Anti-Cancer Drugs 1999, **10**, pp. 889–894

Clinical report

Phase I trial of intoplicine (RP 60475) administered as a 72 h infusion every 3 weeks in patients with solid tumors

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Intoplicine (RP 60475) was selected for a phase I evaluation because it inhibits topoisomerase I and II, and has exhibited antitumor activity against a variety of preclinical solid tumor models. Intoplicine is a 7H-benzo[e]pyrido[4,3-*b*]indole that inhibits DNA nicking and closing reactions by stabilizing the cleavable complex, a transient intermediate in the religation reaction involving topoisomerase I and II and DNA. Twenty-eight patients with refractory advanced malignancies who met standard phase I eligibility criteria were enrolled in a dose-escalation study of intoplicine, ranging from 7 to 420 mg/m^2/day administered as a continuous 72 h i.v. infusion. Fifty-three courses were administered and evaluated. Myelosuppression (four patients, grade 3; two patients, grade 4) and hepatic toxicity (one patient, grade 3) were dose limiting at 336 mg/m^2/day. No objective antitumor responses were observed. The pharmacokinetic parameters of intoplicine were investigated in 11 patients at dose levels of 112 (n=1), 224 (n=3), 336 (n=6) and 420 (n=1) mg/m^2/day. Both the area under the plasma concentration versus time curves and the maximum plasma concentrations increased linearly within the dose range studied. Intoplicine content measured in whole blood exceeded that found in plasma by 3- to 7-fold, indicating that red blood cells may serve as a drug reservoir. Preclinical cytotoxic concentrations were not achieved at the dose levels studied. [© 1999 Lippincott Williams & Wilkins.]

Key words: Intoplicine, NSC 645008, pharmacokinetics, phase I, RP 60475.

Introduction

Intoplicine (RP 60475, Figure 1) inhibits topoisomerase I and II activity in mammalian cells, and is thus a promising anticancer agent.[1,2] The drug reversibly stabilizes a covalent complex of enzyme and DNA, and induces double- and single-strand breaks in cellular DNA.[3–5] In *in vitro* trials on tumor cell suspensions, intoplicine was observed to possess significant activity against breast, colon, renal cell, ovarian and non-small cell lung carcinomas as well as carcinoma of an unknown primary.[6,7] This antitumor effect is augmented when the tumor cells receive continuous exposure to 2.5 μg/ml of intoplicine.[8] In addition, cell suspension studies suggest that tumor lines that are resistant to doxorubicin, cisplatin, fluorouracil, etoposide, vinblastine and 4-hydroxycyclophosphamide may be sensitive to intoplicine.[8] Intoplicine also has activity against tumors with resistance to topoisomerase I and II inhibitors.[9,10] *In vivo* murine studies demonstrated antitumor activity against transplanted tumors including colon carcinoma, mammary carcinoma and pancreatic ductal carcinoma.[11] Lesser activity was found against Lewis lung carcinoma and Glasgow osteogenic

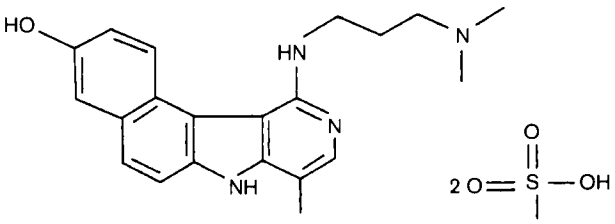

Figure 1. Structure of intoplicine: 11-(3-dimethylaminopropyl-amino)-3-hydroxy-8-methyl-7H-benzo[e]pyrido[4,3-*b*]indole; RP 60475.

This study was supported in part by a contract from Rhone-Poulenc Rorer SA, Antony, France.

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sarcoma.

Because of the above data and an acceptable preclinical toxicity profile, a phase I dose-escalating trial of continuous infusion intoplicine was performed. The purpose of the clinical trial was to determine the maximum tolerated dose of intoplicine, to evaluate intoplicine toxic effects in humans and to analyze the pharmacokinetic properties in patients with solid tumors.

Patients and methods

Patient eligibility

Twenty-eight patients who had a solid malignancy of any histologic type, were older than 15 years, had an estimated life expectancy of at least 12 weeks and had recovered from the toxic effects of prior treatment were enrolled in the study. Patients were required to have a Zubrod performance status of 0–2 and to have adequate bone marrow function (granulocyte count $> 1500/\mu l$, platelet count $> 100\,000/\mu l$), liver function (bilirubin $\leqslant 1.5$ mg/dl) and kidney function (serum creatinine $\leqslant 1.5$ mg/dl). Patients with malignancy of the central nervous system or metastases were ineligible, as were patients with active coronary disease, neurologic or psychiatric disorders, active infection, or any other serious intercurrent medical illness. No pregnant patients were enrolled in the study and women of child-bearing age were required to use contraception. All patients had signed a written informed consent, in accordance with the institutional and federal guidelines. The protocol and consent form were approved by the Institutional Review Board.

