Analysis Program Based on Finite Element Method, MULTI(FEM), for Evaluation of Dose-Dependent Local Disposition of Drug in Liver

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Abstract □ A curve-fitting program based on the Finite Element Method, MULTI(FEM), was developed to model nonlinear local disposition of a drug in the liver under non-steady-state conditions. The program was written in FORTRAN on an IBM-compatible personal computer. The validity of MULTI(FEM) was confirmed by analyzing the outflow kinetics of oxacillin (a model drug) following a pulse input to isolated, perfused rat livers, according to both linear and nonlinear dispersion models. Four dose levels (300, 1000, 3000, and 5000 μg) of oxacillin were administered to observe the dose-dependency in the hepatic local disposition. First, the individual outflow time-profiles at the same dose were averaged, and the average time-profile was analyzed by MULTI(FEM) based on linear dispersion models to yield a single curve fit. The fitted parameters at each dose level were compared with parameters estimated using MULTI(FILT), a program based on fast inverse Laplace transform, to analyze linear pharmacokinetics. The estimated parameters by MULTI(FEM) were in good agreement with those by MULTI(FILT). The apparent elimination rate constant (k_e) decreased with an increase in dose, whereas other parameters showed no discernible dependency on an increase of dose. Second, the average outflow time-profiles at the four dose levels were simultaneously analyzed by MULTI(FEM) based on dispersion models featuring Michaelis–Menten elimination. The outflow time-profiles of oxacillin were well approximated by a two-compartment dispersion model with central Michaelis–Menten elimination. The maximum elimination rate constant (V_{max}) and the Michaelis constant (K_m) were estimated to be 1520 μg/mL/min and 41.3 μg/mL, respectively. Thus, the capability of MULTI(FEM) was demonstrated in evaluating capacity-limited local disposition in the liver.

Introduction

The liver is an essential organ for the metabolism and elimination of drugs. After oral administration, a drug absorbed into the portal system may be eliminated to a large extent during its first pass through the liver. Thus, the liver shields the body from the excess inflow of xenobiotics into the systemic circulation. However, hepatic clearance can exhibit capacity-limited kinetics. Therefore, when the portal drug concentration is extremely high and/or metabolic function is compromised by a hepatic malfunction or drug−drug interaction, a greater fraction of the absorbed dose escapes into the systemic circulation, bypassing first-pass hepatic elimination. Thus, the evaluation of capacity-limited (nonlinear) disposition in the liver is crucial for understanding the influence of dose and absorption kinetics on drugs which undergo significant first-pass metabolism.

In situ liver perfusion experiments following infusion or bolus injection have often been used to assess hepatic local disposition of drugs in vivo. The time-profile of drug outflow from the liver following a pulse input is more informative of intrahepatic disposition than data obtained at steady state. The well-stirred and parallel-tube models offer straightforward analysis of steady-state kinetic data from perfusion experiments.[1−6] In contrast, the dispersion model[5−11] and the distribution model[5,6,12−15] have been developed to explain the outflow time-profile following a pulse input. The dispersion model equations with flexible initial and boundary conditions can explain a variety of outflow drug kinetics at non-steady-state.[7,9] A local disposition model that features several linear dispersion processes has been proposed to analyze the distribution of a drug in the perfused rat hindlimb.[16] However, the dispersion model equations, which are second-order partial differential equations, are difficult to solve analytically. Linear mass-balance rate equations can readily be solved by the Laplace transform. MULTI(FILT)[9−11,18] is one well-recognized computer program for curve-fitting that is based on the transformed equations. However, no analysis program is available to quantitatively evaluate dose-dependent, non-steady-state, local disposition kinetics which are expressed by nonlinear partial differential equations.

Thus, the purpose of the present investigation is to develop a new curve-fitting program, MULTI(FEM), based on the Finite Element Method which has been used in structural[19,20] and hydrodynamic analyses.[21−23] MULTI(FEM) enables the use of local disposition models described by nonlinear partial differential equations with dispersion terms. To confirm the validity of MULTI(FEM), a rat liver perfusion experiment was performed at several dose levels of oxacillin to represent a typical local disposition system of a drug. First, linear dispersion models were adopted in the fitting of data at each dose level, and the estimated parameters were compared with those by MULTI(FILT). Second, outflow time-profiles at the different doses (300, 1000, 3000, and 5000 μg), which were confirmed to exhibit dose-dependent (nonlinear) kinetics, were simultaneously analyzed by MULTI(FEM) based on dispersion models with Michaelis−Menten elimination.[24]

Theory

Five dispersion models (two linear and three nonlinear) were considered. The linear dispersion models involve one-compartment model and two-compartment models with either central and peripheral elimination. However, it is known that the linear two-compartment models, unlike nonlinear models, are not kinetically distinct, because the parameters in the two-compartment model with central elimination are convertible (mapable) to those with peripheral elimination.[9] Thus, the only central elimination process is adopted in our analysis of the linear two-compartment dispersion model. In contrast, the nonlinear two-compartment model with central elimination is kineti-

