

Original article

The creation of urate under-excretion animal models and the uricosuric effects of dihydropyridine calcium channel blockers *in vivo*

Running head: CCBs induce uricosuria in model mice

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Abstract (193)

The purpose of this study was to create novel urate under-excretion animal models using pyrazinamide and to evaluate whether dihydropyridine calcium channel blockers (CCBs) have uricosuric effects *in vivo*.

Adult male ICR mice were treated with pyrazinamide, vehicle (DMSO), or tap water. Thirty minutes later, pyrazinamide-treated mice were given benzbromarone, losartan, nilvadipine, nitrendipine, nifedipine or azelnidipine. Six hours after the second administration, urine (by urinary bladder puncture) and plasma were collected to measure uric acid and creatinine levels, and fractional excretion of uric acid (FEUA) and creatinine clearance (Ccr) were calculated and evaluated. There was no significant difference in the levels of plasma uric acid, plasma creatinine, Ccr, urinary N-acetyl- β -D-glucosaminidase (NAG) and urinary NAG-creatinine ratio between water, DMSO, and pyrazinamide-treated mice. But the FEUA of pyrazinamide-treated mice was significantly lower than water mice. The FEUA was significantly higher in mice taking the dihydropyridine CCBs (nilvadipine, nitrendipine, nifedipine, and high-dose azelnidipine) than in pyrazinamide-treated mice. There was no significant difference in Ccr.

Thus, a novel animal model created with PZA administration was useful as a urate

under-excretion animal model that was probably URAT1-mediated, and the uricosuric effect of dihydropyridine CCBs was confirmed *in vivo*.

Keywords: uric acid, urate, fractional excretion of uric acid, urate under-excretion animal model, calcium channel blocker

Introduction

In humans, serum uric acid levels are determined by the balance between enzymatic production mainly in the liver by xanthine oxidase and urinary excretion mediated by renal tubular urate transporters [1]. Renal excretion of urate corresponds to 60–70% of total urate excretion from the human body [2, 3]. Human urate transporter 1 (URAT1; *SLC22A12*) was first identified in 2002 [4]. URAT1 exchanges urate and monocarboxylates in the molecular mechanism of urate reabsorption. Later, several transporters, such as sodium phosphate transporter 4 (*SLC17A3*) and glucose transporter 9/voltage-driven urate transporter 1 (*SLC2A9*), were found to be closely associated with reabsorption and excretion of urate [5-7]. It has been reported that hyperuricemia is often associated with hypertension and high mortality [8]. The causes of hyperuricemia are urate over-production, urate under-excretion, and combined type. The hyperuricemia induced by urate under-excretion should be treated with uricosuric drugs, and the pharmacological action target of these drugs is URAT1.

In the Japanese guideline for the management of hyperuricemia and gout, it is noted that losartan and calcium channel blockers (CCBs) have urate-lowering effects, thus decreasing serum uric acid levels [9]. Losartan is one of the angiotensin II receptor blockers, and the molecular mechanism of the urate-lowering effect of losartan

has been reported to be inhibition of URAT1 and increased excretion of urate in the urine [10]. CCBs are common anti-hypertensive drugs that inhibit calcium entry by interacting with voltage-gated calcium channels [11]. Several clinical studies have shown that CCBs have uricosuric actions [12, 13]. In previous studies, it has been reported that CCBs, especially the dihydropyridine group, have uricosuric effects in rats, and the effect was considered to be induced by increases of renal blood flow and the glomerular filtration rate [14]. Recently, we reported that the urate-lowering effect of CCBs, especially the dihydropyridine group, may be associated with inhibition of renal urate reabsorption mediated by renal urate transporters such as URAT1 [15]. Nevertheless, little is known about the molecular mechanism of the urate-lowering effect of CCBs.

Oxonate-treated mice known as an animal model for hyperuricemia are injected intraperitoneally with the uricase inhibitor potassium oxonate [16]. We thought that this hyperuricemia animal model is not appropriate for evaluating the uricosuric action with URAT1 because hyperuricemia in this animal model is induced by the inhibition of the uricase enzyme that oxidizes uric acid to allantoin. A urate under-excretion animal model is needed to evaluate the uricosuric action with URAT1 and other urate transporters, but there was no appropriate animal model.

Pyrazinamide (pyrazine-2-carboxamide) is a well-known antituberculosis drug, and it was previously reported that pyrazinamide induces hyperuricemia as a side effect in clinical use [17, 18]. URAT1 mediates the exchange of urate for several organic anions, inorganic anions, and monocarboxylic acids such as 2-pyrazinoic acid [4]. After taking pyrazinamide, it is presumed that pyrazinamide is converted into pyrazinoic acid, and 5-hydroxypyrazinoic acid in the body [19, 20]. These substrates are monocarboxylic acids. The major pathway of pyrazinamide metabolism is from pyrazinamide to these substrates, which are the excretory products [21]. In the kidney, the main excretion pathway of pyrazinamide is glomerular filtration [21].

The purpose of this study was to create novel animal models using pyrazinamide and to evaluate whether dihydropyridine CCBs have uricosuric effects *in vivo*.

Materials and Methods

Animals

Adult male ICR mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) and used in the present study. Eleven-week-old mice were used in each experiment; their body weights were 40.4 ± 0.2 g. All mice were supplied with a commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*. The animals were housed

under controlled temperature and light conditions (lights on from 7:00 to 19:00). The experimental protocols were approved by the Animal Care and Use Committee, and experiments were carried out according to the Guidelines for Animal Experimentation of Dokkyo Medical University. All efforts were made to minimize the number of animals used and their suffering.

Experimental protocols

Pyrazinamide was obtained from Sigma-Aldrich Co., LLC. (Saint Louis, MO, USA). Benzbromarone, nitrendipine, nifedipine, and dimethyl sulfoxide (DMSO) were obtained from Wako Pure Chemical Industry Ltd. (Osaka, Japan). Nilvadipine and azelnidipine were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Losartan was purchased from Merck & Co. (Kenilworth, NJ, USA). All agents, including pyrazinamide, were dissolved in DMSO and administered per os to mice using a cannula at an injection volume of 2.5 ml/kg body weight. Mice were anesthetized with isoflurane (Pfizer Japan Inc., Tokyo, Japan) and treated with pyrazinamide (400 mg/kg), vehicle, or tap water.

Previous studies investigated rats treated with losartan (3.0 mg/kg, per os) by oral gavage [22], and described mice treated with losartan (3.0 mg/kg) that was dissolved in

drinking water for consumption [23]. Based on these reports, an losartan dose of 3.0 mg/kg was first selected for the present study. A previous *in vivo* study showed the effects of nilvadipine on the cardiovascular system [24]. In the previous study, the doses of nilvadipine used were 1.0 or 3.2 mg/kg in rats. Accordingly, both nilvadipine concentrations (1.0 and 3.2 mg/kg, per os) were used in the present study. Based on the above information, 3.2 mg/kg doses of nitrendipine, nifedipine, and azelnidipine were selected. However, azelnidipine (3.2_mg/kg) failed to give a clear result. In regard to azernidipine, high-dose azelnidipine (10.0_mg/kg) was selected in the present study.

Protocol is shown in Supplemental figure. Thirty minutes after the first administration (pyrazinamide 400 mg/kg), mice were additionally treated with benzbromarone (3.0 or 10.0 mg/kg), losartan (3.0 mg/kg), nilvadipine (1.0 or 3.2 mg/kg), nitrendipine (3.2 mg/kg), nifedipine (3.2 mg/kg), or azelnidipine (10.0 mg/kg [high-dose azelnidipine]). The mice after voiding were placed individually in metabolic cages (KN-645; Natsume Seisakusho Co., Ltd., Tokyo, Japan) to collect urine for six hours. Six hours after the second administration, the mice were anesthetized with isoflurane. Then urine samples were collected by urinary bladder puncture, and blood samples were obtained from the inferior vena cava. The mice were euthanized by bleeding from the inferior vena cava under deep anesthesia. The blood was collected in heparinized sampling tubes, and it

was centrifuged to obtain the plasma for the determination of uric acid and creatinine levels. The collected urine from metabolic cage and urinary bladder puncture were combined as total urine volume (UV).

