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Pharmacokinetic disposition analysis of lipophilic drugs injected with various lipid carriers in the single-pass rat liver perfusion system

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Abstract

The hepatic disposition properties of [^{3}H]retinoic acid with moderate lipophilicity and having a logarithm of the *n*-octanol/water partition coefficient (log PC_{oct}) of 6.61 were analyzed in comparison with those of lipid carrier systems labeled by [^{14}C]cholesteryl oleate in an in situ single-pass rat liver perfusion experimental system. Oil-in-water (o/w) type emulsions with mean diameters of 252 and 85 nm, liposomes with a diameter of 122 nm and a HCO-60 micellar solution were tested as lipid carriers. During a single passage through the liver, the large emulsion showed marked uptake of [^{14}C]cholesteryl oleate incorporated in oil droplets, while the other three carriers showed almost complete recovery in the venous outflow, suggesting their 'stealthy' nature. The outflow patterns of [^{3}H]retinoic acid injected with lipid carriers were analyzed on the basis of moment analysis assuming first-order release from carrier particles, with the data for [^{3}H]retinoic acid injected in the form of an aqueous solution. Injection with micellar solution and the small emulsion demonstrated a rapid rate of release of [^{3}H]retinoic acid, however, the large emulsion and liposomes showed considerable retention and delivered [^{3}H]retinoic acid to the liver non-parenchymal cells and to the venous outflow side, respectively, stably entrapping it.

Keywords: Pharmacokinetic analysis; Hepatic disposition; Rat liver perfusion; Lipophilic drug; Release rate; o/w emulsion; Liposome; Micelle

1. Introduction

Recently, various drug carrier systems have been studied with the aim of precise control of the in vivo disposition of drugs. In the case of water-insoluble and lipophilic drugs or drug candidates, the application of lipid carrier systems is considered to be promising. However, it is still in the development stage and the accumulation of basic information about pharmaceutical and biological characteristics of drug/carrier complexes is required.

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In our previous study, the in vivo biodistribution of highly lipophilic drugs injected with lipid carrier systems such as liposomes, surfactant micelles and conventional o/w emulsion for parenteral nutrition was analyzed (Takino et al., 1993). In addition, an o/w emulsion system with a small particle size and 'stealthy' nature was developed and compared with other carriers with respect to its potential for controlling the disposition of lipophilic drugs (Takino et al., 1994). In these studies, the in vivo disposition characteristics of the carriers themselves and the relationship between lipophilicities and in vivo behavior of drugs was elucidated. These studies concluded that control of biodistribution and precise drug delivery would be realized only for highly lipophilic drugs with log PC_{oct} values greater than 9 or more. This finding offered a theoretical ground for derivatization of lipophilic prodrugs aiming at combination with lipid carrier systems (Hashida and Sezaki, 1984; Sasaki et al., 1985; Taniguchi et al., 1988; Tokunaga et al., 1988; Igarashi et al., 1992).

On the other hand, the distribution properties of moderately lipophilic drugs with a log PC_{oct} value below 9 cannot be effectively controlled and these compounds are considered to move to the tissue, apart from carriers, during the passage through the organs. As for this point, information about the retention capability of lipid carriers for lipophilic drugs and the uptake mechanisms of carriers and drugs by tissues is of interest and an important issue. For analyzing the drug disposition at an organ level, we have established in situ organ perfusion experiment systems, for example, for liver (Nishida et al., 1989; Sato et al., 1989), muscle (Kakutani et al., 1985; Nara et al., 1992), brain (Sakane et al., 1991), and kidney (Mihara et al., 1993). In this report, we studied the disposition of retinoic acid of log PC_{oct} of 6.61 in the liver, since this organ plays the predominant role in the whole body level disposition of retinoic acid (Takino et al., 1993, 1994). The tissue distribution and elimination of retinoic acid and various lipid carrier systems were elucidated in the single-pass rat liver perfusion system and evaluated based on pharmacokinetic analysis.

2. Materials and methods

2.1. Chemicals

[^{3}H]Retinoic acid (2060.9 GBq/mmol) and [^{14}C]cholesteryl oleate (2.2 GBq/mmol) were purchased from Daiichi Radioisotopes, Tokyo, Japan. Retinoic acid was obtained from Sigma Chemical Co., MO, U.S.A. Intralipid® (10%) was purchased from Otsuka Pharmaceutical Co., Tokushima, Japan. Polyoxyethylene derivative of hydrogenated castor oil (HCO-60), egg phosphatidylcholine (PC), and egg sphingomyelin (SM) were obtained commercially from Nikko Chemicals Co., Tokyo, Japan, Nippon Oil & Fats Co., Hyogo, Japan and Sigma Chemical Co., MO, U.S.A., respectively. All other chemicals were of the finest grade available.

2.2. Preparation of injection formulations

Liposomes composed of egg PC and egg SM (0.7:0.3) were prepared by a combination of the controlled dialysis method using a Lipoprep dialyzer® (Diachema) and sequential extrusion method (Tokunaga et al., 1988). A large emulsion was obtained by dilution of the 10% o/w emulsion used for parenteral nutrition (Intralipid®) consisting of egg PC and soybean oil (0.12:1). A small emulsion was prepared from egg PC, egg SM and soybean oil (0.7:0.3:1) by sonication (Otake Works, Japan, output 200 W, 60 min). The final lipid content of each formulation was adjusted to 5% (w/v). In addition, rat serum as a control formulation, which might not affect the original distribution property of retinoic acid, was prepared from fresh rat blood collected without heparin treatment through warming at 37°C for 3 h and sequential overnight storage at 4°C.

