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CPT-11: Population Pharmacokinetic Model and Estimation of Pharmacokinetics Using the Bayesian Method in Patients with Lung Cancer

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In this study, we aimed to develop a population pharmacokinetic model for CPT-11 and to use the Bayesian method to estimate CPT-11 pharmacokinetic parameters in each of 43 patients who received combined therapy consisting of CPT-11 and etoposide. The group was divided into first and second data sets of 30 and 13 patients, respectively. We developed a population pharmacokinetic model of CPT-11 based on the first data set. The individual pharmacokinetic parameters [area under the concentration curve (AUC) and clearance (CL)] were subsequently estimated by using the Bayesian method on the second data set. Plasma CPT-11 concentrations were measured by high-performance liquid chromatography, and compartmental pharmacokinetic models were fitted by the Bayesian method. The population pharmacokinetic model was developed by using the nonlinear mixed effect model. We selected the volume of the central compartment (Vc), CL, and distribution rate constants (K12, K21) as population pharmacokinetic parameters. The population mean values (CV%) of Vc, CL, K12, and K21 were, respectively, 31.8 (15.7%) liter/m², 14.1 (27.8%) liter/h/m², 1.1 (8.4%)/h, and 0.41 (30.3%)/h. Residual intraindividual variability was 22.9%. The optimal sampling regime for estimation of the AUC and CL in using the Bayesian method was the two time points of 1 and 8 h post infusion. The mean predictive error, the mean absolute predictive error, and the root mean squared error were −3.3, 9.4, 3.2% (AUC) and 6.3, 10.0, 3.5% (CL), respectively. We concluded that the AUC and CL of CPT-11 could be estimated from plasma concentrations at two times by using the Bayesian method.

Key words: CPT-11 — Population pharmacokinetics — Bayesian method — Lung cancer

CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin) is one of a series of semi-synthetic camptothecin derivatives that were produced in an attempt to reduce the toxicity and improve the therapeutic efficacy of the parent compound by increasing its water solubility without opening the lactone ring. CPT-11 inhibits topoisomerase I activity through the formation of stable topoisomerase I-DNA cleavable complexes.[1-3] It has strong antitumor activity in a broad spectrum of experimental tumor models.[4, 5]

Phase I and II clinical trials of CPT-11 have been performed in Japan and the United States. Significant antitumor activity has been reported for leukemia,[6] lymphoma,[6] small-cell lung cancer,[7] non-small-cell lung cancer,[8] colorectal cancer,[9] and cervical cancer.[10] However, treatment with CPT-11 was associated with multiple toxicities, including myelosuppression, diarrhea, and pulmonary toxicity.

Previous work with CPT-11 as a single agent has demonstrated substantial interpatient variability in pharmacokinetic parameters and toxicity. However, very little information on the pharmacokinetic parameters and toxicity has been obtained.[11, 12] Investigation of these pharmacokinetic and pharmacodynamic relationships necessitates large-scale studies. One major obstacle to such studies is the inconvenience and high cost involved in obtaining individual pharmacokinetic parameters, which requires multiple blood sampling. An approach for solving this problem would be the Bayesian method.[13] The characterization of population pharmacokinetics of CPT-11 and their incorporation into a Bayesian algorithm may allow the use of a limited number of plasma samples to determine the individual patient's pharmacokinetic parameters.

The objective of this study was to determine the population pharmacokinetic parameters of CPT-11, based on the previously published CPT-11-containing combination phase I studies, and to determine the precision of individual pharmacokinetic parameters, especially of the area under the concentration-time curve (AUC), using a few concentration time points based on the Bayesian method.

PATIENTS AND METHODS

Patients Two separate data sets were used for this study. As the first data set, pharmacokinetic data obtained from the 30 patients previously reported by Karato *et al.*[14] were used to develop a population model of CPT-11 pharmacokinetics. Secondly, we used the data from 13 patients (second data set) who satisfied eligibility criteria similar to those of the first data set to estimate individual pharmacokinetic parameters by using a few concentration time points based on the Bayesian method and to evaluate the predictive performance of the Bayesian method.

