Metabolic Pathways and Pharmacokinetics of BOF-4272, a Sulfoxide-Containing Drug, in the Dog: *In Vivo* and *In Vitro* Studies

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BOF-4272, ($\pm$)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4(1H)-one, is a new drug intended for the treatment of hyperuricemia. This report describes the detailed metabolic pathways of BOF-4272 in the dog. The metabolic pathways were investigated using the metabolites found in plasma, urine, and feces after intravenous or oral administration of BOF-4272, as well as the metabolites found in the liver S9 incubation mixture after the addition of BOF-4272 or BOF-4269. BOF-4269 (the sulfide metabolite of BOF-4272) was the only metabolite detected in plasma and feces after the intravenous or oral administration of BOF-4272. BOF-4269 was detected in dog plasma after a lag time following the oral administration of BOF-4272, and the $C_{\max}$ and AUC_{0-t} of BOF-4269 were higher in fed dogs than in fasted dogs after the oral administration of BOF-4272. A small amount of BOF-4269 was detected in dog plasma immediately after the intravenous administration of BOF-4272. Only BOF-4276 (the sulfone metabolite of BOF-4272) was detected in the S9 incubation mixture after the addition of BOF-4272. Mainly BOF-4272 was detected and small amounts of BOF-4276 and M-1 (the hydroxy metabolite of BOF-4269) were detected in the S9 incubation mixture after the addition of BOF-4269. These findings suggest that BOF-4272 is mainly metabolized to BOF-4269 by the intestinal flora in dogs, whereas little of this drug is metabolized to BOF-4269 in the dog liver. In conclusion, this work has allowed us to formulate the proposed metabolic pathways of BOF-4272 in the dog.

Key words sulfoxide-containing drug; xanthine oxidase inhibitor; BOF-4272; metabolic pathway; pharmacokinetics

BOF-4272, a derivative of pyrazolotriazine, is a new drug that has been developed for the treatment of hyperuricemia and ischemic reperfusion injury.^{1–4)} With a structure that resembles that of xanthine, BOF-4272 potentially inhibits the biosynthesis of uric acid by interfering with the xanthine oxidase/xanthine dehydrogenase system.^{1,2)} It also significantly decreases the concentration of the free radicals generated by xanthine oxidase and consequently reduces cellular necrosis.⁵⁾ On the other hand, BOF-4276, the sulfone metabolite of BOF-4272, shows less potent pharmacological activity than BOF-4272,⁶⁾ whereas BOF-4269 itself has no inhibitory action on the uric acid biosynthesis system (unpublished data). We have recently conducted *in situ* studies using the liver perfusion system in the rat and have found that the liver plays a prominent role in the elimination of BOF-4272,^{7–9)} as this drug is highly bound to serum albumin ($>99\%$).¹⁰⁾ It has also been demonstrated that the linear range of absorption and/or elimination of orally administered BOF-4272 is very wide in the mouse and in the rat.¹¹⁾

The metabolic pathways of BOF-4272 have previously been reported in the rat.¹²⁾ This paper describes the detailed metabolic pathways and pharmacokinetics of BOF-4272 in the dog, and discusses species differences in the biotransformation of this drug.

MATERIALS AND METHODS

Materials BOF-4272 and its metabolites, BOF-4269 (8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4(1H)-one), BOF-4276 (8-(3-methoxy-4-phenylsulfonylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4(1H)-one), M-1 (8-[3-methoxy-4-(4-hydroxyphenylsulfinylphenyl)]pyrazolo[1,5-a]-1,3,5-triazine-4(1H)-one) and M-4 ((+)-2-hydroxy-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo[1,5-a]-1,3,5-triazine-4(1H)-one), used in this study were synthesized at Ot-

suka Pharmaceutical Factory, Inc. (Tokushima, Japan). The chemical purity was not less than 99%. Ethyl acetate and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyethylene glycol 400 (PEG 400) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals and reagents used were of analytical reagent grade.

