Hepatic Local Disposition of a Drug with High Protein Binding and High Hepatic Clearance Using BOF-4272 as a Model Drug

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The local disposition of a drug, which is efficiently eliminated by the liver in spite of very high binding with serum albumin, was investigated using BOF-4272 as a model drug in the hepatic perfusion system. Bovine serum albumin (BSA) labeled with Evans Blue was used as the marker of hepatic blood space. The perfusion experiments were carried out at 37°C and at 4°C to evaluate the local disposition in active and inactive livers, respectively. The perfusates included 0, 0.25, 0.5, 1.0 and 4.0% of BSA in the injection of BOF-4272, and 0 and 1.0% of BSA in the injection of Evans Blue. After an instantaneous injection of BOF-4272 or Evans Blue (labeling reagent of BSA), the outflow time profile from the liver was analyzed by curve-fitting based on two-compartment dispersion model. All estimated parameters of BSA were almost the same between 37°C and 4°C, which showed that the blood space in the liver was unaffected by perfusate temperature. The recovery ratio (F_H) and the mean transit time ($\bar{t}_H$) of BSA were 100% and about 0.1 min, respectively, both with BSA (1.0%) and without BSA in the perfusate. F_H of BOF-4272 increased from 20% to 50% with an increase in the perfusate BSA at 37°C, whereas F_H was almost constant (90%) regardless of BSA concentration at 4°C. $\bar{t}_H$ of BOF-4272 was almost 0.1 min regardless of BSA concentration at 37°C, whereas $\bar{t}_H$ decreased from 0.29 min to 0.17 min at 4°C with an increase in the perfusate BSA. A large elimination (10%) of BOF-4272 was noticed even at 4°C in the presence of perfusate BSA, which demonstrates that the passive transfer of BOF-4272 from BSA to the hepatic tissues is considerably rapid. This efficient transfer is responsible for the large hepatic clearance of BOF-4272 with very high binding with BSA.

Key words BOF-4272; xanthine oxidase inhibitor; dispersion model; MULTI (FILT); perfusion system

Global and local disposition studies have shown that some drugs are effectively eliminated by the liver in spite of their high protein binding in the blood. For example, though organic anions such as bromosulphophthalein, taurocholate and bilirubin are bound more than 95% to the serum albumin, the elimination of these anions by the liver is considerably rapid and large.^{1–4)} The theory of albumin-mediated hepatic transport was proposed to explain large hepatic elimination in spite of the large albumin binding. According to this theory, a conformational change in the serum albumin weakly bound to the hepatic membrane is considered to cause the easy release of the bound drug.^{5–7)}

BOF-4272, whose structure resembles that of xanthine, is a drug under development for the treatment of hyperuricemia by blocking xanthine oxidase/xanthine dehydrogenase in the liver.^{8,9)} Previous perfusion experiments without albumin in the perfusate showed that the hepatic local disposition of BOF-4272 was well described by two-compartment dispersion model,^{10–16)} that the hepatic elimination of BOF-4272 was large at 37°C, and the hepatic uptake (elimination plus distribution) was extensively dependent on the perfusate temperature.^{17,18)} Recently, a protein binding study using high performance frontal analysis (HPFA) demonstrated that BOF-4272 was bound more than 99% to human, bovine and rat serum albumins.¹⁹⁾

The purpose of the present study is to investigate the hepatic local disposition of a drug with high protein binding and high hepatic clearance using BOF-4272 as a model drug, by containing bovine serum albumin (BSA) in the perfusate. The perfusion experiment using rat liver is carried out at 37°C and at 4°C to evaluate the local

disposition of BOF-4272 in active and inactive livers, and to estimate the mechanism of efficient hepatic uptake in spite of the very high protein binding of BOF-4272.

MATERIALS AND METHODS

Animals Male Wistar rats, 190–225 g, with free access to standard rat food and water were supplied by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan).

Chemicals BOF-4272 was synthesized in Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). BSA (Fraction V) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Evans Blue was obtained from Nacalai Tesque (Kyoto, Japan). Dimethyl sulfoxide was supplied by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents for the Krebs-Ringer-bicarbonate buffer were of analytical grade.

