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Clinical pharmacokinetics of the new oral camptothecin gimatecan: The inter-patient variability is related to α_1-acid glycoprotein plasma levels

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ABSTRACT

Aim of the study: To determine the pharmacokinetics of gimatecan, a camptothecin with a lipophilic substitution in position 7, given orally to patients participating in the phase I study.

Methods: Pharmacokinetics was evaluated in 78 patients after oral daily dose for 5 days a week for 1, 2 or 3 weeks by HPLC with a fluorescence detector.

Results: Gimatecan was mainly present in plasma as lactone (>85%), the active form as DNA-topoisomerase I poison. The AUC_{0-24} on the first day of treatment normalised per daily dose (mg/m^2), ranged from 194 to 2909 ng h/mL/mg/m^2. The half-life was 77.1 ± 29.6 h, consequently C_{max} and AUC rose 3–6-fold after multiple dosing. Multivariate analysis indicated the daily dose ($p < 0.0001$) and the α_1-acid glycoprotein (AGP) plasma levels ($p < 0.0001$) as main predictors of gimatecan AUC_{0-24}. In the overall analysis, daily dose and AGP plasma levels explained 85% of the deviance. The hydroxy metabolite ST1698 was present in plasma at low levels with AUC values of 5–15% of gimatecan. In mice, orally treated with gimatecan, plasma and tissue levels were 2-fold higher after treatment with a pro-inflammatory agent causing AGP induction.

Conclusions: Gimatecan is orally absorbed and its variable plasma levels seem to be related to AGP plasma concentrations. Data obtained in mice, together with the fact that AGP levels largely exceeded gimatecan plasma concentrations, suggest that the increased gimatecan levels in patients with high AGP levels are not related to the binding of the drug to AGP with consequent reduced tissue drug distribution, but possibly to other mechanism associated with inflammation being AGP simply a marker of the inflammation process.

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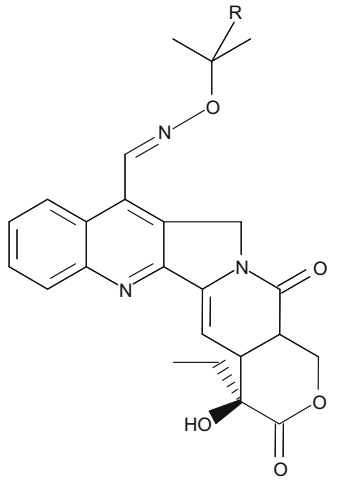
1. Introduction

Camptothecins isolated from the Chinese tree *Camptotheca acuminata*[1] are an effective class of antitumour drugs acting as topoisomerase I poisons.[2] Topotecan and irinotecan are the most widely investigated camptothecins and are currently used in clinical practice.[3–6] The camptothecin molecule has a terminal α-hydroxy-δ-lactone E ring that undergoes reversible, pH dependent hydrolysis in which the closed lactone form is converted to the open carboxylate form under basic and neutral conditions.[7] The high concentration of the closed lactone form in plasma is particularly important as this form is responsible for the pharmacological activity.[7] Gimatecan, the 7-oxyiminomethyl derivative of camptothecin (Fig. 1), was selected as one of a new series of more lipophilic analogues of camptothecin synthesised with the intention to stabilise the lactone ring and improve the pharmacological properties.[8–10] A major feature of gimatecan is its relatively high oral absorption with 40–80% bioavailability in the rat and dog (Sigma Tau internal reports: ST1481-DFM 00-001 and DFM 00-004). When administered orally, it showed strong preclinical antitumour activity against a panel of murine or human tumour xenografts[11,12] and the therapeutic index in rodents appeared to be better than other camptothecins.[13,14] The oral route is a potential alternative method of administration for camptothecin, either for obvious practical reasons but also in consideration of the fact that prolonged exposure to these compounds can improve their antitumour activity.[15–18] The promising preclinical data obtained with oral gimatecan prompted us to undertake a phase I clinical study in which three schedules, i.e. oral treatment for 5 days/week for 1 or 2 or 3 weeks, were investigated. Here we report the detailed pharmacokinetic data of this study. The finding that the high degree of variability appeared to be correlated to AGP plasma levels prompted us to perform some additional preclinical studies in mice to elucidate whether the high AGP plasma levels caused a decreased gimatecan tissue distribution.



Gimatecan: R=H
ST1698: R= OH

Fig. 1 – Chemical structure of gimatecan and its main metabolite ST1698.

2. Materials and methods

2.1. Patients

The plasma pharmacokinetic profile of oral gimatecan was determined in adults with solid tumours at the first cycle of treatment in the phase I trial S003ST1401/ ST1481-DM-00-005.[19] Eligibility criteria, previously described in detail,[19] were age ⩾ 18 years, no surgery affecting drug absorption, ECOG performance status <2, normal haematological tests, adequate hepatic and renal functions, life expectancy of at least 3 months and ability to understand the nature of the trial, giving written informed consent.

The exclusion criteria, detailed in Ref. [19], were related to the presence of concomitant disease involving cardiovascular or gastrointestinal system as well as infective diseases requiring antibiotic therapies.

2.2. Phase I study

Three different durations of treatment based on cycles of 5 days/week schedule (for 1, 2 or 3 weeks) were explored through an open-label dose-finding study in sequential cohorts of patients, with pharmacokinetic evaluation after oral doses of the compound. The different schedules were evaluated concomitantly, increasing the dose and length of treatment inter-dependently to establish three separate MTDs, one for each schedule. The shortest schedule was driving the escalation so that the same total dose per level could be administered over a period of 2 or 3 weeks only after the safety of the same total dose was proven when administered over 1 week. The compound was taken one hour before breakfast as hard gelatine capsules (gelucire 44/14 as diluent), supplied by Sigma–Tau (Pomezia, Italy) containing 0.1, 0.25 or 0.5 mg.

A total of 108 patients were treated during the phase I study; the detailed features of the patients who entered the phase I study were previously reported.[19] The pharmacokinetic studies have involved a total of 88 patients, distributed for three different schedules as illustrated in Table 1.

The pharmacokinetic of gimatecan was evaluable after the first dose in 78 patients. In 53 patients the pharmacokinetics of gimatecan was evaluable both after the first dose and after the last dose (D5, D12 and D19 for the Dx5, Dx10 and Dx15 schedules, respectively).

