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Pharmacological evaluation of dotinurad, a selective urate reabsorption inhibitor

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Running Title Page:

Dotinurad selectively inhibits urate reabsorption transporter

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Abbreviations:

ABCG2, ATP-binding cassette sub-family G member 2; Dotinurad, (3,5-Dichloro-4-hydroxyphenyl)(1,1-dioxo1,2-dihydro-3H-1λ^6-1,3-benzothiazol-3-yl)methanone; FE_{UA}, fractional excretion of urate; HPLC, high performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OAT1, organic anion

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transporter 1; SURI, selective urate reabsorption inhibitor; URAT1, urate transporter 1

Section Assignment:

Metabolism, Transport, and Pharmacogenomics

Abstract

The effect of dotinurad was compared to those of commercially available uricosuric agents, namely benzbromarone, lesinurad, and probenecid. Its effect on urate secretion transporters was evaluated using probe substrates for respective transporters. Dotinurad, benzbromarone, lesinurad, and probenecid inhibited URAT1 with IC_{50} values of 0.0372, 0.190, 30.0, and 165 μmol/l, respectively. Dotinurad weakly inhibited ABCG2, OAT1, and OAT3 with IC_{50} values of 4.16, 4.08, and 1.32 μmol/l, respectively, indicating higher selectivity for URAT1. The hypouricemic effects of dotinurad and benzbromarone were evaluated in Cebus monkeys. Dotinurad, at doses of 1–30 mg/kg, concomitantly decreased plasma urate levels and increased fractional excretion of urate (FE_{UA}) in a dose-dependent manner. On the contrary, benzbromarone, at a dose of 30 mg/kg, showed a modest effect on plasma urate levels. The inhibitory effect of dotinurad on urate secretion transporters was evaluated in Sprague-Dawley rats, with sulfasalazine and adefovir as probe substrates of ABCG2 and OAT1, respectively. Drugs including febuxostat as a reference ABCG2 inhibitor, were administered orally before sulfasalazine or adefovir administration. Dotinurad had no effect on urate secretion transporters *in vivo*, whereas benzbromarone, lesinurad, probenecid, and febuxostat increased the plasma concentrations of probe substrates. These results suggested dotinurad is characterized as a selective urate reabsorption inhibitor (SURI), which is defined as a potent URAT1 inhibitor with minimal effect on urate secretion transporters including

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ABCG2 and OAT1/3, because of its high efficacy in decreasing plasma urate levels compared to that of other uricosuric agents.

Significance statement

Our study on the inhibitory effects of dotinurad on urate transport showed that dotinurad had higher selectivity for URAT1 vs. ABCG2 and OAT1/3 than other uricosuric agents. In Cebus monkeys, dotinurad concomitantly decreased plasma urate levels and increased fractional excretion of urate (FE_{UA}) in a dose-dependent manner. To determine the inhibitory effect of dotinurad on urate secretion transporters, we performed studies in rats using sulfasalazine and adefovir as probe substrates of ABCG2 and OAT1, respectively. Dotinurad had no effect on these urate secretion transporters *in vivo*, whereas the other uricosuric agents increased the plasma concentrations of the probe substrates. These results suggested dotinurad as a potent and selective urate reabsorption inhibitor (SURI), is characterized by increased efficacy with decreasing plasma urate levels compared to the other uricosuric agents studied.

Introduction

Gout is a form of acute arthritis induced by deposition of monosodium urate crystals in the joints. Environmental (food and drink) and genetic (Lesch-Nyhan syndrome and familial juvenile hyperuricemic nephropathy) factors have been reported to play a central role in the etiology of gout (Kuo et al., 2015). Hyperuricemia, a common pathogenetic factor in the development of gout, is typically defined by a serum urate concentration of >6.8 or 7.0 mg/dl (Neogi, 2011; Terkeltaub et al., 2010). Hyperuricemia has been reported as a risk factor for the onset and development of chronic renal diseases, and a predictive factor for metabolic syndrome (Iseki et al., 2004; Obermayr et al., 2008; Yu et al., 2016).

Urate-lowering therapies with either type of urate control drug - urate production inhibitors or uricosuric agents - are indicated for patients with hyperuricemia. Benzbromarone, a commercially available uricosuric agent, effectively lowers serum urate levels. However, because of its rare but severe idiosyncratic hepatotoxic adverse effects, it is not approved in several European Union countries and the United States (Lee et al., 2008). In 2015, lesinurad, another uricosuric agent, was approved for use in the United States. However, it has been approved as 200 mg tablets in combination with urate production inhibitors because of its modest effect and increased serum creatinine at higher doses. There is a need for a novel uricosuric agent because 85% of patients develop hyperuricemia due to insufficient urate excretion (Nakamura et al., 2003); thus, the use of a uricosuric agent is recommended by the

Japanese Guideline for the management of hyperuricemia and gout (2019).

Uricosuric agents are known to exert their pharmacological function by inhibiting the function of URAT1 (solute carrier family 22 member 12: SLC22A12), which is localized in the brush-border membrane of the renal proximal tubules and functions as a urate reabsorption transporter (Enomoto et al., 2002). Other urate reabsorption transporters are also present in the renal tubules: GLUT9 (URATv1 and SLC2A9) in the basolateral membrane and OAT4/10 (SLC22A11/13) in the brush-border membrane (Nigam and Bhatnagar, 2018). Urate secretion transporters are also present in the renal tubules: OAT1/2/3 (SLC22A6/7/8) in the basolateral membrane and ABCG2 (breast cancer resistance protein, BCRP), MRP2/4 (ABCC2/4), and NPT1/4 (SLC17A1/3) in the brush-border membrane (Nigam and Bhatnagar, 2018; Sato et al., 2010). The presence of ABCG2 has also been reported in the brush-border membrane of the intestines, where it functions as an extra-renal urate excretion transporter (Ichida et al., 2012). Inhibition of urate secretion transporters has been reported to cause hyperuricemia. For example, probenecid, a non-selective OAT inhibitor, has been reported to elevate blood urate levels in potassium oxonate-treated rats (Yonetani et al., 1980). In ABCG2-knockout mice, a decrease in urate excretion into the intestines has been reported with a concomitant increase in blood urate levels (Ichida et al., 2012). Furthermore, several urate production inhibitors and benzbromarone were reported to inhibit urate transport by ABCG2 and their interaction probably occurs in the clinical setting (Miyata et al., 2016).

These data suggest that selective urate reabsorption inhibitors (SURIs), which are defined as potent URAT1inhibitors that do not affect urate secretion transporters including ABCG2 and OAT1/3, exhibit more potent hypouricemic effects than non-selective urate reabsorption inhibitor.

In the present study, we first compared the inhibitory effect of dotinurad, a novel agent that exhibits uricosuric effect in rodents, on urate transport in cells overexpressing URAT1, ABCG2, and OAT1/3 with those of benzbromarone, lesinurad, and probenecid. We then investigated the hypouricemic effects of dotinurad and benzbromarone in Cebus monkeys. Finally, the inhibitory effects of these drugs on urate secretion transporters were evaluated in Sprague-Dawley rats, using sulfasalazine and adefovir as probe substrates of ABCG2 and OAT1, respectively. Considering our findings, we discuss the potency of dotinurad as a uricosuric agent and its mechanism of action.

Materials and methods

Drugs and materials

Dotinurad (also called as FYU-981, Fig. 1) was synthesized by Fuji Yakuhin Co., Ltd. (Saitama, Japan). Benzbromarone was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Lesinurad was purchased from Selleck Chemicals, LLC. (Houston, TX, USA). Probenecid and febuxostat were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo,

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Japan). Adefovir was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). Sulfasalazine was purchased from Sigma-Aldrich Co., LLC. (St. Louis, MO, USA). [^{14}C]-urate was synthesized by Moravek, Inc. (Brea, CA, USA). The MicroBeta^2 liquid scintillation counter was purchased from PerkinElmer, Inc. (Waltham, MA, USA). The other drugs that were used in the present study are commercially available.

Determination of xanthine oxidase activity

Xanthine oxidase activity was measured with a spectrophotometer U-3000 (Hitachi High-Technologies Corporation, Tokyo, Japan) at 295 nm by following the absorbance change for initial 1 min. Assay mixture with 100 μmol/l xanthine dissolved in 0.1 mol/l NaOH (1% final) and drugs dissolved in dimethyl sulfoxide (DMSO, 1% final) in 100 mmol/l pyrophosphate buffer, pH 8.5 containing 0.2 mmol/l EDTA was pre-incubated for 5 min, and reactions were started by adding xanthine oxidase (from bovine milk) to the mixture at the final concentration of 3.2 mU/ml under the aerobic conditions at 25°C. Assay was performed in triplicate.

