Local Disposition of a New Xanthine Oxidase/Xanthine Dehydrogenase Inhibitor, BOF-4272, in Rat Liver

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The local hepatic disposition of BOF-4272, a newly developed xanthine oxidase (XO)/xanthine dehydrogenase (XDH) inhibitor, was evaluated in the rat perfusion system following pulse input of the drug into the portal vein. The elution time profiles from the liver into the hepatic vein were analyzed by dispersion models. The disposition of BOF-4272 through the rat liver was represented by a two-compartment dispersion model based on the Akaike’s Information Criterion (AIC). The area under the concentration time curve (AUC0) of BOF-4272 was proportional to the dosing amount, and the mean transit time was constant from 62.5 up to 500 µg/liver, which demonstrates that the local hepatic disposition of BOF-4272 is linear in this dosing range. The local disposition parameters were precisely estimated at the dosing amount of 250 µg/liver using several rats. These parameters in the dispersion model were correlated to the local moment characteristics. The hepatic recovery ratio (Fh) was 22.8±3.2% and the mean transit time (t0) was 0.112±0.008 min, which show that the influx of BOF-4272 into the liver is efficiently large.

Keywords BOF-4272; local disposition; xanthine oxidase inhibitor; perfusion system; two-compartment dispersion model

An inhibitor of xanthine oxidase (XO)/xanthine dehydrogenase (XDH), an enzyme which catalyzes the last step of purine catabolism, is expected to be effective for the medical treatment of hyperuricemia and possibly ischemia reperfusion injury. However, there is no practically useful XO/XDH inhibitor, except for allopurinol, which has been used clinically since 1962.1–9 A derivative of pyrazolotriazine, BOF-4272 ((±)-8-(3-methoxy-4-phenylsulfonylphenyl)pyrazolof[1,5-a]-1,3,5-triazine-4-olate), is a newly developed drug for the treatments of hyperuricemia (Fig. 1). Although the target organ of BOF-4272 is the liver, the local disposition of BOF-4272 in the liver is completely unknown.

Several pharmacokinetic models for the analysis of local drug disposition in organs have been proposed in the pharmacokinetic field. The well-stirred model and the parallel-tube model5–9 are the most simplified models. Other models, such as a distributed model, were proposed by Bass et al.,10 Forker and Luxon.11 A dispersion model was introduced to analyze liver perfusion system by Roberts and Rowland12,13 from the field of chemical engineering. Recently, one-compartment and two-compartment dispersion models have been reported for the analysis of outflow profiles of drugs in the liver perfusion system.14–18 Two-compartment dispersion model analysis offers a means to separately estimate the degree of drug dispersion in the perfusate, the volume of blood space (sinusoidal space and the Disse space), and the extent of drug distribution and elimination processes in liver tissues.

The purpose of the present investigation is to evaluate the hepatic local disposition of BOF-4272 by means of a perfusion experiment through the liver using a single-pass system in situ. The curve fitting was attempted with a nonlinear least squares program MULTI(FILT)19,20 to estimate the local disposition parameters and local moments, such as the recovery ratio, the mean hepatic transit time and the relative variance.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 189–215 g with free access to standard rat food and water were supplied by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan).

Chemicals BOF-4272 used in this study was obtained from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). All other reagents for the Krebs-Ringer-bicarbonate buffer were of analytical grade.

Liver Perfusion Single-pass perfusion experiments using rat liver were performed in situ according to the Mortimore perfusion method.21 Rats were anesthetized with pentobarbital (50 mg/kg i.p., Nembutal, Abbott Lab., U.S.A.) and the common bile duct was cannulated with a polyethylene tube (PE-10, Becton Dickinson and Company, U.S.A.). The portal vein was rapidly catheterized with a polyethylene tube (1.67 mm o.d.), which was attached to the perfusate of Krebs-Ringer bicarbonate buffer with 10 mM glucose, without albumin and red blood cells. 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, Furuse Sci. Co., Ltd., Japan) at a flow rate of 14.8–15.2 ml/min/liver. The

![Fig. 1. Chemical Structure of BOF-4272](image-url)
temperature of the perfusate was kept at 37 °C. A 0.250 ml portion of BOF-4272 solution 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, and the outflow samples were collected at an interval of approximately 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 void time through the inlet and outlet cannula was subtracted from the outflow profile. The variance of the catheter transit time was less than 2.5 × 10^{-5} 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 bile flow rate was monitored throughout the experiments to test hepatic viability. If the flow rate was less than 5 µl/min, the experimental result was not used for the subsequent analysis. As a preliminary experiment, the dosing amount was changed from 62.5 up to 500 µg/liver to confirm the linearity of the local hepatic disposition of BOF-4272. At the dosing amount of 250 µg/liver, the perfusion experiments were repeatedly attempted using several rats.

