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Pharmacokinetics of receptor-mediated hepatic uptake of glycosylated albumin in mice

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Summary

Three types of glycosylated bovine serum albumins (BSA) labeled with ^{111}In were synthesized and their disposition characteristics were studied in mice. At lower doses (0.05–0.1 mg/kg), intravenously injected galactosylated BSA (Gal-BSA) and glucosylated BSA (Glc-BSA) were rapidly eliminated from plasma due to extensive uptake by the liver, and their calculated apparent uptake clearances were fairly close to the hepatic plasma flow and also the total body clearance in each experiment. However, their hepatic uptake was nonlinear and decreased at higher doses. Mannosylated BSA (Man-BSA) also showed preferential hepatic uptake although the maximum rate of uptake was about one quarter of those of Gal-BSA and Glc-BSA. The cellular distribution of Gal-BSA and Man-BSA in the liver was consistent with the localization pattern of receptors recognizing galactose or mannose residues; i.e., preferential accumulation was shown in the parenchymal (Gal-BSA) or non-parenchymal (Man-BSA) cells. The time courses observed for plasma concentration and liver accumulation of glycosylated albumins were satisfactorily described based on a physiological model including hepatic plasma flow. These results suggest a potential and limitation of glycosylated albumins as carriers for hepatic cellular targeting.

Introduction

Most biologically active proteins are glycoproteins which contain oligosaccharide side chains and suffer rapid clearance from the blood circulation by the liver via carbohydrate-receptor mediated endocytosis upon removal of sialic acid (Morell et al., 1968, 1971; Goldwasser et al., 1974; Bose and Hickman, 1977). Therefore, the in vivo behavior of proteins with sugar moieties has become a subject of great interest in a recent development of proteinous drugs.

In a series of investigations, we have developed various kinds of macromolecular carrier systems for small drugs and proteins aiming at controlling their disposition and targeting to the disease site (Hashida et al., 1984; Takakura et al., 1987a,b, 1989; Sezaki et al., 1989; Fujita et al.,

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1990; Yasuda et al., 1990; Noguchi et al., 1991). These approaches include utilization of macromolecules with various modifications of their physicochemical properties, such as molecular size and electric charge (Hashida et al., 1984; Takakura et al., 1987a,b; Sezaki et al., 1989; Fujita et al., 1990), materials with specific biological affinity, e.g., monoclonal antibodies (Noguchi et al., 1991)., and biologically inert substances like polyethylene glycol and carboxymethyldextran (Takakura et al., 1989; Yasuda et al., 1990), as modifiers. In these studies, the effects of chemical modification were evaluated in relation to the biopharmaceutical and pharmacological characteristics of the final products based on pharmacokinetic analysis.

Application of glycosylated macromolecules to drug carriers appears to be an another promising approach to targeting, since cell-specific delivery of drugs would be achieved through this technique (Wu, 1988). However, there is a scarcity of published data on the pharmacokinetic behavior of macromolecules with sugar moieties.

In this paper, we have investigated the effect of the introduction of a sugar moiety on the in vivo fate of a model protein, i.e., bovine serum albumin (BSA). The specific distribution of glycosylated albumins to the liver in mice after intravenous injection was analyzed based on a physiological model. The potential and limitation of modification of proteins with sugar moieties for controlling their in vivo fate or the application of glycosylated proteins as drug carriers is discussed in comparison with other types of modification.

Materials and Methods

Chemicals

Bovine serum albumin (BSA; fraction V), β-D-galactose, β-D-glucose, and α-D-mannose were obtained from Nacalai Tesque, Kyoto, Japan. Collagenase (type I) was obtained from Sigma, St. Louis, U.S.A. [^{111}In]Indium chloride was kindly supplied by Nihon Medi-physics, Co., Takarazuka, Japan. All other chemicals were reagent grade products obtained commercially.

Synthesis of glycosylated albumins

Coupling of monosaccharides to BSA was carried out according to the method of Lee et al. (1976). Briefly, 2-imino-2-methoxyethyl 1-thioglycosides (IME-thioglycosides) were obtained from cyanomethyl 2,3,4,6-tetra-*O*-acetyl-1-thioglycosides (CNM-thioglycosides) by de-*O*-acetylation and conjugated with BSA in 50 mM borate buffer (pH 9.5) for 8 h at room temperature. Three types of BSA conjugates with 1-thiogalactoside (Gal-BSA), 1-thioglucoside (Glc-BSA), and 1-thiomannoside (Man-BSA) were synthesized. The resulting derivatives were washed with distilled water, concentrated by ultrafiltration, and lyophilized. The numbers of sugars incorporated into the albumin were determined by the phenol-sulfuric acid method to be 21.5 (Gal-BSA), 15.3 (Glc-BSA), and 15.8 (Man-BSA). All BSA derivatives were confirmed negative as being charged at pH 7.4 based on a batch method using a CM-Sephadex C-50 cation exchanger and a DEAE-Sephadex A-50 anion exchanger (Pharmacia, Uppsala, Sweden) as described previously (Roos et al., 1984). Glycosylated albumins were labeled with ^{111}In using the bifunctional chelating agent diethylenetriaminepentaacetic acid (DTPA) anhydride (Dojindo Labs, Kumamoto, Japan) according to the method of Hnatowich et al. (1982).

