Anti-Cancer Drugs 1996, 7, pp. 166–174

Phase I and pharmacology study of intoplicine (RP 60475; NSC 645008), a novel topoisomerase I and II inhibitor, in cancer patients

Dany Abigerges, Jean-Pierre Armand, Guy G Chabot,[1] René Bruno,[2] Marie-Christine Bissery,[3] Martine Bayssas,[2] May Klink-Alaki,[2] Michel Clavel[4] and Gilles Catimel[4]

Departments of Medicine and [1]Pharmacology (CNRS URA-147), Institut Gustave-Roussy, Villejuif, 39 rue Camille-Desmoulins, F-94805 Villejuif Cedex, France. Tel: (+33) 1 4559 4909; Fax: (+33) 1 56063 8324. [2]Rhône-Poulenc Rorer SA, Antony and [3]Centre de Recherche de Vitry-Alfortville, Vitry-sur-Seine, France. [4]Centre Léon-Bérard, Lyon, France.

Intoplicine (RP 60475F; NSC 645008) is a novel 7H-benzo[*e*]pyrido[4,3-*b*]indole derivative which interacts with both topoisomerases I and II. Because of its high activity in preclinical cancer models, original mechanism of action and acceptable toxicity profile, intoplicine was further evaluated in a phase I and pharmacology study. Thirty-three (33) patients (24 men and nine women) meeting standard phase I eligibility criteria were included: median age was 56 years, performance status 0–1 in 28 patients and 2 in five patients. Tumor primary sites were head and neck (9), colon (6), lung (3) and various other sites (15). Thirty-one patients had received prior radiotherapy and/or chemotherapy. Sixty-nine courses of intoplicine were administered as a 1 h i.v. infusion at dose levels ranging from 12 to 360 mg/m^2. Dose-dependent and reproducible hepatotoxicity was dose limiting in three out of four patients at 360 mg/m^2: this toxicity was reversible in two of three patients, but was fatal in one. Two sudden deaths occurred in this study at 12 and 48 mg/m^2, and the drug implication could not be excluded. No myelosuppression was noted. Hepatotoxicity is therefore dose limiting at 360 mg/m^2, and the phase II recommended dose is 270 mg/m^2 every 3 weeks with close monitoring of hepatic and cardiac functions. Intoplicine pharmacokinetics was determined in plasma (23 patients) and whole blood (18 patients) at doses ranging from 12 to 360 mg/m^2. Intoplicine plasma concentration decay was either bi- or triphasic with the following pharmacokinetic values (mean ± SEM): half-life α, 0.04 ± 0.004 h; half-life β, 0.61 ± 0.13 h; terminal half-life, 19.4 ± 4.0 h; mean residence time (MRT), 11.3 ± 2.4 h; total plasma clearance (CL), 74 ± 5 l/h; volume of distribution β (V_β), 1982 ± 477 l; volume of distribution at steady state (V_{ss}): 802 ± 188 l. Both the area under the plasma concentration versus time curves (AUC) and the maximum plasma concentrations (C_{max}) increased linearly with the intoplicine dose, indicating linear pharmacokinetics (AUC: r = 0.937; slope = 0.01305; $p < 0.001$; C_{max}: r = 0.847; slope = 0.01115; $p < 0.001$). Plasma AUC was also predicted very accurately by the C_{max} values (r = 0.909; slope = 1.0701; $p < 0.001$). Other plasma pharmacokinetic parameter values increased significantly with dose, e.g. the terminal half-life (r = 0.748, $p < 0.001$) the MRT (r = 0.728, $p < 0.001$), the V_β (r = 0.809, $p < 0.001$), and the V_{ss} (r = 0.804, $p < 0.001$). This was probably due to a longer detectability of the drug in plasma at higher doses. Blood pharmacokinetics was also evaluated in 18 patients since it was found that red blood cells represented a significant drug reservoir for intoplicine. Blood intoplicine disposition curves were either bi- or triphasic with the following pharmacokinetic parameter values (mean ± SEM): half-life α, 0.04 ± 0.01 h; half-life β, 0.94 ± 0.22 h; terminal half-life, 57.1 ± 6.6 h; MRT, 82.2 ± 9.9 h; CL, 18 ± 3 l/h; V_β, 1188 ± 147 l; V_{ss} 1163 ± 138 l. Blood pharmacokinetics was linear, since AUC and C_{max} increased linearly with dose (AUC: r = 0.879; slope = 0.06884; $p < 0.001$; C_{max}: r = 0.835, slope = 0.01223; $p < 0.001$). Blood AUC values could also be determined by the blood C_{max} (r = 0.768; slope = 5.0206; $p < 0.001$). Other blood pharmacokinetic parameter values presented a dose dependence, e.g. the terminal half-life (r = 0.626, p = 0.005), the V_β (r = 0.682, p = 0.002) and the V_{ss} (r = 0.555, p = 0.017). The plasma or blood intoplicine concentrations achieved *in vivo* in humans are potentially cytotoxic levels based on preclinical *in vivo* and *in vitro* data. In conclusion, the phase II recommended dose of intoplicine is 270 mg/m^2 administered as a 1 h i.v. infusion every 3 weeks. Plasma and blood pharmacokinetics were linear within the dose range studied. Potentially cytotoxic concentrations were reached at clinically achievable doses.