After evaporation of mixed solvent (ethanol/benzene = 5:1) containing [^{3}H]retinoic acid (0.75 MBq), retinoic acid (22 μg) and [^{14}C]cholesteryl oleate under reduced pressure, 8 ml of the large emulsion, small emulsion, HCO-60 micellar solution, liposome and rat serum were added and re-dissolved by overnight incubation. In the case of aqueous solution, a trace amount (0.75 MBq)

of [^{3}H]retinoic acid was dissolved in distilled water. Each formulation was filtered through a Millex GV sterile filter (Millipore) prior to animal experiments.

The particle size of each formulation was measured using a laser particle analyzer (LPA-3000, Otuka Electronics Co., Osaka, Japan).

2.3. Liver perfusion experiment

Male Wistar rats (190–210 g) with free access to standard rat foods and water were used. The operative procedure for in situ rat liver perfusion was described previously (Nishida et al., 1989). To avoid the effect of interaction with albumin and to simplify the perfusion system, liver perfusion was carried out with albumin-free perfusate. The perfusate was circulated using a peristaltic pump at an average flow rate of 13.01 ± 0.55 ml/min. Each formulation containing [^{3}H]retinoic acid and [^{14}C]cholesteryl oleate (2.75 μg/ml) was introduced into the portal vein using a six-position rotary valve injector as a pulse function. Venous outflow samples were collected into the previously weighed tubes at intervals of 0.5–4 s for 1 min. The sample volume was calculated from the gain in weight of the tube, assuming the density of the outflow perfusate to be 1.0 The time point of sampling was calculated from each sample volume assuming a constant flow rate. Bile samples were collected into the weighed small test tubes at 10-min intervals for 60 min. After the perfusion experiment, the whole liver was weighed and homogenized. The mean weight of the liver was 8.351 ± 0.902 g.

For determining intercellular localization, perfusion of Ca-free and collagenase-containing perfusate was started 1 min after the bolus injection of drugs and parenchymal cells (PC) were separated from nonparenchymal cells (NPC) by centrifugation (Horiuchi et al., 1985).

2.4. Analytical methods

The radioactivities of ^{3}H and ^{14}C in the effluent perfusate or bile were measured using a liquid scintillation counter (LSC-5000, Beckman, Tokyo, Japan) after addition of scintillation medium (Clear-sol I, Nacalai tesque, Tokyo, Japan). The radioactivity in the homogenized liver or separated cell suspension was measured in the same manner after dissolution with Soluene-350 (Packard, The Netherlands).

2.5. Pharmacokinetic analysis of outflow patterns

The detailed theoretical background of moment analysis for local perfusion experiments is described in our previous paper (Kakutani et al., 1985). This procedure has been applied to the analysis of the hepatic and renal disposition of macromolecular carriers, etc. (Nishida et al., 1989; Sato et al., 1989; Mihara et al., 1993).

The statistical moment parameters for the outflow pattern are defined as follows;

$$\mathrm{AUC}_i = \int_0^\infty C \, \mathrm{d}t \tag{1}$$

$$t_i = \int_0^\infty t \, C \, \mathrm{d}t / \mathrm{AUC}_i \tag{2}$$

where t is time and C represents the concentration of compounds normalized with respect to the injection dose as percentage of the dose per ml. AUC_i and t_i denote the area under the concentration-time curve and mean transit time of the compounds through the liver, respectively. The moment parameters are calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al., 1978; Kakutani et al., 1985). The t_i values were corrected for the lag time of the catheter.

The disposition parameters representing reversible and irreversible processes in the hepatic disposition of lipid carriers or the injected lipophilic drug are calculated from the moment parameters. The methods of derivation of the disposition parameters are summarized as follows;

$$V_i = Qt_i / F_i \tag{3}$$

$$t_{\mathrm{ret},i} = t_i / F_i \tag{4}$$

$$F_i = \mathrm{AUC}_i Q \tag{5}$$

$$E_i = 1 - F_i \tag{6}$$

$$k_{el,i} = E_i / t_i \quad (7)$$

$$CL_{int,i} = k_{el,i} V_i \quad (8)$$

where V_i is the apparent distribution volume, reflecting reversible interaction, $t_{ret,i}$ denotes the retention time, F_i corresponds to the recovery ratio (hepatic availability), E_i denotes the extraction ratio, $k_{el,i}$ is the first-order irreversible elimination rate constant, $CL_{int,i}$ refers to the intrinsic clearance, and Q represents the perfusion rate. The definition of unidirectional uptake without any reverse efflux is valid on the grounds that the hepatic disposition analysis is focused just on the quite early phase in this study. These parameters can be divided into three groups, i.e., parameters representing reversible (V_i and $t_{ret,i}$) and irreversible (E_i, F_i, and $k_{el,i}$) processes and both ($CL_{int,i}$).

