Pharmacological Evaluation of Dotinurad, a Selective Urate Reabsorption Inhibitor

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ABSTRACT

The effect of dotinurad [(3,5-dichloro-4-hydroxyphenyl)(1,1-dioxo-1,2-dihydro-3H-1,3-benzothiazol-3-yl)methanone] was compared with that 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 urate transporter 1 (URAT1) with IC50 values of 0.0372, 0.190, 30.0, and 165 μM, respectively. Dotinurad weakly inhibited ATP-binding cassette subfamily G member 2 (ABCG2), organic anion transporter 1/3 compared to ATP-binding cassette subfamily G member 2 (ABCG2) and organic anion transporter (OAT) 1/3 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 (FEUA) 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 ABCG2 and OAT1/3, because of its high efficacy in decreasing plasma urate levels compared with that of other uricosuric agents.

SIGNIFICANCE STATEMENT

Our study on the inhibitory effects on urate transport showed that dotinurad had higher selectivity for urate transporter 1 (URAT1) versus ATP-binding cassette subfamily G member 2 (ABCG2) and organic anion transporter (OAT) 1/3 compared to other uricosuric agents. In Cebus monkeys, dotinurad decreased plasma urate levels and increased fractional excretion of urate in a dose-dependent manner. To determine the inhibitory effect of dotinurad on urate secretion transporters, we studied the movement of substrates of ABCG2 and OAT1 in rats. Dotinurad had no effect on these transporters, whereas the other uricosuric agents increased the plasma concentrations of the substrates. These results suggested dotinurad is a potent and selective urate reabsorption inhibitor characterized by increased efficacy with decreasing plasma urate levels.

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 (Terkeltaub, 2010; Neogi, 2011). 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.
that selective urate reabsorption inhibitors (SURIs), which are defined as potent URAT1 inhibitors that do not affect urate secretion transporters including ABCG2 and OAT1/3, exhibit more potent hypouricemic effects than nonselective urate reabsorption inhibitor.

In the present study, we first compared the inhibitory effect of dotinurad [(3,5-dichloro-4-hydroxyphenyl) 1,1-dioxo-1,2-dihydro-3H-1H-1,3-benzothiazol-3-y]methanone], a novel agent that exhibits uricosuric effect in rodents, on urate transport in cells overexpressing URAT1, ABCG2, and OAT1/3 with that 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 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). Probenecid and febuxostat were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Adefovir was purchased from LKT Laboratories, Inc. (St. Paul, MN). Sulfasalazine was purchased from Sigma-Aldrich Co., LLC (St. Louis, MO). [3H]-Urate was synthesized by Moravek, Inc. (Brea, CA). The other reagents 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 an initial 1 minute. Assay mixture with 100 μM xanthine dissolved in 0.1 M NaOH (1% final) and drugs dissolved in dimethylsulfoxide (DMSO; 1% final) in 100 mM pyrophosphate buffer, pH 8.5, containing 0.2 mM EDTA was preincubated for 5 minutes, and reactions were started by adding xanthine oxidase (from bovine milk) to the mixture at a final concentration of 3.2 mU/ml under the aerobic conditions at 25°C. Assay was performed in triplicate.

Study of [14C]-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 × 10⁵ cells/well and cultured at 37 ± 1°C and a 5% CO₂ atmosphere in Dulbecco’s modified Eagle’s medium with 4.5 g/l glucose for 24 hours. Before initiating the experiment, culture medium was removed and cells were washed twice in 100 μl of Hanks’ balanced salt solution (HBSS). Uptake experiments were performed at 37 ± 1°C in 50 μl of HBSS containing 20 μM [14C]-urate and test articles dissolved in DMSO. Vehicle control contained 1% DMSO instead of test articles. URAT1-overexpressing MDCKII cells or OAT1-overexpressing HEK293 cells were washed after 10 minutes of incubation, whereas OAT3-overexpressing HEK293 cells were washed after 5 minutes of incubation in 100 μl of ice-cold HBSS, followed by cell lysis with 50 μl of 0.1 M NaOH. Transporter activity was determined by liquid scintillation counting of an aliquot from each well. Assay was performed in triplicate. The amount of translocated [14C]-urate was calculated using the following equation: relative transport of urate (as % of control) = (A - B)/(C - D) × 100, where A represents the amount of translocated [14C]-urate in the presence of

Fig. 1. Chemical structures of dotinurad and commercially available uricosuric agents. Dotinurad (A), benzbromarone (B), lesinurad (C), and probenecid (D).
test article in transfected cells, B represents the amount of translocated \([^{14}\text{C}]\)-urate in the presence of test article in mock cells, C represents the amount of translocated \([^{14}\text{C}]\)-urate in the presence of 1% DMSO in transfected cells, and D represents the amount of translocated \([^{14}\text{C}]\)-urate in the presence of 1% DMSO in mock cells.

