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ORIGINAL ARTICLE

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Preclinical pharmacology of the natural product anticancer agent 10-hydroxycamptothecin, an inhibitor of topoisomerase I

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Abstract *Purpose*: 10-Hydroxycamptothecin (HCPT) is an indole alkaloid isolated from a Chinese tree, *Camptotheca acuminata*, and has a wide spectrum of anticancer activity in vitro and in vivo mainly through inhibitory effects on topoisomerase I. HCPT has been shown to be more potent and less toxic than camptothecin and has recently undergone clinical trials. To determine how HCPT might be best used as an anticancer agent, preclinical studies of the pharmacokinetics, tissue distribution, metabolism and elimination of HCPT in rats were undertaken. *Methods*: HCPT was administered to rats by i.v. bolus injection at doses of 1, 3, and 10 mg/kg body weight. HCPT (lactone and carboxylate) and its metabolites in plasma, urine, feces, and various tissues were quantitated by reversed-phase HPLC. Pharmacokinetic parameters were then estimated. *Results*: Following i.v. administration at doses of 3 or 10 mg/kg, the plasma concentration-time profile for lactone HCPT could be best described by a three-compartment model, with terminal elimination half-lives of 140.4 and 428.6 min, respectively. A two-compartment model was used to fit the plasma concentration-time curve at 1 mg/kg, with a terminal elimination half-life of 30.5 min. Carboxylate HCPT had a longer half-life than the lactone form of HCPT. During the initial 6 h after dosing, urinary excretion was the major route of elimination, and fecal excretion became the major route of elimination thereafter. HCPT was widely distributed to various tissues including the enterohepatic system, kidney, and bone marrow. The lactone form of HCPT was detectable in various tissues examined up to 72 h after dosing at all the three test doses. HCPT glucuronides were present in plasma, urine, feces and various tissues. No significant toxicity was observed at doses of 1 or 3 mg/kg. Polyuria and hematuria were observed only during the initial 3 h after dosing at 10 mg/kg. *Conclusions*: Prolonged elimination of HCPT in vivo may have a significant impact on its therapeutic effects. HCPT is metabolized to its carboxylate form and glucuronides. Dose-dependent toxicity was observed with i.v. administration of HCPT. The results of this study should be useful in the design of future human trials with this anticancer drug.

Key words Anticancer drugs · 10-Hydroxycamptothecin · Pharmacokinetics · Metabolism · Topoisomerase I

Abbreviations *CPT* camptothecin · *CPT-11* irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy camptothecin) · *DMSO* dimethyl sulfoxide · *HCPT* 10-hydroxycamptothecin · *HPLC* high-performance liquid chromatography · *MDR* multidrug resistance · *Topo I* DNA topoisomerase I

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Introduction

DNA topoisomerase I (Topo I) inhibitors represent an exciting and promising new class of anticancer therapeutic agents currently undergoing clinical evaluation. These compounds are structurally related to camptothecin (CPT, Fig. 1), a natural product isolated from a Chinese tree, *Camptotheca acuminata*. Topo I is an

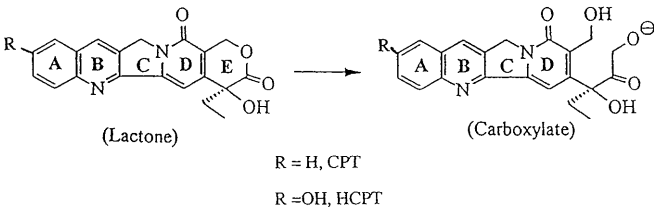


Fig. 1 Structure of water-insoluble camptothecins (*CPT* camptothecin, *HCPT* 10-hydroxycamptothecin)

important nuclear enzyme for various DNA functions including transcription and replication. The mechanisms responsible for the inhibitory effect of CPT on Topo I have been extensively investigated (see references 1 and 2, and references therein). CPT specifically inhibits the breakage/rejoining reaction of Topo I, which leads to the accumulation of the putative covalent reaction intermediate, a reversible Topo I-CPT-DNA ternary complex. The interaction between DNA replication components and the ternary complex may cause cell death among many other cellular responses. CPT induces S-phase-specific cell killing, cell differentiation, and transcription of several growth- and differentiation-related genes. CPT and its natural and synthetic analogs have been shown to have activity both in vitro and in vivo against a broad spectrum of cancers, including leukemias and cancers of the liver, stomach, breast, and colon. In addition, CPT can overcome MDR1-mediated drug resistance completely. Several semisynthetic CPT analogs have been studied extensively for potential clinical use including CPT-11 and topotecan (references 3 and 4, and references therein).

Natural CPT is water insoluble. In order to increase its water solubility, in earlier preclinical and clinical studies, CPT was converted to its sodium salt (CPT-Na^+), yielding limited and, to a certain degree, misleading results regarding its potential clinical application (see references 1 and 5 for review, and references therein). With the development of water-soluble CPT analogs, it has now been demonstrated that the lactone form of CPT is associated with its anticancer activity, and the carboxylate form is much less active and may be responsible for its unwanted toxicity [2]. Recently, CPT given in the lactone form has been shown to have more potent anticancer activity than its water-soluble analogs [6–8]. There now is an increased interest in water-insoluble CPT derivatives, and the clinical studies of CPT have been renewed [3, 9].

Among natural CPTs, 10-hydroxycamptothecin (HCPT) has been shown to be more active and less toxic [5, 10–13]. HCPT has significant anticancer activity against murine leukemia L1210 cells [10, 11], bladder carcinoma MBT-2 cells [13], and experimental animal tumor models [12]. In a Topo I inhibition assay, HCPT has been shown to be more active than either CPT or topotecan, with IC_{50} values (the minimum drug concentration that inhibits cleavable complex formation by 50%) being 0.106 μ*M* for HCPT, 0.677 μ*M* for CPT, and 1.110 μ*M* for topotecan [5]. HCPT has an inhibitory effect on phosphorylation of histone H_1 and H_3 in murine hepatoma cells, which may be related to its specific cell killing effect [14]. Recently, HCPT has undergone clinical trials including evaluation of different schedules of administration as a single agent and in combination therapy [12].

To better understand how HCPT can be used clinically to treat patients with cancer, we investigated the preclinical pharmacology of HCPT in rats following i.v. administration at three different doses of the natural lactone form of HCPT. The plasma pharmacokinetics, tissue distribution, metabolism, and elimination of HCPT (and its metabolites) were determined. The results from this study should be useful in planning future human trials.

