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**INVOLVEMENT OF URIC ACID TRANSPORTER IN INCREASED RENAL CLEARANCE
OF THE XANTHINE OXIDASE INHIBITOR OXYPURINOL INDUCED BY A URICOSURIC
AGENT, BENZBROMARONE**

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Running Title:

Pharmacokinetic interaction of oxypurinol and benzbromarone at uric acid transporter

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Abbreviations: URAT1, uric acid transporter. OAT, organic anion transporter. OCNT, organic cation/carnitine transporter

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ABSTRACT

Benzbromarone has been reported to increase the renal clearance of oxypurinol, an active metabolite of allopurinol. We examined the renal transport of oxypurinol to determine if such a change in renal clearance could be explained by altered transporter-mediated reabsorption. Since the first step of reabsorption takes place at the renal epithelial apical membrane, we focused on membrane transporters. Benzbromarone is an inhibitor of reabsorption of uric acid mediated by the uric acid transporter URAT1 (SLC22A12), which is expressed at the apical membrane of proximal tubular cells in humans. Uptake of oxypurinol by *Xenopus* oocytes injected with complementary RNA of URAT1 was significantly higher than that by water-injected oocytes, and the uptake was saturable with a K_m of about 800 μM . Moreover, benzbromarone inhibited the oxypurinol uptake by URAT1 at concentrations as low as 0.01 μM . The uptake of oxypurinol by another organic anion transporter, OAT4 (SLC22A11), which is also expressed at the apical membrane of proximal tubular epithelial cells, was negligible, while the uptake of [^3H]estrone-3-sulfate by OAT4 was significantly inhibited by oxypurinol. Furthermore, neither of transport activity of OCTN1 nor OCTN2 was affected by oxypurinol or benzbromarone. These results indicate that URAT1 is involved in renal reabsorption of oxypurinol, and the increment of renal clearance of oxypurinol upon concomitant administration of benzbromarone could be due to drug interaction at URAT1.

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INTRODUCTION

Allopurinol is widely used for the treatment of hyperuricemia and gout by inhibiting xanthine oxidase, which is the rate-limiting enzyme for uric acid formation. Allopurinol itself is a substrate of xanthine oxidase and its major metabolite, oxypurinol, is also a potent inhibitor of xanthine oxidase (Rundles et al., 1985; Elion et al., 1966). The inhibitory effect of allopurinol on xanthine oxidase can be largely attributed to oxypurinol, which has a longer plasma half life than allopurinol (Appelbaum et al., 1982; Hande et al., 1972; Elion et al., 1968; Yamamoto et al., 1991). Fractional excretion of oxypurinol is about 20% of glomerular filtration in humans and oxypurinol shows negligible binding to plasma protein (Elion et al., 1966). This observation suggests that about 80% of oxypurinol is reabsorbed after glomerular filtration, though the reabsorption mechanism remains to be clarified (Turnheim et al., 1999; Yamamoto et al., 1991; Colin et al., 1986). Furthermore, concomitant use of benzbromarone and probenecid caused an increase in the renal clearance and a decrease in plasma concentration of oxypurinol (Colin et al., 1986; Muller et al., 1993; Yamamoto et al., 1991). Colin et al. reported that renal clearance of oxypurinol was increased to 0.69 mL/sec from 0.43 mL/sec, and plasma AUC (0- ∞) of oxypurinol was decreased to the half by concomitant use of 20 mg benzbromarone in healthy subjects. Benzbromarone and probenecid are used as uricosuric agents, based on their inhibition of the renal reabsorption of uric acid at proximal tubular epithelial cells (Steel et al., 1973; Sinclair et al., 1975; DeVries et al., 1979). On the other hand, a

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decrease in urinary excretion of oxypurinol was reported in the case of coadministration of pyrazinamide, an antituberculous agent (Yamamoto et al., 1991). It has been reported that pyrazinamide reduces the renal secretion of uric acid via activation of the exchange transport of uric acid with pyrazinecarboxylic acid, which is a metabolite of pyrazinamide, through the uric acid transporter URAT1 (SLC22A12) (De Vries et al., 1979; Enomoto et al., 2002). These observations, i.e., that uricosuric agents increase the renal excretion of oxypurinol, while an anti-uricosuric agent decreases its renal clearance, imply that renal handling of oxypurinol is similar to that of uric acid. As described above, renal reabsorption of uric acid is due to URAT1, which was identified as a member of the OAT family of organic anion transporters and is expressed at the apical membrane of renal tubular epithelial cells (Enomoto et al., 2002). Enomoto and co-workers showed that the uptake of uric acid by URAT1-expressing oocytes was decreased in the presence of uricosuric agents, such as benzbromarone and probenecid, while it was increased by preloading of pyrazinecarboxylic acid in URAT1-expressed oocytes. These findings are similar to those in the case of oxypurinol and further support the idea that renal transport of oxypurinol is similar to that of uric acid. Urinary excretion of uric acid accounts for approximately 70% of the daily production of uric acid. The so-called “four-component hypothesis”, involving glomerular filtration, presecretory reabsorption, secretion, and postsecretory reabsorption, has been proposed to explain the renal handling of uric acid, and approximately 10% of uric acid which is filtered through the glomerulus is finally excreted in

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urine (Sica et al., 2000). It was suggested that URAT1 plays a dominant role in uric acid reabsorption at proximal tubular cells, and it is considered to be one of the sites of action of uricosuric and antiuricosuric agents to regulate blood uric acid level.