Treatment plan CPT-11 was provided by Yakult Honsha Co. Ltd. and Daiichi Pharmaceutical Co. Ltd., and VP-16 was purchased from Bristol Myers Squibb Co. Ltd. The patients received CPT-11 at doses ranging from 40 to 80 mg/m^2 as a 90-min intravenous infusion in combination with VP-16 on days 1 through 3.

Blood sampling and HPLC analysis Plasma samples were collected in heparinized tubes at the following approximate time points: for the first data set, 0 min (pretreatment blank), 15, 30 and 60 min into infusion, end of infusion (EOI), and 0.25, 0.5, 1, 2, 4, 8, 12, 21.5, and 22.5 h post of infusion (POI), and for the second data set, 0 min (pretreatment blank), 30 and 60 min into infusion, EOI, and 0.5, 1, 2, 4, 8, 21.5, and 22.5 h POI. Heparinized blood samples were immediately centrifuged and the plasma obtained was stored at -20°C until analysis. Plasma concentrations of CPT-11 were determined by high-performance liquid chromatography (HPLC) as reported by Kaneda *et al.*[15]

Pharmacokinetic modeling and parameter estimation Modeling of individual concentration data was performed by using weighted nonlinear least-squares regression using the program MULTI[16] on a PC-9801 personal computer (NEC, Tokyo). These data were weighted by 1/concentration. As a result, a two-compartment model was fitted to the first data set (Fig. 1). Thus, a statistical pharmacokinetic program, NONMEM (nonlinear mixed effect model)[17] was used to determine the population pharmacokinetic parameters for a two-compartment model of CPT-11. The population parameters developed were the volume of the central compartment (Vc), clearance (CL), and distribution rate constants (K12, K21).

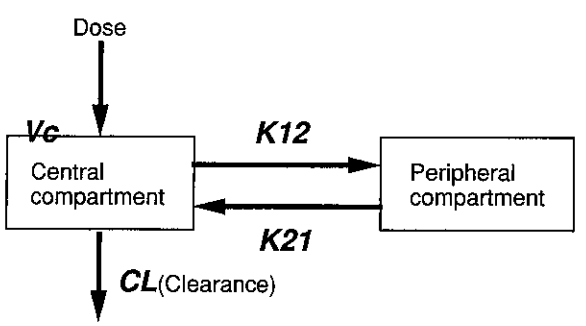

Fig. 1. Schema of the two-compartment model.

We focused on the performance of the Bayesian method to estimate $AUC_{0\text{-}24h}$ and CL. The Bayesian method allows us to estimate individual pharmacokinetic parameters using a very limited number of measurements such as one or two points, and has been reported to be more useful than non-Bayesian methods using a small number of concentrations. The least-squares method based upon a Bayesian algorithm estimates patient-specific pharmacokinetic parameter values which minimize the following sum of squares:

$$SS = \sum_{i=1}^{n} [(Ci - Ci)^2/si^2] + \sum_{j=1}^{m} [(Pj - Pj)^2/wj^2],$$

where Ci is the observed drug concentration in serum plasma, *Ci* is the predicted drug concentration, si is the variance of measured concentrations, n is the number of measurements, m is the number of parameters, Pj is the value of parameter j for an individual, and *Pj* and wj are the mean and variance of population pharmacokinetic parameters (such as Vc, CL, K12 and K21), respectively. This approach has been shown to be reliable for other drugs such as phenytoin and lidocaine even when sampling times do not extend over several half-lives. The Bayesian approach was carried out by using a program, PEDA,[18] on a PC-9801 personal computer.