Quantitative determinations of uric acid and creatinine levels, urinary markers of tubular damage

To measure uric acid levels in plasma (Pua) and urine (Uua), the spectrophotometric method combined with measurement of total antioxidative capacity by ferric reducing anti oxidative power (FRAP) assay with the uricase-reaction (specific elimination of urate) was used [25]. In this method, uric acid levels were calculated from FRAP values. Uricase was obtained from Oriental Yeast Co., Ltd. The QuantiChrom™ Creatinine Assay Kit (BioAssay Systems, Hayward, CA, USA) was used to measure the creatinine levels (mg/dl) in plasma (Pcr) and urine (Ucr).

Urinary N-acetyl- β -D-glucosaminidase (NAG) and urinary β 2 microglobulin were measured as the marker of tubular damage. Urinary NAG was determined by colorimetric method (BML, Inc., Japan). Urinary β 2 microglobulin was determined by latex coagulation method (BML, Inc., Japan). Urinary creatinine has been used to adjust for urinary NAG in many reports. Recently, we have also reported clinical research with

using urinary NAG / urinary creatinine levels in human [26, 27]. According to the above, we showed urinary NAG, and urinary NAG / urinary creatinine in the present study.

Fractional excretion of uric acid and creatinine clearance

Fractional excretion of uric acid (FEUA) was calculated from Pua, Uua, Pcr, and Ucr.

Creatinine clearance (Ccr) was calculated from Ucr, Pcr, and UV (ml/min/kg). There are reports using Ccr in rats, which units composed from urine volume, time, and body weight [28,29]. This same units have been also used in mouse as well [30]. According to the above, we showed Ccr in the present study.

$$\text{FEUA (\%)} = (\text{Uua} \times \text{Pcr} \times 100) / (\text{Pua} \times \text{Ucr})$$

$$\text{Ccr (ml/min/kg)} = (\text{UV} \times \text{Ucr}) / \text{Pcr}$$

Statistical analysis

Statistical tests were performed using JMP (version 11.2.0; SAS Institute Inc., Cary, NC, USA). The statistical method was assessed by the Fisher's least significant difference test. P-values less than 0.05 were considered significant. Firstly, significant differences were determined using one-way analysis of variance (ANOVA). When there

was a significant difference among groups by using ANOVA, followed by Student's t-tests were performed between pyrazinamide group and other drug (benzbromarone and antihypertensive drugs) group. All data are presented as means \pm SE.

Results

Creation of renal urate underexcretion mice

The results for Pua, Pcr, Urinary NAG, and Urinary NAG-creatinine ratio of the Water group, DMSO first (DMSO-1) group, and pyrazinamide first (pyrazinamide-1) group (n = 8, 6, and 6, respectively) are shown in Table 1. One specimen for urinary NAG and urinary NAG-creatinine ratio was failed to obtain, because the amount of the specimen was insufficient. There was no significant difference in Pua, Pcr, urinary NAG, and urinary NAG-creatinine ratio. The results for FEUA and Ccr of the Water, DMSO, and pyrazinamide-1 groups are shown in Figure 1 (n = 8, 6, and 6, respectively). FEUA (%) was 16.44 ± 2.26 (Water group), 12.87 ± 3.92 (DMSO-1 group), and 2.61 ± 0.74 (pyrazinamide-1 group) (Figure 1A). There was a significant difference in FEUA among the 3 groups (p = 0.004). There was no significant difference in FEUA between the Water group and the DMSO-1 group (p = 0.453), but FEUA was significantly lower in the pyrazinamide-1 group than in the Water group (p < 0.001) and the DMSO-1 group

($p = 0.047$). Ccr (ml/min/kg) was 8.19 ± 0.84 (Water group), 7.28 ± 1.17 (DMSO group), and 8.21 ± 1.51 (pyrazinamide-1 group) (Figure 1B). There was no significant difference in Ccr among the 3 groups ($p = 0.820$).

Effects of benzbromarone, losartan, and nilvadipine

Figure 2 shows FEUA and Ccr in the pyrazinamide second (pyrazinamide-2) group, administered with benzbromarone 3.0 mg/kg (benzbromarone3.0) group, administered with benzbromarone 10.0 mg/kg (benzbromarone10.0) group, and administered with losartan 3.0 mg/kg (losartan3.0) group ($n = 5, 6, 5,$ and $8,$ respectively).

FEUA (%) was 3.90 ± 1.32 (pyrazinamide-2 group), 4.90 ± 0.70 (benzbromarone3.0 group), 16.40 ± 3.44 (benzbromarone10.0 group), and 13.04 ± 2.63 (losartan3.0 group) (Figure 2A). There was a significant difference in FEUA among the 4 groups ($p = 0.004$). FEUA was higher in the benzbromarone3.0 group than in the pyrazinamide-2 group, though no significant difference was seen between the two groups ($p = 0.529$). FEUA was significantly higher in the benzbromarone10.0 group than in the pyrazinamide-2 group ($p = 0.019$). FEUA was significantly higher in the losartan3.0 group than in the pyrazinamide-2 group ($p = 0.011$). Ccr (ml/min/kg) was 7.94 ± 0.99 (pyrazinamide-2 group), 9.12 ± 1.20 (benzbromarone3.0 group), 10.10 ± 1.74

(benzbromarone10.0 group), and 7.80 ± 0.82 (losartan3.0 group) (Figure 2B). There was no significant difference in Ccr among the 4 groups ($p = 0.487$).

Figure 3 shows the results for FEUA and Ccr in the pyrazinamide third (pyrazinamide-3) group, administered with nilvadipine 1.0 mg/kg (nilvadipine1.0) group, and administered with nilvadipine 3.2 mg/kg (nilvadipine3.2) group ($n = 6, 9,$ and 9 , respectively). FEUA (%) was 3.67 ± 0.83 (pyrazinamide-3 group), 6.46 ± 0.92 (nilvadipine1.0 group), and 7.29 ± 0.87 (nilvadipine3.2 group) (Figure 3A). There was a significant difference in FEUA among the 3 groups ($p = 0.040$). FEUA was significantly higher in the nilvadipine1.0 group and the nilvadipine3.2 group than in the pyrazinamide-3 group ($p = 0.043$ [nilvadipine1.0 group], 0.011 [nilvadipine3.2 group]). FEUA was higher in the nilvadipine3.2 group than in the nilvadipine1.0 group, but there was no significant difference between them. Ccr (ml/min/kg) was 8.16 ± 0.99 (pyrazinamide-3 group), 10.20 ± 1.51 (nilvadipine1.0 group), and 9.51 ± 1.33 (nilvadipine3.2 group). There was no significant difference in Ccr among the 3 groups ($p = 0.617$).

Effects of calcium channel blockers

The results for FEUA and Ccr in the pyrazinamide fourth (pyrazinamide-4) group (n

= 6) and CCB groups are shown in Figure 4. The CCB groups were administered with nitrendipine 3.2 mg/kg (nitrendipine3.2) group, administered with nifedipine 3.2 mg/kg (nifedipine3.2) group, and administered with azelnidipine 10.0 mg/kg (azelnidipine10.0) group (n = 6, 8, 9, and 10, respectively). FEUA (%) was 2.93 ± 0.91 (pyrazinamide-4 group), 7.21 ± 1.01 (nitrendipine3.2 group), 6.84 ± 1.21 (nifedipine3.2 group), and 13.10 ± 2.33 (azelnidipine10.0 group) (Figure 4A). There was a significant difference among the 4 groups ($p = 0.002$). FEUA was significantly higher in the nitrendipine3.2, nifedipine3.2, and azelnidipine10.0 groups than in the pyrazinamide-4 group ($p = 0.008, 0.023, \text{ and } 0.002$ respectively). Ccr (ml/min/kg) was 7.70 ± 1.47 (pyrazinamide-4 group), 7.69 ± 0.54 (nitrendipine3.2 group), 8.00 ± 0.68 (nifedipine3.2 group), and 5.74 ± 1.05 (azelnidipine10.0 group) (Figure 4B). There was no significant difference in Ccr among the 4 groups ($p = 0.265$).

