral treatment of the drug compared with the metabolic ratio estimated after intravenous infusion of irinotecan, suggesting extensive presystemic metabolism due to high expression levels of irinotecan-converting carboxylesterases in the gastrointestinal tract and liver. It therefore appears that oral administration of irinotecan results in more efficient conversion of irinotecan into SN-38.

Macromolecular drug-delivery systems are an alternative approach to achieve prolonged exposure to anti-cancer agents. It is suggested that a key factor in the success of polymer-based cancer therapies is the unique blood vasculature within solid tumors. Compared with the blood vessels in normal tissue, those of tumors are frequently more leaky to circulating marcomolecules allowing

them easy access to the interior of tumor tissues. Furthermore, macromolecules will accumulate and be retained in tumor tissues due to anomalous lymphatic drainage.

The question which has to be answered is, is there enough evidence for the proposed EPR effect? Likely, the prolonged exposure of high molecular weight macromolecules relates by the impossibility to diffuse passively in and out of cells like low molecular weight drugs do. Their uptake by cells elapses by the process of endocytosis. Afterwards, intracellular lysosomal enzymes need to release the active drug. This means that the polymer-drug conjugate will circulate longer in the general circulation, because the molecular weight is above the renal excretion treshold. Due to temporary inactivation of the active moiety, by linking to the conjugate, the longer systemic exposure of the macromolecular carrier is possible without increasing side effects associated with the active cytotoxic agent.

The design of novel tailor-made synthetic biodegradable polymers (*e.g.*, dendrimers) will be necessary in the next years and this development needs a multidiscplinary approach with collaborations from the field of biology, chemistry, pharmacology, and medicine.

Although the expectations of the potential benefit for improving therapeutic activity from liposomal drug carriers are still high, today's reality in application of novel liposomal drug carriers demands comprehensive knowledge of liposome technology to understand its limitations in the design of efficacious drug carriers.

From a pharmacokinetic point of view, the volume of distribution of liposomes is significantly reduced in comparison with small molecules, and when a drug is stably encapsulated within the a liposomal matrix it displays the pharmacokinetic profile of the liposome rather than of the parental unencapsulated agent. In general, this should achieve a significant increase in the area under the plasma concentration-time curve in the circulation and tumor tissue, and mimick the effect of administering cytotoxic drugs as continuous intravenous infusion, without the inconvience of intravenous devices and toxicities associated with systemic drug exposure. In addition, the maximum plasma concentration and plasma clearance of the liposomes are reduced, and its elimination half-life is prolonged. In theory, all these factors may contribute to an increased therapeutic index of liposomal formulations of cytotoxic drugs.

After an intravenous administration of liposomes, opsonizing blood proteins rapidly associate with the liposome surface. The liposome-protein bound interactions alter the physiochemical properties of liposomes, thereby resulting

in altered stability and clearance properties of liposomes. This opsonization process triggers recognition and liposomes uptake by macrophages of the reticuloendothelial system (RES) through receptor-mediated phagocytosis or endocytosis and clearance by the liver, spleen or lung. The complement system is responsible for the elimination of liposomes from blood circulation, presumably as a consequence of opsonization by activated complement C3 fragments and the membrane attack complex (MAC), *i.e.*, the assembly of C5b-9 complexes that produces a lytic pore, which induce the release of the liposomal contents. The circulation time of pegylated-liposomes (*e.g.*, Caelyx®) is longer than conventional liposomes because the stealth characteristics take more time to be attacked by macrophages. This means that broad suitability of liposomes vehicles as passive targeting for cytotoxic agents in oncological practice may have limited benefits.

