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Pharmacokinetic analysis of uptake process of lactosaminated albumin in rat liver constant infusion experiments

Koyo Nishida, Toichi Takino, Yukiko Eguchi, Fumiyoshi Yamashita, Mitsuru Hashida and Hitoshi Sezaki

Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606 (Japan)

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Summary

The hepatic uptake of ^{111}In-labeled lactosaminated bovine serum albumin (Lac-BSA) was studied in comparison with ^{111}In-labeled cationized albumin (Cat-BSA) in rat liver perfusion experiments. During a single-pass constant infusion mode, [^{111}In]Lac-BSA (0.7–10 μg/ml) was continuously extracted by the liver and its extraction ratio at steady-state (E_{ss}) decreased as the inflow concentration increased. The outflow pattern of [^{111}In]Lac-BSA was significantly different from that of [^{111}In]Cat-BSA. The outflow patterns of [^{111}In]Lac-BSA at various inflow concentrations were simultaneously fitted to a physiological one-organ model, in which the hepatic uptake was represented by division into the processes of binding to the cell surface and internalization, by the use of the MULTI(RUNGE) program. The obtained kinetic parameters (maximum binding amount X_∞, binding constant K and internalization rate constant k_{int}) correlated closely with the experimental data, suggesting the validity of this model. The kinetic parameters for [^{111}In]Lac-BSA and [^{111}In]Cat-BSA clearly characterized the difference in their hepatic transport mechanisms and the in vivo targeting characteristics of these albumin derivatives were discussed based on computer simulation.

Introduction

In our series of investigations (Nishida et al., 1990, 1991a,b), we examined the effect of electric charge and sugar residue on the hepatic disposition of macromolecules in an in vivo study and liver perfusion experiment with a bolus administration mode and demonstrated the possibilities for the use of glycosylated and cationized macromolecules as carriers for hepatic targeting of drugs. In these investigations, the difference in their hepatic disposition characteristics was elucidated with regard to the binding to the cell surface and internalization. In the field of organ perfusion, several model analyses have been advanced for the dilution curve of drug obtained by bolus injection (Goresky, 1963; Tsao et al., 1986; Sato et al., 1988; Yano et al., 1990), while investigations dealing with the outflow pattern of a drug during constant infusion are lacking although important information could also be obtained. In a

Correspondence: H. Sezaki, Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

previous study, we assessed the hepatic uptake of macromolecules during constant infusion in terms of extraction ratio (E_{ss}) and hepatic clearance (CL_h) at steady-state (Nishida et al., 1991a,b). However, these parameters estimated under steady-state conditions are hybrid parameters for the processes of uptake, efflux and subsequent sequestration.

In the present study, we focused on the outflow patterns of [^{111}In]Lac-BSA and [^{111}In]Cat-BSA during constant infusion and analyzed them based on a physiological one-organ model which enabled us to characterize their binding to the cell surface and internalization processes separately. Furthermore, we attempted to simulate the in vivo hepatic disposition of these compounds at both organ and cell levels in order to elucidate their potential in drug targeting to the liver.

Materials and Methods

Animals

Male Wistar rats (190–210 g) maintained on standard rat foods and water ad libitum were used.

Chemicals

BSA (fraction V) was obtained from Sigma Chemical Co. (St. Louis, U.S.A.). [^{111}In]Cl_3 (74 MBq/ml) was kindly supplied by Nihon Mediphysics Co. (Takarazuka, Japan). All other chemicals were of the finest reagent grade available. Lac-BSA was synthesized by the coupling of lactose to the ϵ-NH_2 group of lysine residues of BSA by reductive amination with cyanoborohydride according to the method of Gray (1974). Cat-BSA was synthesized by covalent coupling of hexamethylenediamine to BSA according to the method of Pardridge et al. (1987). The amount of sugar conjugated to Lac-BSA was calculated to be 18:1 (mol/mol) according to the phenol/sulfuric acid method (Gray, 1974) with calibration against galactose. The BSA derivatives have almost the same effective molecular size as the original BSA as judged on the basis of gel-filtration chromatography. The BSA derivatives were labeled with ^{111}In using the bifunctional chelating agent, diethylenetriaminopentaacetic acid anhydride (Dojindo Labs, Kumamoto, Japan), according to the method of Hnatowich et al. (1982). The ^{111}In radioactivity was counted using a well-type NaI scintillation counter (model ARC-500, Aloka, Tokyo, Japan).