Liver Perfusions Single-pass perfusion experiments using a rat liver were performed *in situ* according to the Mortimore perfusion method.²⁰⁾ Rats were anesthetized with pentobarbital (50 mg/kg i.p., Nembutal, Abbot Lab., North Chicago, IL, U.S.A.) and the common bile duct was cannulated with a polyethylene tube (0.61 mm o.d., PE-10, Becton Dickinson & Co., Parsippany, NJ, U.S.A.). The portal vein was rapidly catheterized with a polyethylene tube (1.67 mm o.d., Hayashi Co., Ltd., Kyoto, Japan), attached to the perfusate of Krebs-Ringer bicarbonate buffer with 10 mM glucose. The elution time profiles of Evans Blue (T-1824; marker of BSA) were measured at two BSA concentrations (0 and 1.0%) in the perfusate, and those of BOF-4272 were measured at five BSA concentrations (0, 0.25, 0.5, 1.0 and 4.0%) in the perfusate. During the experiment, a perfusate (pH

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7.4) previously saturated with 95% O₂–5% CO₂ was introduced into the rat liver using a peristaltic pump (RP-3N, Furue Sci. Co. Ltd., Tokyo, Japan) at a flow rate of 15.0 ± 0.16 ml/min per liver. The flow rate of 15.0 ml/min per liver has been adopted by Rowland and coworkers.^{14,15,21} Perfusion experiments of BSA and BOF-4272 were also carried out at two perfusate temperatures (37°C and 4°C). BSA solution (0.250 ml, 40 mg/ml) labeled with Evans Blue (4.0 mg/ml) in the perfusate or BOF-4272 solution (0.250 ml, 1.0 mg/ml) containing 10% dimethyl sulfoxide in the perfusate was injected instantaneously into the liver through the portal vein cannula using a six-way rotary valve injector (Hayashi Co., Ltd., Kyoto, Japan), and the outflow samples were collected at an interval of 1 s from a cannula inserted in the thoracic *vena cava* inferior. The eluent volume was calculated from the weight of each outflow sample. The exact sampling time was calculated from the eluent volume of each outflow sample at a constant flow rate. The delay time through the inlet and outlet catheter was subtracted from the outflow profile. The variance of the catheter transit time was less than $2.5 \times 10^{-5} \text{ min}^2$ which is about 3% or less compared with the variance of the liver perfusion data. Thus, the broadening of the injected sample in the injector loop and the catheter was negligible. The wet liver weight was 9.1 ± 0.8 g. The viability of the liver at 37°C was monitored by bile flow throughout the experiments. When the bile flow rate was less than 5 $\mu\text{l/min}$ at 37°C, the perfusion data were discarded. The bile flow rate was almost negligible at 4°C.

Analytical Procedure The concentration of BSA was determined as a complex of BSA and Evans Blue at 620 nm using a UV-1200 spectrometer (Shimadzu Co., Kyoto, Japan).²² Because of the high affinity of Evans

Blue to BSA, its free fraction was negligible.²³

The maximum UV absorption of BOF-4272 was at 319 nm, where the components in the effluent perfusate from the liver were confirmed to give no interference. Therefore, the concentration of BOF-4272 was determined spectrophotometrically at 319 nm by a UV-1200 spectrometer (Shimadzu Co., Kyoto, Japan). The correlation coefficient of the calibration line for BOF-4272 was more than 0.999 in the concentration range from 0.078 to 20 $\mu\text{g/ml}$.