Therefore, we hypothesized that URAT1 is involved in renal reabsorption of oxypurinol, and that reabsorption of oxypurinol by URAT1 is disturbed by benzbromarone, resulting in increased urinary excretion. Accordingly, we examined the uptake of oxypurinol by URAT1 and the inhibitory effect of benzbromarone on oxypurinol transport by URAT1, using URAT1-expressing oocytes. We also examined the possible involvement in this interaction of organic anion transporter 4 (OAT4, SLC22A11), which is classified into the same SLC22A transporter family as URAT1, and, organic cation/carnitine transporters OCTN1 and OCTN2, all of these transporters are expressed at the apical membrane of proximal tubular epithelial cells and transport xenobiotics (Ekaratanawong et al., 2004; Tamai et al., 2001; 2004).

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Materials and Methods

Chemicals

[¹⁴C]Uric acid (1.92 TBq/mmol), [³H]estrone-3-sulfate (2.12 TBq/mmol), [¹⁴C]tetraethylammonium (2.04 TBq/mmol), and [³H]carnitine (2.96 TBq/mmol) were purchased from Moravek Biochemicals, Inc. (Brea, CA), PerkinElmer Life Sciences (Boston, MA), American Radiolabeled Chemicals, Inc. (Saint Louis, MO), and Amersham Biosciences UK, Ltd., (Buckinghamshire, UK), respectively. Oxypurinol, benzbromarone, and uric acid were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were purchased from Sigma-Aldrich (St Louis, MO), Wako Pure Chemicals (Osaka, Japan) and Kishida Chemicals (Osaka, Japan).

cDNA Cloning of URAT1 and OAT4

The URAT1 gene was PCR-amplified using QUICK-Clone human kidney cDNA (BD Biosciences Clontech, Palo Alto, CA) as a template, with upstream primer 5'-AGGTTCCATGGCATTCTGAAC-3' and downstream primer 5'-CCTGCCAATCAAAGTGCCTT-3', based on the reported URAT1 gene sequence (Enomoto et al., 2002) (GeneBank accession # AB071863). A major 2.0-kilobase polymerase chain reaction product was ligated into pCR 2.1-TOPO TA cloning vector (Invitrogen, Carlsbad, CA) and then URAT1-cDNA was digested with *Bam*HI and *Xba*I, and ligated into the expression gene vector

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pGEMHE, which was kindly provided by Dr. E.R. Liman (Liman et al., 1992). The OAT4 gene was PCR-amplified using Cap Site cDNA dT human kidney (Nippon Gene, Tokyo, Japan) as a template, with upstream primer 5'-GAATTCATGTTTCATGGCGTTCTCGAAG-3' and downstream primer 5'-AAGCTTCTGTATGTGGTGGGGTGAGA-3' (both synthesized by Hokkaido System Science) and Ex taq DNA polymerase (Takara Shuzo Co., Shiga, Japan), based on the reported OAT4 gene sequence (Cha et al., 2000) (GeneBank accession # AB026116). A major 2.2-kilobase polymerase chain reaction product was ligated into the TA cloning vector pGEM-T Easy (Promega, Madison, WI) and then OAT4 cDNA was digested with *EcoRI* and *HindIII*, and ligated to pGEMHE. The obtained cDNA sequences were analyzed and confirmed to be the same as the reported ones.

Uptake Experiments using *Xenopus laevis* oocytes and HEK293 cells expressed with transporter genes

Complementary RNAs (cRNAs) of URAT1 and OAT4 were prepared by *in vitro* transcription with T7 RNA polymerase in the presence of ribonuclease inhibitor and an RNA cap analog using a mMESSEmMACHINE kit (Ambion, Austin, TX). For transport experiments with URAT1, defolliculated oocytes were injected with 25 ng of URAT1-cRNA or the same volume of water, and incubated in modified Barth's solution (MBS; 82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂ and 5 mM HEPES, pH 7.4) containing 50 µg/mL gentamycin at 18 °C for 3 days as we reported previously

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(Nozawa et al., 2005). The oocytes were transferred to a 12-well plate and preincubated in ND96 buffer (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂ and 5 mM HEPES, pH 7.4) at 25 °C for 15 min. After this preincubation, the buffer was replaced with 1 mL of Cl⁻-free buffer (96 mM Na gluconate, 2 mM K gluconate, 1 mM Mg gluconate and 5 mM HEPES, pH 7.4) containing oxypurinol with or without inhibitor (benzbromarone or uric acid) to initiate the uptake reaction at 25°C for the designated time. Uptake was terminated by washing three times with ice-cold Cl⁻ free buffer. For quantitation of oxypurinol, 3 oocytes were collapsed by sonicating them in 200 µL of methanol containing 3-methylxanthine (10 ng/mL) as an internal standard for 10 min, and the precipitated protein was removed by centrifugation (13,000 g, 10 min). The supernatant was dried under nitrogen gas and the residue was dissolved in the mobile phase for high-performance liquid chromatographic analysis. The soluble fraction was separated by centrifugation (13,000 g, 10 min) and the supernatant was used for LC-MS/MS analysis.

For transport experiments with OAT4, defolliculated oocytes were injected with 10 ng of OAT4-cRNA or the same volume of water, and incubated under the same conditions in MBS containing 10 µg/mL gentamycin at 18 °C for 4 days. The oocytes were transferred to a 24-well plate and pre-incubated in ND96 buffer containing 5 mM glutarate at 25 °C for 60 min. After this preincubation, the buffer was replaced with 0.5 mL of ND96 buffer containing [³H]estrone-3-sulfate or oxypurinol with or without inhibitor (benzbromarone, uric acid, estrone-3-sulfate or oxypurinol) to

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initiate the uptake reaction. The oocytes were incubated at 25 °C for 60 min, and then washed three times with 1 mL of ice-cold ND96 buffer to terminate the uptake reaction. The oocytes were solubilized with 5 % sodium dodecyl sulfate solution for 120 min. The radioactivity was measured using a liquid scintillation counter (Perkin Elmer, Boston, MA) after the addition of scintillation cocktail, Clearsol I[®] (Nacalai tesque, Kyoto, Japan).