2.6. Estimation of release rates of [^{3}H]retinoic acid from lipid carriers

Concerning the hepatic uptake of moderately lipophilic [^{3}H]retinoic acid injected with various lipid carrier systems, two possible uptake processes, i.e., uptake in an incorporated form in the carrier particles and uptake in a free form after release from the carriers, are considered. Then, a well-stirred condition-based analysis can be utilized for estimating the contribution of these two kinetic processes to the gross hepatic uptake of [^{3}H]retinoic acid (Fig. 1). Dose injection (100%) is assumed to be performed in a carrier-incorporated form, since dilution by perfusion can be neglected during the initial stage. The two parallel processes, i.e., the direct uptake being incorporated in carriers and the release to the free form compartment (RA,free) are defined by first-order kinetics with rate constants of $k_{RA,car}$ and $k_{RA,rel}$, respectively. The released [^{3}H]retinoic acid in the free form compartment is also taken up with a first-order rate constant of $k_{RA,free}$. The remaining parts of [^{3}H]retinoic acid in the carrier and free form compartments is eluted to the venous outflow with the convective flow. Then, mass balance equations for the concentration of [^{3}H]retinoic acid in the incorporated form and free form compartments, respectively, are expressed as:

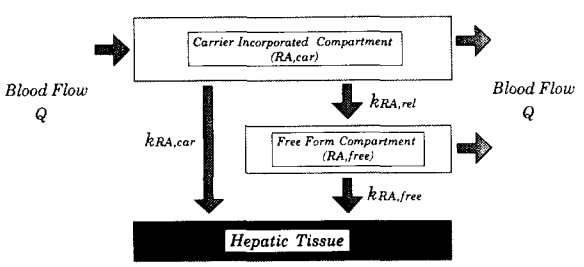

Fig. 1. Schematic diagram of well-stirred condition-based disposition model for [^{3}H]retinoic acid injected with various lipid carrier systems.

$$V_{RA,car}\frac{d}{dt}C_{RA,car} = Q(C_p - C_{RA,car}) - (k_{RA,car} + k_{RA,rel})V_{RA,car}C_{RA,car} \quad (9)$$

$$V_{RA,free}\frac{d}{dt}C_{RA,free} = k_{RA,rel}V_{RA,car}C_{RA,car} - QC_{RA,free} - k_{RA,free}V_{RA,free}C_{RA,free} \quad (10)$$

where C_p, $C_{RA,car}$ and $C_{RA,free}$ denote the concentration of [^{3}H]retinoic acid in the inflow, the incorporated form compartment and the free form compartment, and $V_{RA,car}$ and $V_{RA,free}$ represent the distribution volume of the two compartments, respectively. As for the inflow concentration (C_p), bolus input is considered to be equivalent to a Dirac delta function ($\delta(t)$). Besides, the total concentration of [^{3}H]retinoic acid can be considered as follows:

$$C_{RA} = C_{RA,car} + C_{RA,free} \quad (11)$$

Eq. 9–11 are solved by Laplace transforms, and yield the recovery ratio (F_{RA}) for total [^{3}H]retinoic acid injected with lipid carriers via the zeroth moment. That is:

$$F_{RA} = [(Q + k_{RA,free}V_{RA,free} + k_{RA,rel}V_{RA,car})Q] / [(Q + k_{RA,free}V_{RA,free}) \times \{Q + (k_{RA,car} + k_{RA,rel})V_{RA,car}\}] \quad (12)$$

The release rates of [^{3}H]retinoic acid from various lipid carriers are numerically estimated

from Eq. 12 under the two assumptions; i.e., (i) [^{3}H]retinoic acid incorporated in the carrier particles has the same distribution volume and is also eliminated by the liver in accordance with the same rate constant as those of the carriers (V_{CO}, k_{CO}), which can be estimated from the data of [^{14}C]cholesteryl oleate incorporated in carriers as:

$$V_{RA,car} = V_{CO} \quad (13)$$

$$k_{RA,car} = k_{CO} \quad (14)$$

and (ii) the pharmacokinetic parameters of [^{3}H] retinoic acid in the free form compartment following the release from the carrier ($k_{RA,free}$ and $V_{RA,free}$) are the same as those estimated for [^{3}H]retinoic acid injected in the form of solution ($k_{el,i}$ and V_i).

The mean transit time for [^{3}H]retinoic acid injected with lipid carriers (t_{RA}) can also be given by the first moment from Eq. 9–11, but the equation is too complicated to estimate the release rate from the view point of the accuracy of experimental data. Therefore, we utilize the equation deduced via the zeroth moment (Eq. 12) for the release rate estimation.

To elucidate the contribution of release process to total hepatic uptake, the integrated recovery in each process was calculated on the basis of the ratio of rate constants to their sum.

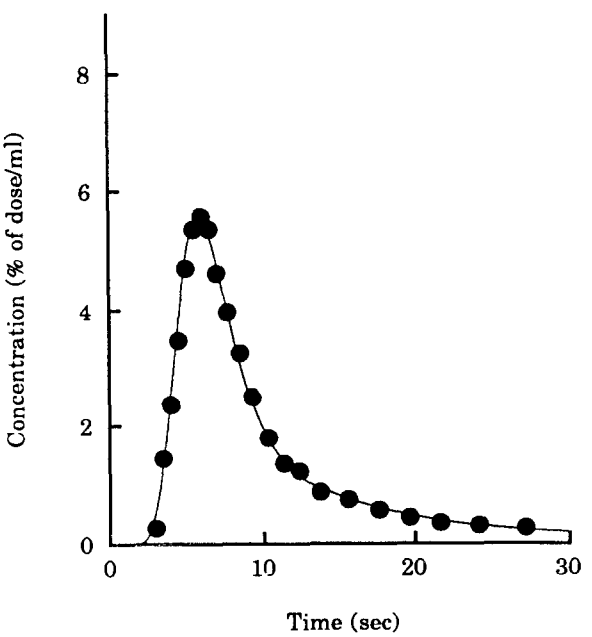

Fig. 2. Representative outflow pattern of [^{3}H]retinoic acid injected in solution in the single-pass rat liver perfusion system.