Materials and methods

Chemicals and the test drug

HCPT (lactone form) was obtained from Midwest Co. (Beijing, China) and the purity was determined by LC/MS to be greater than 98%. Tetrabutylammonium dihydrogen phosphate (TBAP) was purchased from Waters Co. (Milford, Mass.) as a ready-to-use solution, PIC-A (WAT 08510). β-Glucuronidase was purchased from Sigma Co. (St. Louis, Mo.). All other reagents were of analytical grade and double-distilled water was used throughout. All solutions used in HPLC analysis were filtered and degassed using a 0.2 μm filter membrane (Gelman Sciences, Ann Arbor, Mich.) with a filtration system (Millipore, Bedford, Mass.).

Animals

Animal use and care was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (280 ± 10 g; Harlan Laboratories, Indianapolis, Ind.) were utilized in the study. The animals were fed with commercial diet and water *ad libitum* for 1 week prior to the study.

Experimental protocol

HCPT was dissolved in dimethyl sulfoxide (DMSO) at concentrations of 0.5, 1.5, or 5 mg/ml for the studies of 1, 3, and 10 mg/kg, respectively. HCPT was administered to rats by i.v. bolus injection into the caudal tail vein at the dose levels of 1, 3, and 10 mg/kg body weight. Doses were based on the pretreatment body weight and rounded to the nearest 0.01 ml. After i.v. injection, each animal was placed in a metabolism cage and fed with commercial diet and water *ad libitum*. Total voided urine was collected and each metabolic cage was then washed following the collection intervals (0–3, 3–6, 6–9, 9–12, 12–24, 24–48, and 48–72 h after dosing). Total excreted feces were collected from each animal during the same designated intervals as urine collection, and fecal samples were homogenized in a ninefold volume of 0.9% NaCl saline prior to analysis. Blood samples were collected (at 2, 5, 10, 15, 20, 30, and 60 min; and 2, 4, 6, 8, 12, 24, 48, and 72 h after dosing) into heparinized tubes, and plasma was separated by centrifugation.

Animals were euthanized by exsanguination under sodium pentobarbital anesthesia, and the tissues were collected from each animal of the groups designated for 2, 6, 12, and 72 h at the dose of 10 mg/kg, and 72 h at the doses of 1 and 3 mg/kg. Each tissue or organ was immediately blotted on Whatman no. 1 filter paper,

trimmed of extraneous fat or connective tissue, emptied and cleaned of all contents, and weighed. Prior to homogenization in 0.9% NaCl saline (5 ml per gram of wet weight), each tissue or organ was washed using 0.9% NaCl saline. The resultant homogenates were kept at −70 °C until further analysis.

Quantitation of HCPT and metabolites in biological samples

A recently developed reversed-phase HPLC method was used to separate and quantify HCPT and its metabolites in biological samples. The chromatographic system included an HP 1050 HPLC (Hewlett Packard, Palo Alto, Calif.) with a computer-controlled solvent delivery system and an FD 300 dual monochromator fluorescence detector with an Omniscribe recorder. An RP-18 analytical column (250 mm × 4.6 mm ID) was coupled to a guard column (Jones Chromatography, Lakewood, Colo.). The mobile phase was 0.075 *M* ammonium acetate buffer, pH 6.4/acetonitrile (70:30 v/v) to which one vial of PIC-A solution was added to give a final TBAP concentration of 5 m*M*. The column was eluted at a flow rate of 0.8 ml/min, and the effluent monitored spectrofluorometrically with an excitation wavelength of 363 nm and an emission wavelength of 550 nm. Standards of lactone and carboxylate forms of HCPT were prepared by a modification of the method previously described by Rivory and Robert [15], which was used for the preparation of CPT standards in their studies. The lactone form was prepared in acetonitrile 0.01 *M* citric acid, pH 3 (50:50 v/v), and the carboxylate form was prepared in acetonitrile 0.01 *M* sodium tetraborate, pH 9 (50:50 v/v). A standard curve for each form was run on a daily basis. The correlations between peak heights and concentrations of both forms of HCPT were established and were linear in the range of 2–2000 ng/ml.

Blood samples were centrifuged immediately after collection and the resultant plasma (100 µl) was mixed with 100 µl cold methanol/acetonitrile (1:1) for 20 s, and then centrifuged at 9000 *g* for 5 min. The supernatant was stored at −20 °C until analysis.