Study of [^{14}C]-Urate uptake in URAT1-overexpressing MDCKII cells or OAT1- and OAT3-overexpressing HEK293 cells

Cells were seeded into 96-well tissue culture plates at a density of 1×10^5 cells/well and

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cultured at 37 ± 1 °C and a 5% CO_2 atmosphere in Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose for 24 h. Before initiating the experiment, culture medium was removed and cells were washed twice in 100 µl of Hank's balanced salt solution (HBSS). Uptake experiments were performed at 37 ± 1 °C in 50 µl of HBSS containing 20 µmol/l [^{14}C]-urate and test articles dissolved in DMSO. Vehicle control contained 1% DMSO instead of test articles. URAT1-overexpressing MDCKII cells and OAT1-overexpressing HEK293 cells were washed after 10 min of incubation, whereas OAT3-overexpressing HEK293 cells were washed after 5 min of incubation, in 100 µL of ice-cold HBSS, followed by cell lysis with 50 µl of 0.1 mol/l NaOH. Transporter activity was determined by liquid scintillation counting of an aliquot from each well. Assay was performed in triplicate. The amount of translocated [^{14}C]-urate was determined for each well in cpm. Relative urate transport activity was reported as a percentage of the control and was calculated using the following equation: Relative transport of urate (% of control) = (A−B)/(C−D) × 100, where A represent as the amount of translocated [^{14}C]-urate in the presence of test article in transfected cells; B represent as the amount of translocated [^{14}C]-urate in the presence of test article in mock cells; C represent as the amount of translocated [^{14}C]-urate in the presence of 1% DMSO in transfected cells; and D represent as the amount of translocated [^{14}C]-urate in the presence of 1% DMSO in mock cells.

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Study of [^{14}C]-urate uptake by membrane vesicles obtained from ABCG2-overexpressing HEK293 cells

Membrane vesicle preparations from ABCG2-overexpressing cells (SOLVO Biotechnology, Hungary) in the presence of 70 μmol/l urate containing 20 μmol/l [^{14}C]-urate and 4 mmol/l ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. In 96-well plates, 50 μl of membrane vesicle suspended in 75 μmol/l urate and 30 μmol/l [^{14}C]-urate containing ice-cold transport buffer (250 mmol/l sucrose, 10 mmol/l $MgCl_2$, 10 mmol/l Tris-HCl, pH 7.4) were added. Test articles dissolved in DMSO, 0.75 μl, were added to the membrane vesicle mixture. Vehicle control contained 1% DMSO instead of test article. The mixtures were pre-incubated for 15 min at 37 ± 1°C. Reactions were initiated by adding 25 μl of pre-warmed 12 mmol/l MgATP (or 12 mmol/l AMP as a background control) in transport buffer. After 3 min, reactions were quenched by adding 200 μl of ice-cold washing buffer (250 mmol/l sucrose, 100 mmol/l NaCl, 10 mmol/l Tris-HCl, pH 7.4) and immediately filtered using glass fiber filters. The filters were washed 5 times with 200 μl of ice-cold washing buffer and air-dried. The amount of substrate inside the filtrated vesicles was determined by liquid scintillation counting. Assay was performed in triplicate. Relative urate transport activity was reported as a percentage of the control and was calculated using the following equation: Relative transport of urate (% of control) = (A−B)/(C−D) × 100, where A represents the amount of translocated [^{14}C]-urate in the presence of test article and ATP; B

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represents the amount of translocated [^{14}C]-urate in the presence of test article and AMP; C represents the amount of translocated [^{14}C]-urate in the presence of 1% DMSO and ATP; and D represents the amount of translocated [^{14}C]-urate in the presence of 1% DMSO and AMP.

Animals and housing

For the evaluation of hypouricemic effects, 5–10 year-old male Cebus monkeys (Cebus paella, weight range 2.85–3.70 kg)) bred in Shin Nippon Biomedical Laboratories, Ltd. (SNBL, Tokyo, Japan) were used for pharmacological studies. The monkeys were housed individually in stainless steel cages in an air-conditioned animal room under a 12-/12-h light/dark (LD) cycle at 26 ± 3 °C and a relative humidity of 55 ± 20%. Animals were fed a pellet diet (New World Primate Diet 5040, Purina Mills, LCC) and fresh apples daily, except one day before drug administration. Water was provided *ad libitum* throughout the study. The experimental procedures were performed in accordance with the Animal Care and Utilization Guidelines of SNBL.

For the evaluation of inhibitory effects on urate secretion transporters, 7-week-old male Sprague-Dawley rats (weight range 180–220 g) bred in Japan SLC, Inc. (Shizuoka, Japan) were used. The rats were housed in wire-mesh cages in an air-conditioned animal room with a 12-/12-h LD cycle at a temperature of 22 ± 4 °C and a relative humidity of 60 ± 20%. Animals that did not develop abnormalities after a 1-week acclimatization period were

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selected for the study. Rats were fed a CE-2 pellet diet (Crea Japan Inc., Tokyo, Japan) and tap water via automatic stainless steel nozzles *ad libitum* throughout the study. Study protocols were designed and refined taking animal reduction into consideration and were approved by the Animal Care and Utilization Committee of Fuji Yakuhin Research Laboratories.

Study of the hypouricemic effects of dotinurad and benzbromarone in Cebus monkeys

Five Cebus monkeys that were fasted for 18 h before drug administration orally received 1, 5, and 30 mg/kg of dotinurad, 30 mg/kg of benzbromarone, and 0.5% methylcellulose (MC) as control, respectively. Blood samples (about 1 ml) obtained from the saphenous vein at before and 2, 4, 8, and 24 h after drug administration using heparinized needle were kept on ice. Plasma was obtained from the blood samples by centrifugation at 3,000 rpm for 10 min at 4 °C. Urine samples were collected 0–4, 4–8, and 8–24 h after drug administration. Urate and creatinine levels in the samples were measured by a U-3000 spectrophotometer using an Iatro LQ UAII (Mitsubishi Chemical Medience, Corp., Tokyo, Japan) and L-type Wako Creatinine F (Wako Pure Chemical Industries, Ltd. Osaka, Japan). Each treatment was administered in 13-day intervals to wash out drugs, and treatment and sample collection were performed as crossover experiments. FE_{UA} was calculated as the ratio of urate clearance to creatinine clearance. Urinary urate excretion was calculated as urinary urate concentration × urine

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volume.

Determination of drug pharmacokinetics in Cebus monkeys

Plasma drug concentration was measured using the same samples described in the previous section. Plasma samples were deproteinated with thrice volume of methanol and centrifuged at 3,000 rpm for 10 min at 4 °C. Drug concentrations were measured using high-performance liquid chromatography (HPLC) using the Alliance 2695 HPLC system (Waters Corporation. Milford, MA, USA). The concentration of 6-hydroxybenzbromarone, a major benzbromarone metabolite, was also measured in the plasma obtained from benzbromarone-treated animals.

Study of concomitant sulfasalazine and hypouricemic agents treatment in Sprague-Dawley rats

Sprague-Dawley rats that were fasted for 18 h before drug administration orally received 20 mg/kg of febuxostat, 50 mg/kg of benzbromarone, 1.3 mg/kg of dotinurad, and 0.5% MC as control, respectively (n = 4, a total of 16 rats were used). Drug dosages used in the present study were calculated based on their clinically maximal doses detailed in supplemental Table 1. Thirty minutes after the administration of these drugs, sulfasalazine suspended in 0.5% MC was orally administered at a dose of 20 mg/kg. For drug bioavailability (F) calculations, 5

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mg/kg of sulfasalazine dissolved in 50 mmol/l tris-saline was intravenously administered via the tail vein. Blood samples (about 200 μl) were obtained from the jugular vein at 0.083 (i.v. only), 0.25, 0.5, 1, 2, 4, 8, and 12 h after sulfasalazine administration using heparinized needle and kept on ice. Plasma was obtained from the blood samples by centrifugation at 3,000 rpm for 10 min at 4°C. Plasma samples were deproteinated and sulfasalazine concentration was measured as previously described by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent 1100 Series HPLC Value System (Agilent Technologies, Inc. Santa Clara, CA, USA), API3000 (AB SCIEX, LCC. Framingham, MA, USA) (Miyata et al., 2016). F were calculated using the following equation: F (%) = ($AUC_{0\text{-}inf}$ value of oral administration / 20) / ($AUC_{0\text{-}inf}$ value of intravenous administration / 5) ×100.