Discussion

In the present study, without a difference in Ccr between control mice and pyrazinamide-treated mice, FEUA was lower in pyrazinamide-treated mice than in control mice. In addition, FEUA was higher in mice taking nilvadipine, nitrendipine, nifedipine, and high-dose azelnidipine than in pyrazinamide-treated mice. There were

significant differences in FEUA between pyrazinamide-treated mice and mice taking CCBs (nilvadipine, nitrendipine, nifedipine, and high-dose azelnidipine).

Figure 1 and Table 1 show that FEUA was lower in pyrazinamide-treated mice than in control mice, with no significant difference in Ccr, urinary NAG, and urinary NAG-creatinine ratio between the two groups. Urinary β 2-microglobulin was also measured simultaneously as a marker of tubular damage in Water, DMSO-1, and PZA-1 groups. The obtained measurement results of urinary β 2-microglobulin were below the limit of detection (less than 0.3 mg/L) (Data not shown). Moreover, FEUA was higher in mice taking benzbromarone and losartan than in pyrazinamide-treated mice (Figure 2). Benzbromarone is a uricosuric agent used in the treatment of hyperuricemia. As in rodent experiments, benzbromarone has been recently used [31]. It is known that benzbromarone and losartan inhibit urate uptake via URAT1 [4, 32]. Together, these results suggest that the pyrazinamide-treated mice are probably useful as a urate under-excretion animal model. Moreover, the present method is apparently simple and convenient. There was no significant difference in plasma uric acid levels between pyrazinamide-treated mice and control mice (Table 1). For this reason, the present model was not a hyperuricemic animal model, but rather a urate under-excretion animal model. Unfortunately, we only used one dose of pyrazinamide, and the plasma level of

uric acid did not change with this dose. It is not clear how the level of plasma uric acid changes when a higher pyrazinamide dose is used. Uricase in mice has complicated the interpretation of plasma uric acid levels.

We previously reported that the urate-lowering effect of CCBs may be associated with inhibition of renal urate reabsorption mediated by renal urate transporters such as URAT1 [15]. The degree of URAT1 inhibition by CCBs is probably related to their structural differences. In our previous *in vitro* findings, some of the dihydropyridine subgroup of CCBs inhibited URAT1, but the non-dihydropyridine subgroup did not. Based on our previous study, these 4 CCBs (nilvadipine, nitrendipine, nifedipine, and azelnidipine in the dihydropyridine subgroup) were selected for the present *in vivo* study. Nilvadipine was the strongest inhibitor of URAT1 of all of the CCBs investigated in our previous *in vitro* study. For this reason, whether nilvadipine has an inhibitory action of URAT1 was investigated first in the present study. The present study demonstrated that nilvadipine probably inhibits URAT1, and the same doses of nitrendipine and nifedipine also probably do.

In our previous *in vitro* study, azelnidipine did not inhibit URAT1 [15]. However, the present study demonstrated that high-dose azelnidipine increased FEUA significantly. Clinically it has been reported that azelnidipine has a urate-lowering effect

[33]. Miyazaki et al. reported that azelnidipine decreased the serum uric acid level and blood pressure in patients with hyperuricemia and hypertension [33]. In the present study, the data of high-dose azelnidipine provides the interpretation of previous clinical study.

Sodium-dependent monocarboxylate transporter 1 (SMCT1; *SLC5A8*) /2 (SMCT2; *SLC5A12*) is localized at the apical membrane in the renal proximal tubule, and it transport monocarboxylates such as lactate [32, 34, 35]. The proposed model of urate, monocarboxylic acid, and pyrazinamide metabolites transport via URAT1 and SMCT 1/2 at the apical membrane in the proximal renal tubule is shown in Figure 5. Figure 5A shows that urate reabsorption is related with monocarboxylates and transporters in renal proximal tubule cells. First, monocarboxylates are cotransported with Na⁺ via SMCT1/2 from the lumen to the cell cytosol. Then, monocarboxylates and urate are exchange transported as the molecular mechanism of urate reabsorption. Figure 5B shows the molecular mechanism of urate reabsorption with administration of pyrazinamide. In the body, pyrazinamide is converted into pyrazinoic acid and 5-hydroxypyrazinoic acid, which are excreted in urine. Urinary uric acid levels decrease, and this is probably the molecular mechanism of the side effect of hyperuricemia induced by pyrazinamide in humans [4]. In the present study, the plasma uric acid level

of mice did not change significantly with administration of pyrazinamide (Table 1). Mice have the uricase enzyme that oxidizes uric acid to allantoin. Therefore, the authors consider that the plasma uric acid level would not be increased in mice. Figure 5C shows the molecular mechanism of inhibition of URAT1 on proximal renal tubular cells induced by CCBs with administration of pyrazinamide. The pharmacological target of CCBs is URAT1, and they decreased the reabsorption of urate. As a result, the FEUAs of some CCBs were significantly higher than of pyrazinamide.

Recently, it has been reported that various transporters are associated with urate excretion and reabsorption in the proximal tubules. Further experiments are needed to determine whether CCBs act on other urate transporters *in vitro*.

In conclusion, the present study demonstrated that a novel animal model created with pyrazinamide administration was useful as a urate under-excretion animal model that was probably URAT1-mediated, and the uricosuric effect of dihydropyridine CCBs was confirmed *in vivo*. Thus, the advantage of the animal model is that it facilitates the quantitative pharmacological analysis of how urate excretion is affected by drugs.

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Conflicts of interest: None

References

- [1] Anzai N, Endou H. Drug discovery for hyperuricemia. *Expert Opin Drug Discov.* 2007;2:1251–1261.
- [2] Maesaka JK, Fishbane S. Regulation of renal urate excretion: a critical review. *Am J Kidney Dis.* 1998;32:917–933.
- [3] Sorensen LB, Levinson DJ. Origin and extrarenal elimination of uric acid in man. *Nephron.* 1975;14:7–20.
- [4] Enomoto A, Kimura H, Chairoungdua A, et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature.* 2002;417:447–452.
- [5] Jutabha P, Kanai Y, Hosoyamada M, et al. Identification of a novel voltage-driven organic anion transporter present at apical membrane of renal proximal tubule. *J Biol Chem.* 2003;278:27930–27938.
- [6] Otani N, Ouchi M, Hayashi K, Jutabha P, Anzai N. Roles of organic anion transporters (OATs) in renal proximal tubules and their localization. *Anat Sci Int.* 2017;92:200–206.
- [7] Anzai N, Ichida K, Jutabha P, et al. Plasma urate level is directly regulated by a voltage-driven urate efflux transporter URATv1 (SLC2A9) in humans. *J Biol Chem.* 2008;283:26834–26838.

- [8] Zuo T, Liu X, Jiang L, et al. Hyperuricemia and coronary heart disease mortality: a meta-analysis of prospective cohort studies. *BMC Cardiovasc Disord.* 2016;16:207.
- [9] Yamanaka H; Japanese Society of Gout and Nucleic Acid Metabolism. Japanese guideline for the management of hyperuricemia and gout: second edition. *Nucleosides Nucleotides Nucleic Acids.* 2011;30:1018–1029.
- [10] Iwanaga T, Sato M, Maeda T, Ogihara T, Tamai I. Concentration-dependent mode of interaction of angiotensin II receptor blockers with uric acid transporter. *J Pharmacol Exp Ther.* 2007;320:211–217.
- [11] Godfraind T. Calcium channel blockers in cardiovascular pharmacotherapy. *J Cardiovasc Pharmacol Ther.* 2014;19:501–515.
- [12] Ruilope LM, Kirwan BA, de Brouwer S, et al. Uric acid and other renal function parameters in patients with stable angina pectoris participating in the ACTION trial: impact of nifedipine GITS (gastro-intestinal therapeutic system) and relation to outcome. *J Hypertens.* 2007;25:1711–1718.
- [13] Choi HK, Soriano LC, Zhang Y, Rodríguez LA. Antihypertensive drugs and risk of incident gout among patients with hypertension: population based case-control study. *BMJ.* 2012;344:d8190.