2.2.1. Blood sampling

Blood sampling was scheduled on day 1 and on the last day of treatment (D5, D12 and D19).

We considered only those patients from each schedule who had blood samples available after gimatecan on D1 and on the last day* of treatment, in accordance with the following sampling scheme: T0 (pre-dose), 0.5, 1, 1.5, 2, 4, 6, 8 10 (or 12) 24 (36*, optional), 48* and 72* h.

Blood samples (5 ml) were collected in heparinised tubes and immediately centrifuged at 2500 rpm at 4 °C for 10 min. The plasma was separated, collected and divided into 1.5-mL samples in two polypropylene tubes that were stored at –80 °C in the dark until analysis.

Table 1 – Patients' distribution within the three different schedules of the phase I study.

DX5 schedule		DX10 schedule		DX15 schedule	
Dose levels (mg/m^2/cycle)	No. of patients	Dose levels (mg/m^2/cycle)	No. of patients	Dose levels (mg/m^2/cycle)	No. of patients
0.8	3	1.6	2	2.64	3
1.6	3	2.64	3	4	5
2.64	4	4	4	5.6	6
4	4	5	5	6.4	7
4.5	5	5.6	12	7.2	4
5	11	7.2	3		
5.6	4				
Total no. of patients	34	Total no. of patients	29	Total no. of patients	25

2.3. *Preclinical studies*

2.3.1. *Mice*

Female athymic nude CD-1 mice, 8 weeks old were obtained from Charles River Italia (Calco, Italy). They were maintained in laminar flow cabinets with constant temperature and humidity, according to the institutional guidelines.

To obtain the tumour xenografts, 200 μl of A2780 (human ovarian carcinoma) cell suspension containing 10×10^6 cells were injected subcutaneously (s.c.) into the flanks of nude mice. The growing tumour masses were measured with the aid of a caliper, and the tumour weights (1 mm^3 = 1 mg) were calculated by the formula: length × (width)^2/2.

For the pharmacokinetic study, when tumour weights reached approximately 300–350 mg, a group of mice was treated with 100 μl of turpentine oil (Vaillant batch 590425) s.c. to increase AGP plasma levels.[20] Forty-eight hours after turpentine oil administration, pre-treated and control animals were randomised for gimatecan treatment.

For the antitumour activity studies, the mice were randomised in two groups when tumour load achieved about 100 mg. One group of mice was treated with 100 μl turpentine oil. Forty-eight hours after turpentine oil injection each group was divided into two homogeneous groups to receive treatment with gimatecan or its vehicle.

2.3.2. *Pharmacokinetics study*

To assess the pharmacokinetic of gimatecan, the animals were treated p.o. at the dose of 5 mg/kg, a dose previously used for a preclinical pharmacokinetic study in mice,[9] with a volume of 10 ml/kg of the 0.5 mg/mL drug suspension (10% DMSO in sterile water).

The samples were collected at 0, 15, 30 and 45 min and 1, 2, 4, 8 and 24 h after drug administration. Four animals were used for time points. Blood was obtained from the retro-orbital plexus under diethylether anaesthesia and collected in heparinised tubes. The plasma fraction was immediately separated by centrifugation (10 min, 4000 rpm, 4 °C) and stored at –20 °C until analysis. The animals were sacrificed by cervical dislocation.

To evaluate drug distribution, tumour, liver, brain, lung and kidney were removed and frozen at –20 °C until analysis.

2.3.3. *Faecal excretion*

Faeces were obtained from two groups of five mice housed in metabolic cages. One of the two groups was pre-treated with turpentine oil 48 h before administration of 5 mg/kg gimatecan. The samples were obtained 24 and 52 h after gimatecan treatment.

2.3.4. *Antitumour activity studies*

To perform the activity study gimatecan was administered at the dose of 0.6 mg/kg every 4 days for four times (q4dx4). The control mice were treated with the drug solvent. Each treatment group consisted of nine animals. Between the second and the third dose, a further s.c. injection of turpentine oil was necessary to maintain high AGP plasma levels.

Tumour growth was measured two/three times a week by a caliper. Drug efficacy was calculated as T/C%, where T and C were the mean tumour weight of treated and control groups, respectively; T–C (tumour growth delay) where T and C are the mean time to reach 1 g for treated and control tumours and LCK(log cell kill) calculated as $T - C/(Td \times 3.32)$, where Td is the tumour volume doubling time. Toxicity was evaluated on the basis of body weight loss (BWL) and mortality. Toxic deaths were considered as any death in the treated groups occurring before any control death.

2.4. *HPLC determination of gimatecan and ST1698 in plasma and tissue samples*

Total gimatecan (lactone plus carboxylate form), its lactone form and the major metabolite ST1698 (Fig. 1) were assessed, in human plasma, by HPLC with fluorimetric detection as previously described.[21] When we started the pharmacokinetic study of gimatecan, validation of the assay for the metabolite ST1698 had not been completed, so in the first part of the clinical study only the parent compound was determined.

To evaluate drug distribution and elimination, weighed tissues were homogenised in 0.1% acetic acid:acetonitrile 1:4, pH 4.5, added with 100 μl of IS and diluted with 4 ml of 0.1 M formic acid:acetonitrile 90:10. The samples were then centrifuged at 4000 rpm for 10 min and the supernatants were transferred into autosampler vials for the HPLC analysis.

The analytical reference standards of gimatecan (Batch No. 532/68), ST1698 (Batch No. 441/51) were provided by Antibioticos S.p.A. The analytical reference standard of ST 1966 (Batch No. EM/1/1), used as internal standard (IS), was provided by Sigma–Tau.

2.5. Pharmacokinetic analysis

The pharmacokinetic parameters were estimated or derived directly from the individual gimatecan and ST1698 plasma concentration values, by using a not compartmental approach.

All the parameters were estimated by WinNonlin Pro Node 4.1 pharmacokinetic software (Pharsight Co. Mountain View, CA, USA).

2.6. Statistical analysis

Patient's characteristics and dosages were reported using standard descriptive statistics. The relationships between AUC and C_{max} and selected covariates were analysed using univariate or multivariate regression models. In total 12 covariates were tested: daily dose (mg/m^{2}day), age, sex, body surface area (BSA), α_1-acid glycoprotein (AGP), transaminases (ALAT/SGPT, ASAT/SGOT), albumin, alkaline phosphatase (ALP), bilirubin, creatinine and total plasma proteins.