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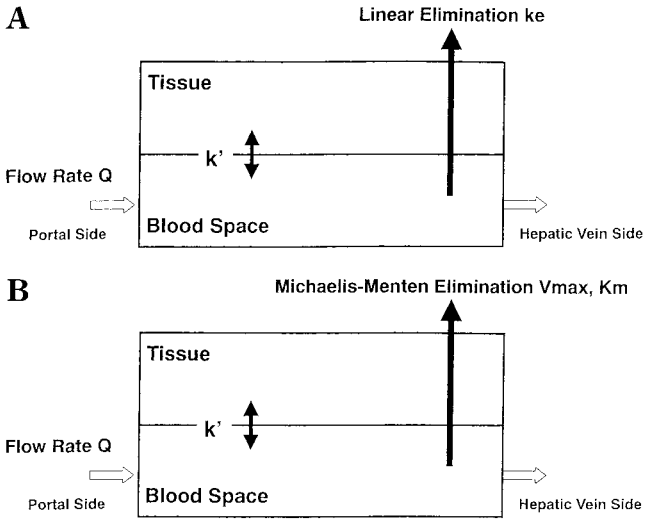

Figure 1—One-compartment dispersion model with linear elimination (A) and nonlinear elimination (B).

cally distinguishable from that with peripheral elimination. Therefore, the one-compartment model with Michaelis–Menten elimination and two-compartment models with central and peripheral Michaelis–Menten elimination were considered as the nonlinear dispersion models.

One-Compartment Dispersion Model—In the one-compartment dispersion model, complete mixing between perfusate and hepatic tissues is assumed as shown in Figure 1A. The equation of this model with linear elimination is described by

$$(1 + k')\frac{\partial C(t,v)}{\partial t} = D_c\frac{\partial^2 C(t,v)}{\partial v^2} - Q\frac{\partial C(t,v)}{\partial v} - k_e C(t,v) \quad (1)$$

where C(t,v) is the perfusate concentration in the liver, Q is the perfusate flow rate, k' is the partition ratio which is the measure of extent of drug partition into the hepatic tissue, D_c is the corrected dispersion coefficient, v is the volume axis, and k_e is the first-order elimination rate constant.

For a pulse input, eq 1 can be solved by Laplace transform under the initial and boundary conditions given by eq 2. The image equation is given by eq 3.[9]

$$C(0,v) = 0, \quad C(t,0) = \frac{M}{Q}\delta(t) \quad \text{and} \quad C(t,\infty) = 0 \quad (2)$$

$$\tilde{C}(s) = \frac{M}{Q}\exp\left[\left\{\frac{Q}{2D_c} - \sqrt{\left(\frac{Q}{2D_c}\right)^2 + \frac{k_e + (1 + k')s}{D_c}}\right\}V_B\right] \quad (3)$$

where V_B is the blood volume which was assumed to be 15.5% of liver weight.[25,26]

If the drug is eliminated according to Michaelis–Menten equation as shown in Figure 1B, the one-compartment dispersion model is described by

$$(1 + k')\frac{\partial C(t,v)}{\partial t} = Dc\frac{\partial^2 C(t,v)}{\partial v^2} - Q\frac{\partial C(t,v)}{\partial v} - \frac{V_{max}}{K_m + C(t,v)}C(t,v) \quad (4)$$

where V_{max} is the maximal elimination rate, and K_m is the Michaelis constant. It is noted here that V_{max} and K_m are the lumped parameters which reflect the transfer of a drug from the sinusoid to the tissue space, the uptake into hepatocytes by active transport, and the enzymatic activity in hepatocytes.

Two-Compartment Dispersion Model—A two-compartment dispersion model with linear elimination from central compartment, as shown in Figure 2A, is described by eq 5. The equilibrium distribution phase, which is kinetically included in the central compartment of the liver, was predicted in the previous paper using BOF-4272, a drug for treatment of hyperuricemia.[26]

$$\begin{cases} \dfrac{\partial C_1(t,v)}{\partial t} = Dc\dfrac{\partial^2 C_1(t,v)}{\partial v^2} - Q\dfrac{\partial C_1(t,v)}{\partial v} - \\ \qquad k_{12}C_1(t,v) + \epsilon k_{21}C_2(t,v) - keC_1(t,v) \\ \dfrac{\partial C_2(t,v)}{\partial t} = \dfrac{1}{\epsilon}k_{12}C_1(t,v) - k_{21}C_2(t,v) \end{cases} \quad (5)$$

where C_1(t,v) and C_2(t,v) are the concentrations in the central and peripheral compartments, respectively, k_{12} and k_{21} are the forward and backward transfer rate constants between central and peripheral compartments, ϵ is the volume ratio of peripheral compartment to central compartment, and k_e is the first-order elimination rate constant. Under the initial and boundary conditions given by eq 6 for a pulse input of drug, the image equation for the central compartment is described by eq 7.[9]

$$C_1(0,v) = 0, \quad C_2(0,v) = 0, \quad C_1(t,0) = \frac{M}{Q}\delta(t), \quad C_1(t,\infty) = 0 \quad \text{and} \quad C_2(t,0) = 0 \quad (6)$$

$$\tilde{C}(s) = \frac{M}{Q}\exp\left[\left\{\frac{Q}{2Dc} - \sqrt{\left(\frac{Q}{2Dc}\right)^2 + \frac{1}{Dc}\left(s + k_{12} + k_e - \frac{k_{12}k_{21}}{s + k_{21}}\right)}\right\}V_B\right] \quad (7)$$

where V_B is the volume of central compartment. It is noted that the volume ratio ϵ disappears in eq 7.