- [14] Sugino H, Shimada H. A comparison of the uricosuric effects in rats of diltiazem and derivatives of dihydropyridine (nicardipine and nifedipine). *Jpn J Pharmacol.* 1997;74:29–36.
- [15] Tsuchiya G, Hori T, Onizawa N, et al. Molecular mechanism of the urate-lowering effects of calcium channel blockers. *Dokkyo J Med Sci.* 2016;43:23–29.
- [16] Wang Y, Zhu JX, Kong LD, et al. Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic xanthine dehydrogenase/oxidase activities in oxonate-treated mice. *Basic Clin Pharmacol Toxicol.* 2004;94:232–237.
- [17] Solangi GA, Zuberi BF, Shaikh S, Shaikh WM. Pyrazinamide induced hyperuricemia in patients taking anti-tuberculous therapy. *J Coll Physicians Surg Pak.* 2004;14:136–138.
- [18] Louthrenoo W, Hongsongkiat S, Kasitanon N, Wangkaew S, Jatuworapruk K. Effect of antituberculous drugs on serum uric acid and urine uric acid excretion. *J Clin Rheumatol.* 2015;21:346–348.
- [19] Ellard GA, Haslam RM. Observations on the reduction of the renal elimination of urate in man caused by the administration of pyrazinamide. *Tubercle.* 1976;57:97–103.

- [20] Sweetman SC. *Martindale: The complete drug reference* 36th ed. London: Pharmaceutical press; 2009.
- [21] Weiner IM, Tinker JP. Pharmacology of pyrazinamide: metabolic and renal function studies related to the mechanism of drug-induced urate retention. *J Pharmacol Exp Ther.* 1972;180:411–434.
- [22] Mohabir R, Young SD, Strosberg AM. Role of angiotensin in pressure overload-induced hypertrophy in rats: effects of angiotensin-converting enzyme inhibitors, an AT1 receptor antagonist, and surgical reversal. *J Cardiovasc Pharmacol.* 1994;23:291–299.
- [23] Vijayapandi P, Nagappa AN. Biphasic effects of losartan potassium on immobility in mice. *Yakugaku Zasshi.* 2005;125:653–657.
- [24] Ohtsuka M, Koibuchi Y, Sakai S, et al. Effects of nilvadipine on the cardiovascular system in experimental animals. *Arzneimittelforschung.* 1988;38:1605–1618.
- [25] Duplancic D, Kukoc-Modun L, Modun D, Radic N. Simple and rapid method for the determination of uric acid-independent antioxidant capacity. *Molecules.* 2011;16:7058–7068.
- [26] Ouchi M, Oba K, Saigusa T, et al. Association between pulse wave velocity and a

marker of renal tubular damage (N-acetyl- β -D-glucosaminidase) in patients without diabetes. *J Clin Hypertens (Greenwich)*. 2015;17:290-297.

[27] Ouchi M, Oba K, Ohara M, et al. Change in urinary N-acetyl- β -D-glucosaminidase levels relevant to postprandial glycemic control conditions in subjects without diabetes mellitus. *Clin Chim Acta*. 2014;433: 88-92.

[28] Friedman M. The creatinine, inulin and hippurate clearance in the rat. *Am J Physiol*. 1947;148:387-391.

[29] Narita Y, Ueda M, Uchimura K, et al. Combination therapy with renin-angiotensin-aldosterone system inhibitor telmisartan and serine protease inhibitor camostat mesilate provides further renoprotection in a rat chronic kidney disease model. *J Pharmacol Sci*. 2016;130:110-116.

[30] Iwashita Y, Kuwabara T, Hayata M, et al. Mild systemic thermal therapy ameliorates renal dysfunction in a rodent model of chronic kidney disease. *Am J Physiol Renal Physiol*. 2016;310,1206-1215.

[31] Lee TM, Lin SZ, Chang NC. Effects of urate-lowering agents on arrhythmia vulnerability in post-infarcted rat hearts. *J Pharmacol Sci*. 2016;131:28-36.

[32] Anzai N, Kanai Y, Endou H. New insights into renal transport of urate. *Curr Opin Rheumatol*. 2007;19:151–157.

- [33] Miyazaki S, Hamada T, Hirata S, et al. Effects of azelnidipine on uric acid metabolism in patients with essential hypertension. *Clin Exp Hypertens*. 2014;36:447–453.
- [34] Gopal E, Fei YJ, Sugawara M, et al. Expression of *slc5a8* in kidney and its role in Na⁺-coupled transport of lactate. *J Biol Chem*. 2004;279:44522–44532.
- [35] Srinivas SR, Gopal E, Zhuang L, et al. Cloning and functional identification of *slc5a12* as a sodium-coupled low-affinity transporter for monocarboxylates (SMCT2). *Biochem J*. 2005;392:655–664.

Figure legends

Figure 1. Effects of pyrazinamide on the fractional excretion of uric acid (FEUA) and creatinine clearance (Ccr) in mice

Each group is comprised of 6 to 8 mice (Water group [No treatment], n = 8; DMSO-1 group, n = 6; PZA-1 group, n = 6). (A), Fractional excretion of uric acid (FEUA) in the pyrazinamide treatment group. FEUA is significantly lower in the PZA-1 group than in the Water group (No treatment) and the DMSO-1 group (**p < 0.001 vs. Water group, #p < 0.05 vs. DMSO-1 group). (B), Creatinine clearance in the pyrazinamide treatment group. There was no significant difference in the Ccr levels among the 3 groups.

FEUA, fractional excretion of uric acid; Ccr, creatinine clearance; DMSO, dimethyl sulfoxide; PZA, pyrazinamide.

Figure 2. Effects of benzbromarone and losartan on the fractional excretion of uric acid (FEUA) and creatinine clearance (Ccr) in mice

Each group is comprised of 5 to 8 mice (PZA-2 group, n = 5; BENZ3.0 group, n = 6; BENZ10.0 group, n = 5; LS3.0 group, n = 8). (A), Fractional excretion of uric acid (FEUA) in the benzbromarone and losartan groups. FEUA is significantly higher in the BENZ10.0 group and the LS3.0 group than in the PZA-2 group (*p < 0.05 vs. PZA-2

group). (B), Creatinine clearance in the benzbromarone and losartan groups. There was no significant difference in the Ccr levels among the 4 groups.

FEUA, fractional excretion of uric acid; Ccr, creatinine clearance; DMSO, dimethyl sulfoxide; PZA, pyrazinamide; BENZ, benzbromarone; LS, losartan.

Figure 3. Effects of nilvadipine on the fractional excretion of uric acid (FEUA) and creatinine clearance (Ccr) in mice

Each group is comprised of 6 to 9 mice (PZA-3 group, n = 6; NV1.0 group, n = 9; NV3.2 group, n = 9).

(A), Fractional excretion of uric acid (FEUA) in the nilvadipine groups. FEUA is significantly higher in the NV1.0 group and the NV3.2 group than in the PZA-3 group (*p < 0.05 vs. PZA-3 group). (B), Creatinine clearance in the nilvadipine groups. There was no significant difference in the Ccr levels among the 3 groups.

FEUA, fractional excretion of uric acid; Ccr, creatinine clearance; PZA, pyrazinamide; NV, nilvadipine.

Figure 4. Effects of nitrendipine, nifedipine, and azelnidipine on the fractional excretion of uric acid (FEUA) and creatinine clearance (Ccr) in mice

Each group is comprised of 6 to 10 mice (PZA-4 group, n = 6; NT3.2 group, n = 8; NF3.2 group, n = 9; AZ10.0 group, n = 10). (A), Fractional excretion of uric acid (FEUA) in the nitrendipine, nifedipine, and azelnidipine groups. FEUA is significantly higher in the NT3.2, NF3.2, and AZ10.0 groups than in the PZA-4 group (*p < 0.05 vs. PZA-4 group). (B), Creatinine clearance in the nitrendipine, nifedipine, and azelnidipine groups. There was no significant difference in the Ccr levels among the 4 groups. CCBs, calcium channel blockers; FEUA, fractional excretion of uric acid; Ccr, creatinine clearance; PZA, pyrazinamide; NT, nitrendipine; NF, nifedipine; AZ, azelnidipine.