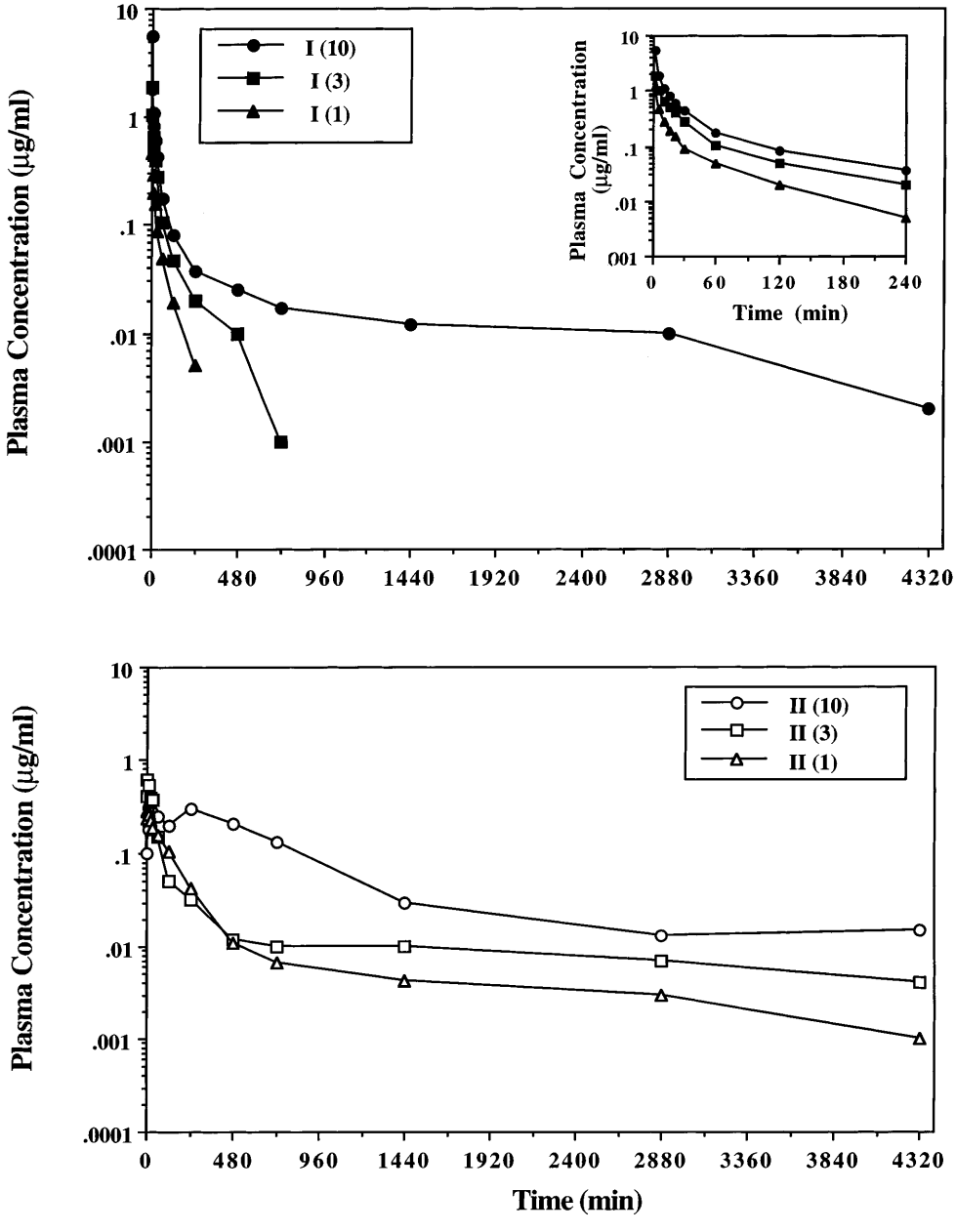


Fig. 2A,B Plasma concentration-time course. Plasma concentrations are expressed as micrograms HCPT equivalents per milliliter after i.v. bolus administration of HCPT to rats at doses of 1, 3, or 10 mg/kg. Three animals were used for each time-point. Plasma concentration was based on the quantitation of the lactone and carboxylate forms of HCPT by reversed-phase HPLC. **A** Lactone form (*insert* expanded time course over the initial 240 min); **B** carboxylate form

Table 1 Pharmacokinetic parameters for HCPT in plasma. Values are means based on the experimental data from rats following i.v. administration of HCPT at various doses (1, 3, 10 mg/kg, three animals per time-point per dose). Concentrations in plasma were based on the HPLC analysis. A three-compartment model of the i.v. bolus injection was fitted to the data of plasma lactone HCPT at doses of 3 and 10 mg/kg. A two-compartment model of the i.v. bolus injection was fitted to the data of plasma lactone HCPT at a dose of 1 mg/kg. A two-compartment model of the first-order absorption was fitted to the data of plasma carboxylate HCPT at doses of 1, 3, and 10 mg/kg (C_{max} maximal concentration, $T_{1/2A}$ absorption half-life, $T_{1/2\alpha}$ distribution half-life, $T_{1/2\beta}$ initial elimination half-life, $T_{1/2\gamma}$ terminal elimination half-life, *AUC* area under the curve, VD_{SS} volume of distribution, *MRT* mean residue time, *CL* clearance)

Dose (mg/kg)	HCPT form	C_{max} (μg/ml)	$T_{1/2A}$ (min)	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	$T_{1/2\gamma}$ (min)	AUC ((μg/ml) · min)	VDss (ml/kg)	MRT (min)	CL (ml/(kg · min))
1	Lactone	1.70	–	2.5 ± 0.6	30.5 ± 12.8	–	14.9	1.97	29.4	0.067
	Carboxylate	0.27	16.5 ± 1.6	–	8.9 ± 0.2	158.4 ± 24.0	113.5	17.57	263.6	0.002
3	Lactone	3.22	–	1.6 ± 0.5	14.3 ± 2.4	140.4 ± 28.7	39.4	6.86	90.0	0.076
	Carboxylate	0.53	13.0 ± 0.4	–	13.0 ± 0.5	765.0 ± 124.2	58.1	35.39	685.8	0.052
10	Lactone	15.93	–	1.1 ± 0.4	16.2 ± 3.5	428.6 ± 87.2	98.3	27.56	271.2	0.101
	Carboxylate	0.31	14.9 ± 4.5	–	3.0 ± 0.3	486.8 ± 99.6	241.9	29.77	719.4	0.041

Urine samples (100 μl) collected at scheduled intervals were also added to 100 μl cold methanol/acetonitrile, vortexed and centrifuged at 9000 *g* for 5 min. The supernatant was diluted by the HPLC mobile phase buffer prior to injection onto the column. Fecal samples were added to physiological saline (0.9% NaCl; 1:9) overnight, homogenized, and centrifuged at 9000 *g* for 30 min, and the supernatant was used for the further analysis. Various tissue samples were homogenized in physiological saline (0.9% NaCl; 1:5) and the resulting homogenates (200 μl) were incubated with 200 μl extraction buffer containing 10% sodium dodecyl sulfate, 5 *M* NaCl, 1 *M* Tris (pH 7.6), 0.5 *M* EDTA and proteinase K (2 mg/ml) at 37 °C for 2 h and then extracted using the methods for plasma sample preparation. The recoveries of HCPT from plasma and urine were greater than 90% and about were 80% for fecal and tissue samples. The detection limit was 2 ng/ml.

Identification of HCPT glucuronides

Following analysis of biological samples from the above-described animal study, two novel metabolite peaks were detected with shorter retention times than the intact HCPT, and these were thought to be the HCPT glucuronides. To confirm the results, an enzyme assay to digest the glucuronide conjugate was conducted. Biological samples (200 μl) were incubated with β-glucuronidase (100 units/100 μl) in a buffer containing 0.025 *M* potassium phosphate (pH 6.8) at 37 °C for 2 h. The reaction was stopped by adding the extraction buffer used for plasma sample preparation and the samples prepared for HPLC analysis as described above. Samples incubated without β-glucuronidase were run simultaneously as controls.