A two-compartment dispersion model with central Michaelis–Menten elimination (Figure 2B) is described by eq 8.

$$\begin{cases} \dfrac{\partial C_1(t,v)}{\partial t} = Dc\dfrac{\partial^2 C_1(t,v)}{\partial v^2} - Q\dfrac{\partial C_1(t,v)}{\partial v} - \\ \qquad k_{12}C_1(t,v) + \epsilon k_{21}C_2(t,v) - \dfrac{V_{max}}{K_m + C_1(t,v)}C_1(t,v) \\ \dfrac{\partial C_2(t,v)}{\partial t} = \dfrac{1}{\epsilon}k_{12}C_1(t,v) - k_{21}C_2(t,v) \end{cases} \quad (8)$$

A two-compartment dispersion model with peripheral Michaelis–Menten elimination (Figure 2C) is described by eq 9.

$$\begin{cases} \dfrac{\partial C_1(t,v)}{\partial t} = Dc\dfrac{\partial^2 C_1(t,v)}{\partial v^2} - Q\dfrac{\partial C_1(t,v)}{\partial v} - \\ \qquad k_{12}C_1(t,v) + \epsilon k_{21}C_2(t,v) \\ \dfrac{\partial C_2(t,v)}{\partial t} = \dfrac{1}{\epsilon}k_{12}C_1(t,v) - k_{21}C_2(t,v) - \\ \qquad \dfrac{V_{max}C(t,v)}{K_m + C_2(t,v)} \end{cases} \quad (9)$$

Although eqs 4, 8, and 9 are not solvable by Laplace transform, a numerical solution can be achieved with MULTI(FEM).

Danckwerts's condition[27] given by eq 10 is adopted as the boundary condition in MULTI(FEM), because it is easily programmed in MULTI(FEM).

$$Dc\left(\frac{\partial C(t,v)}{\delta v}\right)_{v=0} = QC(t,0) \quad \text{and} \quad Dc\left(\frac{\partial c(t,v)}{\partial v}\right)_{v=V_B} = 0 \tag{10}$$

Numerical Procedure

MULTI(FEM) written in Microsoft FORTRAN (ver. 3.2) was newly developed on a IBM-compatible personal computer (PentiumII, 266 MHz and 96 MB). Equations 3 and 7, which are given as Laplace-transformed equations, were numerically solved by fast inverse Laplace transform (FILT).[9−11] Equations 1 and 5, which are given as linear partial differential equations, were numerically solved by MULTI(FEM). The outflow time-profiles following the same dose of oxacillin (300, 1000, 3000, or 5000 μg) were averaged, and the average time-profile at each dose was fitted by MULTI(FILT) and MULTI(FEM) based on the linear dispersion model. Equations 4, 8, and 9, which are given as nonlinear partial differential equations, were fitted by MULTI(FEM) simultaneously to all four average time-profiles at the different doses.

Ten sets of outflow time courses at the four dose levels were generated by adding 10% random normal errors to theoretical curves calculated from parameter values estimated by MULTI(FEM). The generated time courses were fitted by MULTI(FEM), and the estimated parameters were compared with the original parameters to verify the stability of MULTI(FEM).

Materials and Methods

Chemicals—Oxacillin was purchased from Sigma Chemical Co. (St. Louis, MO). Sodium pentobarbital solution (NEMBUTAL forcanimal injection, Abbott Laboratories, Chicago, IL) was used to anesthetize the rats. All other reagents used for preparing the perfusate and HPLC mobile phase were of guaranteed reagents grade or HPLC grade.

Animals—Male Wistar rats weighing 200−240 g were purchased from Shimizu Experimental Materials Co. (Kyoto, Japan). All rats were maintained on standard rat chow, and water was allowed ad libitum before the experiments.

Single-Pass Perfusion Experiment Using Rat Liver—Isolated rat livers were prepared and perfused according to the Mortimore method[28] using Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glucose and saturated with 95% O_2−5% CO_2. The bile duct was cannulated with a polyethylene tube (PE10). The perfusate was maintained at 37 °C and delivered by a roller pump (RP-NP2, Furue Science Co., Ltd., Tokyo, Japan) through a polyethylene cannula (1.67 mm o.d.) placed in the portal vein. The perfusate flow rate was 14.6−15.6 mL/min, and the recoveries of perfusate were greater than 99%. The bile flow rate was measured to monitor the viability of the liver. The liver perfusion data were rejected when the bile flow rate was less than 4 μL/min. At 20 min after the operation, a 250 μL volume of oxacillin in saline solution (1.2 mg/mL, $n = 3$; 4.0 mg/mL, $n = 3$; 12.0 mg/mL, $n = 2$; 20 mg/mL, $n = 3$) was injected into the liver using a six-way valve injector. The outflow sample was collected at intervals of 1 s. After the perfusate collection, the validity of the perfusion experiment was confirmed by uniform coloration and decoloration of the liver after injection of Evans Blue. The lag time was calculated from the total void volume (half volume of the injector tube plus full volume of the catheter) divided by the perfusate flow rate, and was subtracted from sampling times. The liver weights were 8.66−12.0 g.

