Gimatecan, a novel camptothecin with a promising preclinical profile

Graziella Pratesi[a], Giovanni L. Beretta[a] and Franco Zunino[a]

The realization that position 7 of camptothecin allows several options in chemical manipulation of the drug has stimulated a systematic investigation of a variety of substituents in this position. These efforts resulted in the identification of a novel series of 7-oxyiminomethyl derivatives. Among compounds of this series we have selected a promising lipophilic derivative, gimatecan, for further development. The relevant features of gimatecan are: (i) marked cytotoxic potency, likely related to multiple factors, including a potent inhibition of topoisomerase I, a persistent stabilization of the cleavable complex, an increased intracellular accumulation and a peculiar subcellular localization; (ii) lack of recognition by known resistance-related transport systems; (iii) increased lactone stability and favorable pharmacokinetics; (iv) good oral bioavailability; and (v) an impressive antitumor efficacy in a large panel of human tumor xenografts, with various treatment schedules. Phase I clinical studies with oral administration support the preclinical results of the novel camptothecin. Using different schedules and dosing durations, gimatecan exhibited an acceptable toxicity profile, with myelotoxicity being the dose-limiting toxic effect. An appreciable number of tumor responses was achieved and favorable pharmacokinetics with a very long terminal half-life was observed. The clinical development of gimatecan is currently ongoing, with phase II studies in diverse tumor types (colon, lung, breast carcinoma and pediatric tumors). ***Anti-Cancer Drugs*** **15:545–552 © 2004 Lippincott Williams & Wilkins.**

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Introduction

Camptothecins represent an important class of effective antitumor drugs. The introduction into clinical practice of the two synthetic camptothecins, irinotecan and topotecan, represents an important addition to cancer chemotherapy [1]. Camptothecins are characterized by a unique and specific mechanism of action, because they inhibit the function of DNA topoisomerase I, by stabilizing the covalent DNA–enzyme complex to form the ternary complex [2,3]. As a consequence, camptothecins induce DNA breaks by preventing DNA religation [3].

The realization that topoisomerase I is a useful target for pharmacological intervention, the impressive antitumor activity of camptothecins in preclinical systems of human tumor types refractory to conventional agents, and the clinical success of topotecan and irinotecan have stimulated efforts to optimize the efficacy of agents of this class. The initial approach in camptothecin analog development was focused to increase water solubility and obtain compounds with an improved pharmacological profile. Such efforts led to the currently available clinical camptothecins, irinotecan and topotecan, as well as to novel compounds including DX-8951F (exatecan mesylate) and CKD-602, currently in clinical studies [4,5]. More recently, lipophilic analogs have been selected as promising candidates for clinical trials and some of them are already in the early stages of clinical development [6]. Although poorly soluble in water, they were designed and screened to overcome the major limitations of the 'first-generation camptothecins'. Indeed, they present favorable pharmacological properties, such as increased stability of the lactone form, rapid cellular uptake, and enhanced drug–target interaction and stabilization of the ternary complex [7–10]. Among camptothecins of a novel series of lipophilic 7-substituted derivatives, gimatecan (ST1481) was selected as oral camptothecin (Fig. 1) for clinical development on the basis of a combination of several favorable features. This article is an overview of the preclinical profile and preliminary clinical results of gimatecan.

Rationale for development of 7-substituted lipophilic camptothecins

The design of novel camptothecins was aimed at overcoming some relevant limitations of known molecules of this class. Target-specific problems are related to the lack

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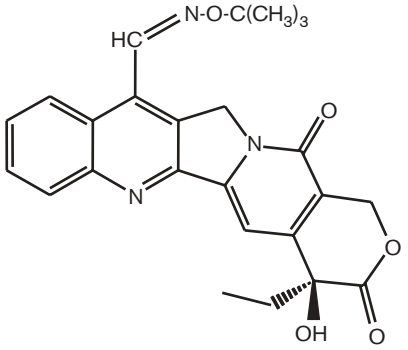
Fig. 1



Chemical structure of gimatecan.

of tumor selectivity of the target and to a variable expression of the target enzyme in different tumor cells. The ubiquitous nature of topoisomerase I is the source of relevant toxicity (in particular, myelotoxicity) and the variable expression may be responsible for the heterogeneous tumor response. Drug-specific limitations include poor water solubility of the natural compound, reversibility of the drug–target interaction and instability of the lactone ring in blood [11]. On the basis of current knowledge, water insolubility does not represent a problem, and lipophilicity may provide some advantages in terms of increased drug–target interaction, lactone stability and oral bioavailability.