Data analysis

The concentrations of HCPT and its metabolites were calculated based on the quantitation of separate peaks using a standard curve, and expressed as HCPT equivalents in either micrograms per milliliter of biological fluid (e.g. plasma and urine) or micrograms per gram of wet tissue or organ. Three determinations for each sample were conducted and the concentration was then calculated. The final concentration for each time-point is expressed as mean ± standard deviation (SD) of the group of three animals.

The pharmacokinetic parameters of HCPT distribution were estimated using the NLIN procedure of SAS programs [16–18]. Functions consisting of the sum of one, two, or three exponential components ($C_t = \sum_{i=1}^{n} A_i e^{-K_i t}, n = 1, 2, 3$) were fitted to the data by a least squares method, where C_t is the concentration at time-point t, A_i is the concentration coefficient, and K_i is the elimination or absorption rate coefficient. Selections of models were based on comparison of Akaikie's Information Criterion (AIC) and standard errors (SE) of estimated parameters. One-, two-, and three-compartment models of i.v. bolus injection were tested for fit for each plasma concentration-time profile for the lactone form of HCPT. For the carboxylate form of HCPT concentration-time curve, it is clearly seen that a formation phase appeared prior to the elimination phase. Therefore, a first-order two-compartment model with a formation/absorption phase was used i.e.

$$C_t = \sum_{i=1}^{n} A_i \left[e^{-K_i t} - e^{-K_a t} \right], \quad n = 1, 2$$

The area under the curve (AUC) was calculated from

$$\sum_{i=1}^{n} A_i / K_i$$

Elimination half-life ($T_{1/2\beta}$ and $T_{1/2\gamma}$) values of HCPT were calculated from $0.693/K_i$. The clearance rate (CL) of HCPT was calculated by dividing the dose by the AUC [18].

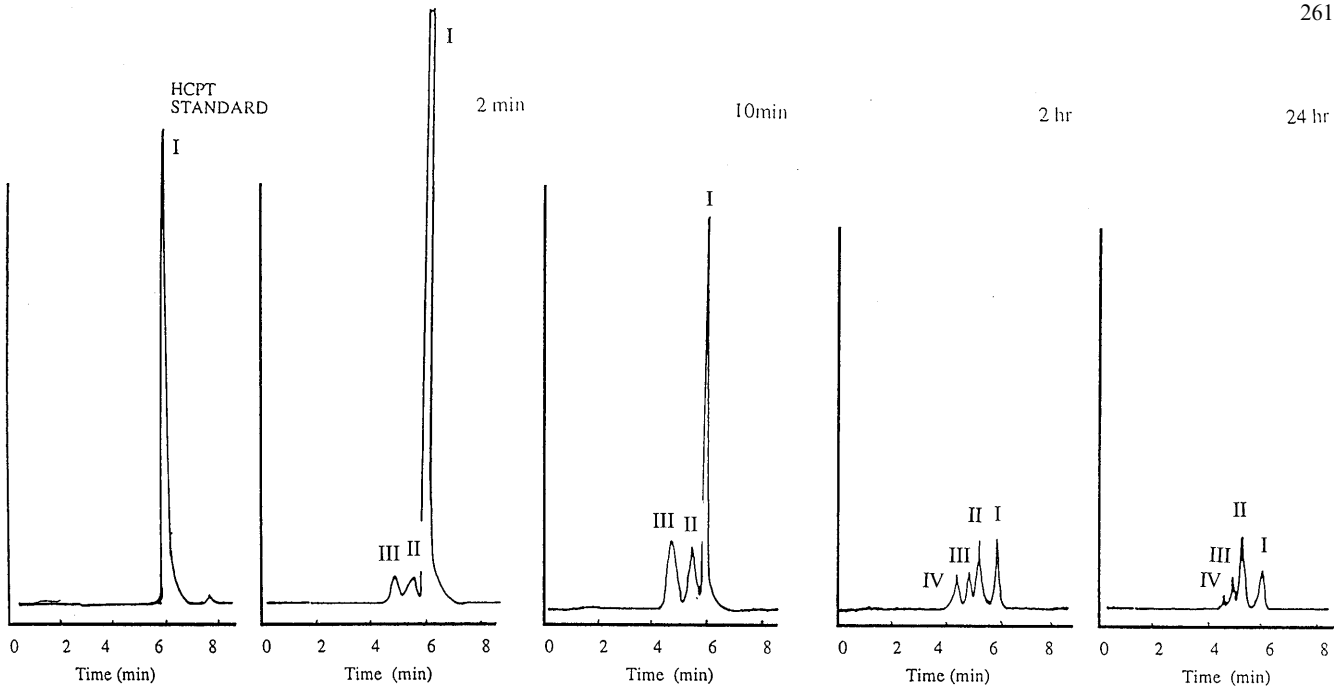
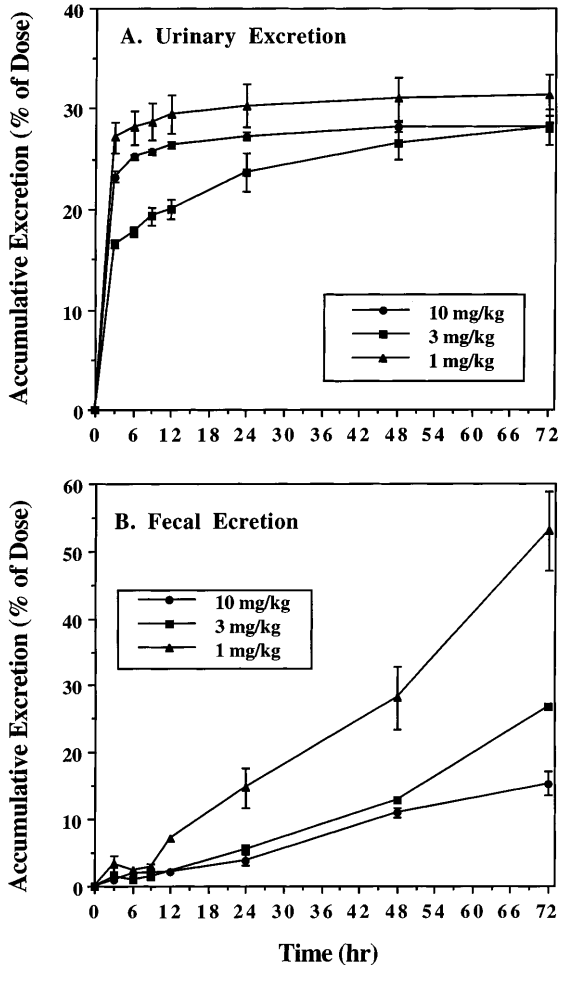


