Influence of Albumin on Enantioselective Local Disposition of BOF-4272, a Xanthine Oxidase Inhibitor with Chiral Sulfoxide, in Rat Liver

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8-(3-methoxy-4-phenylsulfinylphenyl) pyrazolo[1,5-*a*]-1,3,5-triazine-4(1*H*)-one (BOF-4272) blocks xanthine oxidase/xanthine dehydrogenase in the liver. BOF-4272 with a sulfoxide chiral center includes *R*(+)- and *S*(-)-enantiomers. The enantioselectivity in the global disposition of BOF-4272 can be attributed to that in the local disposition of organs, especially the liver. Thus, the enantioselectivity in the hepatic local disposition of BOF-4272 was compared between *R*- and *S*-enantiomers by a hepatic perfusion experiment with a pulse input into the portal vein. The influence of perfusate albumin on the enantioselective local disposition was also investigated. The elution time profile of each BOF-4272 enantiomer from the liver into the hepatic vein was measured at four different bovine serum albumin (BSA) concentrations (0, 0.25, 1.0 and 4.0%) in the perfusate at 37 and 4°C. A crossover test was carried out for *R*- and *S*-enantiomers using one rat liver. In the absence of perfusate BSA at 37°C, hepatic extraction ratios (E_H) of *R*- and *S*-enantiomers of BOF-4272 were $75.6 \pm 4.3\%$ and $71.7 \pm 3.3\%$, respectively, which were statistically the same. In the presence of 4.0% BSA at 37°C, E_H values of *R*- and *S*-enantiomers were $31.7 \pm 4.6\%$ and $19.6 \pm 3.8\%$, respectively, which demonstrated that E_H of *R*-enantiomer was significantly greater than that of *S*-enantiomer ($p < 0.001$). In the absence and presence of perfusate BSA at 4°C, there was no significant difference in E_H between *S*- and *R*-enantiomers. An amplification of stereoselectivity with albumin was observed by the perfusion experiment using BOF-4272 enantiomers.

Key words BOF-4272; stereoselective local disposition; perfusate albumin; xanthine oxidase inhibitor

8-(3-methoxy-4-phenylsulfinylphenyl) pyrazolo[1,5-*a*]-1,3,5-triazine-4(1*H*)-one (BOF-4272), a derivative of pyrazolotriazine, is an inhibitor against xanthine oxidase/xanthine dehydrogenase which catalyzes the last step of purine catabolism.^{1–4)} Since BOF-4272 contains an asymmetric sulfoxide group (Fig. 1), BOF-4272 consists of *R*(+)- and *S*(-)-enantiomers. Recently, an asymmetric synthesis of *S*-enantiomer of BOF-4272 was reported by Matsugi *et al.*⁵⁾ The inhibition mechanism by BOF-4272 was elucidated by an *in vitro* study using milk xanthine oxidase/xanthine dehydrogenase, which demonstrated that *S*-enantiomer exhibited a potency 100 times more active than that of the *R*-enantiomer.⁶⁾ BOF-4272 significantly decreased the concentration of free radicals generated by xanthine oxidase and consequently diminished cell necrosis.⁷⁾ Both *R*- and *S*-enantiomers of BOF-4272 were bound more than 99% to human, bovine and rat serum albumins.⁸⁾ It was also shown in previous studies that the hepatic elimination of BOF-4272 was considerably large, even in the presence of perfusate bovine serum albumin (BSA) at 37°C, in spite of the high protein binding.^{9,10)} Investigations of the stereoselective disposition of drugs which contain a chiral sulfide moiety has been extremely limited.^{11,12)} The stereoselectivity in the global disposition is attributable to that in hepatic local disposition. There has been no report that investigates the influence of serum albumin on the stereoselective local disposition of these drugs in the liver.

The purpose of the present study is to make clear the enantioselective hepatic local disposition of *R*- and *S*-enantiomers in the absence and in the presence of perfusate BSA by means of the rat liver perfusion system with a pulse input. These studies were carried out at 37 and

4°C to estimate the mechanism of elimination of BOF-4272 enantiomers by the liver. The outflow time profile of each enantiomer was evaluated by local moment analysis.