In vivo distribution experiment

Male ddY mice (25–28 g) were obtained from the Shizuoka Agricultural Co-operative Association for Laboratory Animals, Shizuoka, Japan. Mice received 0.05, 0.1, 1, 10 or 20 mg/kg dose of radiolabeled glycosylated albumins in saline by tail vein injection and were then housed in metabolic cages for urine collection. At adequate time periods after injection, blood was collected from the vena cava under ether anesthesia and the mice were killed. The heart, lung, liver, spleen, kidney, intestine, muscle, and iliac lymph nodes (three or four nodes) were excised, rinsed with saline, weighed, and subjected to assay. The distribution of glycosylated albumins in parenchymal (PC) and non-parenchymal cells (NPC) after intravenous injection was determined in different mice by separation of PC and NPC after collagenase perfusion (Horiuchi et al., 1985).

Inhibition of hepatic uptake of glycosylated albumin by simultaneous administration with other glycosylated albumins

Radiolabeled glycosylated albumin (1 mg/kg) was injected into mice simultaneously with different unlabeled glycosylated albumins at a dose of about 10 mg/kg. At 5 min for ^{111}In-Gal-BSA and Glc-BSA or 10 min for ^{111}In-Man-BSA after injection, plasma and liver were sampled and subjected to assay.

Analytical methods

The ^{111}In radioactivities were counted in a well-type NaI scintillation counter (ARC-500, Aloka Co., Tokyo, Japan). Contamination of plasma in tissue samples was corrected using distribution data on ^{14}C-labeled carboxymethyl-dextran (T-70) at 1 h after intravenous injection (Takakura et al., 1990).

Calculation of tissue uptake rate index and organ clearance

The tissue distribution was evaluated using a tissue uptake rate index calculated in terms of clearance (Takakura et al., 1987a). The change in the amount of radioactivity in a tissue with time can be described as follows:

$$dT(t)/dt = Cl_{in}C(t) - K_{out}T(t) \quad (1)$$

where $T(t)$ (% of dose/g) represents the amount of radioactivity in 1 g of the tissue, $C(t)$ (% of dose/ml) is the plasma concentration of radioactivity, Cl_{in} (ml/h per g) denotes the tissue uptake rate index from the plasma to the tissue, and K_{out} (1/h) is the rate constant for efflux from the tissue. In the present study the efflux process can be considered to be negligible during the initial phase of the experiment, since ^{111}In radioactivity was mostly retained in the tissue including the liver even after degradation of compounds (Brown et al., 1987). Ignoring efflux, Eqn 1 integrates to

$$Cl_{in} = X(t_1)/\int_0^{t_1} C(t)\,dt = X(t_1)/AUC_{0-t_1} \quad (2)$$

where t_1 (h) is the time of sampling after injection. According to Eqn 2, the tissue uptake rate index is calculated using the amount of radioactivity in the tissue at an appropriate interval of

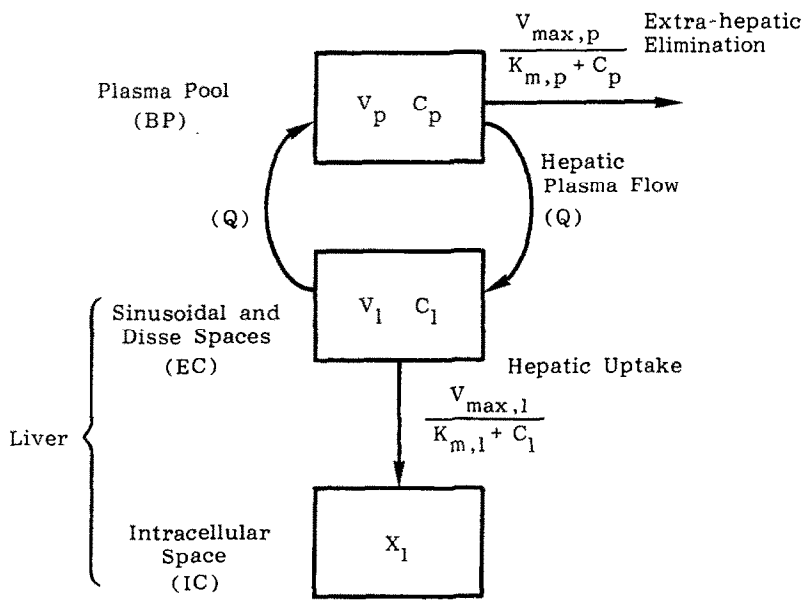

Fig. 1. Physiological pharmacokinetic model for analyzing the disposition of ^{111}In-labeled glycosylated albumins.