Uptake was expressed as the cell-to-medium ratio (μl/oocyte), obtained by dividing the uptake amount by the concentration of substrate in the incubation buffer. In order to estimate the kinetic parameters for the uptake by URAT1 or OAT4, the initial uptake rates by URAT1 or OAT4 (obtained after subtraction of the uptake by water-injected oocytes from that by URAT1- or OAT4-cRNA injected oocytes) were fitted to the following equation by means of nonlinear least-squares regression analysis using Kaleida Graph software version 3.5 (Synergy Software, Reading, PA):

$$v = V_{\max} \times s / (K_m + s)$$

where v is the initial uptake rate of substrate (pmol/min/oocyte), s is the substrate concentration in the medium (μM), K_m is the Michaelis-Menten constant (μM), and V_{\max} is the maximum uptake rate (pmol/min/oocyte).

The inhibition studies for OCTN1- or OCTN2-mediated transport of their typical substrates were examined in the absence or presence of inhibitors (1mM oxypurinol or 1μM benzbromarone). The transport experiments of [¹⁴C]tetraethylammonium by HEK293 cells expressed with human

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OCTN1 was carried out as described previously (Tamai et al., 2004). The transport experiment of [³H]carnitine by HEK293 cells expressed with human OCTN2 was carried out as described previously (Tamai et al., 2001).

LC-MS/MS analysis

LC-MS/MS analysis was carried out using a HPLC system consisting of an Agilent 1100 binary pump, a vacuum degasser, and an autosampler with a 100 µl loop interfaced to an API 3000 SCIEX triple-quadrupole tandem mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada). Oxypurinol, xanthine (oocyte-derived) and the internal standard (3-methylxanthine) were separated on a Mightysil Aqua RP-18 GP 2.0 x 150 mm column (Kanto Chemical, Tokyo, Japan). The mobile phase consisted of 0.1 % acetic acid containing 5 % methanol. The sample was delivered at a flow rate of 0.2 ml/min, and each analysis lasted for 10 min. The mass spectrometer was operated in the turbo ion spray mode with negative ion detection. The turbo ion spray temperature was maintained at 550 °C, and a voltage of -3.5 kV was applied to the sprayer needle. Nitrogen was used as the turbo ion spray and nebulizing gas. The detection and quantification of analytes were performed using the multiple reactions monitoring mode with the transition m/z 151.0 to 107.9 for oxypurinol, and m/z 165.0 to 122.0 for 3-methylxanthine as the internal standard. All raw data were processed with MDS SCIEX Analyst software, version 1.3. Calibration curves were linear from 10

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to 1000 nM for oxypurinol, and the coefficient of variation was less than 15 %.

Statistical analysis

For analysis of differences between the water-injected group and cRNA-injected group, the two tailed unpaired Student's *t* test was used. For the inhibition studies of URAT1 and OAT4, the statistical significance of differences was determined by using one-way analysis of variance with Dunnett's post-hoc test, with $P < 0.05$ as the criterion.

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Results

Uptake of oxypurinol by *Xenopus* oocytes expressing URAT1

Our method to evaluate URAT1 activity using *Xenopus* oocytes was validated with the uptake of [¹⁴C]uric acid (Fig. 1A). Uptake of [¹⁴C]uric acid at 60 min by oocytes injected with URAT1 cRNA was significantly higher than that by oocytes injected with water (control). Then, the time course of oxypurinol uptake by *Xenopus* oocytes injected with URAT1 cRNA or water was examined. The uptake of oxypurinol by *Xenopus* oocytes injected with URAT1-cRNA was linearly increased with time over 60 min (Fig 1B), and was significantly greater than that by water-injected oocytes ($p < 0.05$). Thus, uptake at 60 min was routinely used for initial uptake rate measurement in the subsequent studies. The concentration dependence of oxypurinol uptake by URAT1 was studied in the concentration range from 300 μM to 3000 μM ; all values were corrected by subtraction of the uptake by water-injected oocytes (Fig. 2). The uptake was saturable and the K_m and V_{max} values of oxypurinol transport by URAT1 were $794 \pm 77.9 \mu\text{M}$ (mean and standard error) and 1.37 ± 0.05 pmol/min/oocyte, respectively.

Inhibitory effects of benzbromarone and uric acid on oxypurinol uptake by URAT1

The plasma concentration of oxypurinol is affected by concomitant use of benzbromarone (Colin et al., 1986; Muller et al., 1993; Yamamoto et al., 1991). Therefore, to examine the

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involvement of URAT1 in the drug interaction between oxypurinol and benzbromarone, we examined the inhibitory effects of benzbromarone on oxypurinol uptake by URAT1. Benzbromarone, which is a potent inhibitor of URAT1 (Enomoto et al., 2002), significantly reduced the oxypurinol uptake at a concentration as low as 10 nM and completely blocked it at 1 μ M. Furthermore, uric acid, which is a substrate of URAT1, significantly reduced the uptake of oxypurinol in a concentration-dependent manner (Fig. 3). The IC_{50} value of uric acid for inhibition of oxypurinol uptake was estimated to be about 170 μ M.

Studies for the involvement of OAT4, OCTN1 and OCTN2 in the Interaction between Oxypurinol and Benzbromarone.

The anion exchanger OAT4 is expressed at the apical membrane of proximal tubular epithelial cells (Ekaratanawong et al., 2004) and is a member of the SLC22A family, like URAT1. The amino-acid sequence predicted by the URAT1 cDNA exhibits 42% amino-acid identity with that of OAT4 (Enomoto et al., 2002). We therefore examined whether OAT4 transports oxypurinol and characterized the inhibitory effects of benzbromarone, uric acid, and oxypurinol on the uptake of [3 H]estrone-3-sulfate, a substrate of OAT4 (Fig. 4). Uptake of [3 H]estrone-3-sulfate by OAT4 was reduced in the presence of oxypurinol and uric acid at 1,000 μ M or higher concentration, whereas no decrease in the uptake of estrone-3-sulfate was observed at 1 μ M benzbromarone, which completely

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inhibited URAT1-mediated transport of oxypurinol. Furthermore, uptake of oxypurinol at the concentration of 300 μ M was comparable in oocytes injected with OAT4 cRNA and with water (Fig. 5). Accordingly, although oxypurinol has affinity for OAT4, it does not appear to be transported by OAT4.

