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Pharmacokinetics of BOF-4272, a Xanthine Oxidase Inhibitor, after Single Intravenous or Oral Administration to Male Mice and Rats

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Abstract

BOF-4272, (±)-8-(3-methoxy-4-phenylsulphinylphenyl) pyrazolo [1,5-a]-1,3,5-triazine-4 (1*H*)-one), is a new drug intended for the treatment of hyperuricaemia. This report describes the detailed pharmacokinetics of BOF-4272 in mice and rats after intravenous or oral administration.

Plasma concentrations of BOF-4272 at 2–8 h after intravenous administration were significantly higher in mice than in rats. Plasma concentrations of BOF-4272 after oral administration were significantly higher in fed mice than in fasted mice, but were similar in fasted and fed rats. The elimination half-life of the distribution phase ($t_{1/2}(\alpha)$) was similar in mice (0·158 h) and rats (0·210 h). The elimination half-life of the terminal elimination phase ($t_{1/2}(\beta)$) in mice was 1·936 h, while that in rats was 0·742 h. The volume of the central compartment (V_1) was almost the same in mice (415 mL kg^{-1}) and rats (440 mL kg^{-1}). However, the volume of the peripheral compartment (V_2) in mice was 1068 mL kg^{-1}, while that in rats was 92 mL kg^{-1}. The steady-state volume of distribution (V_{ss}) was 2·8 times larger in mice than in rats. The area under the plasma concentration–time curve (AUC) in mice was 5332 ng h mL^{-1}, while that in rats was 3806 ng h mL^{-1}. The $AUC_{0-24\,h}$ after oral administration was 2·5 times greater in fed mice than in fasted mice, and was 1·4 times greater in fasted rats than in fed rats. The correlation coefficients of C_{max} and $AUC_{0-24\,h}$ in both mice and rats after oral administration were greater than 0·997 in the dose range 1–125 mg kg^{-1}, indicating that the linear range of absorption or elimination (or both) of BOF-4272 is very wide.

The results of the present study demonstrate that the mouse is a suitable animal species for evaluating the clinical pharmacokinetics of BOF-4272.

BOF-4272 ((±)-8-(3-methoxy-4-phenylsulphinylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4(1*H*)-one), a derivative of pyrazolotriazine (Figure 1), is a new drug that has been developed for the treatment of hyperuricaemia and ischaemic reperfusion injury (Sato et al 1991; Yamamoto et al 1993; Iwahara et al 1994; Uematsu & Nakashima 1994). It inhibits the de-novo biosynthesis of uric acid by blocking the xanthine oxidase/xanthine dehydrogenase system, which catalyses the last step of purine catabolism (Sato et al 1991; Uematsu & Nakashima 1994). This mechanism of inhibition by BOF-4272 has been elucidated by in-vitro studies using milk xanthine oxidase/xanthine dehydrogenase (Okamoto & Nishino 1995). BOF-4272 significantly decreases the concentration of free radicals generated by xanthine oxidase and consequently inhibits cell necrosis (Suzuki et al 1994). In a preliminary pharmacokinetic study, we suggested that BOF-4272 is not absorbed from any specific portion of the gastrointestinal tract (Terao et al 1995). It was also demonstrated in previous in-situ studies that the liver plays a prominent role in the elimination of BOF-4272 (Nishimura et al 1994, 1995, 1996).

This paper describes the detailed pharmacokinetics of BOF-4272 in male mice and rats after intravenous or oral administration. The animal findings reported here are of great importance in

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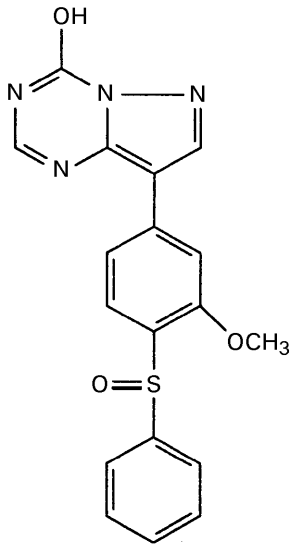

Figure 1. Chemical structure of BOF-4272.

evaluating the clinical pharmacokinetics as well as the pharmacological actions and safety of this drug in humans.

Materials and Methods

Materials

The BOF-4272 used in this study was synthesized at Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Carboxymethyl cellulose sodium salt (CMC) was supplied by Wako Pure Chemical Industries, Ltd (Osaka, Japan). Polyethylene glycol 400 (PEG 400) was supplied by Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals and reagents used were of analytical reagent grade.