Study of concomitant adefovir and uricosuric agents treatment in Sprague-Dawley rats

Sprague-Dawley rats that were fasted for 18 h before drug administration orally received 100 mg/kg of probenecid, 67 mg/kg of lesinurad, 50 mg/kg of benzbromarone, 1.3 mg/kg of dotinurad, and 0.5% MC as control (n = 6, a total of 30 rats were used). Thirty minutes after drug administration, 3 mg/kg of adefovir dissolved in saline was intravenously administered via the tail vein to all animals. Blood samples (about 200 μl) were obtained from the jugular vein at 0.083, 0.25, 0.5, 1, 2, and 4 h after adefovir administration using heparinized needle and kept on ice. Plasma was obtained from blood samples by centrifugation at 3,000 rpm for

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10 min at 4°C. Adefovir was detected in these samples as previously described (Jullien, 2003). Briefly, plasma was derivatized by incubation at 80 °C in 0.34% chloroacetaldehyde solution for 50 min and detected via the excitation and emission spectra at 236 and 420 nm wavelengths, respectively, using the Alliance 2695 HPLC system (Waters Corporation. Milford, MA, USA).

Statistical analysis

In the study of xanthine oxidase activity, IC_{50} values were calculated using probit method. In cell-based urate uptake studies, the concentration–response curves were analyzed using the GraphPad Prism 7.03 software (GraphPad Software, CA, USA) and a 4-parameter logistic equation was applied to calculate the IC_{50} values. In the studies of Cebus monkeys and Sprague-Dawley rats, the mean and standard deviation (SD) were calculated, and differences from the control group were analyzed using the Dunnett's multiple comparison test (at significance levels of 5% and 1%). In the study of Cebus monkeys, $AUC_{0–24h}$ was calculated using the trapezoidal rule in Microsoft Excel 2010 (Microsoft Corporation, DC, USA) and unbound fraction of maximum plasma concentration was calculated using Fu in supplemental Table 3. In the study of Sprague-Dawley rats, pharmacokinetic parameters were analyzed using the Phoenix WinNonlin 6.4 software.

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Results

Effects of uricosuric agents on xanthine oxidase activity

Benzbromarone inhibited xanthine oxidase activity with an IC_{50} value of 15.4 μmol/l. Dotinurad, lesinurad, and probenecid did not have any effect up to 100, 300, and 1000 μmol/l, respectively.

Inhibitory effect of uricosuric agents on urate uptake by URAT1-overexpressing MDCKII cells

Dotinurad, benzbromarone, lesinurad, and probenecid inhibited urate transport by URAT1-overexpressing MDCKII cells in a concentration-dependent manner at respective concentrations of 0.003–3, 0.003–3, 0.3–300, and 10–1000 μmol/l (Fig. 2) and respective IC_{50} values of 0.0372, 0.190, 30.0, and 165 μmol/l (Table 1a).

Inhibitory effect of uricosuric agents on urate uptake by ABCG2-, OAT1-, and OAT3-overexpressing HEK293 cells

Dotinurad inhibited urate transport in a concentration-dependent manner in ABCG2-, OAT1-, and OAT3-overexpressing HEK293 cells at respective concentrations of 0.3–300, 0.1–100, and 0.03–30 μmol/l and respective IC_{50} values of 4.16, 4.08, and 1.32 μmol/l. The ratios of IC_{50} values of ABCG2-, OAT1-, and OAT3-overexpressing cells to that of

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URAT1-overexpressing cells were 112, 110, and 35.5, respectively (Tables 1a and 1b). Benzbromarone inhibited urate transport in ABCG2-, OAT1-, and OAT3-overexpressing cells with respective IC_{50} values of 0.289, 3.14, and 0.967 μmol/l and respective IC_{50} value ratios to that of URAT1-overexpressing cells were 1.52, 16.5, and 5.09. Lesinurad inhibited urate transport with respective IC_{50} values of 26.4, 6.99, and 1.07 μmol/l and respective IC_{50} value ratios to that of URAT1-overexpressing cells were 0.880, 0.233, and 0.0357. Probenecid inhibited urate transport with respective IC_{50} values of 433, 10.9, and 2.37 μmol/l and respective IC_{50} value ratios to that of URAT1-overexpressing cells were 2.62, 0.0661, and 0.0144.

Hypouricemic effects of dotinurad and benzbromarone in Cebus monkeys

Effects of dotinurad and benzbromarone on plasma urate levels, FE_{UA}, and urinary urate excretion were assessed in Cebus monkeys. Dotinurad dose-dependently lowered the plasma urate levels with its maximum effect at 8 h (Fig. 3A). Changes in plasma urate level between 0 and 8 h (ΔP_{UA}) were lower than that of control by 0.28, 0.97 ($p < 0.05$), and 1.79 mg/dl ($p < 0.01$), at doses of 1, 5, and 30 mg/kg, respectively. Furthermore, dotinurad dose-dependently increased FE_{UA} (Fig. 3B). The 0–4 h FE_{UA} increased by 180%, at a dose of 30 mg/kg compared to the control ($p < 0.01$). On the contrary, the effect of benzbromarone at a dose of 30 mg/kg was modest; the ΔP_{UA} was lower than that of control by 0.46 mg/dl and the 0–4 h

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FE_{UA} increased by 30% compared to the control. Figure 3C details the urinary excretion amount of urate in Cebus monkeys 0–8 h after dotinurad and benzbromarone administration. Dotinurad dose-dependently increased the urinary urate excretion to 16.2, 22.8, and 25.3 mg at doses of 1, 5, and 30 mg/kg, respectively, compared with the control, which was 13.7 mg. On the contrary, benzbromarone increased the urinary urate excretion to 19.4 mg at a dose of 30 mg/kg. Results of creatinine clearance and urate clearance were shown in supplemental Table 2.

Pharmacokinetic parameters of dotinurad and benzbromarone in Cebus monkeys

The plasma concentration of dotinurad increased in a dose-dependent manner with C_{max} and $AUC_{0–24h}$ values of 107 μg/ml and 780 μg·h/ml, respectively, at a dose of 30 mg/kg (Table 2). On the contrary, C_{max} and $AUC_{0–24h}$ values of benzbromarone were 20.9 μg/ml and 95.2 μg·h/ml, respectively, at a dose of 30 mg/kg. In the plasma obtained from benzbromarone-treated animals, C_{max} and $AUC_{0–24h}$ value of 6-hydroxybenzbromarone were 12.7 μg/ml and 80.9 μg·h/ml, respectively. Unbound fraction of maximum plasma concentration of dotinurad, at doses of 1, 5, 30 mg/kg, and benzbromarone, at a dose of 30 mg/kg, were 0.0144, 0.0800, 0.750 μg/ml and 0.564 μg/ml, respectively.

Effect of hypouricemic agents on the plasma concentration of sulfasalazine in

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Sprague-Dawley rats

Effects of febuxostat, benzbromarone, and dotinurad on the plasma sulfasalazine concentration were assessed in Sprague-Dawley rats. Febuxostat and benzbromarone significantly increased the plasma sulfasalazine concentration (Fig. 4). Febuxostat increased the $AUC_{0\text{-inf}}$ value to 5012 ng·h/ml ($p < 0.01$) from a control value of 713 ng·h/ml and F to 14.1% from a control value of 2.0%. Benzbromarone also increased the $AUC_{0\text{-inf}}$ value to 2888 ng·h/ml ($p < 0.01$) and F to 8.1%. Dotinurad did not have any effect on the pharmacokinetic parameters of sulfasalazine (Table 3).

Effect of uricosuric agents on the plasma concentration of adefovir in Sprague-Dawley rats

Effects of probenecid, benzbromarone, lesinurad, and dotinurad on the plasma concentration of adefovir were assessed in Sprague-Dawley rats. Probenecid and lesinurad significantly increased the plasma adefovir concentration (Fig. 5). Probenecid increased plasma concentration of adefovir at 0.083–0.5 h ($p < 0.01$) and 1 h ($p < 0.05$). Lesinurad also increased plasma concentration of adefovir at 0.083 h ($p < 0.01$), 0.25 h ($p < 0.05$) and 0.5 h ($p < 0.01$). On the contrary, benzbromarone and dotinurad did not have any effect on the plasma concentration of adefovir.