Liver perfusion experiment

The method for in situ liver perfusion was reported previously (Nishida et al., 1989). The liver was perfused in single-pass mode at a flow rate of 13 ml/min with Krebs-Ringer bicarbonate buffer with 10 mM glucose (oxygenated with 95% O_2-5% CO_2 to pH 7.4 at 37°C). To prevent interactions between macromolecules and blood components, perfusate without red blood cells or albumin was employed. After a stabilization period of 30 min, [^{111}In]Lac-BSA dissolved in the perfusate (0.7–10 μg/ml) was infused for 60 min. The venous outflow and bile were collected into weighed tubes at appropriate intervals. On completion of infusion, perfusate without [^{111}In]Lac-BSA was infused for 5 min to wash out the remaining [^{111}In]Lac-BSA from the intravascular and Disse spaces. In the case of the EDTA-wash experiment, ice-cold Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (pH 5) containing 20 mM EDTA was added to detach surface-bound [^{111}In]Lac-BSA from liver tissue after [^{111}In]Lac-BSA (0.7 μg/ml) had been infused for 5, 20 and 60 min. The amounts of surface-bound and internalized [^{111}In]Lac-BSA were estimated from the radioactivity recovered in the outflow and remaining in the liver, respectively. The viability of the liver was checked with respect to both the bile flow and the glutamic oxaloacetic transaminase activity in the outflow. In all experiments, perfused livers remained viable during the course of the study.

From the venous outflow curves, the extraction ratio (E_{ss}) and the hepatic clearance (CL_h) at steady state for [^{111}In]Lac-BSA are calculated as follows:

$$E_{ss} = (C_{in,ss} - C_{out,ss})/C_{in,ss} \quad (1)$$

$$Cl_h = E_{ss} \cdot Q \quad (2)$$

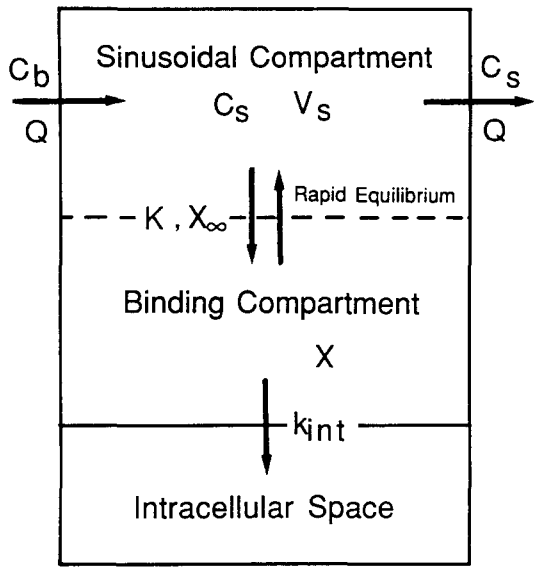

Fig. 1. Physiological pharmacokinetic model for hepatic uptake of [^{111}In]Lac-BSA. Q, flow rate (ml/min); C_b, inflow concentration (μg/ml); C_s, sinusoidal concentration (μg/ml); V_s, sinusoidal volume (ml); X, binding amount (μg); X_∞, maximum binding amount (μg); K, binding constant (ml/μg); k_{int}, internalization rate constant (min^{-1}).

where $C_{in,ss}$ and $C_{out,ss}$ are the respective [^{111}In]Lac-BSA concentrations in the perfusate before and after passing through the liver under steady-state conditions and Q is the perfusion rate.