Chromatography The concentrations of BOF-4272 and its metabolites 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 $\times$ 4.6 mm i.d., Tosoh Co.). The HPLC systems consisted of a system controller (PX-8010), pump (CCPM), autosampler (AS-48), UV detector (UV-8010), and integrated data analyzer (C-R4AX Chromatopac, Shimadzu Co., Kyoto). The detector wavelength and the flow rate were 323 nm and 1.0 ml/min, respectively. The column temperature was ambient. The mobile phase was a mixture of solution A (10 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 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 dogs used were male beagles 8–9 months of age and weighing 8.4–11.0 kg ($n=10$) purchased from Covance Research Products, Inc. (Denver, PA, U.S.A.).

During the experiment, the dogs were housed in individual metabolic cages at a temperature of $23\pm2^\circ\text{C}$ and a relative humidity of $55\pm10\%$ on a 12-h night/day cycle. Food (DS-5; Oriental Yeast Co., Ltd., Tokyo) was provided at 300 g/d after drug administration, except during periods of fasting as described below, and the dogs were allowed free access to water. They were fasted from 24 h before to 4 h after drug administration.

BOF-4272 was dissolved in 50% PEG 400 for intravenous

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administration, and was placed in gelatin capsules for oral administration. BOF-4272 was administered to fasted dogs intravenously or orally at a dose of 5 mg/kg, and to fed dogs orally at a dose of 5 mg/kg.

Blood samples (about 1 ml) were obtained from the cephalic vein using a heparinized syringe. Samples were drawn into test tubes at 11 time-points (0 [pre-dose], 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 h) after bolus intravenous injection and at 11 time-points (0 [pre-dose], 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h) after oral administration. All blood samples were immediately centrifuged to obtain plasma.

Urine and feces samples were collected daily for 2 d after intravenous or oral administration.

Preparation of HPLC Samples from 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 $\text{NH}_4\text{H}_2\text{PO}_4$ (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). An aliquot (500 μl) of the filtrate was injected into the HPLC system.

Preparation of HPLC Samples from Urine Each urine sample (0.5 ml) was mixed with 1 N HCl (0.1 ml) and ethyl acetate (4 ml) and shaken. After centrifugation, 1 ml of the ethyl acetate layer was transferred to another tube and dried under reduced pressure. The dried sample was dissolved in 3 ml of 10 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 3.0) containing 20% acetonitrile. The mixture was then filtered through a membrane filter (pore size, 0.2 μm). An aliquot (500 μl) of the filtrate was injected into the HPLC system.

Preparation of HPLC Samples from Feces Each feces sample was prepared by the successive addition of 10 volumes of acetonitrile at 4 °C with shaking. After centrifugation, the supernatant was transferred to another tube, 10 volumes of 10 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 3.0) were added, and the mixture was shaken again. It was then filtered through a membrane filter (pore size, 0.2 μm), and an aliquot (500 μl) of the filtrate was injected into the HPLC system.

Liver Specimens and Preparation of Liver 9000 g Homogenate Dogs were anesthetized with pentobarbital (50 mg/kg, i.p.; Nembutal, Abbot Laboratories, North Chicago, IL, U.S.A.), and the liver was perfused with saline solution (1000 ml) at 4 °C. The livers were stored frozen at -80 °C until use.

Each liver sample was homogenized in ice-cold 0.01 M phosphate-buffered (pH 7.4) 1.15% potassium chloride (1:3 w/v), and the homogenate was centrifuged (9000 g, 4 °C) for 20 min. The 9000 g supernatant (S9) was used these experiments.

