Pharmacological Correlation between Total Drug Concentration and Lactones of CPT-11 and SN-38 in Patients Treated with CPT-11

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The pharmacokinetics of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) and its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), were examined to establish the pharmacokinetic variability of the active lactones of CPT-11 and SN-38 in comparison with that of the total (lactone and carboxylates) plasma CPT-11 and SN-38. Twelve patients with malignancies were entered in the study. All received 100 mg/m^2 of CPT-11 by intravenous drip infusion over 90 min. Blood was sampled at 10 time points in heparin-containing syringes. Analysis by high-performance liquid chromatography showed that the ratio of CPT-11 lactone to total CPT-11 concentration was highest (66%) just after the end of infusion and gradually decreased to 30% at 24 h. Almost 70% of SN-38 lactone was detected after the end of infusion and this decreased to 50% within 24 h. The standard errors of percent lactone of CPT-11 or SN-38 to total drug concentration at each sampling point were less than 12%. The area under the concentration-time curve (AUC) of total CPT-11 and that of total SN-38 were significantly correlated with the AUCs of the lactone CPT-11 and those of lactone SN-38, respectively. We conclude that, for practical purposes, monitoring of total CPT-11 and SN-38 has essentially the same clinical significance as monitoring of lactone CPT-11 and SN-38.

Key words: CPT-11 — SN-38 — Lactone — Pharmacokinetics

Camptothecin (CPT),[1] obtained from the Chinese tree *Camptotheca accuminata*, is an alkaloid with a novel ring structure. Although CPT has shown promising antitumor effects *in vitro* and *in vivo*, its clinical application has been disappointing because of its low therapeutic efficacy and severe toxicity to the intestine, bladder and bone marrow.[2, 3] To improve the therapeutic index in the clinical setting, various derivatives of CPT have been semisynthesized. 7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) is a promising water-soluble camptothecin derivative developed in Japan.[4–6] CPT-11 is metabolized to the major active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), and both CPT-11 and SN-38 are thought to be responsible for the pharmacodynamics, including antitumor effect and side effects.[7, 8]

The structure-activity relationship of CPT analogues has been reported, and it is known that: (1) hydrolysis of the lactone ring of CPT produces a hydroxy acid, (2) lactone and open-ring hydroxy acid are in equilibrium in aqueous buffers, (3) the lactone predominates at pH 4.5 and the hydroxy acid predominates at pH 7.4, and (4) the open-ring hydroxy acid is a less potent inhibitor of topoisomerase I and a much less potent antitumor agent, implying that the closed lactone ring is important for

activity.[9] However, the assay methodology for lactones at present is complicated and time-consuming. Many clinical investigations hitherto have reported the total concentration of CPT-11, which represents CPT-11 carboxylate and lactone, and that of SN-38, which represents SN-38 carboxylate and lactone.[10, 11] We also reported that the area under the concentration-time curve (AUC) of both total CPT-11 and total SN-38 were significant pharmacokinetic parameters correlating with the pharmacodynamics.[12] However, the clinical significance of monitoring the lactones remains unknown, although the importance of the lactones was suggested in preclinical reports.[13, 14]

We prospectively conducted a pharmacological study on CPT-11 and its metabolite SN-38 and compared the pharmacokinetics of total (carboxylate and lactone) and lactone CPT-11 as well as total and lactone SN-38.

MATERIALS AND METHODS

Selection of patients Twelve patients with inoperable malignancies were entered in the pharmacokinetic study. All patients had to have documented histologic evidence of advanced malignancy for which routine treatments were not or had not been effective, or no standard chemotherapy existed. The requirements were; (1) at least 18 years of age, (2) ambulatory with an ECOG

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performance status of 0, 1 or 2, (3) a life expectancy of at least 3 months, (4) at least 4 weeks since prior cytotoxic chemotherapy and/or radiotherapy (6 weeks for nitrosoureas and mitomycin) or at least 3 weeks since any major surgical procedure, and (5) adequate bone marrow function (leucocytes >3,000/μl, platelets >100,000/μl, Hb>10.0 g/100 ml), renal function (blood urea nitrogen concentration <1.5×normal, creatinine <2.0 mg/dl or creatinine clearance (Ccr) >50 ml/min), cardiac function (normal ECG) and hepatic function (total) bilirubin <2.0 mg/ml, GOT or GPT levels <2× normal). This study was conducted in accordance with the principles of the Declaration of Helsinki and the 1975 Declaration of Tokyo.[15] Written informed consent was obtained from all patients after they had been informed of the procedure to be followed, the experimental nature of the treatment, potential benefits, side effects, risks and discomfort.

