Cancer Chemother Pharmacol (1997) 40: 449–452

SHORT COMMUNICATION

Toshiaki Takahashi · Yasuhiro Fujiwara
Hidetaka Sumiyoshi · Takeshi Isobe · Naoki Yamaoka
Michio Yamakido

Salivary drug monitoring of irinotecan and its active metabolite in cancer patients

Received: 13 July 1996 / Accepted: 15 December 1996

Abstract To assess the clinical usefulness of salivary monitoring of irinotecan (CPT-11) and its active metabolite (SN-38), we examined the clinical pharmacological profile of both drugs in 9 patients with thoracic malignancies who received 60 mg/m^2 CPT-11 (21 courses). Plasma and unstimulated whole saliva were collected over a 24-h period, and concentrations of CPT-11 and SN-38 were measured by high-performance liquid chromatography. Both CPT-11 and SN-38 were detectable in saliva, and the concentration-time curves in plasma and saliva showed a very similar pattern. A good correlation was observed between the saliva concentration (C_s) and the plasma concentration (C_p) for both CPT-11 and SN-38 (r=0.732, $P<0.0001$ and r = 0.611, $P<0.0001$, respectively). The area under the concentration-time curve calculated for saliva (AUC_s) correlated with that generated for plasma (AUC_p) for both CPT-11 and SN-38 (r = 0.531, P = 0.012 and r = 0.611, P = 0.0025, respectively). These results suggest that it may be feasible to use saliva instead of plasma for pharmacokinetics/pharmacodynamics studies of CPT-11.

Key words Saliva · Therapeutic drug monitoring · CPT-11 · SN-38

Introduction

Pharmacokinetics (PK) and pharmacodynamics (PD) studies of anticancer drugs are considered to be essential for the efficient and safe administration of cancer chemotherapy. Although frequent blood sampling is essential for precise PK/PD analysis, it is painful for the patients, and the drawing of blood increases the risk of biohazards for clinicians. In comparison with blood sample collection, the collection of saliva is noninvasive and does not require special skill. Furthermore, monitoring of levels in saliva has been reported to be useful for many therapeutic drugs [8]. However, salivary monitoring of anticancer drugs has received little attention [3, 6, 21].

Irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin (CPT-11), and its active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) have been reported to show promising antitumor activity against a variety of tumor types. Several PK/PD studies using plasma have been conducted [1, 5, 7, 10, 14–19, 22], but there has been no detailed report describing their detectability in human saliva. Only one report has mentioned two cases in which CPT-11 was measured at the end of intravenous infusions [1]. Herein we report on the clinical pharmacological profile of CPT-11 and SN-38 in saliva.

T. Takahashi · Y. Fujiwara (✉) · H. Sumiyoshi · T. Isobe
N. Yamaoka · M. Yamakido
Second Department of Internal Medicine, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan

Y. Fujiwara
Department of Integrated Medicine, Hiroshima University School of Medicine, Hiroshima, Japan

Patients and methods

Nine patients with inoperable lung tumors were included in this study. The characteristics of the patients were as follows: median age, 67 (range 46–74) years; M/F ratio, 8/1; Eastern Cooperative Oncology Group (ECOG) performance status of 0/1/2, 4/3/2 patients; no previous chemotherapy, 6 patients; and histology of small/squamous/mesothelioma, 7/1/1 patients. All patients met the standard phase-II-study entry criteria, informed written consent was obtained from them before the start of the treatment, and the study was approved by the institutional review board.

CPT-11 was infused intravenously at 60 mg/m^2 over 90 min weekly for 3 weeks followed by a 1-week rest. On day 1 (week 1), only 60 mg/m^2 cisplatin was given over 60 min just after the end of the CPT-11 infusion. Patients were required to remain on therapy as long as their disease was responding, unless toxicity prevented this. Blood samples were obtained and subjected to drug measurement by high-performance liquid chromatography (HPLC) as reported elsewhere [23]. In brief, blood samples were collected into heparinized tubes before the infusion; at 30, 60, and 90 min during the infusion; and at 30 min and 1, 2, 3, 5, 7, 10, and 24 h after the end of the infusion. After centrifugation, 200 µl plasma was extracted with 750 µl methanol

Table 1 Comparison of pharmacokinetic parameters of CPT-11 and SN-38 between plasma and saliva