time and the area under the plasma concentration-time curve (AUC) up to the same time point. Then, the organ clearance (CL_{org}) is expressed as follows:

$$CL_{org} = Cl_{in}W \quad (3)$$

where W (g) is the total weight of the organ. When the tissue uptake process followed non-linear kinetics and Cl_{in} was not constant, the calculated Cl_{in} values would represent an average value for the overall experimental period. We have also calculated total body clearance (CL_{total}) from AUC for infinite time (AUC_∞) according to the following equation:

$$CL_{total} = \text{dose}/AUC_\infty \quad (4)$$

The total body clearance also represents an average value if the total disposition does not follow linear kinetics.

The AUC for any experimental period or for infinite time was estimated by fitting a two-exponential equation to the plasma concentration data for the first 30 min (Gal-BSA and Glc-BSA) or 1 h (Man-BSA) during which efflux of ^{111}In radioactivity from the liver was negligible.

Pharmacokinetic analysis based on a physiological model

The time courses of plasma concentration and liver accumulation of ^{111}In-labeled glycosylated albumins were analyzed based on the model shown in Fig. 1. In this model, the body is represented by three compartments, i.e., the plasma pool (BP), the sinusoidal and Disse spaces in the liver (EC), and the intracellular space in the liver (IC). The BP and EC compartments have apparent volumes of distribution, V_p and V_1, respectively. The BP compartment represents all plasma spaces within blood vessels of the tissue except the liver. This compartment is connected with EC by the hepatic plasma flow (Q). The uptake of the glycosylated albumins from EC to IC is expressed as a Michaelis-Menten type of saturable process with maximum rate of uptake, $V_{max,1}$, and Michaelis constant, $K_{m,1}$. The extra-hepatic elimination from BP is also assumed to be a saturable process represented by $V_{max,p}$ and $K_{m,p}$. At time zero, the injected substance is assumed to be distributed in the BP and EC compartments at the same concentration. Mass balance equations for the concentration of glycosylated albumins in BP, EC, and IC, respectively, are expressed as:

$$dC_p/dt = \left(QC_1 - QC_p - V_{max,p}C_p/(K_{m,p} + C_p)\right) /V_p \quad (5)$$

$$dC_1/dt = \left(QC_p - QC_1 - V_{max,1}C_1/(K_{m,1} + C_1)\right) /V_1 \quad (6)$$

$$dX_1/dt = V_{max,1}C_1/(K_{m,1} + C_1) \quad (7)$$

The values of V_p, V_1, and Q were assumed to be 1.5, 0.15 ml and 85 ml/h, respectively, on referring the work of Gerlowski and Jain (1983). To determine the kinetic parameters, these equations were fitted to the experimental data by use of the non-linear least-squares method MULTI (Yamaoka et al., 1981) associated with the Runge-Kutta-Gill method [MULTI(RUNGE)] (damping Gauss Newton method; weight $1/C_p$) on the M-382 mainframe computer of the Kyoto University Data Processing Center.

Estimation of the initial values of the parameters was performed as follows: When the hepatic uptake process is saturated, i.e., during the initial stages after injection of high doses of glycosylated albumins, the integration of Eqn 7 gives:

$$X_1(t) = V_{max,1}t \quad (8)$$

where $X_1(t)$ (μg) denotes the amount of glycosylated albumins in the liver. Therefore, the initial value of $V_{max,1}$ was estimated from plotting $X_1(t)$ vs t (h). In contrast, when the concentration of glycosylated albumins is low compared with $K_{m,1}$, $X_1(t)$ is expressed as:

$$X_1(t) = V_{max,1}/K_{m,1}AUC_{0-t} \quad (9)$$

$$Cl_{1,int} = V_{max,1}/K_{m,1} \quad (10)$$

where $CL_{1,int}$ (ml/h) represents intrinsic hepatic clearance. Therefore, $CL_{1,int}$ can be estimated from the plotting $X_1(t)$ vs AUC_{0-t}. However, in the cases of Gal-BSA and Glc-BSA, the intrinsic hepatic clearance is very large compared with the hepatic plasma flow, and the uptake of glycosylated albumins is limited by the flow. In this case, we estimated $CL_{1,int}$ based on the following equation:

$$CL_{1,int} = QCL_{1,app}/(Q - CL_{1,app}) \quad (11)$$

where $CL_{1,app}$ (ml/h) is the apparent hepatic clearance calculated from Eqn 3. The initial $K_{m,1}$ was subsequently estimated uisng $V_{max,1}/CL_{1,int}$. The initial values of the parameters of extrahepatic elimination were also determined in the same manner.