Fig. 3 HPLC analysis of plasma HCPT and its metabolites. HPLC analysis was carried out as described in Methods. Extracted sample (20 µl) was injected onto the column. To increase the determination sensitivity, 100 µl was injected for the sample at 24 h after dosing (*peak I* lactone form, *peak II* carboxylate form, *peaks III and IV* HCPT glucuronides)



Results

Pharmacokinetics and stability of HCPT in plasma

The pharmacokinetics of HCPT were evaluated following i.v. bolus administration of HCPT to rats at doses of 1, 3, and 10 mg/kg body weight. The mean plasma concentrations over time of the lactone and carboxylate forms of HCPT are illustrated in Fig. 2. The pharmacokinetic parameters for the two forms of HCPT are shown in Table 1. Pharmacokinetic analysis revealed that, following i.v. administration, the plasma disappearance curve for lactone HCPT at 3 and 10 mg/kg could be best described by a three-compartment model, and a two-compartment model could be used to describe the plasma pharmacokinetics of lactone HCPT at a dose of 1 mg/kg. For the carboxylate form of HCPT, the plasma disappearance curve at the three dose levels could be best described by a first-order absorption two-compartment model. The "absorption half-life" ($T_{1/2A}$) could be used to describe the mean half-life of formation of the carboxylate form of HCPT, mainly the conversion of lactone HCPT to its carboxylate form. As illustrated in Table 1, both lactone and carboxylate forms of

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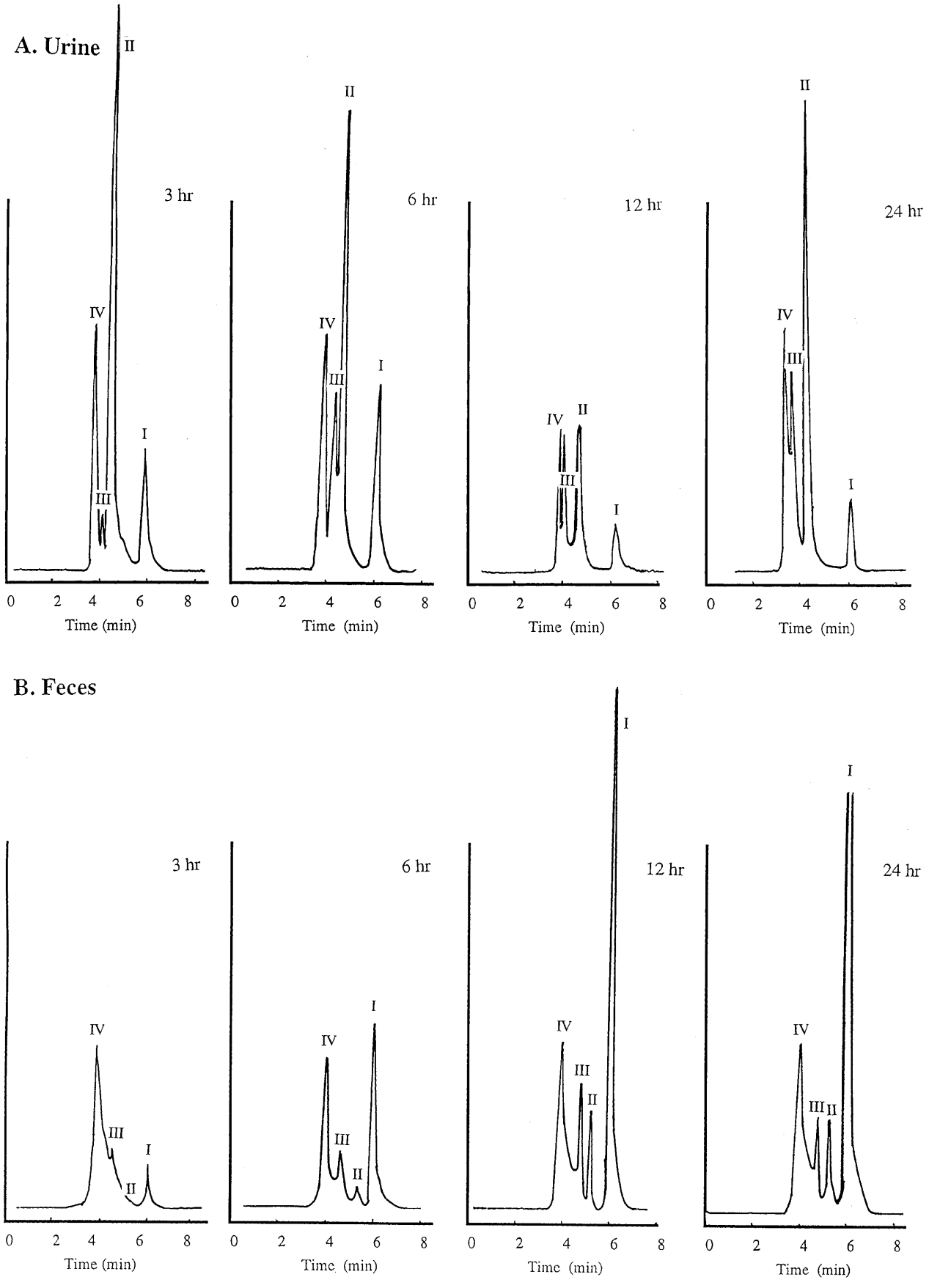
Fig. 4A,B Cumulative urinary and fecal excretion of HCPT and its metabolites. Urinary and fecal excretions of HCPT equivalents are expressed as mean ± SD of the cumulative percentage of administered dose excreted over time. Total excretion was based on the quantitation of HCPT and its metabolites by reversed-phase HPLC


A. Urine
3 hr
6 hr
12 hr
24 hr
I
II
III
IV
0 2 4 6 8
Time (min)
B. Feces
3 hr
6 hr
12 hr
24 hr
I
II
III
IV
0 2 4 6 8
Time (min)

HCPT had a prolonged terminal elimination half-life, which may be important to the therapeutic effects and the dosing schedule of the drug. In addition, the pharmacokinetics of HCPT appeared to be nonlinear at various doses.