Incubation with S9 A typical incubation mixture, in a final volume of 12 ml, contained 2 ml of S9, 6 μg of BOF-4272 or BOF-4269, 1.25 mM NADP, 2.5 mM glucose-6-phosphate, 0.75 U/ml glucose-6-phosphate dehydrogenase, and 6.25 mM MgCl_2 in 0.1 M potassium phosphate buffer (pH 7.4). All mixtures were incubated at 37 °C under open air in a shaking water bath (80 oscillations/min). Aliquots (0.5 ml) of the incubation mixture were collected at 0, 0.25, 0.5, 0.75, 1, 2, 4, and 6 h and mixed with 1 ml of acetonitrile to terminate the enzymatic reaction. The acetonitrile mixture was then vortexed for 1 min. After centrifugation, the supernatant was transferred to another tube, 2 volumes of 10 mM

Fig. 1. Plasma Concentration Profiles for BOF-4272 and Its Metabolites after the Intravenous Administration of BOF-4272 at 5 mg/kg to Fasted Dogs

Each point is mean $\pm$ S.D. of 5 dogs.

$\text{NH}_4\text{H}_2\text{PO}_4$ (pH 3.0) were added, and the mixture was shaken, then filtered through a membrane filter (pore size, 0.2 μm). An aliquot (500 μl) of the filtrate was injected into the HPLC system.

Data Analysis The concentration at time 0 (C_0) for intravenous administration was estimated by the residual method. 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 plasma concentration data. The elimination half-life ($t_{1/2}$) after intravenous or oral administration was calculated using the equation $t_{1/2} = \ln 2/K$, where K is the slope of the terminal portion of the natural logarithm of the plasma concentration-time curve obtained by linear regression. The area under the plasma concentration-time curve from time 0 to the measurement time-point (AUC_{0-t}), the total area under the plasma concentration-time curve ($\text{AUC}_{0-\infty}$), and the mean residence time (MRT) after intravenous or oral administration were calculated according to the trapezoidal rule.¹³ Total systemic clearance (CL) and volume of distribution (V_{dss}) were calculated according to the following equations:

$$CL = D/\text{AUC}$$

$$V_{\text{dss}} = D \cdot \text{MRT}/\text{AUC}_{0-\infty}$$

where D is the administered dose. The bioavailability (F) was assessed by comparing AUC values after oral and intravenous administration.

RESULTS

Figure 1 shows the plasma concentration profiles for BOF-4272 and its metabolites after the intravenous administration of the drug at 5 mg/kg to fasted dogs. The elimination curve of BOF-4272 from dog plasma shows a biexponential pattern, with a rapid-elimination phase up to 1 h and a slow-elimination phase for 1–8 h after intravenous administration. BOF-4269, a sulfide metabolite of BOF-4272, appeared in plasma 15 min after the intravenous injection of BOF-4272, and was the only metabolite detected in dog plasma up to 24 h after administration. The increase in the BOF-4269 plasma concentration seemed to consist of two peaks as shown in Fig. 1. The first peak and the second peak appeared at about 2 h and 6–8 h after injection, respectively. Table 1

Table 1. Pharmacokinetic Parameters for BOF-4272 and Its Metabolites in Plasma after the Intravenous Administration of BOF-4272 to Fasted Dogs

Parameter	BOF-4272	BOF-4269
Fasted dogs, 5 mg/kg (n=5)		
C_0 (ng/ml)	12970±2002	
C_{max} (ng/ml)		27±9
t_{max} (h)		6.02±3.96
$t_{1/2}$ (h)	1.25±0.13	
AUC_{0-t} (ng·h/ml)	5098±1576	210±216
$AUC_{0-\infty}$ (ng·h/ml)	5140±1591	
MRT (h)	1.12±0.20	
CL (ml/h/kg)	1056±316	
CL^* (ml/h/kg)	1048±314	
V_{dss} (ml/kg)	1147±327	

C_0 , Plasma concentration at time 0; C_{max} , maximum plasma concentration; t_{max} , time to maximum plasma concentration; $t_{1/2}$, elimination half-life; AUC_{0-t} , area under the plasma concentration-time curve from time 0 to the measurement time-point; $AUC_{0-\infty}$, total area under the plasma concentration-time curve; MRT , mean residence time; CL , clearance=dose/ AUC_{0-t} ; CL^* , dose/ $AUC_{0-\infty}$; V_{dss} , volume of distribution. Values are mean±S.D.