Results

Tissue distribution of ^{111}In-labeled glycosylated albumins

Fig. 2 shows plasma concentration- and liver accumulation-time curves of Gal-BSA, Glc-BSA, and Man-BSA at various doses after intravenous injection into mice. At lower doses (0.05–1 mg/kg), Gal-BSA was rapidly cleared from the circulation and accumulated in the liver to an extent of more than 80% of the dose within 10 min irrespective of the dose. However, with an increase in dose to higher than 1 mg/kg, plasma elimination and liver accumulation of Gal-BSA were delayed. Glc-BSA showed similar behavior to Gal-BSA. On the other hand, the disappearance from plasma and the accumulation in liver of Man-BSA were slower than those of Gal-BSA and Glc-BSA at doses below 1 mg/kg. Dose

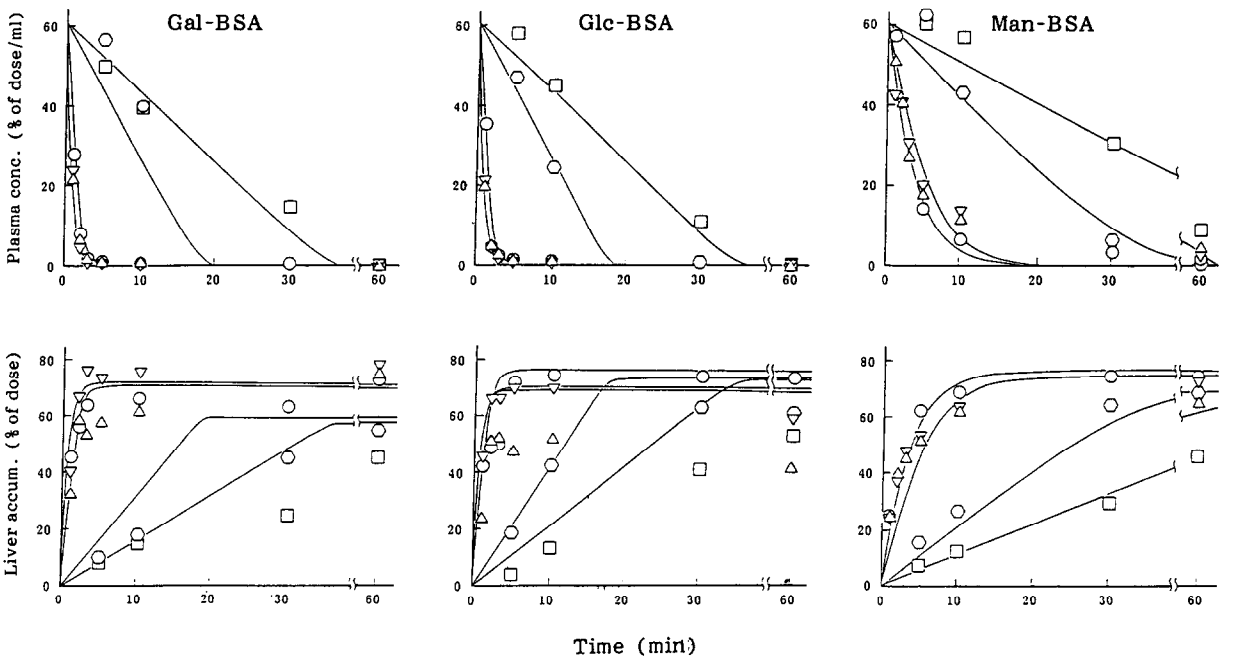

Fig. 2. Plasma concentration and liver accumulation of ^{111}In-labeled glycosylated albumins after intravenous injection into mice at doses of 0.05 (△), 0.1 (▽), 1 (○), 10 (⬡), and 20 (□) mg/kg. Results are expressed as means of four mice. Fitted curves were drawn using the values listed in Table 3.

dependency in hepatic uptake was also observed in Man-BSA. In any other tissues, the radioactivity counted was not significant except in the spleen in the case of Man-BSA (data not shown). Radioactivity in the liver was retained for longer than 1 h but thereafter gradually decreased.