Chromatography

BOF-4272 concentrations in plasma were measured using high-performance liquid chromatography (HPLC) systems (CCP & 8010 Series, Tosoh Co., Tokyo, Japan) with a stationary phase of TSKgel ODS-120T (250 × 4·6 mm i.d., Tosoh Co.). The HPLC system consisted of a system controller (PX-8010), pump (CCPM), autosampler (AS-48), UV detector (UV-8010) and integrated data analyser (C-R4AX Chromatopac, Shimadzu Co., Kyoto, Japan). The detector wavelength and the flow rate were 323 nm and 1·0 mL min^{-1}, respectively. The column temperature was ambient. The mobile phase was a mixture of solution A (10 mM $NH_4H_2PO_4$, pH 3·0) and solution B (acetonitrile and solution A, 80 : 20 v/v) with a gradient from 70%/30% to 0%/100% over 31 min.

Animal studies

The mice used were male ICR mice, 7 weeks of age, weighing 31·1–43·1 g (n = 280) supplied by Japan SLC, Inc. (Shizuoka, Japan). The rats used were male Wistar rats, 7 weeks of age, weighing 194–252 g (n = 328) supplied by Charles River Japan (Kanagawa, Japan).

During the experiment, the mice and rats were housed individually in metabolic cages at a temperature of 23 ± 2°C and a relative humidity of 55 ± 10% with a 12-h night–day cycle. Animals were allowed free access to food (CRF-1-R, Oriental Yeast Co., Ltd, Tokyo, Japan), except during periods of fasting, and drinking water. The mice and rats were fasted from 16 h before to 4 h after drug administration. BOF-4272 was dissolved in 50% PEG 400 for intravenous administration, and in 0·5% CMC solution for oral administration. BOF-4272 was administered to fasted mice and rats intravenously or orally at a dose of 5 mg kg^{-1}, and to fed mice and rats orally at doses of 1, 5, 25 and 125 mg kg^{-1}. The mice and rats were anaesthetized with pentobarbital (50 mg kg^{-1}, i.p., Nembutal, Abbot Laboratories, North Chicago, IL) and blood samples were obtained from the abdominal inferior vena cava. Blood samples were drawn into heparinized test tubes at 0 (pre-dose), 0·083, 0·25, 0·5, 1, 2, 4, 8, 24 and 96 h after bolus intravenous injection and at 0 (pre-dose), 0·25, 0·5, 1, 2, 4, 6, 8, 16, 24, 48 and 96 h after oral administration. All blood samples were immediately centrifuged to obtain plasma.

Preparation of HPLC samples from mouse and rat plasma

Each plasma sample was prepared by the successive addition of 2 volumes of acetonitrile at 4°C with shaking. After centrifugation, the supernatant was transferred to another tube, 2 volumes of 10 mM $NH_4H_2PO_4$ (pH 3·0) were added and the mixture was shaken again. The mixture was then filtered through a membrane filter (pore size 0·2 μm). A sample (500 μL) of the filtrate was injected into the HPLC system.

Data analysis

All plasma concentrations are given as mean ± s.d. of 4 animals, except those for fed rats that received 5 or 25 mg kg^{-1} orally, which are given as mean ± s.d. of 6 animals. The time profile of plasma concentrations after intravenous administration was fitted to a two-compartment model using a nonlinear least-squares method on a microcomputer (MULTI) (Yamaoka et al 1981).

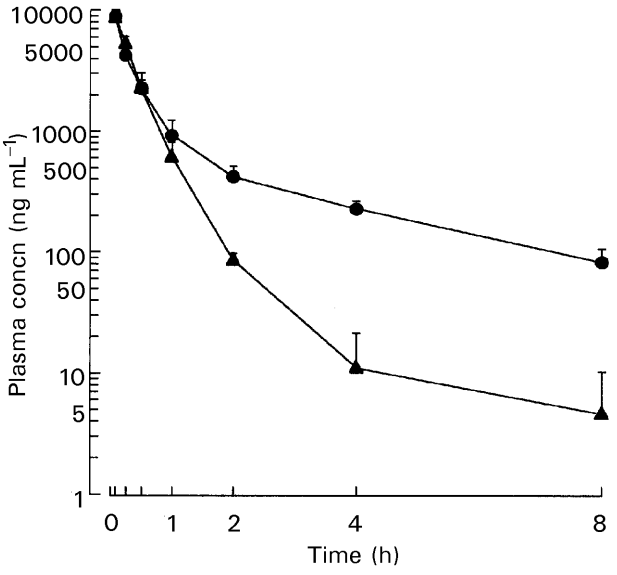

Figure 2. Time courses of plasma BOF-4272 concentrations after bolus intravenous administration to fasted mice (●) and rats (▲) at a dose of 5 mg kg^{-1}. Each point represents mean ± s.d. of 4 animals.