Data Analysis All the drug disposition processes of BSA and BOF-4272 were assumed to be linear. The outflow time profiles of BSA and BOF-4272 after a bolus input were evaluated in one- and two-compartment dispersion models. When a drug is injected into the liver as a pulse input, the image equations for one- and two-compartment dispersion models with central elimination are given by Eqs. 1 and 2, respectively.^{10–12}

$$\tilde{C}(s) = \frac{M}{Q} \exp \left[\frac{QV_B}{2D_e} \left\{ 1 - \sqrt{1 + \frac{4D_e}{Q^2} (k_e + (1+k')s)} \right\} \right] \quad (1)$$

$$\tilde{C}(s) = \frac{M}{Q} \exp \left[\frac{QV_B}{2D_e} \left\{ 1 - \sqrt{1 + \frac{4D_e}{Q^2} \left(s + k_{12} + k_e - \frac{k_{12}k_{21}}{s + k_{21}} \right)} \right\} \right] \quad (2)$$

where M is the amount of dose injected, Q (ml/min per liver) is the flow rate of perfusate, D_e (ml²/min) ($= D_e A^2$) is the corrected dispersion coefficient, D_e is the effective dispersion coefficient, A is the cross-sectional area of blood space, V_B (ml) is the volume of hepatic blood space which is the sum of the volume of the sinusoid and the space of Disse, k_{12} and k_{21} are the forward and backward transfer rate constants, respectively, and k_e is the irreversible transfer rate constant (elimination rate constant) from the perfusate, mainly into the hepatic

Fig. 1. The Hepatic Outflow Time Profiles of BSA Labeled with Evans Blue at Two BSA Concentrations (0 and 1.0%) in the Perfusate. The left and right panels show the time profiles at 37°C and at 4°C, respectively.

Fig. 2. The Hepatic Outflow Time Profiles of BOF-4272 at five BSA Concentrations (0, 0.25, 0.5, 1.0 and 4.0%) in the Perfusate. The left and right panels show the time profiles at 37°C and at 4°C, respectively.

tissues. The ratio $k' (=k_{12}/k_{21})$ is the partition ratio, which is an index of the extent of drug distribution between the perfusate and hepatic tissues. Equations 1 and 2 have been rearranged from the original equations.¹⁰⁾ The sum of $k_{12} + k_e$ is an index for the rate of hepatic uptake (elimination plus distribution) in the central elimination model. The peripheral elimination model was demonstrated to be kinetically equivalent to the central elimination model.¹⁰⁾ In the central dispersion model, "elimination" means irreversible transfer into the hepatic tissues, and "distribution" means reversible movement between the blood space and the hepatic tissues.

According to the dispersion theory,¹⁰⁾ the hepatic recovery ratio (F_H) and the mean transit time ($\bar{t}_H$) for both one- and two-compartment models are given by

$$F_H = \exp \left[\frac{Q V_B}{2 D_c} \left\{ 1 - \sqrt{1 + \frac{4 D_c k_e}{Q^2}} \right\} \right] \quad (3)$$

$$\bar{t}_H = \frac{V_B}{Q} (1 + k') \sqrt{1 + \frac{4 D_c k_e}{Q^2}} \quad (4)$$

The dispersion number (D_N), the efficiency number (R_N) and the hepatic distribution volume (V_H) are calculated by Eqs. 5, 6 and 7, respectively.

$$D_N = D_c / Q V_B \quad (5)$$

$$R_N = k_e V_B / Q \quad (6)$$

$$V_H = V_B (1 + k') \quad (7)$$

The curve fittings of Eqs. 1 and 2 to the outflow profiles of BSA and BOF-4272 from the liver were carried out by means of MULTI(FILT)^{24,25)} on a mainframe computer (M-1800/30) in the Kyoto University Data Processing Center. In the curve-fitting to BSA data, the elimination rate constant, k_e , was assumed to be zero.²²⁾ The optimum dispersion model (whether one- or two-compartment) was tested by Akaike's information criterion (AIC),²⁶⁾ and the dispersion model with a smaller AIC value was adopted. Consequently, time profiles of BSA and BOF-4272 were both well described by two-compartment dispersion model. F_H and $\bar{t}_H$ were calculated from the estimated parameters according to Eqs. 3 and 4, respectively.

All experimental results are given as the mean $\pm$ S.D. Statistical analysis was performed by ANOVA at a significant level of 5% to assess the difference among the groups.