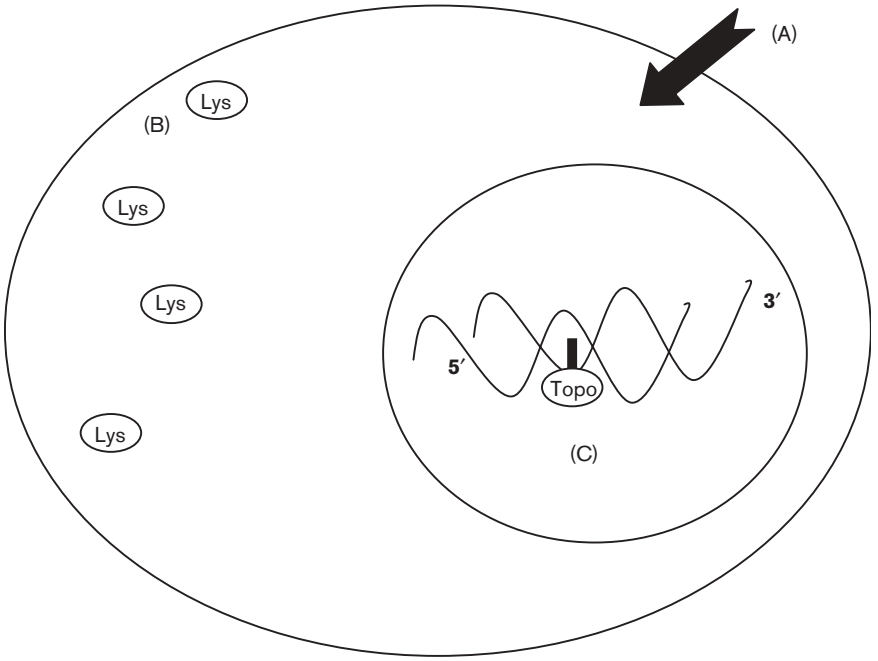
Since the therapeutic potential of topoisomerase I poisons results from the stabilization of the cleavable complex, a full understanding of the molecular determinants of the recognition of the DNA–enzyme complexes by inhibitors is fundamental for a rationale design of more effective poisons. In the past years, structure–activity studies of camptothecins have demonstrated a few salient points of the structure of camptothecins which are essential for the poisoning activity. These studies revealed that positions 7, 9 and 10 in the A and B rings of camptothecins are tolerant of modifications with a variety of substitutions allowing the anti-topoisomerase I activity to be conserved [12–15]. Recently, the X-ray crystal structure of human topoisomerase I covalently joined to double-stranded DNA and bound to topotecan has been solved [16]. Topotecan mimics a DNA base pair and binds by intercalation at the site of DNA cleavage. The intercalation process displaces the 5′-OH of the cleaved strand, thus preventing re-ligation. The structural model demonstrates that the positions 7, 9 and 10 face into the major groove of the DNA, and modifications in these positions, aimed at improving the solubility and stability of the compounds, would not sterically interfere with drug binding. The available models of the drug interaction in the ternary complex indicated a wide space for chemical manipulations in position 7 of camptothecin. On the basis of this rationale, a systematic investigation of several substituents at position 7 was performed at the National Cancer Institute (Milan) in collaboration with the University of Milan (DISMA Department). Among the tested compounds, the cytotoxic potency was critically dependent on the type of substituents [9,17]. On the basis of its promising preclinical profile, a 7-oxyiminomethyl derivative (ST1481, gimatecan) was selected for development.