Fig. 2. Plasma Concentration Profiles for BOF-4272 and Its Metabolites after the Oral Administration of BOF-4272 at 5 mg/kg to Fed and Fasted Dogs

The closed and open symbols indicate fed and fasted dogs, respectively. Each point is mean±S.D. of 6 dogs.

shows the pharmacokinetic parameters for BOF-4272 and its metabolites in plasma after the intravenous administration of BOF-4272 to fasted dogs. The V_{dss} of BOF-4272 was calculated to be 1147 ml/kg, indicating higher volume of distribution.

Figure 2 shows the plasma concentration profiles for BOF-4272 and its metabolites after the oral administration of BOF-4272 at 5 mg/kg to fed and fasted dogs. Irrespective of the feeding status, there were no detectable metabolites except for BOF-4269 in the plasma after the oral administration of BOF-4272. The C_{max} of BOF-4272 was observed at 2 h after oral administration, while the C_{max} of BOF-4269 was observed at 8 h. The parent drug was not detectable in dog plasma at 48 h after oral administration. The pharmacokinetic parameters for BOF-4272 and its metabolites in plasma after its oral administration to fasted and fed dogs are summarized in Table 2. In fasted dogs, the AUC_{0-t} of BOF-4272 was about 2 times greater than that of BOF-4269, while the AUC_{0-t} of the parent drug in fed dogs was approximately one half that of BOF-4269. Regarding BOF-4269, both C_{max} and AUC_{0-t} values in fed dogs were twice as great as those in fasted dogs.

Table 3 shows the urinary and fecal excretion of BOF-

Table 2. Pharmacokinetic Parameters for BOF-4272 and Its Metabolites in Plasma after the Oral Administration of BOF-4272 to Fasted and Fed Dogs

Parameter	BOF-4272	BOF-4269
Fasted dogs, 5 mg/kg (n=6)		
C_{max} (ng/ml)	133±45	43±86
t_{max} (h)	2.17±0.98	8.00±0.00
$t_{1/2}$ (h)	1.28±0.16	
AUC_{0-t} (ng·h/ml)	550±153	281±484
$AUC_{0-\infty}$ (ng·h/ml)	580±157	
MRT (h)	3.69±0.38	
F (%)	10.8	
F^* (%)	11.3	
Fed dogs, 5 mg/kg (n=6)		
C_{max} (ng/ml)	101±31	88±59
t_{max} (h)	2.50±1.00	9.00±2.00
$t_{1/2}$ (h)	1.35±0.20	
AUC_{0-t} (ng·h/ml)	357±172	639±489
$AUC_{0-\infty}$ (ng·h/ml)	380±184	
MRT (h)	3.21±0.59	
F (%)	7.0	
F^* (%)	7.4	

F , Bioavailability based on AUC_{0-t} values; F^* , bioavailability based on $AUC_{0-\infty}$ values. Values are mean±S.D.

Table 3. Urinary and Fecal Excretion of BOF-4272 and Its Metabolites after the Intravenous and Oral Administration of BOF-4272 to Fasted and Fed Dogs

Time (d)	BOF-4272 (% of dose)	BOF-4269 (% of dose)
Intravenous administration		
Fasted dogs, 5 mg/kg (n=5)		
Urine 1	4.97±1.20	ND
2	0.11±0.11	ND
Feces 1	31.7±18.7	29.0±22.7
2	0.78±0.53	0.35±0.51
Oral administration		
Fasted dogs, 5 mg/kg (n=6)		
Urine 1	1.24±0.35	ND
2	0.12±0.11	ND
Feces 1	43.1±17.4	17.6±15.8
2	2.37±3.21	4.34±9.41
Fed dogs, 5 mg/kg (n=6)		
Urine 1	1.14±0.79	ND
2	ND	ND
Feces 1	22.8±11.7	50.8±9.8
2	0.31±0.37	1.58±1.69

ND, not detected. Values are mean±S.D.