Validation of the model and statistical method We compared the AUC and CL estimated by the Bayesian method and the actual AUC and CL using the second data set. Predictive performance of the model using the Bayesian method was evaluated on the basis of the following values. The bias, accuracy and precision were measured by calculating, respectively, the mean predictive error (ME) and its percentage (ME%), the mean absolute prediction error (MAE) and its percentage (MAE%), and the root mean squared error (RMSE) and its percentage (RMSE%)[19] according to the following formulas:

$$ME = \frac{\Sigma(Est - Act)}{n}, \quad MAE = \frac{\Sigma|Est - Act|}{n},$$

$$RMSE^2 = \frac{\Sigma(Est - Act)^2}{n}$$

Est: estimated value, Act: actual value

The relationships between estimated and actual values were evaluated by linear regression and correlation (StatView II; Abacus Concepts Inc., Berkeley, CA).

RESULTS

Population parameters We calculated the population pharmacokinetic parameters of CPT-11 in 30 patients

enrolled in a combination phase I study (first data set) by NONMEM analysis. First we decided what compartment model best fitted the concentration-time curves in the first data set. All concentration-time curves were fitted by a two-compartment model, and so we calculated the population pharmacokinetic parameters of CPT-11 in a two-compartment model. The residual error after fitting the data to this model was 22%. Table I lists the mean values and the coefficients of variation for the population pharmacokinetic parameters derived by this analysis.

Individual pharmacokinetic parameter estimation using the Bayesian method

One-point Bayesian method: First we estimated individual pharmacokinetic parameters (AUC, CL) using population pharmacokinetic parameters and a single blood concentration (one-point Bayesian method). For this estimation we used the concentration at one of five time points from EOI to 8 h POI because at time points after 8 h POI CPT-11 was not detectable in five patients. Tables II and III summarize the bias (ME%), accuracy (MAE%), and precision (RMSE%) of the AUC and CL estimated by the one-point Bayesian method. The ME, MAE, and RMSE of AUC and CL estimation depended upon the sampling time and they were relatively poor at earlier sampling times. The best sampling time was 8 h POI. The MAE%, ME%, and RMSE% of the AUC at that time were 14, −9.8, and 4.6, respectively (Table II). Those of the CL were 17.6, 17.6, and 5.7, respectively (Table III). In Fig. 2, the actual versus estimated AUC values for the one-point (8 h POI) Bayesian method are shown.

Two-point Bayesian method: In the one-point Bayesian method the best sampling time was 8 h POI. So we calculated the AUC and CL of CPT-11 using the concentrations at two time points including the 8 h POI point. Tables IV and V summarize the MAE%, ME%, RMSE% of the AUC and CL estimated by the two-point Bayesian method. The best sampling set was the 1 h POI and 8 h POI time points. In that sampling set the MAE%, ME%, and RMSE% of the estimated AUC and CL were lower and the correlations between the actual and estimated AUC and CL were higher than those estimated by the one-point Bayesian method. In all the

Table I. Population Pharmacokinetic Parameters for CPT-11 Derived by NONMEM Analysis: Two-compartment Model

Pharmacokinetic parameter	Mean	Coefficient of variation (%)
CL (liter/h/m^2)	14.1	27.8
Vc (liter/m^2)	31.8	15.7
K12 (/h)	1.1	8.4
K21 (/h)	0.4	30.3

Table II. AUC Estimated by the One-point Bayesian Method

Time (h)	MAE (%)	ME (%)	RMSE (%)	R-squared
0	81.6	78.6	56.6	0.16
0.5	39.3	37.6	14.1	0.56
1	44.7	44.2	16.3	0.56
2	36.7	36.7	12.4	0.79
4	17.4	12.3	5.9	0.76
8	14.0	−9.8	4.6	0.84

Table III. Clearance Estimated by the One-point Bayesian Method

Time (h)	MAE (%)	ME (%)	RMSE (%)	R-squared
0	27.2	−26.0	8.7	0.24
0.5	28.5	−27.9	8.9	0.52
1	31.0	31.0	9.5	0.62
2	29.1	−29.1	8.7	0.74
4	16.9	16.9	3.6	0.71
8	17.6	17.6	5.7	0.80