Treatment regimen CPT-11 was supplied by Daiichi Pharmaceutical Co., Ltd. (Tokyo) as a solution in vials (40 mg/2 ml or 100 mg/5 ml). These vials were kept at room temperature in a shaded place. The contents of the vial were reconstituted in 250 ml of 5% glucose solution in a plastic bottle. CPT-11 was administered at the dose of 100 mg/m^2 by intravenous drip infusion over 90 min using an electric infusion pump (Termo, Tokyo). The CPT-11 administrations were repeated in the same patient once a week for 4 consecutive weeks. After a two-week rest, the patients received the same courses of treatment until disease progression was documented or intolerable side effects were observed.

Blood sampling and analysis Blood samples in heparinized tubes were collected before infusion, at 45 and 90 min after the start of the infusion, and at 15, 30, 60 120, 240, 480, 720 and 1440 min after the end of the infusion. Specimen processing, extraction and high-performance liquid chromatography (HPLC) for quantitation of both lactone and total concentration of CPT-11 as well as SN-38 were performed using a modified method[16] that was previously reported for topotecan as well as CPT-11.[17-20] Briefly, plasma was isolated by low-speed (3,000 rpm for 10 min at −5°C) centrifugation as soon as possible following sample collection. Two (2) ml of plasma was immediately mixed with 8 ml of MeOH, and the mixture was stored at −40°C, then vortexed, and centrifuged at 3,000 rpm for 5 min. The upper layer was stored at −40°C until CPT-11 and SN-38 analysis. Twenty (20) μl of water was added to 200 μl of the supernatant (upper layer) and aliquots of 20 μl were applied to the HPLC system for the detection of CPT-11 lactone and SN-38 lactone. For total CPT-11 and total SN-38 quantitation, 20 μl of HCl was added to 200 μl of the supernatant and 20 μl of the mixture was applied to the HPLC system. A C18 reversed-phase column, TSK gel ODS-80Ts (4.6 mm

ID×150 mm) (Tosoh Co., Tokyo), was used for separation of CPT-11 and SN-38. The mobile phases consisted of THF/MeOH/50 mM phosphate, 5 mM heptane sulfate (pH 6.0) (10/30/60, v/v) and THF/50 mM phosphate, 5 mM heptane sulfate (pH 6.0) (40/60, v/v) for CPT-11 and SN-38, respectively. A Shimadzu LC-10AD liquid chromatograph (Shimadzu, Kyoto) was used, with a Hitachi 650-10LC fluorospectrometer (Hitachi, Tokyo). The flow rates were 1.0 ml/min for CPT-11 and SN-38. The detector was set at an excitation wavelength of 370 nm and an emission wavelength of 430 nm for CPT-11, and at 380 nm and 556 nm for SN-38. The concentration of open-ring CPT-11 or SN-38 was calculated by use of the following formula:

$$\text{open ring form} = \text{total drug} - \text{lactone form}$$

The detection limits of CPT-11 and SN-38 were 5 ng/ml and 0.5 ng/ml, respectively.

Pharmacokinetic analysis Pharmacokinetic parameters of CPT-11 and SN-38 were determined by a non-compartmental model (model-independent method). The AUC was calculated by the linear trapezoidal method up to the last measured point, with extrapolation to infinity. Clearance (Cl) was calculated by dividing the total dose of CPT-11 by the AUC.

Statistical analysis Simple regression models were used to determine the correlation between the AUC of total CPT-11 and that of lactone CPT-11 or between the AUC of the total SN-38 and that of lactone SN-38 by the computer program "FASTAT" (Systat Inc., Evanston, IL, USA).

RESULTS

Twelve patients were entered in the present pharmacological study at the National Cancer Center Hospital East (Table I). The majority of patients had good performance status, but had various kinds of malignancies including lung cancer, breast cancer, colon cancer, head and neck cancer, pancreas cancer and malignancy with unknown primary sites. Most of the patients received the planned dose of CPT-11 with tolerable toxicities. The median number of courses of CPT-11 administration per patient was two. Planned blood sampling was achieved for all the patients (Table II), and all the patients were fully evaluable for toxicity and response.

Just after the end of drug infusion, the peak plasma concentration of total CPT-11 was observed and the level declined thereafter according to a bi-exponential mode with the mean T1/2 of 6.8±0.4 h. The maximum plasma concentration of lactone CPT-11 was also detected at the end of infusion and the mean T1/2 of lactone CPT-11 was also similar, 6.4±0.3 h (Fig. 1). The peak plasma concentrations of total SN-38 and lactone SN-38 were detected within 2 h after the end of drug infusion.

