Effect of Temperature in Perfusate on Local Hepatic Disposition of BOF-4272, a New Xanthine Oxidase Inhibitor

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The effect of temperature on the local hepatic disposition of BOF-4272 [(±)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4-olate], a new drug used to treat hyperuricemia, was investigated by means of a perfusion experiment following the pulse input into the portal vein of rat, in which the temperature of the perfusate was changed from 37°C down to 4°C. The same perfusion experiment was also attempted using bovine serum albumin (BSA) as a reference substance to compare with the hepatic disposition of BOF-4272. The elution time profiles of BOF-4272 and BSA from the liver into the hepatic vein were evaluated by moment analysis. The recovery ratio (F_H) and the mean transit time ($\bar{t}_H$) of BOF-4272 were $22.8 \pm 3.3\%$ and 0.111 ± 0.008 min at 37°C, respectively. Both F_H and $\bar{t}_H$ significantly increased with the decrease in the temperature of the perfusate, 3 times and 2 times greater at 4°C than at 37°C, respectively. The F_H and $\bar{t}_H$ of BSA were $98.3 \pm 4.5\%$ and 0.129 ± 0.013 min at 37°C, respectively. These parameters of BSA were independent of temperature, while those of BOF-4272 showed a definite dependency on temperature. A new estimation method for the elimination rate constant (k_e) and the partition ratio (k') in the dispersion model was developed by rearranging the theoretical equations of F_H and $\bar{t}_H$. The index for the elimination (k_e) of BOF-4272 decreased with the decrease in temperature, while the index for the distribution (k') increased with the decrease in temperature. This result shows that the metabolism (or the biliary excretion) decreased and the distribution increased with a decrease in temperature, indicating that the hepatic metabolizing pathway which is presumably temperature-dependent is blocked, and the blocked portion of BOF-4272 thus returns back to the perfusate at the low temperature.

Key words BOF-4272; local moment; xanthine oxidase inhibitor; perfusion system; temperature

BOF-4272, (±)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4-olate, is a newly developed drug for the treatment of hyperuricemia (Fig.1). BOF-4272 inhibits the *de novo* biosynthesis of uric acid by blocking the xanthine oxidase/xanthine dehydrogenase system, which catalyzes the last step of purine catabolism.¹⁾ It is suggested that the elimination of BOF-4272 by the liver is extremely elevated compared with that of β -lactam drugs such as ampicillin, oxacillin and cefixime.²⁻⁴⁾ However, there exists only very limited information on the hepatic uptake of BOF-4272.

In the study of the membrane transport of drugs through hepatocytes, the temperature of the medium is often changed to examine the carrier-mediated transport mechanism.⁵⁻⁷⁾ The effect of the temperature on the influx of α -amanitin into hepatocytes was tested by changing the medium temperature down to 4°C, at which the uptake of α -amanitin by the basolateral membrane of rat liver was considerably blocked.⁵⁾ Taurocholate efflux from the canalicular membrane was studied at temperatures of 37, 25, and 4°C, and the efflux from the membrane was completely stopped at 4°C.⁶⁾

The purpose of this report has been to investigate the effect of temperature on the local hepatic disposition of BOF-4272 and to obtain information on the hepatic elimination and distribution of BOF-4272 between the blood space and liver tissues. The hepatic elimination can be evaluated, but the hepatic distribution is difficult to estimate in the perfusion experiment under a steady-state condition. Therefore, we attempted to estimate the local hepatic disposition of BOF-4272 by means of a non-steady

state perfusion experiment with the pulse input of BOF-4272. Bovine serum albumin (BSA) was used as the reference substance in comparison with the disposition of BOF-4272. The elution time profiles of BOF-4272 and BSA into the hepatic vein were assessed by moment analysis. A new method is proposed to calculate the elimination rate constant (k_e) and the partition ratio (k') in the dispersion model from the recovery ratio (F_H) and the mean transit time ($\bar{t}_H$).

MATERIALS AND METHODS

Animals Male Wistar rats weighing 189–239 g, with free access to standard rat foods and water, were supplied by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan).

Chemicals BOF-4272 used in this study was synthe-

Fig. 1. Chemical Structure of BOF-4272

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sized from 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). 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.⁸⁾ Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p., Nembutal, Abbott Lab., North Chicago, IL, U.S.A.) and the 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, without albumin and red blood cells, to eliminate the effects of these substances. During the experiment, the perfusate (pH 7.4) was saturated with 95% O₂-5% CO₂ and was introduced into the rat liver using a roller pump (RP-3N, Furue Sci. Co., Ltd., Tokyo, Japan) at a flow rate of 15.0 ± 0.1 ml/min/liver. Temperatures of the perfusate in the experiments using BSA and BOF-4272 were kept at 4 or 37°C and 4, 10, 20, 30 or 37°C, respectively. A 0.250 ml portion of BSA solution (40 mg/ml) labeled with Evans blue of 4.0 mg/ml in the perfusate or the BOF-4272 solution (1.0 mg/ml) in the perfusate containing 10% dimethyl sulfoxide were 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 intervals of approximately 1 s from a cannula inserted in the hepatic vein. 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 catheters was subtracted from the outflow profile. The variance of the catheter transit time was less than 2.5 × 10⁻⁵ min², which was about 3% or less than the variance of the liver perfusion data. Thus, broadening of the injected sample in the injector loop and the catheter was negligible. The wet liver weight was 9.1 ± 1.1 g. The bile flow rate was monitored throughout the experiments to test hepatic viability.