4272 and its metabolites after the intravenous and oral administration of BOF-4272 to fasted and fed dogs. The bioavailability based on the AUC_{0-t} following the oral administration of 5 mg/kg of BOF-4272 was 10.8% in fasted dogs and 7.0% in fed dogs. When the absorption rate of BOF-4272 was calculated from the total urinary excretion of the drug, it was estimated to be 26.8% and 22.4% in fasted and fed dogs, respectively. Irrespective of the administration route or the feeding status, both BOF-4272 and BOF-4269 were mainly excreted in the feces within 1 d after administration. In all the dogs used in the experiments, BOF-4269 was the only metabolite of BOF-4272 detected in feces, but was not detected in the urine. After oral administration, the fecal excretion of BOF-4272 in fasted dogs was about twice that of the metabolite. On the other hand, exactly the opposite finding was observed in fed dogs that received BOF-4272

Fig. 3. *In Vitro* Metabolism of BOF-4272 (A) and BOF-4269 (B) in Liver S9 Fraction Obtained from Dogs

The closed and open symbols indicate substrates and their metabolites, respectively. Values are data for 1 study in 1 dog, which are representative results from 3 separate studies in 3 dogs.

orally. As for BOF-4269 excreted in the feces for 2 d after the oral administration of BOF-4272, the amount of fecal metabolite detected was twice as great in fed dogs as in fasted dogs. The total recovery rates of BOF-4272 and BOF-4269 for 2 d after the administration of BOF-4272 were about 70–80% in the present experiments.

In order to investigate the *in vitro* metabolism of BOF-4272 and BOF-4269, each compound was incubated at 37°C with the liver S9 fraction obtained from dogs. As shown in Fig. 3-A, BOF-4276 was detected as the only metabolite in the S9 incubation mixture, and was produced as a function of the incubation time after the addition of BOF-4272. After BOF-4269 was added to the S9 incubation mixture, the concentration of BOF-4272 in the mixture increased time-dependently with decreasing concentrations of BOF-4269 (Fig. 3-B). Very small amounts of BOF-4276 and M-1 were also detected after the incubation of S9 and BOF-4269 (Fig. 3-B).

DISCUSSION

In the present study, BOF-4269, a sulfide form of BOF-4272, was detected as a unique metabolite in the plasma and feces of dogs that received BOF-4272 intravenously or orally. However, the *in vitro* experiments showed that BOF-4269 was not detected in the incubation mixture containing BOF-4272 and dog liver S9. It was previously demonstrated that the sulfoxide drugs sulfinpyrazone and sulindac are metabolized to sulfides by the intestinal flora,^{14–16} but not by the intestinal epithelium.¹⁴ We suggested in a previous study that BOF-4272, a sulfoxide drug, is also reduced to the sulfide

form by the intestinal flora of rats, whereas little BOF-4272 is metabolized to BOF-4269 in the liver.¹²

BOF-4269 was detected in dog plasma with a lag time of a few hours after the oral administration of BOF-4272 (Fig. 2). The $t_{\max}$ of BOF-4272 was approximately 2 h, while the $t_{\max}$ of BOF-4269 was 8–9 h (Fig. 2 and Table 2). The difference in the $t_{\max}$ values of BOF-4272 and its sulfide metabolite was probably due to the time required for the compound to pass through the gut to undergo reduction. Judging from the delay time, reduction may occur in the distal digestive tract of the dog. In our previous study using the loop method in the rat gastrointestinal tract, we reported that the relative absorption rates of BOF-4272 from the duodenum, small intestine, and colon were 31%, 14–22%, and 6%, respectively.¹⁷ As compared with BOF-4272, the relative absorption rates of BOF-4269 from the duodenum, small intestine, and colon were 72%, 57–68%, and 56%, respectively (unpublished data). Therefore, BOF-4269 is thought to be absorbed from the intestine, and particularly the colon, much more readily than BOF-4272. These findings suggest that orally administered BOF-4272 is mainly metabolized to BOF-4269 by the intestinal flora in the dog, after which the metabolite is absorbed and enters the plasma, as reported in the rat.¹²