Calculation of basic pharmacokinetic parameters and tissue uptake rate index

Table 1 summarizes the AUC, total body, hepatic, and urinary clearances, and tissue uptake rate index for representative tissues after intravenous administration of glycosylated albumins at various doses. In every experiment, hepatic clearance was found to be the major contributor to total body clearance, suggesting that these glycosylated albumins were selectively taken up by the liver. At lower doses of 0.05 and 0.1 mg/kg, the hepatic uptake clearances of these three glycosylated albumins were constant and, except for Man-BSA, approximately equal to the hepatic plasma flow (Gerlowski and Jain, 1983). The hepatic clearance decreased with increasing dose. In accordance with this, total body clearance also decreased.

In all experiments, urinary clearance was very small and the total amounts excreted remained approximately constant (0.12–1.3% of dose). Tissue uptake rates in the kidney and muscle were low and did not display differences, irrespective of the types of sugar moieties. However, tissue uptake rate in the spleen was largely different between glycosylated albumins and Man-BSA showed a considerable, dose-dependent uptake rate (Table 1).

Cellular localization of ^{111}In-labeled glycosylated albumins in the liver

Fig. 3 shows the distribution of glycosylated albumins in parenchymal (PC) and non-parenchymal cells (NPC) at 30 min after intravenous injection at a dose of 1 mg/kg. Gal-BSA and Glc-BSA

TABLE 1

AUC, clearance, and tissue uptake rate index for ^{111}In-labeled glycosylated albumins in mice at various doses

Compound	Dose (mg/kg)	AUC (% of dose h per ml)	Clearances (μl/h)				Tissue uptake rate index (μl/h per g)			
			CL_{total}	CL_{liver}	CL_{other} [b]	CL_{urine}	Liver	Spleen	Kidney	Muscle
BSA	1	1430	69.7	15.9	53.8	7.1	15.1	7.5	28.0	1.5
Gal-BSA	0.05	0.97	103000	72700	30300	520	65300	n.d. [a]	n.d.	n.d.
	0.1	1.03	97100	83400	13700	120	60000	110	860	100
	1	1.34	74600	56700	17900	570	30200	82.4	500	55.1
	10	19.1	5240	3220	2020	54.3	2090	29.4	95.8	24.5
	20	20.6	4860	2380	2480	20.4	2130	n.d.	n.d.	n.d.
Glc-BSA	0.05	0.91	110000	65000	45000	380	60900	n.d.	n.d.	n.d.
	0.1	0.95	105000	84700	20300	170	62500	1600	790	89.2
	1	1.62	61700	51500	10200	390	31500	1030	310	59.4
	10	11.8	8460	6110	2350	410	4550	410	120	16.8
	20	21.9	4560	2670	1890	24.6	2440	n.d.	n.d.	n.d.
Man-BSA	0.05	3.36	27300	18000	9300	360	17700	9450	n.d.	n.d.
	0.1	3.67	27200	19300	7900	100	11300	5930	300	19.6
	1	3.98	25100	19200	5900	130	16000	7970	460	30.7
	10	24.8	4040	3200	840	41.6	2710	1320	110	13.2
	20	41.1	2430	1300	1130	3.5	1170	750	n.d.	n.d.

[a] Not determined.

[b] $CL_{other} = CL_{total} - CL_{liver}$.

TABLE 2

Competitive inhibition of hepatic uptake of ^{111}In-labeled glycosylated albumins by other types of glycosylated albumins [a]

Compound	Inhibitor	Plasma concentration (% of dose/ml)	Liver accumulation (% of dose)
Gal-BSA	none	0.88 ± 0.27 [b]	81.1 ± 3.0
	Glc-BSA	26.8 ± 4.4 [c]	58.6 ± 1.5 [c]
	Man-BSA	0.37 ± 0.05	75.2 ± 2.9
Glc-BSA	none	1.12 ± 0.20	80.9 ± 5.1
	Gal-BSA	25.8 ± 1.5 [c]	49.5 ± 2.5 [c]
Man-BSA	none	6.22 ± 1.01	59.2 ± 2.4
	Gal-BSA	8.24 ± 1.50	61.1 ± 2.9
	Glc-BSA	20.3 ± 4.7 [c]	51.4 ± 2.8

[a] Glycosylated albumin (1 mg/kg) was injected with other albumin derivatives (10 mg/kg), and plasma concentration and liver accumulation were compared at 5 min for Gal-BSA and Glc-BSA or at 10 min for Man-BSA after injection.
[b] Results are expressed as the means ± SD of at least three animals.
[c] Statistically significant difference based on Student t-test ($P < 0.01$) as compared with each control.

were recovered preferentially in the PC, while Man-BSA was accumulated in the NPC to a greater extent than PC.