	AUC_p (ng/ml×h)	AUC_s (ng/ml×h)	$Cmax_p$ (ng/ml)	$Cmax_s$ (ng/ml)	$Tmax_p$ (h)	$Tmax_s$ (h)
CPT-11						
Mean	2440	2299	545	442	1.5	1.7
SD	469	875	176	185	0.3	0.4
Maximum	3903	4180	1138	797	2.0	2.5
Minimum	1778	1091	357	203	1.0	1.0
SN-38						
Mean	75.2	18.1	9.7	2.2	2.0	2.0
SD	28.8	9.5	3.3	0.6	0.7	0.4
Maximum	147.2	55.9	16.8	3.6	4.5	2.5
Minimum	45.5	10.4	6.3	1.4	1.5	1.0

Abrreviations: AUC_p, area under the concentration-time curve for plasma; AUC_s, area under the concentration-time curve for saliva; $Cmax_p$, peak plasma concentration; $Cmax_s$, peak saliva concentration; $Tmax_p$, time to reach $Cmax_p$; $Tmax_s$, time to reach $Cmax_s$.

PK parameters were determined on the basis of a non-compartmental model using the computer program MULTI (12) AUC was calculated by the trapeziodal method to the last point of sampling, 25.5 h after the the start of CPT-11 infusion. All the values were calculated from 21 data sets (12 data sets with cisplatin co-administration and 9 data sets without co-administration) from nine patients.

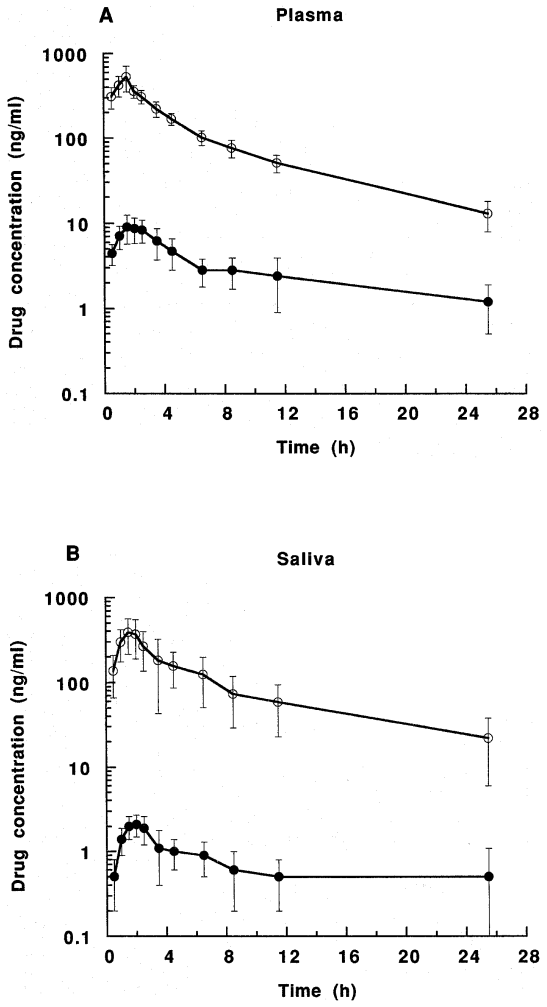

Fig. 1A, B Plasma concentration-time curves generated for CPT-11 and SN-38 in **A** plasma and **B** saliva in nine patients with thoracic malignancy (*Points* mean values ± SD, *white circles* CPT-11, and *black circles* SN-38)

and centrifuged, and the supernatant was evaporated to dryness. The dried extract was reconstituted with 400 µl mobile phase solution [acetonitrile-50 m*M* disodium hydrogen phosphate (28:72) containing 5 m*M* heptanesulfonate, adjusted to pH 2.0 with orthophosphoric acid]. After further centrifugation, 100 µl of the supernatant solution was injected into the HPLC system for fluoroescence detection (excitation wavelength 380 nm, emission wavelength 556 nm).

Although several procedures, such as parafilm chewing [2, 12] or citric acid stimulation [20], have been used for saliva collection, we collected at least 2-ml samples of unstimulated whole saliva. Aliquots of saliva (1.5 ml) were centrifuged at 10,000 rpm for 5 min at 20 °C, and 400 µl of the supernatant was subjected to drug extraction and measurement.

PK parameters were determined on the basis of a noncompartmental model using the computer program MULTI [24]. The area under the concentration-time curve (AUC) was calculated by the trapezoidal method to the last point of sampling, i.e., 25.5 h after the start of the CPT-11 infusion.