Study design

Rhone-Poulenc Rorer (Antony, France) provided intoplicine in a 10 mg/ml solution. The appropriate dose was then obtained by dilution of the stock preparation in 5% dextrose; the maximum concentration of intoplicine was no more than 0.3 mg/ml. Drug solutions were prepared on the day of each 24 h infusion; these were infused on three consecutive days using a portable pump. This regimen was repeated every 3 weeks. The starting dose was 7 mg/m^2/day, which represents 1/10 of the LD_{10} in mice when animals were administered for each of five consecutive days. A minimum of three patients were entered at each dose level with an additional three patients tested at the maximum tolerated dose. The dose levels were 14, 28, 56, 112, 224, 336 and 420 mg/m^2/day. The dose could be increased to the next specified level if none of three patients or one of six patients experienced a dose-limiting toxicity at the prior dose level.

Pretreatment evaluation included a complete medical history and a physical examination, a complete blood count with differential counts, a reticulocyte count, coagulation studies, urinalysis, a serum chemistry analysis, a direct Coombs' test, an electrocardiogram (EKG) and a chest X-ray. Staging radiologic studies were obtained on an individual basis. During continuous infusion on the first day of treatment, blood pressure and pulse were evaluated every 15 min for the first hour, then at 2 h and finally at 4 h. EKG testing was performed at baseline, and then at 24, 48 and 72 h after the start of i.v. infusion. Following drug therapy on day 1, complete blood counts, platelet counts, differential counts and reticulocyte counts were obtained twice weekly. Weekly chemistries including liver enzyme studies and urinalysis were performed. Before the second course, the coagulation studies, EKG, chest X-ray, Coombs' testing, medical history and physical examination were repeated. Tumor measurements were obtained prior to the initiation of intoplicine and after every two courses.

Blood sample collection. Serial blood samples (5 ml) were collected in tubes containing heparin as anticoagulant. Samples were drawn from an indwelling i.v. catheter placed in the contralateral arm to that receiving the drug infusion. Blood samples were obtained at the following times: predose; during the infusion at 24, 48 and 71 h, upon completion of the infusion at 72 h, and after the infusion at 5, 10, 15 and 30 min, and at 1, 2, 3, 4, 8, 10 and 24 h. An aliquot of whole blood (1 ml) from each sample was immediately stored in a polypropylene tube at -20 C and the remaining blood was centrifuged at 2000 g for 20 min. The resulting plasma was transferred to a polypropylene tube and was also stored at -20 C until analysis.

Analytic assay. A high-performance liquid chromatography (HPLC)-based analytical assay was developed and validated for determination of intoplicine in human plasma and whole blood.[12] To the plasma or whole blood samples was added an aliquot of RP 60257 (provided by Rhone-Poulenc Rorer) as an internal standard. This material is an analog of intoplicine with similar extraction and chromatographic behavior. An aliquot (0.5 ml) of plasma or whole blood was added to prepared C2 end-capped solid-phase extraction cartridges (Waters, Milford, MA). Cartridges were washed sequentially with water (2 ml) followed by 0.5 ml of acetonitrile:water (10:90, v/v) and the washes were discarded. Drug was eluted

from the cartridges using 250 μl 0.1 M Na_2HPO_4:acetonitrile (70:30, v/v) at a pH of 2.0. An aliquot (100 μl) of the eluant was used directly for injection into the HPLC.

The HPLC equipment consisted of a Model 510 pump (Waters) and a Model 990 Photodiode array detector (Waters). A Taxil' (MetaChem Technologies, Torrance, CA) 5 μm diameter, 25 cm long analytical HPLC column was used. The isocratic mobile phase consisted of 0.1 M NaH_2PO_4:acetonitrile (78:22) at a pH of 4.2. A flow rate of 1 ml/min was used. The lower limit of quantitation of this assay was 5 ng/ml and was linear over a range of 5–500 ng/ml.

Pharmacokinetic analyses. Pharmacokinetic parameters were estimated using standard methodology by both non-compartmental as well as compartmental analyses. For model-dependent analysis, intoplicine plasma or whole blood concentrations were fitted to the appropriate pharmacokinetic model using a non-linear least-square weighted regression analysis program (WinNonlin; Scientific Consulting, Apex, NC). The program also permits model-independent determination of appropriate pharmacokinetic parameters. The following parameters were determined: the maximum plasma concentration (C_{max}), area under the concentration-time curve (AUC) and mean residence time (MRT). Other parameters were not determined because of the limited number of patients from whom pharmacology samples could be obtained.