Discussion

The kidneys play an important role in urate elimination. After filtration through the glomerulus, urate undergoes complex bidirectional steps of reabsorption and secretion in the proximal tubules, resulting in a net urinary excretion of <10% of the filtered amount (Chonko and Grantham, 1981). Because renal handling of urate is reabsorption-dominant, the reabsorption process is the target of uricosuric agents. In the renal tubules, several transporters are involved in urate reabsorption. Particularly, URAT1 in the brush-border membrane and GLUT9 in the basolateral membrane of renal tubules are the main urate reabsorption transporters. Aberrant function of these transporters caused by single nucleotide polymorphisms (SNPs) results in insufficient urate reabsorption, increased urinary urate excretion, and remarkable hypouricemia (<1 mg/dl; Kikuchi et al., 2000; Dinour et al., 2010). Uricosuric agents are known to exert their pharmacological effect via URAT1 inhibition, by which almost maximum effect is obtained without affecting other urate reabsorption transporters. On the contrary, urate secretion transporters are also present in the renal tubules: OAT1/2/3 are localized in the basolateral membrane, whereas ABCG2, MRP2/4, and NPT1/4 are localized in the brush-border membrane (Nigam and Bhatnagar, 2018; Sato et al., 2010). SNPs in ABCG2 result in a higher risk of gout caused by dysfunctional urate excretion (extra-renal excretion) from the intestines (Matsuo et al., 2009; Miyata et al., 2016). Furthermore, gene association studies performed in a patient cohort with chronic kidney

disease (CKD) showed that SNPs in ABCG2, MRP4, and OAT3 are associated with increased serum urate levels, although the association of SNPs in ABCG2 with serum levels was modest in the non-CKD cohort (Bhatnagar et al., 2016). In humans, the contribution of other urate secretion transporters on serum urate level has not been reported. Our findings suggested that SURIs had superior hypouricemic effects to those of non-selective urate reabsorption inhibitors.

Our previous study showed that in rats treated with topitoxostat, a urate production inhibitor, dotinurad decreases plasma urate levels and increases FE_{UA} in a dose-dependent manner without inhibiting xanthine oxidase or uricase (Taniguchi et al., 2016; Matsumoto et al. 2011). In studies in pyrazinamide-treated rats and brush-border membrane vesicles, dotinurad inhibits urate uptake at the brush-border membrane of the renal tubules with pyrazinecarboxylic acid as an exchange substrate (Taniguchi et al., 2017). It was postulated that this effect is mediated by urate transporters; however, the detailed underlying mechanisms are unknown. Based on these findings, we next evaluated the inhibitory effect of dotinurad on urate uptake by MDCKII or HEK293 cells overexpressing transporters. Dotinurad inhibited urate transport mediated by URAT1 with an IC_{50} value of 0.0372 μmol/l. The effect of dotinurad was approximately 5.11, 806, and 4440 times more potent than that of benzbromarone, lesinurad, and probenecid, respectively, because the IC_{50} value of these compounds was 0.190, 30.0, and 165 μmol/l, respectively (Table 1a). Dotinurad modestly

inhibited urate transport by ABCG2 and OAT1/3 with IC_{50} values of 4.16, 4.08, and 1.32 μmol/l, which were 112, 110, and 35.5 times higher than its IC_{50} values for URAT1, respectively (Tables 1a and 1b). By contrast, the IC_{50} ratios of benzbromarone, lesinurad, and probenecid for ABCG2 and OAT1/3 to URAT1 were 1.52–16.5, 0.0357–0.880, and 0.0144–2.62 for, respectively. These findings showed that dotinurad inhibited URAT1 more selectively than the other uricosuric agents. Several lines of evidence showed the inhibitory effect of benzbromarone, lesinurad, and probenecid on urate transport by URAT1, ABCG2, OAT1, and OAT3 (Miner et al., 2016; Miyata H et al., 2016; Ichida et al., 2003), although the inhibitory effects of benzbromarone and probenecid on urate transport by OAT3 are not reported. Our data were consistent with these reports, except for the URAT1 inhibitory effect of lesinurad and probenecid (the IC_{50} values are 3.53 and 13.23 μmol/l, respectively). We could not evaluate the inhibitory effect of uricosuric agents on MRP4, because urate transport by MRP4-overexpressing cells was not reported. These data also suggested that dotinurad did not affect ABCG2 and OAT1/3 in clinical use because its unbound fraction in plasma appeared to be extremely low (Supplemental Table 3; FDA, 2018; PMDA, 2019).

Subsequently, we compared the hypouricemic efficacy of dotinurad with that of benzbromarone in Cebus monkeys. Dotinurad at doses of 1, 5, and 30 mg/kg decreased plasma urate levels and increased FE_{UA} in a dose-dependent manner, and its effect at 1 mg/kg was approximately equal to that of 30 mg/kg benzbromarone (Fig. 3A and 3B). However, the

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stringency of URAT1 inhibition (the IC_{50} values of dotinurad and benzbromarone were 0.0372 and 0.190 μmol/l, respectively) did not sufficiently account for the difference in their hypouricemic effects. We hypothesized that the pharmacokinetic profiles of these drugs possibly contribute to their hypouricemic effects. Indeed, dotinurad had higher C_{max} and $AUC_{0–24h}$ values than those of benzbromarone (Table 2). The unbound fraction of maximal plasma concentration of dotinurad at doses of 1, 5, 30 mg/kg, and that of benzbromarone at a dose of 30 mg/kg, which are considered to correlate with their concentration at the renal proximal tubules, were calculated to be 0.0144, 0.0800, 0.750, and 0.564 μg/ml, respectively (Table 2). Because these values are 1.1-, 6.0-, 56- and 7.2-times higher than the IC_{50} values of URAT1, respectively, an equal hypouricemic effect was expected to be obtained by 5 mg/kg of dotinurad and 30 mg/kg of benzbromarone (Table 2 and Supplemental Table 3). Considering that 6-hydroxybenzbromarone, a major metabolite of benzbromarone, also inhibited URAT1 and contributed to the hypouricemic effect in addition to benzbromarone itself (Shin et al., 2011), another factor was assumed to explain the difference in hypouricemic effect. Thus, we speculated that property as SURIs, defined as potent URAT1 inhibitors with minimal effect on urate secretion transporters, ABCG2 and OAT1/3, generated the difference of hypouricemic effect apart from non-selective inhibitor.

In order to estimate the effect of uricosuric agents on urate secretion *in vivo* accurately, we evaluated whether dotinurad and other uricosuric agents interact with ABCG2 and OAT1

using their probe substrates in Sprague-Dawley rats. Sulfasalazine is known as a typical ABCG2 substrate with low absorption because it is excreted on the intestinal brush-border membrane (Dahan and Amidon, 2009). In our rat study using sulfasalazine as a probe substrate, benzbromarone and the positive control agent febuxostat markedly increased the plasma concentration and F of sulfasalazine (Fig. 4 and Table 3), these increases were assumed to be caused by inhibition of intestinal ABCG2, which appeared to be important for urate excretion (Ichida et al., 2012). Adefovir, a well-known OAT1 substrate, is excreted in urine (as adefovir itself), and its fraction of drug elimination in urine (fe) is approximately 90% in humans (Cundy et al., 1995). In our rat study using adefovir as a probe substrate, lesinurad and probenecid increased the plasma concentration of adefovir (Fig. 5); this increase was assumed to be caused by inhibition of renal OAT1, which mediates urate secretion (Wu et al., 2017). Because these drugs showed more potent inhibition on OAT3 than on OAT1, further experiments should be conducted using a probe substrate for OAT3. In our rat studies, dotinurad did not affect the plasma concentration of probe substrates, indicating that dotinurad hardly affected urate secretion transporter *in vivo*.

We must consider that the probe substrates did not move exactly in the same manner as urate. It can be predicted by evaluating the inhibitory effect of a compound on urate and probe substrates. For example, another ABCG2 inhibitor, rifampicin, inhibits both urate and sulfasalazine transport by ABCG2 at a similar concentration (Hosomi et al., 2012; Kosa et al.,

2018). In terms of OAT1, probenecid inhibits adefovir transport with an inhibition constant (Ki) of 18.6 μmol/l (Maeda et al., 2014), suggesting a similarity to our result. These reports indicated that sulfasalazine and adefovir were transported in the same manner as urate. Furthermore, species differences between rats and humans have to be taken into account. The $AUC_{0–48h}$ of sulfasalazine was three-fold higher for functional ABCG2 SNPs, and a similar effect was observed in ABCG2-knockout mice (Gotanda et al., 2015; Miyata et al., 2016). The fe of adefovir in rodents is approximately 80%, which is nearly the same as that for humans (Naesens et al., 1996). Therefore, we concluded that the data obtained from our rat studies could predict urate handling in humans.

Studies on Cebus monkeys showed that the hypouricemic effect of UR-1102, a selective inhibitor of URAT1 vs. OAT1/3, showed a higher estimated E_{max} of urinary urate excretion than that shown by benzbromarone (Ahn et al., 2016). In this report, authors discussed about URAT1 selectivity, defined as potent URAT1 inhibition without effect on OAT1/3, is important for producing potent hypouricemic effect. In fact, UR-1102, in a dose-dependent manner, lowered serum urate in humans at doses (0.25–2 mg) much lower than that of benzbromarone (Jun et al., 2017).