Analytical Procedure—The perfusate sample (0.2 mL) was diluted up to 10-fold with blank perfusate, when oxacillin concentration was too high. Acetonitrile (0.3 mL) was added to the sample (0.2 mL) to precipitate proteins, and an aliquot of the supernatant (15 μL) obtained by centrifugation at 2000 rpm was injected into HPLC. An HPLC system (LC-10A series, Shimadzu Company, Kyoto, Japan) was equipped with the Chemcosorb 5-ODS−H reversed-phase column (5 μm, 150 × 4.6 mm i.d., Chemco Scientific Company, Ltd., Osaka, Japan). The HPLC conditions described by Yano et al.[9] were modified as follows. The mobile phase consisted of a 1:1 (v/v) mixture of 100 mM sodium acetate buffer (pH5.2)/methanol. Mobile phase flow rate, detection wavelength, and column temperature were 1.0 mL/min, 220 nm, and 40 °C, respectively. The peak area was recorded on Chromatopack C-R6A (Shimadzu Company). A linear calibration plot was made over the oxacillin concentration range of 1.0−300 μg/mL, according to the method of the variance-stabilizing transformation.[29] Accuracy and precision were within 10% at all concentrations.

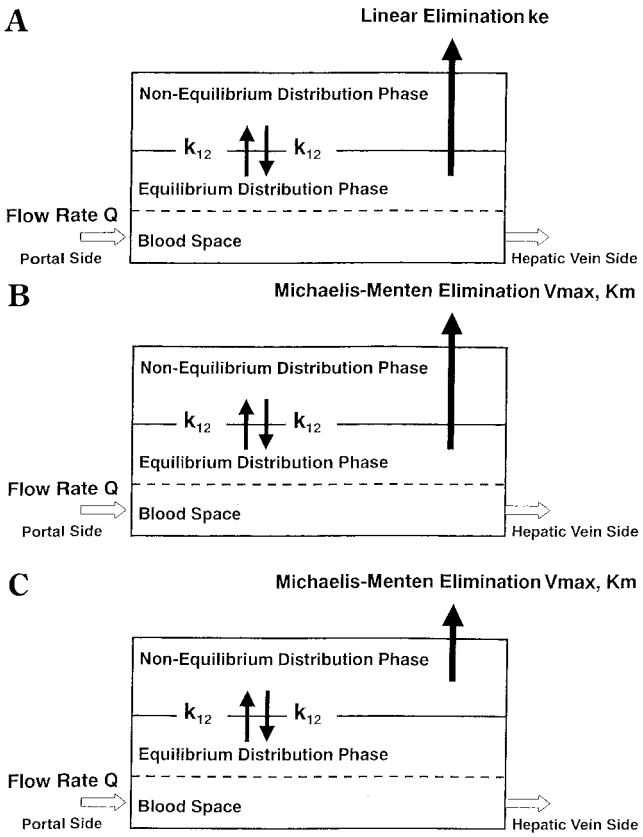

Figure 2—Two-compartment dispersion model with linear elimination (A), with central Michaelis–Menten elimination (B), and with peripheral Michaelis–Menten elimination (C).

Results and Discussion

Figure 3 presents the average outflow time-profiles and the predicted time courses by MULTI(FILT) and MULTI(FEM) based on a two-compartment dispersion model with linear elimination. Table 1 shows the parameter estimates. The curve fittings based on the one-compartment dispersion model gave much larger Akaike's Information Criterion (AIC)[30] values than those based on the two-compartment model and were deemed unacceptable. The predicted time courses by MULTI(FILT) and MULTI(FEM) agreed well with the experimental data points. The pharmacokinetic parameters by MULTI(FEM) coincided well with those by MULTI(FILT), which demonstrates the validity of MULTI(FEM). It is noted in Table 1 that k_e increases with a decrease in dose from 5000 to 300 μg, whereas other parameters show no systematic change with dose. Thus, this suggested that the average outflow time-profiles at the four dose levels would be better described by a dispersion model with capacity-limited elimination.