Multivariate models were built using the covariates that showed a significant association with the dependent variables in the univariate analysis.

The R^2 coefficient was taken as a measure of variability explained by the chosen model. Assumptions of linear regression were assessed through graphic and analytical analysis of residuals.

In all the analyses, the alpha levels were set to 0.05 (two tail). Given the explorative nature of the analysis, no adjustment was made for multiple testing.

SAS version 8.2 statistical software (SAS Institute Inc., Cary, NC, USA) was used.

3. Results

Complete pharmacokinetic data were available from 53 of the 88 patients involved in the study, 22 enrolled in the Dx5 schedule, 14 in the Dx10 schedule and 17 in the Dx15. The main pharmacokinetic parameters are reported in Table 2, panels A–C. The time of absorption appeared to be independent from the dose and the duration of treatment since most of the patients achieved the C_{max} within 4 h from drug administration. Moreover in most patients, after the absorption phase, gimatecan concentrations declined and then rose again. In 14 cases, the second peak corresponded to the C_{max} and occurred between 6 and 24 h from drug administration (except in one case at 72 h). This was probably due to enterohepatic recirculation.

C_{max} and AUC varied widely regardless of the different schedules and doses with CV% ranging between 6.6% and 85%.

Drug accumulation resulted in 2–6 times higher plasma levels after the last dose (D5, D10 or D15) than on D1. The elimination half-life of the drug was determined in patients sampled at least up to 72 h after the last dose given. Despite the sampling scheme was inappropriate to estimate accurately the half-life of gimatecan, it appeared very long with a mean value of 77.1 ± 29.6 h in the total 53 patients with no apparent differences related to the schedule.

The intact lactone form was assayed in 11 patients, two on the Dx5 schedule, five on the Dx10 schedule and four on the Dx15 schedule. Gimatecan was present in the plasma of each patient, almost completely as the intact lactone form, amounting to 92.3 ± 12.2% of the total AUC. Fig. 2 shows a representative comparison of total gimatecan and intact lactone form plasma levels in four patients on D1 and on the last day of treatment.

3.1. Pharmacokinetic variability

An analysis in 78 patients for whom the individual C_{max} and AUC_{0-24} values on the first day of treatment were normalised per daily dose expressed in mg/m^2 and in mg showed a long inter-individual variability with mean ± sd normalised AUCs of 753.8 ± 482.2 ng h/mL/mg/m^2 (range 194.0–2909) and 445.3 ± 305.5 ng h/mL/mg (range 92.4–1891).

To investigate the reasons for the pharmacokinetic variability we conducted a statistical analysis including 12 covariates (Table 3 panel A) to clarify whether between subject differences in the pharmacokinetic parameters could be attributed to specific factors. The results of the univariate analysis are shown in Table 3 panel A. The main predictors of AUC_{0-24} on day 1 were the daily dose ($p < 0.0001$), the AGP plasma levels ($p < 0.0001$) and albumin ($p = 0.014$). These three covariates account for, respectively, 25.0%, 28.8% and 7.8% of the deviance.

For the AUC_{0-72} on the last day of treatment the analysis was conducted separately for each schedule. AGP and dose were significant predictors in all three schedules and albumin in the Dx5 and Dx15 schedules. When the same analysis was done taking the three schedules together, the daily dose ($p = 0.001$) accounted for 21.5% of the deviance, AGP plasma levels ($p < 0.0001$) for 65.5% and albumin ($p < 0.0001$) for 31.8%.

Multivariate analysis was carried out to predict the AUC in relation to several covariates (Table 3 panel B). The variability of AUC_{0-24} on day 1 was explained by the daily dose ($p < 0.0001$) and the AGP plasma levels ($p < 0.0001$) which accounted together for 59.8% of the deviance. When the analysis was done considering each of the three schedules separately, AGP was the only significant predictor of the AUC_{0-72}, except in the Dx5 schedule where the daily dose was also significant. In the overall analysis, daily dose (<0.0001) and AGP plasma levels ($p < 0.0001$) explained 85.0% of the deviance. Albumin was never significant in the multivariate model.

Fig. 3 shows the plot of AUC_{0-72} residuals adjusted for the dose alone (panel A) or the dose and AGP (panel B), according to the bivariate model, without taking account of the different schedules.

3.2. ST1698 pharmacokinetics

Plasma levels of ST1698, the polar hydroxy metabolite of gimatecan previously identified in rats and dogs (Sigma–Tau internal report: ST 1481-DFM 00-004) were measured in 26 patients, seven on the Dx5 schedule, eight on the Dx10 schedule and eleven on the Dx15 schedule. After the first exposure to gimatecan (D1), ST1698 plasma levels in most patients were undetectable, or at any rate negligible at several time points

Table 2 – Main pharmacokinetic parameters of gimatecan on day 1 and day last in patients treated with the Dx5 (panel A), Dx10 (panel B) and Dx15 (panel C) schedules.