Figure 6 shows a scheme of urate pool reduction by SURIs or non-selective inhibitors. SURIs effectively reduce urate pool by increasing renal excretion of urate. On the contrary, non-selective inhibitors modestly reduce urate pool because of their inhibitory effect on urate

secretion transporters. For non-selective inhibitors to reduce urate pool at a similar level as SURIs, they require more URAT1 inhibition at higher doses, which results in excessively elevated renal excretion of urate and pose a risk factor for urolithiasis (Yu and Gutman, 1967). In the present study, benzbromarone did not exert an apparent effect on plasma urate levels in Cebus monkeys (Fig. 3A), although it increased urinary urate excretion more potently than 1 mg/kg of dotinurad (Fig. 3C). The phenomenon appeared to be mediated by inhibition of urate secretion transporters, especially ABCG2 in the intestine. These findings suggested that non-selective inhibitors can only reduce urate pool. Furthermore, our findings proved that SURIs were not associated with a risk of drug-drug interaction under clinical settings.

In conclusion, we showed that dotinurad, a SURI, could be an effective therapeutic option for the treatment of hyperuricemia because of its more effective hypouricemic action than that of commercially available uricosuric agents. Furthermore, dotinurad could be a useful agent in studies on urate transporters because of its ability to selectively and potently inhibit URAT1.

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References

Ahn SO, Ohtomo S, Kiyokawa J, Nakagawa T, Yamane M, Lee KJ, Kim KH, Kim BH, Tanaka J, Kawabe Y, and Horiba N (2016) Stronger uricosuric effects of the novel selective URAT1 Inhibitor UR-1102 lowered plasma urate in tufted capuchin monkeys to a greater extent than benzbromarone. J Pharmacol Exp Ther 357: 157–166.

Bhatnagar V, Richard EL, Wu W, Nievergelt CM, Lipkowitz MS, Jeff J, Maihofer AX, and Nigam SK (2016) Analysis of ABCG2 and other urate transporters in uric acid homeostasis in chronic kidney disease: potential role of remote sensing and signaling. Clin Kidney J 9: 444–453.

Chonko AM, and Grantham JJ (1981) Disorders of urate metabolism and excretion, in The Kidney, 2nd ed. (Brenner BM, and Rector FC eds) pp 1023–1055, WB Saunders, Philadelphia.

Cundy KC, Barditch-Crovo P, Walker RE, Collier AC, Ebeling D, Toole J, and Jaffe HS (1995) Clinical pharmacokinetics of adefovir in human immunodeficiency virus type 1-infected patients. Antimicrob Agents Chemother 39: 2401–2405.

Dahan A, and Amidon GL (2009) Small intestinal efflux mediated by MRP2 and BCRP shifts sulfasalazine intestinal permeability from high to low, enabling its colonic targeting. Am J Physiol Gastrointest Liver Physiol 297: G371–377.

Dinour D, Gray NK, Campbell S, Shu X, Sawyer L, Richardson W, Rechavi G, Amariglio N,

Ganon L, Sela BA, Bahat H, Goldman M, Weissgarten J, Millar MR, Wright AF, and Holtzman EJ (2010) Homozygous SLC2A9 mutations cause severe renal hypouricemia. J Am Soc Nephrol 21: 64–72.

Enomoto A, Kimura H, Chairoungdua A, Shigeta Y, Jutabha P, Cha SH, Hosoyamada M, Takeda M, Sekine T, Igarashi T, Matsuo H, Kikuchi Y, Oda T, Ichida K, Hosoya T, Shimokata K, Niwa T, Kanai Y, and Endou H (2002) Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature 417: 447–452.

Gotanda K, Tokumoto T, Hirota T, Fukae M, and Ieiri I (2015) Sulfasalazine disposition in a subject with 376C>T (nonsense mutation) and 421C>A variants in the ABCG2 gene. Br J Clin Pharmacol 80: 1236–1237.

Hosomi A, Nakanishi T, Fujita T, and Tamai I (2012) Extra-renal elimination of uric acid via intestinal efflux transporter BCRP/ABCG2. PLoS One 7: e30456.

Ichida K, Hosoyamada M, Kimura H, Takeda M, Utsunomiya Y, Hosoya T, and Endou H (2003) Urate transport via human PAH transporter hOAT1 and its gene structure. Kidney Int 63: 143–155.

Ichida K, Matsuo H, Takada T, Nakayama A, Murakami K, Shimizu T, Yamanashi Y, Kasuga H, Nakashima H, Nakamura T, Takada Y, Kawamura Y, Inoue H, Okada C, Utsumi Y, Ikebuchi Y, Ito K, Nakamura M, Shinohara Y, Hosoyamada M, Sakurai Y, Shinomiya N, Hosoya T, and Suzuki H (2012) Decreased extrarenal urate excretion is a common cause of

hyperuricemia. Nat Commun 3: 764.

Iseki K, Ikemiya Y, Inoue T, Iseki C, Kinjo K, and Takishita S (2004) Significance of hyperuricemia as a risk factor for developing ESRD in a screened cohort. Am J Kidney Dis 44: 642–650.

Japanese society of gout and nucleic acid metabolism (2019): Guideline for the management of hyperuricemia and gout, 3rd ed. (in Japanese) (Hisatome I ed) pp 110–113, Shindan to Chiryo-sha, Tokyo, Japan.

Jullien V, Tréluyer JM, Pons G, and Rey E (2003) Determination of tenofovir in human plasma by high-performance liquid chromatography with spectrofluorimetric detection. J Chromatogr B Analyt Technol Biomed Life Sci 785: 377–381.

Jun JB, Lee H, Suh CH, Lee CK, Kim DW, Choe JY, Lee SH, Kim SH, Hong SJ, Bang SY, Choi SJ, Park YB, Onohara M, Choi J, Song JS, and Park W (2017) A series of double-blind, placebo-controlled, randomized, multicenter, phase 2 studies to evaluate the efficacy, safety, and dose–response relationship of orally administered URC102, a novel URAT1 inhibitor, in Korean patients with gout. American college of rheumatology annual meeting. Abstract number 2074.

Kikuchi Y, Koga H, Yasutomo Y, Kawabata Y, Shimizu E, Naruse M, Kiyama S, Nonoguchi H, Tomita K, Sasatomi Y, and Takebayashi S (2000) Patients with renal hypouricemia with exercise-induced acute renal failure and chronic renal dysfunction. Clin Nephrol 53: 467–

472.

Kosa RE, Lazzaro S, Bi YA, Tierney B, Gates D, Modi S, Costales C, Rodrigues AD, Tremaine LM, and Varma MV (2018) Simultaneous assessment of transporter-mediated drug–drug interactions using a probe drug cocktail in Cynomolgus monkey. Drug Metab Dispos 46: 1179–1189.

Kuo CF, Grainge MJ, Zhang W, and Doherty M (2015) Global epidemiology of gout: prevalence, incidence and risk factors. Nat Rev Rheumatol 11: 649–662.

Lee MH, Graham GG, Williams KM, and Day RO (2008) A benefit-risk assessment of benzbromarone in the treatment of gout. Was its withdrawal from the market in the best interest of patients? Drug Saf 31: 643–665.

Maeda K, Tian Y, Fujita T, Ikeda Y, Kumagai Y, Kondo T, Tanabe K, Nakayama H, Horita S, Kusuhara H, and Sugiyama Y (2014) Inhibitory effects of p-aminohippurate and probenecid on the renal clearance of adefovir and benzylpenicillin as probe drugs for organic anion transporter (OAT) 1 and OAT3 in humans. Eur J Pharm Sci 59: 94–103.

Matsumoto K, Okamoto K, Ashizawa N, Nishino T (2011) FYX-051: a novel and potent hybrid-type inhibitor of xanthine oxidoreductase. J Pharmacol Exp Ther 336: 95–103.

Matsuo H, Takada T, Ichida K, Nakamura T, Nakayama A, Ikebuchi Y, Ito K, Kusanagi Y, Chiba T, Tadokoro S, Takada Y, Oikawa Y, Inoue H, Suzuki K, Okada R, Nishiyama J, Domoto H, Watanabe S, Fujita M, Morimoto Y, Naito M, Nishio K, Hishida A, Wakai K,

Asai Y, Niwa K, Kamakura K, Nonoyama S, Sakurai Y, Hosoya T, Kanai Y, Suzuki H, Hamajima N, and Shinomiya N (2009) Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. Sci Transl Med 1: 5–11.

Miner JN, Tan PK, Hyndman D, Liu S, Iverson C, Nanavati P, Hagerty DT, Manhard K, Shen Z, Girardet JL, Yeh LT, Terkeltaub R, and Quart B (2016) Lesinurad, a novel, oral compound for gout, acts to decrease serum uric acid through inhibition of urate transporters in the kidney. Arthritis Res Ther 18: 214.