Figure 4 shows the outflow time profiles of oxacillin and the curves predicted by MULTI(FEM) fit to the two-

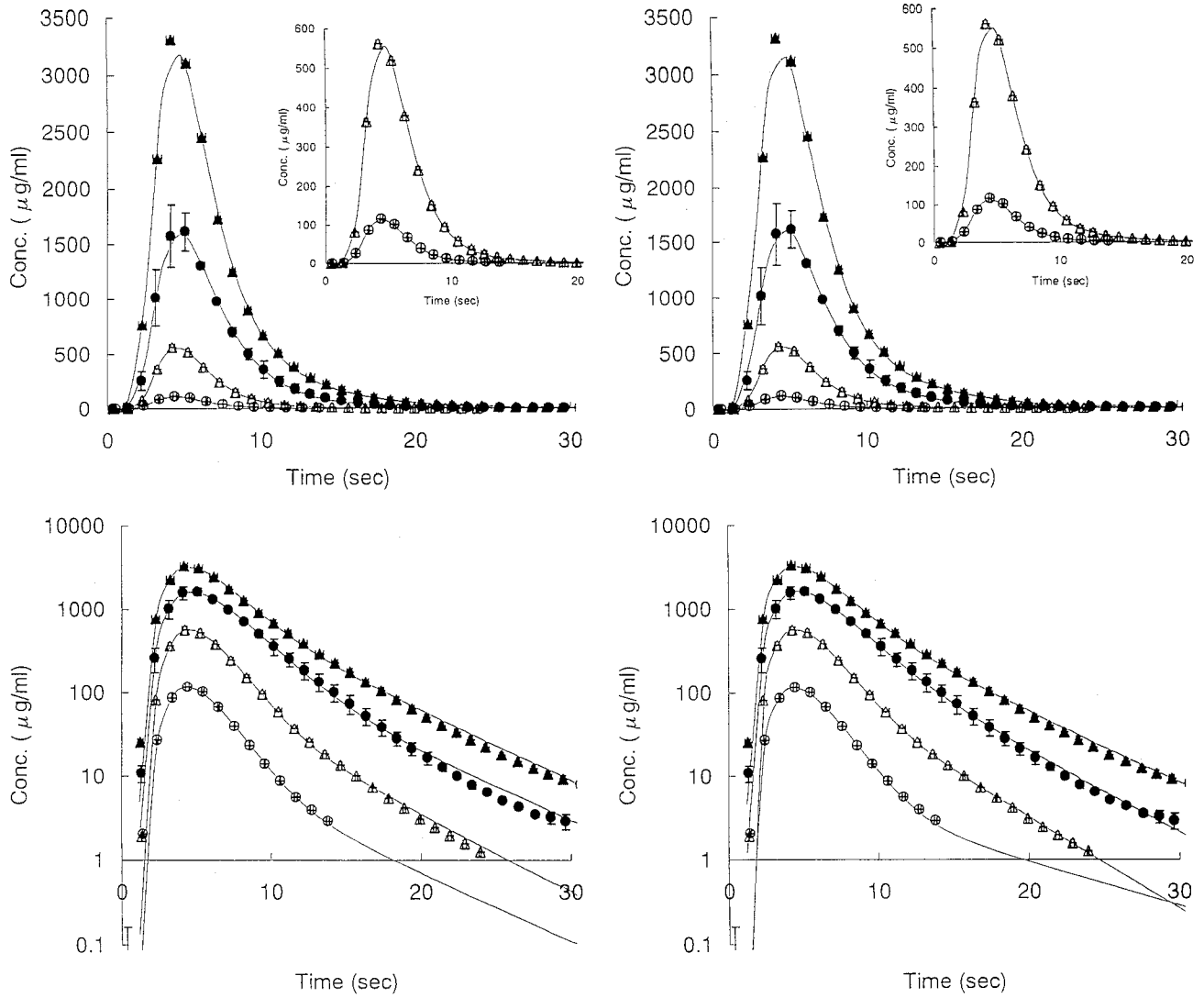

Figure 3—Outflow time-profiles averaged at 300 μg (○, $n = 3$), 1000 μg (△, $n = 3$), 3000 μg (●, $n = 2$) and 5000 μg (▲, $n = 3$) doses of oxacillin and theoretical curves predicted by MULTI(FILT) (left) and MULTI(FEM) (right). The time-profiles are given by means and standard deviations in two-compartment dispersion model with linear elimination. The bottom figures and the inserts at the top show the semilogarithmic and the expanded plots, respectively.

Table 1—Kinetics Parameters of Oxacillin Calculated Using MULTI(FILT) and MULTI(FEM) in Two-Compartment Dispersion Model with Linear Elimination

	dose (μg)			
	5000	3000	1000	300
	MULTI(FILT)			
D_c (mL^2/min)	1.93	2.37	1.63	1.71
V_B (ml)	1.46	1.63	1.49	1.51
k_{12} (min^{-1})	2.19	0.929	1.19	1.02
k_{21} (min^{-1})	13.4	11.7	13.8	11.7
k' (= k_{12}/k_{21})	0.163	0.0796	0.0856	0.0871
k_e (min^{-1})	1.01	2.52	4.14	8.80
	MULTI(FEM)			
D_c (mL^2/min)	2.10	2.40	1.68	1.95
V_B (mL)	1.45	1.58	1.47	1.53
k_{12} (min^{-1})	2.31	1.53	1.54	0.881
k_{21} (min^{-1})	13.9	15.2	16.4	7.44
k' (= k_{12}/k_{21})	0.167	0.101	0.0942	0.118
k_e (min^{-1})	1.05	2.58	4.24	8.65