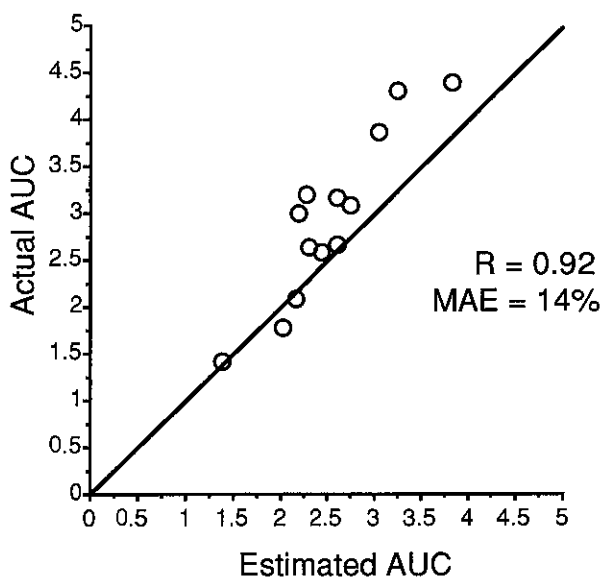

Fig. 2. Relationship between actual AUC and estimated AUC by the one-point Bayesian method. The horizontal axis is estimated AUC (mg h/ml), and the vertical axis represents actual AUC. The solid line is the line of identity.

Table IV. AUC Estimated by the Two-point Bayesian Method

Time (h)	MAE (%)	ME (%)	RMSE (%)	R-squared
0/8	12.9	−12.9	4.0	0.94
0.5/8	11.5	−7.1	3.6	0.91
1/8	9.4	−3.3	3.2	0.93
2/8	9.9	−3.5	3.3	0.91
4/8	11.6	−7.1	3.8	0.95

Table V. Clearance Estimated by the Two-point Bayesian Method

Time (h)	MAE (%)	ME (%)	RMSE (%)	R-squared
0/8	16.3	16.3	5.0	0.96
0.5/8	13.6	10.3	4.4	0.80
1/8	10.0	6.3	3.5	0.85
2/8	10.3	6.6	3.7	0.84
4/8	10.1	10.1	4.5	0.83

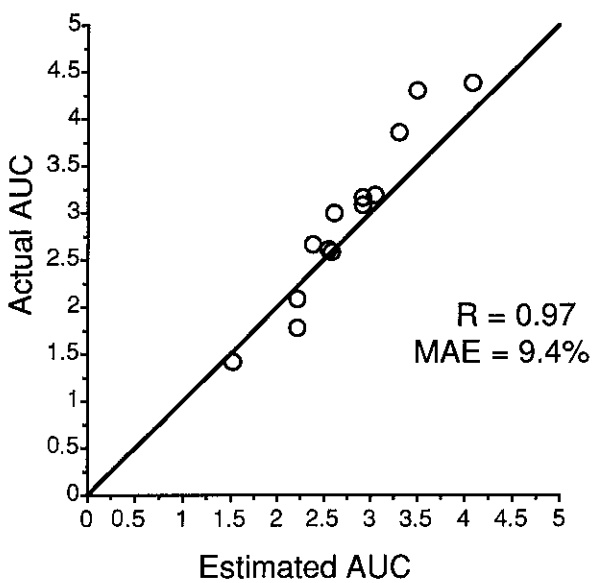

Fig. 3. Relationship between the actual AUC and the estimated AUC by the two-point (1, 8 h POI) Bayesian method. The axes and the solid line are the same as in Fig. 2.

sampling sets in which the 8 h POI point was included, the MAE%, ME%, and RMSE% were almost equivalent. The range of MAE%, of the AUC using the two-point Bayesian method was from 9.4% to 12.9%. In Fig. 3 the actual versus estimated AUC values for the two-point (1 h, 8 h POI) Bayesian method are shown.