Miyata H, Takada T, Toyoda Y, Matsuo H, Ichida K, and Suzuki H (2016) Identification of febuxostat as a new strong ABCG2 inhibitor: potential applications and risks in clinical situations. Front Pharmacol 7: 518.

Naesens L, Balzarini J, Bischofberger N, and De Clercq E (1996) Antiretroviral activity and pharmacokinetics in mice of oral bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, the bis(pivaloyloxymethyl) ester prodrug of 9-(2-phosphonylmethoxyethyl)adenine. Antimicrob Agents Chemother 40: 22–28.

Nakamura T (2003) Pathogenesis and pathophysiology of hyperuricemia (in Japanese), in Nakamura T, editor. Treatment of hyperuricemia and gout. Medical Review, Osaka, Japan. 21–39.

Neogi T (2011) Clinical practice. Gout. N Engl J Med 364: 443–452.

Nigam SK, and Bhatnagar V (2018) The systems biology of uric acid transporters: the role of remote sensing and signaling. Curr Opin Nephrol Hypertens 27: 305–313.

Obermayr RP, Temml C, Gutjahr G, Knechtelsdorfer M, Oberbauer R, and Klauser-Braun R (2008) Elevated uric acid increases the risk for kidney disease. J Am Soc Nephrol 19: 2407–2413.

Pharmaceuticals and medical devices agency (PMDA) (2019): Guideline on drug interaction for drug development and appropriate provision of information.

Sato M, Mamada H, Anzai N, Shirasaka Y, Nakanishi T, and Tamai I (2010) Renal secretion of uric acid by organic anion transporter 2 (OAT2/SLC22A7) in human. Biol Pharm Bull 33: 498–503.

Shin HJ, Takeda M, Enomoto A, Fujimura M, Miyazaki H, Anzai N, and Endou H (2011) Interactions of urate transporter URAT1 in human kidney with uricosuric drugs. Nephrology (Carlton) 16: 152–162.

Taniguchi T, Ashizawa N, Matsumoto K, Iwanaga T, and Saitoh K (2016) Uricosuric agents decrease the plasma urate level in rats by concomitant treatment with topiroxostat, a novel xanthine oxidoreductase inhibitor. J Pharm Pharmacol 68: 76–83.

Taniguchi T, Ashizawa N, Matsumoto K, and Iwanaga T (2017) Enhancement of pharmacological effects of uricosuric agents by concomitant treatment with pyrazinamide

in rats. Naunyn Schmiedebergs Arch Pharmacol 390: 253–260.

Terkeltaub R (2010) Update on gout: new therapeutic strategies and options. Nat Rev Rheumatol 6: 30–38.

USA food and drug administration (FDA) (2017): Guidance for industry. In vitro metabolism and transporter mediated drug–drug interaction studies.

Wu W, Bush KT, and Nigam SK (2017) Key role for the organic anion transporters, OAT1 and OAT3, in the in vivo handling of uremic toxins and solutes. Sci Rep 7: 4939.

Yonetani Y, Ishii M, and Iwaki K (1980) Hyperuricemia induced by some antihypertensives and uricosuric drugs in oxonate-treated rats. Jpn J Pharmacol 30: 829–840.

Yu T, and Gutman AB (1967) Uric acid nephrolithiasis in gout. Predisposing factors. Ann Intern Med 67: 1133–1148.

Yu TY, Jee JH, Bae JC, Jin SM, Baek JH, Lee MK, and Kim JH (2016) Serum uric acid: A strong and independent predictor of metabolic syndrome after adjusting for body composition. Metabolism 65: 432–440.

Footnotes:

Thesis information:

A part of Cebus monkey studies in the work was previously presented at the 86 th annual meeting of the Japanese pharmacological society (abstract number. P2-143).

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Figure Legends:

Fig. 1 Chemical structures of dotinurad and commercially available uricosuric agents.

A: Dotinurad, B: Benzbromarone, C: Lesinurad, D: Probenecid

Fig. 2 Concentration-response curve of dotinurad and commercially available uricosuric agents on urate transport by URAT1-overexpressing MDCKII cells.

Each value is the mean ± SD of urate transport relative to the control (n = 3) and described by GraphPad Prism 7.03 software (GraphPad Software, CA, USA).

Fig. 3 Effects of dotinurad and benzbromarone on plasma urate level, FE_{UA}, and urinary urate excretion in Cebus monkeys.

FE_{UA}: fractional excretion of urate; MC: methylcellulose.

Data are represented as change in plasma urate level (A), FE_{UA} at 0–4, 4–8, and 8–24 h after dosing (B) and the amount of urinary urate excretion for 8 h after dosing (C). Each value is the means ± SD of four to five animals. *P < 0.05, **P < 0.01 for significant differences from the control at each time point using the Dunnett's multiple comparison test.

Fig. 4 Effect of dotinurad and other hypouricemic agents on the plasma concentration of

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sulfasalazine in Sprague-Dawley rats

MC: methylcellulose.

Hypouricemic agents or their vehicle as control (0.5% MC) were orally administered to Sprague-Dawley rats fasted for 18 h. After 30 min, sulfasalazine was orally administered. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, and 12 h after sulfasalazine administration. Plasma samples were deproteinated and sulfasalazine concentrations were measured using LC-MS/MS. Each value is the mean ± SD of three to four animals.

**P < 0.01 shows significant differences from the 0.5% MC control group after Dunnett's multiple comparison test.

Fig. 5 Effect of dotinurad and other uricosuric agents on the plasma concentration of adefovir in Sprague-Dawley rats

MC: methylcellulose.

Hypouricemic agents or their vehicle as control (0.5% MC) were orally administered to Sprague-Dawley rats fasted for 18 h. After 30 min, adefovir was intravenously administered. Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 12 h after adefovir administration. Plasma samples were derivatized and adefovir concentrations were measured using HPLC. Each value is the mean ± SD of six animals.

*P < 0.05 and **P < 0.01 show significant differences from the 0.5% MC control group after

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Dunnett's multiple comparison test.

Fig. 6 Comparison of the potency of selective and non-selective urate reabsorption inhibitors in decreasing urate pool and the concomitant effects of non-selective inhibitors.

The upper figure shows the usual state of urate handling showing urate excretion from the renal tubules and the small intestine. SURI increases urate excretion from the renal tubules due to URAT1 inhibition, leading to a potent reduction of urate pool (A). A non-selective inhibitor decreases urate excretion from the renal tubules and/or the small intestine thorough inhibition of ABCG2 and/or OAT1/3. For a non-selective inhibitor to obtain the same effect at a level similar to SURI, more potent inhibition of URAT1 and additional renal excretion are required (B). Arrow width represents the quantity of urate handling, which is affected by inhibition of URAT1, ABCG2, and OAT1/3.

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Table 1a. IC_{50} values of dotinurad and commercially available uricosuric agents against urate transport by URAT1 and urate secretion transporters

Test article	IC_{50} (μmol/l)			
	URAT1	ABCG2	OAT1	OAT3
Dotinurad	0.0372 + 0.0065	4.16 + 2.49	4.08 + 1.02	1.32 + 0.07
Benzbromarone	0.190 + 0.029	0.289 + 0.074	3.14 + 1.59	0.967 + 0.071
Lesinurad	30.0 + 3.9	26.4 + 4.7	6.99 + 1.94	1.07 + 0.27
Probenecid	165 + 14	433 + 218	10.9 + 0.5	2.37 + 0.94

IC_{50}: half maximal inhibitory concentration

For measurement of transport activities, MDCKII-URAT1 (passage number 3–11), HEK293-OAT1 (passage number 8), HEK293-OAT3 (passage number 5), and HEK293-ABCG2 membrane vesicles (SOLVO Biotechnology, Hungary) were used.

IC_{50} was calculated using four-parameter logistic equation. Each value is the mean + SEM (n = 2–3).

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Table 1b. IC_{50} ratios for ABCG2 and OAT1/3 to URAT1 of dotinurad and commercially available uricosuric agents

Test article	IC_{50} ratio to URAT1			
	URAT1	ABCG2	OAT1	OAT3
Dotinurad	1	112	110	35.5
Benzbromarone	1	1.52	16.5	5.09
Lesinurad	1	0.880	0.233	0.0357
Probenecid	1	2.62	0.0661	0.0144

Each value was calculated as the IC_{50} of each compound for ABCG2, OAT1, and OAT3/IC_{50} for URAT1.