compartment dispersion models with Michaelis–Menten elimination from the central compartment and from the peripheral compartment. The local disposition parameters estimated from curve-fittings are shown in Table 2. Because AIC assumed a smaller value for the central elimination model (AIC = 854) than for the peripheral elimination model (AIC = 996), the two-compartment dispersion model with central capacity-limited elimination was considered to better represent the outflow time-profiles of oxacillin. The maximum elimination rate constant (V_{max}) and the Michaelis constant (K_m) were estimated to be 1520 μg/mL/min and 41.3 μg/mL, respectively. Since the AIC based on the nonlinear one-compartment model was much larger than those based on two-compartment models, the results were not presented. To verify the stability of MULTI(FEM) fits, 10 sets of outflow time courses at four dose levels were generated by adding 10% random normal errors to theoretical curves calculated using mean parameter estimates from the earlier data fitting. The generated time courses were fitted by MULTI(FEM). The parameter values from the fit of noise-added data (D_c: 2.20 ± 0.18 mL^2/min, V_B: 1.50 ± 0.02 mL, k_{12}: 3.45 ± 0.21min^{-1}, k_{21}: 10.8 ± 0.49min^{-1}, k′: 0.319 ± 0.026, V_{max}: 1520 ± 120 μg/mL/min and K_m: 41.5 ± 6.8 μg/mL) were in good agreement with the original parameter estimates (D_c: 2.14 mL^2/min, V_B: 1.49 mL, k_{12}: 3.45 min^{-1}, k_{21}: 10.7 min^{-1}, k': 0.322, V_{max}: 1520 μg/mL/min and K_m: 41.3 μg/mL), respectively.

In conclusion, dose-dependency kinetics in local organ disposition kinetics at steady state[2,3] has been well-