RESULTS

Figures 1 and 2 present the elution time courses of BSA and BOF-4272, respectively, at 37°C and 4°C. Table 1 presents the perfusion conditions and the estimated kinetic parameters of BSA labeled with Evans Blue in two-compartment dispersion model. F_H and $\bar{t}_H$ subsequently calculated from the kinetic parameters are also shown in Table 1. R_N and F_H are zero and 100%, respectively, because the hepatic elimination of BSA was assumed to be negligible.²²⁾ All kinetic parameters and moment values are almost the same between 37°C and 4°C by ANOVA (5% level). It was also found that the local disposition of BSA labeled with Evans Blue was not

affected by BSA (1%) in the perfusate. k' values of BSA were 0.1 to 0.25, which show the small distribution of BSA to the hepatic tissues with BSA (1%) and without BSA in the perfusate. The dispersion numbers (D_N) of BSA were 0.05 to 0.1 which almost agreed with those in the literature.¹⁶⁾ V_B values were 16% to 18% which were almost the same as those estimated by the stereological method.²⁷⁾ Thus, it was concluded that BSA has slight interaction with the hepatic tissues at 37°C and at 4°C, which is the reason why we adopted BSA as the blood space maker. The mean transit time ($\bar{t}_H$) was almost independent (0.12 min to 0.135 min) of the perfusate temperature and of the perfusate BSA concentration.

Table 2 presents the experimental conditions and the estimated kinetic parameters of BOF-4272 in two-compartment dispersion model as well as moment values (F_H and $\bar{t}_H$). The efficiency number (R_N) of BOF-4272 decreased from 1.7 to 0.65 with an increase in perfusate BSA at 37°C, whereas R_N was almost independent (0.12) of BSA concentration at 4°C. As an exception, R_N without BSA in the perfusate was slightly larger than others at 4°C. At 37°C and at 4°C, D_N values of BOF-4272 were almost constant (0.05 to 0.1), which also were the same as those of BSA (Table 1). Therefore, it is reconfirmed

Table 1. Experimental Conditions, Moment Values and Kinetic Parameters of BSA (10 mg/liver) in Two-Compartment Dispersion Model by MULTI (FILT)

Parameter	BSA Concentration (%)	
	0	1.0
Liver perfusion at 37°C		
Body weight (g)	214 $\pm$ 3	214 $\pm$ 11
Liver (g)	9.83 $\pm$ 1.16	10.2 $\pm$ 1.2
Flow (ml/min/liver)	15.0 $\pm$ 0.2	15.0 $\pm$ 0.2
D_c (ml ² /min)	1.36 $\pm$ 0.32	2.51 $\pm$ 1.17
V_B (ml)	1.57 $\pm$ 0.18	1.61 $\pm$ 0.10
k_e (min ⁻¹)	0	0
k_{12} (min ⁻¹)	3.32 $\pm$ 1.05	1.80 $\pm$ 1.07
k_{21} (min ⁻¹)	12.8 $\pm$ 3.1	22.0 $\pm$ 11.0
$k' (=k_{12}/k_{21})$	0.259 $\pm$ 0.051	0.109 $\pm$ 0.090
$V_H (=V_B(1+k'))$ (ml)	1.98 $\pm$ 0.24	1.78 $\pm$ 0.14
R_N	0	0
D_N	0.0576 $\pm$ 0.0100	0.102 $\pm$ 0.042
V_B (%) to liver	16.1 $\pm$ 0.4	15.9 $\pm$ 1.0
F_H (%)	100	100
$\bar{t}_H$ (min)	0.132 $\pm$ 0.017	0.119 $\pm$ 0.011
Liver perfusion at 4°C		
Body weight (g)	209 $\pm$ 6	220 $\pm$ 3
Liver (g)	10.1 $\pm$ 1.5	9.10 $\pm$ 0.46
Flow (ml/min/liver)	14.9 $\pm$ 0.2	14.9 $\pm$ 0.2
D_c (ml ² /min)	2.80 $\pm$ 1.81	1.31 $\pm$ 0.52
V_B (ml)	1.79 $\pm$ 0.17	1.65 $\pm$ 0.20
k_e (min ⁻¹)	0	0
k_{12} (min ⁻¹)	1.55 $\pm$ 0.99	3.73 $\pm$ 2.07
k_{21} (min ⁻¹)	11.1 $\pm$ 2.2	17.1 $\pm$ 0.9
$k' (=k_{12}/k_{21})$	0.130 $\pm$ 0.072	0.220 $\pm$ 0.124
$V_H (=V_B(1+k'))$ (ml)	2.02 $\pm$ 0.06	2.00 $\pm$ 0.19
R_N	0	0
D_N	0.101 $\pm$ 0.053	0.0529 $\pm$ 0.0168
V_B (%) to liver	17.9 $\pm$ 2.5	18.2 $\pm$ 3.0
F_H (%)	100	100
$\bar{t}_H$ (min)	0.135 $\pm$ 0.002	0.134 $\pm$ 0.011