A		Day						
		1			5			
Dose (mg/m^2)		T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	AUC_{0-72} (ng h/mL)
2.64	N	1	1	1	1	1	1	1
	Mean	1.5	35.50	391.0	4.0	74.60	1580	3533
	SD	–	–	–	–	–	–	–
	Range	–	–	–	–	–	–	–
4.0	N	4	4	4	4	4	4	4
	Mean	2.6	52.01	715.9	1.4	129.5**	2299***	5666
	SD	3.6	18.93	395.5	0.6	29.03	569.4	1468
	Range	0.5–8.0	31.30–76.30	467.1–1300	0.5–2.0	101.4–162.6	1857–3118	4807–7863
4.5	N	5	5	5	5	5	5	5
	Mean	1.8	34.22	513.3	17.7	79.74*	1575*	3812
	SD	1.4	7.63	199.0	30.5	30.89	775.7	1771
	Range	0.5–4.0	22.67–40.59	287.0–818.0	2.0–72.0	61.34–133.5	1045–2928	2817–6946
5.0	N	9	9	9	9	9	9	8
	Mean	3.9	33.06	491.8	2.8	103.41**	2027**	5819
	SD	7.5	18.79	378.4	1.8	68.70	1634	4913
	Range	0.8–24.0	18.48–73.77	193.1–1346	0.5–6.0	48.41–247.4	859.8–5743	1907–16,054
5.6	N	3	3	3	3	3	3	3
	Mean	12.7	42.35	853.7	3.7	156.5	3317	9018
	SD	11.0	15.25	343.5	2.5	61.96	1538	3951
	Range	2.0–24.0	24.89–53.10	465.0–1116	1.0–6.0	107.6–226.2	2228–5076	6094–13,512
B		Day						
		1			12			
Dose (mg/m^2)		T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	AUC_{0-72} (ng h/mL)
5.0	N	2	2	2	2	2	2	1
	Mean	1.5	21.40	249.9	1.8	78.41	1521	5683
	SD	0.7	13.48	13.36	0.4	25.47	710.5	–
	Range	1.0–20	11.87–30.93	240.5–259.4	1.5–2.0	60.40–96.42	1018–2023	–
5.6	N	9	9	9	9	9	9	7
	Mean	6.3	30.23	388.4	2.5	73.27***	1360***	3565
	SD	10.1	19.81	268.9	2.2	34.30	712.5	1877
	Range	1.0–24.0	10.85–67.52	117.3–886.3	0.6–6	35.55–130.4	717.1–2614	1804–6412
7.2	N	3	3	3	3	3	3	2
	Mean	1.2	23.61	342.3	1.2	59.37	1052	2638
	SD	0.3	3.88	22.50	0.6	9.60	201.5	516.9
	Range	1.0–1.5	20.52–27.97	319.9–364.9	0.5–1.5	48.88–67.72	849.0–1252	2272–3003
C		Day						
		1			19			
Dose (mg/m^2)		T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	AUC_{0-72} (ng h/mL)
4.0	N	4	4	4	4	4	4	4
	Mean	1.0	25.42	257.6	2.1	71.29	1348	3379
	SD	0.4	14.88	181.3	1.4	48.17	1077	2865
	Range	0.6–1.5	10.46–38.34	130.5–524.8	0.5–4.0	33.22–136.9	467.2–2849	940.4–7323
5.6	N	4	4	4	4	4	4	4
	Mean	6.9	22.02	244.1	1.0	45.92	810.9	1975
	SD	11.4	12.31	153.1	0.4	20.61	490.7	1312
	Range	1.0–24	8.10–35.95	67.73–440.5	0.5–1.5	25.25–69.51	373.7–1363	863.0–3492

(*continued on next page*)

Table 2 – (*continued*)

		Day						
		1			19			
		T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	AUC_{0-72} (ng h/mL)
6.4	N	6	6	6	6	6	6	5
	Mean	2.1	21.59	283.90	1.8	69.78*	1195*	2392
	SD	2.0	9.40	122.92	1.1	34.91	681.2	1680
	Range	0.5–6.0	11.39–34.19	140.5–470.1	1.0–4.0	36.04–113.9	549.3–2146	1165–5292
7.2	N	3	3	3	3	3	3	1
	Mean	1.3	26.97	378.7	2.2	98.70**	1671**	4421
	SD	0.3	7.55	181.1	1.8	18.94	315.0	–
	Range	1.0–1.5	18.32–32.21	217.2–574.4	0.5–4	79.76–117.6	1318–1923	–

