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SHORT COMMUNICATION

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Pharmacokinetics of irinotecan and its metabolites in human blood, bile, and urine

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Abstract Two patients were treated with CPT-11 for colorectal cancer and had a percutaneous biliary catheter for extrahepatic biliary obstruction. The first patient was treated with CPT-11 according to the 100-mg/m^2 weekly therapeutic schedule, and the second patient was treated every 3 weeks, with a dose of 350 mg/m^2 being given at the first course, after which it was decreased to 300 mg/m^2 for the following courses. In plasma, the active identified metabolite of CPT-11, SN-38, occurred mainly in the form of a glucuronide conjugate. CPT-11 was mainly excreted in bile and urine as CPT-11. The cumulative biliary and urinary excretion of CPT-11 and its metabolites (SN-38 and SN-38 glucuronide conjugate) over a period of up to 48 h ranged from 25% (100 mg/m^2 weekly) to 50% (300 mg/m^2 every 3 weeks). This means that CPT-11 can be excreted under other, not yet identified metabolite forms.

Key words CPT-11 · SN-38 · Metabolism

Introduction

Irinotecan (CPT-11) is a new water-soluble semisynthetic derivative of camptothecin (7-ethyl-10-[4{1-piperidino}-1-piperidino]carbonyloxycamptothecin) synthesized by Yakult Honsha Central Institute in Tokyo, Japan, which acts as an inhibitor of DNA topoisomerase I [6]. Although CPT-11 is active in vivo, the intensity of its in vitro activity seems rather low. It has been suggested that its major identified metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38) plays a key role in the antitumor activity of CPT-11 [4]. Some in vitro data suggest that SN-38 is 250- to 1,000-fold as potent as CPT-11 in the inhibition of topoisomerase I activity [5]. Although a glucuronide of SN-38 has been found in the bile and urine of rats [3], data have not been reported on humans. However, only Rothenberg et al. [10] have studied the bile concentrations of CPT-11 and SN-38. This report summarizes the pharmacokinetics of CPT-11 and SN-38 and their glucuronide metabolites in the blood, bile, and urine of two patients treated with CPT-11.

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Patients and methods

Patients

Two patients, a 48-year-old man and a 53-year-old woman, were admitted with an extrahepatic biliary obstruction due to liver metastatic colon carcinoma. A percutaneous biliary catheter was placed to enable a quick decrease in the total bilirubin level from 17.1 mg/dl to a stable 3.4 mg/dl in the first patient and a normalization of this parameter in the second. Prior to chemotherapy, plasma levels of proteins and transaminases were normal. Plasma γGT levels were 20- and 4-fold the normal limit, respectively, for the two patients. In both patients, the biological parameters remained stable between two infusions of CPT-11.

Drug administration

The first patient (male) received two infusions of CPT-11 according to the 100-mg/m^2 weekly therapeutic schedule, and the second patient (female) received 350 mg/m^2 in the first course and 300 mg/m^2 in the second and third courses (these courses were separated by a 3-week interval). CPT-11 was given as a 30-min infusion.

Blood, bile, and urine collection

The CPT-11 pharmacokinetics and metabolism study was performed after each infusion of the drug. At least four blood samples were

Table 1 Plasma pharmacokinetic parameters of CPT-11 and SN-38 (*ND* Not done, C_{max} maximal concentration, *AUC* area under the curve extrapolated to infinity, *Clt* total body clearance [dose/AUC])

		Patient 1	Patient 2
CPT-11 dose	Course 1	100 mg/m^2	350 mg/m^2
	Course 2	100 mg/m^2	300 mg/m^2
	Course 3	ND	300 mg/m^2
CPT-11 C_{max}	Course 1	1.60 mg/l	9.71 mg/l
	Course 2	1.92 mg/l	7.25 mg/l
	Course 3	ND	8.09 mg/l
CPT-11 Clt	Course 1	14.8 l h^{-1} m^{-2}	14.9 l h^{-1} m^{-2}
	Course 2	11.7 l h^{-1} m^{-2}	10.6 l h^{-1} m^{-2}
	Course 3	ND	6.41 l h^{-1} m^{-2}
CPT-11 terminal half-life	Course 1	12.6 h	12.2 h
	Course 2	13.8 h	6.5 h
	Course 3	ND	6.5 h
SN-38 C_{max}	Course 1	70 µg/l	76 µg/l
	Course 2	74 µg/l	117 µg/l
	Course 3	ND	39 µg/l
SN-38 AUC	Course 1	114 µg l^{-1} h	347 µg l^{-1} h
	Course 2	419 µg l^{-1} h	546 µg l^{-1} h
	Course 3	ND	374 µg l^{-1} h

collected for determination of the pharmacokinetic parameters of CPT-11 and SN-38 using a "limited sampling strategy" [7]. Blood samples were centrifuged immediately after collection and the plasma was removed and stored at –20 °C until analysis. Bile was collected at least ten times over a period of up to 48 h and urine was also collected for up to 48 h. Bile and urine samples were stored at –20 °C until analysis. The pharmacokinetic CPT-11 and SN-38 analysis was performed using SIPHAR and MICROPHARM programs (Simed and S. Urien, Créteil, France).

