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EFFECT OF BOF-4272 ON THE OXIDATION OF ALLOPURINOL AND PYRAZINAMIDE *IN VIVO*

IS XANTHINE DEHYDROGENASE OR ALDEHYDE OXIDASE MORE IMPORTANT IN OXIDIZING BOTH ALLOPURINOL AND PYRAZINAMIDE?

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Abstract—Allopurinol or pyrazinamide was administered to rats treated with BOF-4272 (a potent xanthine oxidase inhibitor) to investigate to what degree xanthine dehydrogenase participates in the oxidation of these agents. BOF-4272 markedly decreased the plasma concentration and the urinary excretion of both oxypurinol and 5-hydroxypyrazinamide. It also decreased the sum of the urinary excretion of allopurinol and oxypurinol and that of pyrazinamide and its metabolites, although it did not affect the sum of the plasma concentrations of allopurinol and oxypurinol at 105 min after administration of allopurinol or the plasma concentration of pyrazinamide during the period after the administration of pyrazinamide. These results suggested that BOF-4272 almost completely inhibited the oxidation of allopurinol and pyrazinamide and had some effect on the excretion and/or the tissue incorporation of these two compounds. Since the *in vitro* study demonstrated that BOF-4272 did not inhibit the activity of aldehyde oxidase, which oxidized both allopurinol to oxypurinol and pyrazinamide to 5-hydroxypyrazinamide, the results suggested that xanthine dehydrogenase was the more important enzyme in converting allopurinol to oxypurinol and pyrazinamide to 5-hydroxypyrazinamide.

Allopurinol is widely used in the treatment of gout. The main metabolic pathway of allopurinol is its oxidation to oxypurinol. However, whether the main enzyme oxidizing allopurinol is aldehyde oxidase [1–3] or xanthine oxidase (most of which is present in the form of xanthine dehydrogenase *in vivo*) is unknown. It is important to determine the main oxidizing enzyme of allopurinol in view of the metabolism of allopurinol and the treatment for hyperuricemia. On the other hand, pyrazinamide is an antituberculous drug, and is also used to investigate the mechanism of the renal transport of uric acid in patients with renal hypouricemia [4–6], since it completely inhibits the renal secretion of uric acid. One of the main metabolic pathways of pyrazinamide is its oxidation to 5-hydroxypyrazinamide [7, 8]. This conversion has been shown to be due to xanthine oxidase [7, 9] but has also been suggested as being due to aldehyde oxidase [10]. Therefore, we were interested in the degree to which xanthine dehydrogenase participates in the oxidation of allopurinol or pyrazinamide *in vivo*. However, since there is no potent xanthine oxidase inhibitor that could be used *in vivo* except for both allopurinol and oxypurinol, no study on the *in vivo* effect of a xanthine oxidase inhibitor on the oxidation of allopurinol or pyrazinamide has been feasible. Recently, however, a new potent inhibitor of xanthine oxidase (BOF-4272) [11] was developed by the Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). It is metabolized to BOF-4269 by intestinal flora and to M-4 by an unidentified enzyme(s) in humans and rats. These metabolites are also xanthine oxidase inhibitors, although their action is weak. Therefore, by employing BOF-4272 in rats, we were able to investigate to what extent xanthine dehydrogenase takes part in the oxidation of allopurinol or pyrazinamide.

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MATERIALS AND METHODS

Chemicals. Allopurinol and oxypurinol were prepared by the Tanabe Pharmaceuticals Co. (Osaka, Japan). Pyrazinamide and pyrazinoic acid were prepared by the Sankyo Pharmaceuticals Co. (Tokyo, Japan) and BOF-4272, BOF-4269 and M-4 by the Otsuka Pharmaceutical Factory, Inc. 5-Hydroxypyrazinamide and 5-hydroxypyrazinoic acid were obtained by a method described previously [12]. Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Subjects and protocol. The study was conducted on 30 Wistar male rats, approximately 300 g in weight, which were fed *ad lib.* at the vivarium of our College. Catheterization in the femoral vein, the femoral artery and the urinary bladder of these

