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Clinical report

Topoisomerase I/II inhibitor intoplicine administered as a 24 h infusion: phase I and pharmacologic study

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Intoplicine, an antitumor drug which interacts with both topoisomerase enzymes I and II, has demonstrated a broad spectrum of activity in preclinical studies. This indicates further clinical evaluation. In the present phase I study, with the primary objective to determine the maximum tolerated dose, intoplicine was administered by a 24 h continuous infusion every 21 days to 32 patients with solid malignant tumors. The patients received 12–640 mg/m^2 by a central venous catheter. Liver toxicity was dose limiting. One patient died in a hepatic coma after the first course (dose 640 mg/m^2), which was associated with intoplicine treatment. Other side effects were sporadic and mild. Myelotoxicity was virtually absent. Twenty-two patients had stable disease for four to six courses of treatment. The plasma concentration–time curves were compatible with standard linear pharmacokinetic models, with a protracted terminal half-life (mean 115 h). Although one sudden death occurred probably due to intoplicine toxicity, we nevertheless feel that research with intoplicine should continue, mainly because of its preclinical activity and its unique mechanism of action. The recommended dose for phase II studies with intoplicine administered as a 24 h infusion is 384 mg/m^2. Liver toxicity, also seen in studies employing other dosages and infusion durations, should be investigated extensively in further clinical studies. [© 1999 Lippincott Williams & Wilkins.]

Key words: Intoplicine, pharmacokinetics, phase I.

Introduction

Intoplicine (RP60475F, NSC 645008, 11-(3-dimethylaminopropyl-amino)-3-hydroxy-8-methyl-7H-benzo[*e*]-pyrido[4,3-*b*]-indole dimethanesulfonate, Figure 1), belongs to a new class of antitumor drugs which interact with both nuclear DNA modifying enzymes topoisomerase I and II.[1] During the process of winding and unwinding of DNA, torsional stresses and topological problems occur. Topoisomerase enzymes transiently break and rejoin DNA, by which they change the conformation of a segment of DNA, and resolve the mechanical obstacles that hamper processes such as replication and transcription. Intoplicine stabilizes both the DNA topoisomerase I and II cleavable complexes, which can result in lethal DNA damage during DNA replication.[2] The dual activity on DNA topoisomerase I and II is virtually unique, since other topoisomerase-inhibiting drugs selectively inhibit either topoisomerase I or II enzymes.[1] Selective inhibition of the topoisomerase I enzymes can induce a reactive increase in topoisomerase II enzyme levels and vice versa.[3] This mechanism is associated with the occurrence of drug resistance.[3] The dual activity of intoplicine may overcome this problem. Furthermore, in both *in vitro* and *in vivo* preclinical models the drug demonstrated a broad spectrum of activity against a variety of tumors, including breast, non-small cell lung, colon and ovarian cancer.[4] This activity was generally higher than that observed with topoisomerase inhibitors selectively inhibiting either topoisomerase I or II.[1] Toxicity studies revealed that the LD_{10} (lethal dose for 10% of the animals tested) in mice was 117 mg/m^2 on a single i.v. bolus schedule, with myelotoxicity and hematotoxicity being the main toxicities. Other toxicities were seen in the gastrointestinal tract, lymphoid tissues and kidneys.[4–6] Pharmacokinetic studies performed in mice and dogs revealed a bi-exponential decay and linear pharmaco-

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kinetics of intoplicine. Investigations with i.v. administration of radiolabeled intoplicine demonstrated that the radioactivity was mainly recovered in feces.[4]

Based on intoplicine's strong preclinical antitumor activity, its acceptable toxicity profile and its unique mechanism of action, we conducted a phase I and pharmacologic study of i.v. intoplicine given to cancer patients. *In vitro*, cell cultures continuous exposure to intoplicine yielded higher responses than following a short exposure time, indicating schedule-dependent activity.[7,8] This result is in line with the presumed mechanism of action of topoisomerase enzymes. Since prolonged exposure may enhance activity, intoplicine was administered to patients as a 24 h infusion.