We also examined the involvement of organic cation/carnitine transporters OCTN1 and OCTN2, since they are present at the apical membrane of the renal tubular epithelial cells (Tamai et al., 2001; 2004) and are known to transport xenobiotics. As a result, uptakes of the typical substrate of OCTN1 (tetraethylammonium) by the HEK293 cells expressed with human OCTN1 in the presence of 1 mM oxypurinol and 1 μ M benzbromarone were 103% and 96.7% of control, respectively. Similarly, the uptakes of typical substrate of OCTN2 (carnitine) by the HEK293 cells expressed with human OCTN2 in the presence of those two compounds were 95.0% and 111% of control, respectively. Accordingly, neither of OCTN1 nor OCTN2 is involved in the interaction between oxypurinol and benzbromarone.

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Discussion

We examined the renal transport mechanism of oxypurinol, an active metabolite of the xanthine oxidase inhibitor allopurinol, in order to clarify the mechanism of the pharmacokinetic interaction between oxypurinol and the uricosuric agent benzbromarone, since concomitant use of benzbromarone causes increased renal clearance of oxypurinol, which may lead to reduced efficacy of allopurinol/oxypurinol (Colin et al., 1986; Muller et al., 1993; Yamamoto et al., 1991). Previous observations that approximately 80% of glomerularly filtered oxypurinol is reabsorbed (Turnheim et al., 1999) and that benzbromarone is a strong inhibitor of URAT1-mediated transport of uric acid (Enomoto et al., 2002), suggest the involvement of a transporter such as URAT1 in the reabsorption of oxypurinol, and inhibition of the transporter by benzbromarone could explain the drug interaction between oxypurinol and benzbromarone. Accordingly, the present study was mainly focused on URAT1, though attention was also paid to another apical membrane transporter, OAT4, which is classified into the SLC22A family (as is URAT1) and is expressed at the apical membrane of renal tubular epithelial cells (Ekaratanawong et al., 2004) as well as organic cation/carnitine transporters OCTN1 and OCTN2, that are also expressed at the apical membrane and transport xenobiotics (Tamai et al., 2001; 2004).

The uptake of oxypurinol by oocytes injected with URAT1 cRNA was significantly higher than that by water-injected oocytes (Fig. 1B). The initial uptake of oxypurinol mediated by URAT1 was

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saturable with a Michaelis constant of 794 μM (Fig. 2). In addition, this URAT1-mediated uptake of oxypurinol was inhibited by benzbromarone in a concentration-dependent manner (Fig. 3).

Accordingly, it was demonstrated that oxypurinol is a substrate of URAT1, and that the transport was inhibited by benzbromarone.

On the other hand, although oxypurinol inhibited the transport of estrone-3-sulfate by OAT4 (Fig. 4), it appeared not to be a substrate of OAT4, at least in *Xenopus* oocytes expressing OAT4 (Fig. 5). Furthermore, we examined the involvement of organic cation/carnitine transporters OCTN1 and OCTN2, since they are present at the apical membrane of the renal tubular epithelial cells (Tamai et al., 2001; 2004) and are known to transport xenobiotics. However, we could not see any effect of oxypurinol or benzbromarone on the transport activity of OCTN1 or OCTN2. Therefore, we conclude that the contribution of OAT4, OCTN1 and OCTN2 to the renal reabsorption of oxypurinol is negligible, and that URAT1 plays a key role in the renal reabsorption of oxypurinol. Since the clinical concentration of oxypurinol is as high as 30 μM in plasma and the plasma protein binding of oxypurinol is negligible (Elion et al., 1966), the estimated clinical concentration of oxypurinol in plasma or glomerular filtrate is 30 μM or less. Here, the K_m value of oxypurinol to URAT1 (794 μM) is higher than the clinical concentration (30 μM). Accordingly, it was suggested that URAT1 is not saturated at the clinical concentration of oxypurinol and oxypurinol can be transported efficiently by URAT1.

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According to the basic product information of benzbromarone from the pharmaceutical company (Urinorm, Torii Pharmaceutical Co. Ltd., Tokyo, Japan), the maximum plasma concentration of benzbromarone reached 5.4 μM after oral administration of the clinical dose of 100 mg in humans, and at 24 h after the administration, 0.5 μM of benzbromarone remained in plasma (Oikawa et al, 2004). Since plasma protein binding of benzbromarone is approximately 97% (basic product information of benzbromarone Urinorm, Torii Pharmaceutical Co. Ltd.), the concentration of benzbromarone in glomerularly filtered urine could be in the range of 16 to 160 nM, assuming that the plasma free concentration is equal to the concentration in the glomerular filtrate. Our results showed that 10 nM benzbromarone reduced uptake of oxypurinol by URAT1 to be less than half of the control (Fig. 3). Therefore, it is possible that benzbromarone can reduce the reabsorptive transport of oxypurinol mediated by URAT1 to be one third or less in clinical situation. Furthermore, our result suggested that oxypurinol could be transported efficiently by URAT1 *in vivo*, as described above. Accordingly, benzbromarone should be able to reduce the reabsorptive transport of oxypurinol mediated by URAT1 at clinical doses of the two drugs.