Inhibition of hepatic uptake by simultaneous administration with a different type of glycosylated albumin

Table 2 summarizes the data on the plasma concentration and liver accumulation of glycosylated albumins (1 mg/kg) with or without simultaneous administration with other glycosylated albumin (10 mg/kg). When Gal-BSA was administered with Glc-BSA, liver accumulation of Gal-BSA was reduced from 81.1 to 58.6% of dose at 5 min after injection. Similarly, the uptake of Glc-BSA by the liver was inhibited by coadministration of Gal-BSA. On the other hand, no significant effect was observed among Gal-BSA and Man-BSA. Glc-BSA partly inhibited the liver accumulation of Man-BSA.

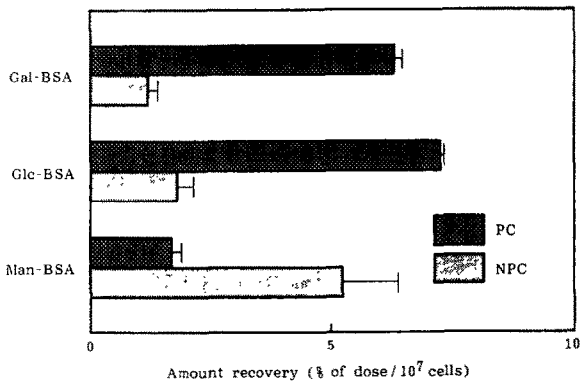

Fig. 3. Cellular localization of ^{111}In-labeled glycosylated albumins after intravenous injection into mice at a dose of 1 mg/kg. Results are expressed as the means ± SD of three mice.

Pharmacokinetic analysis based on a physiological model

Eqns 5–7 were simultaneously fitted on the experimental data for plasma concentration and liver accumulation of each glycosylated albumin at five doses and the values of $K_{m,l}$, $V_{max,l}$, $K_{m,p}$, and $V_{max,p}$ were estimated (Table 3). The simulated plasma concentration and liver accumulation curves demonstrate a good agreement with the experimental data (Fig. 2). Thus, the disposition, especially the liver uptake, of glycosylated

TABLE 3

Pharmacokinetic parameters of ^{111}In-labeled glycosylated albumins in mice calculated based on a physiological model

Compound	Liver		Blood pool	
	$K_{m,l}$ (μg/ml)	$V_{max,l}$ (μg/h)	$K_{m,p}$ (μg/ml)	$V_{max,p}$ (μg/h)
Gal-BSA	0.308 ± 0.0175	489 ± 25.9	11.0 ± 3.10	399 ± 66.7
Glc-BSA	0.382 ± 0.0022	608 ± 3.03	5.57 ± 0.115	271 ± 31.1
Man-BSA	12.3 ± 1.86	344 ± 31.8	29.7 ± 14.3	195 ± 58.7

Parameter values are expressed as the means ± computer-calculated SD. The values of V_p, V_l, and Q were assumed to be 1.5, 0.15 ml and 85 ml/h, respectively, as referred to Gerlowski and Jain (1983).

albumins was analyzed based on the physiological model. The $K_{m,l}$ of Gal-BSA was very low and approximately equal to that of Glc-BSA, reflecting the same distribution pattern (Table 3). However, the $K_{m,l}$ of Man-BSA was greater than those of Gal-BSA and Glc-BSA. In all glycosylated albumins, the $K_{m,l}$ values were very low compared with those of $K_{m,p}$, indicating that the process of liver uptake had an extremely high affinity compared with that of extra-hepatic elimination.

Discussion

Glycosylated macromolecules, such as asialoglycoproteins and synthetic glycoconjugates, are well known to be taken up by the liver via receptor-mediated endocytosis (Meijer and Van der Sluijs, 1989). Since they are promising candidates for use as drugs or drug carriers, the ability to predict their behavior after administration is of considerable importance from a pharmacokinetic viewpoint. In the present study, therefore, we studied the in vivo fate of glycosylated albumins as model compounds. The process of liver uptake was analyzed in detail using a physiological model.