The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (t_{max}) after oral administration were read directly from the mean plasma concentration data. The area under the plasma concentration–time curve from 0 to 24 h ($AUC_{0-24\,h}$) after oral administration was calculated according to the trapezoidal rule (Yamaoka et al 1978).

Results

Figure 2 shows the plasma concentration–time profiles of BOF-4272 after intravenous administration (5 mg kg^{-1}) to fasted male mice and rats. The plasma concentration–time profiles of BOF-4272 in both mice and rats were assessed for both the distribution (α) phase and the elimination (β) phase. Plasma concentrations of BOF-4272 at 2–8 h after administration were significantly higher in mice than in rats. BOF-4272 was not detected in plasma at 24 h or 96 h after administration in mice or rats. Figure 3 shows the plasma concentration profiles of BOF-4272 after oral administration (5 mg kg^{-1}) to fasted and fed male animals. Plasma concentrations of BOF-4272 were significantly higher in fed mice than in fasted mice, whereas plasma concentrations of BOF-4272 in rats were almost the same in the fasted and fed groups. BOF-4272 was not detected in plasma at 48 h and 96 h after administration in mice or rats at any dose (1–125 mg kg^{-1}).

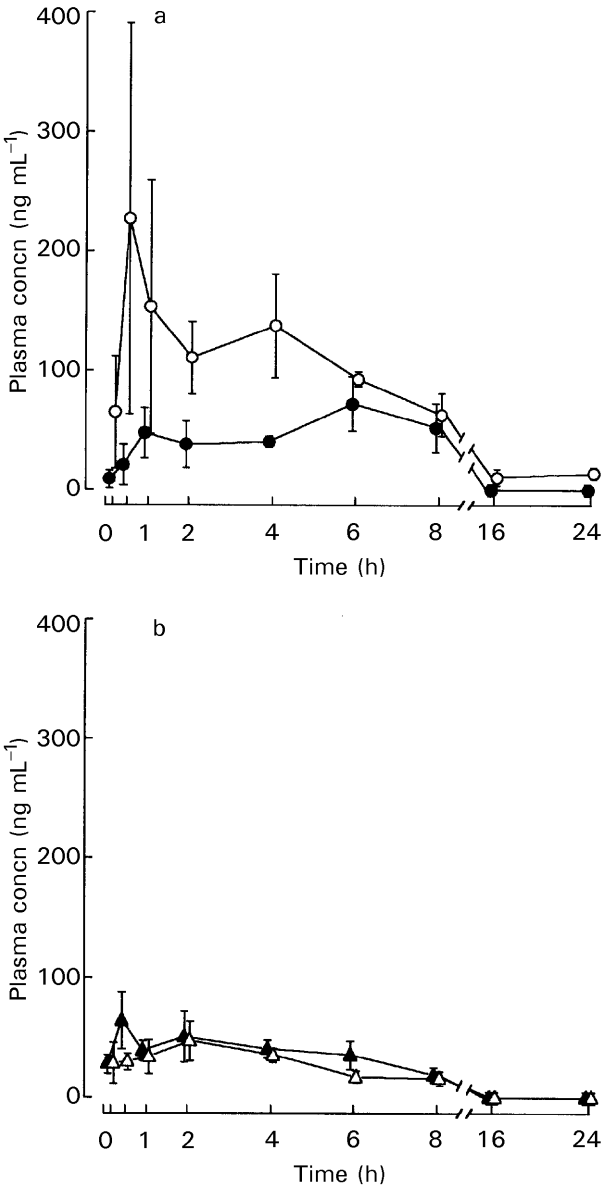

Figure 3. Time courses of plasma BOF-4272 concentrations after oral administration to fed (○) or fasted (●) mice (a) and fed (△) or fasted (▲) rats (b) at a dose of 5 mg kg^{-1}. Each point represents mean ± s.d. of 4 or 6 animals.