On the other hand, URAT1-mediated uptake of oxypurinol was inhibited by uric acid, which is a substrate of URAT1. In “Guideline for the management of hyperuricaemia and gout” in Japan, hyperuricaemia is defined as a plasma concentration of uric acid of 7 mg/dL (417 μM) or more. Since uric acid exhibits negligible plasma protein binding, its concentration in the proximal tubular

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lumen is expected to be comparable with that in plasma. Accordingly, renal reabsorption of oxypurinol might also be decreased by uric acid under physiological conditions.

In conclusion, oxypurinol was clarified to be a substrate of URAT1, but not of OAT4, OCTN1 and OCTN2, and URAT1 is likely to be involved in the reabsorption of oxypurinol. The uricosuric agent benzbromarone is a strong inhibitor of URAT1, and URAT1-mediated transport of oxypurinol is influenced by benzbromarone at clinically relevant concentrations. We consider that the interaction of oxypurinol and benzbromarone at URAT1 is likely to be a major contributor to the altered renal handling of oxypurinol upon coadministration of benzbromarone. Accordingly, pharmacological effect of allopurinol may be reduced when benzbromarone or other agents that have inhibitory effect on URAT1 were coadministered.

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References

- Appelbaum SJ, Mayersohn M and Dorr TR (1982) Allopurinol kinetics and bioavailability: Intravenous, oral and rectal administration. *Cancer Chemother Pharmacol* **8**: 93-98
- Breithaupt H and Tittel M (1982) Kinetics of allopurinol after single intravenous and oral doses. Noninteraction with benzbromarone and hydroxychlorothiazide. *Eur J Clin Pharmacol* **22**: 77-84
- Colin JN, Farinotti R, Fredj G, Tod M, Clavel JP, Vignon E and Dietlin F (1986) Kinetics of allopurinol and oxypurinol after chronic oral administration. Interaction with benzbromarone. *Eur J Clin Pharmacol* **31**: 53-58
- De Vries A and Sperling O (1979) Inborn hyperuricaemia due to isolated renal tubular defect. *Biomedicine* **30**: 75-80
- Ekaratanawong S, Anzai N, Jutabha P, Miyazaki H, Noshiro R, Takeda M, Kanai Y, Sophasan S and Endou H (2004) Human organic anion transporter 4 is a renal apical organic anion/dicarboxylate exchanger in the proximal tubules. *J Pharmacol Sci* **94**: 297-304

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Enomono A, Kimura H, Chairoungdua A and Endou H (2002) Molecular identification of a renal urate-anion exchanger that regulates blood urate levels. *Nature* **417**: 447-452

Elion GB, Yu TF, Gutmann AB and Hitchings GH (1968) Renal clearance of oxypurinol, the chief metabolite of allopurinol. *Am J Med* **45**: 69-77

Elion GB, Kovensky A and Hitchings GH (1966) Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. *Biochem Pharmacol* **15**: 863-880

Hande K, Reed E and Cabner B (1972) Allopurinol kinetics. *Clin Pharmacol Ther* **23**: 598-605

Liman ER, Tytgat J and Hess P (1992) Subunit stoichiometry of a mammalian K⁺ channel determined by construction of multimetric cDNAs. *Neuron* **9**: 861-871

Muller FO, Schall R, Groenewoud G, Hundt HKL, Merwe JC and Dyk M (1993) The effect of benzbromarone on allopurinol/oxypurinol kinetics in patients with gout. *Eur J Clin Pharmacol* **44**: 69-72

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Nozawa T, Minami H, Sugiura S, Tsuji A and Tamai I (2005) Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. *Drug Metab Dispos* **33**: 434-439

Oikawa T, Kunishima C, Adachi Y, Sato Y, Okamura Y, Tanaka N and Hosoya T (2004) Metabolism study of benzbromarone: In vitro metabolism and pharmacokinetics in healthy volunteers. *J New Rem Clin* **53**: 682-691

Rundles RW (1985) The development of allopurinol. *Arch Intern Med* **145**: 1492-1503

Sica DA and Schoolwerth AC (2000) Renal handling of organic anions and cations: Excretion of uric acid. *The Kidney, 6th ed.* 680-700

Sinclair DS and Fox IH (1975) The pharmacology of hypouricemic effect of benzbromarone. *J Rheumatol* **2**: 437-445

Stamp L, Gow P, Sharples K and Raill B (2000) The optimal use of allopurinol: an audit of allopurinol

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use in South Auckland. *Aust N Z J Med* **30**: 567-572

Steele TH and Boner G. (1973): Organic of the uricosuric response. *J Clin Invest* **52**: 1368-1375

Tamai I, Nakanishi T, China K, Kosugi Y, Kobayashi D, Nezu J, Sai Y, Tsuji A (2004) Involvement of OCTN1 (SLC22A4) in pH-dependent transport of organic cations. *Mol Pharm* **1**: 57-66

Tamai I, China K, Sai Y, Kobayashi D, Nezu J, Kawahara E, Tsuji A (2001) Na⁺-coupled transport of L-carnitine via high-affinity carnitine transporter OCTN2 and its subcellular localization in kidney. *Biochim Biophys Acta* **1512**: 273-284

Turnheim K, Krivanek P and Oberbauer R (1999) Pharmacokinetics and pharmacodynamics of allopurinol in elderly and young subjects. *Br J Clin Pharmacol* **48**: 501-509

Yamamoto T, Moriwaki Y, Takahashi Y, Suda M and Higashino K (1991) Effects of pyrazinamide, probenecid, and benzbromarone on renal excretion of oxypurinol. *Ann Rheum Dis* **50**: 631-633

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Legend for figures

Fig. 1

Uptake of Uric Acid and Oxypurinol by URAT1-Expressing Oocytes.

A) The uptake of [³H]uric acid at the concentration of 55 μM by water-injected (open column) and URAT1-cRNA-injected (closed column) oocytes was measured for 60 min. B) Time course of uptake of oxypurinol by water-injected (open) and URAT1-cRNA-injected (closed) oocytes was measured at a concentration of 300 μM.