Analytical Procedure The concentration of BSA was determined to be a complex of BSA and Evans blue (T-1824) at 620 nm.⁹⁾ The maximum absorption of BOF-4272 was 319 nm, and the effluent perfusate into the hepatic vein gave 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 (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 µg/ml.

Data Analysis According to the theory of the dispersion model with a central elimination model,²⁾ the hepatic recovery ratio (F_H) and the mean transit time ($\bar{t}_H$) are given by

$$F_H = \frac{auc_H}{M/Q} = \exp\left(\frac{QV_B}{2D_c}\left(1 - \sqrt{1 + \frac{4D_ck_e}{Q^2}}\right)\right) \quad (1)$$

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

where auc_H is the area under the curve, M is the dose, Q is the flow rate of perfusate, V_B is the blood volume which corresponds to the sum of the volume of the sinusoid and the space of Disse, D_c is the corrected dispersion coefficient, k_e is the elimination rate constant and k' is the partition ratio. It is noted that k_e and k' are the indexes for the elimination and distribution in the liver, respectively. The peripheral elimination model is demonstrated to be kinetically equivalent to the central elimination model.²⁾

It was shown in the previous paper²⁾ that the term $4D_ck_e/Q^2$ is far less than 1, even for drugs with a large hepatic clearance. Thus, Eqs. 1 and 2 are reduced to Eqs. 3 and 4, respectively.

$$F_H = \exp\left(-\frac{k_e V_B}{Q}\right) \quad (3)$$

$$\bar{t}_H = \frac{V_B(1 + k')}{Q} \quad (4)$$

It is noted here that F_H is independent of k' , and $\bar{t}_H$ is independent of k_e . Both parameters are independent of D_c .

Rearranging Eqs. 3 and 4,

$$k_e = -\frac{Q}{V_B} \ln F_H \quad (5)$$

$$k' = \frac{Q\bar{t}_H}{V_B} - 1 \quad (6)$$

V_B was assumed to be 15.5% of the liver weight according to the stereological study by Blouin *et al.*¹⁰⁾ auc_H and $\bar{t}_H$ were calculated by standard trapezoidal integration with the extrapolation to infinite time.^{11,12)} Using Eqs. 5 and 6, k_e and k' were calculated from the statistical moments.

RESULTS

The typical hepatic outflow profiles of BSA labeled with Evans blue and BOF-4272 are shown in Figs. 2 and 3, respectively. The open and closed circles specify the experimental data between 37 and 4°C, respectively. The top panel shows ordinal time course plots and the bottom panel shows semilogarithmic plots. It is known that the outflow profiles of BSA nearly coincide between 37 and 4°C (Fig. 2), while the profile of BOF-4272 is broader at 4°C than that at 37°C (Fig. 3). Table 1 presents the F_H , $\bar{t}_H$, k_e and k' of BSA and BOF-4272. The F_H of BOF-4272 at 37°C was 22.8 ± 3.3%, while the F_H of BSA was 98.3 ± 4.5%, that is, close to unity. The F_H of BOF-4272 steadily increased with the decrease in the temperature of the perfusate from 37 to 10°C, and there was no significant difference between 10 and 4°C at the 5% level of ANOVA. The F_H of BSA was, of course, independent of the temperature. The $\bar{t}_H$ of BOF-4272 was about 0.1 min at 37°C and increased with a decrease in tem-

Fig. 2. The Typical Hepatic Outflow Profile of BSA Concentration vs. Time after Pulse Input into a Perfused Rat Liver

The bottom panel is the semilogarithmic plot of the same data. The open and closed circles are experimental points at 37 and 4°C, respectively.

perature. The $\bar{t}_H$ of BSA was about 0.13 min at 37°C and was independent of the temperature.

DISCUSSIONS

Since the liver is an important organ from the viewpoints of metabolism, excretion and physiological effect, analysis of local hepatic disposition is required to understand the behavior of a drug in the liver. However, a complicated dispersion model or distribution model (MID method), along with curve fitting, have always been needed to estimate local hepatic parameters such as the elimination rate constant, k_e , and partition ratio, k' .^{4,9)} From Eqs. 3 and 4, k_e and k' in the dispersion model can simply be estimated from the moments F_H and $\bar{t}_H$ calculated by standard trapezoidal integration.