Keywords: Intoplicine, NSC 645008, pharmacokinetics, phase I, RP 60475.

During the course of this study, our friend and colleague oncologist Dr Michel Clavel died on 28 February 1993. This article is dedicated to his memory.

This study was supported in part by Rhône-Poulenc Rorer SA, Antony, France.

Correspondence to J-P Armand

Introduction

Inhibitors of topoisomerases I or II have emerged as very attractive anticancer drug targets.[1] These enzymes interfere with the DNA nicking/closing reactions by stabilizing a transient intermediate of the reaction—the cleavable complex—whose formation is thought to lead to cell death.[1] The topoisomerase II inhibitors, such as anthracyclines, ellipticines, acridines, anthracenediones and epipodophyllotoxins, have shown clinical activity in a variety of cancers.[2–8] More recently, inhibitors of topoisomerase I, such as camptothecin and its derivatives, have also shown clinical activity.[9–13]

Considering the clinical activity of the topoisomerase inhibitors, a collaborative work has been undertaken at the Institut Curie (Orsay, France) and the Centre National de la Recherche Scientifique (CNRS), to synthesize analogs of the 7-H-benzo[*e*]pyrido[4,3-*b*]-indole series in order to find new DNA topoisomerase inhibitors.[14,15] Among these analogs, intoplicine (RP 60475; NSC 645008, Figure 1) emerged as the most interesting and the most active.[16,17] Intoplicine induces stabilization of DNA cleavable complex mediated by both topoisomerase I or topoisomerase II, and inhibits the relaxation and decatenation activities catalyzed by these enzymes.[18] Until the synthesis of intoplicine, only actinomycin D was shown to possess dual activity on topoisomerases I and II.[4,19]

Intoplicine has demonstrated good antitumor activity in a wide variety of *in vivo* preclinical models including colon carcinomas C38, C51 and C26; pancreatic ductal adenocarcinoma PO3; mammary tumors MA16/C, MA13/C, MA14/A and MA44; Glasgow osteogenic sarcoma GOS; B16 melanoma; P388 and L1210 leukemias; and Lewis lung carcinoma.[16]

Intoplicine is also active *in vitro* against many human tumors. Using the tumor soft-agar cloning assay, activity of intoplicine was demonstrated in breast, non-small-cell lung and ovarian cancers.[17] Also of interest, incomplete cross-resistance with doxorubicin, cisplatin, fluorouracil, 4-hydroperoxycyclophosphamide, vinblastine and etoposide was also observed.[17]

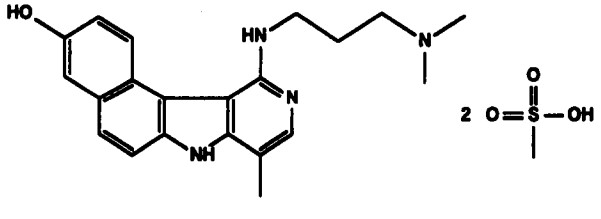

Figure 1. Chemical structure of intoplicine: 11-(3-dimethylaminopropyl-amino)-3-hydroxy-8-methyl-7H-benzo[*e*]pyrido[4,3-*b*]indole; RP 60475; NSC 645008.