Table VI. AUC and Clearance Estimated by the Three-point Bayesian Method

	MAE (%)	ME (%)	RMSE (%)	R-squared
AUC	10.9	−10.0	3.7	0.93
Clearance	13.0	12.8	4.5	0.95

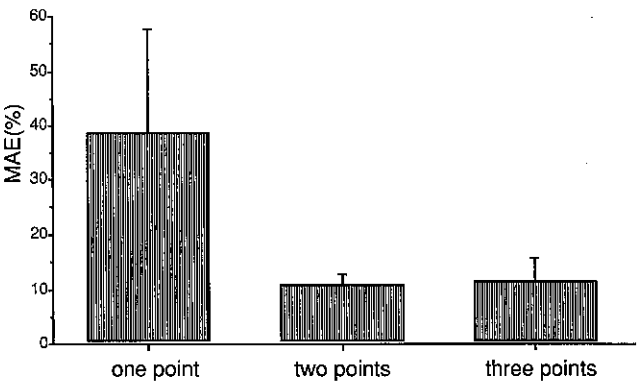

Fig. 4. Relationship between MAE% and the number of sampling points. MAE% of two points was significantly less than that of one point. The error bar represents the 95% confidence interval.

Three-point Bayesian method: Next, we selected three sampling points, EOI, 4, and 8 h POI. These reflect the peak, median, and trough plasma concentrations of CPT-11, respectively. Table VI shows the MAE%, ME%, and RMSE% of the three-point Bayesian method. The results show that the three-point Bayesian method is not superior to the two-point Bayesian method in this case.

DISCUSSION

In this report we described the population pharmacokinetics of CPT-11 and the selection of suitable sampling points for estimation of the AUC and CL using the Bayesian method. The Bayesian method has wide applicability in drug therapy because it enables us to perform pharmacokinetics-assisted individualization of treatment with limited sampling data. We demonstrated that it is possible to estimate the plasma CPT-11 AUC and CL by the Bayesian method with as few as one or two drug concentrations. First, we estimated the AUC and CL using the one-point Bayesian method. The AUC and CL estimation dependent upon sampling time and we could obtain an acceptable estimate of the CPT-11 AUC by using the sampling point at 8 h POI (MAE% 14%, RMSE% 4.6%). The use of the two-point Bayesian

method markedly improved the ME%, MAE%, and RMSE%, and the AUC and CL values estimated by all sampling sets that included the 8 h POI were not significantly different. The three-point Bayesian method did not improve these parameters (Fig. 4). Therefore we decided that the best sampling set was the two time points at 1 h and 8 h POI.

The MAE% of predicted values in previous studies using the Bayesian method were between 10% and 15%,[20-22] and the MAE% of the AUC and CL with this study were 9.4% and 10%, respectively. This result indicates that the predicted values in this study are useful, as was the case in previous studies.

Preclinical studies suggested that CPT-11 may behave primarily as a prodrug *in vivo* and that most of its antitumor activity may be attributable to its more active metabolite, 7-ethyl-10-hydroxycamptothecin, SN-38. We have made no attempt to estimate the SN-38 concentration. To examine the pharmacodynamics of CPT-11, it is necessary to investigate not only the pharmacokinetics of CPT-11 but also that of SN-38. However, the model for estimation of CPT-11 and SN-38 pharmacokinetics at the same time is very complicated. Nevertheless, as SN-38 is the major metabolite of CPT-11 it is possible to obtain the relationship between the clearance of CPT-11 and its pharmacodynamics. So we selected the AUC and CL as estimated pharmacokinetic parameters in this study.