MATERIALS AND METHODS

Animals Male Wistar rats ($n=40$, 182–230 g), supplied by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan), were allowed free access to standard rat food and water.

Chemicals *R*-enantiomer (>99%e.e.) and *S*-enantiomer (>99%e.e.) of BOF-4272 used in this study were synthesized at Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). BSA (Fraction V) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 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 Perfusion A single-pass perfusion experiment

Fig. 1. Chemical Structure of BOF-4272

Left and right panels show structures of *S*(-)- and *R*(+)-enantiomers of BOF-4272, respectively.

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using rat livers was performed *in situ* according to the Mortimore perfusion method.¹³⁾ 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 and 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. Each elution time profile of BOF-4272 enantiomers from the liver was measured at four different BSA concentrations (0, 0.25, 1.0 and 4.0%) of the perfusate. During the experiment, the perfusate (pH 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 14.9 ± 0.13 ml/min/liver. This flow rate has been adopted by Rowland and coworkers.^{14,15)} The perfusion experiment was also carried out at two perfusate temperatures (37 and 4°C). It was shown that the structure of the blood space in the liver at 4°C was the same as that at 37°C.^{9,10)} After a stabilization period of 15 min, a 0.25 ml portion of the BOF-4272 solution (1.0 mg/ml) dissolved in the perfusate containing 10% dimethyl sulfoxide was injected instantaneously into the liver through the portal vein cannula using a six-way rotary valve injector (Hayashi Co., Ltd., Kyoto, Japan). The effect of dimethyl sulfoxide on the liver was predicted to be negligible, because the administration amount (0.025 ml) of dimethyl sulfoxide was less than 0.37% of the wet liver weight. The outflow samples were collected at an interval of approximately 1 s from a cannula inserted in the thoracic inferior *vena cava*. The crossover test was carried out for *R*- and *S*-enantiomers using one rat liver at an administration interval of 15 min, after which the effect of the first administration is considered to be negligible. The eluent volume was calculated from the weight of each outflow sample. The exact sampling times were evaluated 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 mean wet liver weight was 8.43 ± 1.15 g ($n=40$). The viability of the liver at 37°C was monitored by bile flow throughout the experiments. The bile flow rate in all rats was greater than 5 μ l/min at 37°C, whereas the bile flow rate was almost negligible at 4°C.

Analytical Procedure BOF-4272 had a maximum UV absorption at 319 nm, and the effluent perfusate into the hepatic vein presented no interference at 319 nm. Therefore, the concentration of BOF-4272 in the effluent perfusate was determined spectrophotometrically at 319 nm using 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 of 0.078 to 20 μ g/ml. Effluent samples with a concentration higher than 20 μ g/ml were diluted further with the perfusate.

Data Analysis Maximum perfusate concentration ($C_{\max}$) and the time at maximum perfusate concentration ($t_{\max}$) were obtained directly from the perfusate concentration data.

Fig. 2. The Typical Hepatic Outflow Profile of BOF-4272 Enantiomers at Various BSA Concentrations

The BSA concentrations of A, B, C and D are 0, 0.25, 1.0 and 4.0%, respectively. The open and closed symbols specify 37 and 4°C, respectively. Circle and triangle show *S*- and *R*-enantiomers of BOF-4272, respectively. The insert is the semilogarithmic plot of the same data.

Table 1. Experimental Conditions, $C_{\max}$, $t_{\max}$, Local Moments and V_H (%) to Liver of BOF-4272 Enantiomers (250 $\mu\text{g/liver}$)