Data are shown as mean $\pm$ S.D. ($n=3$). Moment values of F_H and $\bar{t}_H$ are calculated by Eqs. 3 and 4, respectively. V_B (%) to liver estimated from V_B and wet liver weight.

Table 2. Experimental Conditions, Moment Values and Kinetic Parameters of BOF-4272 (250 µg/liver) in Two-Compartment Dispersion Model by MULTI (FILT)

Parameter	BSA Concentration (%)				
	0	0.25	0.5	1.0	4.0
Liver perfusion at 37 °C					
Body weight (g)	207 ± 4	214 ± 12	207 ± 16	221 ± 2	210 ± 8
Liver (g)	9.83 ± 0.64	9.03 ± 0.81	9.20 ± 0.40	9.60 ± 0.46	9.13 ± 0.45
Flow (ml/min/liver)	14.9 ± 0.2	15.1 ± 0.2	14.9 ± 0.1	15.0 ± 0.2	14.9 ± 0.2
D_c (ml ² /min)	1.45 ± 0.29	1.32 ± 0.35	1.35 ± 0.67	1.36 ± 0.11	1.34 ± 0.17
V_B (ml)	1.65 ± 0.14	1.74 ± 0.04	1.65 ± 0.05	1.65 ± 0.10	1.49 ± 0.20
k_e (min ⁻¹)	15.0 ± 4.3	10.5 ± 1.8	9.18 ± 0.44	6.66 ± 0.29	6.68 ± 1.80
k_{12} (min ⁻¹)	3.80 ± 5.67	1.23 ± 0.91	1.67 ± 1.12	2.44 ± 2.79	3.23 ± 1.96
k_{21} (min ⁻¹)	34.2 ± 23.2	30.8 ± 3.2	18.0 ± 4.7	20.8 ± 15.2	13.3 ± 2.1
$k' (=k_{12}/k_{21})$	0.0903 ± 0.0909	0.0407 ± 0.0299	0.0857 ± 0.0510	0.0943 ± 0.0665	0.264 ± 0.207
$V_H (=V_B(1+k'))$ (ml)	1.79 ± 0.06	1.81 ± 0.10	1.79 ± 0.09	1.80 ± 0.06	1.86 ± 0.07
R_N	1.68 ± 0.61	1.21 ± 0.21	1.01 ± 0.03	0.733 ± 0.071	0.650 ± 0.082
D_N	0.0587 ± 0.0117	0.0502 ± 0.0131	0.0544 ± 0.0342	0.0550 ± 0.0029	0.0604 ± 0.0045
V_B (%) to liver	16.9 ± 2.5	19.4 ± 1.5	17.9 ± 0.3	17.2 ± 1.3	16.3 ± 1.5
F_H (%)	23.4 ± 10.9	32.1 ± 6.5	38.2 ± 2.0	49.5 ± 3.3	53.6 ± 4.1
$\bar{t}_H$ (min)	0.102 ± 0.003	0.108 ± 0.009	0.109 ± 0.011	0.111 ± 0.003	0.116 ± 0.005
Liver perfusion at 4 °C					
Body weight (g)	211 ± 1	208 ± 12	199 ± 10	215 ± 10	204 ± 13
Liver (g)	9.70 ± 0.61	9.23 ± 0.81	7.87 ± 0.47*	8.80 ± 1.31	9.00 ± 0.98
Flow (ml/min/liver)	15.1 ± 0.1	14.9 ± 0.1	15.1 ± 0.1	14.9 ± 0.1	14.9 ± 0.1
D_c (ml ² /min)	4.53 ± 0.48*	2.54 ± 0.81	2.04 ± 0.11	1.81 ± 0.65	1.61 ± 0.04
V_B (ml)	3.54 ± 0.33*	2.50 ± 0.16*	2.09 ± 0.39	2.09 ± 0.24*	1.92 ± 0.33
k_e (min ⁻¹)	1.65 ± 0.24*	0.752 ± 0.294*	0.934 ± 0.728*	0.808 ± 0.481*	1.08 ± 0.94*
k_{12} (min ⁻¹)	2.51 ± 0.17	2.52 ± 0.75	3.35 ± 0.51	3.47 ± 2.21	4.12 ± 2.91
k_{21} (min ⁻¹)	8.27 ± 1.05	10.0 ± 4.3*	9.94 ± 1.84	10.8 ± 3.1	11.3 ± 1.1
$k' (=k_{12}/k_{21})$	0.305 ± 0.034*	0.263 ± 0.058*	0.339 ± 0.030*	0.306 ± 0.114	0.380 ± 0.301
$V_H (=V_B(1+k'))$ (ml)	4.62 ± 0.37*	3.15 ± 0.10*	2.80 ± 0.54*	2.71 ± 0.14*	2.59 ± 0.15*
R_N	0.387 ± 0.056*	0.127 ± 0.051*	0.124 ± 0.091*	0.118 ± 0.076*	0.128 ± 0.111*
D_N	0.0855 ± 0.0129	0.0685 ± 0.0234	0.0659 ± 0.0089	0.0573 ± 0.0151	0.0575 ± 0.0111
V_B (%) to liver	36.4 ± 1.2*	27.1 ± 1.2*	26.7 ± 6.0	24.4 ± 6.1	21.8 ± 5.9
F_H (%)	68.8 ± 3.5*	88.3 ± 4.5*	88.7 ± 7.7*	89.1 ± 6.8*	88.5 ± 10.0*
$\bar{t}_H$ (min)	0.288 ± 0.028*	0.208 ± 0.007*	0.183 ± 0.036*	0.180 ± 0.009*	0.172 ± 0.010*