Samenvatting en conclusies

In dit proefschrift worden de resultaten besproken van klinische en farmacologische fase I studies, die enerzijds met tweede en derde generatie topoisomerase I remmers en anderzijds met het cytostaticum (antikankermiddel) liposomaal geformuleerd paclitaxel zijn verricht. De topoisomerase I remmers irinotecan en exatecan werden respectievelijk oraal als capsule en intraveneus als polymeer - door koppeling aan een biologisch afbreekbare polyalcoholstructuur - toegediend. Het antimicrotubulaire cytostaticum paclitaxel werd intraveneus als een liposomale formulering toegediend. Het doel van dit proefschrift was om de toepasbaarheid van verlengde expositieduur van deze cytostatica en formuleringen te onderzoeken. Deze studies werden uitgevoerd op basis van de resultaten van preklinisch onderzoeken, waarbij aangetoond werd dat de effectiviteit van celdoding door zowel topoisomerase I remmers als paclitaxel groter werd naarmate de expositieduur van deze middelen kon worden verlengd.

In ***Hoofdstuk 1*** wordt een overzicht gegeven van semi-synthetische camptothecine analoga, die inmiddels zijn geregistreerd voor dagelijkse klinische toepassing of die zich nog in een (pre-)klinisch ontwikkelingsstadium bevinden. Ten einde antitumoractiviteit te vergroten en bijwerkingen te verminderen, zijn verschillende strategieën toegepast om de systemische toepassing van deze analoga te verbeteren. Drie manieren van aanpak worden hierbij besproken, (*a*) de farmaceutische wijziging van formuleringsvehikels en structuurformule en het maken van meer wateroplosbare prodrugs, (*b*) wijziging van toedieningsroutes en van infusieduur, en (*c*) de biomodulatie van de farmacodynamiek en farmacokinetiek.

Het oraal innemen van irinotecan is een patientvriendelijke benadering om de continue intraveneuze toediening van dit cytostaticum na te bootsen ten einde een verlengde expositieduur van dit antikankermiddel te bewerkstelligen. In ***Hoofdstuk 2*** worden de resultaten van een klinische en farmacokinetische fase I studie met oraal toegediend irinotecan, geformuleerd als semi-solid matrix (SSM) capsules, bij kankerpatiënten beschreven. In deze studie werd eveneens het effect van voedselinname op de biologische beschikbaarheid onderzocht, alsmede farmacogenetische aspecten van irinotecan. Het doel van deze studie was om de maximaal verdraagbare dosering, de aanbevolen dosering voor verder onderzoek in fase II studies, het bijwerkingen- en het farmacokinetische profiel

van irinotecan vast te stellen. Irinotecan werd door de patiënten 's ochtends op de nuchtere maag éénmaal daags gedurende vijf achtereenvolgende dagen in een periode van drie weken ingenomen. Eénmalig werd het cytostaticum ingenomen na een vet- en koolhydraatrijk ontbijt om het effect daarvan op de biologische beschikbaarheid van het cytostaticum vast te stellen. Randomisatie hiervoor vond plaats om te bepalen of de medicatie met het ontbijt tijdens de eerste of de tweede kuur moest worden ingenomen. Aan vijfentwintig patiënten werden 101 kuren (mediaan 2 kuren, interval 1 - 15) toegediend. De toegepaste doseringen waren 70 en 80 mg/m^2/dag. Ernstige diarree (graad 3) en koorts (graad 3) waren de dosis-limiterende bijwerkingen (DLT) bij de dosering van 80 mg/m^2/dag in drie van vijf patiënten. In vergelijking met intraveneuze toediening van irinotecan is de blootstelling aan de actieve metaboliet SN-38 relatief hoog, maar er werd geen relevante accumulatie van SN-38 in onze studie vastgesteld. Het gelijktijdig innemen van de irinotecan capsules met voedsel had geen significant effect op de farmacokinetiek van irinotecan. In de patiëntengroep, die eerdere behandeld was geweest met andere cytostatica, werd bij één patiënt een partiële remissie vastgesteld, terwijl bij tien patiënten stabilisatie van de ziekte als respons werd waargenomen. Tussen UGT1A1*28-genotypering en het risico op het onstaan van ernstige bijwerkingen door irinotecan (i.h.b. diarree en neutropenie) kon in onze studie geen verband worden vastgesteld. De aanbevolen dosering is 70 mg/m^2/dag gedurende vijf achtereenvolgende dagen tijdens een periode van drie weken. De studie bevestigt dat de orale toediening van irinotecan goed haalbaar is en mogelijk verbeterde farmacokinetische kenmerken heeft in vergelijking met intraveneuze toediening van dit cytostaticum.