The ratio of closed lactone of CPT-11 to total plasma concentration was highest, 66%, just after the end of infusion and decreased to almost 30% at 24 h (Fig. 1, Fig. 2A, Table II). On the other hand, the ratio of SN-38 lactone to the total SN-38 was almost 70% after the end of infusion and decreased to around 50% within 24 h (Fig. 1, Fig 2B, Table III). When the interpatient variability of the ratio of the CPT-11 lactone or SN-38 lactone to the total CPT-11 or total SN-38 concentrations was analyzed at all sampling points, the standard errors of the percent lactones of both CPT-11 and SN-38 proved to be less than 12% (Tables II and III). The pharmacokinetic parameters of CPT-11 and SN-38 were analyzed, and similar values were obtained for peak plasma concentration (Cmax) and total body clearance of both CPT-11

and SN-38 to those found in our previous study (Table IV). The ratios of the AUC of CPT-11 lactone to that of the total CPT-11 and the AUC of the SN-38 lactone to that of the total SN-38 were 37% and 53%, respectively. The half-lives of CPT-11 lactone and SN-38 lactone tended be shorter than that of the total CPT-11 and SN-38, although the differences were not significant. The total body clearance of CPT-11 lactone was larger than that of the total CPT-11. The relationship between the AUC of the total CPT-11 or total SN-38 and that of CPT-11 or SN-38 lactone was analyzed, because our previous study had indicated that the AUCs of CPT-11 and SN-38 were the most informative pharmacokinetic parameters in relation to the pharmacodynamics, including the incidence of leukocytopenia and diarrhea.[12] Good correlations were observed between the AUC of

Table I. Characteristics of Patients

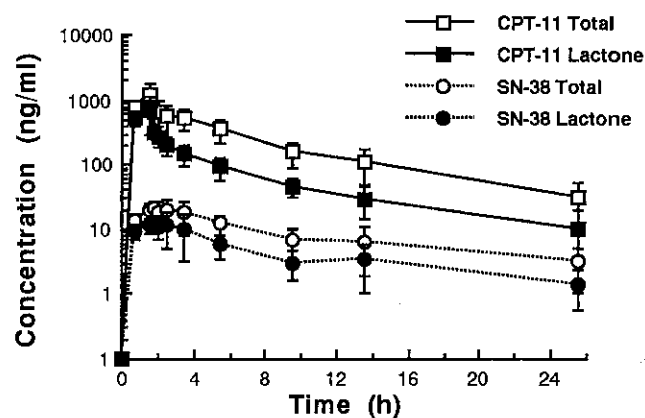| No. of patients	12
Sex (M/F)	7/5
Median age, years (range)	56 (43–69)
ECOG performance status	
0	3
1	6
2	3
Previous therapy	
Chemotherapy	7
Radiotherapy	2
Chemotherapy + radiotherapy	2
No	3
Disease site	
Lung (non-small cell)	3
Lung (small cell)	1
Breast	2
Colon	2
Head and neck	1
Pancreas	1
Unknown primary site	2

Fig. 1. Plasma concentration-time profiles of total and lactone CPT-11 and SN-38 determined by HPLC in 12 patients treated with CPT-11 at a dose of 100 mg/m² per 90 min. Each point represents the mean ± SD.

Table II. Plasma Concentration of Total and Lactone CPT-11

Time (h)	Total (ng/ml)	±SE	Lactone (ng/ml)	±SE	% Lactone	±SE
0.75	790	37	520	42	66	5.2
1.5	1170	176	720	125	62	10.6
1.75	680	78	320	21	47	3.1
2.0	650	80	260	21	39	3.2
2.5	560	84	190	19	35	3.4
3.5	530	58	140	15	27	2.8
5.5	350	41	90	11	27	3.1
9.5	150	18	40	4	29	2.7
13.5	100	18	20	4	27	3.8
25.5	30	17	10	2	33	9.2