Using the recently developed reversed-phase HPLC system, the chemical forms of HCPT and its metabolites in the plasma were determined in comparison with the standard HCPT. Lactone HCPT was detectable in the plasma up to 72 h after dosing at the dose of 10 mg/kg. Owing to the determination limit, the lactone form of HCPT was detectable only up to 8 h and 12 h after dosing at the doses of 1 and 3 mg/kg, respectively. Representative HPLC profiles over time are illustrated in Fig. 3, and these demonstrate that the major HCPT in plasma was the lactone form in the initial phase, and thereafter mainly as the carboxylate form and glucuronide conjugates.

Elimination of HCPT via urine and feces

Following i.v. administration of HCPT, urinary excretion was the major pathway for elimination of HCPT in the first 6 h, but became a minor excretion pathway thereafter (Fig. 4A). During the first 12 h after dosing, fecal excretion was the minor pathway for elimination of HCPT, but became the major elimination pathway thereafter (Fig. 4B). HPLC analysis indicated that minimal amounts of the lactone form of HCPT were excreted via the urine, with the major chemical forms being the carboxylate form of HCPT and its glucuronide conjugates (Fig. 5A). Significant amounts of the lactone form of HCPT were detected in the feces together with other metabolites (Fig. 5B).

Distribution and metabolism of HCPT in various tissues

In this study, the stability and distribution of HCPT were determined in various tissues between 2 and 72 h following i.v. administration. HCPT had a wide tissue distribution, with detectable levels of HCPT in all the tissues examined after i.v. bolus administration. Representative profiles of tissue concentrations of lactone HCPT and its metabolites are presented in Table 2. At 2 h after dosing and thereafter, most tissues had significantly

Table 2 Concentrations of HCPT (lactone form and total) in plasma (μg/ml) and tissues (μg/g). Values are means ± SD, based on HPLC analyses of biological samples from rats following i.v. administration of HCPT at various doses (1, 3, 10 mg/kg, three animals per time-point per doze) (*Total* concentrations of HCPT equivalents including lactone HCPT, carboxylate HCPT, and HCPT glucuronides, *ND* not detectable)

		1 mg/kg 72 h	3 mg/kg 72 h	10 mg/kg			
				2 h	6 h	12 h	72 h
Plasma	Lactone	ND	ND	0.110 ± 0.020	0.030 ± 0.010	0.030 ± 0.010	0.002 ± 0.001
	Total	0.001 ± 0.001	0.004 ± 0.001	0.140 ± 0.030	0.060 ± 0.020	0.070 ± 0.020	0.032 ± 0.020
Liver	Lactone	0.020 ± 0.005	0.050 ± 0.010	1.940 ± 0.690	0.800 ± 0.006	1.030 ± 0.450	0.260 ± 0.090
	Total	0.095 ± 0.120	1.670 ± 0.430	10.19 ± 1.640	6.650 ± 0.760	6.460 ± 0.980	6.520 ± 0.920
Kidney	Lactone	0.050 ± 0.020	0.280 ± 0.100	7.630 ± 0.820	0.310 ± 0.080	0.370 ± 0.060	0.330 ± 0.070
	Total	0.780 ± 0.110	1.520 ± 0.180	15.85 ± 1.720	8.400 ± 0.730	8.790 ± 0.650	8.770 ± 0.670
Bone marrow	Lactone	0.020 ± 0.010	0.050 ± 0.003	0.520 ± 0.030	0.680 ± 0.010	8.880 ± 0.570	0.320 ± 0.080
	Total	0.080 ± 0.010	0.130 ± 0.020	1.810 ± 0.040	2.050 ± 0.010	11.41 ± 0.170	1.370 ± 0.040
Spleen	Lactone	0.002 ± 0.003	0.009 ± 0.003	0.672 ± 0.031	0.321 ± 0.040	0.441 ± 0.031	0.002 ± 0.006
	Total	0.047 ± 0.009	0.067 ± 0.002	0.889 ± 0.049	1.010 ± 0.035	0.578 ± 0.008	0.112 ± 0.024
Lung	Lactone	ND	ND	0.837 ± 0.059	0.121 ± 0.003	0.037 ± 0.040	0.003 ± 0.001
	Total	0.046 ± 0.006	0.104 ± 0.009	1.012 ± 0.040	0.243 ± 0.004	0.123 ± 0.005	0.012 ± 0.003
Heart	Lactone	ND	0.019 ± 0.010	0.026 ± 0.010	0.037 ± 0.001	0.042 ± 0.014	0.027 ± 0.001
	Total	0.058 ± 0.015	0.079 ± 0.013	0.154 ± 0.019	0.259 ± 0.008	0.056 ± 0.014	0.138 ± 0.034
Stomach	Lactone	ND	0.018 ± 0.002	0.114 ± 0.008	0.075 ± 0.011	0.093 ± 0.004	ND
	Total	0.043 ± 0.004	0.181 ± 0.011	0.271 ± 0.030	0.236 ± 0.005	0.270 ± 0.007	0.275 ± 0.012
Small intestine	Lactone	ND	ND	0.289 ± 0.006	0.023 ± 0.002	0.047 ± 0.004	ND
	Total	0.021 ± 0.004	0.033 ± 0.030	0.292 ± 0.010	0.039 ± 0.002	0.186 ± 0.014	0.312 ± 0.034
Large intestine	Lactone	ND	ND	ND	ND	ND	ND
	Total	0.027 ± 0.002	0.100 ± 0.014	0.470 ± 0.039	1.380 ± 0.075	0.642 ± 0.008	1.233 ± 0.019