Data are shown as mean ± S.D. ($n=3$). Moment values of F_H and $\bar{t}_H$ are calculated by Eqs. 3 and 4, respectively. V_B (%) to liver estimated from V_B and wet liver weight. * Significant between 37 °C and 4 °C by analysis of variance (ANOVA) at 5% level.

that D_N is independent of the properties of drugs and is dependent on the structure of blood space (sinusoid plus the space of Disse). V_B values were almost independent (16% to 19%) of the perfusate BSA at 37 °C, whereas V_B decreased from 36% to 22% with an increase in perfusate BSA at 4 °C. Besides, V_B at 4 °C was unusually larger than those of BSA (Table 1), as is discussed below.

The index for the rate of nonequilibrium distribution (k_{21}) of BOF-4272 at 37 °C decreased from 35 min⁻¹ to 13 min⁻¹ with an increase in perfusate BSA, whereas k_{21} values at 4 °C were almost independent (8 min⁻¹ to 10 min⁻¹) by ANOVA (5% level). The hepatic recovery ratio (F_H) at 37 °C increased from 23% to 54% with an increase in the perfusate BSA concentration, whereas F_H at 4 °C was roughly independent (90%) of BSA concentration in the perfusate. As an exception, F_H without BSA in the perfusion was smaller (70%) than the others at 4 °C. The mean hepatic transit time ($\bar{t}_H$) of BOF-4272 at 37 °C was almost independent (0.10 min to 0.12 min) of BSA concentration in the perfusate, whereas $\bar{t}_H$ at 4 °C decreased from 0.29 min to 0.17 min with an increase in the perfusate concentration. $\bar{t}_H$ values of BOF-4272 at 37 °C were almost the same as those of BSA, the blood space maker (Table 1).