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

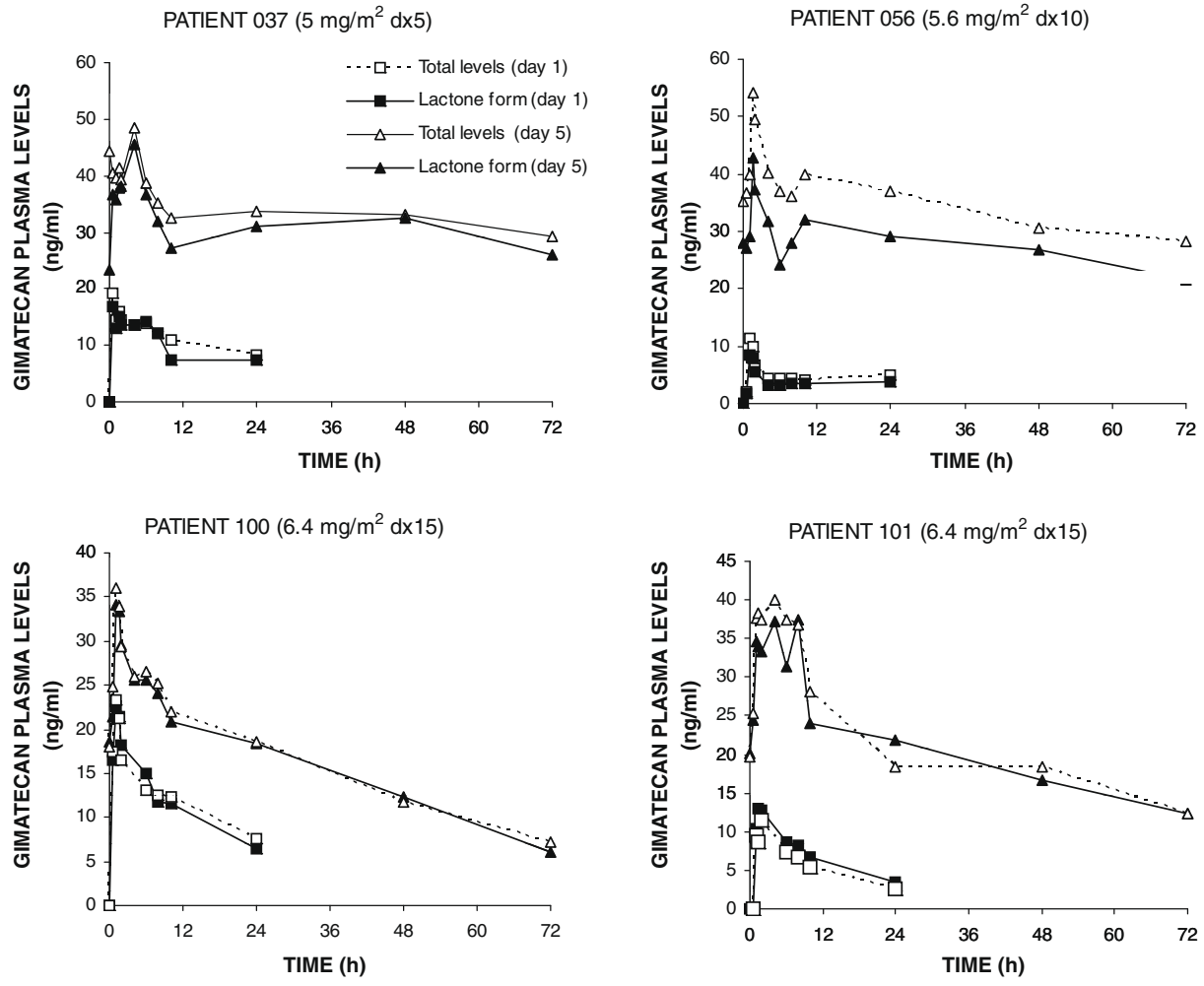


Fig. 2 – Typical plasma decay curves of ST1481 in four patients. Comparison of total gimatecan (□ and Δ for day 1 and day last, respectively) and the intact lactone form (■ and ▲ for day 1 and day last, respectively).

compared to the concentrations of the parent drug. As previously mentioned, after multiple dosing gimatecan accumulated in the plasma. Similarly, ST1698 plasma levels rose after repeated gimatecan doses. The AUC_{0-72} of the metabolite after the last dose amounted to 5–15% of the parent drug. The mean ± sd AUC_{0-72} in five cases on D5 at the dose of

Table 3 – Summary of the statistical analysis. Panel A: univariate analysis; panel B: multivariate analysis.

A		Univariate analysis AUC_{0-24} (day 1)			Univariate analysis AUC_{0-72}											
		Overall			Schedule Dx5			Schedule Dx10			Schedule Dx15			Overall		
Covariates	Mean (Range)	*p*-value	R^2	N	*p*-value	R^2	N	*p*-value	R^2	N	*p*-value	R^2	N	*p*-value	R^2	N
Daily dose (mg/m^2 day)	0.58 (0.16–1.12)	<0.0001	0.250	78	0.177	0.0938	21	0.287	0.140	10	0.8808	0.002	14	0.001	0.215	45
Age (years)	56.8 (18–85)	0.070	0.043	78	0.476	0.027	21	0.578	0.0403	10	0.909	0.0011	14	0.752	0.002	45
Sex (M/F)	44/41	0.878	0.0003	78	0.925	0.0005	21	0.3393	0.1143	10	0.628	0.0202	14	0.776	0.002	45
AGP (mg/mL)	1.1 (0.4–3.9)	**<0.0001**	**0.288**	73	**<0.0001**	**0.820**	18	**0.0009**	**0.7658**	10	**0.0003**	**0.6711**	14	**<0.0001**	**0.655**	42
ALAT-SGPT (U/I)	20.8 (3–63)	0.268	0.016	78	0.868	0.0015	21	0.5085	0.0565	10	0.513	0.0364	14	0.437	0.014	45
Albumin (g/dl)	3.95 (2.8–4.8)	0.014	0.078	78	0.0038	0.380	21	0.2228	0.1792	10	0.0038	0.3798	14	<0.0001	0.318	45
ALP (U/I)	204.8 (46–727)	0.995	0.000	78	0.185	0.0906	21	0.352	0.109	10	0.471	0.0441	14	0.266	0.029	45
ASAT-SGOT (U/I)	28.1 (11–106)	0.267	0.016	78	0.599	0.0148	21	0.761	0.0122	10	0.988	0.000	14	0.711	0.003	45
Bilirubin (mg/dl)	0.57 (0.18–1.52)	0.406	0.009	78	0.862	0.0016	21	0.719	0.0171	10	0.116	0.194	14	0.567	0.008	45
Creatinine (mg/dl)	0.98 (0.50–1.49)	0.355	0.011	78	0.229	0.0751	21	0.818	0.0071	10	0.4233	0.0542	14	0.574	0.007	45
Protein (g/dl)	7.3 (5.8–8.7)	0.297	0.014	78	0.517	0.0224	21	0.9465	0.0006	10	0.3261	0.0804	14	0.862	0.001	45
BSA (m^2)	1.79 (1.41–2.18)	0.075	0.041	78	0.903	0.0008	21	0.7561	0.0127	10	0.8264	0.0042	14	0.81	0.001	45

B	Multivariate analysis AUC_{0-24} (day 1)				Multivariate analysis AUC_{0-72}															
	Overall				Schedule Dx5				Schedule Dx10				Schedule Dx15				Overall			
Variable	Parameter	*p*-value	R^2	N	Parameter	*p*-value	R^2	N	Parameter	*p*-value	R^2	N	Parameter	*p*-value	R^2	N	Parameter	*p*-value	R^2	N
Intercept	–218.69		0.598	73	–9140.01		0.881	18	–1317.88		0.775	10	–1765.82		0.674	14	–3776.05		0.850	42
Daily dose	546.88	<0.0001			10254	0.0142			2640.26	0.6161			1354.24	0.7552			5066.59	<0.0001		
AGP	**262.79**	**<0.0001**			**4241.16**	**<0.0001**			**3353.45**	**0.003**			**3455.52**	**0.0006**			**4056.91**	**<0.0001**		