Study of \([^{14}\text{C}]\)-Urate Uptake by Membrane Vesicles Obtained from ABCG2-Overexpressing HER293 Cells. Membrane vesicles were prepared from ABCG2-overexpressing cells (SOLVO Biotechnology, Hungary), and assay was performed in the presence of 70 \(\mu\)M \([^{14}\text{C}]\)-urate containing 20 \(\mu\)M \([^{14}\text{C}]\)-urate and 4 mM ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. In 96-well plates, 50 \(\mu\)l of membrane vesicle suspended in 75 \(\mu\)M urate and 30 \(\mu\)M \([^{14}\text{C}]\)-urate containing ice-cold transport buffer (250 mM sucrose, 100 mM MgCl2, 10 mM Tris-HCl, pH 7.4) were added. Test articles dissolved in DMSO, 0.75 \(\mu\)l were added to the membrane vesicle mixture. Vehicle control contained 1% DMSO instead of test article. The mixtures were preincubated for 15 minutes at 37 ± 1°C. Reactions were initiated by adding 25 \(\mu\)l of prewarmed 12 mM MgATP (or 12 mM AMP as a background control) in transport buffer. After 3 minutes, reactions were quenched by adding 200 \(\mu\)l of ice-cold washing buffer (250 mM sucrose, 100 mM NaCl, 10 mM Tris-HCl, pH 7.4) and immediately filtered using glass fiber filters. The filters were washed five times with 200 \(\mu\)l 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.

Animals and Housing. For the evaluation of hypouricemic effects, 5- to 10-year-old male Cebus monkeys (\textit{Cebus paella}, weight range 2.85–3.70 kg) bred in Shin Nippon Biomedical Laboratories, Ltd. (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-hour light/dark cycle at 26 ± 3°C and a relative humidity of 55% ± 20%. Animals were fed a pelleted diet (New World Primate Diet 5040; Purina Mills, LCC) and fresh apples daily, except 1 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 Shin Nippon Biomedical Laboratories, Ltd.

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-hour light/dark 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 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 Benz bromorone in Cebus Monkeys. Five Cebus monkeys that were fasted for 18 hours before drug administration orally received 1, 5, and 30 mg/kg dotinurad; 30 mg/kg benz bromorone; and 0.5% methy cellulose (MC) as control, respectively. Blood samples (about 1 ml) obtained from the saphenous vein at; before; and 2, 4, 8, and 24 hours after drug administration using a heparinized needle were kept on ice. Plasma was obtained from the blood samples by centrifugation at 3000 rpm for 10 minutes at 4°C. Urine samples were collected 0–4, 4–8, and 8–24 hours after drug administration. Urate and creatinine levels in the samples were measured by a U-3000 spectrophotometer using an Iatro LQ UAFII (Mitsubishi Chemical Medience, Corp., Tokyo, Japan) and L-type Wako Creatinine F (Wako Pure Chemical Industries, Ltd.). Each treatment was administered in 13-day intervals to wash out drugs, and treatment and sample collection were performed as crossover experiments. Fractional excretion of urate (\(\text{FEUA}_{\text{C2}}\)) was calculated as the ratio of urate clearance to creatinine clearance. Urinary urate excretion was calculated as urinary urate concentration × urine 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 deproteinized with thrice volume of methanol and centrifuged at 3000 rpm for 10 minutes at 4°C. Drug concentrations were measured using high-performance liquid chromatography (HPLC) using the Alliance 2695 HPLC system (Waters Corporation, Milford, MA). Area under the plasma concentration-time curve 0–24h (\(\text{AUC}_{0-24\text{h}}\)) was calculated using the trapezoidal rule. The concentration of 6-hydroxybenzbromorone, a major benzbromorone metabolite, was also measured in the plasma obtained from benzbromorone-treated animals.

Study of Concomitant Sulfasalazine and Hypouricemic Agent Treatment in Sprague-Dawley Rats. Sprague-Dawley rats that were fasted for 18 hours before drug administration orally received 20 mg/kg febuxostat, 50 mg/kg benzbromorone, 1.3 mg/kg 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 mg/kg sulfasalazine dissolved in 50 mM Tris-saline was intravenously administered via the tail vein. Blood samples (about 200 \(\mu\)l) were obtained from the jugular vein at 0.083 (intravenous only), 0.25, 0.5, 1, 2, 4, 8, and 12 hours after sulfasalazine administration using a heparinized needle and kept on ice. Plasma was obtained from the blood samples by centrifugation at 3000 rpm for 10 minutes at 4°C. Plasma samples were deproteinated, and sulfasalazine concentration was measured after cooling and centrifugation. Plasma sulfasalazine concentration was measured using LC-MS/MS on an Agilent 1100 Series HPLC Value System (Agilent Technologies, Inc., Santa Clara, CA) and tandem mass spectrometry (LC-MS/MS) on an Agilent 1100 Series HPLC system (Waters Corporation, Milford, MA). Area under the plasma concentration-time curve at 0–24h (\(\text{AUC}_{0-24\text{h}}\)) was calculated using the trapezoidal rule. Concentration-time profiles of sulfasalazine were calculated using the trapezoidal rule. The concentration of 6-hydroxybenzbromorone, a major benzbromorone metabolite, was also measured in the plasma obtained from benzbromorone-treated animals.