Pharmacokinetic analysis

Fig. 1 shows a physiological one-organ model employed for the present pharmacokinetic analysis. The sinusoidal compartment including the Disse space is assumed to be under conditions of thorough stirring with a concentration similar to that in the outflow (C_s). The terms C_s and C_b shown in Fig. 1 correspond to C_{out} and C_{in}, respectively. The binding compartment is characterized by a maximum binding amount X_∞ and binding constant K and instantaneous equilibration is assumed to occur between the sinusoidal and binding compartments. This assumption was validated by our previous experimental data showing that the amount of [^{111}In]Lac-BSA bound to the surface was almost constant 2–5 min after the start of perfusion (Nishida et al., 1991b). V_s represents the sum of the volumes of the sinusoid and Disse spaces and as its value we took the figure of 0.180 ml/g liver which was determined in the indicator dilution experiment (Nishida et al., 1990).

Assuming first-order kinetics for the internalization process, the internalization rate (dX/dt) is expressed as a product of binding amount X and its rate constant k_{int}. Since we employed ^{111}In for radiolabeling, efflux from the intracellular space can be neglected. Then, in the sinusoidal and binding compartments, mass-balance equations are defined as follows:

$$V_s \cdot (dC_s/dt) + dX/dt = Q \cdot C_b - Q \cdot C_s - k_{int} \cdot X \quad (3)$$

Binding of BSA derivatives to the cell surface is consistent with the Langmuir equation and the following expression holds.

$$X = (X_\infty \cdot K \cdot C_s)/(1 + K \cdot C_s) \quad (4)$$

If Eqn 4 is differentiated with respect to t, the following equation is obtained:

$$dX/dt = K \cdot X_\infty/(K \cdot C_s + 1)^2 \cdot (dC_s/dt) \quad (5)$$

By substituting Eqn 3 with Eqn 5, the following mass-balance equation is obtained:

$$(dC_s/dt) \cdot \left(V_s + (K \cdot X_\infty)/(K \cdot C_s + 1)^2\right) = Q \cdot C_b - Q \cdot C_s - (k_{int} \cdot X_\infty \cdot K \cdot C_s)/(1 + K \cdot C_s) \quad (6)$$

The differential equations derived from Eqn 6 for various inflow concentrations are numerically solved using the Runge-Kutta-Gill method. The initial condition is $C_s = 0$ when $t = 0$. The fitting was performed by use of the MULTI(RUNGE) program (Yamaoka and Nakagawa, 1983), which was developed on the M-382 main-frame computer of the Kyoto University Data Processing Center. This program is written in Fortran 77.

In the simulation of in vivo hepatic disposition of [^{111}In]Lac-BSA and [^{111}In]Cat-BSA, the blood pool compartment is connected to the physiological one-organ model shown in Fig. 1. In this model, elimination is considered to take place via both the liver and extrahepatic routes. If extrahepatic clearance is considered to be constant and represented by CL_{others}, the mass-balance equation for the blood pool compartment is defined as follows:

$$V_b \cdot (dC_b/dt) = Q \cdot C_s - Q \cdot C_b - CL_{others} \cdot C_b \quad (7)$$

where V_b (ml) is the blood pool volume (20 ml) (Gerlowski and Jain, 1983), Q represents the hepatic blood flow rate (15 ml/min) (Lin et al., 1982), and C_b and C_s respectively denote the concentration in blood and hepatic sinusoidal space (μg/ml). Values of CL_{others} (ml/min) for Lac-BSA and Cat-BSA are estimated from the previous results (Nishida et al, 1991a,b) to be 0.15 and 0.45 ml/min, respectively. From Eqns 6 and 7, the amounts of macromolecules in the total liver, surface of the liver and intracellular space were calculated using the Runge-Kutta-Gill method. The initial condition is $C_b = \text{dose}/V_b$ and $C_s = 0$ when $t = 0$.