3. Results

3.1. Characterization of model compounds and lipid carriers

The partitioning coefficients between *n*-octanol and water (PC_{oct}) of [^{3}H]retinoic acid, model lipophilic compound, and [^{14}C]cholesteryl oleate, a marker of lipid carriers, were calculated according to the fragment method of Hansch et al. (1973) and Leo et al. (1975), and compared with their lipophilic indices (log k'_0) which were experimentally determined using a reversed-phase HPLC system (Yamana et al., 1977; Takino et al., 1994). Cholesteryl oleate has a log PC_{oct} value of 18.3 and a log k'_0 value of 11.0 which support the assumption that it behaves with lipid carriers as their marker during the passage of the liver. Retinoic acid is moderately lipophilic with a PC_{oct} value of 6.61 and log k'_0 value of 2.68.

The four injection formulations were the same as tested in the previous in vivo distribution study (Takino et al., 1993, 1994). The mean diameters of the large emulsion, small emulsion, HCO-60 micelle and liposomes were 252, 85.4, 12.3 and 122 nm, respectively.

3.2. Outflow patterns of lipid carriers and [^{3}H]retinoic acid injected with carriers

Fig. 2 illustrates a representative outflow concentration-time curve of [^{3}H]retinoic acid injected in the form of an aqueous solution. The outflow concentration was very low and its peak concentration was less than 10% of dose/ml. The outflow pattern of [^{3}H]retinoic acid injected with rat serum was comparable to that in aqueous solution (data not shown). Fig. 3 shows outflow patterns of [^{3}H]retinoic acid and carriers ([^{14}C]cholesteryl oleate) injected with various for-

mulations. Liposome and HCO-60 micelles showed extremely high peak concentrations of more than 80% of dose/ml, while the large emulsion resulted in a low peak concentration of about 30% of dose/ml. The peak concentrations of [^{3}H]retinoic acid were lower than those of its carriers, and the differences between each dilution curve of [^{3}H]retinoic acid were smaller than

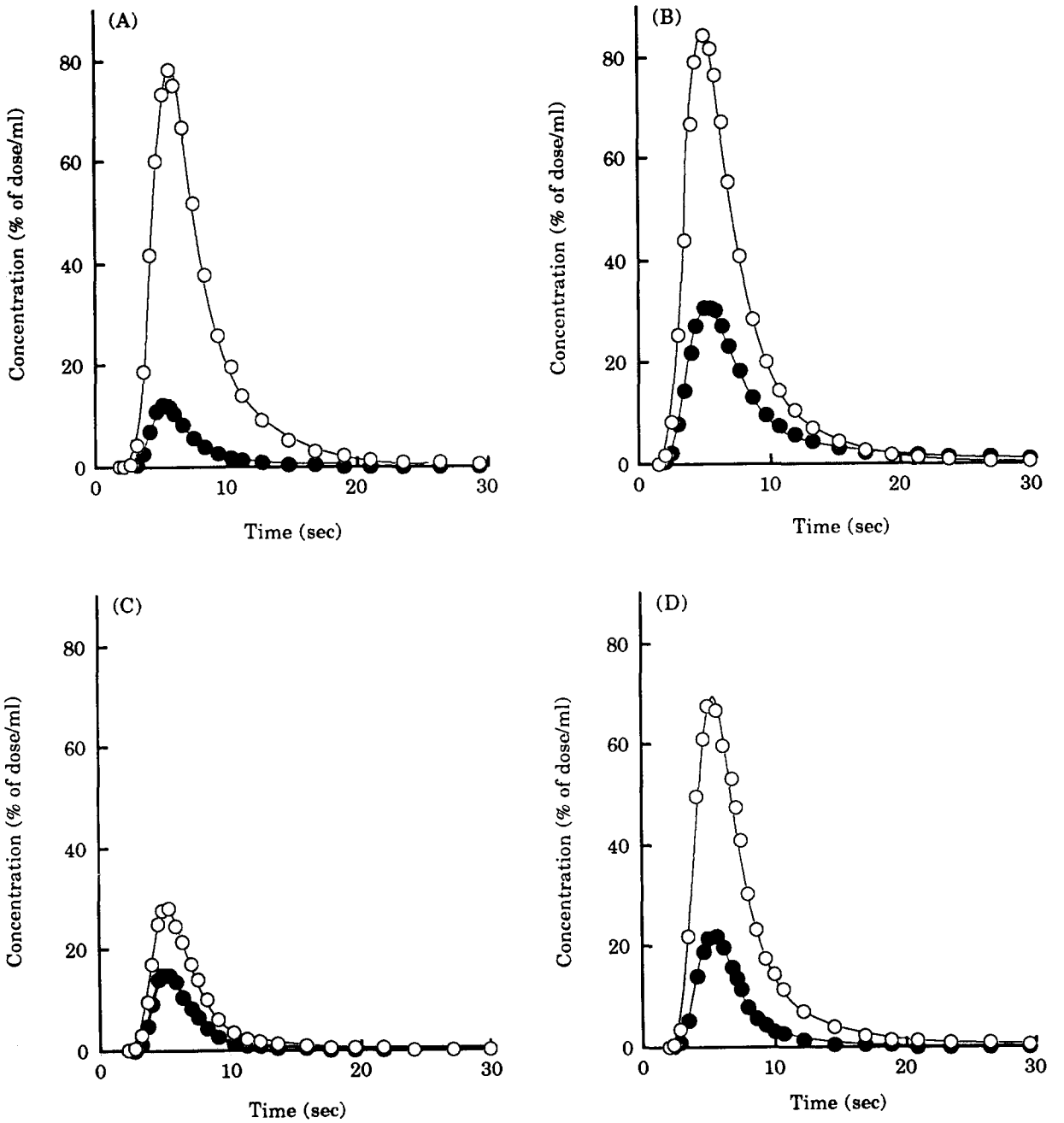

Fig. 3. Representative outflow patterns of (A) HCO-60 micellar solution, (B) liposomes, (C) large emulsion, and (D) small emulsion (○) labeled with [^{14}C]cholesteryl oleate and of [^{3}H]retinoic acid (●) injected with them.