The primary objective of the conducted phase I study was to determine the maximum tolerated/acceptable dose (MTD/MAD) of intoplicine given as a 24 h continuous infusion every 3 weeks. The secondary objectives of this phase I study were: (i) to determine the qualitative and quantitative toxic effects of intoplicine, and to study the predictability, duration, intensity, onset and reversibility of the toxic side effects; (ii) to propose a recommended dose close to the MTD for phase II efficacy evaluation; (iii) to determine the pharmacokinetics of intoplicine in man; and (iv) to document any possible antitumor activity.[4]

Patients and methods

Patient selection

Eligibility criteria included a histologically confirmed diagnosis of a solid malignant tumor no longer amenable to established forms of effective therapy. All patients had acceptable bone marrow function [white blood cells (WBC) $>4\times10^9$/l and platelets $\geqslant100\times10^9$/l), serum bilirubin, serum alanine amino transferase (ALAT), serum aspartate amino transferase (ASAT) and serum creatinine $\leqslant1.25$ times the upper normal limit, WHO performance status $\leqslant2$, anticipated life expectancy of $\geqslant12$ weeks, and age between 18 and 75 years. Previous anticancer chemotherapy had to be discontinued for at least 4 weeks before entry into the study, or for 6 weeks in case of pretreatment with a nitrosourea derivative or mitomycin C. The study protocol was approved by the Medical Ethical Committees of the hospitals (i.e. the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital and the Free University Hospital in Amsterdam) and all patients gave written informed consent.

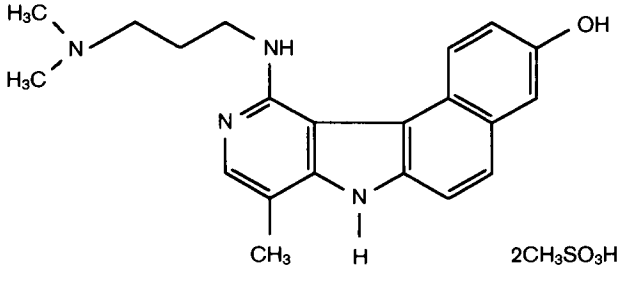

Figure 1. Chemical structure of intoplicine.

Drug administration and dose escalation

Intoplicine was supplied by Rhône-Poulenc Rorer (Antony Cedex, France) as a dimethanesulfonate salt in ampoules containing 100 mg of the base in 5 ml of Water for Injection, USP. The formulation of the drug was diluted in 500 ml of 5% dextrose or saline 0.9% solutions and administered i.v. through a central or peripheral access over a period of 24 h. In these infusion fluids the drug is chemically stable for at least 7 days at room temperature.[9]

Dose escalation was performed according to a modified Fibonacci scheme (if no significantly toxicity is observed the dose is increased with 100% steps; if significantly toxicity is observed, the dose may increased by 40-67% initially followed by 16-33% steps) with at least three patients entered at each dose level. Intrapatient dose escalation was not permitted. The starting dose was 12 mg/m^2 every 3 weeks, representing 1/10 of the LD_{10} for mice. To determine the hematological and non-hematological toxicities, patients were weekly evaluated by clinical history, physical examination, serum chemistry and hematology screen. Tumor measurements were performed every other cycle. All toxicities observed were graded according to WHO criteria.

Pharmacokinetic sample collection

Clinical pharmacokinetic studies were performed in at least three patients per dose level. Blood samples, taken from an indwelling i.v. cannula, were collected in heparinized tubes at 17 time points: pre-infusion, at 6, 12 and 24 h during the infusion, and at 5, 10, 20, 60 and 90 min, and 2, 3, 6, 8, 12, 24, 36 and 48 h after the end of infusion. From patients treated with intoplicine at dose levels of 192-640 mg/m^2 additional blood samples were drawn on days 7, 14 and 20. From each

blood sample an aliquot (1 ml) of whole blood was immediately taken from the collection tube and stored in a polypropylene tube at −30°C until analysis. Next, the collection tube was centrifuged at 3000 *g* for 5 min, and plasma was transferred into a polypropylene tube and stored at −30°C until analysis. Urine was collected over 3 × 24 h from the start of infusion and samples were stored at −30°C until analysis. From a selected group of patients, receiving intoplicine at a dose level of 640 mg/m^2, the separated portions of feces produced over a maximal period of 120 h were stored at −30°C until analysis. Intoplicine concentrations in plasma, whole blood and feces were determined by a validated high-performance liquid chromatography (HPLC) method with fluorescence detection as developed in our laboratory.[11,12]