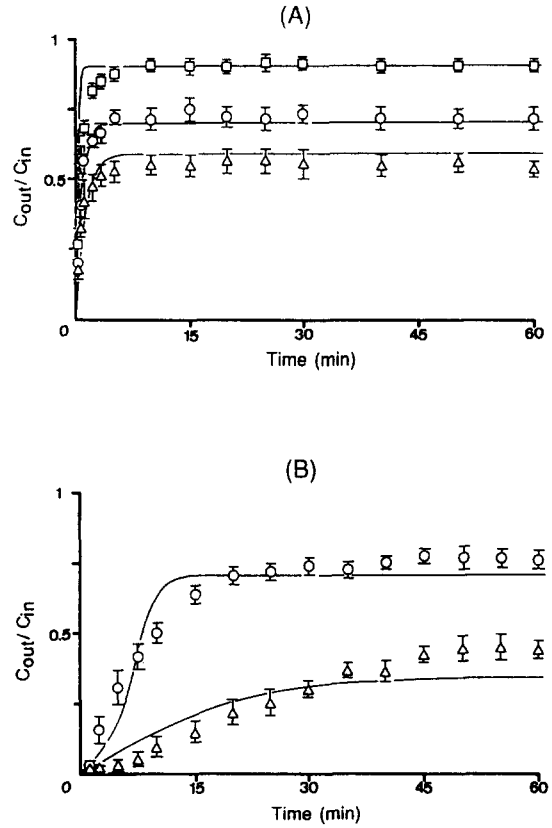

Fig. 2. Hepatic recovery ratio (C_{out}/C_{in})-time profiles of [^{111}In]Lac-BSA (A) and [^{111}In]Cat-BSA (B) in the single-pass liver perfusion experiment. (A) Inflow concentrations: (△) 0.7 μg/ml, (○) 2 μg/ml and (□) 10 μg/ml. (B) Inflow concentrations: (△) 10 μg/ml and (○) 50 μg/ml. Values are means of at least three experiments with the bars indicating the S.D. Curves show simulated functions obtained based on the parameters shown in Table 2.

Results

Hepatic uptake of [^{111}In]Lac-BSA during constant infusion

Fig. 2A shows the hepatic recovery ratio (C_{out}/C_{in})-time profiles of [^{111}In]Lac-BSA at various inflow concentrations (0.7–10 μg/ml) in the constant infusion experiment. In all cases, the recovery ratio reached plateau levels within 5 min and [^{111}In]Lac-BSA was continuously extracted

TABLE 1

Hepatic uptake data for [^{111}In]Lac-BSA at different inflow concentrations in the constant infusion experiment

Inflow concentration (μg/ml)	Outflow		Amount	
	E_{ss} (%)	CL_h (μl/min)	Liver (μg)	Bile (μg)
0.7	44.6 ± 4.0	5691 ± 863	149.9 ± 41.5	1.47 ± 0.58
2.0	28.1 ± 4.5	3335 ± 558	261.5 ± 95.4	2.87 ± 1.17
10.0	9.9 ± 2.2	1217 ± 240	818.2 ± 204.0	10.26 ± 5.43

Values are means ± S.D. of at least three experiments.

TABLE 2

Pharmacokinetic parameters for hepatic uptake of [^{111}In]Lac-BSA and [^{111}In]Cat-BSA

Compound	Method	X_∞ (μg)	K (ml/μg)	k_{int} (min^{-1})
[^{111}In]Lac-BSA	model analysis [a]	38.8	0.868	0.344
	previous experiment [b]	40.1	0.586	0.463
[^{111}In]Cat-BSA	model analysis [a]	4186.3	0.176	0.050
	previous experiment [b]	49212.4	0.066	0.015

[a] Parameters were obtained from simultaneous multiple curve fitting to the experimental results at various inflow concentrations by use of the MULTI(RUNGE) program.