DISCUSSION

V_B in two-compartment dispersion model has been considered to be the sum of the sinusoidal space plus the space of Disse. In the present experiment, V_B values of BSA at 37 °C and 4 °C were 16% to 18% of the liver weight, respectively (Table 1), which almost agreed with those in the literature.^{22,27,28} V_B values of BOF-4272 at 37 °C were 16% to 19% which were roughly the same as those of BSA at 37 °C, whereas V_B values of BOF-4272 at 4 °C were 20% to 40% which were unusually greater than those of BSA at 4 °C. V_B values of BSA at 4 °C were about 18%, which demonstrates that the structure of blood space is unchanged by the temperature in the perfusate. Therefore, the unusually large V_B values may indicate that V_B actually consists of the volume of hepatic blood space plus the volume of *equilibrium* distribution in the hepatic tissues. This concept is similar to the classical 2-compartment model for global disposition,²⁹ where the volume of distribution at steady-state (V_{ss}) is given by

$$V_{ss} = V_c(1+k') \quad (8)$$

where V_c is the volume of distribution in the central compartment and k' is the partition ratio between the central and peripheral compartments. Equation 8 is noted

to be similar to Eq. 7. It has been known that V_c is usually greater than that of the blood space and includes the volume of equilibrium distribution.²⁹⁾

D_e is given by $D_e A^2$ where D_e is the effective dispersion coefficient and A is a cross-sectional area, whereas V_B is given by $L \cdot A$ where L is the length of blood space.¹⁰⁾ D_e decreased more rapidly than V_B did with an increase in the perfusate BSA at 4°C. The dispersion number (D_N) that is independent of A was almost independent of the perfusate concentration at 4°C. Therefore, the decreases in D_e and V_B were possibly explained by the decrease in A with an increase in the perfusate BSA, which may also suggests the existence of the phase of equilibrium distribution.

At 37°C, F_H of BOF-4272 increased with an increase in BSA in the perfusate, whereas $\bar{t}_H$ was almost constant. It was shown in the previous study that F_H and $\bar{t}_H$ are indices exclusively for elimination and distribution, respectively.¹⁶⁾ Beside, $\bar{t}_H$ values of BOF-4272 were almost the same as those of BSA (marker of hepatic blood space), which demonstrated that BOF-4272 taken up by the liver almost completely disappeared at 37°C. On the other hand, F_H of BOF-4272 at 4°C was almost independent of the perfusate BSA and $\bar{t}_H$ decreased with an increase in the perfusate BSA concentration, which demonstrated that the large portion of BOF-4272 taken up by the liver returned in an intact form to the perfusate at 4°C. It is reported that BOF-4272 is bound more than 99% to bovine serum albumin at 37°C.¹⁹⁾ Since the thermodynamic principle demonstrates that the protein binding at a low temperature is higher than that at a high temperature, the protein binding of BOF-4272 is predicted to be very high at 4°C.

Thus, it is concluded that BOF-4272 was eliminated at 4°C not by the hepatic metabolism but possibly by a strong physical adsorption on the hepatic tissues, whose capacity was calculated to be about 25 μg ($=0.1 \times 250 \mu\text{g}$) per liver. As a postulation, V_B , which has been considered to be the hepatic blood space, actually includes the volume due to the equilibrium distribution. The bound BOF-4272 in the perfusate is rapidly transferred from BSA to hepatic tissues without a spatial active transport system. Because the transferred BOF-4272 is very efficiently eliminated through the metabolic system by the liver at 37°C, the hepatic elimination is large, dependent on the perfusate BSA concentration. Because the transferred BOF-4272 was not further eliminated at 4°C, 10% of BOF-4272 was constantly eliminated, independent of the perfusate BSA concentration.

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