Based on intoplicine's good preclinical antitumor activity against solid tumors and leukemias in mice,[16] *in vitro* activity against human tumors,[17] its acceptable toxicity profile, and its original mechanism of action, this compound was selected for clinical development. The purpose of the present phase I study was to determine the toxic effects of intoplicine, the maximum tolerated dose (MTD), the phase II recommended dose and the pharmacokinetics of this drug in cancer patients.

Patients and methods

Patients

Between August 1991 and May 1993, 33 patients from two cancer centers were included in this study: 21 patients were entered at the Gustave-Roussy Institute (Villejuif, France) and 12 patients at the Centre Anticancéreux Léon-Bérard (Lyon, France). Inclusion criteria included the following: (i) a histologically confirmed malignant solid tumor refractory to conventional effective therapies or for which no established therapy exists; (ii) age between 18 and 75 years; (iii) life expectancy of at least 12 weeks; (iv) a performance status of 0–2 according to the WHO grading system; (v) off prior anticancer therapy for at least 4 weeks (6 weeks for nitrosoureas and mitomycin C); (vi) adequate bone marrow reserve (hemoglobin ≥ 11 g/dl, leucocytes $\geq 4000/\mu l$, granulocytes $\geq 1500/\mu l$, platelets $\geq 100\,000/\mu l$); (vii) adequate renal function (serum creatinine < 1.25 times the upper normal limits of the institutional normal values; (viii) normal hepatic function (serum total bilirubin $< 1.25 \times$ normal values, SGPT or SGOT $< 2 \times$ normal values, normal prothrombin and thromboplastin; (ix) a signed informed consent form reviewed by the French Committee of Ethics was obtained from each patient.

Treatment evaluation

Prior to the first course, each patient had a complete medical history, physical examination, chest X-ray, complete blood cell count, direct Coombs test, serum chemistry, renal and hepatic function assessment, and urinalysis. During drug administration, blood pressure and pulse were measured

every 15 min for the first 90 min, and an electrocardiogram was recorded at 1, 4, 24, 48 and 72 h after drug infusion. In addition, Holter monitoring was performed starting 24 h before and lasting for 24 h after drug administration. Toxic effects and antitumor activity were evaluated according to the WHO criteria.[20]

Intoplicine administration

Intoplicine was provided by Rhône-Poulenc Rorer SA (Antony, France) as vials containing a solution of 20 mg/2 ml or 100 mg/5 ml. The appropriate amount of the drug was diluted in 250 ml of 5% dextrose or 0.9% sodium chloride solution and administered to the patient as a 1 h i.v. infusion every 3 weeks.

Dose escalation

The starting dose was 12 mg/m^2 every 3 weeks. This dose represents 1/10 the lethal dose for 10% of mice. The dose was escalated in decreasing increments, depending on the clinical judgment of the investigator and after discussion with the clinical monitor. If no significant toxicity was observed, doses could be escalated with a 100% step. If significant toxicity was observed, doses could be escalated 40–67% initially, then with 33% increase steps. Each dose level included at least three evaluable patients. At a given dose level, at least a 1 week interval should have elapsed between the entry of the first patient and the next two patients, and two patients had to be observed for acute toxicity for a minimum of 2 weeks before escalating to the next dose level. No dose reduction was planned. The MTD was defined as the highest dose which can be safely administered, while producing tolerable, manageable and reversible toxicity.

Pharmacokinetics

Biological sample collection. Serial blood samples (5 ml) were collected in heparinized tubes from an indwelling i.v. catheter placed in the arm contralateral to that receiving the drug. Blood samples were harvested at the following times: before the start of the i.v. infusion (time 0), during the infusion at 30 min, and at the end of infusion at 60 min; after the infusion at 5, 10, 20, 60 and 90 min, and at 2, 3, 6, 8, 12, 24, 36 and 48 h; sampling was prolonged beyond 48 h in many patients. An aliquot of whole blood (1 ml) was immediately stored in a polypropylene tube at −20°C, and the remaining blood was centrifuged at 3000 *g* for 15 min and plasma was transferred to a polypropylene tube and stored at −20°C until analysis.