[b] X_∞ and K were obtained from previous in vitro experimental data (Nishida et al., 1990, 1991a,b). k_{int} was evaluated via the constant infusion experiment (Nishida et al., 1991a,b).

by the liver at steady state. The E_{ss}, CL_h and recoveries in the liver and bile are summarized in Table 1. As the inflow concentration of [^{111}In]Lac-BSA increased, the apparent hepatic clearance at steady state decreased considerably. Furthermore, the absolute amount of [^{111}In]Lac-BSA recovered in the liver was not proportional to the inflow concentration, indicating that saturation of the hepatic uptake of [^{111}In]Lac-BSA had occurred. Hepatic recovery ratio-time profiles of [^{111}In]Cat-BSA observed in the same way (Nishida et al., 1991a) are also shown in Fig. 2B. The E_{ss} values for [^{111}In]Cat-BSA at inflow concentrations of 10 and 50 μg/ml were 54 and 23%, respectively. Although extensive hepatic uptake was observed for both [^{111}In]Lac-BSA and [^{111}In]Cat-BSA, the E_{ss} and time required to attain the steady state differed greatly. This phenomenon appeared to be explained in terms of differences in the affinity and capacity of binding to the cell surface and/or in the internalization rate.

Simultaneous multiple fitting of outflow pattern

Kinetic parameters representing the binding to the cell surface and internalization were calculated according to the simultaneous multiple curve fitting to the experimental results at various inflow concentrations of [^{111}In]Lac-BSA. The obtained kinetic parameters for [^{111}In]Lac-BSA are summarized with those for [^{111}In]Cat-BSA in Table 2. A considerable difference was observed between parameters for [^{111}In]Lac-BSA and [^{111}In]Cat-BSA. The X_∞ for [^{111}In]Cat-BSA was about 100-fold larger than that for [^{111}In]Lac-BSA. On the other hand, the K for [^{111}In]Lac-BSA was 5-times larger than that for [^{111}In]Cat-BSA. The k_{int} for [^{111}In]Lac-BSA was about 7-times greater than that for [^{111}In]Cat-BSA. These kinetic parameters basically corresponded well to those obtained during an in vitro experiment using isolated rat hepatocytes and/or liver perfusion with the EDTA-wash treatment in previous reports (Nishida et al., 1991a,b) (Table 2).

Fig. 2A and B also shows simulation curves for the hepatic recovery ratio-time profiles of [^{111}In]Lac-BSA and [^{111}In]Cat-BSA which have been reconstructed employing the estimated parameters. In general, good agreement was observed between fitted lines and experimentally observed data at all inflow concentrations in both compounds. As for [^{111}In]Lac-BSA, its surface-bound and internalized amounts at respective times of 5, 20 and 60 min after the start of perfusion of [^{111}In]Lac-BSA (0.7 μg/ml) obtained with the EDTA-wash experiment, correlated well with those calculated from the present kinetic parameters (Table 2). This finding might support the validity of the present fitting procedure. In the initial stage of hepatic uptake within 5 min after the start of infusion, however, some differences are observed between them.

Simulation of in vivo hepatic disposition

The simulated time courses of the amounts of Lac-BSA and Cat-BSA existent in blood, surface-bound and internalized following an intravenous bolus injection at doses of 100 (low