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Table 2. Pharmacokinetic parameters of dotinurad and benzbromarone in Cebus monkeys

Administered article	Dose (mg/kg)	Measured article	Pharmacokinetic parameters			
			C_{max} (μg/ml)	Fu × C_{max} (μg/ml)	T_{max} (h)	$AUC_{0\text{-}24h}$ (μg·h/ml)
Dotinurad	1	Dotinurad	2.09 ± 0.78	0.0144	3.2 ± 1.1	20.3 ± 8.6
	5		11.4 ± 4.8	0.0800	2.8 ± 1.1	109 ± 53
	30		107 ± 22	0.750	2.0 ± 0.0	780 ± 265
Benzbromarone	30	Benzbromarone	20.9 ± 9.0	0.564	2.0 ± 0.0	95.2 ± 28.1
		6-Hydroxybenzbromarone	12.7 ± 4.4	-	2.0 ± 0.0	80.9 ± 33.9

C_{max}: maximum plasma concentration; Fu: unbound fraction in plasma; Fu × C_{max}: unbound maximum plasma concentration; T_{max}: time to reach maximum plasma concentration; $AUC_{0–24h}$: area under the plasma concentration-time curve at 0–24 h. "-" means not calculated. Plasma samples were deproteinated and drug concentrations were measured using HPLC. $AUC_{0–24h}$ was calculated using the trapezoidal rule. Each value is the mean ± SD of five animals.

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Table 3. Pharmacokinetic parameters of sulfasalazine and effects of concomitant treatment with hypouricemic agents in Sprague-Dawley rats

Test article	Dose (mg/kg)	C_{max} (ng/ml)	$T_{1/2}$ (h)	AUC_{o-t} (ng·h/ml)	AUC_{inf} (ng·h/ml)	CL_T (ml/h/kg)	F (%)
0.5% MC	-	422 ± 182	1.49 ± 0.28	665 ± 94	713 ± 90	28395 ± 3800	2.0
Febuxostat	20	3138** ± 272	1.30 ± 0.13	5004** ± 851	5012** ± 848	4067** ± 607	14.1
Benzbromarone	50	1520** ± 202	1.24 ± 0.46	2858** ± 750	2888** ± 729	7294** ± 1989	8.1
Dotinurad	1.3	415 ± 68	1.88 ± 1.56	708 ± 166	885 ± 264	24126 ± 7777	2.5

MC: methylcellulose; C_{max}: maximum plasma concentration; $T_{1/2}$: elimination half-life; $AUC_{0–t}$: area under the plasma concentration-time curve at 0–12 h; AUC_{inf}: AUC from zero to infinity; CL_T: total clearance; F: bioavailability.

Pharmacokinetic parameters were calculated using Phoenix WinNonlin (Pharsight Corp. Sunnyvale, CA, USA). F were calculated using the following equation: F = ($AUC_{0\text{-}inf}$ value of oral administration / 20) / ($AUC_{0\text{-}inf}$ value of intravenous administration / 5) ×100. Values are reported as the mean ± SD of three to four animals.

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**P < 0.01 shows significant differences from the 0.5% MC control group, as analyzed by Dunnett's multiple comparison test.

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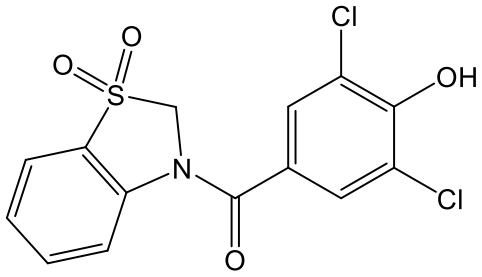
Fig. 1

A



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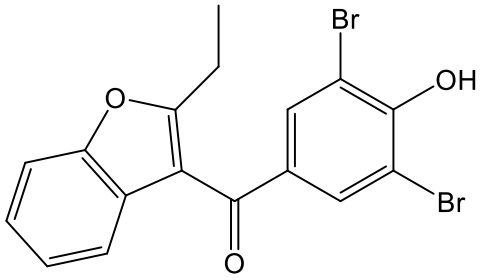

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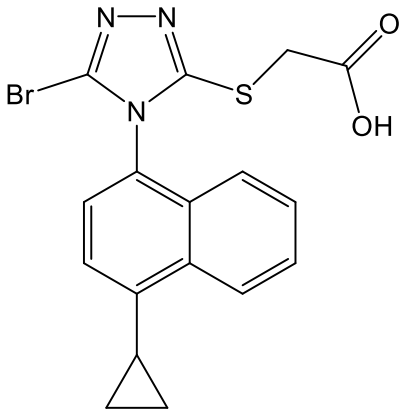

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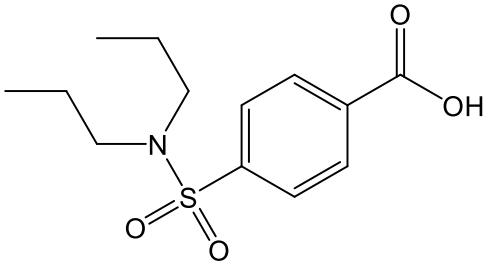

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Fig. 2

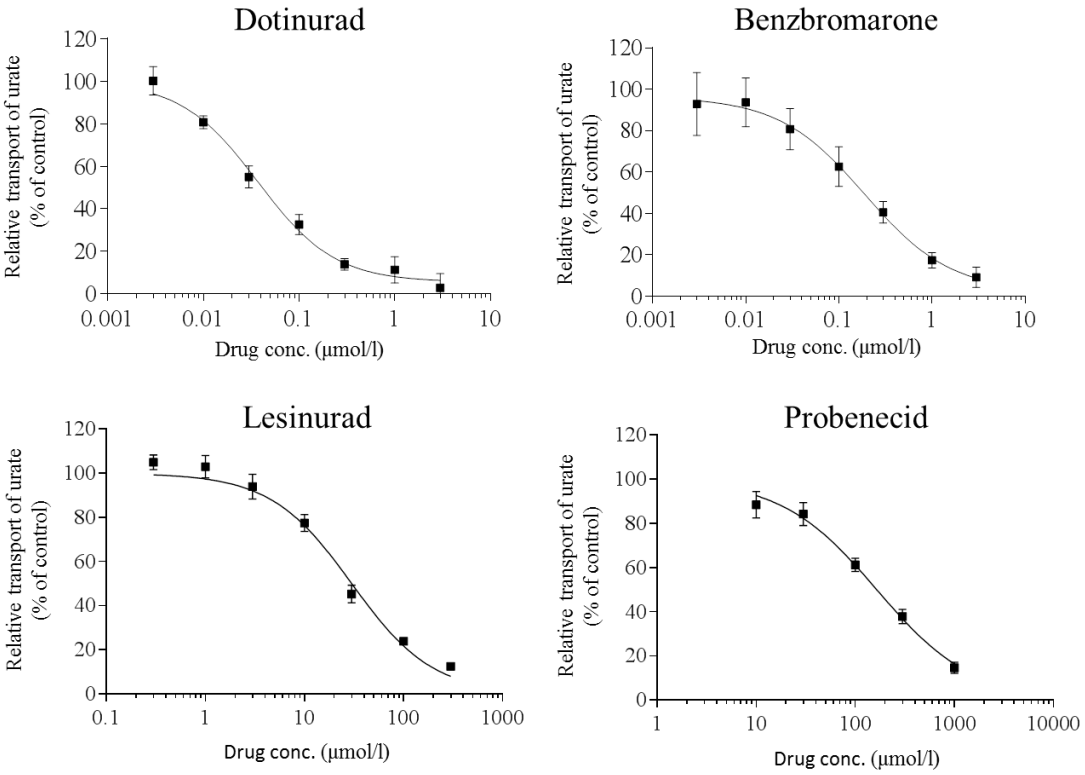

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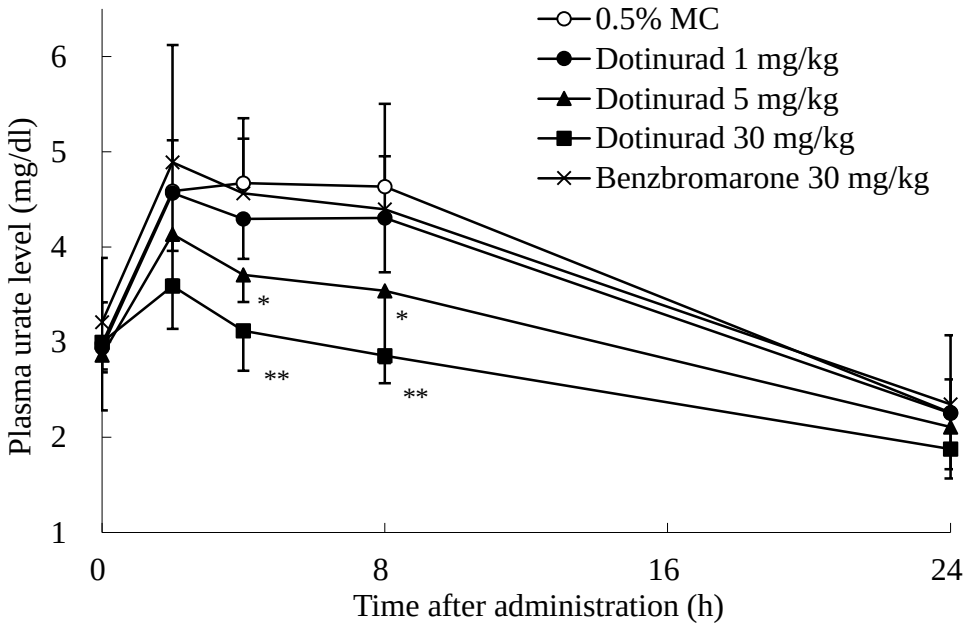
Fig. 3