Table 1. Patient characteristics (N = 27)

Variable	No. of patients
Median age in years (range)	55 (36-75)
Sex	
female	14
male	13
Performance status	
0	14
1	13
Race	
Black	2
Hispanic	5
White	20
Histology	
adenocarcinoma	19
adenocarcinoma, mucinous	1
adenoid cystic carcinoma	1
leiomyosarcoma	1
myxoid liposarcoma	1
renal cell carcinoma	1
serous carcinoma	2
transitional carcinoma of ovary	1
Prior therapy	
chemotherapy	26
hormones	4
immunotherapy	17
radiotherapy	4
surgery	26

Results

Patient characteristics are summarized in Table 1. Twenty-seven of the 28 patients that were enrolled were assessable. One patient was excluded from analysis because he never received treatment with intoplicine after registration. The median age was 55 years (range 36–75 years). The male:female ratio was 13:14. All assessable patients had a Zubrod performance status of 0 or 1. Seventeen of the assessable patients had adenocarcinoma of the colon, two had adenocarcinomas of the pancreas and two had serous ovarian carcinomas. There was one each of the following tumors: mucinous adenocarcinoma of the appendix, submandibular adenoid cystic carcinoma, myxoid liposarcoma, adenocarcinoma of the lung, transitional cell carcinoma of the ovary, renal cell carcinoma and leiomyosarcoma.

Dosing

Fifty-three courses of intoplicine were administered as 72 h i.v. infusions. The doses ranged from 7 to 420 mg/m^2/day. The dose escalation format along with the number of patients at each dose level is depicted in Table 2.

Toxicity

At all dose levels, the most consistent grade 1 or 2 toxicity was nausea. Other toxic effects were encountered sporadically.

Table 2. Dose escalation

Level	Dose (mg/m^2/day)	No. of courses	No. of patients
0	7	8	3
1	14	6	3
2	28	4	3
3	56	5	3
4	112	6	3
5	224	6	3
6	336	14	7
7	420	4	3

Grade 3 and 4 toxic effects observed in this study are summarized in Table 3. Intoplicine toxic effects appeared consistently at a dose level of 336 mg/m^2/day. These toxicities principally involved the hepatic and hematopoietic systems. Grade 4 and grade 3 granulocytopenia were encountered in one patient and two patients, respectively. One patient developed a grade 3 thrombocytopenia. One patient and two patients developed a grade 3 and a grade 4 leukopenia, respectively. Grade 3 hepatic toxicity evident by elevation in transaminases was observed in one patient. Therefore, myelosuppression and hepatic toxicity were dose limiting at the 336 mg/m^2/day dose level. At the 420 mg/m^2/day dose level, a grade 3 granulocytopenia was encountered in one patient. In addition, there were no adverse cardiac events at these intoplicine dose levels. None of the courses of treatment with intoplicine at 336 or 420 mg/m^2/day were associated with fever, headache, anorexia or dizziness.

Antitumor activity

No objective antitumor responses were observed.

Pharmacology

Because the pharmacology of intoplicine had been previously described in detail,[13,14] drug levels in plasma and whole blood were determined in relatively few patients. Blood samples were collected from one patient who was receiving intoplicine at 112 mg/m^2/day, three patients at 224 mg/m^2/day, six patients at 336 mg/m^2/day and one patient at 420 mg/m^2/day. Representative patient data are shown in Figure 2, which presents the mean drug concentration data from the 112 and 224 mg/m^2/day dose levels.

Table 3. Grade 3 and 4 toxicities of intoplicine

Dose, (mg/m^2/day)	Toxicities	Grade 3[a]	Grade 4[a]
56	transaminase increase	1	0
112	anemia	1	0
224	transaminase increase	0	1
	anemia	1	0
336	granulocytopenia	2	1
	leukopenia	1	1
	transaminase increase	1	0
	thrombocytopenia	1	0
420	transaminase increase	1	0

[a]Values in column = no. of patients.

Pharmacokinetic parameters for intoplicine in blood samples obtained from these patients are presented in Table 4. Although limited data are available, it is apparent that both intoplicine plasma concentration and AUC increased linearly as a function of dose. Limited blood sampling after the end of the final day of intoplicine infusion did not permit accurate determination of many pharmacokinetic parameters such as terminal half-life and clearance; however, these parameters have been previously described.[13] Drug levels in whole blood were 3- to 7-fold higher than those in plasma, indicating that the blood cell may function as a drug reservoir (see Table 4 and Figure 2).

Discussion

Intoplicine was chosen for this phase I trial because of its novel mechanism of action as an inhibitor of topoisomerase I and II.[1,2] Also, the drug has shown significant action *in vitro* against human tumor cell lines and *in vivo* against mouse malignancies.[6,11] The drug was administered as a 72 h continuous i.v.