Each point represents the mean ± S.E. from 6-8 oocytes. An asterisk (*) indicates a significant difference from the uptake by water-injected oocytes (p<0.05).

Fig. 2

Concentration Dependence of URAT1-Mediated Uptake of Oxypurinol.

The uptake rates of oxypurinol by water-injected or URAT1-cRNA-injected oocytes at various concentrations (300-3000 μM) was measured for 60 min. URAT1-mediated uptake was determined by subtracting the uptake in water-injected oocytes from that in URAT1-cRNA-injected oocytes. Each point represents the mean ± S.E. from 8 oocytes. Inset, Eadie-Hofstee plots for URAT1-mediated initial uptake rate of oxypurinol.

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Fig. 3

Inhibitory Effects of Benzbromarone and Uric Acid on the URAT1-Mediated Uptake of Oxypurinol.

The uptake of 300 μ M oxypurinol by water-injected or URAT1-cRNA-injected oocytes was measured in the presence or absence (control) of inhibitors for 60 min. URAT1-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that in URAT1-cRNA-injected oocytes. An asterisk (*) indicates a significant difference from the control uptake ($p < 0.05$).

Fig. 4

Inhibitory Effects of Benzbromarone, Uric Acid and Oxypurinol on the OAT4-Mediated Uptake of [³H]Estrone-3-Sulfate.

The uptake of [³H]estrone-3-sulfate by water-injected or OAT4-cRNA-injected oocytes was measured in the presence or absence (control) of inhibitors for 60 min. OAT4-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that in OAT4-cRNA injected oocytes. An asterisk (*) indicates a significant difference from the control uptake ($p < 0.05$). E-3-S: estrone-3-sulfate.

Fig. 5

Uptake of Oxypurinol by OAT4 expressed in *Xenopus* Oocytes.

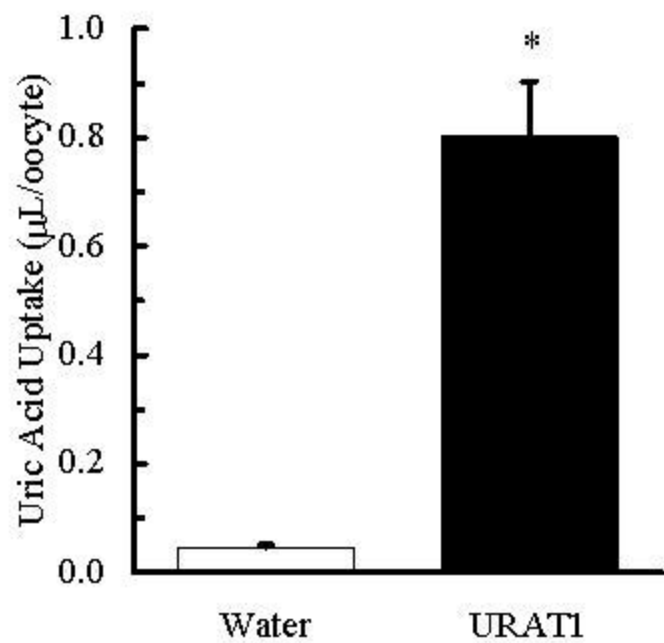
DMD6056

Uptake of 300 μ M oxypurinol by water-injected (open column) and OAT4-cRNA injected (closed column) oocytes was measured for 60 min.

Each point represents the mean \pm S.E. from 8 oocytes

Fig.1

A)



B)