Molecular pharmacology

Modification of the DNA topology is essential for critical cell functions and survival. Because of the size of the eukaryotic chromosome and the complex structure of DNA, removal of the supercoils can only be accomplished by introducing transient breaks into the DNA helix [18]. The cleavage of one DNA strand with covalent attachment of topoisomerase I to the 3′ terminus of the nicked DNA leads to a topoisomerase I covalent complex (cleavable complex) and occurs through a tyrosine hydroxyl group of the enzyme. In the 'controlled rotation model', the intact strand is held stationary while the cleaved strand undergoes rotation. DNA religation of the cleaved DNA strand is the last step. The rate of religation is normally much faster than the rate of cleavage, and this ensures a low steady-state level of the covalent topoisomerase I–DNA complex [19]. Some compounds are able to interfere with the enzyme activity by transforming the enzyme into a DNA-damaging agent [20]. The drug–enzyme–DNA complex leads to reversible, single-strand nicks that, according to the fork collision model, are converted to irreversible and lethal double-strand DNA breaks during replication. Therefore, due to the specific mechanism of cytotoxicity, camptothecins are S phase specific, indicating that they are preferentially toxic to cells that are undergoing DNA synthesis [21]. Since the enzyme-mediated primary DNA lesions are potentially reversible before cellular processing of the cleavable complex, it is evident that the stability of the drug interaction in the ternary complex is a critical determinant of drug-inhibitory activity. Considering the quite divergent cytotoxic effects of 7-substituted analogs [9,17], it is likely that the nature of the substituent at position 7 plays an important role in influencing the drug–target interaction and the pharmacological properties of the drug.

Among the 7-oxyiminomethyl derivatives, gimatecan was found to be one of the most potent compounds. The increased cytotoxic potency of gimatecan could be ascribed to several favorable events, including (i) increased intracellular accumulation related to the lipophilicity, (ii) a peculiar subcellular distribution and (iii) an increased and persistent stabilization of the covalent topoisomerase I–DNA complex (Fig. 2). In

Fig. 2



Relevant events in determining the cytotoxic potency of gimatecan: (A) uptake, (B) lysosomal localization and (C) stabilization of the cleavable complex.

contrast to the distribution of topotecan that involves mainly mitochondria and endoplasmic reticulum, gimatecan exhibits a peculiar behavior at the subcellular level with a lysosomal localization [22]. Since the lactone form is stabilized in the acidic environment of lysosomes, this compartment may represent a store allowing intracellular release of the active drug. Indeed, single-strand DNA breaks induced by gimatecan, which are potentially reversible lesions, were found to be substantially more persistent than those induced by topotecan following removal of extracellular drug [23]. Moreover, the persistence of DNA damage produced by gimatecan likely reflects the enhanced potency at the target level and stabilization of cleavage. Indeed, as reported in Figure 3, the study of the cleavage persistence after NaCl-mediated disruption of the ternary complex indicated a more stable complex in the presence of gimatecan in comparison with SN-38 and camptothecin. A plausible explanation for the enhanced stabilization of the enzyme–DNA complex is a favorable fitting of the drug in the ternary complex, rather than a different mechanism of topoisomerase poisoning. However, the exact mode of drug interaction in the ternary complex remains to be determined. Indeed, gimatecan shares a common binding site with camptothecin in the DNA cleavable complex, as documented by a similar pattern of DNA cleavage (Fig. 3).