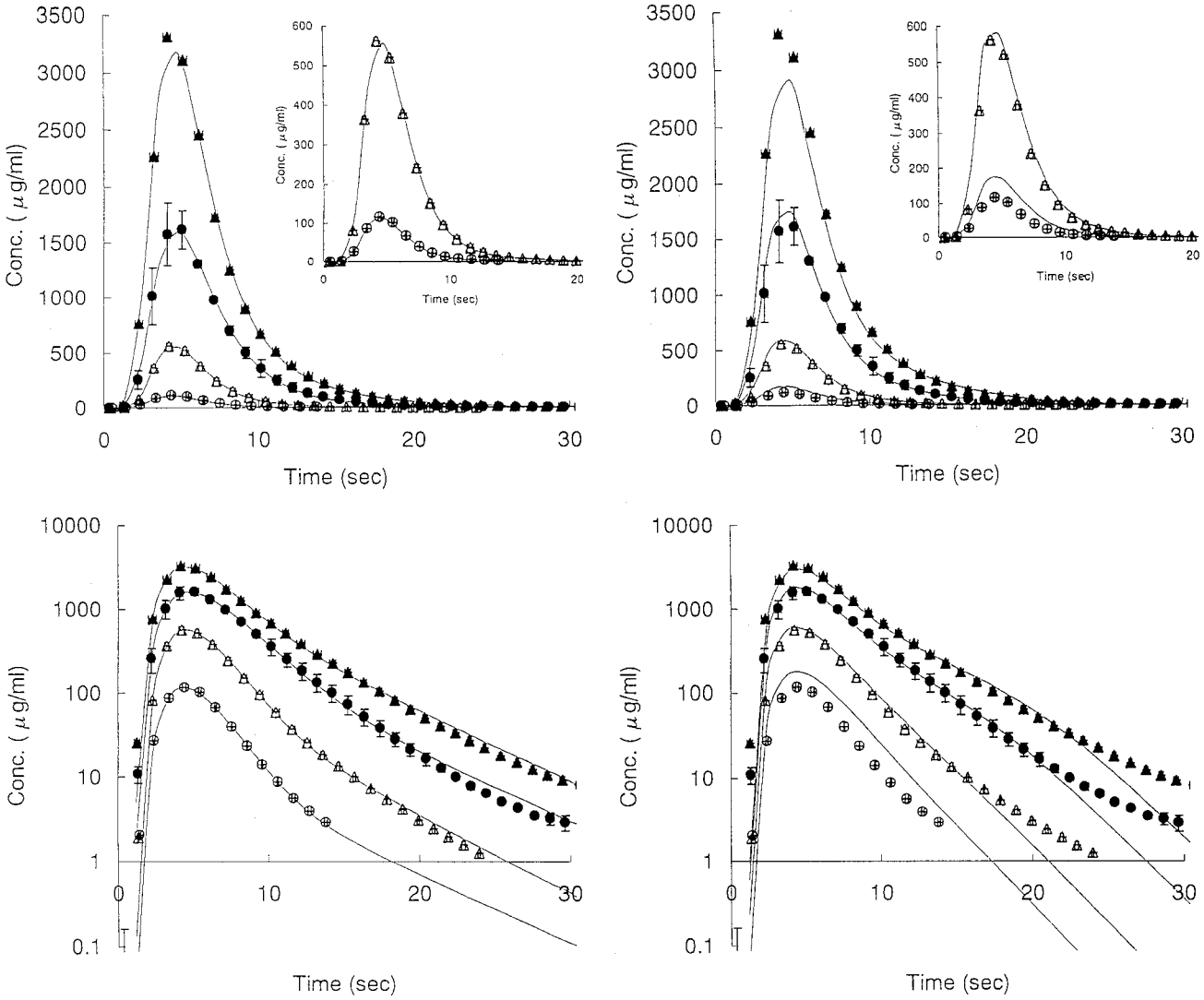

Figure 4—Outflow time-profiles of oxacillin (300 μg (○, $n = 3$), 1000 μg (○, $n = 3$), 3000 μg (●, $n = 2$), 5000 μg (▲, $n = 3$)) and theoretical curves predicted by MULTI(FEM) based on two-compartment dispersion models with Michaelis–Menten elimination from central compartment (left) and from peripheral compartment (right). The time-profiles are given by means and standard deviations. The bottom figures and the inserts at the top show the semilogarithmic and the expanded plots, respectively.

Table 2—Kinetics Parameters of Oxacillin Calculated Using MULTI(FEM) in Two-Compartment Dispersion Model with Central and Peripheral Michaelis–Menten Elimination

	central	peripheral
D_c (mL^2/min)	2.14	1.84
V_B (mL)	1.49	1.45
k_{12} (min^{-1})	3.45	4.93
k_{21} (min^{-1})	10.7	7.45
k' (= k_{12}/k_{21})	0.322	0.662
V_{max} (μg/mL/min)	1520	4250
K_m (μg/mL)	41.3	200
AIC	854	996

characterized, but investigations of the disposition under transient or non-steady-state conditions have been scarce. MULTI(FEM) enables the evaluation of dose-dependent drug clearance kinetics in the hepatic perfusion system following a pulse input, i.e., to allow detailed description of intrahepatic Michaelis−Menten disposition kinetics in the liver. The present analysis further showed that the central and peripheral elimination models can be distinguished based on perfusate outflow data under the nonlinear condition. With the availability of MULTI(FEM), the dispersion model may be applicable to analyzing the dose-dependency in other perfused organ experiments, such as those with intestine and kidney.

References and Notes

1. Wilkinson, G. R.; Shand, D. G. A Physiological Approach to Hepatic Drug Clearance. *Clin. Pharmacol. Ther.* **1975**, *18*, 377−390.
2. Ishida, R.; Suzuki, K.; Masubuchi, Y.; Narimatsu, S.; Fujita, S.; Suzuki, T. Enzymatic Basis for the Non-Linearity of Hepatic Elimination of Propranolol in the Isolated Perfused Rat Liver. *Biochem. Pharmacol.* **1992** *44*, 2281−2288.
3. Kukan, M.; Woolf, T. F.; Meluš, M.; Bezek, Š. Characterization of Nonlinear Elimination of the Xanthine-Related Drug Ethimizol in 3-Methylcholanthrene-Induced Rat Liver by the "Parallel-Tube" Model. *Drug Metab. Dispos.* **1993**, *21*, 547−550.
4. Pang, K. S.; Rowland, M. Hepatic Clearance of Drugs. II. Experimental Evidence for Acceptance of the "Well-Stirred" Model over the "Parallel Tube" Model Using Lidocaine in the Perfused Rat Liver in Situ Preparation. *J. Pharmacokin. Biopharm.* **1977**, *5*, 655−680.
5. Roberts, M. S.; Donaldson, J. D.; Rowland, M. Model of Hepatic Elimination: Comparison of Stochastic Models to Describe Residence Time Distributions and to Predict the Influence of Drug Distribution, Enzyme Heterogeneity, and