In het algemeen geldt, dat een combinatie van cytostatica effectiever is dan monotherapie met een cytostaticum. Irinotecan en capecitabine hebben verschillende werkingsmechanismen en tonen synergistische en toegevoegde effectiviteit van celdoding in experimentele diermodellen. Verder hebben beide middelen slechts gedeeltelijk overlappende bijwerkingsprofielen. Tegen deze achtergrond, hebben wij een klinische en farmacokinetische fase I studie verricht met de combinatie van orale irinotecan en capecitabine bij kankerpatiënten, zoals beschreven in ***Hoofdstuk 3***. Achtentwintig patiënten kregen in totaal 155 kuren (mediaan 5 kuren, interval 1 - 18) toegediend. Zij kregen éénmaal daags gedurende vijf achtereenvolgende dagen irinotecan SSM capsules (dosering 50 - 60 mg/m^2/dag) gelijktijdig met capecitabine (dosering 800 - 1000 mg/m^2) tweemaal daags gedurende veertien achtereenvolgende dagen. Een kuur werd elke drie weken herhaald. Bij de dosering van irinotecan

60 mg/m^2/dag en capecitabine 2 x 800 mg/m^2/dag waren ernstige diarree (graad 3) in combinatie met braken (graad 2), ondanks maximale bescherming met anti-emetica, alsmede in combinatie met ernstige eetlustverlies (anorexie) (graad 3) en colitis (graad 3), dosis-limiterende bijwerkingen (DLT) tijdens de eerste kuur in twee van zes patiënten. De bijwerkingen op het niveau van de aanbevolen dosering (c.q. irinotecan 50 mg/m^2/dag en capecitabine 2 x 1000 mg/m^2/dag) waren over het algemeen mild tot matig en reversibel. Farmacokinetische analyse toonde geen interactie tussen irinotecan en capecitabine. Drie patiënten hadden een partiële remissie als respons en zestien patiënten behielden stabilisatie van hun ziekte tijdens deze behandeling. De studie toont aan dat de combinatiebehandeling van orale irinotecan en capecitabine haalbaar is en goed verdragen wordt. De behandeling met orale cytostatica geeft de mogelijkheid om patiënten poliklinisch te behandelen. Mede daarom is het aan te bevelen om deze combinatie verder te onderzoeken in fase II studies.

Een andere benadering om meer continue expositie aan een cytostaticum te bereiken, vormt het toepassen van een zogenaamd 'macromolecular drug-delivery system' (macromoleculair geneesmiddelafleveringssysteem), waarbij bijvoorbeeld een actief werkzame topoisomerase I remmer wordt gekoppeld aan een polyalcoholstructuur (*i.e.*, polymeer). Er wordt verondersteld dat macromoleculen bij voorkeur in tumorweefsel kunnen accumuleren door een fenomeen, genaamd *enhanced permeability and retention* (EPR) effect, dat wordt veroorzaakt door de gewijzigde structuur van bloedvaten in tumorweefsel, waarbij het endotheel niet als een continue aaneengesloten laag is aangelegd, maar daartussen grote poriën bevat. Als gevolg hiervan zouden macromoleculen gemakkelijker in tumorweefsel kunnen lekken dan in normale weefsels. Bovendien voorkomt de gebrekkige aanleg van lymfvaten in tumorweefsel dat macromoleculen daar efficiënt uit kunnen worden geklaard. Ten gevolge van deze beide tumorweefselkarakteristieken wordt verondersteld dat accumulatie van een 'macromolecular drug-delivery system' in tumorweefsel plaatsvindt en dat hiermee een hogere concentratie van een actief werkzaam cytostaticum in de tumor kan worden bereikt.