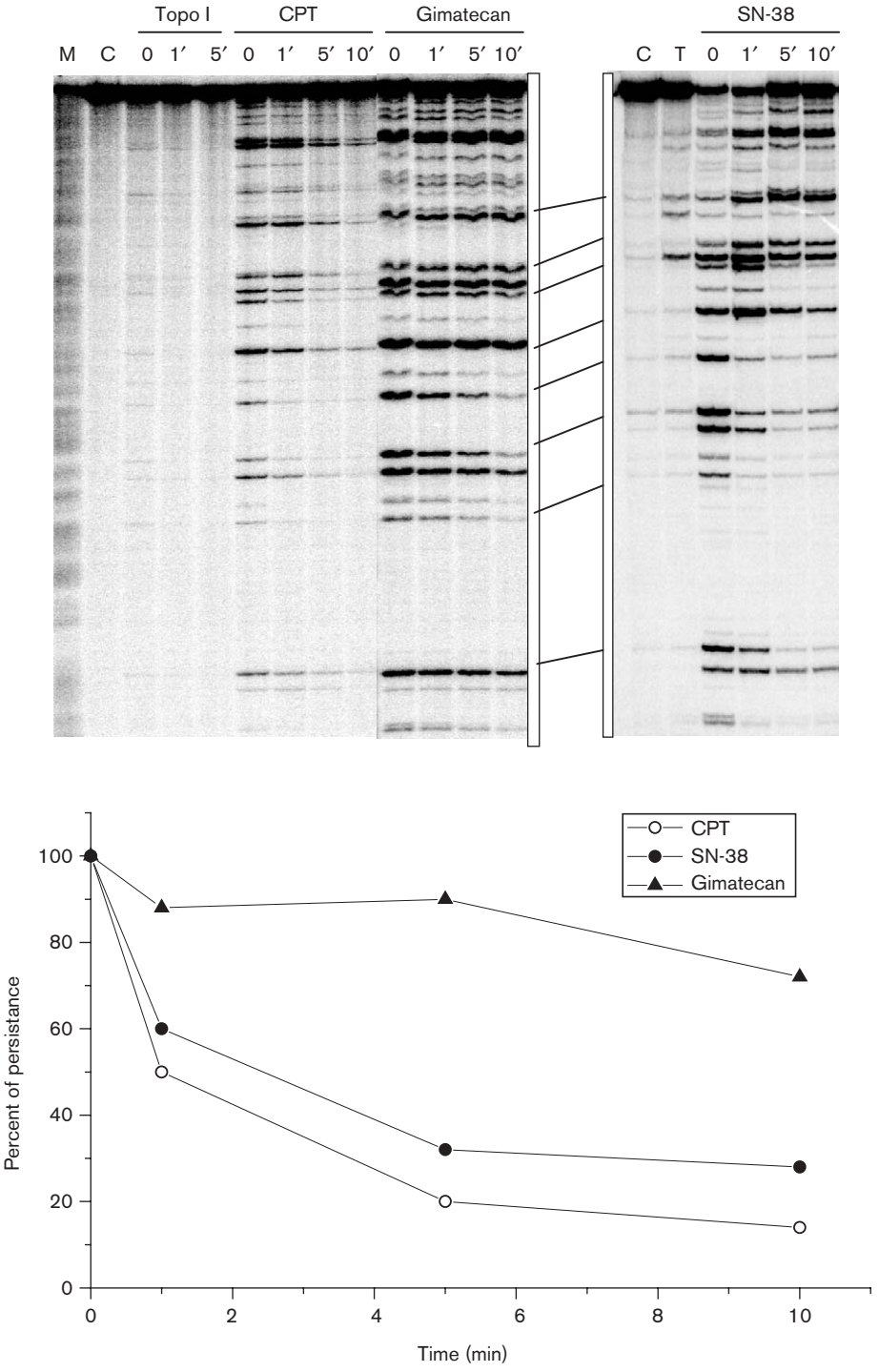
Cellular pharmacology

Cytotoxicity and pattern of cross-resistance

In a large panel of human tumor cell lines of diverse tumor type, gimatecan exhibited a potent cytotoxic effect with IC_{50} values in the range between 10 and 50 ng/ml following a 1-h exposure [23–26]. The cytotoxic potency of gimatecan was at least 10-fold superior to that of topotecan and was reflected in an increased ability to induce apoptosis [23].