those of carriers. The outflow peak concentration of [^{3}H]retinoic acid injected with HCO-60 micellar solution was almost the same as that with the large emulsion irrespective of the large difference in those of the carriers, while the former was much lower than that with liposomes in spite of the similarity in those of carriers.

3.3. Disposition parameters of lipid carriers

Each dilution curve was analyzed by moment analysis and the parameters are summarized in Table 1. The results for ^{51}Cr-RBC and ^{131}I-HSA are referred as vascular reference substance (VRS) from our previous report (Nishida et al., 1989). The parameters of [^{14}C]cholesteryl oleate are considered to represent those of injection carriers. [^{14}C]Cholesteryl oleate in HCO-60 micelles and liposomes showed almost equal AUC values to those of VRS and their hepatic availabilities (F_i) were close to 100%. The V_i values for ^{51}Cr-RBC and ^{131}I-HSA correspond to the volume of the sinusoidal space (0.209 ml/g liver) and sinusoidal space plus the space of Disse (0.252 ml/g liver), respectively. Since the V_i values of HCO-60 micelles and liposomes were comparable to that of ^{131}I-HSA, they are considered to simply distribute within the sinusoidal space plus the space of Disse without any interaction with the tissue. The large emulsion resulted in a rather low availability ($F_i = 52.2\%$), large distribution volume ($V_i = 0.801$ ml/g liver), and rapid elimination ($CL_{int,i} = 3.99$ ml/min per g liver), probably due to its reversible and irreversible interaction with the liver. However, the small emulsion gave almost the same values as VRS.

Table 1
Moments and disposition parameters for [^{3}H]retinoic acid and [^{14}C]cholesteryl oleate with various formulations in the single-pass liver perfusion system

Formulations	Moment parameter		Disposition parameter					Biliary [a] recovery (%)	Recovery [b] in liver (%)
	AUC_i (% of dose s/ml)	$\bar{t}_i$ (s)	V_i (ml/g)	$\bar{t}_{ret,i}$ (min)	E_i (%)	$k_{el,i}$ (/min)	$CL_{int,i}$ (ml/min/g)		
^{51}Cr-RBC [c]	471 ± 25	8.89 ± 0.83	0.209	0.148	0	–	0	–	–
^{131}I-HSA [c]	484 ± 14	9.33 ± 1.09	0.252	0.156	0	–	0	–	–
[^{3}H]Retinoic acid solution	41.3 ± 4.9	16.8 ± 2.94	5.04	3.24	91.1	3.36	16.6	13.1 ± 5.1	84.7 ± 8.5
[^{3}H]Retinoic acid									
Large emulsion	73.2 ± 18.5	7.80 ± 1.37	1.41	0.855	84.0	6.70	9.28	9.29 ± 1.22	51.6 ± 5.2
HCO-60 micellar solution	73.2 ± 15.5	8.67 ± 1.34	1.43	0.999	85.1	6.03	8.66	11.9 ± 2.1	56.9 ± 0.1
Liposomes	221 ± 17.7	22.7 ± 0.28	1.14	0.794	52.2	1.38	1.58	15.6 ± 1.1	14.2 ± 0.6
Small emulsion	89.2 ± 6.3	9.65 ± 1.72	1.37	0.839	80.4	5.14	6.80	10.1 ± 2.7	56.3 ± 2.2
Rat serum	48.7 ± 27.0	12.2 ± 2.41	4.97	3.05	89.3	4.51	20.7	7.23 ± 1.17	68.2 ± 5.2
[^{14}C]Cholesteryl oleate									
Large emulsion	135 ± 6.7	8.45 ± 1.80	0.801	0.479	70.7	5.32	3.99	0.260 ± 0.05	60.9 ± 1.7
HCO-60 micellar solution	420 ± 27.7	8.30 ± 1.59	0.237	0.158	12.2	0.889	0.207	0.262 ± 0.05	11.3 ± 3.3
Liposomes	438 ± 16.2	9.74 ± 2.05	0.248	0.171	5.33	0.352	0.0790	0.149 ± 0.18	0.374 ± 0.222
Small emulsion	407 ± 40.4	9.26 ± 0.57	0.286	0.174	11.2	0.757	0.222	0.143 ± 0.06	11.8 ± 6.5
Rat serum	264 ± 30.3	11.5 ± 1.32	0.557	0.339	42.2	2.20	1.23	0.354 ± 0.15	45.5 ± 5.2

[a] Totally recovered amount in bile until 60 min.
[b] Recovered amount in liver at 60 min.
[c] Data are refered from our previous report (Nishida et al., 1989).
Values are means ± S.D. of at least three experiments.