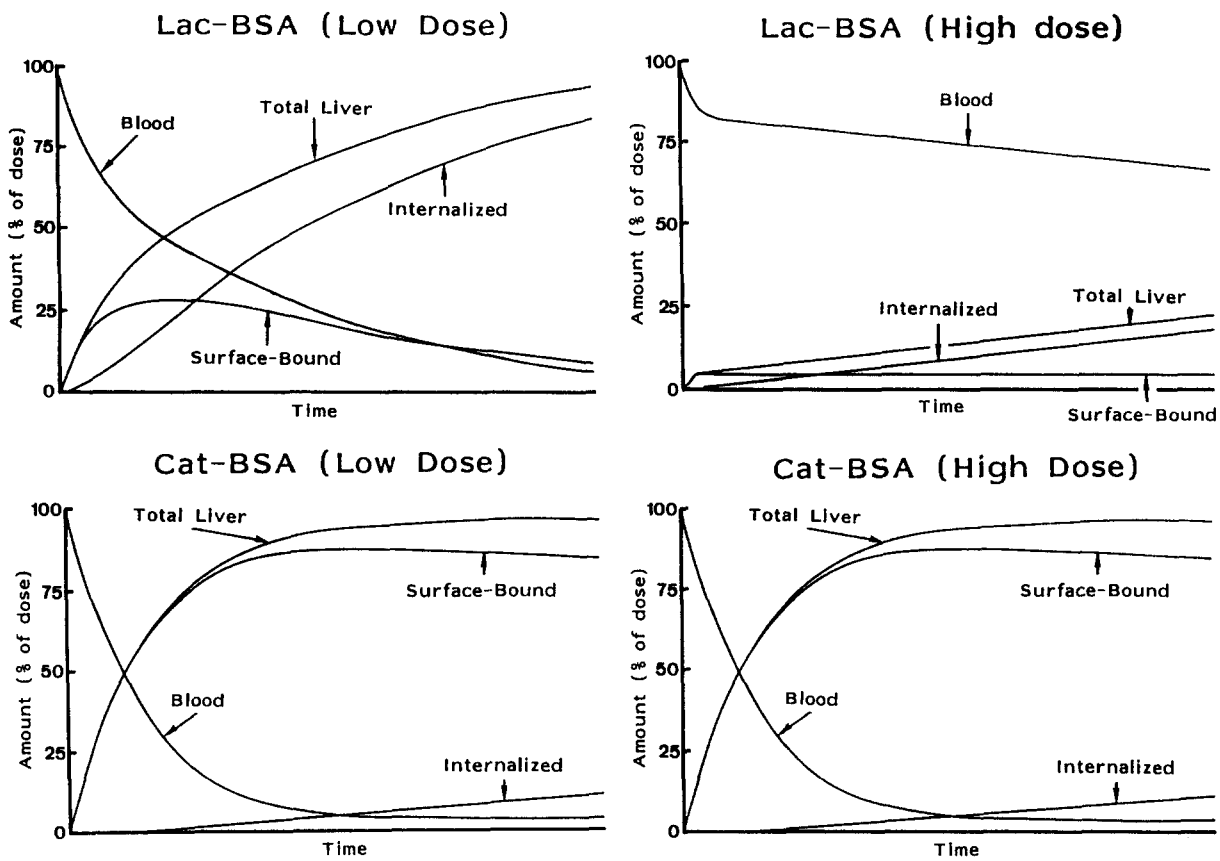

Fig. 3. Results of computer simulation for time courses of amount in blood and liver (total, surface-bound and internalized) of Lac-BSA and Cat-BSA following intravenous injection to rats. Simulation was performed by use of the parameters given in Table 2 and physiological parameters reported elsewhere.

dose) and 1000 μg/rat (high dose) are shown in Fig. 3. In the case of a low dose of Lac-BSA, the surface-bound amount rapidly reaches a plateau after intravenous injection and the internalized amount increases with time. In contrast, the greater part of the injected Cat-BSA exists on the liver cell surface. At high doses, the amounts of Lac-BSA bound to the cell surface and internalized were significantly decreased, while that of Cat-BSA was unchanged. These results clearly explain the differences in the behavior of in vivo hepatic disposition of Lac-BSA and Cat-BSA at the cell level.