Pharmacokinetic parameters were calculated using standard non-compartmental analysis.[12] The area under the curve (AUC) was calculated with the linear trapezoidal rule and extrapolated to infinity. The elimination half-life was calculated from the slope of the final log-linear part of the concentration-time curve. The clearance (CL) was calculated by R_{inf}/C_{ei}, where R_{inf} is the rate of infusion and C_{ei} the observed plasma/whole blood concentration at the end of infusion (ei), and the apparent volume of distribution V by $[(\text{dose} \times \text{AUMC})/(\text{AUC}^2)] - (\text{dose} \times T_{inf})/(2 \times \text{AUC})]$, where T_{inf} is the infusion time, and AUMC the area under the first moment curve and calculated to infinity.

Table 1. Patient characteristics

Total number of patients	32
Men/women	17/15
Median age [years (range)]	65 (35–75)
Prior therapy	
chemotherapy	28
radiotherapy	15
immunotherapy	3
hormonal therapy	2
no prior therapy	2
Primary tumors	
colorectal	10
ovary	5
lung	4
stomach	4
bladder	2
melanoma	2
oropharynx	2
soft tissue sarcoma	1
unknown primary	2

Table 2. Drug administration

Intoplicine dose level (mg/m^2)	No. of patients per dose level	Total no. of courses
12	4	7
24	3	5
48	4	8
96	3	6
192	3	9
384	6	8
640	9	30

Results

A total of 32 patients was enroled in this trial. Median age was 65 (range 35–75) years and median performance status was 1 (range 0–2). Additional patient characteristics are outlined in Table 1. The intoplicine starting dose was 12 mg/m^2, and the dose escalation was performed in six steps to, respectively 24, 48, 96, 192, 384 and 640 mg/m^2 (Table 2). At the dosages of 12, 24, 48 and 96 mg/m^2, severe phlebitis occurred at the vein of infusion. Dilution of the formulation of the drug in the consecutive courses with 5% dextrose or 0.9% saline solutions from 500 to 1000 ml per infusate could not prevent this side effect. Although the phlebitis appeared to be reversible, the use of a central venous access for drug administration was required at higher dosages.

A total of 73 courses was given, with a mean of 2.3 (range 1–6) courses per patient. The mean total dose per patient was 786 (range 20–1408) mg. Ten patients received only one course, due to rapidly progressive disease (nine patients), and one patient died of intoplicine-associated toxicity.

The dose-limiting toxicity of intoplicine was liver toxicity, which was generally characterized by transient elevation of hepatic transaminases (Table 3). This toxicity was clinically asymptomatic, dose dependent and the onset of this toxicity generally was detected at day 3, and the values returned to normal by day 9. Overall, 22% of the courses resulted in a grade I, 32% was grade II, 10% was grade III and 12% had a grade IV elevation of the hepatic transaminases ASAT and ALAT. At a dose level of 384 mg/m^2 four out of six patients experienced a grade 2 hepatic toxicity; this toxicity was reversible and further dose escalation with 67% was performed. One patient died in a hepatic coma 3 days after the first cycle (640 mg/m^2). This patient had progressive ovarian carcinoma stage IV with intrathoracal metastases, previously treated with multiple courses of carboplatin and 10 courses of paclitaxel. Before her first course of intoplicine, a pleural effusion was drained (1.5 l) and an abdominal parcenthesis had

Table 3. Toxicity

Dose (mg/m^2)	12				24				48				96				192				384				640			
Total no. of courses	7				5				8				6				9				8				30			
WHO grade	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Phlebitis	1	4			1	2				5				2												3		
Vomiting		3								1								2			2	3				8		
Nausea		2			1					2			1				2				4	1			12	1		
ALAT					2		1						1								3	2			5	7	1	5
ASAT		1			1	2							2									4			2	7	5	4
SGGT		2			2			1							3	1		1					3	1	9	4	4	6
Anemia	2				1	1			1				1				4	1	1		2				17	1		
Fatigue		3				1															2	1			2			
Headache	1																2				3							

been performed (5.5 l). One day after the infusion, she developed fever, hypotension and oliguria, which only briefly responded to parenteral fluid administration and antibiotic treatment. Massive liver damage was seen, with ALAT and ASAT values of over 100 times the upper normal limit. At autopsy cytotoxic liver injury was found, that was ascribed to intoplicine treatment. The pharmacokinetic parameters, however, were comparable to those obtained from the other patients.