It is considered that the blood volume, V_B , has a constant ratio to the total weight of the liver at 37°C. There is a possibility that this ratio may change in the low temperature in the perfusate. In the perfusion experiment using BSA as a reference substance, the outflow profiles of BSA almost coincided between 37 and 4°C. BSA is

Fig. 3. The Typical Hepatic Outflow Profile of BOF-4272 Concentration vs. Time after Pulse Input into a Perfused Rat Liver

The bottom panel is the semilogarithmic plot of the same data. The open and closed circles are experimental points at 37 and 4°C, respectively.

Table 1. The Local Hepatic Moments (F_H , $\bar{t}_H$) and Local Hepatic Disposition Parameters (k_e , k') of Time Profiles in Perfusate at Several Temperatures

Temperature (°C)	F_H (%)	$\bar{t}_H$ (min)	k_e (min ⁻¹)	k'
BSA				
4	97.9 ± 0.8	0.132 ± 0.003	0.20 ± 0.07	0.27 ± 0.18
37	98.3 ± 4.5	0.129 ± 0.013	0.14 ± 0.43	0.28 ± 0.08
BOF-4272				
4	63.9 ± 7.4	0.241 ± 0.034	4.56 ± 1.00	1.46 ± 0.47
10	71.4 ± 2.5	0.218 ± 0.024	4.08 ± 0.64	1.63 ± 0.38
20	45.7 ± 11.9	0.157 ± 0.028	8.62 ± 2.21	0.69 ± 0.20
30	30.6 ± 4.7	0.146 ± 0.027	12.52 ± 1.13	0.52 ± 0.18
37	22.8 ± 3.3	0.111 ± 0.008	16.63 ± 2.41	0.24 ± 0.08

Each value of BSA and BOF-4272 represents the mean ± S.D. of three and five rats, respectively.

therefore not metabolized in the liver, and the partition of BSA into the liver is small. If V_B changes with the temperature in the perfusate, the time profile of BSA should also change with the temperature. Thus, the blood volume is predicted to be unaffected by the temperature.

Fig. 4. The Correlations of the Temperature to the Elimination (Irreversible Transfer) Rate Constant (k_e) (Top Panel) and the Partition Ratio (k') (Bottom Panel)

The open and closed circles are values of BOF-4272 and BSA, respectively. Each point of BOF-4272 and BSA represents the mean $\pm$ S.D. of five and three rats, respectively.

Accordingly, V_B is fixed to be 15.5% on the basis of stereological study.¹⁰ Since Roberts *et al.* also estimated V_B to be about 15% by means of a perfusion experiment using BSA,¹³ the adopted value of V_B in the present study is expected to be proper.

The top and bottom panels on Fig. 4 present k_e and k' versus the temperature in the perfusate, respectively. The open and closed circles specify the values of BOF-4272 and BSA, respectively. The k_e value of BOF-4272 decreased with the decrease in temperature from 37 to 10 °C and was almost constant below 10 °C. The k_e value of BSA was almost zero. In contrast to k_e , the k' of BOF-4272 increased with the decrease in the temperature from 37 °C down to 10 °C, and was almost constant below 10 °C. The k' value of BSA is independent of temperature and is very close to that of BOF-4272 at 37 °C. These

results indicate that the metabolism (or the biliary excretion) of BOF-4272 decreased with the decrease in the temperature. It is suggested that the hepatic metabolizing pathway, which is presumably temperature-dependent, is blocked at the low temperature, and BOF-4272, which has already been moved into the hepatocytes, returns back into the perfusate in an intact form at the low temperature.

Equation 6 expresses the proportional relationship between $\bar{t}_H$ and $(1+k')$. Accordingly, it is suggested that a drug with a large $\bar{t}_H$ has a large distribution into hepatic tissues. BOF-4272 is extensively eliminated by the liver and slightly distributed into hepatic tissues, because the k_e and k' of BOF-4272 are large and small, respectively. It is reported that the $\bar{t}_H$ of allopurinol, whose structure resembles BOF-4272, is about two-thirds smaller than that of oxipurinol, a metabolite of allopurinol.¹⁴ The $\bar{t}_H$ value of BOF-4272 is about half that of allopurinol. The smallest $\bar{t}_H$ of BOF-4272 shows that BOF-4272 which moves to the hepatic tissues is effectively eliminated by the metabolism or biliary excretion at 37 °C.

In conclusion, this study indicates that the liver perfusion experiment at a low temperature is important in evaluating the mechanisms involved in the local disposition of a drug metabolized in the liver, and the proposed method using moments offers a simple means to estimate the elimination and the distribution of drugs in the liver.

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