Discussion

Hepatic transport of drug is usually studied with several experimental systems such as the in vivo, isolated liver perfusion and in vitro systems with isolated hepatocytes. In a previous study (Nishida et al., 1991b), we assessed the hepatic uptake of [^{111}In]Lac-BSA by means of calculation of the apparent hepatic uptake clearance (total accumulation/plasma AUC) in the in vivo experiments. Since hepatic uptake followed non-linear kinetics, we only had an average value of clearance for the experimental period in this case. In

addition, a significant contribution of blood flow to the apparent clearance was suggested, since the clearance values were rather close to hepatic plasma flow (Lin et al., 1982). On the other hand, we have examined the binding characteristics of macromolecules using isolated hepatocytes, although this approach also involves a number of problems such as that of the observed binding including not only that with the apical side but also that with the basolateral side of the hepatocytes. From these viewpoints, the isolated liver perfusion system appears to be an appropriate tool for analyzing the hepatic transport characteristics of drugs, since experiments can be performed independently of any influence by other body parts while the spatial architecture between cells and capillaries is maintained.

The liver perfusion experiment is carried out by using several injection modes such as bolus input, constant infusion and recirculation methods. The bolus input experiment (indicator dilution analysis) is particularly useful for examining the initial stages of hepatic uptake of drugs, or in other words, rapid uptake processes. In the recirculation experiment, only the overall elimination rate of drug from the perfusate is obtained. On the other hand, constant infusion gives direct information about the uptake behavior of drugs at steady state. In particular, slow processes such as endocytosis of macromolecules can be clearly characterized in this manner. We have performed liver perfusion experiments with bolus injection and constant infusion modes based on these considerations and made several interesting observations (Nishida et al., 1990, 1991a,b). However, we were unable to distinguish the binding and internalization processes based on a single experimental and analytical system in these cases.

In the present study, therefore, hepatic uptake of [^{111}In]Lac-BSA was studied in the constant infusion experiment and results were analyzed based on a physiological one-organ model in which binding and internalization were assessed separately. As shown in Fig. 2A and B, the computer fitting yielded good results at any inflow concentration, suggesting the validity of this model. However, in the early phase after the start of infusion, the model did not fit the data well. In this analysis, it is assumed that the sinusoidal space is under conditions of thorough stirring and that the binding to the cell surface occurs instantaneously. In a strict sense, such a discrepancy might be caused by the inapplicability of these conditions. Hepatic extraction of Lac-BSA in the early phase may be so extensive that the sinusoidal concentrations declined with distance along blood flow. Also, it has been reported that pericentral hepatocytes incorporate [^{125}I]asialoorosomucoid more avidly than do periportal cells (Sluijs et al., 1988). Thus, another kinetic model such as the parallel-tube, distribution and dispersion models might be more appropriate to describe the precise shape of the outflow pattern in the initial stages.

In the present study, the obtained binding parameters should be reliable, since the liver perfusion experiment retained the blood flow and original architecture of the liver. With respect to k_{int}, the present approach would afford its direct estimation without any additional treatment such as detachment by EDTA washing (Nishida et al., 1991b).

In comparison with [^{111}In]Lac-BSA, relatively large differences in the kinetic parameters of [^{111}In]Cat-BSA are observed between the present (liver perfusion) and previous (in vitro) experiments. Among the kinetic parameters, the discrepancy in X_∞ was remarkable. The total surface area of hepatocytes isolated by collagenase perfusion was rather larger than that of the hepatocytes in the intact liver and estimation of the latter from the former value might incur some errors. Regarding this point, the present approach seems to afford more precise information.

The calculated kinetic parameters of [^{111}In]Lac-BSA and [^{111}In]Cat-BSA were characteristic and largely different between the compounds. In comparison with [^{111}In]Cat-BSA, the binding site affinity and internalization rate of [^{111}In]Lac-BSA for binding to hepatocytes were very much higher, whereas the binding capacity to the hepatocytes was approx. 1/100 of that of [^{111}In]Cat-BSA. Consequently, the hepatic uptake behavior of these BSA derivatives differed greatly at the suborgan level and a precise understanding of these differences would lead to more effective drug

targeting using these macromolecules as carriers. These characteristic targeting potentials of Lac-BSA and Cat-BSA were more clearly demonstrated in the in vivo simulation (Fig. 3). From the viewpoint of drug targeting, Lac-BSA is concluded to be a good carrier for the delivery of attached drug to the cytosol of hepatocytes, while Cat-BSA results in preferential delivery to the cell surface of the liver.