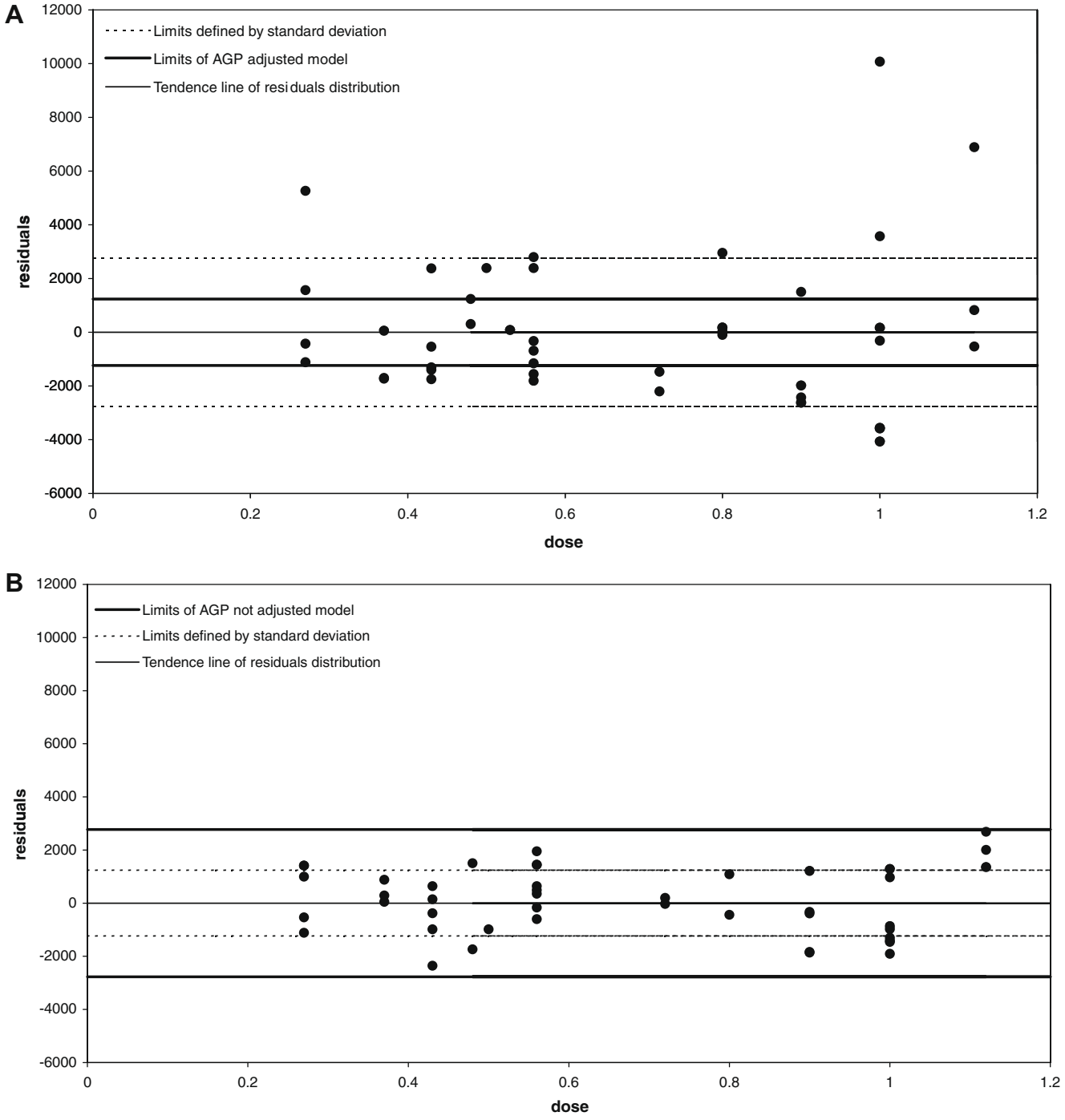


Fig. 3 – Residuals of the AUC_{0-72h} of gimatecan determined by univariate analysis adjusted for daily dose (panel A) and for dose and AGP (panel B), according to the bivariate model.

4.5 mg/m^2 was 349.2 ± 160.5 ng h/mL, ranging from 183.7 to 607.4 ng h/mL. In three patients on the Dx10 schedule at 5 mg/m^2 and in five on the Dx15 schedule at 6.4 mg/m^2 they were, respectively, 394.1 ± 298.2 ng h/mL (range: 119.3–711.3 ng h/mL) and 308.0 ± 276.9 ng h/mL (range 46.90–711.9 ng h/mL). The half-life in 14 of the 26 patients sampled, with plasma available at least up to 72 h, was 79.6 ± 22.1 h (range 41.6–113 h), similar to that of the parent compound (66.6 ± 20.4 h) in the same patients and 77.1 ± 29.4 h in a total of 53 patients.

3.3. *Preclinical studies*

To clarify the role of AGP plasma concentrations on gimatecan pharmacological behaviour, we assessed the pharmacokinetic and the antitumour activity of this drug in an experimental model in which AGP plasma levels were increased by injecting 100 μl of turpentine oil subcutaneously in A2780 tumour-bearing mice (398.2 ± 169.2 μg/mL and 1625 ± 101.3 μg/mL in control and turpentine oil pre-treated mice, respectively).

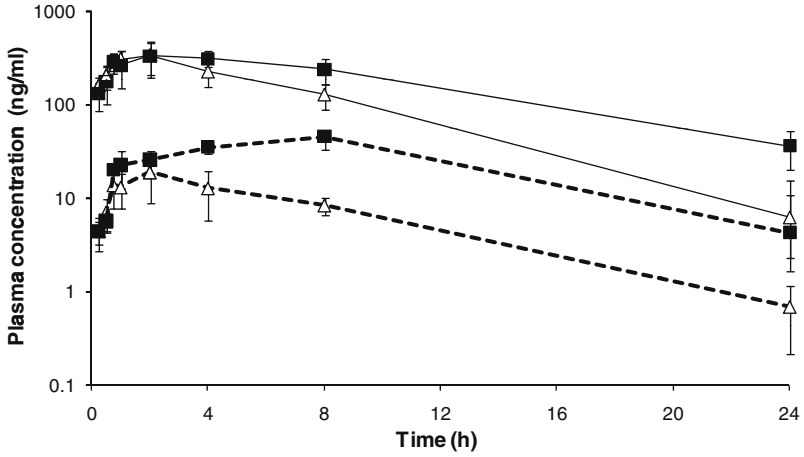


Fig. 4 – Concentration time profiles of gimatecan (solid lines) and its main metabolite ST1698 (dotted lines) in plasma of turpentine oil pre-treated mice (■) compared to control mice (Δ).

Table 4 – Main pharmacokinetic parameters of gimatecan (5 mg/kg p.o.) and its main metabolite ST1698 in nude mice bearing A2780 tumour with or without turpentine oil pre-treatment.