DE-310 is een nieuwe macromoleculaire prodrug van de topoisomerase I remmer exatecan (DX-8951f). Om de distributie van deze macromoleculaire prodrug en zijn enzymatische afsplitsingsmetabolieten (*i.e.*, glycyl-DX-8951 en DX-8951) te bestuderen, werden hoge druk vloeistofchromatografie (HPLC) bepalingen in menselijk bloed, rode bloedcellen en speeksel ontwikkeld en gevalideerd, zoals beschreven in ***Hoofdstuk 4***. De voorbehandeling van de monsters bestond

uit een enkelvoudige onteiwitingsstap, gevolgd door een deconjugatie van het hoofdmolecuul. Scheiding van de verschillende componenten werd bereikt door gebruik te maken van een HPLC methode met een reversed-phase kolom en isocratische elutie. De kolomeffluent werd gemonitored door middel van fluorescentie. De laagst adequaat en precies meetbare concentraties bij deze methode was 0.5 ng/mL voor glycyl-DX-8951 en DX-8951, alsmede 6.9 ng/mL voor geconjugeerd DX-8951 (*i.e.*, het aan de polyalcoholstructuur gekoppelde molecuul). De toepasbaarheid van de methode voor klinisch gebruik werd aangetoond door de bepaling van DE-310 en de twee enzymatische afsplitsingsprodukten in rode bloedcellen en speeksel afkomstig van acht kankerpatiënten, die behandeld werden met DE-310 gedoseerd met een dosis van 7.5 mg/m^2 éénmaal per 6 weken. De concentraties van DE-310, glycyl-DX-8951 en DX-8951 in alle geanalyseerde monsters van de rode bloedcellen en speeksel waren lager dan de laagst adequaat en precies meetbare concentratie. Deze bevinding komt overeen met de hypothese van preferentiële opname van macromoleculen in tumorweefsel en niet in normale weefsels. Op grond hiervan kan worden gesteld, dat de biodistributie van DE-310 wezenlijk anders is dan van andere topoisomerase I remmers, zoals irinotecan en topotecan.

In ***Hoofdstuk 5*** rapporteren wij een klinische en farmacokinetische fase I studie met DE-310, intraveneus toegediend als een 3-uur durend infuus éénmaal per 2 of 6 weken bij kankerpatiënten. Zevenentwintig patiënten werden behandeld en er werden in totaal 86 toedieningen (overeenkomend met 61 kuren) gegeven. Aanvankelijk werden de patiënten éénmaal per 2 weken in een 4-wekelijks toedieningsschema behandeld (doseringsinterval 1.0 - 2.0 mg/m^2). In verband met de lange halfwaardetijd, die bijna gelijk bleek aan het toedieningsinterval en veel langer was dan de verwachte halfwaardetijd op basis van preklinisch onderzoek, was verder onderzoek van dit 2-wekelijkse toedieningsschema niet meer opportuun wegens het te verwachten risico op accumulatie dit cytostaticum. Derhalve werd het studieprotocol geamendeerd tot een 6-wekelijks toedieningsschema (doseringsinterval 6.0 - 9.0 mg/m^2). Zeer ernstige beenmergonderdrukking (graad 4) en ernstige levertoxiciteit (graad 3), gepaard gaande met veno-occlusive disease (VOD), waren de belangrijkste dosis-limiterende bijwerkingen. Overige hematologische en niet-hematologische bijwerkingen waren mild tot matig en waren reversibel. Farmacokinetische analyse kon in alle patiënten worden verricht. De halfwaardetijden van geconjugeerd DX-8951, glycyl-DX-8951 en DX-8951 waren respectievelijk 13, 12 en 13 dagen over het gehele doseringstraject. De plasmaconcentraties van alle