Figure 5. Proposed model of urate and monocarboxylic acid transport in the proximal renal tubule

(A), Urate reabsorption and transport of MCs in the proximal renal tubule. Under normal conditions, urate reabsorption and transport of MCs on the lumen side in the proximal renal tubule cell. Urate (tubular lumen) and MCs (cytosol) are exchanged via URAT1. Sodium and MCs cotransport via SMCT1/2. (B), Urate reabsorption and transport of PZA in the proximal renal tubule. PZA is converted into PA and 5-OHPA in the body. Administration of PZA promotes this exchange transport. (C), Urate

reabsorption and transport of PZA in the proximal renal tubule with administration of BENZ, LS, and CCBs (NV, NT, NF, and AZ). CCBs (NV, NT, NF, and AZ) probably inhibit URAT1, and urinary excretion of urate increases.

UA, urate; CCBs, calcium channel blockers; MCs, monocarboxylic acids; Na, sodium; PZA, pyrazinamide; URAT1, urate transporter 1; SMCT, sodium-dependent monocarboxylate transporter; PA, pyrazinoic acid; 5-OHPA, 5-hydroxypyrazinoic acid; BENZ, benzbromarone; LS, losartan; NV, nilvadipine; NT, nitrendipine; NF, nifedipine; AZ, azernidipine.

Supplemental figure. Protocols for the induction of urate under-excretion mice and its treatment (BENZ, LS, or CCBs).

Eleven-week-old mice were randomly allocated to the Water, DMSO, PZA, and treatment (BENZ, LS, or CCBs [NV, NT, NF, or AZ]). DMSO, dimethyl sulfoxide; PZA, pyrazinamide; BENZ, benzbromarone; LS, losartan; CCBs, calcium channel blockers; NV, nilvadipine; NT, nitrendipine; NF, nifedipine; AZ, azelnidipine; p.o., per os.

Table 1: Results of plasma uric acid levels and renal function.

	Water group	DMSO-1 group	PZA-1 group	P-value
Plasma uric acid (Pua) (mg/dL)	1.17 ± 0.12	1.59 ± 0.23	1.55 ± 0.24	0.240
Plasma creatinine (Pcr) (mg/dL)	0.17 ± 0.01	0.23 ± 0.04	0.18 ± 0.02	0.237
Urinary NAG (U/L)	87 ± 18	125 ± 20	88 ± 19	0.347
Urinary NAG–creatinine ratio (U/mg Cr)	0.60 ± 0.12	0.69 ± 0.21	0.64 ± 0.12	0.919

DMSO, dimethyl sulfoxide; PZA, pyrazinamide; NAG, N-acetyl- β -D-glucosaminidase.

Figure 1.

Figure 1A.

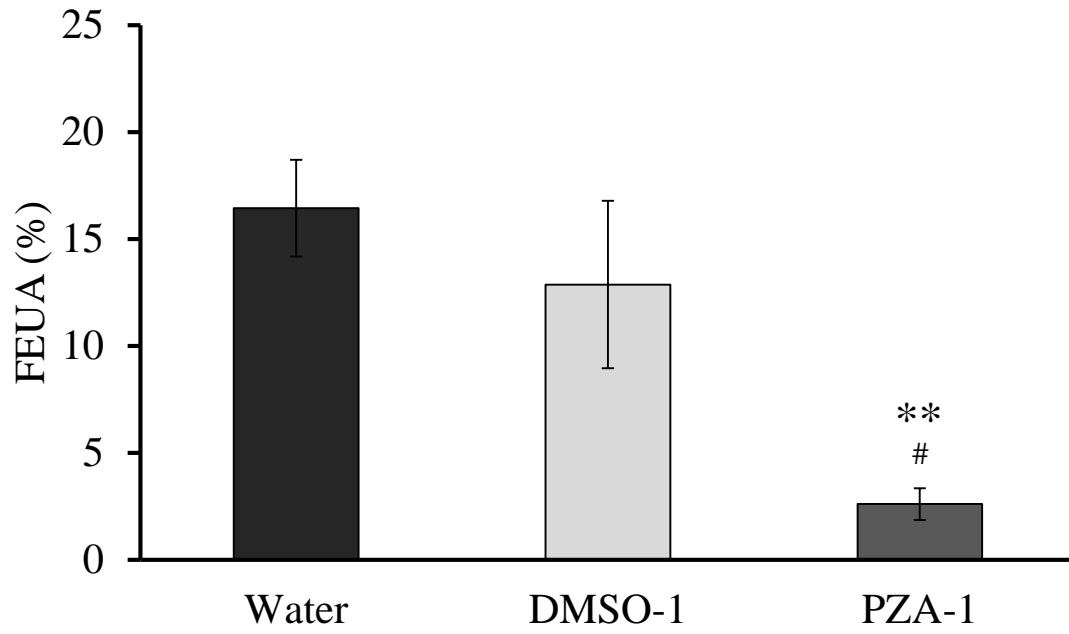


Figure 1B.

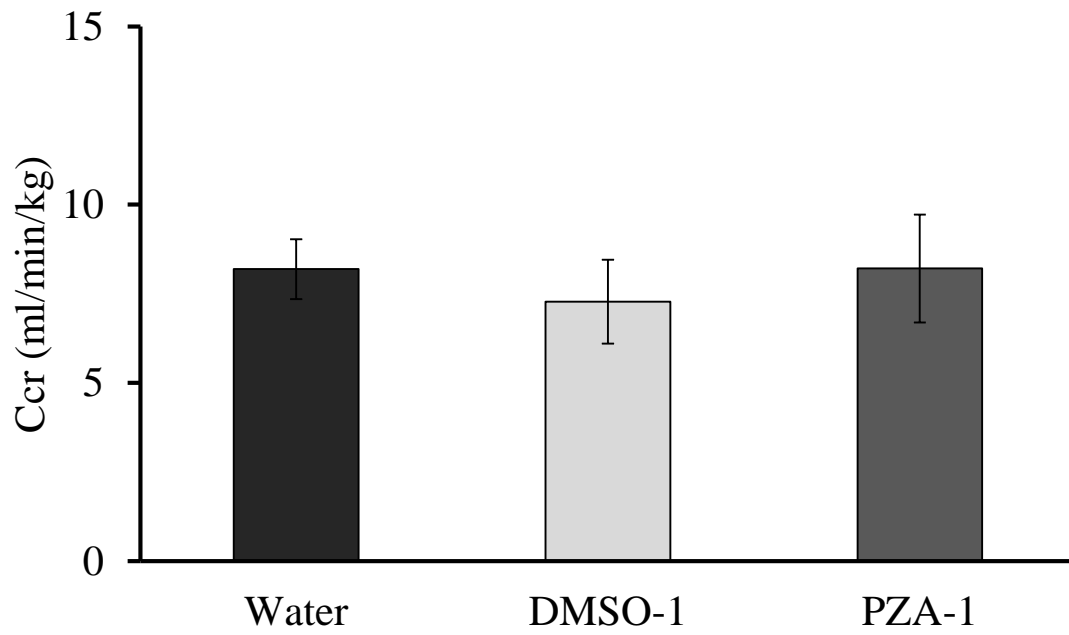


Figure 2.

Figure 2A.