Parameters	BSA Concentration (%)			
	0	0.25	1.0	4.0
37 °C				
Body weight (g)	218 $\pm$ 12	198 $\pm$ 8	205 $\pm$ 11	193 $\pm$ 3
Liver (g)	8.93 $\pm$ 1.70	9.07 $\pm$ 0.58	8.40 $\pm$ 0.73	8.30 $\pm$ 1.13
Flow (ml/min/liver)	14.9 $\pm$ 0.2	15.0 $\pm$ 0.2	14.9 $\pm$ 0.1	14.9 $\pm$ 0.2
S(-)-BOF-4272				
$C_{\max}$ ($\mu\text{g/ml}$)	53.9 $\pm$ 7.7	90.5 $\pm$ 3.4	116 $\pm$ 7	148 $\pm$ 16
$t_{\max}$ (min)	0.0972 $\pm$ 0.0104	0.0878 $\pm$ 0.0091	0.0873 $\pm$ 0.0072	0.0743 $\pm$ 0.0132
F_H (%)	28.3 $\pm$ 3.3	44.8 $\pm$ 5.7	60.0 $\pm$ 2.7	80.4 $\pm$ 3.8
E_H (%)	71.7 $\pm$ 3.3	55.2 $\pm$ 5.7	40.0 $\pm$ 2.7	19.6 $\pm$ 3.8
$\bar{t}_H$ (min)	0.123 $\pm$ 0.010	0.110 $\pm$ 0.010	0.113 $\pm$ 0.007	0.128 $\pm$ 0.013
V_H (%) to liver	21.0 $\pm$ 3.2	18.4 $\pm$ 2.5	20.1 $\pm$ 1.8	23.4 $\pm$ 4.0
R(+)-BOF-4272				
$C_{\max}$ ($\mu\text{g/ml}$)	50.8 $\pm$ 9.7	82.9 $\pm$ 7.5*	104 $\pm$ 10**	134 $\pm$ 23*
$t_{\max}$ (min)	0.0966 $\pm$ 0.0091	0.0866 $\pm$ 0.0074	0.0804 $\pm$ 0.0092	0.0809 $\pm$ 0.0102
F_H (%)	24.4 $\pm$ 4.3	39.7 $\pm$ 5.5*	50.8 $\pm$ 4.0***	68.3 $\pm$ 4.6****
E_H (%)	75.6 $\pm$ 4.3	60.3 $\pm$ 5.5*	49.3 $\pm$ 4.0***	31.7 $\pm$ 4.6****
$\bar{t}_H$ (min)	0.117 $\pm$ 0.009*	0.106 $\pm$ 0.006	0.106 $\pm$ 0.010	0.116 $\pm$ 0.012*
V_H (%) to liver	20.0 $\pm$ 3.1*	17.6 $\pm$ 1.4	18.9 $\pm$ 1.5	21.1 $\pm$ 3.6*
4 °C				
Body weight (g)	199 $\pm$ 6	199 $\pm$ 8	203 $\pm$ 8	199 $\pm$ 20
Liver (g)	7.65 $\pm$ 0.37	8.55 $\pm$ 0.95	8.40 $\pm$ 1.26	7.68 $\pm$ 1.82
Flow (ml/min/liver)	14.9 $\pm$ 0.1	14.9 $\pm$ 0.1	14.8 $\pm$ 0.0	14.9 $\pm$ 0.1
S(-)-BOF-4272				
$C_{\max}$ ($\mu\text{g/ml}$)	55.7 $\pm$ 4.8	101 $\pm$ 7	123 $\pm$ 7	132 $\pm$ 11
$t_{\max}$ (min)	0.144 $\pm$ 0.008	0.110 $\pm$ 0.014	0.0934 $\pm$ 0.0150	0.0980 $\pm$ 0.0204
F_H (%)	77.3 $\pm$ 3.4	91.9 $\pm$ 2.8	95.1 $\pm$ 4.4	94.6 $\pm$ 2.7
E_H (%)	22.7 $\pm$ 3.4	8.1 $\pm$ 2.8	4.9 $\pm$ 4.4	5.4 $\pm$ 2.7
$\bar{t}_H$ (min)	0.275 $\pm$ 0.016	0.195 $\pm$ 0.013	0.169 $\pm$ 0.015	0.161 $\pm$ 0.028
V_H (%) to liver	53.5 $\pm$ 3.7	34.1 $\pm$ 3.9	30.4 $\pm$ 7.1	31.6 $\pm$ 3.8
R(+)-BOF-4272				
$C_{\max}$ ($\mu\text{g/ml}$)	54.4 $\pm$ 3.2	98.0 $\pm$ 7.9	115 $\pm$ 12	128 $\pm$ 10
$t_{\max}$ (min)	0.144 $\pm$ 0.009	0.116 $\pm$ 0.013	0.0999 $\pm$ 0.0064	0.0936 $\pm$ 0.0179
F_H (%)	74.6 $\pm$ 2.1	94.4 $\pm$ 4.4	90.0 $\pm$ 7.7	92.8 $\pm$ 2.9
E_H (%)	25.4 $\pm$ 2.1	5.6 $\pm$ 4.4	10.0 $\pm$ 7.7	7.2 $\pm$ 2.9
$\bar{t}_H$ (min)	0.278 $\pm$ 0.016	0.220 $\pm$ 0.010**	0.182 $\pm$ 0.008	0.158 $\pm$ 0.021
V_H (%) to liver	54.0 $\pm$ 2.1	38.6 $\pm$ 4.6**	32.4 $\pm$ 4.1	31.1 $\pm$ 3.9