Total and lactone concentrations of CPT-11 are the mean concentrations for 12 patients. Percent (%) lactone means the percentage of lactone concentration with respect to total CPT-11 concentration.
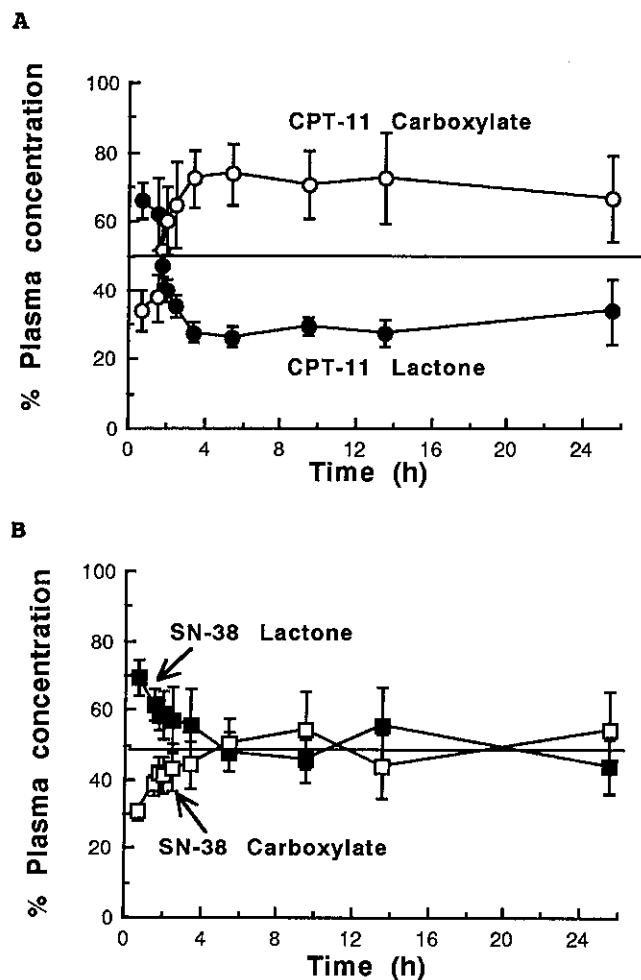
A

B

Fig. 2. Percent plasma concentration-time profile of lactone CPT-11 and carboxylate CPT-11 (A) and lactone SN-38 and carboxylate SN-38 (B) in 12 patients treated with CPT-11 at a dose of 100 mg/m² per 90 min. Each point represents the mean±SD.
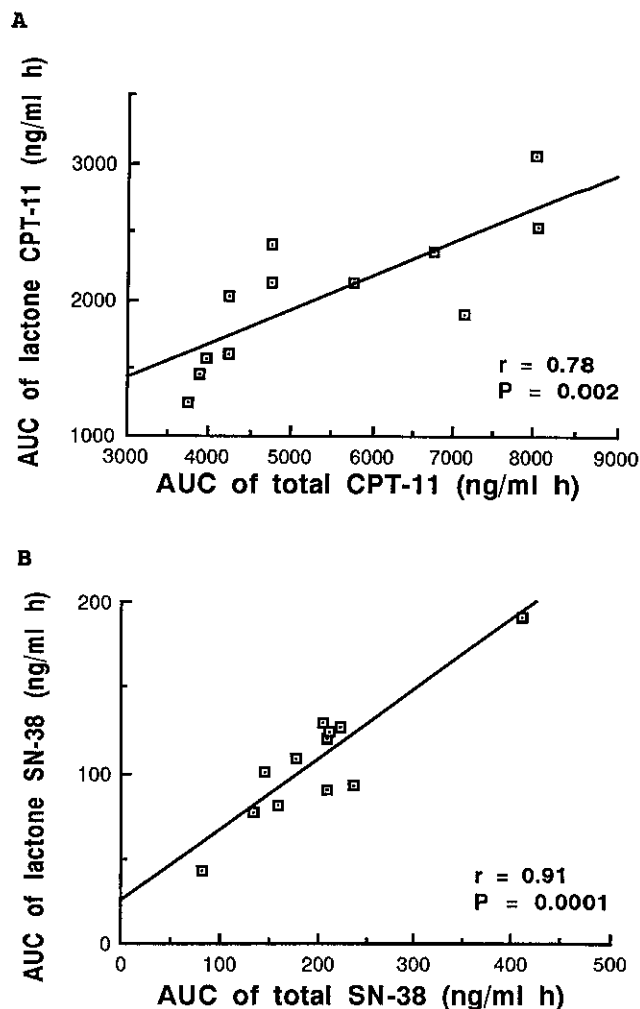
A

B

Fig. 3. The relationship between AUC of total CPT-11 and AUC of lactone CPT-11 (A) or AUC of total SN-38 and AUC of lactone SN-38 (B) in 12 patients treated with CPT-11 at a dose of 100 mg/m² per 90 min.