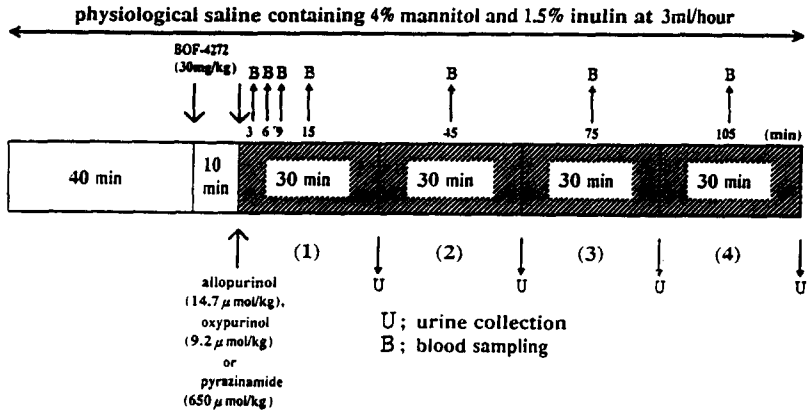

Fig. 1. Protocol of the study. The respective control studies were performed without the administration of BOF-4272.

rats was performed as described previously [13]; then physiological saline containing 4% mannitol and inulin was administered intravenously from the femoral vein at 3 mL/hr. To investigate the effect of BOF-4272 on allopurinol metabolism, BOF-4272 (30 mg/kg) was administered intravenously, 40 min after the beginning of the infusion of mannitol and inulin, to 5 rats (BOF-4272-treated rats) as a solution of 10 mg/mL BOF-4272 in physiological saline, and then 10 min later BOF-4272 (30 mg/kg) was administered again. Immediately after the second administration of BOF-4272, allopurinol (14.7 μmol/kg) was administered as a solution of 7.4 μmol/mL allopurinol in physiological saline (Fig. 1). At 3, 6, 9, 15, 45, 75 and 105 min after the administration of allopurinol, 0.3 mL of blood was drawn from the femoral artery with a heparinized syringe (Fig. 1). In addition, after the administration of allopurinol, urine was collected four times at 30-min intervals (Fig. 1). A control study was performed in 5 rats using the same method as described above except for the administration of plain physiological saline instead of physiological saline containing BOF-4272. To investigate the effect of BOF-4272 on the plasma concentration and the urinary excretion of oxypurinol, 5 rats were also treated with BOF-4272 as described above after catheterization, and then 9.2 μmol/kg oxypurinol was administered intravenously as a solution of 6.6 μmol/mL oxypurinol in physiological saline. Blood and urine samples were obtained at the same times as described above for allopurinol (Fig. 1). In addition, a control study was performed in 5 rats under the same protocol except for the administration of physiological saline without oxypurinol (Fig. 1). Next, to investigate the effect of BOF-4272 on pyrazinamide metabolism, the following study was performed. After catheterization, 5 rats were treated with BOF-4272 as described above and then pyrazinamide (650 μmol/kg) was administered as a solution of 163 μmol/mL in physiological saline (Fig. 1). At 3, 6, 15, 45, 75 and 105 min after the administration of pyrazinamide, 0.3 mL of blood was drawn from the femoral artery and urine was collected as described above (Fig. 1). A control study was also performed in 5 rats by the administration of physiological saline without BOF-4272 (Fig. 1).

Analysis of samples. Allopurinol and oxypurinol in both plasma and urine were determined by HPLC [14], using external standards, and pyrazinamide and its metabolites in both plasma and urine were measured at 410/265 nm (emission/excitation) by the method of Yamamoto *et al.* [12, 15], using a fluorescence HPLC monitor. The concentration of inulin in plasma and urine was determined as described previously [13]. The plasma concentration of BOF-4272 was determined by HPLC as follows. Five microliters of plasma was placed in a small sample tube containing 195 μL of acetonitrile, with thorough mixing for 10 sec. After centrifugation, at 1500 *g* for 10 min, 20 μL of supernatant was loaded onto the column. The HPLC system consisted of an LC-9A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan), an RF 530 fluorescence HPLC monitor (Shimadzu) and a C-R3A Chromatopac Recorder (Shimadzu). The mobile phase was 0.02 M KH_2PO_4 (pH 3.0) and acetonitrile (1 : 1), and the flow rate was 1.0 mL/min. The column used was a Wakosil 5C 18-200 (4.6 × 150 mm). A fluorescence detector was used at 410/315 nm. The activity of xanthine oxidase + xanthine dehydrogenase was measured as follows. The reaction mixture consisted of 0.25 mL of 100 mM Tris–HCl buffer (pH 7.8) containing 0.1 mM EDTA, 50 μL of 10 mM potassium oxonate, 50 μL of 10 mM NAD, 0.1 mL of 360 μM xanthine and 50 μL of the rat liver homogenate. The reaction was initiated by the addition of 0.1 ml of 360 μM xanthine to the test tube containing 0.25 ml of 100 mM Tris–HCl buffer including 0.1 mM EDTA, 50 μl of 1 mM potassium oxonate, 10 mM NAD and the rat liver homogenate or its heat-treated supernatant after preincubation for 15 min. At 0 and 5 min after the initiation, 0.2 mL of reaction mixture was taken, the enzyme reactions