Analysis of CPT-11 and its metabolites

The plasma, bile, and urine samples were analyzed according to the high-performance liquid chromatographic (HPLC) technique described by Barilero et al. [1], which permits the simultaneous assay of CPT-11 and SN-38. Briefly, the samples were processed using a solid-phase (C_{18}) extraction step. The extracts were chromatographed on a C_{18} reversed-phase column with a mobile phase consisting of acetonitrile/phosphate buffer (34:66, v/v) containing 3 m*M* heptanesulfonic acid (adjusted to pH 4 with 1 *M* HCl) using fluorescence detection (excitation, 380 nm; emission, 500 nm). Due to acidification of the samples, only total CPT-11 and SN-38 were assayed. For quantification of the glucuronide conjugates, 100-µl samples of plasma, bile, and urine were treated with 1000 Sigma units of β-glucuronidase (*Escherichia coli*, type IX; Sigma Chemical, St. Louis, USA) overnight at 37 °C [4, 9]. CPT-11 and SN-38 samples were then reanalyzed as previously described and the difference between the first analysis (before β-glucuronidase treatment) and the second analysis (after β-glucuronidase treatment) gave the amount of glucuronide conjugates.

Results

The main plasma pharmacokinetic parameters obtained for CPT-11 and SN-38 are summarized in Table 1. If the pharmacokinetic parameters of CPT-11 seemed to be close in the two cycles analyzed for the first patient, a high intraindividual variability was noticed for the second patient: the total body clearance decreased from 14.9 to 6.4 l h^{-1} m^{-2}. The other plasma pharmacokinetic parameters obtained either for CPT-11 or for SN-38 differed considerably from one course to another. No glucuroconjugate of CPT-11 was found in plasma. Glucuronide SN-38 was the

Table 2 Plasma SN-38 and SN-38 glucuronide conjugate parameters

		Patient 1	Patient 2
CPT-11 dose	Course 1	100 mg/m^2	350 mg/m^2
	Course 2	100 mg/m^2	300 mg/m^2
	Course 3	ND	300 mg/m^2
SN 38 C_{max}	Course 1	70 µg/l	76 µg/l
	Course 2	74 µg/l	117 µg/l
	Course 3	ND	39 µg/l
SN 38 glucuronide C_{max}	Course 1	120 µg/l	258 µg/l
	Course 2	75 µg/l	390 µg/l
	Course 3	ND	286 µg/l
SN-38 AUC	Course 1	114 µg l^{-1} h	347 µg l^{-1} h
	Course 2	419 µg l^{-1} h	546 µg l^{-1} h
	Course 3	ND	374 µg l^{-1} h
SN-38 glucuronide C_{max}	Course 1	1098 µg l^{-1} h	4061 µg l^{-1} h
	Course 2	1137 µg l^{-1} h	5696 µg l^{-1} h
	Course 3	ND	5174 µg l^{-1} h

Table 3 Biliary excretion of CPT-11 and SN-38, expressed as a percentage of the delivered dose of CPT-11

		Patient 1	Patient 2
CPT-11 dose	Course 1	100 mg/m^2	350 mg/m^2
	Course 2	100 mg/m^2	300 mg/m^2
	Course 3	ND	300 mg/m^2
CPT 11	Course 1	3.5%	18.1%
	Course 2	2.3%	23.7%
	Course 3	ND	23.6%
SN-38	Course 1	0.15%	0.17%
	Course 2	0.06%	1.72%
	Course 3	ND	0.86%
SN-38 glucuronide	Course 1	0.85%	1.43%
	Course 2	0.44%	1.11%
	Course 3	ND	0.80%

main form of SN-38 present in plasma (Table 2). The ratio of the area under the concentration-time curves (AUCs) for SN-38 and its glucuroconjugate varied from 3 to 10 according to the patient. The SN-38 glucuronide conjugate AUCs remained stable throughout the treatment for the two patiens and were close to the dose ratio, whereas the SN-38 AUC varied according to the cycle. No relationship was found between C_{max} and AUC for either SN-38 or its glucuronide conjugate.