In this paper, glycosylated albumins were radiolabeled with ^{111}In using DTPA anhydride and tissue uptake was estimated by counting radioactivity in the tissue. Radioiodination is the most commonly used method for protein labeling. However, once the labeled protein taken up by the organ has been degraded, radioactivity may return to the blood circulation (Brown et al., 1987; Taylor et al., 1987). This disadvantage of radioiodination may complicate the analysis of data. On the other hand, ^{111}In has been reported to be accumulated in the organ by exchange into an iron-binding protein (Brown et al., 1987) after intracellular degradation. In the present study, the radioactivity in the organ was retained for a relatively long time even in the liver. Fig. 4 shows the relationship between $X_l(t)$ and AUC_{0-t} in the case of Glc-BSA. During the early phase of the experiment, a linear relation was observed between liver accumulation ($X_l(t)$) and AUC_{0-t} passing through the origin at each administered dose, indicating that the efflux of radioactivity from the liver was negligible within this interval. This also demonstrates the validity of calculation of apparent hepatic clearance by counting the radioactivity in the organ using Eqns 2 and 3. More precisely, the slopes of the plots for the 10

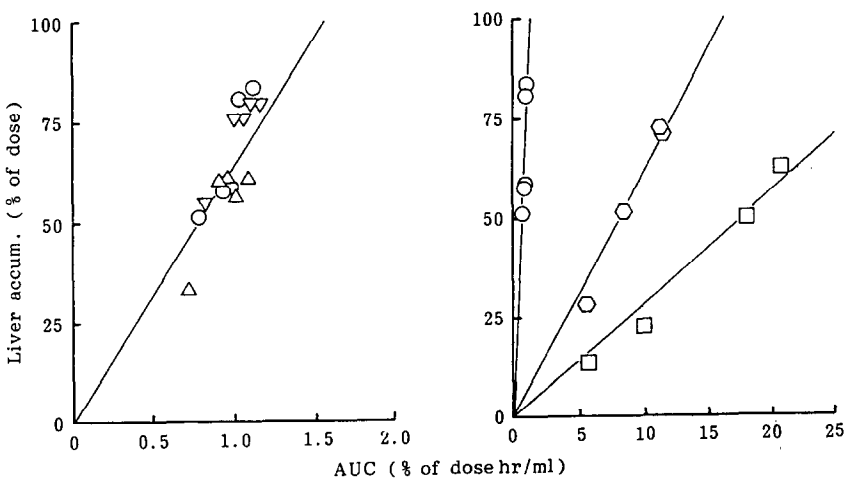

Fig. 4. Relationship between the liver accumulation at time t and the area under the plasma concentration-time curve after intravenous administration of ^{111}In-labeled Glc-BSA from time 0 to t at doses of 0.05 (△), 0.1 (▽), 1 (◇), 10 (○), and 20 (□) mg/kg. Results are expressed as the means of four mice.

and 20 mg/kg doses increased with AUC_{0-t}, indicating that the process of hepatic uptake follows saturable and non-linear kinetics. During the later phase of the experiment, however, the radioactivity of liver was found to decrease gradually, suggesting slight release of ^{111}In from the tissue (data not shown). Therefore, the pharmacokinetic analysis was performed during a relatively early period when the plasma concentration had decreased by less than 1% of dose/ml and efflux of ^{111}In radioactivity from the liver was negligible.

It is well known that liver parenchymal cells have a receptor for galactose-terminated glycoproteins, which are rapidly incorporated from the circulation (Ashwell and Morell, 1974; Ashwell and Harford, 1982). Kupffer and liver endothelial cells are also known to possess a receptor for mannose-terminated glycoproteins (Jones and Summerfield, 1988; Smedsrød et al., 1990). Except for the liver, some organs such as bone marrow and spleen are reported to take up glycoprotein (Regoeczi et al., 1980; Summerfield et al., 1982). In the present study, Gal-BSA disappeared rapidly from the plasma and accumulated in the liver at lower doses (Fig. 2). This rapid disappearance was in accordance with data on the ligands for the asialoglycoprotein receptor such as asialoorosomucoid (Ashwell and Morell, 1974) and galactosylated albumin (Vera et al., 1984a). Therefore, these glycosylated BSA derivatives would appear to be good models of glycosylated proteins delivered to liver parenchymal or non-parenchymal cells or to be suitable candidates for use as carriers for delivery of conjugated drugs.

In this study, Glc-BSA was also taken up by the liver as rapidly as Gal-BSA (Fig. 2). This finding is consistent with the in vitro observation reported by Kawaguchi et al. (1981) that glucosylated albumin synthesized by this procedure was incorporated by isolated rat hepatocytes via the same receptor as Gal-BSA. However, Glc-BSA partly inhibited the liver uptake of Man-BSA (Table 2) and had a relatively rapid rate of uptake in the spleen (Table 1). These results suggested that Glc-BSA synthesized via this procedure might be recognized by both galactose- and mannose-terminated glycoprotein receptors.