were terminated by the addition of 0.4 mL of 0.2 M perchloric acid, and then the mixture was centrifuged at 1500 *g*. After the supernatant was neutralized with 1 M K_2CO_3, 40 μL of the neutralized supernatant was injected onto a reversed-phase HPLC described previously [14]. The activity of aldehyde oxidase was measured spectrophotometrically as described previously [16]. In brief, the reaction mixture consisted of 1.475 mL of 67 mM phosphate buffer (pH 7) containing 25 μM *p*-dimethylaminocinnamaldehyde (DMAC)* and 25 μL of the homogenate. The reaction was initiated by the addition of 25 μL of the homogenate, and the decrease in absorbance due to disappearance of the substrate (DMAC) was monitored at 398 nm.

In vitro *study*. The rat livers were weighed and then homogenized with a Potter–Elvehjem homogenizer in 3 vol. of ice-cold 0.1 M phosphate buffer (pH 7.4) solution. To investigate the effect of BOF-4272 on the activities of xanthine oxidase and aldehyde oxidase in the homogenate, 0.05 to 20 μg/mL BOF-4272 (final concentration) was added to the assay systems described above, and the activities were expressed as percent of control. In addition, to determine whether aldehyde oxidase oxidizes allopurinol and pyrazinamide, partial purification of aldehyde oxidase was performed as follows. The rat liver homogenate was heated rapidly to 55–60° on a water bath for 10 min and then centrifuged (14,000 *g*, 45 min, 4°). Ammonium sulfate was added to the supernatant, and ammonium sulfate precipitate (30–50%) was obtained. The precipitate obtained by centrifugation was dissolved in 0.01 M phosphate buffer (pH 7.4) and dialyzed against 0.01 M phosphate buffer at 4°. The dialyzed solution was applied on a DEAE-cellulose column (2.5 × 15 cm) with a linear gradient of 0.01 to 0.2 M potassium phosphate buffer at 4°, a flow rate of 50 mL/hr, and a fraction size of 10 mL. This procedure partially separated aldehyde oxidase from xanthine oxidase. Fractions containing aldehyde oxidase were then collected. To exclude xanthine oxidase completely, the batch method was performed using hydroxyapatite. In brief, the collected fractions were diluted 2-fold with 0.01 M phosphate buffer and were mixed with hydroxyapatite at a ratio of 2:1 (v/w). After centrifugation at 1700 *g* for 20 min, the supernatant containing aldehyde oxidase was collected, and then the residual aldehyde oxidase was eluted from hydroxyapatite by the addition of 3 mL of 100 mM phosphate buffer to 1 g of hydroxyapatite; after centrifugation, the supernatant containing aldehyde oxidase was collected. These supernatants were combined and concentrated 10-fold, and the concentrated factions were dialyzed against 10 mM phosphate buffer (pH 7.4). Fifty microliters of the dialyzed fraction (1.2 mg protein/mL) was added to the mixture which consisted of 250 μL of 100 mM phosphate buffer (pH 7.4) containing 2.6 μmol/mL allopurinol, 2.6 μmol/mL allopurinol + 6 μg/mL BOF-4272, 2.6 μmol/mL allopurinol + 1.5 mg/mL benzamidine, 5.7 μmol/mL pyrazinamide, 5.7 μmol/mL pyrazinamide + 6 μg/mL BOF-4272 or 5.7 μmol/mL pyrazinamide + 1.5 mg/mL benzamidine, and the reaction mixtures were incubated at 37° for 30 min. The reaction was terminated by adding 60 μL of 20% perchloric acid, and then the mixture was centrifuged at 1500 *g*. After the supernatant was neutralized with 1 M K_2CO_3, 20 μL of the neutralized supernatant containing allopurinol was injected onto the reversed-phase HPLC described previously [14], and 20 μL of the neutralized supernatant containing pyrazinamide was injected onto reversed-phase HPLC at 410/265 nm [12]. To examine the effect of BOF-4272 on hypoxanthine guanine phosphoribosyl transferase (HGPRT) in rat erythrocytes, 20 μg/mL was added to the HGPRT assay system described previously [17], and its activity was compared with that of a control incubation.

* Abbreviations: DMAC, *p*-dimethylaminocinnamaldehyde; and HGPRT, hypoxanthine guanine phosphoribosyl transferase.