The tested cell lines included variants selected for resistance to cisplatin or doxorubicin and exhibited cross-resistance to several agents including topotecan. No cross-resistance to gimatecan was observed in two ovarian tumor cell sublines selected for resistance to doxorubicin and characterized by the MDR phenotype, whereas a marked cross-resistance to topotecan was observed in one of them, i.e. IGROV-1/DX cells, which exhibited a high degree of resistance to doxorubicin [24]. Gimatecan was highly cytotoxic against a human colon carcinoma cell line, HT29/MIT, selected by exposure to mitoxantrone and exhibiting overexpression of BCRP. In contrast to the marked cross-resistance to the clinically relevant camptothecins, topotecan and SN38, the pattern of cytotoxic response in the parental and in the resistant cells indicated a lack of cross-resistance to gimatecan [26]. A collateral sensitivity to gimatecan was observed in a small

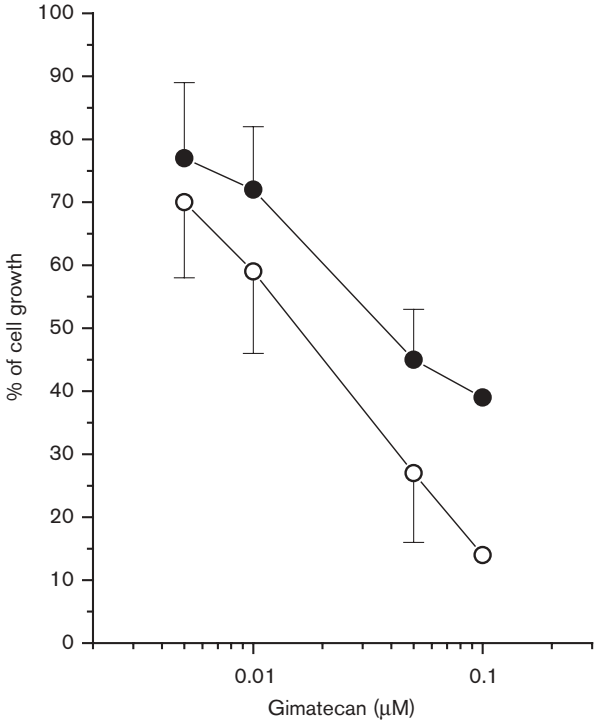
Fig. 3



Persistance of topoisomerase I-mediated DNA cleavage in the presence of camptothecin (CPT), SN-38 and gimatecan. Samples were reacted with 10 μM drug, DNA cleavage was then reversed at the indicated time by adding 0.6 M NaCl, and stopped by 0.5 SDS and 0.3 mg/ml of proteinase K before loading on a denaturing 7% polyacrylamide gel. The 100% value refers to the extent of DNA cleavage at 30 min of incubation. The diagram represents the cleavage persistence versus time. C, Control DNA; T, reaction without drug; M, purine markers.

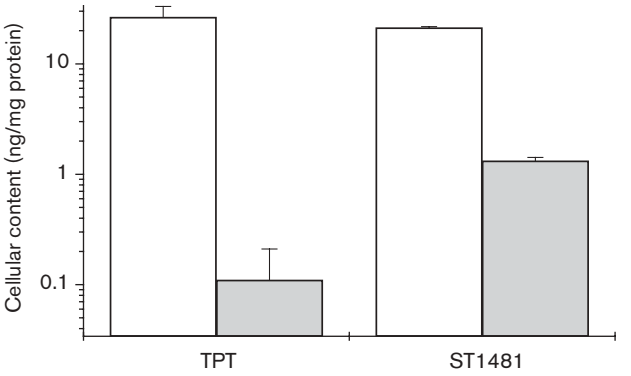
cell lung cancer cell line, POGB/DX, with acquired resistance to doxorubicin and characterized by over-expression of MRP (Fig. 4). Thus, in contrast to topotecan and irinotecan, which are to a variable extent substrates for transport systems (e.g. P-glycoprotein, MRP, BCRP) implicated in the MDR phenotype [27],

Fig. 4



Dose–response curves for cytotoxicity of gimatecan in the parental POGB (solid circles) and in the doxorubicin-resistant POGB/DX (open circles) cell lines. Cells were exposed to drug for 72 h. Values are the mean ± SD (bars) of three experiments.