componenten konden gedurende enkele weken worden aangetoond. De parallel verlopende verlaging van de concentraties van de metabolieten suggereert dat de eindfase niet gerelateerd is aan een werkelijke uitscheiding van de DE-310-metabolieten, maar bepaald wordt door de langzame enzymatische afsplitsing van DE-310. Aanzienlijke interindividuele variabiliteit (met variatiecoëfficiënt tot 92%) werd waargenomen bij een dosering van 7.5 mg/m^2. De verhouding van de oppervlakte van de curve, die het verloop tussen de plasmaconcentratie en de tijd weergeeft (AUC; area under the curve), van geconjugeerd DX-8951 en van DX-8951 was ongeveer 600. De AUC's van geconjugeerd DX-8951, glycyl-DX-8951 en DX-8951 bleken in goede verhouding te staan met toename van de dosering. Glycyl-DX-8951 en DX-8951 konden in lage concentraties worden aangetoond in tumorbiopten afkomstig van vier patienten, terwijl in normale huidbiopten afkomstig van negen patiënten geen DX-8951 kon worden aangetoond. Bij één patient met gemetastaseerd pancreascarcinoom, die behandeld werd met DE-310 in een dosis van 6.0 mg/m^2 (q6w), konden concentraties van de drie componenten in ascites worden aangetoond. Eén patiënt met gemetastaseerd adenocarcinoom van onbekende primaire origine bereikte een histologisch bewezen complete remissie met een follow-up duur van ruim één jaar. Een partiële remissie werd vastgesteld bij een andere patiënt, terwijl stabilisatie van ziekte als respons werd waargenomen bij veertien andere patiënten. De aanbevolen dosering van DE-310 voor fase II studies is 7.5 mg/m^2 éénmaal per 6 weken intraveneus toegediend. De studie toonde aan dat langdurige concentraties van de topoisomerase I remmer exatecan (DX-8951) konden worden verkregen door middel van een eenmalige korte infusie van de macromoleculaire prodrug DE-310, dat slechts éénmaal per 6 weken hoeft te worden toegediend. Vanuit farmacokinetische gezichtspunt kon hiermee het principe van langdurige expositieduur van een topoisomerase I remmer bevestigd worden.

Liposomen zijn macromoleculaire vehikels om geneesmiddelen te transporteren. Ook liposomen zijn in staat om in tumorweefsel te lekken als gevolg van het eerdergenoemde EPR effect. Een klinische en farmacokinetische fase I studie met paclitaxel geformuleerd (c.q. ingepakseld) in liposomen (LEP; liposome encapsulated paclitaxel) werd door ons uitgevoerd zoals beschreven in ***Hoofdstuk 6***. Veertien kankerpatiënten werden behandeld met LEP (doseringsinterval 90 - 150 mg/m^2/week), waarbij het cytostaticum zesmaal wekelijks als kortdurend infuus van 45 minuten werd toegediend in een totale periode van acht weken. Eén patiënt kreeg een dosis-limiterende bijwerking op het doseringsniveau van 150 mg/m^2/week, ten gevolge van het feit dat minder

dan 70% van de bedoelde cumulatieve dosering aan deze patiënt kon worden gegeven. In deze studie werden geen cumulatieve bijwerkingen vastgesteld. In twee patiënten werd stabilisatie van hun ziekte gedurende 8 weken als respons waargenomen. De klaring van totaal paclitaxel uit bloed was identiek voor LEP (15.3 ± 8.98 L/h/m^2) als voor Taxol® (17.5 ± 3.43 L/h/m^2). Op latere tijdstippen van de farmacokinetische bloedafnames kon geen onderscheid meer gemaakt worden tussen extraliposomaal paclitaxel en totaal paclitaxel. Deze studie werd voortijdig beëindigd na voltooiing van de patiënteninstroom voor het doseringscohort 150 mg/m^2/week, op basis van farmacokinetische en klinische resultaten, die suggereerden dat LEP geen voordeel zou hebben ten opzichte van Taxol®. Derhalve werd geconcludeerd dat deze liposomale formulering van paclitaxel (LEP) niet zou kunnen voorzien in verbeteringen ten opzichte van de taxanen, die momenteel klinisch worden toegepast.