Another method for estimation of the AUC is the limited sampling model by linear regression (LSM). Several authors have reported the utility of the LSM, and LSM has been developed for several anti-cancer agents.[23-25] However, there are some problems with this strategy. The first is that the sampling points are fixed. Another possible source of error is the selected time points. The second weak point is that we estimate only one parameter in this strategy. By using the Bayesian method, we can estimate several pharmacokinetic parameters and, as shown in this report, the sampling points are flexible to some extent. The only disadvantage of the Bayesian method for estimating individual pharmacokinetics lies in the difficulty of applying it in the routine clinical setting, since it requires a computer equipped with the proper software, whereas the LSM can be calculated manually. However, if the purpose is dose adjustment based on the measured plasma concentration, it is necessary to use the Bayesian method. Therefore, we consider the Bayesian method to be more useful than the LSM.

In conclusion, the feasibility and validity of estimation of the AUC and CL of CPT-11 using the Bayesian method were demonstrated. The optimal sampling regime was two sampling points including the 8 h POI, especially 1 h and 8 h POI. It is hoped that use of population pharmacokinetics and the Bayesian method will lead to elucidation of the relationships between individual pharmacokinetic parameters, toxicity and response, and thus help to improve the therapeutic index of CPT-11 for large numbers of patients.

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REFERENCES

1) Hsiang, Y. H., Hertzberg, R., Hechit, S. and Liu, L. F. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.*, **260**, 14873–14878 (1985).
2) Hsiang, Y. H. and Liu, L. F. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res.*, **48**, 1722–1736 (1988).
3) Hertzberg, R. P., Caranfa, M. J. and Hechit, S. M. On the mechanism of topoisomerase I inhibition by camptothecin: evidence for binding to an enzyme-DNA complex. *Biochemistry*, **28**, 4629–4638 (1989).
4) Kunimoto, T., Nitta, K., Tanaka, T., Uehara, N., Baba, H., Takeuchi, M., Yokokura, T., Sawada, S., Miyasaka, T. and Murai, M. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res.*, **47**, 5944–5947 (1987).
5) Matsuzaki, T., Yokokura, T., Murai, M. and Tsuruo, T. Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice. *Cancer Chemother. Pharmacol.*, **21**, 308–312 (1988).
6) Ohno, R., Okada, K., Masaoka, T., Kuramoto, A., Arima, T., Yoshida, Y., Ariyoshi, H., Ichimaru, M., Sakai, Y., Oguro, M., Ito, Y., Morishima, Y., Yokomaku, S. and Ota, K. An early phase II study of CPT-11: a new derivative of camptothecin, for the treatment of leukemia and lymphoma. *J. Clin. Oncol.*, **8**, 1907–1912 (1990).
7) Masuda, N., Fukuoka, M., Kusunoki, Y., Matsui, K., Takifuji, N., Kudoh, S., Negoro, S., Nishioka, M., Nakagawa, K. and Takada, M. CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. *J. Clin. Oncol.*, **10**, 1225–1229 (1992).
8) Fukuoka, M., Niitani, H., Suzuki, A., Motomiya, M., Hasegawa, K., Nishiwaki, Y., Kuriyama, T., Ariyoshi, Y.,

Negoro, S., Masuda, N., Nakajima, S. and Taguchi, T. Phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J. Clin. Oncol.*, **10**, 16–20 (1992).