Table 2
Pharmacokinetic first-order rate constants of [^{3}H]retinoic acid injected with various lipid carriers in the single-pass rat liver perfusion experiment system

Formulations	$k_{RA,rel}$ [a,c]		$k_{RA,car}$		$Q/V_{RA,car}$ [c]	
	(min^{-1})	(%) [b]	(min^{-1})	(%) [b]	(min^{-1})	(%) [b]
Large emulsion	10.4	(59.0)	5.32 ± 1.40	(30.1)	1.95	(11.0)
HCO-60 micellar solution	83.7	(91.8)	0.89 ± 0.26	(1.0)	6.57	(7.2)
Liposomes	7.91	(54.4)	0.35 ± 0.14	(2.4)	6.28	(43.2)
Small emulsion	38.0	(86.0)	0.76 ± 0.55	(1.7)	5.45	(12.3)
Rat serum	99.8	(95.2)	2.20 ± 0.13	(2.1)	2.80	(2.7)

[a] Experimentally obtained parameters utilized for the estimation of $k_{RA,rel}$ are as follows: $Q = 13.01 \pm 0.55$ (ml/min); liver wet weight = 8.35 ± 0.90 (g); $k_{RA,free} = 3.36 \pm 0.61$ (min^{-1}); $V_{RA,free} = 5.04 \pm 0.92$ (ml).
[b] Percentage of each first-order rate constant to the sum of them.
[c] Parameters were calculated with utilizing the mean values of the other parameters.
Each parameter value represents the value calculated form mean values of at least three experiments.

3.4. Disposition parameters of [^{3}H]retinoic acid injected with carriers

The V_i value of retinoic acid injected in aqueous solution ($V_i = 5.04$ ml/g liver) was much larger than that of VRS and its rapid elimination ($CL_{int,i} = 16.6$ ml/min per g liver) was recognized. These values were similar to those in the case of rat serum injection. The disposition parameters of [^{3}H]retinoic acid led to an area of $V_i = 1.14$–1.43 ml/g liver and $CL_{int,i} = 1.58$–8.66 ml/min per g by injection with lipid carriers. Liposomes were most influential on the elimination process of retinoic acid ($CL_{int,i} = 1.58$ ml/min per g liver). Total recoveries of [^{3}H]retinoic acid were rather low in the case of injection with

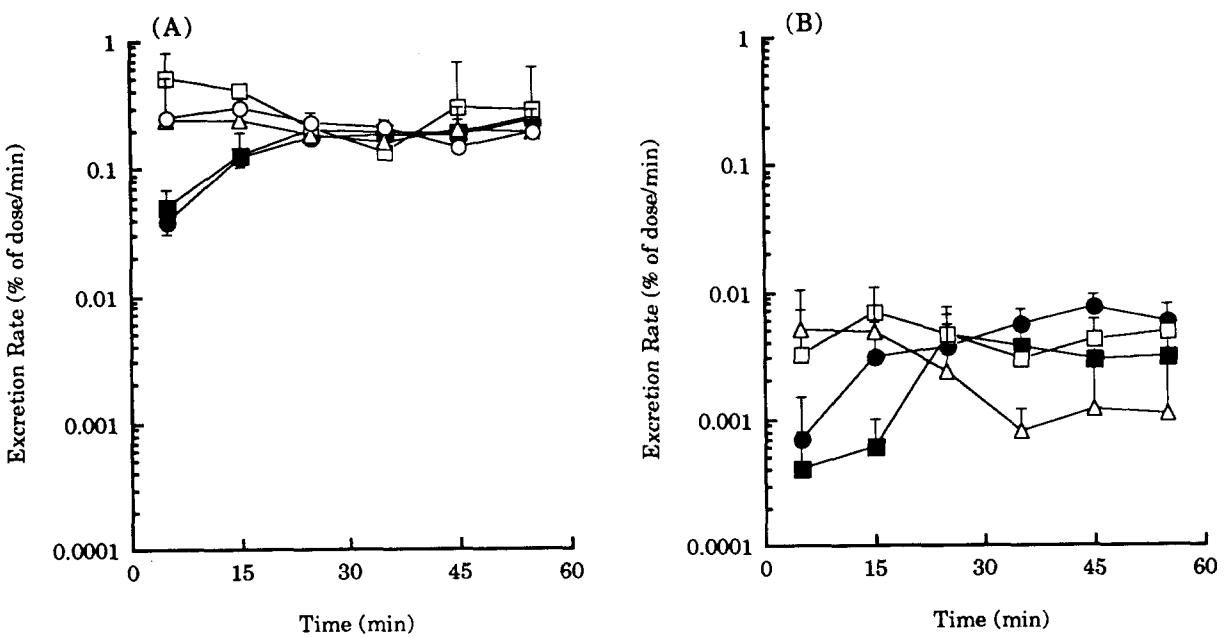

Fig. 4. Biliary excretion rate-time curves of (A) [^{3}H]retinoic acid and (B) [^{14}C]cholesteryl oleate injected with various lipid carriers in the single-pass rat liver perfusion system. Results are expressed as the mean ± S.D. of at least three experiments. (○) Solution; (●) large emulsion; (△) HCO-60 micellar solution; (□) liposome; (■) small emulsion.

lipid carriers, and this might result partly from later efflux of metabolites into the venous outflow.

3.5. Release rates of [^{3}H]retinoic acid from lipid carriers

In addition to the uptake with carriers ($k_{RA,car}$) and the release from carriers ($k_{RA,rel}$), convective movement of [^{3}H]retinoic acid to the venous outflow ($Q/V_{RA,car}$) is compared in Table 2.