Although the *in vitro* experiments with dog liver S9 were not able to prove the generation of BOF-4269 from BOF-4272 (Fig. 3-A), a small amount of BOF-4269 was found in dog plasma immediately after the intravenous administration of BOF-4272 (Fig. 1). The rat liver is known to show only slight activity in generating the sulfide from sulfinpyrazone, and is 100 times less active than the cecal flora.¹⁴ It is possible that a small amount of BOF-4272 was metabolized to BOF-4269 in the dog liver immediately after the intravenous injection of BOF-4272.

BOF-4272 was detected in the dog S9 incubation mixture after the addition of BOF-4269. It is known that sulfides are oxidized to their sulfoxides by a flavin-containing monooxidase^{18,19} and cytochrome P-450.^{18,20} Therefore, a portion of the BOF-4269, which is generated from BOF-4272 by the gut flora and absorbed from the intestinal tract, may have been converted back to the parent compound by the hepatic enzymes in the dog. These results suggest that some form of enterohepatic recycling must occur in the dog after the administration of BOF-4272.

When BOF-4272 was administered intravenously to the dog, a biexponential pattern was observed in the elimination of BOF-4272 from plasma (Fig. 1). Enterohepatic recycling may account for the second phase with a relatively low elimination rate of BOF-4272 as shown in Fig. 1. Furthermore, the absorption of BOF-4272 estimated from total urinary excretion was higher than the bioavailability based on the AUC_{0-t} in dogs that received BOF-4272 orally (Table 2). This discrepancy may be partially explained by the enterohepatic recycling of BOF-4272, in which BOF-4272 is regenerated in the liver from the BOF-4269 absorbed from the intestinal tract and then excreted in the urine.

BOF-4276, a sulfone form of BOF-4272 which is, however, not found in dog plasma, was detected in the incubation mixture containing dog liver S9 and BOF-4272. Sulfoxides are metabolized to their sulfones by rat liver cytochrome P-450.²⁰ Therefore, BOF-4272, a sulfoxide, may possibly be metabolized to BOF-4276 by cytochrome P-450 in the dog

Fig. 4. Proposed Metabolic Pathways of BOF-4272 in the Dog

liver. The results of the present study lead us to propose the metabolic pathways for BOF-4272 in the dog as shown in Fig. 4.

Regarding the species differences in the metabolic pathways for BOF-4272, the drug is metabolized to the sulfide BOF-4269 mainly by the intestinal flora in rats and dogs. In the rat, BOF-4269 is further metabolized mostly to the sulfate conjugate of M-1 via M-1,¹²⁾ whereas little BOF-4269 is metabolized to M-1 in the dog. In man, M-4 (the hydroxy metabolite of BOF-4272) is the main metabolite and BOF-4269 is a minor metabolite which has been detected in the plasma of healthy volunteers.²⁾ With respect to the hydroxylation of the moieties of BOF-4272 and/or BOF-4276, the dog differs from other species due to the absence of hydroxylation. The results of the present study, when taken together with previous findings, indicate that BOF-4272 is a drug that shows large species differences in metabolism.