9) Shimada, Y., Yoshino, M., Wakui, A., Nakao, I., Futatsuki, K., Sakata, Y., Kambe, M., Taguchi, T. and CPT-11 gastrointestinal cancer study group. Phase II study of CPT-11, new camptothecin derivative, in the patients with metastatic colorectal cancer. *Proc. Am. Soc. Clin. Oncol.*, **10**, 135 (1991).
10) Takeuchi, S., Takamizawa, H., Takeda, Y., Okada, T., Tamaya, T., Noda, K., Sugawa, T., Sekiba, K., Yakushiji, M., Taguchi, T. and CPT-11 study group on gynecologic malignancy. Clinical study of CPT-11, a camptothecin derivative, on gynecological malignancy. *Proc. Am. Soc. Clin. Oncol.*, **10**, 189 (1991).
11) Sasaki, Y., Morita, M., Miya, T., Shinkai, T., Eguchi, K., Tamura, T., Ohe, Y. and Saijo, N. Pharmacokinetic (PK) and pharmacodynamic (PD) analysis of CPT-11 and its active metabolite SN-38. *Proc. Am. Soc. Clin. Oncol.*, **11**, 111 (1992).
12) Ohe, Y., Sasaki, Y., Shinkai, T., Eguchi, K., Tamura, T., Kojima, A., Kunikane, H., Okamoto, H., Karato, A., Omatsu, H., Kanzawa, F. and Saijo, N. Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. *J. Natl. Cancer. Inst.*, **84**, 972–973 (1992).
13) Peck, C. C. and Rodman, J. H. Analysis of clinical pharmacokinetics data for individualizing drug dosage regimen. *In* "Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring," ed W. E. Evans, J. J. Schentag and W. J. Jusko, pp. 55–82 (1986). Applied Therapeutics, Inc., Spokane, WA.
14) Karato, A., Sasaki, Y., Eguchi, K., Tamura, T., Ohe, Y., Ohita, F., Nishio, M., Kunikane, H., Arioka, H., Ohmatsu, H., Nakajima, H., Shiraishi, J. and Saijo, N. Phase I study of CPT-11 and etoposide in patients with refractory solid tumors. *J. Clin. Oncol.*, **11**, 2030–2035 (1993).
15) Kaneda, N., Nagata, H., Furuta, T. and Yokokura, T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res.*, **50**, 1715–1720 (1990).
16) Yamaoka, K. and Tanigawara, Y. Program of automated pharmacokinetic analysis system. *In* "Introduction of Pharmacokinetic Analysis," pp. 141–157 (1983). Nankodo, Tokyo (in Japanese).
17) Beal, S. L. and Sheiner, L. B. "NONMEM Users Guide Part I: Users Basic Guide" (1979). University of California, San Francisco.
18) Higuchi, S., Aoyama, T. and Horioka, M. PEDA: A microcomputer program for parameter estimation and dosage adjustment in clinical practice. *J. Pharmacobio-Dyn.*, **10**, 703–718 (1987).
19) Sheiner, L. B. and Beal, S. L. Some suggestions for measuring predictive performance. *J. Pharmacokinet. Biopharm.*, **9**, 503–512 (1981).
20) Conley, B. A., Forrest, A., Egorin, M. J., Zuhowski, E. G., Sinibaldi, V. and Van Echo, D. A. Phase I trial using adaptive control dosing of hexamethylene bisacetamide (NSC 95580). *Cancer Res.*, **49**, 3436–3440 (1989).
21) Scher, H. I., Jodrell, D. I., Iversen, J. M., Curley, T., Tong, W., Egorin, M. J. and Forrest, A. Use of adaptive control with feedback to individualize suramin dosing. *Cancer Res.*, **52**, 64–70 (1992).
22) Rodman, J. H., Furman, W. L., Sunderland, M., Rivera, G. and Evans, W. E. Escalating teniposide systemic exposure to increase dose intensity for pediatric cancer patients. *J. Clin. Oncol.*, **11**, 287–293 (1993).
23) Ratain, M. J., Staubus, A. E., Schilsky, R. L. and Malspeis, L. Limited sampling models for amonafide (NSC 308847) pharmacokinetics. *Cancer Res.*, **48**, 4127–4130 (1988).
24) Ratain, M. J., Robert, J. and van der Vijgh, W. J. F. Limited sampling models for doxorubicin pharmacokinetics. *J. Clin. Oncol.*, **9**, 871–876 (1991).
25) Egorin, M. J., Forrest, A., Belani, C. P., Ratain, M. J., Abrams, J. S. and Van Echo, D. A. A limited sampling strategy for cyclophosphamide pharmacokinetics. *Cancer Res.*, **49**, 3129–3133 (1989).