HPLC assay. To 100 µl of plasma or whole blood was added 1 ml of 2-propanol to precipitate proteins. The sample was vortex mixed (30 s) and centrifuged (9500 *g*). The clear supernatant was directly injected into the HPLC system. Chromatographic analyses were performed according to a selective and sensitive normal-phase HPLC assay with fluorescence detection.[21] This assay was validated, and the stability of the analyte in plasma, in whole blood and in the extraction fluid was at least 2 months at −20°C.[21] The lower limit of quantitation of this assay is 1 ng/ml.

Pharmacokinetic analyses. Pharmacokinetic parameters were estimated using standard methodology by non-compartmental and compartmental analyses.[22,23] The following pharmacokinetic parameters were determined by non-compartmental analysis: C_{max}, the actual concentration at the end of i.v. infusion; $AUC_{0-\infty}$, area under the plasma or blood concentration versus time curve calculated by the trapezoidal method from time zero to infinity; AUMC, area under the first moment curve calculated to infinity; MRT, mean residence time calculated by the model-independent approach (corresponds to the time for 63% of the drug to be eliminated); MRT_{inf} = AUMC/AUC;MRT_{iv} = $MRT_{inf} - T/2$, where T is the infusion time; V_{ss}, apparent volume of distribution at steady state calculated as [(dose × AUMC)/(AUC^2)] − [(dose × T)/(2 × AUC)]; CL, total body clearance calculated as: total dose/$AUC_{0-\infty}$; V_β, apparent volume of distribution of the terminal phase calculated as: dose/(terminal decay constant × AUC).

For model-dependent analysis, intoplicine plasma or blood concentrations were fitted to the appropriate pharmacokinetic model using nonlinear least square weighted regression analysis (SIPHAR program, version 4; SIMED, Créteil, France; or, APIS program, version 3.04; MIIPS, Marseille, France). Individual concentration data were fitted to either two- or three-compartment models (corresponding to two- or three exponential equations). The best model was selected on the basis of the Akaike information criterion. The half-lives were calculated from the model-derived elimination constants (λ) as $0.693/\lambda$.

Statistical considerations

Results are presented as means with their associated standard error of the mean (SEM). The correlation between dose and pharmacokinetic parameters was evaluated using the Pearson product moment correlation test.

Results

Patients

The characteristics of the 33 patients (24 men and nine women) entered in this phase I study are presented in Table 1. The median age was 56 years, performance status 0 or 1 in 28 patients, and five patients had a performance status of 2. Tumor primary sites were head and neck (9), colon (6), lung (3) and various other sites (15). With the exception of two patients, all patients had previously received radiotherapy and/or chemotherapy.

Dose escalation

Sixty-nine courses of intoplicine were administered as a 1 h i.v. infusion at dose levels ranging from 12 to 360 mg/m^2. The number of patients treated at each dose level and the number of courses administered are presented in Table 2.

Table 1. Patient characteristics

Median age (range)	56 (32–72)
Men/women	24/9
Performance status	
0	12
1	16
2	5
Primary sites	
head and neck	9
colon	6
unknown	3
lung	3
others (one of each): uterus, cervix, bile duct, bladder, melanoma, esophagus, urinary tract, kidney, pancreas, pleura, breast, stomach	
Prior therapy	
radiation	21
surgery	27
chemotherapy	31
immunotherapy	1
hormonotherapy	2

Table 2. Dose escalation scheme

Dose (mg/m^2)	No. of patients	No. of infusions
12	5	6
24	3	7
48	4	6
80	4	9
120	4	15
180	4	8
270	5	13
360	4	5

Toxicity

Hepatic toxicity. In this phase I study the dose-limiting toxicity was the increase in hepatic transaminases. This toxicity appeared to increase with dose and it was also reproducible. Indeed, at the 360 mg/m^2 dose level, three out of four patients presented an increase in transaminases, which is suggestive of hepatocellular damage (Table 3). The onset of this disorder was detected at day 2 and returned to normal values by day 9. This toxicity was clinically asymptomatic. Unfortunately, death occurred in one of these patients apparently due to morphine intoxication related to decreased metabolism by the damaged liver. Adjustment of the morphine dosage did not improve the patient's condition. It is also noteworthy that these significant increases in transaminases were always accompanied by increases in LDH, which is suggestive of hepatocellular damage.