Results

Both CPT-11 and SN-38 were detectable in saliva during and after drug administration. The concentration-time (c-t) curves generated for CPT-11 (Fig. 1A) and SN-38 (Fig. 1B) in plasma and saliva showed a very similar pattern. The PK parameters are listed in Table 1. For CPT-11 the time taken for the peak saliva concentration to be attained (T_{maxs}) was approximately 15 min longer than that observed in plasma, and the c-t curve showed a biexponential mode of decline, with the half-life ($T_{1/2\beta s}$) being 9.8 h, longer than that seen in plasma (7.1 h). T_{maxs} and $T_{1/2\beta s}$ (33.0 h) values recorded for SN-38 showed the same pattern observed in plasma (plasma $T_{1/2\beta p}$ = 13.9 h).

Good correlation was observed between the saliva concentration (C_s) and the plasma concentration (C_p) for both CPT-11 and SN-38 (r = 0.732, $P < 0.0001$ and r = 0.611, $P < 0.0001$, respectively; Fig. 2A). The ratio of C_s to C_p (C_s/C_p ratio) was approximately 65% for CPT-11 and 20% for SN-38. In addition, there was no change in PK parameters in the presence or absence of cisplatin coadministration, suggesting no interaction between cisplatin and CPT-11.

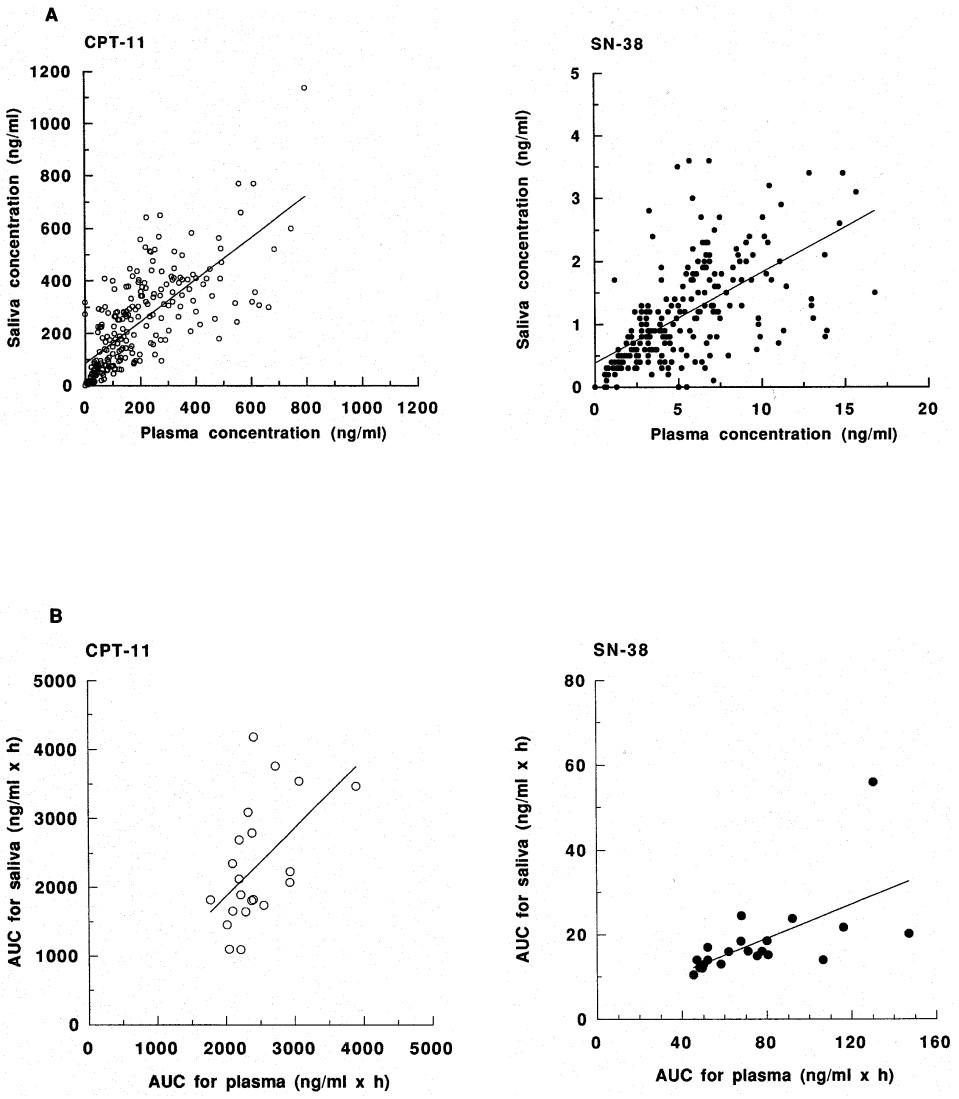

Fig. 2A Relationship between the saliva drug concentration and the plasma drug concentration for all data sets used in the present study. **B** Relationship between the AUC_s and the AUC_p for all data sets (21 courses in 9 patients) used in the present study. (*Lines* Linear regression lines)