A



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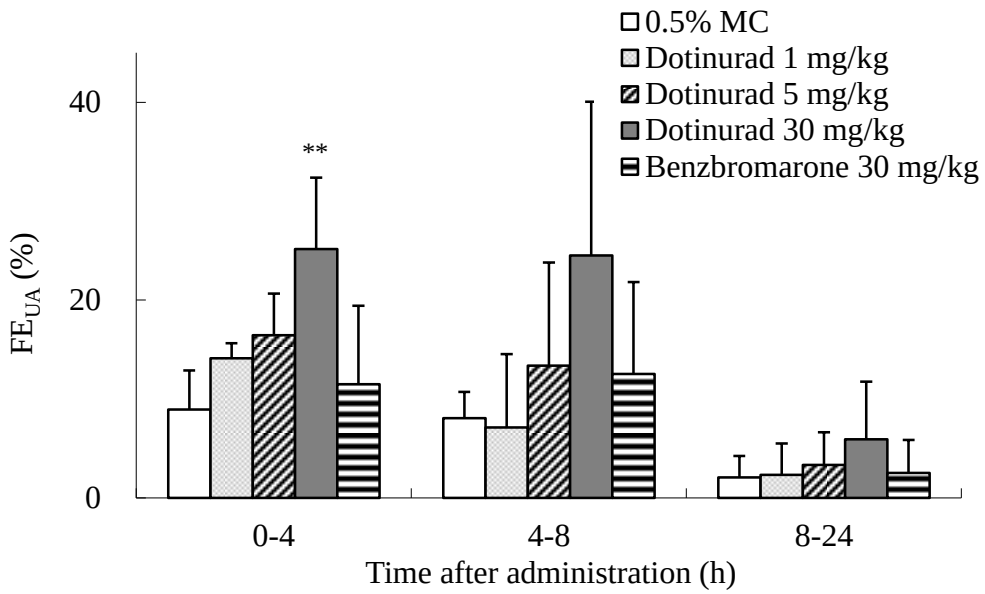

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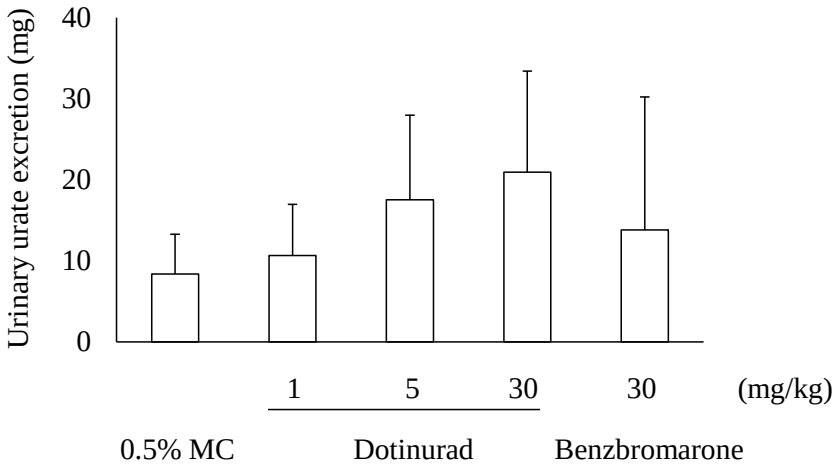

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Fig. 4

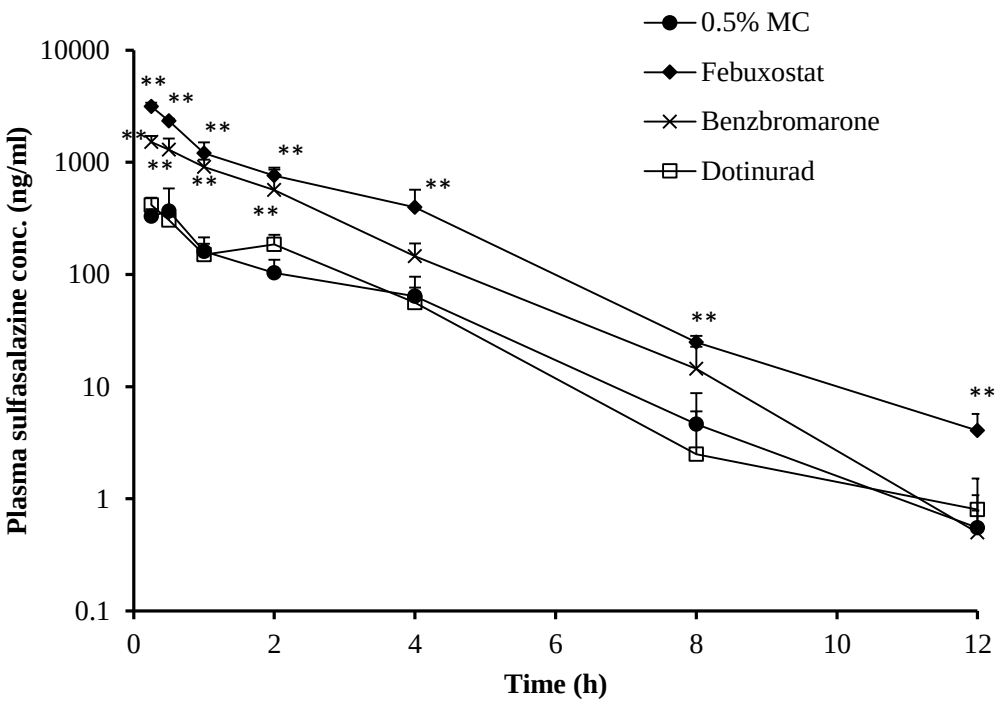

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Fig. 5

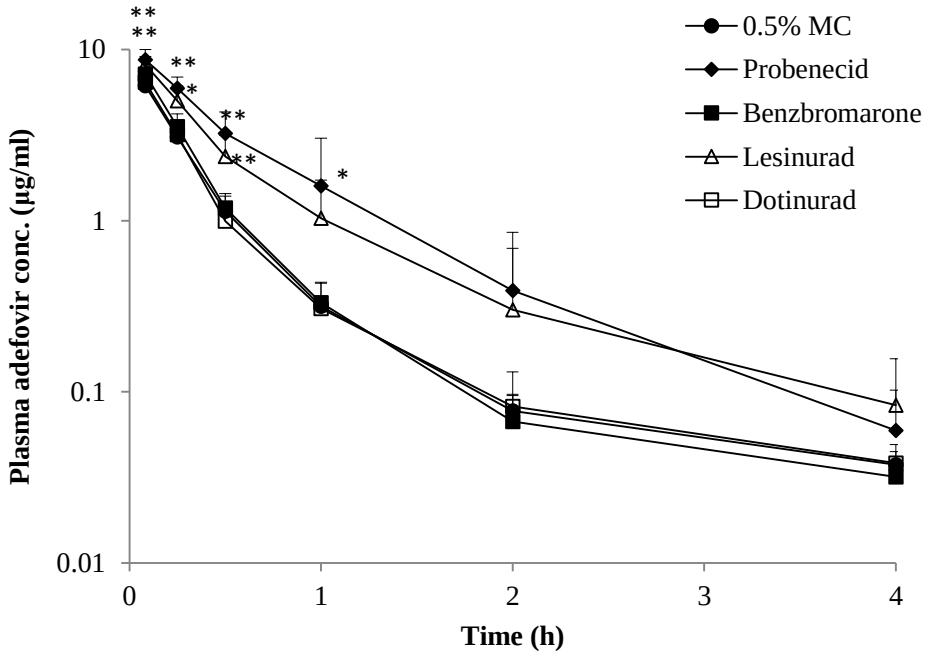

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Fig.6