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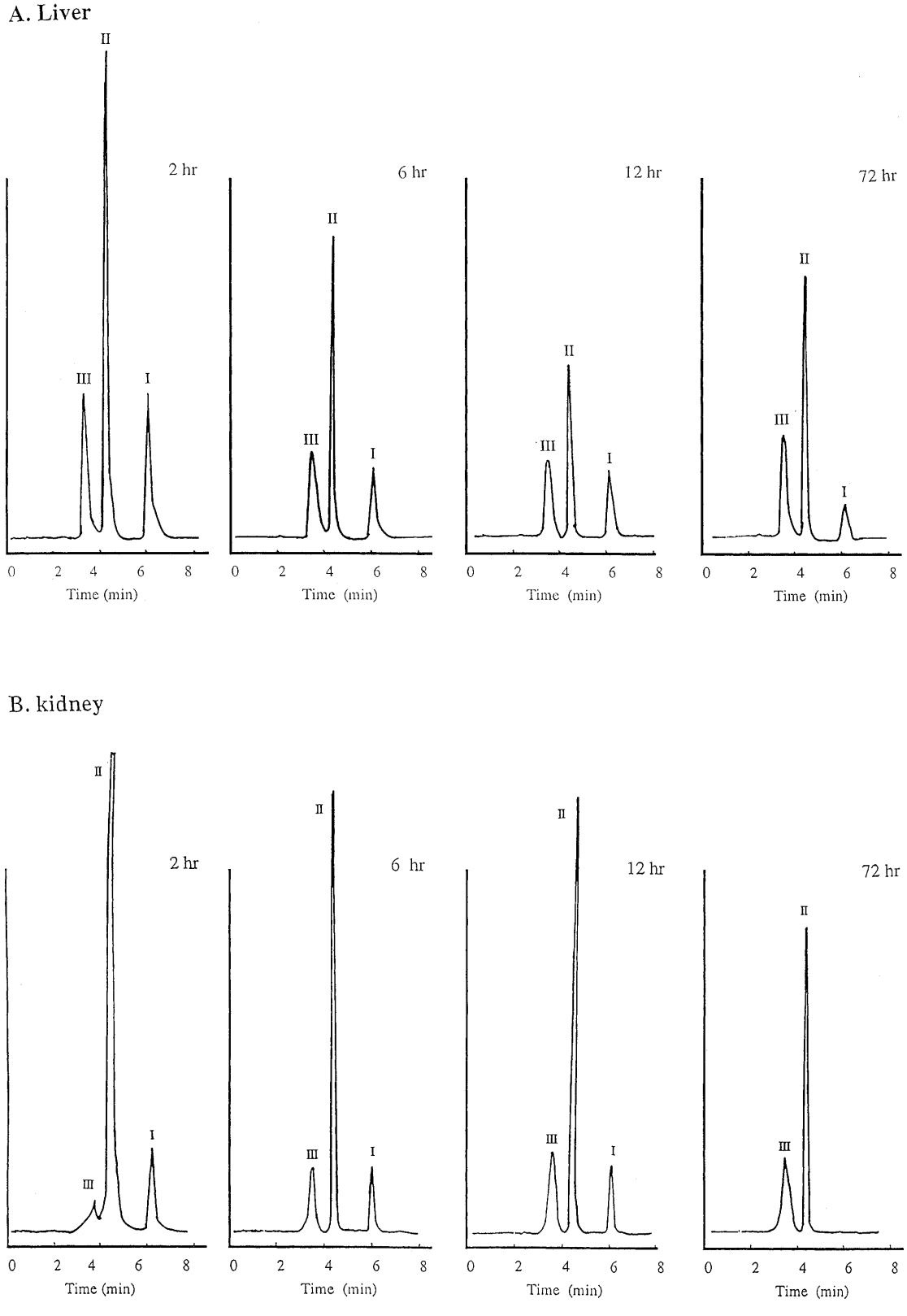
Fig. 5A,B HPLC analysis of HCPT and its metabolites in urine (**A**) and feces (**B**) HPLC analysis was carried out as described in Methods. Extracted urine samples (20 μl) were injected onto the column. The urine sample at 3 h after dosing was diluted fivefold. To increase the determination sensitivity, 100 μl was injected for the sample at 24 h after dosing. For all fecal samples, 10 μl of extracted sample was used (*peak I* lactone form, *peak II* carboxylate form, *peaks III and IV* HCPT glucuronides)

higher concentrations (10–300-fold) than did plasma. The in vivo stability of HCPT in various tissues was evaluated by HPLC, and this demonstrated that HCPT was present largely as the carboxylate form in most tissues, e.g. the liver and kidneys, except bone marrow which had large amounts of lactone HCPT (Fig. 6). Interestingly, the lactone form of HCPT was detectable in all tissues at all the three doses up to 72 h after dosing,



C. Bone Marrow

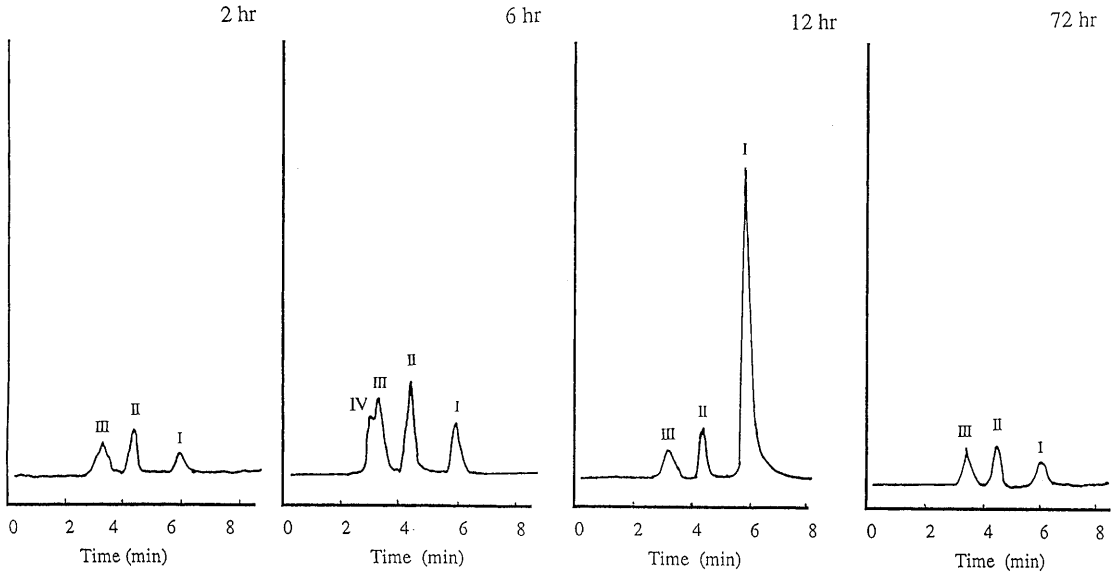


Fig. 6A–C HPLC analysis of HCPT and its metabolites in liver (**A**), kidney (**B**) and bone marrow (**C**). HPLC analysis was carried out as described in Methods. Extracted samples (50 µl) were used for homogenized liver and kidney samples, and 100 µl for bone marrow samples (*peak I* lactone form, *peak II* carboxylate form, *peaks III and IV* HCPT glucuronides)

although the plasma level was very low or not detectable at that time (Table 2).