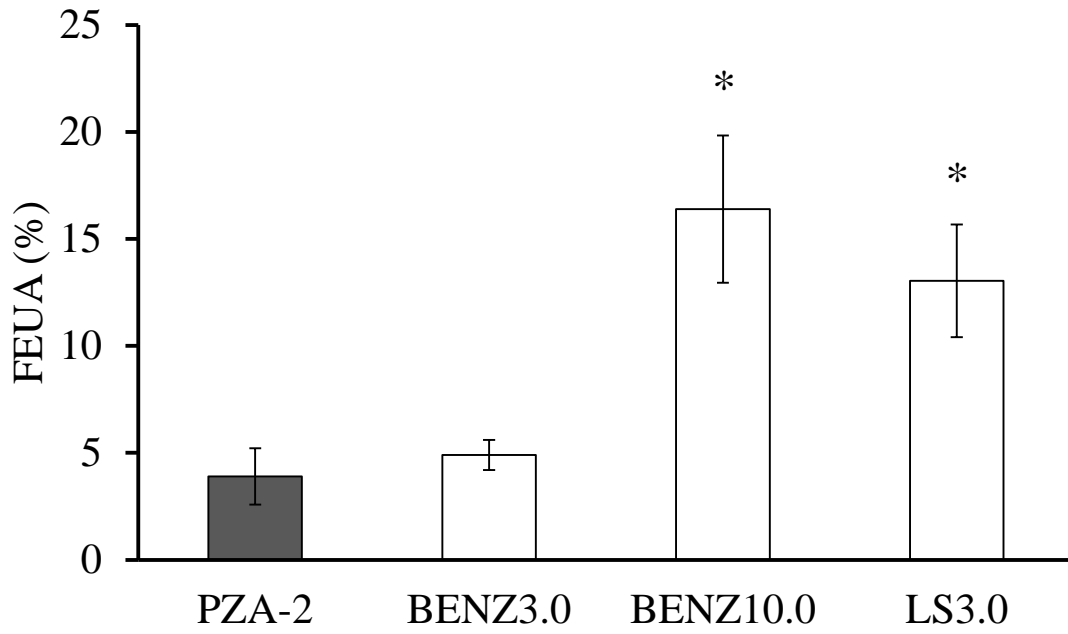


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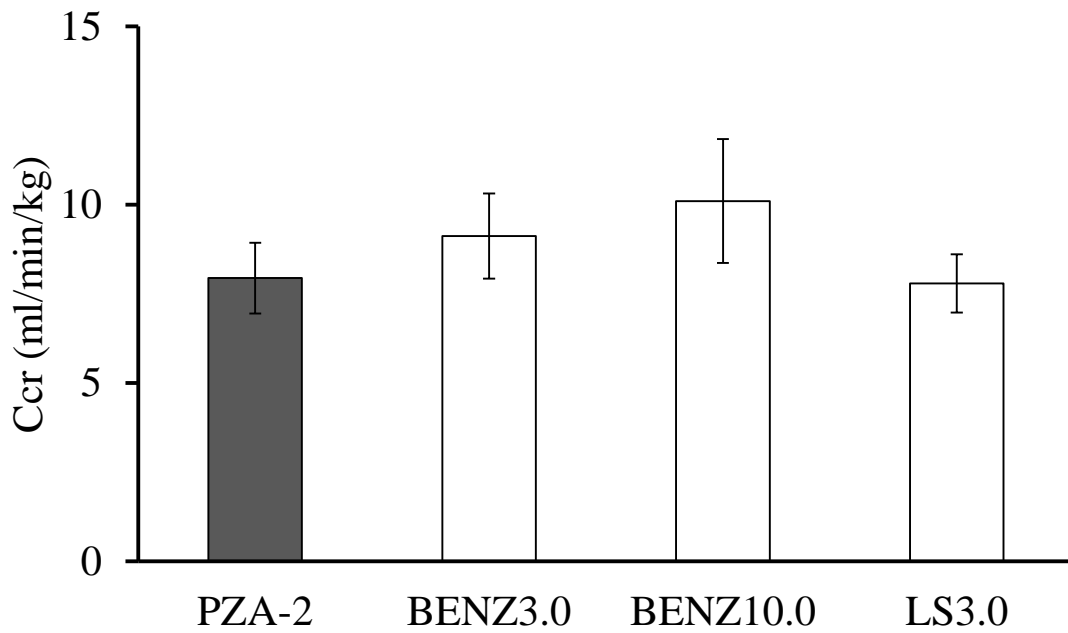


Figure 3.

Figure 3A.

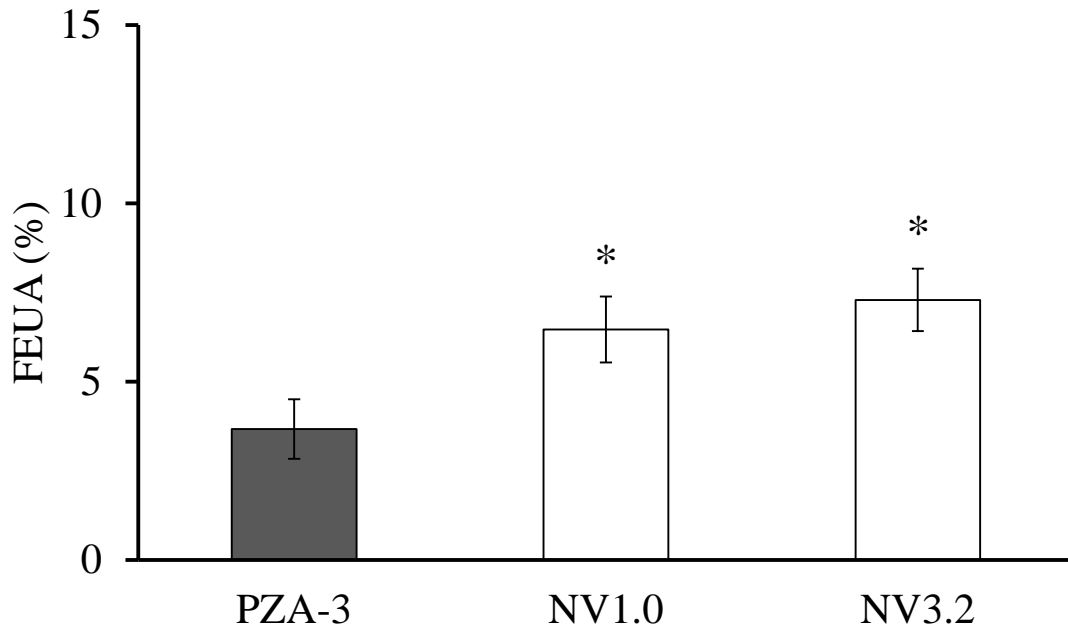


Figure 3B.

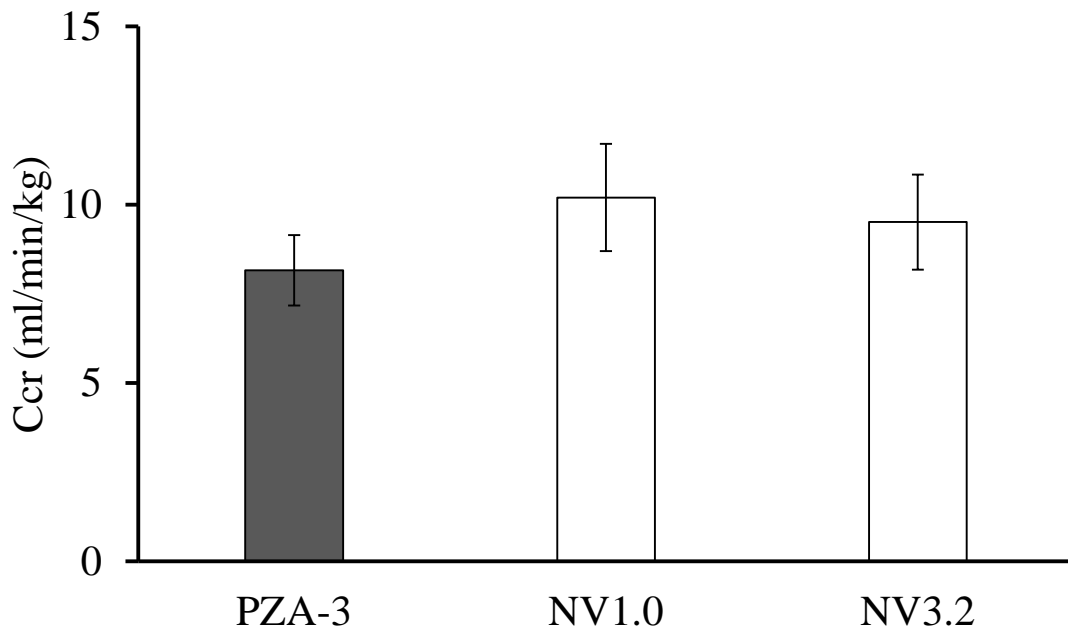


Figure 4.