Eindconclusies en toekomstperspectieven

In preklinische modellen laat verlengde expositie aan lage concentraties van cytostatica - een concept dat vooral toepasbaar is voor topoisomerase I remmers en paclitaxel, vanwege hun unieke werkingsmechanismen - een betere antitumoractiviteit zien in vergelijking met korte expositie aan hoge concentraties cytostatica, die als bolus werden toegediend. Echter, de optimale behandeling en doseringsschema's, zoals bijvoorbeeld door middel van continue intraveneuze infusie, langdurige orale toediening, toediening via liposomale of gepolymeriseerde formuleringen, dient nog nader te worden uitgekristalliseerd in meer onderzoeken. Tot nu toe suggereren de gegevens, ofschoon slechts afkomstig van klinische fase II studies, dat verlengde expositie aan cytostatica tenminste even effectief en minder toxisch kan zijn dan conventionele schema's met intermitterende, intraveneuze toediening van hoge doseringen cytostatica als een bolus. Traditioneel wordt continue intraveneuze infusie van cytostatica voor dit concept toegepast, echter deze toedieningsvorm heeft een aantal nadelen, zoals bijvoorbeeld catheter-gerelateerde infecties. De orale toedieningsvorm is in principe een mogelijkheid voor continue toediening van cytostatica en de meerderheid van patiënten geeft hieraan de voorkeur, mits de effectiviteit van het cytostaticum door de orale toedieningsvorm niet wordt verminderd.

Vanuit een farmacologisch gezichtspunt gezien, heeft het gebruik van oraal irinotecan een aantal nadelen. Tot deze nadelen behoren o.a. het behoud van absorptie van het cytostaticum door de dunne darm tegen een biochemische

barrière van efflux-pompen (*i.e.*, drug transport-eiwitten) in, wijziging van het cytostaticum door cytochroom P-450 isoenzymen in het dunne darmepitheel, het 'first pass'-effect in de lever en de uitscheiding in de gal. Deze factoren beïnvloeden de relatief lage en hoog variabele biologische beschikbaarheid van dit cytostaticum. Bovendien zorgt de smalle therapeutische breedte enerzijds voor een potentieel risico van incidentele overdosering en daarmee de mogelijkheid van onvoorspelbare ernstige bijwerkingen, en anderzijds van suboptimale expositie van het cytostaticum en daarmee een kans op vermindering van de effectiviteit. Aan de andere kant wordt een hogere metabole ratio, gedefinieerd als de AUC-verhouding van SN-38 ten opzichte van irinotecan, waargenomen na orale toediening van irinotecan in vergelijking met de metabole ratio na intraveneus toegediende irinotecan. Deze verhoogde metabole ratio wordt veroorzaakt door uitgebreide presystemische metabolisatie van irinotecan ten gevolge van hoge expressie in de darmen en in de lever van carboxylesterases, enzymen die irinotecan kunnen metaboliseren. Op grond hiervan kan verondersteld worden dat de orale toediening van irinotecan resulteert in efficiëntere omzetting van irnotecan in zijn actieve metaboliet SN-38.

Met het gebruik maken van macromolecular drug-delivery systems wordt een andere manier om verlengde expositieduur van cytostatica te bewerkstelligen toegepast. In de literatuur wordt gesuggereerd dat een sleutelfactor in het succes van op polymeren-gebaseerde antikankerbehandelingen wordt bepaald door de unieke structuur van de bloedvaten van tumorweefsels. In vergelijking met bloedvaten in normale weefsels zijn de bloedvaten in tumorweefsels meer doorlaatbaar voor circulerende macromoleculen. Hierdoor zijn macromoleculen in staat om in tumorweefsel te accumuleren. Bovendien kunnen de macromoleculen daarna niet uit tumorweefsel worden geklaard in verband met de gebrekkige aanleg van lymfvaten in tumorweefsels.