Under the current chromatographic conditions, two additional peaks were eluted earlier than the carboxylate and lactone forms of HCPT. These probably represented glucuronide conjugates of HCPT in the lactone and carboxylate forms based on the assay of incubation of biological samples with β-glucuronidase. Figure 7 illustrates the HPLC analysis of plasma samples incubated with or without β-glucuronidase, and shows that the two peaks disappeared after the enzyme digestion.

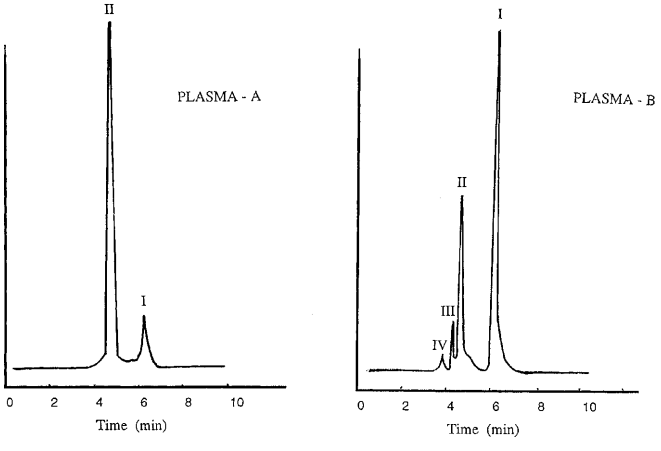


Fig. 7A,B HPLC analysis of plasma HCPT metabolites. **A** Plasma samples were incubated with β-glucuronidase (100 unit/100 µl plasma) at 37 °C for 2 h; **B** plasma samples were incubated without β-glucuronidase at 37 °C for 2 h (*peak I* lactone form, *peak II* carboxylate form, *peaks III and IV* HCPT glucuronides)

Toxicity

No significant toxicity was observed following i.v. administration of HCPT at doses of 1 and 3 mg/kg. At the dose of 10 mg/kg, polyuria and hematuria were observed only during the initial 3 h after dosing at the level of 10 mg/kg. No gastrointestinal toxicity was found at the three doses.

Discussion

The present study establishes five major points regarding the pharmacokinetics and metabolism of HCPT, the natural product anticancer agent. (1) Following i.v. administration the plasma disappearance curve for lactone HCPT (active form) could be best described by a two- or three-compartment model, with a very short distribution half-life and a prolonged terminal elimination half-life. (2) Lactone HCPT was rapidly converted to the carboxylate form (less active form) which had a significantly prolonged elimination half-life. (3) HCPT had a wide tissue distribution with the lactone form being detectable up to 72 h after dosing, which is important to

its Topo I inhibition and therefore anticancer activity. (4) Urinary excretion was the major elimination pathway in the initial phase (first 6 h) and fecal excretion was the major pathway thereafter. (5) The host toxicity was dose-dependent with polyuria and hematuria being the major acute toxicity at the highest dose (10 mg/kg); no significant gastrointestinal toxicity was observed. These results should be useful in the future design of human studies with this novel anticancer drug.

Pharmacokinetic parameters for HCPT were estimated based on the experimental data in rats following i.v. bolus injection at three dose levels. The short distribution half-life illustrates that HCPT was rapidly distributed to other compartments (tissues) outside the plasma and/or rapidly converted to its carboxylate form in the blood. The prolonged elimination half-life reflects the retention of HCPT in the body, which may be associated with: (1) binding to tissue and plasma protein; (2) reversible conversion of lactone and carboxylate forms of HCPT; (3) redistribution among various tissues including potential enterohepatic circulation; and (4) delayed elimination of the parent drug and its metabolites.

Urinary and fecal excretions of HCPT were examined up to 72 h following administration. Urinary excretion was the major pathway of elimination of HCPT and its metabolites within the initial 3 h after i.v. administration, with the chemical forms in urine being mainly the carboxylate form and glucuronides. Fecal excretion, a minor pathway during this initial period, became a major elimination pathway 12 h after administration and thereafter. Interestingly, significant amounts of lactone and carboxylate forms of HCPT were found in the feces, indicating that HCPT is conjugated in the liver and excreted into the intestinal lumen as conjugates which may be deconjugated and reabsorbed or excreted via the feces. The possible enterohepatic circulation of HCPT and its metabolites and the significance of the circulation on the disposition of HCPT are currently under investigation in this laboratory.

Following i.v. administration of HCPT, there was a wide distribution of HCPT and its metabolites in various tissues, including the liver, kidney, and bone marrow. HPLC analysis of tissue extracts demonstrated that the compound was present as the lactone as well as the carboxylate and conjugated forms in the tissues. Metabolites were identified by reversed-phase HPLC analysis, and had shorter retention times than intact HCPT. Initial analysis indicated that the two peaks represented the glucuronide conjugates of HCPT. Further studies to identify these metabolites are in progress.

Previous pharmacokinetic studies with CPT and its water-soluble analogs [1, 3, 6, 9, 12, 19–21] indicate that these drugs undergo significant metabolism in the body. The present study is the first published study on the novel CPT analog, HCPT. Although it is not always possible to extrapolate data from animals to humans, this study suggests that because HCPT has a short distribution half-life and a prolonged elimination half-life, it can be best administered as a loading dose followed by a maintenance dose every other day. Significant accumulation in the liver and the gastrointestinal tract, as well as fecal excretion of HCPT, indicate that enterohepatic circulation of HCPT (and possibly its metabolites) may occur. Therefore, careful monitoring of liver function and possible gastrointestinal side effects will be necessary. Since large amounts of drug were excreted within a short period following i.v. administration, the possible side effects on renal function, especially in the early phase of drug disposition, need to be carefully investigated. Other alternative routes of drug administration, e.g. oral dosing, should be explored. Additional pharmacological analysis of human samples obtained after HCPT chemotherapy should shed further light on how HCPT can be best administered to achieve maximal anticancer effect.

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