Data at 37 °C and 4 °C are shown as mean $\pm$ S.D. of 6 and 4 rats, respectively. F_H and $\bar{t}_H$ calculated by trapezoidal integration with the extrapolation into infinite time. V_H (%) was calculated from V_H and wet liver weight. The difference of parameters between *R*- and *S*-enantiomers of BOF-4272 is significant at levels (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$).

Local moment characteristics such as the hepatic recovery ratio (F_H) and the mean transit time ($\bar{t}_H$) of time profiles of *R*- and *S*-enantiomers in the perfusate were calculated by trapezoidal integration with the extrapolation into infinite time.^{16,17)} The hepatic extraction ratio $E_H (= 1 - F_H)$ was also calculated from the recovery ratio. The hepatic distribution volume V_H (%) is calculated by Eq. 1.¹⁸⁾

$$V_H (\%) = \bar{t}_H \cdot Q / Wt \quad (1)$$

where Q is the flow rate of the perfusate and Wt is the weight of wet rat liver. All experimental results are given as mean $\pm$ S.D. in Table 1, and statistical analysis was performed using the paired Student's *t*-test (two-tailed) with a significance level of $p < 0.05$.

The variance in catheter transit time was less than $2.5 \times 10^{-5} \text{ min}^2$, which was about 3% or less compared with the variance of the liver perfusion data. Thus, the diffusion of the injected sample in the injector loop and the catheter was considered to be negligible.

RESULTS

Typical hepatic outflow profiles of *R*- and *S*-enantiomers are shown in Fig. 2. Table 1 presents the experimental conditions, $C_{\max}$, $t_{\max}$, local moments and V_H (%) of the enantiomers. $C_{\max}$ values of *R*- and *S*-enantiomers were the same in the absence of BSA at 37 °C, whereas $C_{\max}$ of *R*-enantiomer was smaller than that of *S*-enantiomer in the presence of BSA at 37 °C. There was no significant difference between $C_{\max}$ values of *R*- and *S*-enantiomers in either the absence or presence of BSA at 4 °C (Fig. 2, Table 1). $t_{\max}$ was the same between *R*- and *S*-enantiomers under all experimental conditions (Table 1). F_H values of *R*- and *S*-enantiomers at 37 °C increased with an increase in the perfusate BSA concentration, whereas F_H values of enantiomers at 4 °C were roughly independent of BSA concentration in the perfusate (Table 1). Fig. 3 shows E_H ratio (*R*/*S*) of *R*- and *S*-enantiomers of BOF-4272 versus BSA concentration in the perfusate. E_H ratio (*R*/*S*) at 37 °C was almost unity without perfusate BSA and in-

Fig. 3. E_H Ratio (R/S) of R - and S -Enantiomers of BOF-4272 versus BSA Concentration in the Perfusate at 37 (A) and 4°C (B)

Each point at 37 and 4°C is shown as the data of 6 and 4 rats, respectively.

creased linearly with an increase in the perfusate BSA concentration, whereas R/S at 4°C was independent of the BSA concentration and is almost unity. V_H values at 37°C were almost the same between BOF-4272 enantiomers and were independent of the perfusate BSA concentration. V_H values at 4°C were roughly the same between R - and S -enantiomers and decreased with an increase in the perfusate BSA concentration (Table 1).