Hematological toxicity was virtually absent. Mild anemia (grade II) occurred in only one patient at the highest dose level. Serious infections, neutropenic fever or sepsis did not occur. Cumulative toxicity was not observed. Nausea and vomiting of mild to moderate severity was experienced by the majority of patients, but could easily be controlled by standard antiemetic medication. All patients developed alopecia (grade II or III). Fatigue was a common side effect of the treatment, but was usually mild (Table 3).

In this phase I trial, no clinical partial or complete responses were seen, although 22 patients remained stable, during four to six cycles of therapy.

Pharmacokinetics

Complete plasma concentration-time curves were obtained from 23 patients during their first course. Typical examples of the plasma and whole blood concentration-time curves at drug dosage of 12 and 640 mg/m^2, respectively, are shown in Figures 2 and 3. The pharmacokinetic parameters of intoplicine are listed in Table 4. During the 24 h infusion period, the concentrations of intoplicine in both plasma and whole blood increased, and steady-state levels were not reached during the infusion period. Already in

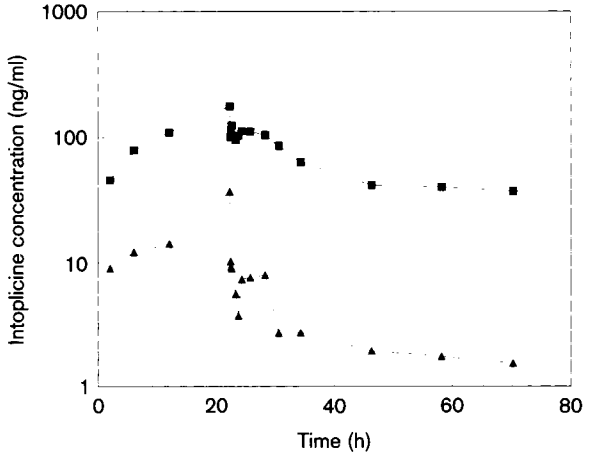

Figure 2. Typical plasma (▲) and whole blood (■) concentration versus time plot of intoplicine at a dosage of 12 mg/m^2.

the first sample (at 2 h) the concentration of intoplicine in whole blood exceeded the concentration in plasma. The highest levels of intoplicine were 5408 and 1347 ng/ml (dose 640 mg/m^2) in whole blood and plasma, respectively. After cessation of the 24 h infusion the intoplicine concentrations rapidly declined in parallel in plasma and whole blood (Figures 2 and 3) with a short distribution phase, followed by a protracted terminal half-life period with a mean half-live of about 115 h for both plasma and whole blood (the ratio of the terminal half-life in whole blood/plasma is 1.1 ± 0.7, F-test: $p < 0.0001$). However, all the pharmacokinetic curves indicated a re-distribution phase after the end of the infusion. This phase was observed 4-5 h after the end of the infusion. The AUCs of intoplicine in whole blood were consistently higher than those in

plasma, yielding a whole blood/plasma ratio of 3.84 (range 20.0-1.61, r=0.70; $p<0.0001$). These AUC values were linearly related (F-test for lack of fit; α=0.05) to the administered dose (r=0.92 for plasma and r=0.89 for whole blood) (Figure 4). Also, a linear relationship (F-test for lack of fit; α=0.05) between C_{ei} and dose level was seen over the range of 12-640 mg/m^2 (r=0.78 for plasma and r=0.86 for whole blood). The mean apparent distribution volumes (V) were 10.7 (range 0.86-41.2) $\times 10^3$ l and 4.56 (range 0.36-12.8) $\times 10^3$ l, and the mean total body clearance (CL) was 67.6 (range 17.7-150.4) l/h and 27.9 (range 4.82-65.3) l/h, as calculated from plasma and whole blood levels, respectively. The cumulative urine excretion for intoplicine averaged only 5.3% (range 2.7-11.6%) of the total administered dose. However, the major part of the administered dose (up to 65%) was excreted unchanged in feces within 5 days after the infusion, with most of the intoplicine excreted during the 24 h period following discontinuation of the infusion. No evidence for the presence of any circulating metabolites of intoplicine was found.