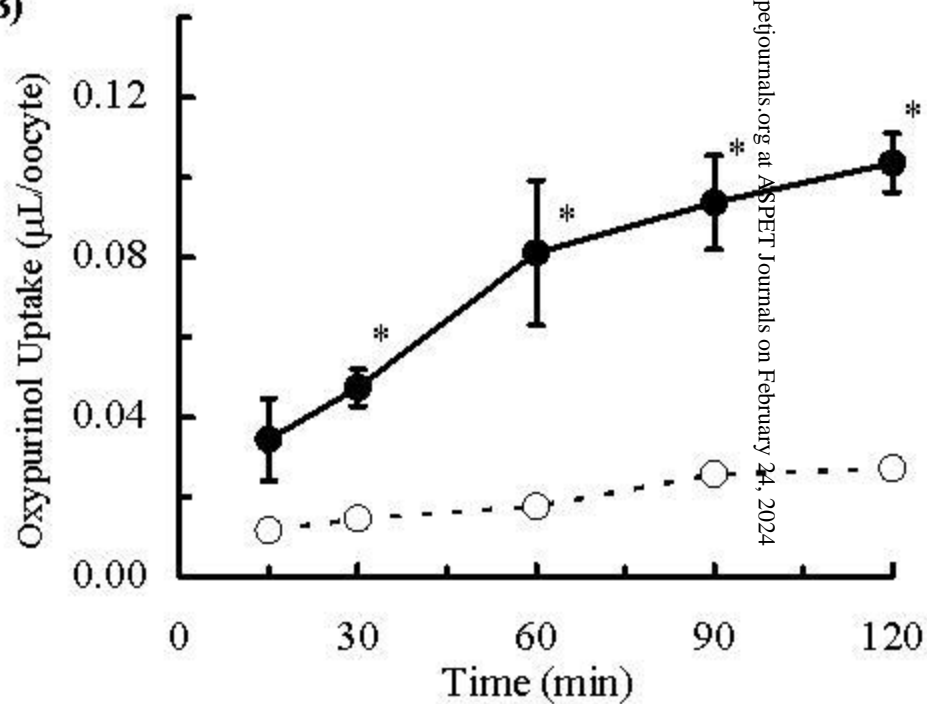


Fig.2

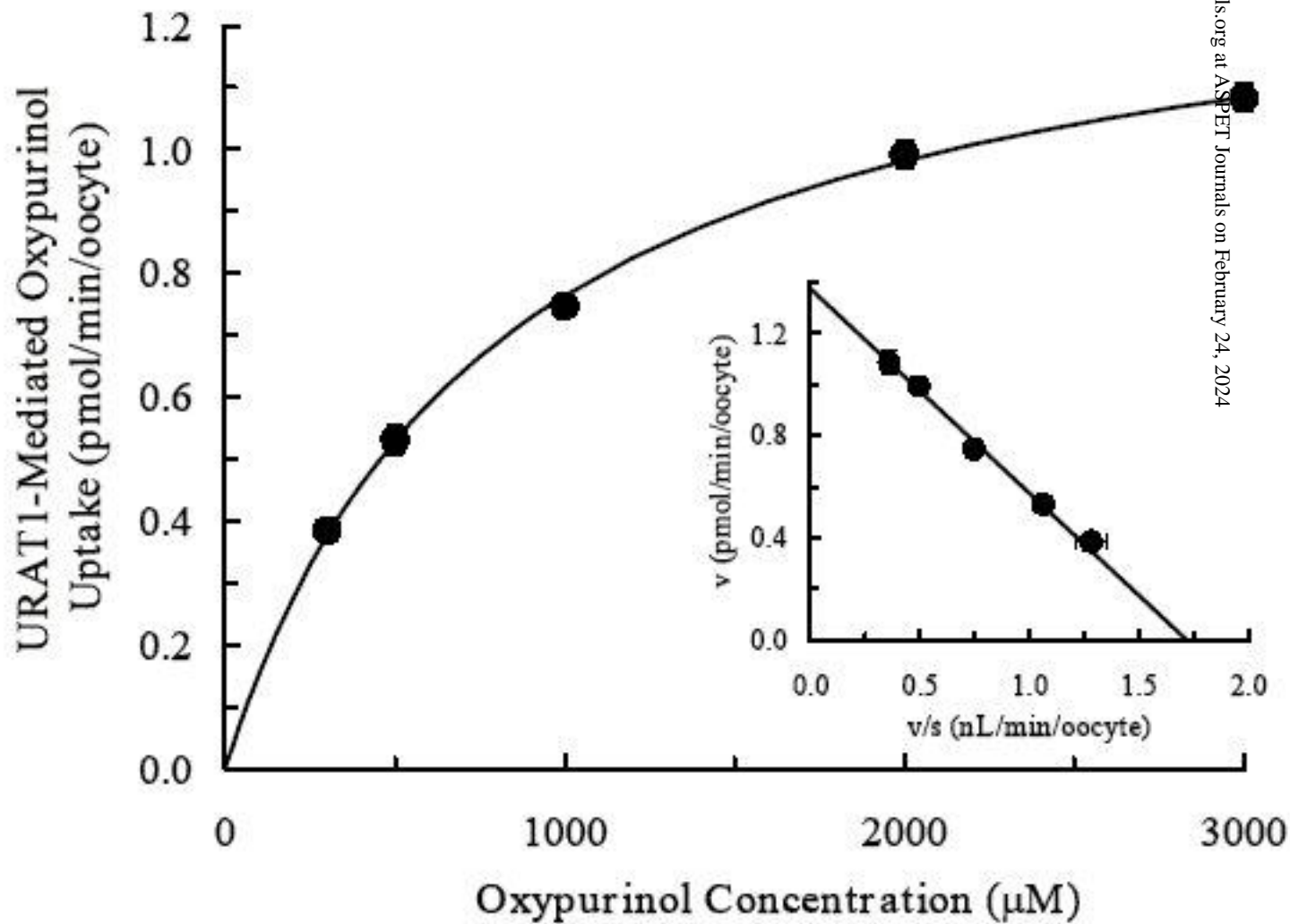


Fig.3