Cardiac toxicity. Cardiac-related toxicity was observed in three patients at different dose levels. A patient at 180 mg/m^2 developed angina pectoris on day 2, which responded to i.v. nitrate derivatives after admission in the coronary care unit. This patient was not rechallenged with the drug. Two other patients developed an increase in QT interval at the 270 and 360 mg/m^2 dose levels. These EKG changes were clinically asymptomatic and no treatment was required.

Table 3. Hepatic toxicity

	No. of patient(s) per grade					
	SGcOT grade			SGPT grade		
Dose level (mg/m^2)	2	3	4	2	3	4
180	0	0	1	0	1	0
270	1	0	0	0	0	0
360	1	1	1	1	0	1

Other toxicities. No myelosuppression, such as leuco or thrombocytopenia, was encountered in this phase I study. One case of extravascular hemolytic anemia was observed at 80 mg/m^2 which was in the context of progressive disease with possible microangiopathy; although it appears unlikely that the drug alone was the unique cause of this toxicity, it cannot be excluded; no other similar event was observed.

Hypoaldosteronism–hyporeninism with increased partial thromboplastin time was also seen in one patient with head and neck carcinoma. This symptom responded well to treatment with fluorocortisone acetate.

Three patients developed veinitis along the infusion tract accompanied with mild pain. This was not reproducible at each course and only local treatment was needed. This toxicity was encountered at 80, 120 and 270 mg/m^2 dose levels. No sequellae was observed.

Mortality. Two unexplained sudden deaths were encountered at low doses (12 and 48 mg/m^2), which occurred on days 4 and 15 after drug administration, respectively. No autopsy was performed at the request of the patient's family.

Response. No objective tumor response was observed in this phase I study.

Pharmacology

During this phase I study, intoplicine pharmacokinetics was determined in the plasma of 23 patients at doses ranging from 12 to 360 mg/m^2. When it was found that erythrocytes represented a significant drug reservoir, whole blood pharmacokinetics was also performed in 18 patients at doses ranging from 24 to 360 mg/m^2.

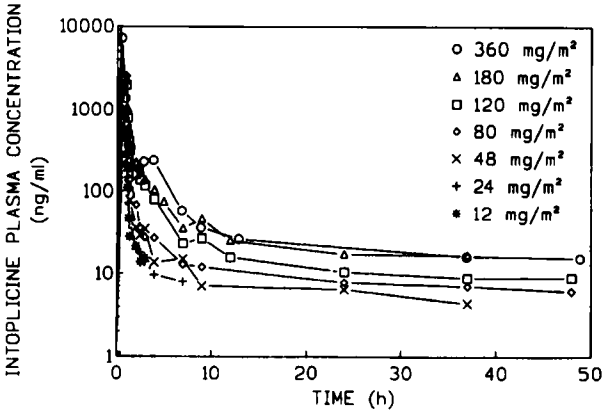

Figure 2. Mean intoplicine plasma levels in patients after drug administration at the indicated dose levels. Error bars not shown for legibility. Drug levels were assayed by HPLC with fluorescence detection as described in Patients and methods. Dose levels as indicated.

Plasma pharmacokinetics. Mean plasma pharmacokinetic profiles achieved after administration of intoplicine at various dose levels are presented in Figure 2. Intoplicine plasma disposition was either bi- or triphasic. Table 4 presents the pharmacokinetic parameters values for each dose levels. The area under the plasma concentration versus time curves (AUC; Figure 3) and the maximum plasma concentrations (C_{max}) increased linearly with the

Table 4. Intoplicine plasma pharmacokinetics in cancer patients (mean ± SEM)