De vraag die beantwoord dient te worden, is of er voldoende bewijs is om aan te nemen dat het zogenaamde EPR effect bestaat? De verlengde expositieduur van macromoleculen met een hoog moleculair gewicht wordt bereikt door de onmogelijkheid van deze macromoleculen om passief in cellen te diffunderen zoals stoffen met een laag moleculair gewicht dat wel kunnen doen. De opname van macromoleculen geschiedt via het proces van endocytose. Intracellulair wordt de actief werkzame stof vrijgemaakt van de polymeerstructuur met behulp van lysosomale enzymen. Dit tijdkostende opnameproces van macromoleculen in cellen impliceert dat de macromoleculen langdurig in de bloedsomloop blijven circuleren, mede gelet op het feit dat macromoleculen, vanwege hun hoge moleculaire gewicht, niet door de nieren kunnen worden uitgescheiden.

Een actief werkzaam cytostaticum dat aan een polymeerstructuur gekoppeld is, is hierdoor tijdelijk inactief gemaakt en geeft daardoor minder bijwerkingen bij verblijf in de bloedsomloop.

Het ontwerp van nieuwe op maat gemaakte synthetische biologisch afbreekbare polymeren (bijvoorbeeld dendrimeren) zal in de komende jaren noodzakelijk zijn en een succesvolle ontwikkeling hiervan noopt tot intensieve multidisciplinaire samenwerking vanuit verschillende disciplines, o.a. biologie, biochemie, farmacologie en geneeskunde.

Hoewel de verwachtingen van het potentiële voordeel voor het bereiken van een verbeterde therapeutische activiteit door liposomen nog steeds hoog gespannen zijn, eist de realiteit dat het toepassen van liposomen als vehikels voor cytostatica een uitgebreide kennis van de liposomentechnologie noodzakelijk maakt, om de beperkingen van liposomen als effectieve vehikels van cytostatica te kunnen begrijpen.

Vanuit een farmacokinetisch gezichtspunt gezien, is het verdelingsvolume van liposomen significant verminderd in vergelijking met kleine moleculaire stoffen. Het farmacokinetische profiel wordt weerspiegeld door het liposoom zelf en niet door het erin opgenomen cytostaticum, indien dit cytostaticum stabiel genoeg in de liposomale matrix is opgenomen. In het algemeen wordt een significante stijging van de AUC in de bloedsomloop en in tumorweefsel bereikt, waarmee het effect van een continue infusie van een cytostaticum kan worden nagebootst zonder het ongemak van langdurige intraveneuze infusie of bijwerkingen ten gevolge van de systemische blootstelling van het cytostaticum. Bovendien zijn de maximale plasmaconcentratie en plasmaklaring van liposomen verminderd en is de halfwaardetijd verlengd. In theorie dragen al deze farmacokinetische factoren bij tot een verhoogde therapeutische breedte van liposomale formuleringen voor cytostatica.

Na intraveneuze toediening van liposomen, wordt het liposomale oppervlak snel door bloedeiwitten geopsoniseerd. Deze liposomale-eiwitbindinginteracties wijzigen de fysicochemische eigenschappen van liposomen, resulterend in gewijzigde stabiliteit en klaring van de lipsomen. Dit proces van opsonisatie zorgt ervoor dat liposomen worden herkend en opgenomen door macrofagen van het reticuloendotheliale systeem (RES) door middel van receptoren-gemedieerde fagocytose en vervolgens door de lever, milt en longen worden geklaard. Het complement systeem is verantwoordelijk voor de eliminatie van liposomen uit de bloedsomloop, voornamelijk als een gevolg van opsonisatie door geactiveerd complement C3-fragmenten en het 'membrane attack complex' (MAC), *i.e.*, de verzameling van C5b-9 complementcomplexen. Het MAC zorgt ervoor dat

een porie in het liposoom kan ontstaan, als gevolg waarvan het vrijkomen van de liposomale inhoud wordt bewerkstelligd. De circulatietijd van pegylated-liposomen (bijvoorbeeld Caelyx®) is langer dan van conventionele liposomen, vanwege het feit dat macrofagen meer tijd nodig hebben om pegylated-liposomen te fagocyteren. Een en ander impliceert dat de brede toepasbaarheid van liposomen als vehikels voor passief transport van cytostatica in de oncologische praktijk slechts beperkt mogelijk is.

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