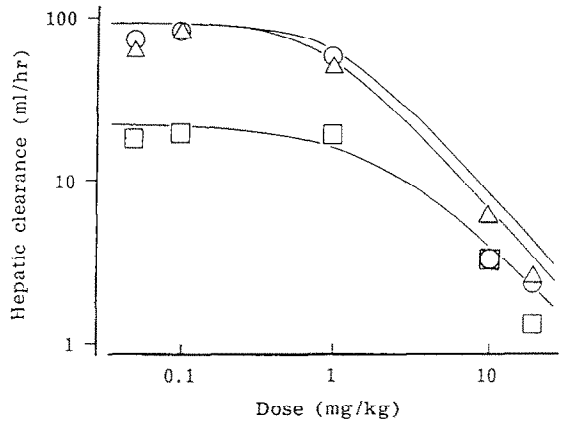

Fig. 5. Effect of dose on hepatic uptake clearances of ^{111}In-labeled glycosylated albumins in mice. Each point represents an apparent clearance value directly calculated from the accumulated amount in the liver and AUC, for Gal-BSA (○), Glc-BSA (△), and Man-BSA (□). Simulated curves were obtained from pharmacokinetic parameters estimated by curve fitting to the time courses of plasma concentration and liver accumulation.

In the present investigation, the hepatic uptake process and whole body distribution of glycosylated BSA were analyzed according to the physiological model shown in Fig. 1. The hepatic plasma flow, Q, was taken from the literature (Gerlowski and Jain, 1983) in order to estimate the intrinsic (free from the plasma flow) values of $K_{m,l}$ and $V_{max,l}$ for liver uptake. The saturable process of elimination from the plasma pool would represent the uptake by organs such as bone marrow and spleen. Data fitting was performed using the results recorded within the initial 1 h during which efflux from the liver was negligible (Fig. 4). Fig. 5 compares the hepatic clearances against dose determined by curve simulation using the estimated values of $K_{m,l}$, $V_{max,l}$, $K_{m,p}$, and $V_{max,p}$ listed in Table 3 and by model-independent calculation. Good agreement is observed between the data. Pharmacokinetic analysis revealed that Gal-BSA and Glc-BSA had extremely high affinities in the liver uptake process (Fig. 5). Apparently, liver uptake of Gal-BSA and Glc-BSA was limited by the plasma flow (85 ml/h) at doses below 0.1 mg/kg (Fig. 5), indicating that these compounds were almost completely taken up by the liver during a single passage. Compared

with the equilibrium constant determined on the basis of in vitro experiments (Vera et al., 1984b), the $K_{m,l}$ value obtained for Gal-BSA is about 10-times larger. This discrepancy can be explained as a result of the difference in the experimental conditions. Therefore, this approach to estimate the disposition parameters from in vivo data should provide more appropriate information for predicting the disposition of glycosylated macromolecules in man as well as in other animals.

The affinity of glycosylated BSA for the liver might be affected by the degree of substitution of the sugar moieties. It has been shown previously that the clustering of sugar residues enhances binding to cell surface carbohydrate receptors of the liver (Baenziger and Maynard, 1980; Connolly et al., 1982; Lee et al., 1983). Vera et al. (1984b) studied in vitro binding to liver membranes of galactosylated BSA synthesized via the same method and found that the equilibrium constant between the cell membrane and galactosylated albumins was altered by the galactose densities. In the present study, the numbers of sugars incorporated into the albumin were 16, 22, and 15 for Man-BSA, Gal-BSA, and Glc-BSA, respectively. The numbers of sugar moieties in Gal-BSA and Glc-BSA appeared to suffice for liver targeting, since the rate of uptake achieved a similar value to that of hepatic plasma flow.

The present study has demonstrated the feasibility of using glycosylated albumins to deliver drugs to specific liver cells. In addition, it has been reported that this method of modification does not impair the activity of the protein (Lee et al., 1976), and thus biologically active proteins might also be delivered to the liver using this procedure. In comparison with ^{111}In-labeled cationized BSA (Cat-BSA), which also shows considerable hepatic clearance (Takakura et al., 1990) due to electrostatic interaction with the cell surface (Nakane et al., 1988), these glycosylated albumins have a strong affinity and high specificity for liver cell types. However, the dose dependency in liver uptake is marked in glycosylated albumins whereas Cat-BSA shows constant hepatic clearance between 1 and 20 mg/kg (unpublished data). Therefore, glycosylated BSA should be utilized to target drugs selectively to liver parenchymal or non-parenchymal cells at relatively lower doses while Cat-BSA should be useful for delivering high drug loads nonspecifically to the liver.

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