Dose (mg/m^2)	(N)	C_{max} (μg/ml)	Half-lives (h)			MRT (h)	$AUC_{0-\infty}$ (μg.h/ml)	CL (l/h)	V_β (l)	V_{ss} (l)
			First	Second	Last					
12	(5)	0.28	–	0.05	3.7	3.0	0.32	67	367	206
		±0.06	–	±0.01	±0.9	±1.0	±0.03	±6	±97	±65
24	(3)	0.55	0.02	0.26	8.9	5.2	0.50	80	787	321
		±0.05	–	±0.16	±5.2	±3.2	±0.1	±10	±382	±161
48	(4)	0.73	0.03	0.29	9.6	6.7	0.83	95	1032	500
		±0.07	±.01	±0.13	±3.6	±2.7	±0.12	±15	±304	±152
80	(3)	3.37	0.04	0.73	25.4	10.6	2.68	49	1541	438
		±0.48	±.01	±0.25	±12	±5.6	±0.09	±5	±622	±205
120	(3)	2.63	0.04	1.03	30.9	14.1	3.33	72	3543	1062
		±0.68	±0.01	±0.11	±4.4	±1.0	±0.6	±19	±1397	±345
180	(4)	2.54	0.04	1.17	31.8	23.2	4.03	74	3322	1684
		±0.08	–	±0.37	±9.0	±6.9	±0.1	±4	±966	±513
360	(1)	7.19	–	1.80	67.8	36.1	7.11	89	8716	3219
Mean	(23)		0.04	0.61	19.4	11.3	–	74	1982	802
			±0.01	±0.13	±4.0	±2.4	–	±5	±477	±188

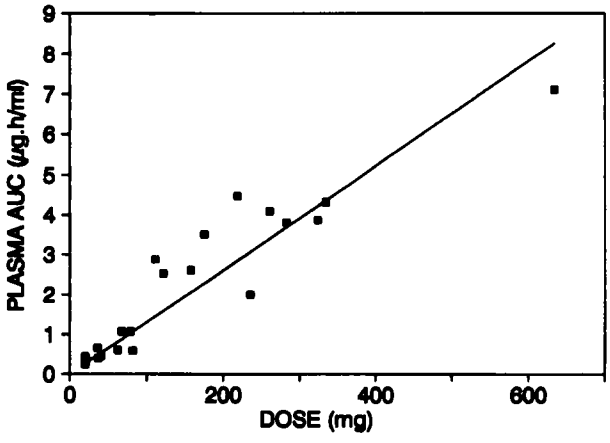

Figure 3. Linear increase in intoplicine area under the plasma concentration versus time curve (AUC) as a function of intoplicine dose (mg) ($r=0.937$; slope $=0.01305$; $p<0.001$).

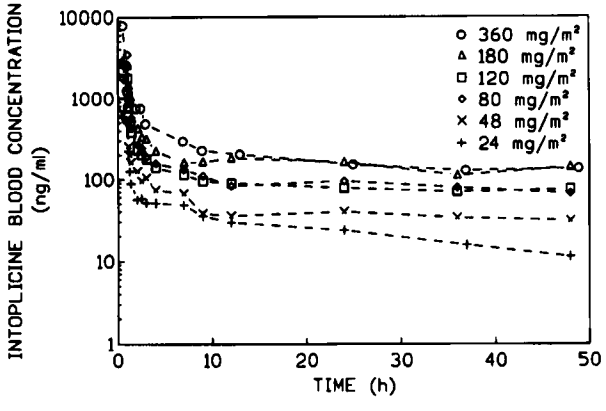

Figure 4. Mean intoplicine blood levels in patients after drug administration at the indicated dose levels. Error bars not shown for legibility. Drug levels were assayed by HPLC with fluorescence detection as described in Patients and methods. Dose levels as indicated.

intoplicine dose, indicating linear pharmacokinetics within the dose range studied (AUC: $r = 0.937$; slope $= 0.01305$; $p<0.001$; C_{max}: $r = 0.847$; slope $= 0.01115$; $p<0.001$). Also of interest for future clinical studies, the plasma AUC (μg.h/ml) was accurately predicted by the C_{max} values (μg/ml), with a slope almost equal to 1 ($r = 0.909$; slope $= 1.0701$; $p<0.001$). Also indicative of linear pharmacokinetics, the total plasma clearance did not vary significantly as a function of intoplicine dose.

Unexpectedly, other plasma pharmacokinetic parameters appeared to increase as a function of intoplicine dose (Table 4). These dose-dependent increases were significant for the terminal

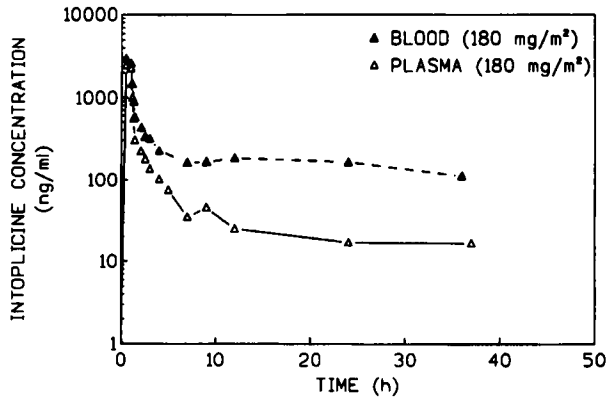

Figure 5. Mean intoplicine levels in blood and plasma in patients after administration of a 180 mg/m^2 dose.