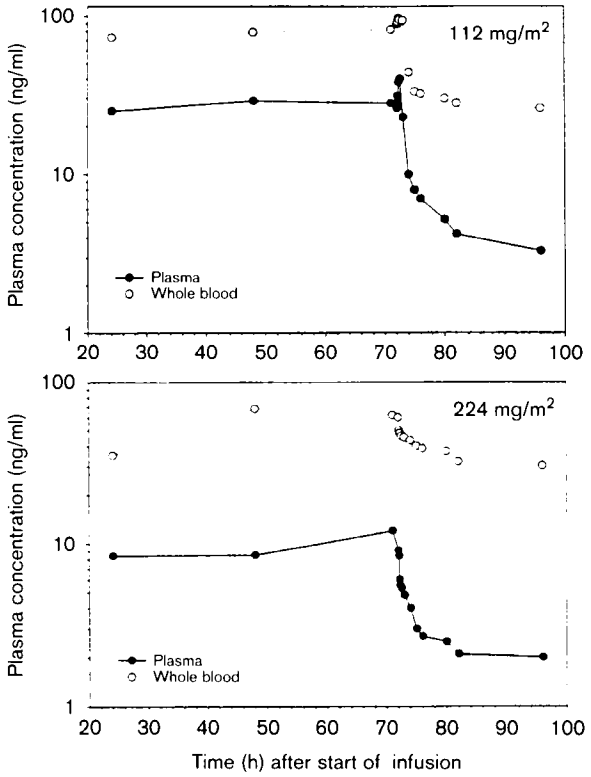

Figure 2. Intoplicine: plasma and whole-blood concentration versus time curves at two dose levels (112 and 224 mg/m^2/day). The higher drug levels in whole blood at every dose level indicate a significant retention of agent within red blood cells.

Table 4. Pharmacokinetic parameters derived from a 24 h infusion of intoplicine

Parameter	112 mg/m^2/day		224 mg/m^2/day		336 mg/m^2/day		440 mg/m^2/day	
	Plasma	Whole blood	Plasma	Whole blood	Plasma	Whole blood	Plasma	Whole blood
C_{max} (ng/ml)	42	145	120	803	188	574	325	910
$AUC_{24-96\ h}$ (μg/ml·h)	2.09	7.76	6.43	35.44	10.54	25.87	13.89	37.2
MRT (h)	47	49	50	50	54	50	52	52

infusion and the dose was gradually increased to a maximum tolerated dose of 336 mg/m^2/day. At this dose, grade 3 and 4 granulocytopenia and elevations in the hepatic transaminases associated with nausea and vomiting were noted. Prior trial of intoplicine[13] ceased dose escalation because hepatocellular damage, indicated by rising levels of liver enzymes, resulted in death in one patient. In that study by Abigerges *et al.*[13] intoplicine was administered as a 1 h i.v. infusion. In another study of intoplicine by van Gijn *et al.*,[15] administered as a 24 h continuous i.v. infusion, liver toxicity was also dose limiting and death in one patient due to liver failure was associated with intoplicine treatment. In our study, an increase in hepatic transaminases was evident at the dose level of 336 mg/m^2/day. This prompted us to halt dose escalation at the dose level of 440 mg/m^2/day and enter four more patients at the dose level of 336 mg/m^2/day. Therefore, liver toxicity appears to be independent of infusion duration.

In general, the continuous infusion of intoplicine was well tolerated and there were no deaths due to toxicity in this group of patients. In our population of patients, the majority of whom had gastrointestinal malignancies, no antitumor effects from intoplicine were witnessed. Most of our patients had progressive disease following two courses of the drug.

Although the pharmacology of intoplicine was examined in relatively few patients, the accumulation of drug in red blood cells may be a property of this compound. Our data obtained from analyses of whole blood are similar to those previously reported by Abigerges *et al.*[13] and van Gijn *et al.*[15] In their studies and the present trial, there was considerable variation in whole-blood levels of drug among patients at any particular dose level, and the relationship between C_{max} and dose were not as evident in blood as they were in plasma. Plasma drug concentrations during the 72 h i.v. infusion period rarely exceeded 0.2 μg/ml; the relatively low concentrations observed in this present study are, unfortunately, below those levels (0.25–25 μg/ml) reported to be necessary to produce cytotoxicity against cells in culture when exposed to intoplicine on a continuous basis.[7] Therefore, the plasma drug concentration achieved in this study may not have been high enough to be effective. The dose-limiting toxicities of intoplicine as a 72 h continuous i.v. infusion are elevation in transaminases and myelosuppression occurring at doses of 336 mg/m^2/day and higher. The suggested phase II starting dose of this schedule is 224 mg/m^2/day with close observation of liver enzymes.

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(Received 22 June 1999; revised form accepted 26 August 1999)