Tissue	Variable	Treatment	C_{max} (ng/ml)	T_{max} (h)	C_{last} (ng/ml)	T_{last} (h)	AUC_{last} (ng/ml h)	AUCINF (ng/ml h)	T1/2 (h)
Plasma	Gimatecan	Controls	336.8	2.0	6.3	24.0	2861	2896	3.8
		Turpentine oil	333.2	2.0	36.3	24.0	4458	4804	6.6
	ST1698	Controls	19.0	2.0	0.7	24.0	171.3	175.9	4.6
		Turpentine oil	45.2	8.0	4.3	24.0	649.9	n.e.	n.e.
Brain	Gimatecan	Controls	312.4	1.0	95.0	8.0	1457	2016	4.1
		Turpentine oil	237.0	0.8	26.3	24.0	2990	3239	6.6
	ST1698	Controls	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
		Turpentine oil	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Kidney	Gimatecan	Controls	1574	1.0	51.6	24.0	12732	13079	4.7
		Turpentine oil	1528	0.8	169.2	24.0	18822	20374	6.4
	ST1698	Controls	82.1	2.0	3.8	24.0	845.5	872.5	4.9
		Turpentine oil	117.8	8.0	12.4	24.0	1742 I	n.e.	n.e.
Liver	Gimatecan	Controls	3081	1.0	50.1	24.0	19742	20029	4.0
		Turpentine oil	2054	2.0	214.1	24.0	23767	25868	6.8
	ST1698	Controls	135.3	2.0	4.7	24.0	1363	1394	4.6
		Turpentine oil	193.2	8.0	18.5	24.0	2721 I	n.e.	n.e.
Lung	Gimatecan	Controls	1141	1.0	408.6	8.0	6064	9107	5.2
		Turpentine oil	846.2	4.0	105.1	24.0	11318	12314	6.6
	ST1698	Controls	40.9	4.0	14.7	8.0	231.0	n.e.	n.e.
		Turpentine oil	50.3	8.0	3.4	24.0	703.0	n.e.	n.e.
Tumour	Gimatecan	Controls	354.4	2.0	148.5	8.0	2102	3082	4.6
		Turpentine oil	317.6	4.0	34.7	24.0	4191	4500	6.2
	ST1698	Controls	16.1	2.0	8.0	8.0	85.2	155.7	6.1
		Turpentine oil	22.8	8.0	2.3	24.0	330.8 I	n.e.	n.e.

BLQ: below limit of quantitation.
n.e.: not evaluable.

After oral administration of 5 mg/kg gimatecan in mice pre-treated with turpentine oil, different pharmacokinetic profiles were observed compared to control mice. As shown in Fig. 4, drug plasma levels appeared super imposable during the absorption phase, then the curves differed with a slower elimination phase in mice with higher AGP levels (T1/2 of 3.8

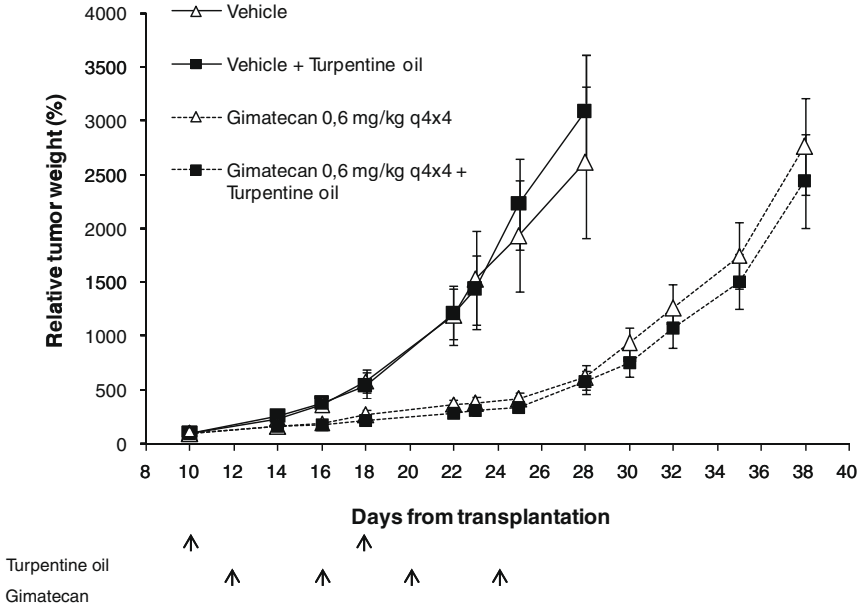


Fig. 5 – Antitumour activity of gimatecan administered p.o. q4x4 at the dose of 0.6 mg/kg to control and turpentine oil pre-treated mouse (black arrows: treatment days).

and 6.6 h, respectively). As a consequence, a higher AUC_{last} was found in plasma of mice pre-treated with turpentine oil than in controls (4458 versus 2861 ng/mL h, respectively).

A similar trend was observed in all tissues examined: in tumours the AUC_{last} in pre-treated mice was almost double than in control mice (4191 versus 2102 ng/mL h).

ST1698 behaviour reflected that of the parental drug, with differences in tissue distribution even more evident. ST1698 brain levels were below the lowest limit of quantitation of the analytical method indicating that this metabolite does not cross the Blood–Brain Barrier. Pharmacokinetic parameters of all tissues investigated were summarised in Table 4.

To evaluate if the altered pharmacokinetics could be due to the differences in drug elimination, we assessed the faecal excretion of gimatecan and its metabolite. No significant differences were observed being the percentage of the dose eliminated as parental drug in faeces of 34.7% and 32.3% in turpentine oil pre-treated and non-pre-treated mice, respectively. The amount of ST1698 found in faeces was very low (2.78% and 2.39 % of the administered dose, respectively).

The impact of the altered pharmacokinetic on gimatecan antitumour activity and toxicity was tested. The drug was administered p.o. at the dose of 0.6 mg/kg q4dx4. Fig. 5 shows the tumour growth curves obtained. Tumour growth was super imposable in vehicle-treated mice with or without turpentine oil pre-treatment. Gimatecan treatment resulted very effective and well tolerated – no toxic death and body weight loss- in both pre-treated (T/C 14.9%, T–C 12.0 and LCK 1.03) and non-pre-treated mice (T/C 23.3% , T–C 10.2 and LCK 0.87).

4. Discussion

We report the detailed data on the clinical pharmacokinetics of gimatecan, in patients participating in the phase I studies in which the drug was given orally daily for 5 days per week for 1, 2 or 3 weeks.