RESULTS

Plasma concentration of BOF-4272 in BOF-4272-treated rats. Three minutes after the second administration of BOF-4272, the plasma concentration of BOF-4272 was 19.3 ± 4.1 μg/mL (mean ± SEM); this concentration decreased gradually to 0.5 ± 0.2 μg/mL (mean ± SEM) after 105 min in the BOF-4272-treated rats (Fig. 2A). During the study, the plasma concentration of BOF-4272 did not differ from that of the allopurinol-, oxypurinol- or pyrazinamide-administered rats that had been treated with BOF-4272 (data not shown).

Plasma concentrations and urinary excretion of oxypurinol and allopurinol after administration of allopurinol. The plasma concentration of oxypurinol was decreased markedly in the BOF-4272-treated rats, as compared with that in the control rats (Fig. 2B). In the control rats, the concentration of oxypurinol gradually decreased from 3 to 105 min after the administration of allopurinol. However, in the BOF-4272-treated rats, there was less change over time. The plasma concentration of allopurinol was higher in the BOF-4272-treated rats than in the controls (Fig. 2C); allopurinol was still detected in the plasma of the BOF-4272-treated rats 45 min after its administration, although it was not detected in the plasma of the control rats. After the intravenous administration of allopurinol in the control rats (Fig. 2C), the plasma concentration of allopurinol decreased rapidly, and in 3 min that of oxypurinol was at a level comparable with the plasma concentration of allopurinol in the BOF-treated rats (Fig. 2B). Inulin clearance during the study neither changed nor differed between the BOF-4272-treated rats and the controls (Table 1). However, the urinary excretion of oxypurinol in the BOF-4272-treated rats decreased to 0.2 of the value in the control rats (Table 1). In contrast to a decrease in the urinary excretion of oxypurinol in the BOF-4272-treated rats, the urinary excretion of allopurinol increased 1.9-fold in the BOF-4272-treated rats, as compared with the control rats (Table 1). These results suggested that BOF-4272 inhibited the rapid oxidation of allopurinol to oxypurinol, resulting in decreases in the plasma concentration and the urinary excretion of oxypurinol and increases in those of allopurinol, compared with those of the