Systemic Recycling on Hepatic Elimination. *J. Pharmacokin. Biopharm.* **1988**, *17*, 41−83.
6. Saville, B. A.; Gray, M. R.; Tam, Y. K. Models of Hepatic Drug Elimination. *Drug Metab. Reviews* **1992**, *24*, 49−88.
7. Roberts, M. S.; Rowland, M. Hepatic Elimination−Dispersion Model. *J. Pharm. Sci.* **1985**, *74*, 585−587.
8. Roberts, M. S.; Rowland, M. A Dispersion Model of Hepatic Elimination: 1. Formulation of the Model and Bolus Considerations. *J. Pharmacokin. Biopharm.* **1986**, *14*, 227−260.
9. Yano, Y.; Yamaoka, K.; Aoyama, Y.; Tanaka, H. Two-Compartment Dispersion Model for Analysis of Organ Perfusion System of Drugs by Fast Inverse Laplace Transform (FILT). *J. Pharmacokin. Biopharm.* **1989** *17*, 179−202.
10. Yano, Y.; Yamaoka, K.; Minamide, T.; Nakagawa, T.; Tanaka, H. Evaluation of Protein Binding Effect on Local Disposition of Oxacillin in Rat Liver by a Two-Compartment Dispersion Model. *J. Pharm. Pharmacol.* **1990** *42*, 632−636.
11. Yano, Y.; Yamaoka, K.; Yasui H.; Nakagawa, T. Effect of Perfusion Rate on the Local Disposition of Cefixime in Liver Perfusion System Based on Two-Compartment Dispersion Model. *Drug Metab. Dispos.* **1991**, *19*, 1022−1027.
12. Goresky, C. A.; Bach, G. G.; Nadeau, B. E. On the Uptake of Materials by the Intact Liver. *J. Clin. Invest.* **1973**, *52*, 991−1009.
13. Tsao, S. C.; Sugiyama, Y.; Sawada, Y.; Nagase, S.; Iga, T.; Hanano, M. Effect of Albumin on Hepatic Uptake of Warfarin in Normal and Analbuminemic Mutant Rats: Analysis by Multiple Indicator Dilution Method. *J. Pharmacokin. Biopharm.* **1986**, *14*, 51−64.
14. Miyauchi, S.; Sugiyama, Y.; Sawada, Y.; Morita, K.; Iga, T.; Hanano, M. Kinetics of Hepatic Transport of 4-Methylumbelliferone in Rats. Analysis by Multiple Indicator Dilution Method. *J. Pharmacokin. Biopharm.* **1987**, *15*, 25−38.
15. Tsao, S. C.; Sugiyama, Y.; Sawada, Y.; Iga, T.; Hanano, M. Kinetic Analysis of Albumin-Mediated Uptake of Warfarin by Perfused Rat Liver. *J. Pharmacokin. Biopharm.* **1988**, *16*, 165−181.
16. Oliver, R. E.; Heatherring, A. C.; Janes, A. F.; Rowland, M. A Physiologically Based Pharmacokinetic Model Incorporating Dispersion Principles to Describe Solute Distribution in the Perfused Rat Hindlimb Preparation. *J. Pharmacol. Biopharm.* **1997**, *25*, 389−412.
17. Sato, H.; Sugiyama, Y.; Sawada, Y.; Iga, T.; Sakamoto, S.; Fuwa, T.; Hanano, M. Dynamic Determination of Kinetic Parameters for the Interaction between Polypeptide Hormones and Cell-Surface Receptors in the Perfusate Rat Liver by the Multiple-Indicator Dilution Method. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8355−8359.
18. Yano, Y.; Yamaoka, K.; Tanaka, H. A Nonlinear Least Squares Program, MULTI(FILT), Based on Fast Inverse Laplace Transform for Microcomputers. *Chem. Pharm. Bull.* **1989**, *37*, 1035−1038.
19. Turner, M. J.; Clough, R. W.; Martin, H. C.; Topp, L. J. Stiffness and Deflection Analysis of Complex Structures. *J. Aeronaut. Sci.* **1956**, *23*, 805−823.
20. McMeeking, R. M.; Rice, J. R. Finite Element Formulations for Problems of Large Elastic-Plastic Deformation. *Int. J. Solids Struct.* **1975**, *11*, 601−616.
21. Hughes, T. J. R.; Liu, W. K.; Brooks, A. Finite Element Analysis of Incompressible Viscous Flows by the Penalty Function Formulation. *J. Comput. Phys.* **1979**, *30*, 1−60.
22. Gresho, P. M.; Chan, S. T.; Lee, R. T.; Upson, C. D. A Modified Finite Element Method for Solving the Time-Dependent, Incompressible Navier−Stokes Equations. Part 1: Theory and Part 2: Applications. *Int. J. Numer. Methods Fluids* **1984**, *4*, 557−598, 619−640.
23. Baker A. J.; Pepper D. W. *Finite Elements 123*; McGraw-Hill: New York, 1991.
24. Michaelis, L.; Menten, M. L. Die Kinetik der Invertinwirkung. *Biochem. Z.* **1913**, *49*, 333−369.
25. Blouin, A.; Bolender, R. P.; Welbel, E. R. Distribution of Organelles and Membranes between Hepatocytes and Nonhepatocytes in The Rat Liver Parenchyma. A Stereological Study. *J. Cell Biol.* **1977**, *72*, 441−455.
26. Nishimura, M.; Yamaoka, K.; Naito, S.; Nakagawa, T. Effect of Temperature in Perfusate on Local Hepatic Disposition of BOF-4272, a New Xanthine Oxidase Inhibitor. *Biol. Pharm. Bull.* **1996**, *19*, 1197−1202.
27. Danckwerts, P. V. Continuous Flow Systems Distribution and Residence Time. *Chem. Eng. Sci.* **1953**, *2*, 1−13.
28. Mortimore, G. E.; Tietze, F.; Stetten, D., Jr. Metabolism of Insulin-I^{131} Studies in Isolated, Perfused Rat Liver and Hindlimb Preparations. *Diabetes* **1959**, *8*, 307−314.
29. McLean, A. M.; Ruggirello, D. A.; Banfield, C.; Gonzalez, M. A.; Bialer, M. Application of a Variance-Stabilizing Transformation Approach to Linear Regression of Calibration Lines. *J. Pharm. Sci.* **1990**, *79*, 1005−1008.
30. Yamaoka, K.; Nakagawa, T.; Uno, T. Application of Akaike's Information Criterion (AIC) in the Evaluation of Linear Pharmacokinetic Equations. *J. Pharmacokin. Biopharm.* **1978**, *6*, 165−175.

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