Table III. Plasma Concentration of Total and Lactone SN-38

Time (h)	Total (ng/ml)	±SE	Lactone (ng/ml)	±SE	% Lactone	±SE
0.75	13	0.8	9	0.6	69	5.0
1.5	19	1.3	11	0.8	61	4.5
1.75	20	1.7	11	0.8	58	4.3
2.0	18	2.0	10	1.2	58	6.7
2.5	19	2.7	11	1.8	57	9.3
3.5	17	2.7	9	1.8	55	10.6
5.5	12	1.2	5	0.7	47	5.6
9.5	6	1.0	3	0.4	45	6.3
13.5	6	1.2	3	0.7	55	11.4
25.5	3	0.5	1	0.2	43	7.8

Total and lactone concentrations of SN-38 are the mean concentrations for 12 patients. Percent (%) lactone means the percentage of lactone concentration with respect to total SN-38 concentration.

Table IV. Pharmacokinetic Parameters of CPT-11 and SN-38

	AUC (ng/ml h)	Tmax (h)	Cmax (ng/ml)	T1/2 (h)	Cl total (1/h/m^2)
CPT-11					
Total±SE	5437.3±472.9	1.3±0.1	1231.6±159.4	6.8±0.4	18.8±1.6
Lactone±SE	2034.1±148.6	1.3±0.1	789.1±108.4	6.4±0.3	49.5±4.2
SN-38					
Total±SE	200.1±22.9	2.4±0.4	23.3±2.2	11.3±1.6	
Lactone±SE	107.4+10.4	2.2+0.4	14.5±1.5	9.1±1.5	

the total CPT-11 and that of CPT-11 lactone ($r=0.78$) and between the AUC of total SN-38 and that of SN-38 lactone ($r=0.91$) (Fig. 3). No definitive conclusion could be drawn on the pharmacodynamics because of the insufficient number of patients entered in the present study. Severe adverse effects, including myelosuppression and diarrhea, were not experienced in the present cohort (data not shown).

DISCUSSION

A scientific and rational approach to establish the optimum administration method of drugs, especially anticancer agents, should be based on pharmacological information, including cellular and clinical pharmacology. The basic approach is to measure the pharmacokinetic parameters, to evaluate the pharmacodynamic outcome and to analyze the pharmacokinetic/pharmacodynamic relationship. It is also essential to identify the active forms of anticancer drugs that inhibit cancer cell growth. The importance of measuring the lactone form of CPT and its derivatives, which is thought to be responsible for the anticancer effects, has been reported by several investigators.[17-20] Although CPT-11 was developed in Japan, no pharmacokinetic analyses have been performed on CPT-11 lactone or SN-38 lactone in the clinical setting in Japan. This may have been because; (1) an assay procedure for measuring lactones of CPT-11 and SN-38 was not available in Japan when the initial phase I study started, (2) the assay procedure and plasma separation are too complicated to be clinically convenient, (3) good pharmacological correlations between total concentration of CPT-11 or SN-38 and side effects were obtained without measurement of the lactones.[10-12] The open-ring hydroxy acid is a less potent inhibitor of topoisomerase I and a much less potent antitumor agent, and the closed lactone ring appears to be important for activity. However, whether or not the measurement of the lactone form of CPT or its derivatives would provide more precise pharmacodynamic information than the measurement of total concentration remains unclear in the clinical setting. Two separate groups performed phase I and pharmacological analysis of CPT-11 in the

U.S. by measurement of the lactones of CPT-11 and SN-38, but neither of them concluded that the measurement of the lactone form was of greater clinical significance than that of the total concentration of CPT-11 and SN-38 in describing the precise pharmacodynamics.[19, 20] In addition, there was reported to be no pharmacodynamic correlation for the lactone of topotecan.[17, 18] The clinical significance of the measurement of lactones of CPTs to predict pharmacodynamics is uncertain for the following reasons; (1) the AUCs of total CPTs are significantly correlated with those of lactone CPTs, as demonstrated in the present study, (2) the assay procedure used in the present study as well as that used by other investigators may not always reflect the true lactone concentration in the clinical setting, (3) the carboxylates of SN-38 as well as CPT-11 may have high anticancer activity, and (4) other factors including protein binding are also important and it is impossible to measure them separately for lactone and carboxylate forms at present. Although it has very promising anticancer activity, CPT-11 also has a very complex pharmacokinetic behavior (CPT-11 vs. SN-38, lactone vs. carboxylates and free vs. protein-bound). A more precise and more accurate pharmacodynamic model should be establised. The standard errors of percent lactone to total drug concentration of CPT-11 and SN-38 at all sampling points in our study were less than 12%, with very small interpatient variability, and the AUCs of total CPT-11 and total SN-38 were significantly correlated with those of lactone CPT-11 and lactone SN-38. We conclude that, taking account of simplicity and convenience, monitoring of total CPT-11 and SN-38 is sufficient to determine the pharmacodynamics in patients treated with CPT-11.

ACKNOWLEDGMENTS

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