Fig. 5



Intracellular accumulation and retention of topotecan (TPT) and gimatecan (ST1481) in DU-145 cells. Cells were exposed for 1 h to equitoxic concentrations of the drugs (20 and 2 μg/ml for topotecan and ST1481, respectively) and the cellular drug content was determined immediately after exposure (white column) or after 5 h of incubation in drug-free medium (grey column). Values are the mean ± SD (bars) of quadruplicate determinations.

the available evidence indicates that gimatecan is not recognized by such transporters. Thus, at present, the mechanism of resistance to gimatecan remains unknown, but it is likely that the conventional mechanisms of defense play a marginal (if any) role. The variable sensitivity of tumor cells probably reflects specific alterations in recognition mechanisms of drug-induced DNA damage and in cellular response.

It is conceivable that common alterations in cellular pathways in response to genotoxic stress, involving activation of cell cycle checkpoints and/or apoptosis pathways, could be implicated in determining sensitivity to camptothecins. For example, the effect of gimatecan on cell cycle progression may differ in different cell lines characterized by different cell cycle checkpoints status that may influence tumor response [23,25]. The cytotoxic effect of gimatecan in DU145 prostate carcinoma cells was associated with arrest in S phase and early induction of apoptosis, whereas PC-3 prostate carcinoma cells responded by a persistent block in G_2 phase, late apoptosis and an increased tumor responsiveness. The role of cell cycle arrest at different checkpoints likely depends on the specific biological context. Indeed, arrest at the S phase checkpoint may allow reversal of transient single-strand DNA breaks. G_2 arrest may allow the cells to repair DNA prior to mitosis, but could also trigger apoptotic signals in the case of persistent arrest and insufficient repair. GBM glioma cells, which exhibited arrest in G_2, were found highly sensitive *in vitro* and *in vivo* to gimatecan. p53 gene status has a well-known role in DNA damage response and is involved in activation of cell cycle checkpoints. Apparently, p53 mutation is not a determinant of resistance to gimatecan [23].

Cellular pharmacokinetics

The increased cytotoxic potency of gimatecan over topotecan observed in the cell systems was supported by the cellular pharmacokinetics of the drug. The cellular accumulation studies performed in diverse tumor cell lines were designed to compare gimatecan and topotecan at equimolar or equitoxic concentrations of each drug [23,24]. Exposure (1 h) to equimolar drug concentrations produced a substantially higher intracellular accumulation of gimatecan. This behavior reflects the lipophilic nature of gimatecan. Equitoxic concentrations resulted in a comparable cellular drug content, but the retention of gimatecan following removal of extracellular drug was substantially higher than that of topotecan (Fig. 5). The slow release of the drug could be consistent with the peculiar subcellular distribution of gimatecan [22].

Antitumor activity

Under optimal treatment conditions (i.e. protracted daily schedules) camptothecins may be curative against several human tumor xenografts [28]. However, such a schedule of treatment is unsuitable for evaluation of efficacy in a large panel of tumors used in early phases of preclinical drug development. Thus, most *in vivo* studies with

Table 1 Profile of tumor responsiveness to gimatecan and topotecan (p.o., q4d × 4) in a panel of human tumor xenografts

Tumor type	No. of tumor lines	Responsiveness of tumor lines to			
		Topotecan (15 mg/kg)		ST1481 (2-3 mg/kg)	
		Responsive[a]	Highly responsive[b]	Responsive[a]	Highly responsive[b]
Lung	3	1/3	0/3	2/3	2/3
Melanoma	3	2/3	0/3	2/3	2/3
Brain	3	1/2	0/2	2/3	1/3
Ovary	2	1/2	0/2	1/2	0/2
Colon	3	1/3	0/3	2/3	0/3
Prostate	3	2/3	1/3	2/3	2/3
Osteosarcoma	2	2/2	2/2	2/2	2/2

[a]Number of tumor lines with at least one complete response (CR, i.e. tumor disappearance for at least 10 days)/total number of tumor lines investigated.
[b]Number of tumor lines with more than 50% of CR/total number of tumor lines investigated.

gimatecan investigated a less effective intermittent (q4d × 4) schedule. In a large panel of human tumor xenografts the drug was delivered p.o., using oral topotecan as reference drug. Maximum tolerated doses were 15 and 2–3 mg/kg/injection for topotecan and gimatecan, respectively.