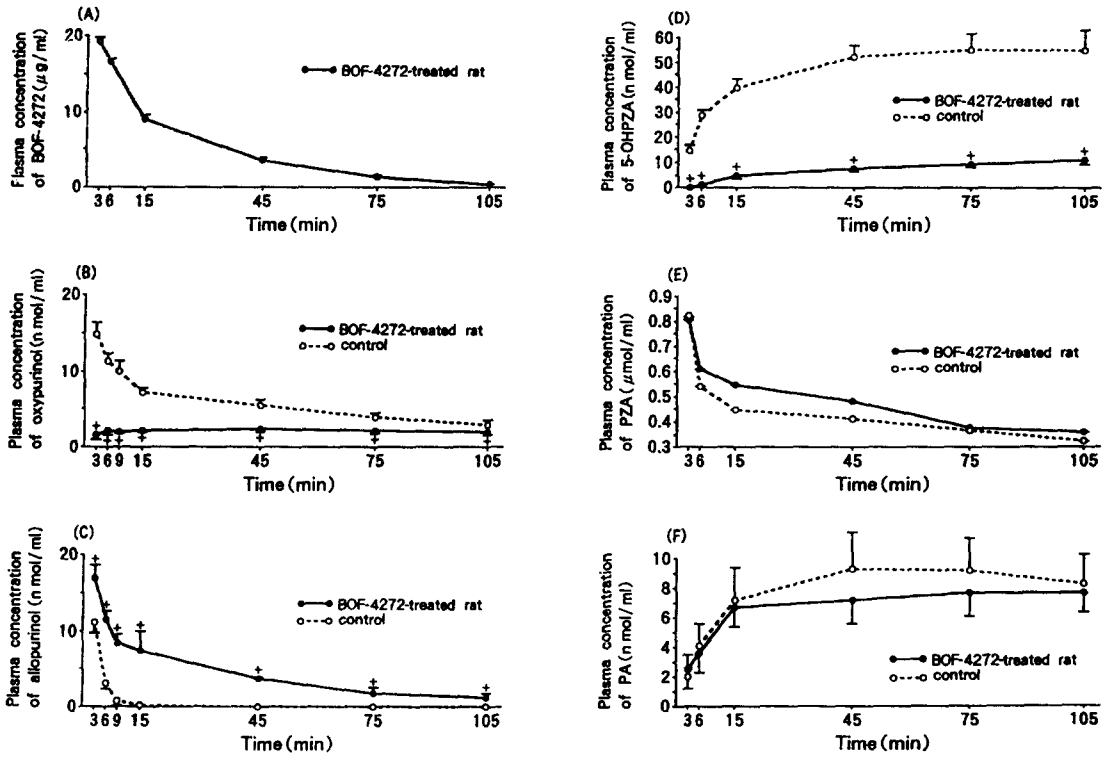

Fig. 2. Plasma concentrations of BOF-4272, oxypurinol, allopurinol, 5-hydroxypyrazinamide (5-OHPZA), pyrazinoic acid (PZA) and pyrazinamide (PA). Values are means ± SEM, N = 5. (A) Plasma concentration of BOF-4272 in BOF-treated rats. (B) Plasma concentration of oxypurinol in BOF-4272-treated and control rats after the administration of allopurinol. (C) Plasma concentration of allopurinol in BOF-4272-treated and control rats after the administration of allopurinol. The plasma concentration of allopurinol at 45, 75 and 100 min in the BOF-treated rats was compared with the detection limit of allopurinol (0.05 nmol/mL). (D) Plasma concentration of 5-OHPZA in BOF-4272-treated and control rats after the administration of pyrazinamide. (E) Plasma concentration of PZA in BOF-4272-treated and control rats after the administration of pyrazinamide. (F) Plasma concentration of PA in BOF-4272-treated and control rats after the administration of pyrazinamide. Key: (+) significantly different from control: $P < 0.05$.

controls, since BOF-4272 affected neither HGPRT activity *in vitro* nor orotate phosphoribosyl transferase activity *in vivo*, as described below. However, during the first period, the urinary excretion of allopurinol did not increase (Table 1) despite the higher plasma concentration of allopurinol in the BOF-treated rats than in the control rats (Fig. 2C), suggesting a BOF-4272-induced decrease in the renal clearance of allopurinol. In addition, the sum of allopurinol and oxypurinol excretion decreased 0.5-fold in the BOF-treated rats compared with that in the control rats (Table 1), and the sum of the concentrations of allopurinol and oxypurinol at 105 min was not different between BOF-4272-treated rats (3.3 ± 0.5 nmol/mL) and the control rats (3.0 ± 0.4 nmol/mL) (mean ± SEM) (Fig. 2, B and C). These results suggested that BOF-4272 induced an increase in the extrarenal excretion and/or the tissue incorporation of allopurinol since BOF-4272 did not affect the metabolism of oxypurinol, as described below.

Plasma concentration and urinary excretion of oxypurinol in BOF-4272-treated rats after administration of oxypurinol. Three minutes after the administration of oxypurinol, the plasma concentration of oxypurinol was 15 ± 1 nmol/mL in the BOF-4272-treated rats and 14 ± 2 nmol/mL (mean ± SEM) in the control rats, indicating no difference between the two groups. In addition, there was no difference in the plasma concentrations of oxypurinol between the BOF-4272-treated rats and the control rats at 6, 9, 15, 45, 75 and 105 min (data not shown). Total urinary excretion of oxypurinol was also similar in both groups [3.5 ± 0.5 and 3.6 ± 0.4 μmol (mean ± SD) in the BOF-4272-treated rats and the control rats, respectively]. In addition, neither the urinary excretion of oxypurinol nor the inulin clearance was different between the BOF-4272-treated rats and the control rats at the first, second, third and fourth periods (data not shown). These results suggested that BOF-4272 did not affect the *in vivo* metabolism of oxypurinol, including the conversion of oxypurinol to oxypurinol nucleotide by orotate phosphoribosyl transferase.

Plasma concentrations and urinary excretion of pyrazinamide and its metabolites after administration

Table 1. Urinary excretions of allopurinol, oxypurinol, pyrazinamide, pyrazinamide metabolites and clearance of inulin in BOF-4272-treated rats (BOF) and control rats