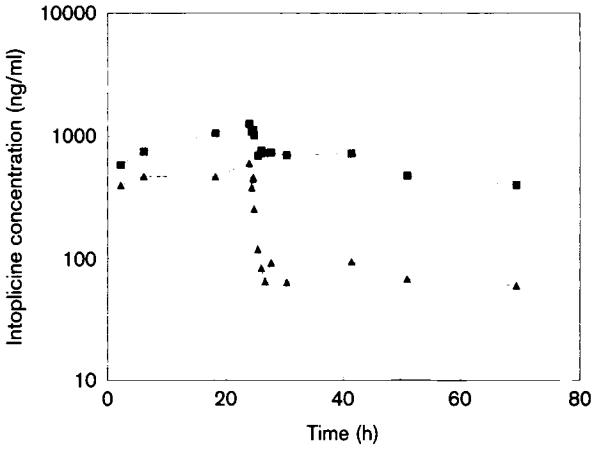

Figure 3. Typical plasma (▲) and whole blood (■) concentration versus time plot of intoplicine at a dosage of 640 mg/m^2.

Table 4. Pharmacokinetic parameters of intoplicine

Total no. of patients	Dose	C_{ei} (ng/ml)	AUC (μg/ml·h)	CL (l/h)	$t_{1/2\ elm}$ (h)	V (l)
4	12	13.2	0.32	66.6	80.0	6769
		(2.2)	(0.09)	(12.5)	(75.2)	(6083)
2	24	26.8	0.44	81.7	67.9	6240
		(14.2)	(0.14)	(44.5)	(54.9)	(2119)
2	48	75.1	1.42	46.8	67.1	4288
		(21.0)	(0.11)	(9.1)	(35.9)	(1541)
2	96	94.1	2.04	79.7	197.3	20932
		(33.2)	(0.03)	(12.4)	(195.3)	(18917)
3	192	424.8	5.52	37.0	103.8	8566
		(408.1)	(3.36)	(34.7)	(31.6)	(6683)
4	384	499.2	9.74	60.7	67.4	5696
		(212.7)	(2.72)	(15.2)	(22.8)	(1896)
6	640	844.0	12.05	76.4	171.5	18103
		(436.9)	(2.52)	(48.3)	(81.1)	(13078)
Whole blood						
4	12	42.2	2.12	24.6	103.5	3718
		(19.6)	(0.79)	(13.9)	(3.7)	(2222)
2	24	156.0	5.53	12.3	48.1	887
		(31.8)	(1.13)	(2.7)	(16.3)	(478)
2	48	183.5	8.16	18.6	155.2	4244
		(5.4)	(0.42)	(1.1)	(100.5)	(2933)
2	96	269.7	8.74	27.3	203.1	7743
		(71.8)	(0.59)	(1.8)	(193.6)	(7090)
3	192	745.7	19.10	25.7	89.9	3635
		(481.1)	(5.11)	(14.4)	(37.5)	(3350)
4	384	2001.7	39.41	32.3	58.1	2266
		(2312.0)	(26.5)	(25.6)	(27.7)	(1378)
6	640	1357.8	35.7	36.2	143.2	7262
		(234.3)	(7.18)	(8.3)	(77.2)	(3903)

Standard deviation between parentheses.

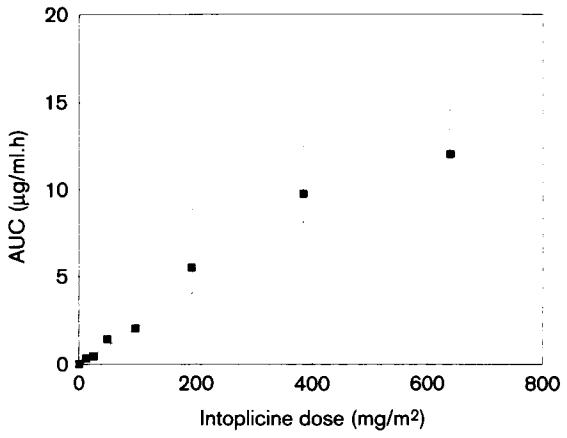

Figure 4. Relation between the intoplicine plasma AUC (μg/ml.h) and the dose (mg/m^2). The solid line is the corresponding regression line (r=0.92). The error bars represent the SEM.