half-life ($r = 0.748$, $p<0.001$), the MRT ($r=0.728$, $p<0.001$), the V_β ($r=0.809$, $p<0.001$), and the V_{ss} ($r=0.804$, $p<0.001$) (Pearson product moment correlation test).

Blood pharmacokinetics. Since it was found that red blood cells represented a significant drug reservoir for intoplicine, whole blood pharmacokinetics was also performed in 18 patients at doses ranging from 24 to 360 mg/m^2

Mean blood pharmacokinetic levels achieved after administration of intoplicine at various dose levels are presented in Figure 4. Blood intoplicine disposition curves were either bi- or triphasic. The intoplicine concentrations achieved in whole blood were similar to the plasma concentration values during and immediately after the 1 h i.v. infusion. However, 8–10 h after cessation of i.v. infusion, blood levels were about 10-fold higher compared with plasma levels, which illustrates the importance of erythrocytes as a drug reservoir (Figure 5).

Blood pharmacokinetics (Table 5) disclosed changes in certain parameters as a function of dose level. As expected, the area under the blood concentration versus time curves (AUC) and the maximum blood concentrations (C_{max}) increased linearly with the intoplicine dose, indicating linear pharmacokinetics within this dose range (AUC: $r = 0.879$; slope $= 0.06884$; $p<0.001$; C_{max}: $r = 0.835$, slope $= 0.01223$; $p<0.001$). As observed above for plasma, blood AUC values could also be determined by the blood C_{max} ($r = 0.768$; slope $= 5.0206$; $p< 0.001$).

Linear blood pharmacokinetics is also demonstrated by the dose independence of the total blood clearance (Table 5; $r = -0.118$; $p = 0.641$, not significant).

Table 5. Intoplicine blood pharmacokinetics in cancer patients

Dose (mg/m^2)	(N)	C_{max} ($\mu g/ml$)	Half-lives (h)			MRT (h)	$AUC_{0-\infty}$ ($\mu g.h/ml$)	CL (l/h)	V_β (l)	V_{ss} (l)
			First	Second	Last					
24	(3)	0.65	0.06	1.43	45.8	104	2.7	15	932	1437
		±0.10	±0.01	±0.94	±17.05	±28	±0.3	±2	±285	±338
48	(4)	0.68	0.02	0.36	32.1	44	3.9	27	767	730
		±0.03	±0.001	±0.15	±10.5	±13	±0.9	±10	±245	±209
80	(3)	3.78	0.04	1.23	68.4	78	13.3	10	1077	819
		±0.63	±0.01	±0.46	±6.7	±5	±1.4	±2	±311	±179
120	(3)	2.18	0.03	0.86	63.7	80	12.5	18	1551	1334
		±0.13	±.001	±0.11	±12.3	±17	±2.3	±2	±207	±190
180	(4)	3.10	0.08	0.73	63.3	90.2	23.7	14	1209	1138
		±0.33	±0.02	±0.30	±9.0	±20.0	±5.0	±2	±61	±63
360	(1)	7.92	0.02	2.0	111.8	154.8	36.4	17	2806	2693
Mean	(18)		0.04	0.94	57.1	82.2	–	18	1188	1163
			±0.01	±.22	±6.6	±9.9	–	±3	±147	±138

As noted above for plasma pharmacokinetics, other blood pharmacokinetic parameters appeared to increase as a function of intoplicine dose. These dose-dependent increases in blood pharmacokinetic parameters were significant for the terminal half-life ($r = 0.626$, $p = 0.005$), the V_β ($r = 0.682$, $p = 0.002$) and the V_{ss} ($r = 0.555$, $p = 0.017$).