The effect of feeding status on the absorption of BOF-4272 was also investigated in the present study. The plasma concentrations of BOF-4272 after oral administration were higher in fasted dogs than in fed dogs. Furthermore, the bioavailability based on the AUC_{0-4} following the oral administration of 5 mg/kg of BOF-4272 was 10.8% in fasted dogs and 7.0% in fed dogs. This suggests that the absorption of BOF-4272 from the digestive tract may be decreased in the presence of food in dogs. On the other hand, it was suggested in previous studies that the absorption of BOF-4272 from the digestive tract is increased in the presence of food in mice and healthy human subjects.¹¹⁾ Therefore, it is apparent that the absorption of BOF-4272 is influenced by the feeding status. However, the reason for the species differences observed in the absorption of BOF-4272 in the fed state has not yet been identified.

REFERENCES

- 1) Sato S., Tatsumi K., Nishino T., *Adv. Exp. Med. Biol.*, **309A**, 135—138 (1991).
- 2) Uematsu T., Nakashima M., *J. Pharmacol. Exp. Ther.*, **270**, 453—459 (1994).
- 3) Iwahara R., Emoto S., Kaga J., Imura Y., Aoki M., Asakawa K., *Iyakuhin Kenkyu* (in Japanese), **25**, 426—437 (1994).
- 4) Yamamoto T., Moriaki Y., Suda M., Nasako Y., Takahashi S., Hiroishi K., Nakano T., Hada T., Higashino K., *Biochem. Pharmacol.*, **46**, 2277—2284 (1993).
- 5) Suzuki H., Suematsu M., Ishii H., Kato S., Miki H., Mori M., Ishimura Y., Nishino T., Tsuchiya M., *J. Clin. Invest.*, **93**, 155—164 (1994).
- 6) Okamoto K., Iwamoto T., Nishino T., "New Tight Binding Inhibitors of Xanthine Oxidase, in *Flavins and Flavoproteins 1993*," ed. by Yagi K., Walter de Gruyter, Berlin, 1994, pp. 731—734.
- 7) Nishimura M., Yamaoka K., Yasui H., Naito S., Nakagawa T., *Biol. Pharm. Bull.*, **17**, 1301—1304 (1994).
- 8) Nishimura M., Yamaoka K., Yasui H., Naito S., Nakagawa T., *Biol. Pharm. Bull.*, **18**, 980—983 (1995).
- 9) Nishimura M., Yamaoka K., Naito S., Nakagawa T., *Biol. Pharm. Bull.*, **19**, 1197—1202 (1996).
- 10) Shibukawa A., Kadohara M., He J., Nishimura M., Naito S., Nakagawa T., *J. Chromatogr. A*, **694**, 81—89 (1995).
- 11) Naito S., Nishimura M., Nogawa H., *J. Pharm. Pharmacol.*, **51**, 347—351 (1999).
- 12) Naito S., Nishimura M., *Pharm. Pharmacol. Commun.*, **5**, 123—131 (1999).
- 13) Yamaoka K., Nakagawa T., Uno T., *J. Pharmacokinet. Biopharm.*, **6**, 547—558 (1978).
- 14) Renwick A. G., Evans S. P., Sweatman T. W., Cumberland J., George C. F., *Biochem. Pharmacol.*, **31**, 2649—2656 (1982).
- 15) Strong H. A., Warner N. J., Renwick A. G., George C. F., *Clin. Pharmacol. Ther.*, **38**, 387—393 (1985).
- 16) Strong H. A., Renwick A. G., George C. F., Liu Y. F., Hill M. J., *Xenobiotica*, **17**, 685—696 (1987).
- 17) Terao T., Matsubara T., Kaga J., Kobatake H., Naito S., *Yakuzaigaku*, **55**, 36—43 (1995).
- 18) Buronfosse T., Moroni P., Benoit E., Riviere J. L., *J. Biochem. Toxicol.*, **10**, 179—189 (1995).
- 19) Rettie A. E., Meier G. P., Sadeque A. J., *Chem. Biol. Interact.*, **96**, 3—15 (1995).
- 20) Benoit E., Buronfosse T., Delatour P., *Chirality*, **6**, 372—377 (1994).