Treatment	Urinary excretion				
	First period	Second period	Third period	Fourth period	Total
	After administration of allopurinol				
	Excretion of oxypurinol (μmol/kg body weight)				
BOF	0.09 ± 0.04*	0.24 ± 0.04*	0.21 ± 0.08*	0.21 ± 0.06*	0.69 ± 0.09*
Control	1.00 ± 0.40	1.00 ± 0.40	0.90 ± 0.40	0.60 ± 0.10	3.70 ± 0.30
	Excretion of allopurinol (μmol/kg body weight)				
BOF	0.75 ± 0.25	0.46 ± 0.16*	0.23 ± 0.10*	0.18 ± 0.09*	1.50 ± 0.40*
Control	0.73 ± 0.08	0.08 ± 0.07	ND	ND	0.81 ± 0.05
	Inulin clearance (mL/min/kg body weight)				
BOF	8.4 ± 0.6	8.1 ± 0.3	8.5 ± 0.4	8.3 ± 0.4	
Control	8.1 ± 0.7	8.1 ± 1.0	7.5 ± 1.0	8.6 ± 1.9	
	After administration of pyrazinamide				
	Excretion of 5-OHPA (μmol/kg body weight)				
BOF	ND*	ND*	0.05 ± 0.06*	0.09 ± 0.08*	0.14 ± 0.10*
Control	0.28 ± 0.20	2.10 ± 0.40	2.50 ± 1.30	3.50 ± 1.50	7.50 ± 2.90
	Excretion of 5-OHPZA (μmol/kg body weight)				
BOF	0.42 ± 0.23*	0.6 ± 0.24*	1.63 ± 0.8*	2.50 ± 1.2*	4.80 ± 1.30*
Control	7.90 ± 6.10	22.00 ± 10.00	23.00 ± 11.00	25.00 ± 11.00	78.00 ± 35.10
	Excretion of PA (μmol/kg body weight)				
BOF	0.42 ± 0.25	0.86 ± 0.38	1.10 ± 0.40	1.30 ± 0.50	3.9 ± 1.0
Control	0.46 ± 0.28	1.10 ± 0.40	1.30 ± 0.40	1.30 ± 0.70	4.10 ± 1.10
	Excretion of PZA (μmol/kg body weight)				
BOF	2.20 ± 0.70	1.50 ± 0.30	1.30 ± 0.20	1.40 ± 0.4	6.40 ± 0.90
Control	1.70 ± 0.80	1.40 ± 0.50	1.30 ± 0.50	1.10 ± 0.30	5.60 ± 1.80
	Inulin clearance (mL/min/kg body weight)				
BOF	8.4 ± 0.9	7.3 ± 0.9	8.1 ± 1.1	8.3 ± 1.3	
Control	8.0 ± 0.6	8.0 ± 1.1	7.6 ± 1.1	7.3 ± 1.0	

Values are means ± SD, N = 5. Abbreviations: 5-OHPA, 5-hydroxypyrazinoic acid; 5-OHPZA, 5-hydroxypyrazinamide; PA, pyrazinoic acid; PZA, pyrazinamide; and ND, not detected.
* Significantly different from control, $P < 0.05$.

of pyrazinamide. The plasma concentration of 5-hydroxypyrazinamide was decreased markedly in the BOF-4272-treated rats, as compared with the control rats (Fig. 2D), suggesting that BOF-4272 markedly inhibited the oxidation of pyrazinamide to 5-hydroxypyrazinamide, resulting in a decrease in the plasma concentration of 5-hydroxypyrazinamide. 5-Hydroxypyrazinoic acid was not detected in the plasma of either BOF-4272-treated or control rats during the study, suggesting that the renal clearance of 5-hydroxypyrazinoic acid was very high and/or 5-hydroxypyrazinoic acid was not sufficiently produced from pyrazinoic acid since the plasma concentration of pyrazinoic acid, to which pyrazinamide was metabolized by microsomal deamidase, was low. Inulin clearance neither changed during the study nor differed between the BOF-4272-treated and the control rats (Table 1). The urinary excretions of pyrazinamide and pyrazinoic acid did not differ between the BOF-4272-treated and the control rats (Table 1), whereas the urinary excretions of 5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid decreased markedly in the BOF-4272-treated rats, as compared with the control rats (Table 1). Their decreases were 0.06-and 0.02-fold, respectively. These results suggested that BOF-4272 almost completely inhibited the production of the 5-hydroxymetabolites (5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid) by xanthine oxidase, resulting in decreases in the plasma concentration and the urinary excretion of the 5-hydroxymetabolites in the BOF-4272-treated rats. Although the sum of the urinary excretion of pyrazinamide and its metabolites decreased 0.14-fold in the BOF-treated rats, compared with that in the control rats (Table 1), the plasma concentrations of pyrazinamide and pyrazinoic acid did not differ between the BOF-4272-treated and the control rats (Fig. 2, E and F). Since the conversion of pyrazinoic acid to 5-hydroxypyrazinoic acid seemed to be small in the control rats, pyrazinoic acid may not have increased in the BOF-4272-treated rats despite the conversion being inhibited by BOF-4272, while the plasma concentration of pyrazinamide should have increased in BOF-4272-treated rats since the conversion of pyrazinamide to 5-hydroxypyrazinamide was inhibited by BOF-4272. However, the plasma concentration of pyrazinamide did not increase in the BOF-4272-treated rats. Therefore, these results suggested a BOF-4272-induced increase in the

Table 2. *In vitro* effect of BOF-4272 on the activities of xanthine oxidase + xanthine dehydrogenase, aldehyde oxidase in homogenate and HGPRT in hemolysate