Table 1 shows the pharmacokinetic parameters of BOF-4272 after intravenous administration of BOF-4272 to mice and rats at a dose of 5 mg kg^{-1}. The elimination half-life of the distribution phase ($t_{1/2}(\alpha)$) was similar in mice and rats, whereas the elimination half-life of the terminal elimination phase ($(t_{1/2}(\beta))$) was 2·6 times longer in mice than in rats. The volume of the central compartment (V_1) was almost the same in mice and rats, whereas the volume of the peripheral compartment (V_2) was 11·6 times larger in mice than in rats. The steady-state volume of distribution (V_{ss}) was 2·8 times larger in mice than in rats. The area under the

Table 1. Pharmacokinetic parameters of BOF-4272 after bolus intravenous administration to mice and rats at a dose of 5 mg kg^{-1}.

Parameter	Fasted mice	Fasted rats
$t_{1/2}(\alpha)$ (h)	0·158	0·210
$t_{1/2}(\beta)$ (h)	1·936	0·742
V_1 (mL kg^{-1})	415	440
V_2 (mL kg^{-1})	1068	92
V_{ss} (mL kg^{-1})	1483	532
AUC (ng h mL^{-1})	5332	3806
Cl_{tot} (mL h^{-1} kg^{-1})	938	1314

$t_{1/2}(\alpha)$ = elimination half-life of distribution phase, $t_{1/2}(\beta)$ = elimination half-life of terminal elimination phase, V_1 = volume of central compartment, V_2 = volume of peripheral compartment, V_{ss} = steady-state volume of distribution, AUC = area under the plasma concentration–time curve, CL_{tot} = total clearance.

plasma concentration–time curve (AUC) was 1·4 times larger in mice than in rats.

Table 2 shows C_{max}, t_{max} and $AUC_{0-24\,h}$ values of BOF-4272 after single oral administration of BOF-4272 to mice and rats at a dose of 5 mg kg^{-1}. C_{max} and $AUC_{0-24\,h}$ in fed mice were 3·2 times and 2·5 times greater, respectively, than those in fasted mice. Both C_{max} and $AUC_{0-24\,h}$ in fasted rats were 1·4 times greater than those in fed rats. Both C_{max} and $AUC_{0-24\,h}$ after oral administration were almost the same in fasted mice and rats, whereas both C_{max} and $AUC_{0-24\,h}$ after oral administration were higher in fed mice than in fed rats. Figure 4 shows the correlation between C_{max} or $AUC_{0-24\,h}$ and dose in fed mice and rats following single oral administration. The correlation coefficients between C_{max} and $AUC_{0-24\,h}$ in both mice and rats were greater than 0·997 in the dose range 1–125 mg kg^{-1}. The t_{max} values for doses of 1, 5, 25 and 125 mg kg^{-1} were 1, 0·5, 0·5 and 1 h, respectively, in mice and 0·25, 2, 0·5 and 1 h, respectively, in rats.