DISCUSSION

The considerably large elimination of BOF-4272 at 4°C may possibly be due to a strong physical adsorption and/or passive transport on the hepatic tissues, because the active transport was predicted to be completely blocked at 4°C.^{19,20} There was no significant difference in E_H between R - and S -enantiomers either in the absence or presence of BSA at 4°C, and E_H was independent of BSA concentration in the perfusate. Therefore, a strong physical adsorption and/or passive transport of BOF-4272 was not enantioselective.

At 37°C, there was no significant difference in E_H be-

tween R - and S -enantiomers in the absence of BSA, whereas E_H ratio (R/S) significantly increased with an increase in the perfusate BSA concentration (an amplification of enantioselectivity), as shown in Fig. 3. The unbound fraction of R -enantiomer to BSA was slightly smaller than that of S -enantiomer at 4% BSA concentration.⁸) If stereoselective protein binding plays an important role in the elimination of BOF-4272, S -enantiomer would be eliminated more rapidly than R -enantiomer. There is a theory that attempts to explain the high hepatic uptake of drugs with very high binding to albumin.²¹⁻²³) The theory is based on the concept that a conformational change in the albumin bound to the hepatic membrane causes the easy release of bound drugs.^{23,24}) If physical interaction of the perfusate albumin with the hepatocytes is important in the elimination of BOF-4272 enantiomers, the enantioselectivity at 4°C would also be observed in the presence of BSA. The partition ratio (k') of BSA was 0.1 to 0.2 and almost the same between 37 and 4°C, which demonstrates that BSA has little interaction with the liver.¹⁰) Therefore, an amplification of stereoselectivity with BSA is not sufficiently explained by the stereoselective protein binding of BOF-4272 enantiomers. The unbound fractions of R - and S -enantiomers to BSA were reported to be about 0.2% in 4% BSA concentration at 37°C.⁸) The experiment using rat hepatocytes suspended in a medium demonstrated that the uptake rate of R -enantiomer of BOF-4272 was greater than that of S -enantiomer at a low concentration, whereas R - and S -enantiomers were eliminated at almost the same rate at a high concentration.²⁴) Thus, the elimination of BOF-4272 enantiomers is predicted to be capacity-limited. The result that no stereoselectivity in the elimination of BOF-4272 was observed in the absence of perfusate albumin may be explained by the unbound concentration being high; consequently, the uptake rate of the enantiomers was almost the same due to the saturation in the hepatic elimination. The fact that the enantioselective elimination became obvious with an increase in the perfusate albumin may be explained by the unbound concentration of BOF-4272 being low; consequently, R -enantiomer is eliminated more rapidly than S -enantiomer.

V_H (%), which is an important parameter regarding distribution between hepatic blood space and hepatic tissues, was used to evaluate the hepatic distribution of allopurinol,²⁵) oxacillin²⁶) and cefixime.²⁷) At 37°C, V_H (%) values of R - and S -enantiomers were very small both in the absence and presence of the perfusate BSA, and were nearly the same as that of BSA.^{10,26,28}) Therefore, the distribution of BOF-4272 enantiomers was negligible, which suggests that the hepatic uptake system of BOF-4272 is efficient, and BOF-4272, once it moves into the hepatic tissues, never returns into the perfusate at 37°C. V_H (%) values of R - and S -enantiomers were almost the same both in the absence and in the presence of BSA at 37°C.¹⁰) At 4°C, V_H (%) values of R - and S -enantiomers were large even in the presence of 4.0% BSA of the perfusate, which shows that a considerable portion of R - and S -enantiomers, once moved into the hepatocytes, returns to the perfusate in an intact form at 4°C. Stereoselectivity in the hepatic distribution was not generally noticed at either 37°C or 4°C, with an exception that V_H

(%) of *R*- enantiomer was slightly larger than that of *S*-enantiomer in 0.25% perfusate BSA at 4 °C.

It is shown in this study that the enantioselectivity in the hepatic elimination of BOF-4272 increases with an increase in albumin concentration in the perfusate. This amplification of stereoselectivity is not sufficiently explained by the enantioselective protein binding of BOF-4272 to BSA, but may be explained by the capacity-limited elimination of BOF-4272. The free concentration of BOF-4272 becomes low with an increase in perfusate BSA concentration; consequently, the stereoselectivity in hepatic elimination becomes obvious.

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