BOF-4272 concentration in reaction mixture (μg/mL)	Xanthine oxidase + xanthine dehydrogenase activity (% of control)	Aldehyde oxidase activity (% of control)	HGPRT activity (% of control)
0.05	25	102	
0.25	6	98	
0.5	0	100	
10	0	101	98
20	0	100	101

Values are the means of 3 experiments. Control values of the activities of xanthine oxidase + xanthine dehydrogenase, aldehyde oxidase, and HGPRT were 13.9 μmol/mg protein/min, 22.7 μmol/mg protein/min, and 90 nmol/g Hb/min, respectively.

extrarenal excretion and/or the tissue incorporation of pyrazinamide.

Effect of BOF-4272 on the activities of aldehyde oxidase, xanthine oxidase and hypoxanthine guanine phosphoribosyl transferase. More than 0.5 μg/mL BOF-4272 inhibited xanthine oxidase activity completely during a 5-min incubation; 0.05 μg/mL BOF-4272 also inhibited xanthine oxidase activity although not completely (Table 2). In contrast, 20 μg/mL BOF-4272 did not affect the activity of aldehyde oxidase or the activity of erythrocyte HGPRT, which converts allopurinol to allopurinol nucleotide. At 20 μg/mL neither M-4 nor BOF-4269 (metabolites of BOF-4272 and weak inhibitors of xanthine oxidase) affected aldehyde oxidase or HGPRT (data not shown).

Oxidation of allopurinol and pyrazinamide by aldehyde oxidase. Since the lower detection limits of oxypurinol and 5-hydroxypyrazinamide were 0.05 and 0.06 nmol/mL, respectively, the reaction mixture was incubated for 30 min. During the 30-min incubation study using the partially purified aldehyde oxidase, 1.8 nmol of 5-hydroxypyrazinamide was produced in the reaction mixture (0.3 mL) containing pyrazinamide and 1.8 nmol of oxypurinol was produced in the reaction mixture (0.3 mL) containing allopurinol. However, their production was inhibited to 10 and 15% of control, respectively, by the addition of 1.5 mg/mL of benzamidine (an aldehyde oxidase inhibitor) to the reaction mixture, but not by the addition of 20 μg/mL of BOF-4272 (a xanthine oxidase inhibitor); in the presence of BOF-4272, the oxidation of pyrazinamide to 5-hydroxypyrazinamide was 99% of control and the oxidation of allopurinol to oxypurinol was 101% of control. (The control values for the production of oxypurinol and 5-hydroxypyrazinamide were both 1 nmol/mg protein/min.) These results indicated that aldehyde oxidase converted pyrazinamide and allopurinol to 5-hydroxypyrazinamide and oxypurinol, respectively.

DISCUSSION

Allopurinol is a potent inhibitor of xanthine oxidase but is itself metabolized to oxypurinol by xanthine oxidase, and is also metabolized to oxypurinol by aldehyde oxidase [2, 3] as shown in the present *in vitro* study. Pyrazinamide and pyrazinoic acid (a metabolite of pyrazinamide) are oxidized to 5-hydroxypyrazinamide [7–9] and 5-hydroxypyrazinoic acid [9], respectively, by xanthine oxidase. In addition, it has been suggested that pyrazinamide is oxidized to 5-hydroxypyrazinamide by aldehyde oxidase [10] (Fig. 3). In the present study, we demonstrated that pyrazinamide was metabolized to 5-hydroxypyrazinamide by aldehyde oxidase. However, the contribution of xanthine dehydrogenase or aldehyde oxidase to the oxidation of these two agents has not been determined *in vivo*. In the present study, we demonstrated that BOF-4272 inhibited the activity of xanthine oxidase as described previously [11] but did not inhibit the activity of aldehyde oxidase or HGPRT *in vitro* (Table 2). In addition, neither M-4 nor BOF-4269 (both major metabolites of BOF-4272) affected the activity of aldehyde oxidase (data not shown), although these major metabolites are weak xanthine oxidase inhibitors. Therefore, using BOF-4272, we investigated the contribution of xanthine dehydrogenase to the oxidation of allopurinol and pyrazinamide *in vivo* and demonstrated that BOF-4272 almost completely inhibited the oxidation of allopurinol to oxypurinol, that of pyrazinamide to 5-hydroxypyrazinamide and that of pyrazinoic acid to 5-hydroxypyrazinoic acid *in vivo*. Even if the plasma concentration of BOF-4272 used in the study may not have completely inhibited the activity of xanthine dehydrogenase *in vivo*, these results strongly suggest that xanthine dehydrogenase is the most important enzyme in the conversion of allopurinol to oxypurinol, pyrazinamide to 5-hydroxypyrazinamide, and pyrazinoic acid to 5-hydroxypyrazinoic acid, respectively, and that aldehyde oxidase plays a minor role in the oxidation of allopurinol to oxypurinol and that of pyrazinamide to 5-hydroxypyrazinamide *in vivo*. Although in BOF-4272-treated rats, the decreases in the plasma concentration and the urinary excretion of oxypurinol (Table 1) and the concomitant increases in the plasma concentration and the urinary excretion of allopurinol (Fig. 2C, Table 1) all seem to be mainly ascribable to the inhibition of xanthine