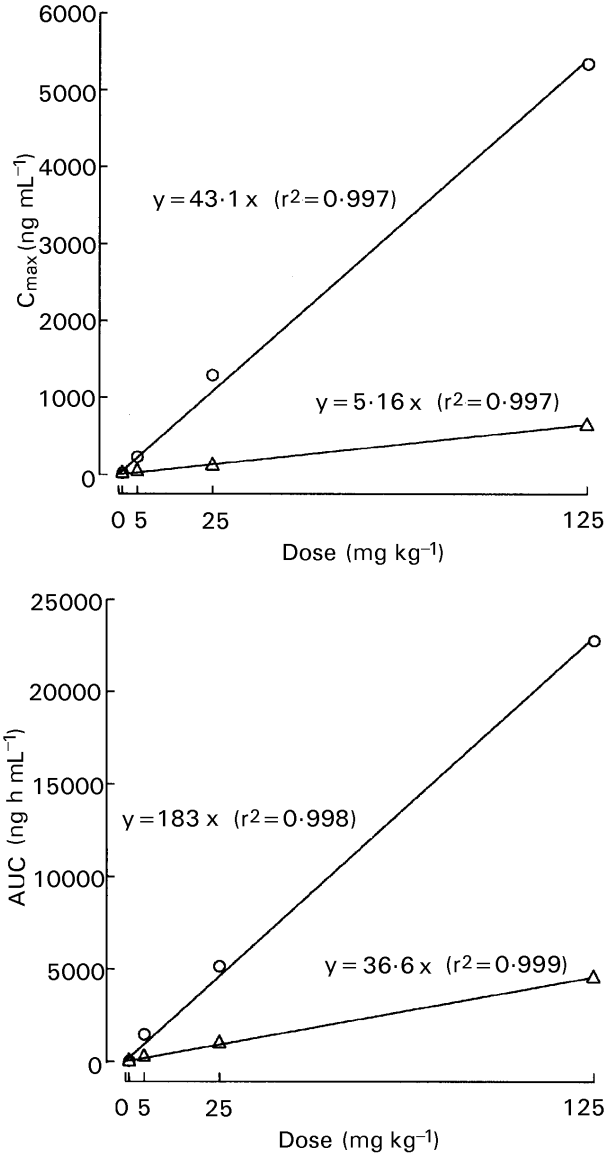

Figure 4. Correlation between C_{max} or $AUC_{0-24\,h}$ and dose in fed mice (○) and rats (△) following single oral administration of BOF-4272.

Discussion

Studies conducted in healthy men have shown that plasma concentrations of BOF-4272 after oral administration are markedly higher in fed subjects than in fasted subjects (unpublished data), and that the plasma concentrations of uric acid are reduced

Table 2. C_{max}, t_{max} and $AUC_{0-24\,h}$ of BOF-4272 after single oral administration of BOF-4272 to mice and rats at a dose of 5 mg kg^{-1}.

	Fasted mice	Fed mice	Fasted rats	Fed rats
Cmax (ng mL^{-1})	72	227	64	47
tmax (h)	6	0·5	0·5	2
$AUC_{0-24\,h}$ (ng h mL^{-1})	586	1459	381	268

C_{max} = maximum plasma concentration, t_{max} = time to maximum plasma concentration, $AUC_{0-24\,h}$ = area under the plasma concentration–time curve from 0 to 24 h.

both during and after the period showing high plasma BOF-4272 levels (Uematsu & Nakashima 1994). In the present study, the plasma concentrations of BOF-4272 after oral administration in fed mice were significantly higher than those in fasted mice. On the other hand, plasma concentrations of BOF-4272 in rats were almost the same in the fasted and fed groups. Furthermore, the bioavailability based on the AUC following oral administration of 5 mg kg^{-1} of BOF-4272 was 11% in fasted mice, 27% in fed mice, 10% in fasted rats and 7% in fed rats. This suggests that the absorption of BOF-4272 from the digestive tract is increased in the presence of food in mice, which is in agreement with the findings of studies involving healthy men.

The plasma concentration of an orally administered drug is affected not only by absorption but also by clearance. The total clearance (CL_{tot}), a parameter of metabolic clearance, excretion clearance, or both, was so high in both mice and rats that this parameter was assumed not to be regulated by food intake. In both animal species, C_{max} values could not be firmly established after oral administration because plasma concentrations of BOF-4272 were maintained at about the same level until 8 h after administration. This finding is consistent with a report suggesting that BOF-4272 is not absorbed from a specific portion of the gastrointestinal tract (Terao et al 1995). It also appears that the linear range of absorption or elimination (or both) of BOF-4272 is very wide, because both C_{max} and $AUC_{0-24\,h}$ after oral administration to mice and rats increased linearly over the dose range 1–125 mg kg^{-1}. Absorption is therefore thought to be a major factor responsible for the significant increase in the bioavailability of orally administered BOF-4272 in fed mice.

BOF-4272 is both hydrophobic and acidic (pKa = 6·6), and is therefore likely to be absorbed from the gastrointestinal tract by passive transport (Terao et al 1995). The lipophilicity, charge and size of this compound have major effects on its passage across the gastrointestinal wall, thus affecting its absorption and bioavailability. Although dissolution in the gastric fluid is a prerequisite for the absorption process, the gastric pH during fasting is as low as pH 1–2, so the relatively insoluble BOF-4272 may precipitate, thereby limiting further dissolution. After a meal, the gastric pH may rise to about pH 5, as reported in healthy men and women (Russell et al 1993). This increase in gastric pH results in increased dissolution of BOF-4272, and thus the dissociated form is transferred to the intestine, where it is converted into the molecular form and efficiently absorbed. This may be the reason for the higher bioavailability of BOF-4272 in fed mice and humans, but not in fed rats. However, the reason for the species differences in the kinetics of BOF-4272 after a meal has not yet been identified.

The results of the present study demonstrate that the mouse is a suitable animal species for evaluating the clinical pharmacokinetics of BOF-4272.

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