**Analytical Procedure** 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** The outflow profile of BOF-4272 was analyzed by one-compartment and two-compartment dispersion models. The pharmacokinetic parameters in the dispersion models were estimated by MULTI(FILT) on the mainframe computer of the Kyoto University Data Processing Center. The model with a smaller AIC value was assumed to be better. Since the time profiles except for one rat were well described by the two-compartment dispersion model, all data were uniformly analyzed by the two-compartment dispersion model. The adopted perfusion model was a two-compartment dispersion system with central elimination which is represented in the Laplace-transformed as

\[ C_p(s) = \frac{M}{Q} \exp\left(\frac{Q}{2D_0} - \sqrt{\frac{Q}{2D_0}}\left(\frac{1}{D_0} + \frac{k_1 + k_2}{s + k_1 + k_2}\right)\right) \]

where \( M \) is the amount injected into the portal vein, \( Q \) is the flow rate of the perfusate, \( D_0 \) is the corrected dispersion coefficient, \( V_B \) is the volume of blood space which is the sum of the volume of sinusoid and the Disse space, \( k_e \) is the elimination rate constant from the perfusate into the hepatic tissues, and \( k_{12} \) and \( k_{21} \) are the forward and backward transfer rate constants, respectively. The partition ratio (\( k' \)), which is the index of the reversible distribution into the liver tissues, is given by \( k_{12}/k_{21} \). In the central elimination model, \( k_{12} + k_{12} \) and \( k_{21} \) are indexes for the influx into and the efflux from the hepatic tissues, respectively. It has been demonstrated that the central elimination model is kinetically equivalent to the peripheral elimination model.

These parameters are correlated to the distribution volume (\( V_M \)), dispersion number (\( D_0 \)) and efficiency number (\( R_E \)) as

\[ V_M = V_p(1+k') \]
\[ D_0 = D_C/(Q - V_B) \]
\[ R_E = k_e - V_B/Q \]

The hepatic local moments, that is, the area under the concentration time curve (\( AUC_{125} \)), the mean transit time (\( \bar{t}_{125} \)) and the relative variance (\( \sigma_{125}^2/I_{125} \)) were correlated with the parameters in the dispersion model by the following equations.

\[ AUC_{125} = \frac{M}{Q} \exp\left[\frac{Q}{2D_0} - \sqrt{\frac{Q}{2D_0}}\left(\frac{k_e}{D_0}\right)\right] \]
\[ \bar{t}_{125} = \frac{V_B}{Q} \left(1 + k'\right) \]
\[ \sigma_{125}^2/I_{125} = \left(\frac{V_B}{Q} \right)^2 \left(1 + k'\right) \]

The recovery ratio (\( F_{125} \)) is given by

\[ F_{125} = Q/M \cdot AUC_{125} \]

**RESULTS**

Figure 2 shows the typical hepatic outflow profiles of BOF-4272 (250 µg/liver). The closed circles are the experimental points and the line is the time curve predicted by MULTI(FILT). The hepatic transit time of BOF-4272 is shorter than 0.3 min, and the descending portion on the outflow profile slightly deviates from a mono-exponential decay after about 0.2 min.

The \( AUC_{125} \) estimated by the curve fitting is proportional to the dosing amount from 62.5 up to 500 µg/liver with a good correlation coefficient (\( r = 0.990 \)). Also, the line slightly deviates from the origin and has a positive intercept across the abscessa, which may suggest the existence of extremely rapid elimination at a low concentration, as shown in Fig. 3A. The \( \bar{t}_{125} \) is almost independent of the dosing amount, as shown in Fig. 3B. These results demonstrate that the local hepatic disposition of BOF-4272 is regarded as linear in this dosing range.

Table I presents the body and liver weight of five rats, and the estimated parameters in a two-compartment dispersion model by MULTI(FILT). The moments (\( F_{125}, \bar{t}_{125}, \sigma_{125}^2/I_{125} \)) calculated by the two-compartment dispersion model are also shown in Table I. The mean
values of $F_H$ and $\bar{r}_H$ calculated by the two-compartment dispersion model are 22.8% and 0.112 min, respectively.

**DISCUSSION**

Goresky indicated that the $V_H$ in dog liver was 15% of the liver weight.\(^{22}\) Roberts et al. also reported that the $V_H$ in rat liver at a flow rate of 10ml/min was 15% of the liver weight.\(^{23}\) The $V_H$ (%) estimated with BOF-4272 per the liver weight was slightly greater than that cited above.

The outflow profiles of ampicillin, oxacillin and cefixime were described better by a two-compartment dispersion model than by a one-compartment dispersion model, because the descending portion on the elution profile showed an obvious biexponential phase.\(^{14} - {16}\) In the present experiment, it was suggested that a two-compartment dispersion model was also able to describe the outflow profile of BOF-4272, because of the slight deviation of the descending portion of the time profile from monoexponential decay. The $F_H$ of BOF-4272 was significantly smaller than that of the drugs cited above. The sum of $k_e$ and $k_{12}$ is an index of the influx into the hepatic tissues in a two-compartment dispersion model with central elimination. The parameter $k_{21}$ related mass-transfer coefficient is an index for non-equilibrium partition.\(^{14}\) The large $k_e$ and the large $k_{21}$ values of BOF-4272 made the descending portion of the time profile close to a monoexponential, which presumably explains
the large standard deviation of $k_{21}$ in Table I. This result may suggest that the distribution process of BOF-4272 in the liver is rather close to an equilibrium state, compared with those of the $\beta$-lactam drugs.

The $F_H$ of ampicillin, oxacillin and cefixime were 95%, 50% and 95%, respectively. The $F_H$ of BOF-4272 was down to 22.8 ± 3.2%. The extraction ratio of BOF-4272 by the liver was significantly larger than those of the $\beta$-lactam drugs. Though the concentration of BOF-4272 in hepatic tissue depends closely on hepatic clearance, that is, the rates of metabolism and bile excretion, it is concluded that BOF-4272 has a strong kinetic interaction with the hepatic tissues.

REFERENCES