The elimination half-life of gimatecan was between 32 and 147 h, and this explains why when the drug was given daily marked accumulation was observed with all three schedules. In most patients the drug was rapidly absorbed, with peak levels within a few hours, after which there was a decline of the drug concentrations followed by a second peak around 24 h or later, probably related to enterohepatic recirculation.

Gimatecan was present in plasma almost totally in the active lactone form. As previously reported by Sessa and colleagues[19] and further confirmed by Zhu and colleagues,[22] this is a distinct property of gimatecan compared to other clinically used camptothecins where 30–75% in the lactone form is found in plasma.[7]

In keeping with the data showed by Zhu and colleagues,[22] gimatecan plasma levels varied widely in different patients. Since gimatecan was given orally variable gastro-intestinal absorption and intestinal and liver metabolism are possible reasons for the AUC variability.

The low water solubility of gimatecan has been an obstacle for the development of an injectable formulation. Therefore it has been impossible to compare the pharmacokinetics after oral and intravenous (i.v.) doses in the same patients to determine the absolute bioavailability of the drug. However, preclinical pharmacokinetic data in rats and dogs suggested that more than 50% of the drug was absorbed (Sigma–Tau internal reports: ST1481-DFM 00-001 and DFM 00-004).

It is possible that the metabolism of gimatecan differs in different subjects. This requires further studies. However, the major metabolite so far identified, ST1698, which is the product of hydroxylation of the *t*-butyl group of gimatecan, was present in plasma at relatively low concentrations (only about 10% of the concentration of gimatecan) reflecting those

of the parent compound, thus certainly not explaining the inter-patient pharmacokinetic variability.

In order to find the reasons for the variable pharmacokinetics in different patients, we did a statistical analysis to see whether any available clinical or biochemical factors correlated with the AUC. Factors such as age, sex, BSA, transaminases (ALAT/SGPT, ASAT/SGOT), ALP, bilirubin, creatinine and total plasma protein levels did not show any significant correlation, but the plasma levels of AGP were closely related to gimatecan AUC. Analysis of each of the three schedules (i.e. Dx5, Dx10 and Dx15) indicated a strong statistical direct proportionality between AGP plasma levels and gimatecan AUC values.

Since the number of patients in each of the three groups was small for multivariate analysis, we then pooled all patients, taking the AUC values on the last day, without taking account of the schedule. This analysis would have been correct if we had achieved steady-state gimatecan plasma levels in all three schedules, but since this was not the case the findings must be taken as purely indicative. Nevertheless the 65.5% of the variability could be explained by AGP plasma levels alone. This value rises to 85% when dose and AGP were considered together.

Although these data need to be confirmed in phase II trials with much larger numbers of patients receiving the same doses, it seems likely that the main reason for the variability of plasma levels in different patients lies in the AGP levels.

However since the relevance of this finding was not clear, we tried to unravel the relationship between AGP plasma concentration and gimatecan pharmacokinetics, antitumour activity and toxicity in a mouse model with high AGP plasma levels induced by s.c. injection of turpentine oil.

The plasma pharmacokinetic profile and tissue distribution of gimatecan were found different in mice treated with turpentine oil compared to control mice with higher AUC and longer T1/2 in mice with high AGP plasma levels. It is to note that drug levels were increased also in the neoplastic tissue where the drug exerts its antitumour effects. Consistent with these data, the antitumour activity of gimatecan was not decreased in mice with high AGP plasma levels.

The data on ST1698 reflected those obtained on gimatecan, indicating that higher drug levels in turpentine oil pre-treated mice were not due to a reduced metabolism, unless there is a decreased biotransformation of gimatecan in other unknown metabolites, undetectable in our analytical conditions. Detailed studies on gimatecan and ST1698 excretion in faeces showed no differences in drug elimination, nevertheless only a complete mass balance assessment with radioactive-labeled drug might address this point.

It is worthy to discuss whether the differences observed are due to the binding of gimatecan to AGP, or to other mechanisms involved in the inflammation. It is a matter of fact that mice treated with turpentine oil have an acute inflammation leading to different changes in the body,[23] that involve factors, like, for example, cytokines, that might have profound effects on the host physiology and thus, presumably also on the disposition of gimatecan.

The system used does not allow to separate the importance of AGP from many other factors associated with the inflammation (e.g. alteration in the expression and activity of MDR transporters and reduced activity of CYP3A4). Nevertheless, in our opinion, the increase in AGP plasma levels that is associated to acute inflammation might mimic the clinical situation in which the advanced tumour may be associated inflammatory reaction.[24,25] Therefore in the clinic too the highly significant correlation between gimatecan AUC values and AGP levels is not necessarily due to a direct mechanism that involves the binding of gimatecan to AGP, but possibly to other mechanisms and AGP simply represents a marker of the acute phase reaction phenomenon occurring in patients.

In this respect, it may be of interest in future studies to evaluate not only AGP but also other inflammation markers whose levels might be related to gimatecan plasma levels.

In our opinion the hypothesis that the change in gimatecan pharmacokinetics is not related to a direct mechanism involving AGP binding is supported by the fact that the concentration of the protein in plasma (10–25 μM) is much higher than that of the drug (<1 μM) even when it is in the normal range and thus an increase in AGP concentration should not have any influence on the ratio free/bound gimatecan and on the drug disposition. Ultrafiltration experiments conducted in human plasma showed that more than 99% of the total gimatecan is bound to plasma proteins (data not shown), but unfortunately a precise determination of the affinity of gimatecan to AGP , necessary to demonstrate our hypothesis, was not feasible due to the instability of the lactone form in vitro at physiologic pH and temperature.

In conclusion this study provides detailed information on the pharmacokinetic properties of gimatecan, a new oral camptothecin under clinical development. Gimatecan has some distinct pharmacological properties that make it different from other camptothecins: it is present in plasma in the active lactone form and it has a long elimination half-life, thus ensuring continuous exposure of tumour cells to active levels of the drug. Acute inflammation present in a fraction of patients or in mice treated with a pro-inflammatory agents associated with high AGP plasma levels, influence gimatecan pharmacokinetics, but at least in mice not reducing the antitumour activity. Ongoing phase II studies and more detailed preclinical studies will help to confirm these findings and to unravel the relationship between gimatecan pharmacokinetic parameters and drug pharmacodynamics.

5. Conflict of interest statement

Claudio Pisano and Paolo Carminati are Sigma Tau employers.

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