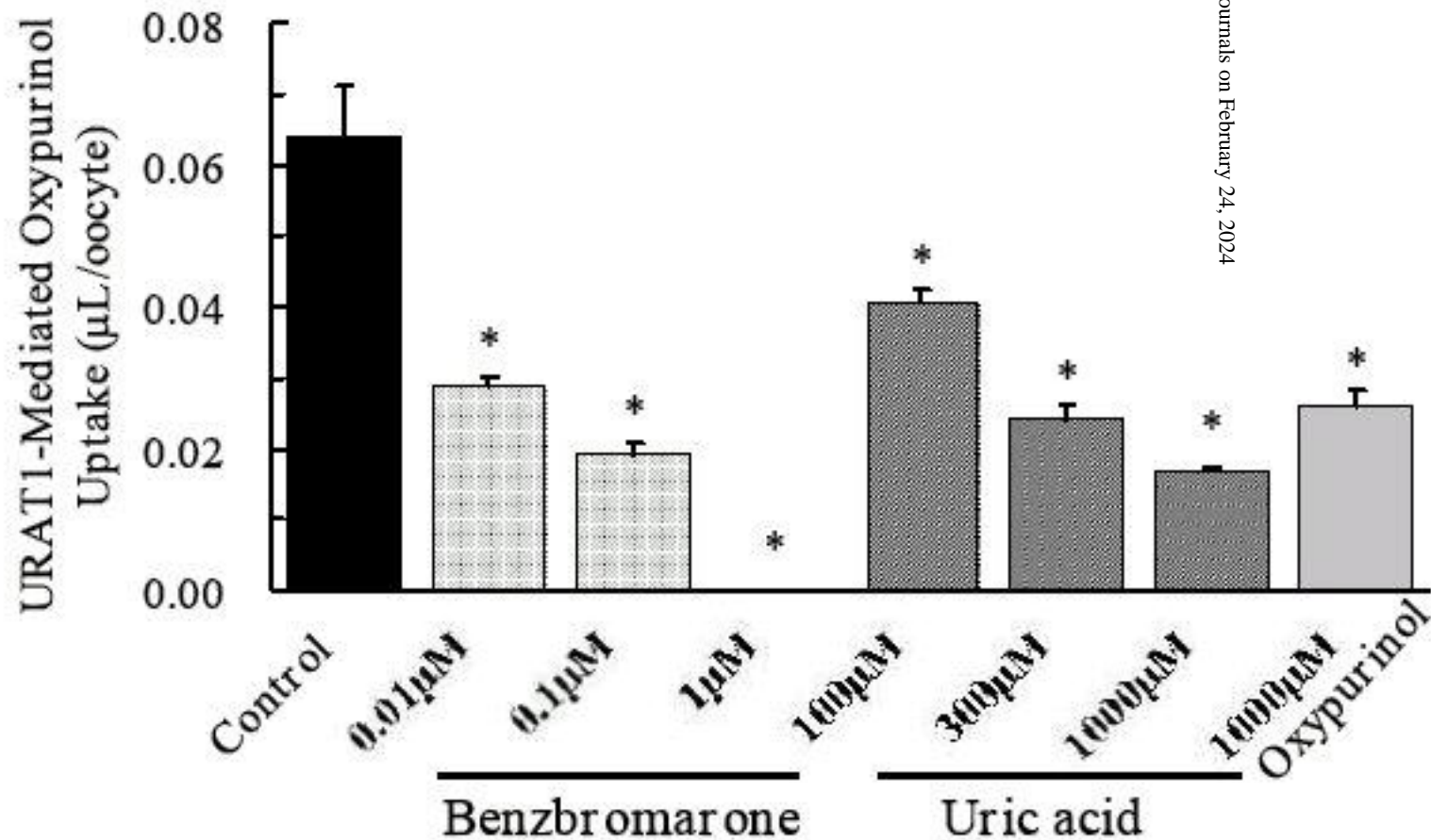


Fig.4

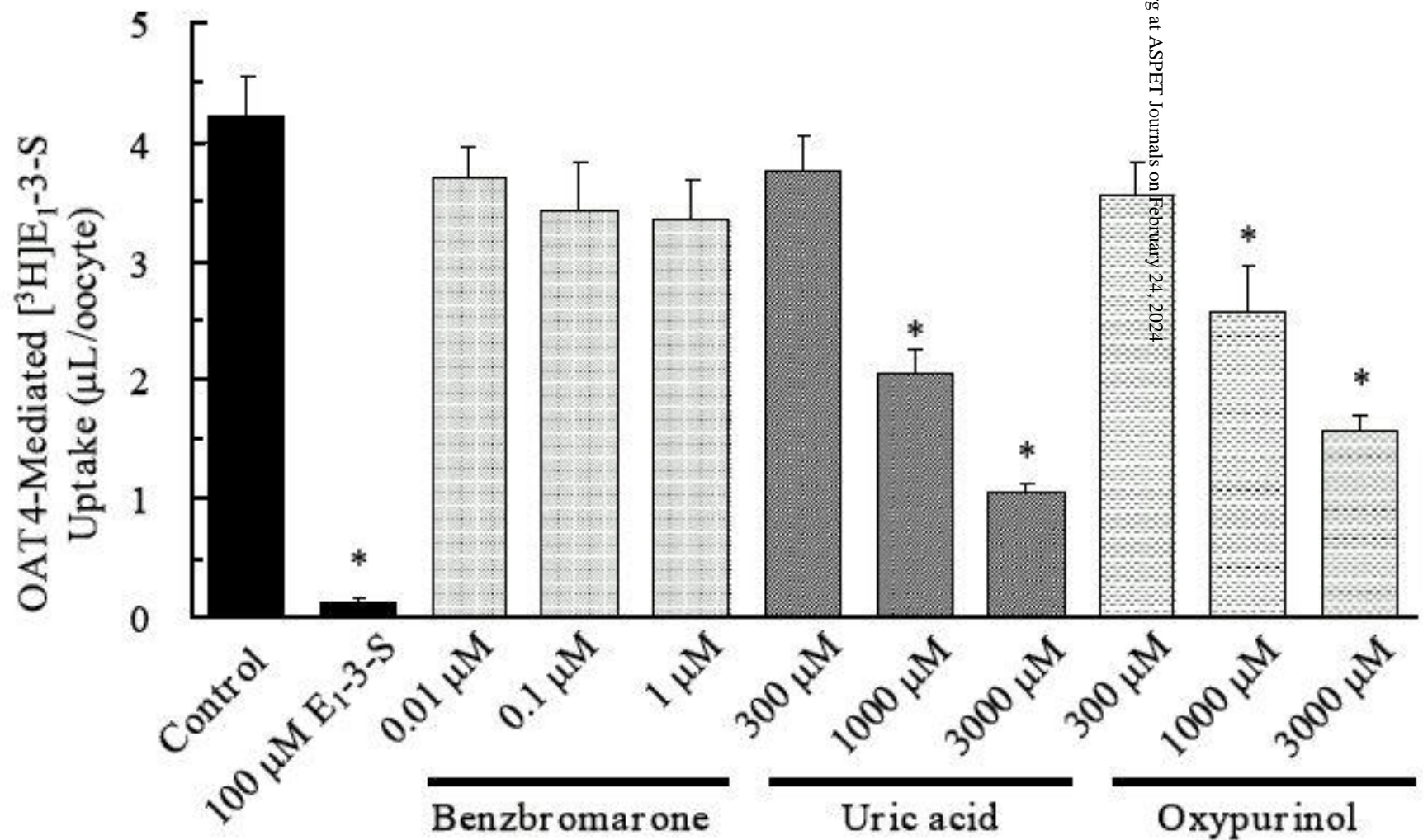


Fig.5