The results obtained in bile are summarized in Tables 3. As in plasma, no CPT-11 glucuronide conjugate was present, whereas SN-38 appeared mostly as a glucuronide conjugate. The percentage the delivered dose of CPT-11 recovered in bile seemed to remain stable for the two patients, being around 3% for the patient receiving 100 mg/m^2 of CPT-11 and around 20% for the patient receiving the highest dose. In contrast, the value obtained for SN-38 or its glucuroconjugate varied with the course. Simultaneous bile-to-plasma ratios were higher in the patient treated with the low dose (88:1 for CPT-11 and 41:1 for SN-38) than in the patient treated with the high dose (30:1 and 24:1, respectively).

Urinary excretion of CPT-11 and SN-38 is summarized in Table 4. As in plasma and in bile, CPT-11 in urine was present only as the parent drug, which was not the case for SN-38. In urine, SN-38 occurred predominantly in the glucuroconjugate form. No relationship between the excretion values obtained for SN-38 and its glucuroconjugate and between the SN-38 excretion values and the injected dose of CPT-11 was noticed.

Respectively, 26.1% and 23.6% of the delivered dose was recovered from the bile and urine of the patient treated with the weekly schedule for the two courses studied, and during the same 48-h period, 52.5% and 40.1% of the dose, respectively, was recovered from the bile and urine of the patient treated with the highest CPT-11 dose.

Discussion

For the two patients treated according to a 100-mg/m^2 (every week) or 350-mg/m^2 (every 3 weeks) schedule, the plasma pharmacokinetic results obtained for CPT-11 or SN-38 were similar to those obtained at the same dose levels by other authors [7, 10]. Wide intra-patient variability of the pharmacokinetic parameters obtained for both CPT-11 and its major active metabolite has been reported, and the results we obtained agree with such variability [2, 7, 8]. As demonstrated by Rivory and Robert [9], SN-38 glucuronide conjugate is the main form of SN-38 present in patients' blood. The concentrations of SN-38 glucuronide conjugate were about 10-fold those of SN-38. However, the clinical meaning of this glucuronide conjugate remains unknown.

In bile, CPT-11, SN-38, and SN-38 glucuronide conjugate were found. The percentage of the injected dose of

Table 4 Urinary excretion of CPT-11 and SN-38, expressed as a percentage of the delivered dose of CPT-11

		Patient 1	Patient 2
CPT-11 dose	Course 1	100 mg/m^2	350 mg/m^2
	Course 2	100 mg/m^2	300 mg/m^2
	Course 3	ND	300 mg/m^2
CPT 11	Course 1	18.5%	ND
	Course 2	14.2%	22.6%
	Course 3	ND	12.5%
SN-38	Course 1	0.5%	ND
	Course 2	1.6%	0.31%
	Course 3	ND	0.30%
SN-38 glucuronide	Course 1	2.6%	ND
	Course 2	5.4%	3.1%
	Course 3	ND	2.0%

CPT-11 recovered in bile was much higher for the patient receiving the high CPT-11 dose than for the other patient. As described in rats, CPT-11 was most extensively metabolized into SN-38, but this biotransformation was saturable, leading to an increase in CPT-11 excretion in bile at high doses [3]. Nevertheless, this percentage remained constant for the two patients, whatever the number of courses.

By contrast, the percentage of biliary SN-38 and SN-38 glucuronide conjugate varied with the number of courses for both CPT-11 regimens. The ratio of SN-38 glucuronide to SN-38 was apparently constant for the patient receiving 100 mg/m^2 but rather variable for the patient receiving the high dose. In the latter case the ratio varied from 0.6 to 8. In rats [3], SN-38 is also conjugated into glucuronide (perhaps by conjugation on the hydroxy radical corresponding to the leaving group of CPT-11). In urine, the excretion of CPT-11, SN-38, and SN-38 glucuronide conjugate varied according to the course.

The cumulative biliary and urinary excretion of all the compounds studied as determined over a period of up to 48 h was 26% and 24%, respectively, for the patient receiving 100 mg/m^2 and 53% and 40%, respectively, for the patient receiving 300 mg/m^2. The most accurate explanation for these percentages is that CPT-11 is probably excreted under not yet identified metabolites. The pharmacokinetic parameters and percentage of excretion variabilities were not correlated with the biological status of the patients or the comedications they received at each course.

The metabolism of CPT-11 seems to differ between rats and humans, and further human studies are necessary for a better understanding of CPT-11 metabolic behavior and of metabolites other than those thus far identified and, especially, for the determination of a possible pharmacokinetic-pharmacodynamic relationship with the SN-38 glucuronide conjugate.

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