References

Gerlowski, L.E. and Jain, R.K., Physiologically based pharmacokinetic modeling. Principles and applications. *J. Pharm. Sci.*, 72 (1983) 1103–1126.

Goresky, C.A., A linear method for determining liver sinusoidal and extravascular volumes. *Am. J. Physiol.*, 204 (1963) 626–640.

Gray, G.R., The direct coupling of oligosaccharides to proteins and derivatized gels. *Arch. Biochem. Biophys.*, 163 (1974) 426–428.

Hnatowich, D.J., Lane, W.W. and Childs, R.L., The preparation and labeling of DTPA-coupled albumin. *Int. J. Appl. Isot. Radiat.*, 33 (1982) 327–332.

Lin, J.H., Sugiyama, Y., Awazu, S. and Hanano, M., Physiological pharmacokinetics of ethoxybenzamide based on biochemical data obtained in vitro as well as on physiological data. *J. Pharmacokinet. Biopharm.*, 10 (1982) 649–661.

Nishida, K., Tonegawa, C., Nakane, S., Takakura, Y., Hashida, M. and Sezaki, H., Effect of electric charge on the hepatic uptake of macromolecules in the rat liver. *Int. J. Pharm.*, 65 (1990) 7–17.

Nishida, K., Mihara, K., Takino, T., Nakane, S., Takakura, Y., Hashida, M. and Sezaki, H., Hepatic disposition characteristics of electrically charged macromolecules in rat in vivo and in the perfused liver. *Pharm. Res.*, 8 (1991a) 437–444.

Nishida, K., Eguchi, Y., Takino, T., Takakura, Y., Hashida, M. and Sezaki, H., Hepatic disposition characteristics of ^{111}In-labeled lactosaminated bovine serum albumin in rats. *Pharm. Res.*, 8 (1991b) 1253–1257.

Pardridge, W.M., Kumagai, A.K. and Eisenberg, J.B., Chimeric peptides as a vehicle for peptide pharmacokinetical delivery through the blood-brain barrier. *Biochem. Biophys. Res. Commun.*, 146 (1987) 307–313.

Sato, H., Sugiyama, Y., Sawada, Y., Iga, T., Sakamoto, A., Fuwa, T. and Hanano, M., Dynamic determination of kinetic parameters for the interaction between polypeptide hormones and cell surface receptors in the perfused liver by the Multiple-indicator dilution method. *Proc. Natl. Acad. Sci. USA*, 85 (1988) 8355–8359.

Sluijs, P.V.D., Braakman, I., Meijer, D.K.F. and Groothuis, G.M.M., Heterogeneous acinar localization of the asialoglycoprotein internalization system in rat hepatocytes. *Hepatology*, 8 (1988) 1521–1529.

Tsao, S.C., Sugiyama, Y., Sawada, Y., Nagase, S., Iga, T. and Hanano, M. Effect of albumin on hepatic uptake of warfarin in normal and analbuminemic mutant rats. Analysis by multiple indicator dilution method. *J. Pharmacokinet. Biopharm.*, 14 (1986) 61–64.

Yamaoka, K. and Nakagawa, T., A nonlinear least squares program based on differential equations, MULTI (RUNGE), for microcomputers. *J. Pharmacobio-Dyn.*, 6 (1983) 595–606.

Yano, Y., Yamaoka, K., Minamide, T., Nakagawa, T. and Tanaka, H., Evaluation of protein binding effect on local disposition of oxacillin in rat liver by a two-compartment dispersion model. *J. Pharm. Pharmacol.*, 42 (1990) 632–636.