$k_{RA,rel}$ values were relatively larger than $k_{RA,car}$ and $Q/V_{RA,car}$, and dominated the fate of [^{3}H]retinoic acid injected with lipid carriers. Injection with HCO-60 micellar solution and rat serum resulted in quite large release rate constants of 80–100 min^{-1}, whereas those of large emulsion and liposome were smaller (about 10 min^{-1}). As for $k_{RA,car}$, the large emulsion showed a somewhat greater value (5.32 min^{-1}), but the other formulations gave values below 1 min^{-1}. Regarding the convective removal rate ($Q/V_{RA,car}$), the larger distribution volume tends toward a smaller value as shown in the large emulsion (1.95 min^{-1}).

3.6. Biliary excretion of the radioactivities

In Fig. 4, biliary excretion of [^{3}H]retinoic acid and [^{14}C]cholesteryl oleate during 60 min after bolus injection is compared. Biliary excretion of [^{3}H]retinoic acid was relatively rapid in all formulations, whereas only a small amount of [^{14}C] cholesteryl oleate was recovered in the bile. In the case of emulsion carriers, the biliary excretion of [^{14}C]-cholesteryl oleate as well as that of [^{3}H]retinoic acid was delayed. In all cases, it was difficult to extrapolate the terminal phases to calculate the biliary recoveries over an infinite period.

3.7. Intercellular localization of the radioactivities

The cellular uptake of [^{14}C]cholesteryl oleate injected with lipid carriers except for the large emulsion was very small, in accordance with their

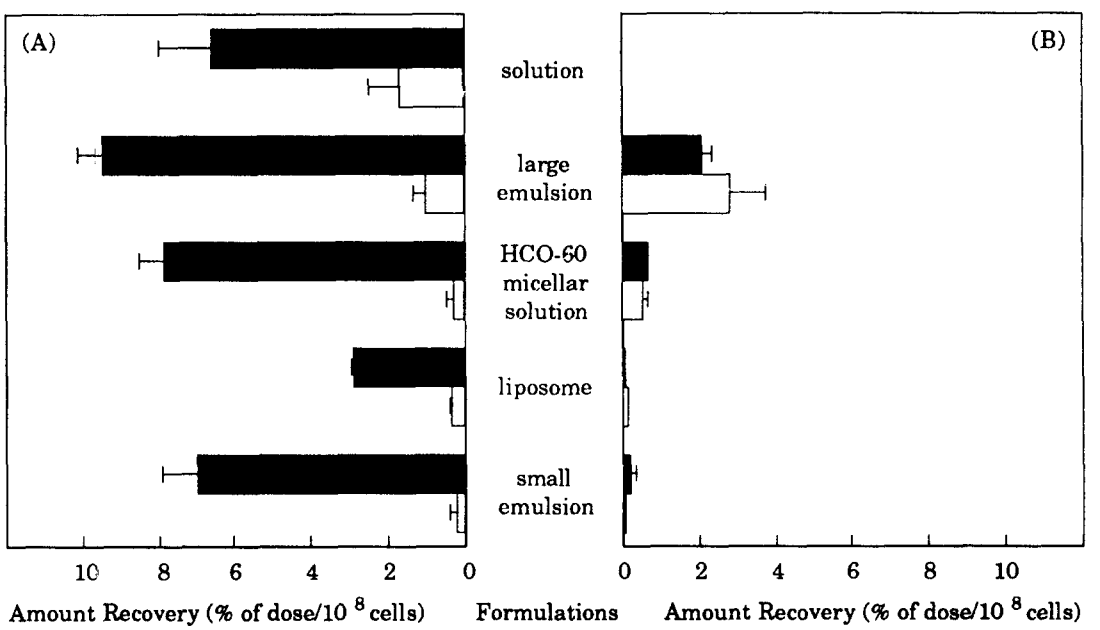

Fig. 5. Intercellular distribution of (A) [^{3}H]retinoic acid and (B) [^{14}C]cholesteryl oleate injected with various lipid carriers in the single-pass rat liver perfusion systems. Results are expressed as the mean ± S.D. of at least three experiments. (■) Parenchymal cells; (□) nonparenchymal cells.