Subcutaneously growing human tumor xenografts

The great majority of the studies was performed on s.c. growing tumors in adult athymic mice. In total, more than 20 tumor lines of various tumor types were investigated, with particular reference to intrinsically resistant tumors [24,25]. In no tumor line was gimatecan less effective than topotecan. The results reported in Table 1 summarize the profile of responsiveness of tumor lines investigated in terms of complete response (CR, meaning disappearance of tumors lasting at least 10 days). Both drugs achieved CRs in several tumor lines of different tumor types, including lung and prostate carcinomas, and melanoma and osteosarcoma. Moreover, in most tumor lines an impressive antitumor effect of gimatecan, resulting in tumor CR, was observed in the majority of treated mice (more than 50%). In contrast, only occasional CRs were achieved by topotecan. Thus, due to the high efficacy of both camptothecins against human tumor xenografts, only a quantitative difference could be found. Complete tumor regression in 100% of mice has been reported for gimatecan even in a neuroblastoma xenograft [29]. A further advantage of gimatecan was a high therapeutic index in mice bearing human tumor xenografts [24].

The effect of protracted daily treatment (qd × 5/week) was investigated in a few tumor lines, including the fast-growing NCI-H460 lung carcinoma and the two slow-growing tumors, A549 lung carcinoma and HT29 colon carcinoma. In all cases, such a treatment schedule was more effective than the q4d × 4 schedule. Moreover, even though the single dose level was lower than in the intermittent schedule, higher cumulative doses of the drug were tolerated [24,25]. Unexpectedly for a camptothecin, gimatecan was very active even when high doses (5–6 mg/kg) were administered by a very spaced schedule, q8–10d, for a long time (10 treatments). More than 50% of the treated NCI-H460 tumor-bearing mice were tumor-free 100 days after cell inoculum [24].

Consistently with the results in cell systems, gimatecan was highly effective against human tumor xenograft with the MDR phenotype (due to P-glycoprotein or BCRP overexpression) or resistant to cisplatin [24–26].

Intracranially growing human tumor xenografts

Due to the high lipophilicity of the molecule, the ability to overcome P-gp170-mediated resistance and a favorable pharmacokinetic behavior [24], gimatecan was tested against intracranially growing human tumor xenografts. The tumor lines investigated were representative of central nervous system tumors (glioblastoma) and brain-metastasizing tumors (melanoma). In all cases, gimatecan was active in increasing mice survival time, the best efficacy being achieved against the SW1783 glioblastoma [30].

Experimental metastases

The marked antitumor activity achieved by gimatecan in primary tumors suggested testing the drug against experimental metastasis models. Gimatecan was administered q4d × 4 to nude mice inoculated i.v. (lung metastases) or intrasplenically (liver metastases) with human tumor cells of different cell lines. In such experimental models, metastases induce mouse death in the controls by 2–4 months. Treated mice were sacrificed about 1 month after; all controls were dead. All treated mice were still alive and most of them were free of metastasis. The remaining mice presented a low number of metastases, only marginally affecting the organ weight ([24] and INT, unpublished results).

Antiangiogenic activity

In addition to the direct cytotoxic effect, camptothecins have been described as possessing antiangiogenic activities [31–34]. Cytotoxic drugs of various classes have shown antiangiogenic potential in preclinical systems, in particular when delivered by a continuous low-dose

schedule. Such therapeutic approach has been named 'metronomic chemotherapy'. The antiangiogenic effects of gimatecan *in vitro* and *in vivo* have been reported in a recent paper [35]. The drug showed an antimotility effect on endothelial cells, *in vivo* inhibition of vascularization in the Matrigel assay and down-regulation of the expression of the pro-angiogenic basic fibroblast growth factor in tumor cells. Moreover, a strong inhibition of microvessel density was induced by gimatecan in a human tumor xenograft and such inhibition paralleled the antitumor effect of the drug [35].