Discussion

Intoplicine is a novel antitumor agent of the 7-H-benzo[*e*]pyrido[4,3-b]indole series that has been recently synthesized in order to find new DNA topoisomerase inhibitors.[14,15] The mechanism of action of this compound is original because it possesses dual activity on DNA topoisomerases I and II,[18] like actinomycin D.[4,19] Intoplicine can in fact stabilize DNA cleavable complexes mediated by both topoisomerase I or topoisomerase II, and can inhibit the relaxation and decatenation activities catalyzed by these enzymes.[18] Not only the mechanism of action of this compound is original, but intoplicine has shown a broad spectrum of antitumor activity at the preclinical level and is also effective against some resistant sublines.[16,17] Based on the original mechanism of action of this compound, its activity in preclinical models and its acceptable preclinical toxicity profile, intoplicine was therefore evaluated in a clinical phase I and pharmacokinetic study.

Using a single 1 h i.v. administration every 3 weeks, the intoplicine maximum tolerated dose was 270 mg/m^2, the dose at which only one of four patients developed reversible hepatic toxicity. In fact, at 360 mg/m^2, three of four patients presented serious hepatic toxicity. This hepatic toxicity appears to be dose related, but its etiology remains unclear because no such toxicity was encountered in preclinical studies. In addition, there was no preclinical toxicity data that could have prevented the two sudden deaths observed in this study. Organ biopsies (lung, liver, kidney, gall bladder) available in one patient did not disclose any specific pathological lesions.

Intoplicine pharmacokinetics revealed that plasma levels can reach potentially cytotoxic concentrations at the phase II recommended dose (5.1 $\mu g/ml$ at 270 mg/m^2 or 459 mg), which are similar to efficacious plasma levels achieved in mice at the highest non- toxic dose (2.4 $\mu g/ml$ at 68.4 mg/m^2 or 22.8 mg/kg, 1 h i.v.; Rhône-Poulenc Rorer, unpublished data: Reference IBP/Biodyn. 1532s, 19 April, 1991). *In vitro*, Eckardt and colleagues[17] recently reported that 26 and 54% of human tumor specimens responded to intoplicine at concentrations of 2.5 and 10 $\mu g/ml$, respectively. With continuous exposure to intoplicine, lower concentrations in the range of 0.25 and 2.5 $\mu g/ml$ were needed to obtain positive responses in 16 and 71% of the specimens, respectively.[17] These data are impressive, given the fact that a subgroup of tumors insensitive *in vitro* to standard antineoplastic compounds were sensitive to intoplicine. It is also noteworthy that the plasma concentrations achieved clinically are well above the concentrations needed to obtain topoisomerases I and II inhibition, which are about 1 μM (0.35 $\mu g/ml$). These *in vitro* data also suggest that intoplicine activity is schedule-dependent and that longer infusion times are worth testing in humans.

Also of potential importance, the high whole blood levels observed in this study, compared to plasma levels, suggest that this compartment may act as an important drug reservoir, that probably prolongs the residence time of this compound in the body. As a consequence, whole blood clearance values are 4-fold lower than plasma clearance. These high blood concentrations appear to be due to binding of intoplicine to red blood cells.

The fact that intoplicine pharmacokinetics was linear, based on the proportional increase in AUC and C_{max} as a function of dose, and that other pharmacokinetic parameters, e.g. half-life, MRT and volumes of distribution, increased with dose may appear intriguing, but this could be explained by the longer detection time of the drug in biological fluids as the dose was increased.

Although no anticancer responses were observed in this phase I study, probably because too few patients were evaluable at the recommended dosages, we feel that the clinical development of intoplicine should be pursued because of its unique preclinical activity and its original mechanism of action. For example, if means are developed to alleviate or circumvent the hepatic toxicity of this compound, e.g. by longer infusion time, intoplicine could eventually lead to meaningful anticancer activity in man.

In conclusion, the phase II recommended dose of intoplicine is 270 mg/m^2 administered as a 1 h i.v. infusion every 3 weeks. Plasma and blood pharmacokinetics were linear within the dose range studied, and potentially cytotoxic concentrations were reached at clinically achievable doses.

Acknowledgments

We are grateful to the devoted assistance of the nursing staff that made this study possible (Ms Pascale Dielenseger, Ms Catherine Lepain and Ms Danielle Leuleu). We thank Mr A Renard and Mr JC Vergniol for expert pharmacokinetic analyses.

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(Received 5 December 1995; accepted 3 January 1996)