The AUC value recorded for plasma (AUC_p) correlated with that noted for saliva (AUC_s) for both CPT-11 and SN-38 (r = 0.531, P = 0.012 and r = 0.611, P = 0.0025, respectively; Fig. 2B).

Discussion

This study demonstrated for the first time the clinical pharmacological profile of CPT-11 and its active metabolite, SN-38, in human saliva. Statistically significant correlations were observed between C_s and C_p, and between AUC_s and AUC_p for both CPT-11 and SN-38.

Although for the whole patient-data set the coefficient of correlation between C_s and C_p was not very high in the present study, reflecting considerable interpatient variability, the relationship between C_s and C_p in each patient was clearly linear (data not shown). If the C_s/C_p ratio for an individual can be determined by collecting both plasma and saliva at one time point, sampling at the other time points can be done using only saliva; the C_p value can then be estimated from the calculated C_s/C_p ratio. There are several factors that influence the C_s/C_p ratio for drugs, such as plasma protein binding, molecular weight, lipid solubility, p*K*a, and salivary pH [11]. The differences observed between CPT-11 and SN-38 with regard to the ratios of AUC_p/AUC_s and C_{maxp}/C_{maxs} may be attributable to differences in the protein-binding characteristics of the two drugs [4, 13]. Changes in salivary pH have been shown to be wholly dependent on changes in the salivary flow rate [11]. Several investigators have reported that the correlation between C_s and C_p becomes stronger after saliva secretion has been stimulated [2, 12, 20], although we collected unstimulated whole saliva samples. In addition, CPT-11-induced cholinergic effects might have influenced saliva secretion [9]. Therefore, we think that further studies are needed to examine whether stimulation of saliva secretion might reduce the inter- and intrapatient variability of the C_s/C_p ratio.

Relationships between PK parameters in plasma, such as AUC_p, for CPT-11 and/or SN-38 and their toxicities have

been reported [1, 5, 7, 10, 14, 18, 19, 22]. Although in our present preliminary study we could not perform satisfactory PD analysis, one patient experienced ECOG grade 2 diarrhea and WHO grade 3 vomiting, and her AUC_s values for CPT-11 (4180 ng.h ml^{-1}) and SN-38 (55.9 ng.h ml^{-1}) were higher than those of other patients who did not experience severe toxicity. If data from future studies using larger sample sizes confirm the usefulness of saliva sampling in patients receiving CPT-11, it should be feasible to use saliva instead of blood samples for PK/PD studies. This will be of great benefit to both patients and clinicians.

Acknowledgements This research was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare, Japan, and grants from the Uehara Memorial Foundation, Yokoyama Clinical Pharmacology Foundation, Daiichi Pharmaceutical Co. Ltd., and Yakult Honsha Co. Ltd. Y. Fujiwara is the recipient of a Fellowship from the Japan Society for the Promotion of Science for Research at Centers of Excellence Abroad, and part of this research analysis was performed at the University of Maryland Cancer Center. We express our appreciation to Dr. Merrill J. Egorin for his helpful critique of the manuscript and to Drs. Kenji Niitani and Ken Okusaki and the Nishi-2 ward staff for their help during the study.