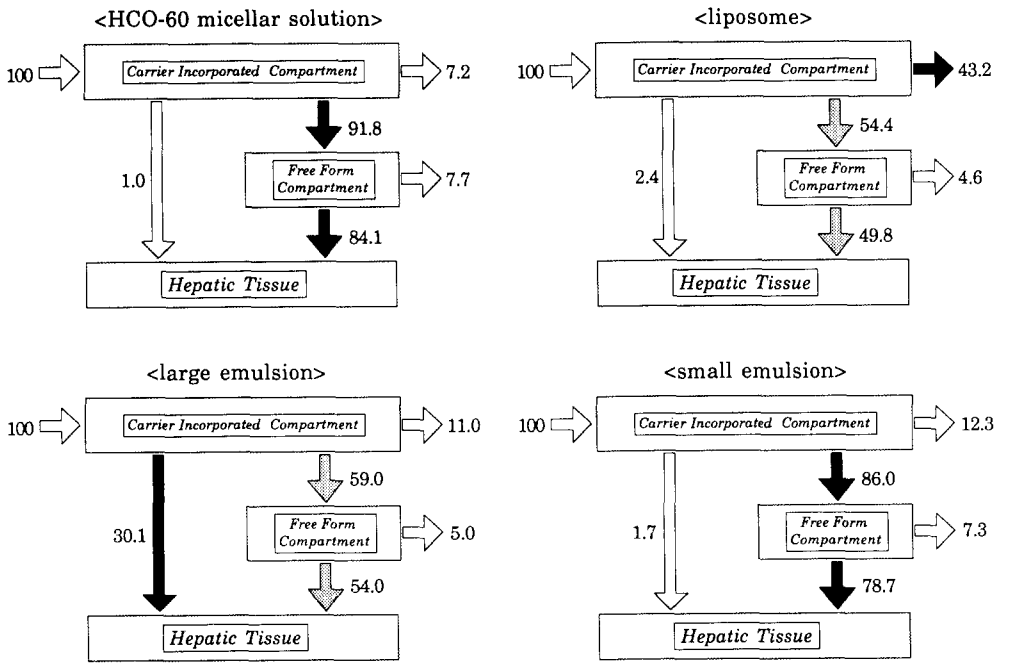

Fig. 6. A scheme representing contribution of each intrahepatic pharmacokinetic process of [^{3}H]retinoic acid injected with various lipid carrier systems estimated by integration of their rate constants.

high recovery in the venous outflow (Fig. 5). In the case of the large emulsion, [^{14}C]cholesteryl oleate was largely taken up by non-parenchymal cells. The radioactivity of ^{14}C was also detected in the parenchymal cells, which may suggest intercellular transport during collagenase perfusion and cell separation process. In contrast, [^{3}H]retinoic acid was preferentially recovered in the parenchymal cells in all lipid carriers, suggesting its rapid distribution therein from carriers. As for the large emulsion, carrier particles themselves were considerably taken up by non-parenchymal cells, so that [^{3}H]retinoic acid might be excreted into the bile somewhat slower.

4. Discussion

Organ perfusion experiments are widely employed for analyzing the disposition characteristics of drugs at an organ level. In previous reports (Takino et al., 1993, 1994), the total body disposition of lipid carriers and entrapped lipophilic drugs with different lipophilicities was systematically investigated. In the present investigation, their detailed disposition characteristics in the liver, a major elimination organ, were analyzed employing the single-pass rat liver perfusion experiment and moment analysis-based approach. Discussion was focused on comparisons of disposition patterns between different formulations.

As shown in Table 1, lipid carriers with stealthy nature such as small liposomes with SM, small SM emulsions, and HCO-60 micelles have similar distribution characteristics to those of the vascular reference substance ^{131}I-HSA, and their irreversible elimination rates are extremely slow. On the other hand, the large emulsion showed considerable distribution and rapid elimination, suggesting its interaction with hepatic tissue. Nonparenchymal cells such as Kupffer cells and endothelial cells on the vascular capillary bed would contribute to this irreversible uptake as shown in Fig. 5. These disposition profiles of various lipid carriers are consistent with the previous results obtained in the in vivo experiments. As for [^{3}H]retinoic acid with moderate lipophilicity, injection with lipid carriers resulted in apparently

similar disposition profiles regardless of the formulations. This is also in good agreement with previous in vivo data, however, attention should be paid to the fact that the apparent dilution curves in the outflow perfusate result from the balance of uptake rates for carriers and [^{3}H]retinoic acid and the release rate of the latter from the former.

In the present analysis, disposition parameters for [^{3}H]retinoic acid and lipid carriers were estimated based on the moment analysis and the release rates of [^{3}H]retinoic acid were obtained from these parameters. Dilution curves of drugs obtained after a single passage through the organ in a local perfusion experiment are frequently analyzed by the dispersion model (Roberts and Rowland, 1986; Yano et al., 1989) and distributed model (Tsao et al., 1988). However, curve fitting in such model analyses requires high accuracy in experimental data for estimating secondary parameters such as the release rate of drug from carriers. In order to estimate this kind of parameter correctly, we applied a novel analytical method based on moment analysis in this study. Through the present procedures, the release rates of [^{3}H]retinoic acid from carriers were obtained and relative contributions of different disposition processes to gross hepatic uptake could be elucidated.

Fig. 6 shows a schematic representation of total disposition patterns of [^{3}H]retinoic acid injected with four types of lipid carrier systems. The large emulsion showed particular features and 30% of [^{3}H]retinoic acid was taken up and incorporated. In the case of the small emulsion and HCO-60 micellar solution, approx. 80% of the injected dose was taken up by the liver in the free form. Thus, the hepatic disposition of [^{3}H]-retinoic acid was proved to be affected by the nature of lipid carriers through the release rate and their intrinsic uptake rate, although it apparently looks similar regardless of the formulations.

The large emulsion could achieve the potential delivery of moderately lipophilic drugs such as retinoic acid to the liver by incorporating into it, whereas the HCO-60 micellar solution acts only as a simple solubilizer and has no effect on the pharmacokinetic control of their disposition. Liposomes prevent such drugs from partitioning to the liver and keep them in the blood circulation or in the next tissue in an incorporated form. The small emulsion has similar characteristics to those of the HCO-60 micellar solution, i.e., poor retention ability, although it has a stealthy nature. Irrespective of their fundamentally common structure as o/w emulsions, there is a great difference in their retention abilities (release rates) between a large emulsion and a small emulsion. This should be explained by differences in the total surface area of particles between them (approx. 3-fold). There is also a difference between a small emulsion and liposomes in spite of the similar particle size and surface lipid composition of SM. This might be due to two physicochemical factors, i.e., liquid and multilamellar structures and fluidization of PC/SM layer by the presence of soybean oil. The information for each lipid carrier system obtained in this study would be useful for selecting lipid carrier systems for moderately and highly lipophilic drugs.

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