Clinical studies

Three phase I studies with oral gimatecan are currently ongoing in Europe and the US. The European study explored the schedule dependency of gimatecan by escalating doses in three schedules of different dosing duration: daily × 5/week for 1, 2 or 3 weeks, every 28 days. The qualitative toxicity pattern was similar in all schedules, i.e. myelotoxicity, asthenia, mild diarrhea and mucositis. Late-onset thrombocytopenia followed by granulocytopenia was dose limiting in all schedules. Partial responses were documented in non-small cell lung cancer, breast cancer and rhabdomiosarcoma. Gimatecan has a long terminal half-life (83 h) with plasma levels increasing from day 1 to 5 on each week of administration. Recommended phase II doses were defined for 1- and 2-week schedules, and are still under study for the 3-week schedule [36].

A phase I/II trial in recurrent malignant glioma patients is ongoing in the US, using the daily × 5 schedule, every 28 days. Due to the specific type of tumor, one group of patients (five patients) received enzyme-inducing antiepileptic drugs (EIAEDs) and the other group (seven patients) did not. In non-EIAED patients, a terminal half-life of 71 h was reported for gimatecan, much longer than in EIAED patients (6 h). Moreover, C_{max} and AUC_{0-24} values were higher in non-EIAED patients, thus indicating that gimatecan clearance is markedly enhanced by EIAEDs. Radiographic response or disease stabilization was observed only in non-EIAED patients. Dose escalation is still ongoing [37].

A phase I trial of an alternative schedule to administer oral gimatecan once a week for 3 or 4 weeks is also ongoing in patients with refractory solid tumors. Dose levels up to 1.32 mg/m^2 were well tolerated. Disease stabilization was observed in one patient with melanoma and one with colon carcinoma. The pharmacokinetic analysis indicated a rapid absorption and a slow elimination with an apparent terminal half-life of 108 h. Importantly, the compound exists in plasma almost entirely as the active, intact lactone species [38]. High levels of the lactone form have been observed even for the daily × 5 schedule [39].

Phase II studies are planned for gimatecan in non-small cell lung, colorectal, breast and pediatric cancer.

Conclusions

The intense research activity in the field of topoisomerase I inhibitors has provided valuable insights to better understand the mechanism of action, optimize the design of novel agents and exploit the therapeutic potential of camptothecins. An appreciable result of these efforts was the finding that appropriate substituents at the position 7 confer favorable features in terms of potency, stability of the active lactone, oral bioavailability and pharmacological profile [7,9,17].

Gimatecan, the lead compound of a novel series of 7-oxyiminomethyl derivatives, was selected for clinical development on the basis of the promising preclinical profile in terms of efficacy and therapeutic index. The cytotoxic potency of gimatecan likely reflects the contribution of multiple factors, including a peculiar cellular pharmacokinetic behavior (not solely related to its lipophilic nature), potent and persistent inhibition of topoisomerase I, and ability to overcome resistance mechanisms mediated by transport systems, including P-glycoprotein, MRP and BCRP. Additional pharmacological advantages of gimatecan are good oral bioavailability, and favorable distribution and pharmacokinetics in animal studies [25].

Early clinical studies with oral administration of gimatecan are promising and support the preclinical results even in terms of favorable pharmacokinetics, i.e. long half-life of the closed lactone form in plasma [38,39]. It is too soon to predict the clinical success of oral gimatecan, since the clinical applications are not only dependent on the antitumor activity, but also on the toxicity profile. Relevant to this point is the observation that in the phase I studies, the compound was well tolerated and active according to different schedules and dosing durations. Moreover, the dose-limiting toxicity is myelotoxicity, which could be prevented by a variety of support strategies. Phase II studies, currently ongoing, will possibly support the clinical interest in gimatecan.

In conclusion, it is noteworthy that a number of camptothecin derivatives have recently entered clinical studies. In particular, novel 7-modified lipophilic derivatives, including gimatecan, originally designed to overcome some clinical limitations of conventional camptothecins, appear to exhibit a therapeutic/pharmacological behavior more comparable in preclinical and clinical settings, thus suggesting a better prediction of their preclinical profiles.

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