Figure 4A.

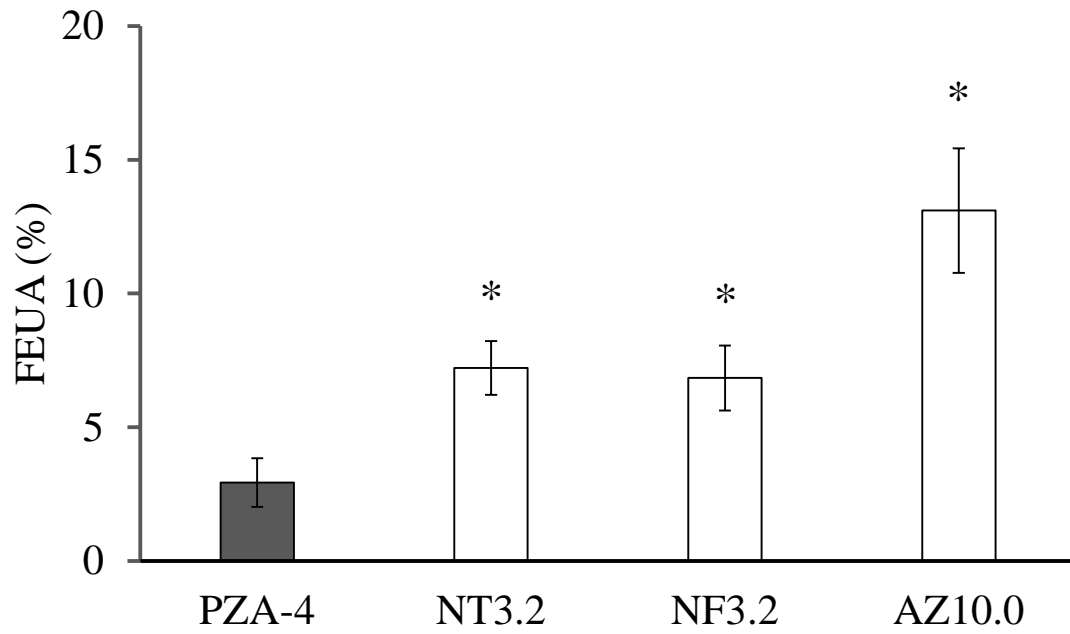


Figure 4B.

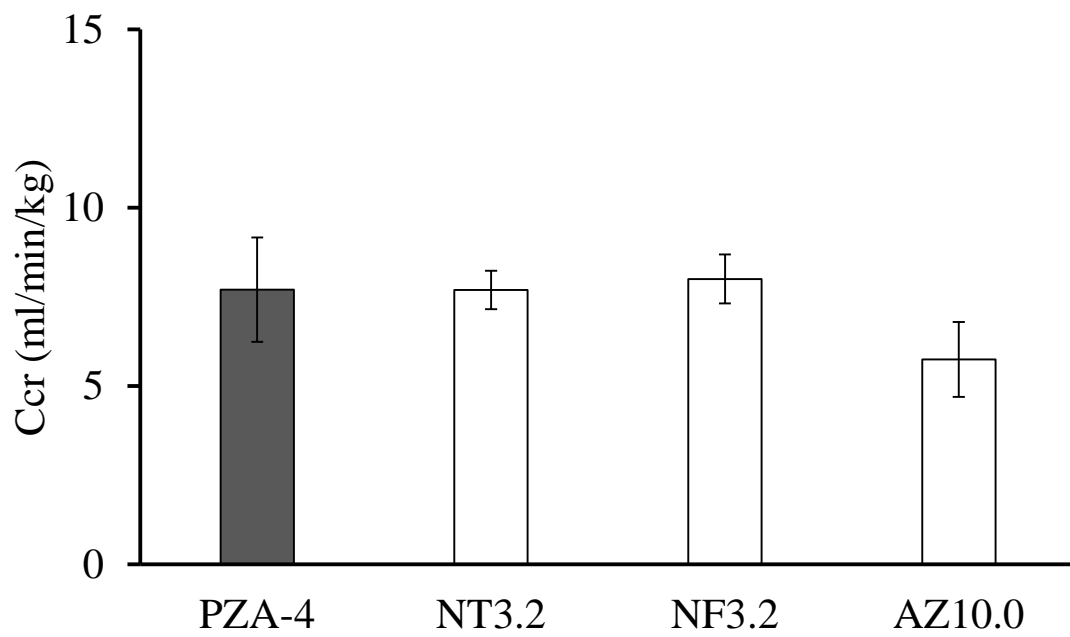


Figure 5.

Figure 5A.

Proximal renal tubule cell

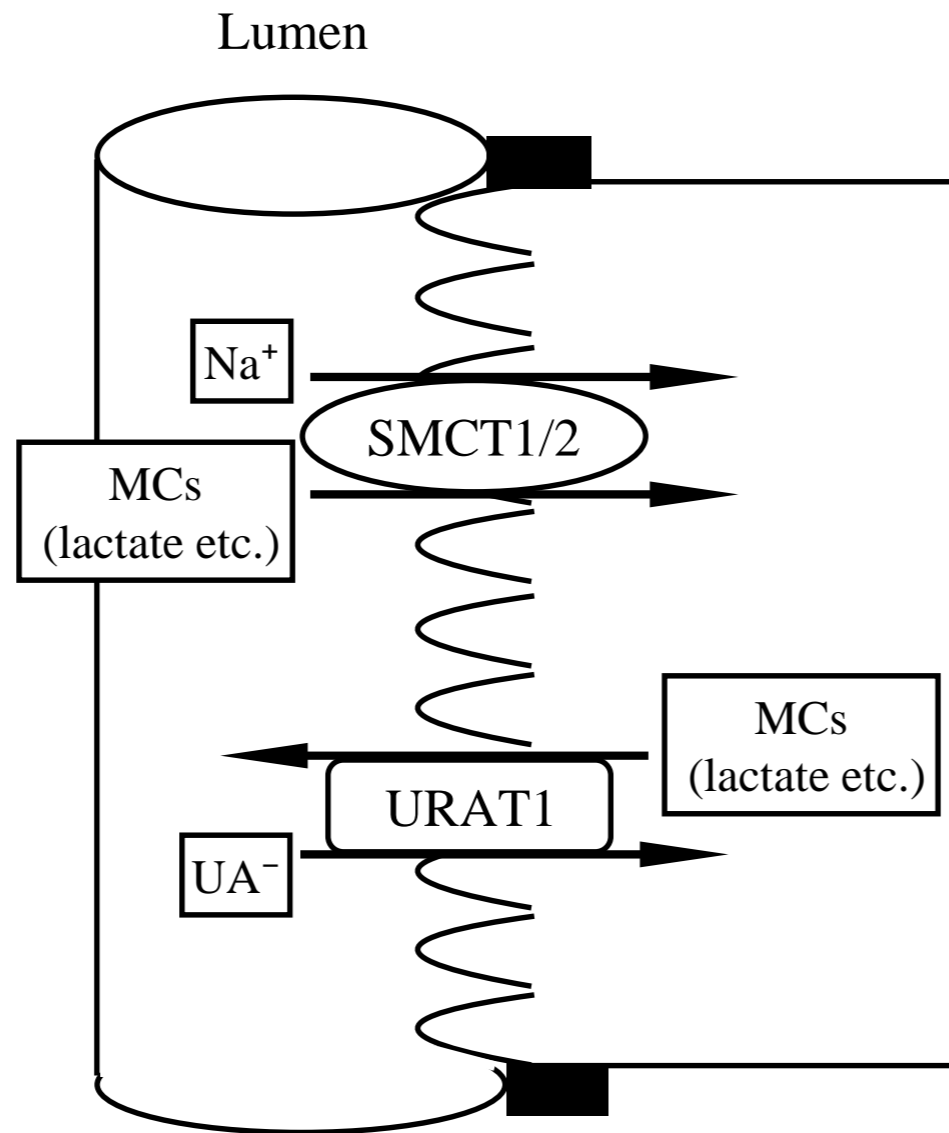


Figure 5B.