Discussion

The MTD in this phase I study of intoplicine was 384 mg/m^2. The highest given dose was 640 mg/m^2, at which all patients experienced hepatic toxicity, being grade IV in six out of nine patients. Unlike what is seen with most other cytotoxic agents or topoisomerase inhibitors, myelotoxicity was virtually absent. Although the liver enzyme elevation appeared to be dose related, one patient died unexpectedly probably as a result of intoplicine treatment. In this patient, the values of the pharmacokinetic parameters measured were comparable to those obtained from the other patients. Thus, a pharmacokinetic cause (e.g. a high exposure or AUC) is not likely.

The observed re-distribution period in the pharmacokinetic curves may be due to enterohepatic cycling. It cannot be explained by hepatic dysfunction of the liver, as it was observed already at the starting dose of 12 mg/m^2. No indication of any hepatic toxicity was found at this dose level. Another remarkable toxicity was the reversible phlebitis, occurring at the infusion vein, which necessitated the use of a central venous access catheter. In another intoplicine phase I study, that employed a daily × 5 schedule, the main toxicities at a dosage of 270 mg/m^2 × 5 were leucopenia and reversible liver enzyme elevation.[13] When intoplicine was administered over 1 h every 3 weeks, hepatic toxicity without bone marrow suppression was found to be dose limiting. In that study, the MTD was 360 mg/m^2. Two patients died unexpectedly at 12 and 48 mg/m^2, and drug toxicity could not be excluded as the cause. At the highest dose level, three out of four patients experienced grade IV liver toxicity, which was fatal in one patient.[14]

Intoplicine pharmacokinetics indicated a linear behavior, which is in accordance with another phase I study, in which a 1 h infusion was applied. The achieved plasma concentrations of intoplicine, already at the second dose level of 24 mg/m^2, were above 35 μg/l (0.1 μM) at which concentration both topoisomerase I- and II-induced DNA breaks decrease.[15] However, concentrations of intoplicine in the range 2.5–10 mg/l for 1 h, at which specific activity in non-small cell lung cancer, breast and ovarian cancer was found, were not achieved.[7] This could explain in part the absence of antitumor activity seen in this study.

The AUCs of intoplicine in plasma were about 25% of those in whole blood, indicating a storage phenomenon probably in erythrocytes. Furthermore, the elimination curves in whole blood and plasma declined in a parallel manner with identical slopes. Since intoplicine eventually has to be eliminated from the central (plasma) compartment, this indicates that the actual elimination half-life of intoplicine in plasma is shorter than that from erythrocytes. This erythrocyte storage effect can also explain the long elimination half-life of intoplicine from the body, as well as the relatively high volumes of distribution calculated from plasma levels. Fecal excretion of intoplicine was high, indicating that hepato-biliary excretion was the most important route for elimination. No indication for metabolite formation was found. According to the hypothesis that mouse pharmacokinetics predict for human toxicity, an AUC of 4.4 μg/ml·h would be expected to represent the MTD of intoplicine in man (calculated from the AUC in mice given at 0.6 LD_{10} and assuming pharmacokinetic linearity).[4,16,17] Comparison of the pharmacokinetic data in mice and man showed a 2.2-fold higher AUC at the MTD in humans than at the LD_{10} in mice and an elimination half-life of intoplicine in man which was much longer than the elimination half-life in mice. Because there was no liver toxicity observed in mice, this relatively long elimination half-life of intoplicine in man in combination with higher AUCs could be related to the observed liver toxicity.

In conclusion, although one sudden death occurred, probably due to intoplicine toxicity, we feel that clinical evaluation of intoplicine should continue because of its strong and broad preclinical activity, and its unique mechanism of action. The recommended dose for phase II studies with intoplicine administered as a 24 h infusion is 384 mg/m^2. However, liver toxicity, which is also seen in studies employing other dosages and infusion durations, should be studied extensively and in-depth in further

clinical studies with intoplicine. Hopefully more insight can be obtained into the cause of the liver enzyme elevation and means to circumvent this toxicity.

Intoplicine is now undergoing clinical phase I evaluation in the US, with the drug administered as a 5 day continuous infusion.

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