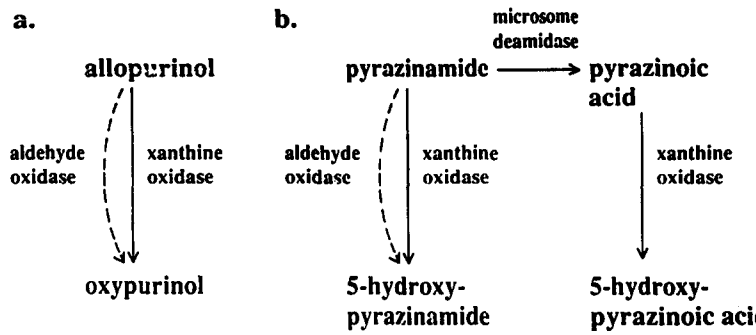

Fig. 3. Metabolism of (a) allopurinol and (b) pyrazinamide by xanthine oxidase and aldehyde oxidase. The dashed line indicates metabolism by aldehyde oxidase.

dehydrogenase by BOF-4272, the present *in vivo* study (Table 1) suggested some effects of BOF-4272 on the excretion and/or the tissue incorporation of allopurinol and pyrazinamide. These effects (BOF-4272-induced increase in the extrarenal excretion and/or the tissue incorporation of allopurinol and pyrazinamide and BOF-induced decrease in the renal clearance of allopurinol) seem to be indicated by the following reasons: (1) Both the sum of the excretion of allopurinol and oxypurinol in urine and that of pyrazinamide and its metabolites in urine decreased considerably in the BOF-treated rats, compared with that in the control rats (Table 1), although a decrease in the urinary excretion of oxypurinol and the 5-hydroxymetabolites of pyrazinamide should be accompanied by a comparable increase in the respective urinary excretion of allopurinol and pyrazinamide in the BOF-4272-treated rats. (2) BOF-4272 affected neither the plasma concentration nor the urinary excretion of oxypurinol after the administration of oxypurinol. (3) The sum of the plasma concentrations of allopurinol and oxypurinol at 105 min was not different between the BOF-4272-treated rats and the control rats despite a decrease in the sum of the urinary excretion of allopurinol and oxypurinol. (4) BOF-4272 affected neither the plasma concentration nor the urinary excretion of pyrazinamide despite the inhibition by BOF-4272 of the conversion of pyrazinamide to 5-hydroxypyrazinamide. (5) In the first period, the urinary excretion of allopurinol did not increase in the BOF-4272-treated rats, compared with that in the control rats (Table 1) despite the higher plasma concentration of allopurinol in the BOF-4272-treated rats than in the control rats (Fig. 2C).

In previous studies [10, 18], it was suggested that aldehyde oxidase plays a major role in the oxidation of allopurinol *in vivo* in humans and rodents, and that in xanthinuric patients who can metabolize neither allopurinol to oxypurinol nor pyrazinamide to 5-hydroxypyrazinamide, there was a combined deficiency of the activities of xanthine oxidase and aldehyde oxidase. In xanthinuric patients able to metabolize both allopurinol to oxypurinol and pyrazinamide to 5-hydroxypyrazinamide, there was only a deficiency of xanthine oxidase activity. Although the present study in rats suggested that aldehyde oxidase plays only a minor role in the oxidation of allopurinol and pyrazinamide *in vivo*, it is difficult to extrapolate the present results to humans *in vivo* for the following reasons: (1) Allopurinol and pyrazinamide were administered intravenously in the rats, but they are usually administered orally. With oral administration, they may be metabolized at the level of the intestinal mucosa. (2) The distribution of the two enzymes in rats is different from that in humans. Xanthine oxidase, in particular, has a wide tissue distribution in rats, whereas in humans it is confined to the liver and intestinal mucosa. (3) The renal clearance of oxypurinol in rats is also different from that in humans. Therefore, further study is needed to investigate the effects of BOF-4272 on the metabolism of allopurinol and pyrazinamide in humans.

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