References

1. Abigerges D, Chabot GG, Armand J-P, Herait P, Gouyette A, Gandia D (1995) Phase I and pharmacologic studies of the camptothecin analog irinotecan administered every 3 weeks in cancer patients. J Clin Oncol 13:210
2. Blanchard J, Harvey S, Morgan WJ (1991) Serum/saliva correlations for theophylline in asthmatics. J Clin Pharmacol 31:565
3. Bressolle F, Jacquet J-M, Galtier M, Jourdan J, Donadio D, Rossi J-F (1992) Doxorubicin and doxorubicinol plasma concentrations and excretion in parotid saliva. Cancer Chemother Pharmacol 30: 215
4. Burke TG, Mi A (1994) The structural basis of camptothecin interactions with human serum albumin: impact on drug sensitivity. J Med Chem 37:40
5. Chabot GG, Abigerges D, Catimel G, Culine S, De Forni M, Extra J-M, Mahjoubi M, Herait P, Armand J-P, Bugat R, Clavel M, Marty ME (1995) Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during phase I trials. Ann Oncol 6:141
6. Conley BA, Egorin MJ, Zuhowski EG, Sinibaldi VJ, Peterson DE (1988) Salivary concentrations of hexamethylene bisacetamide (HMBA) in patients receiving 5-day continuous infusions. Cancer Chemother Pharmacol 22:65
7. De Forni M, Bugat R, Chabot GG, Culine S, Extra J-M, Gouyette A, Madelaine I, Marty ME, Mathieu-Boue A (1994) Phase I and pharmacokinetic study of the camptothecin derivative irinotecan, administered on a weekly schedule in cancer patients. Cancer Res 54:4347
8. Drobitch RK, Svensson CK (1992) Therapeutic drug monitoring in saliva. Clin Pharmacokinet 23:365
9. Gandia D, Abigerges D, Aramnd J-P, Chabot GG, Costa LD, De Forni M (1993) CPT-11-induced cholinergic effects in cancer patients. J Clin Oncol 11:196
10. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ (1994) Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. Cancer Res 54:3723
11. Haeckel R (1993) Factors influencing the saliva/plasma ratio of drugs. Ann NY Acad Sci 694:128
12. Kirk JK, Dupuis RE, Miles MV, Gaddy GD, Miranda-Massari JR, Williams DM (1994) Salivary theophylline monitoring: reassessment and clinical consideration. Ther Drug Monit 16:58
13. Kurono Y, Miyajima M, Ikeda K (1993) Interaction of camptothecin derivatives with human plasma proteins in vitro (in Japanese). Yakugaku Zasshi 113:167
14. Mick R, Gupta E, Vokes EE, Ratain MJ (1996) Limited-sampling models for irinotecan pharmacokinetics-pharmacodynamics: prediction of biliary index and intestinal toxicity. J Clin Oncol 14:2012
15. Negoro S, Fukuoka M, Masuda N, Takada M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Niitani H, Taguchi T (1991) Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small cell lung cancer. J Natl Cancer Inst 83:1164
16. Ohe Y, Sasaki Y, Shinkai T, Eguchi K, Tamura T, Kojima A, Kunikane H, Okamoto H, Karato A, Ohmatsu H, Kanzawa F, Saijo N (1992) Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. J Natl Cancer Inst 84:972
17. Rothenberg ML, Kuhn JG, Burris HA, Nelson J, Eckardt JR, Tristan-Marales M, Hilsenbeck SG, Weiss GR, Smith LS, Rodriguez GI, Rock MK, Von Hoff DD (1993) Phase I pharmacokinetic trial of weekly CPT-11. J Clin Oncol 11:2194
18. Rowinsky EK, Grochow LB, Ettinger DS, Sartorius SE, Lubejko BG, Chen T-L, Rock MK, Donehower RC (1994) Phase I and pharmacological study of the novel topoisomearase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin (CPT-11) administered as a ninety-minutes infusion every 3 weeks. Cancer Res 54:427
19. Sasaki Y, Hakusui H, Mizuno S, Morita M, Miya T, Eguchi K, Shinkai T, Tamura T, Ohe Y, Saijo N (1995) A pharmacokinetic and pharmacodynamic analysis of CPT-11 and its active metabolite SN-38. Jpn J Cancer Res 86:101
20. Siegal IA, Ben-Aryeh H, Gozal D, Colin AA, Szargel R, Laufer D (1990) Comparison of unbound and total serum theophylline concentrations with those of stimulated and unstimulated saliva in asthmatic children. Ther Drug Monit 12:460
21. Slavik M, Wu J, Riley C (1993) Salivary excretion of anticancer drugs. Ann NY Acad Sci 694:319
22. Sumiyoshi H, Fujiwara Y, Ohashi N, Egusa Y, Yamaoka N, Yamakido M (1995) Clinical pharmacological evaluation of CPT-11 and cisplatin (CDDP) in patients with small cell lung cancer (SCLC) (abstract 1470). Proc Am Soc Clin Oncol 14:457
23. Sumiyoshi H, Fujiwara Y, Ohune T, Yamaoka N, Yamakido M (1995) High-performance liquid chromatographic determination of irinotecan(CPT-11) and its active metabolite (SN-38) in human plasma. J Chromatogr B 670:309
24. Yamaoka K, Tanigawara Y, Nakagawa T, Uno T (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J Pharmacobio-Dyn 4:879