Proximal renal tubule cell

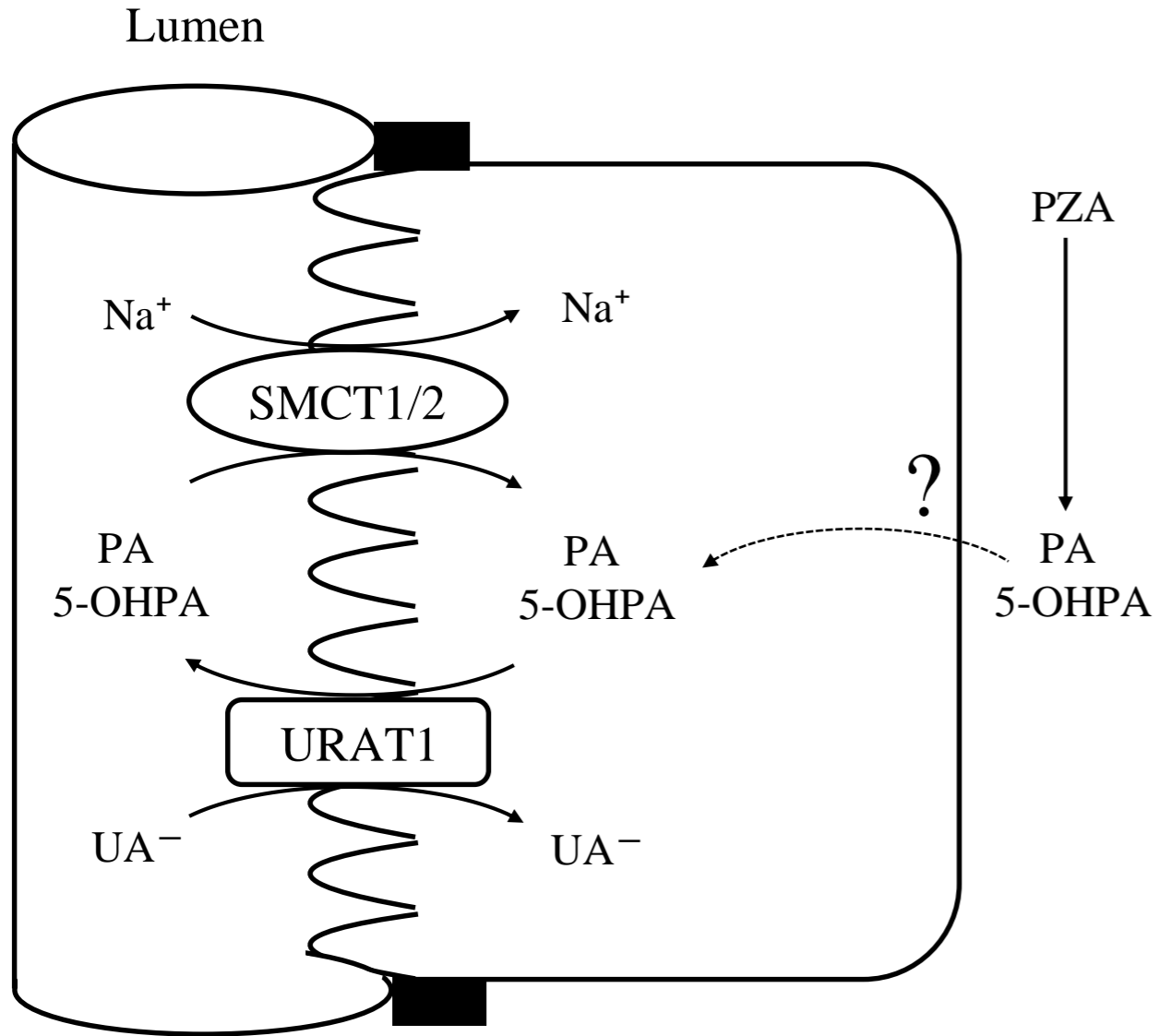
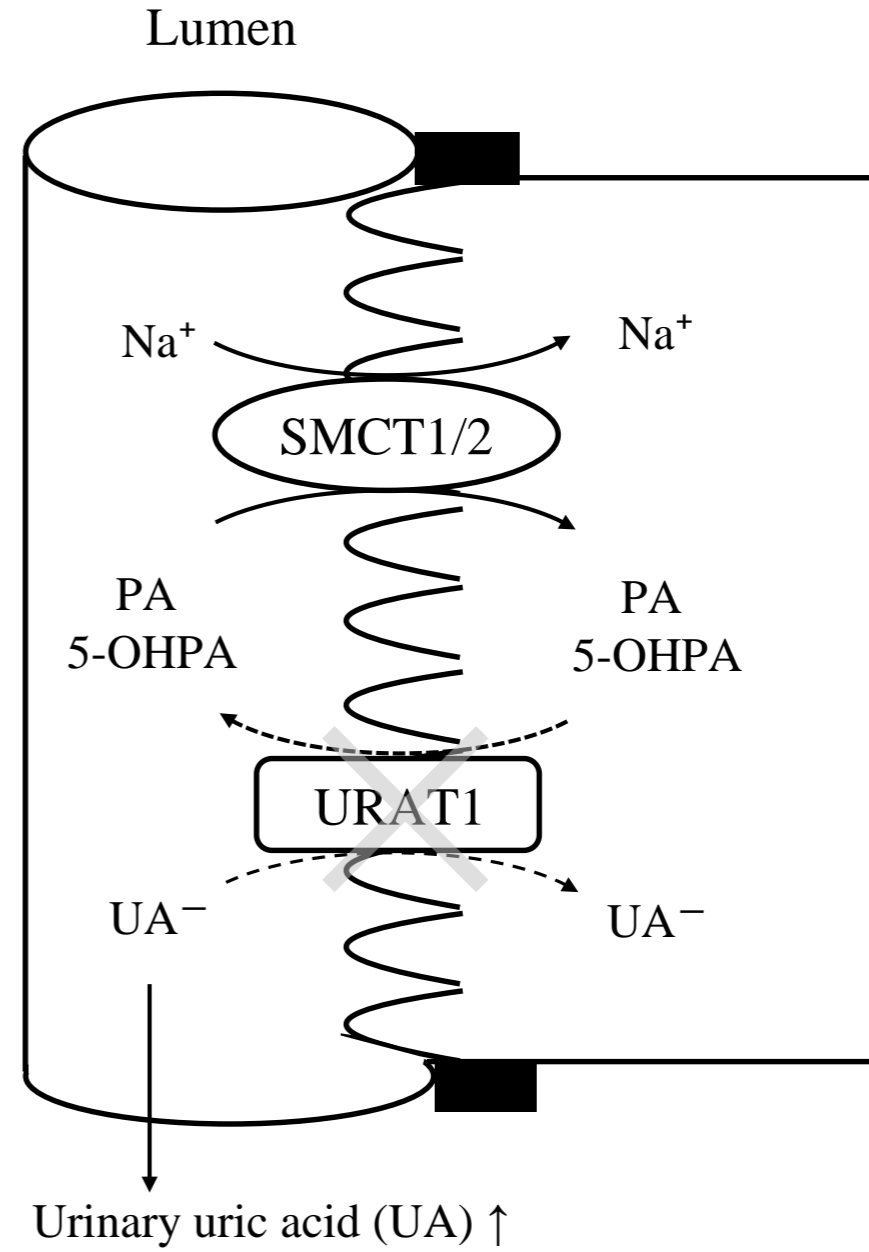


Figure 5C.

Proximal renal tubule cell



Supplemental figure.