Study of Concomitant Adefovir and Uricosuric Agent Treatment in Sprague-Dawley Rats. Sprague-Dawley rats that were fasted for 18 hours before drug administration orally received 100 mg/kg probenecid, 67 mg/kg lesinurad, 50 mg/kg benzbromorone, 1.3 mg/kg dotinurad, and 0.5% MC as control, respectively (\(n = 6\), a total of 30 rats were used). Thirty minutes after drug administration, 3 mg/kg adefovir dissolved in saline was intravenously administered via the tail vein to all animals. Blood samples (about 200 \(\mu\)l) were obtained from the jugular vein at 0.083, 0.25, 0.5, 1, 2, 4, and 4 hours after adefovir administration using a heparinized needle and kept on ice. Plasma was obtained from blood samples by centrifugation at 3000 rpm for 10 minutes at 4°C. Plasma samples were deproteinated, and adefovir concentration was measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS) on an Agilent 1100 Series HPLC system (Waters Corporation, Milford, MA). Area under the plasma concentration-time curve 0–24h (\(\text{AUC}_{0-24\text{h}}\)) was calculated using the trapezoidal rule. The concentration of adefovir was calculated as the area under the adefovir concentration × urine volume.

Statistical Analysis. In the study of xanthine oxidase activity, \(\text{IC}_{50}\) 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, La Jolla, CA).
Effects of Uricosuric Agents on Xanthine Oxidase Activity. Benzobromarone inhibited xanthine oxidase activity with an IC_{50} value of 15.4 μM. Dotinurad, lesinurad, and probenecid did not have any effect up to 100, 300, and 1000 μM, respectively.

Inhibitory Effect of Uricosuric Agents on Urate Uptake by URAT1-Overexpressing MDCKII Cells. Dotinurad, benzobromarone, lesinurad, and probenecid inhibited urate transport by URAT1-overexpressing MDCKII cells in a concentration-dependent manner at concentrations of 0.003–3, 0.003–3, 0.3–300, and 10–1000 μM (Fig. 2) and IC_{50} values of 0.0372, 0.190, 30.0, and 165 μM, respectively (Table 1).

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 concentrations of 0.3–300, 0.1–100, and 0.03–30 μM and IC_{50} values of 4.16, 4.08, and 1.32 μM, respectively. The ratios of IC_{50} values of ABCG2-, OAT1-, and OAT3-overexpressing cells to that of URAT1-overexpressing cells were 112, 110, and 35.5, respectively (Tables 1 and 2). Benzobromarone inhibited urate transport in ABCG2-, OAT1-, and OAT3-overexpressing cells with IC_{50} values of 0.289, 3.14, and 0.967 μM, respectively, and IC_{50} value ratios to that of URAT1-overexpressing cells were 1.52, 16.5, and 5.09, respectively. Lesinurad inhibited urate transport with IC_{50} values of 26.4, 6.99, and 1.07 μM, respectively, and IC_{50} value ratios to that of URAT1-overexpressing cells were 0.880, 0.233, and 0.0357, respectively. Probenecid inhibited urate transport.

**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 ± S.D. of urate transport relative to the control (n = 3) and described by GraphPad Prism 7.03 software (GraphPad Software).
transport with IC\textsubscript{50} values of 433, 10.9, and 2.37 \mu M, respectively, and IC\textsubscript{50} value ratios to that of URAT1-overexpressing cells were 2.62, 0.0661, and 0.0144, respectively.

**Hypouricemic Effects of Dotinurad and Benzbro- 
marone in Cebus Monkeys.** Effects of dotinurad and benzbro- 
mareone on plasma urate levels, FE\textsubscript{UA}, and urinary urate excretion were assessed in Cebus monkeys. Dotinurad dose-dependently lowered the plasma urate levels, with its maximum effect at 8 hours (Fig. 3A). Changes in plasma urate level between 0 and 8 hours (\Delta P\textsubscript{UA}) were lower than that of control by 0.28, 0.97 (\textit{P} < 0.05), and 1.79 mg/dl (\textit{P} < 0.01) at doses of 1, 5, and 30 mg/kg, respectively. Furthermore, dotinurad dose-dependently increased FE\textsubscript{UA} (Fig. 3B). The 0- to 4-hour FE\textsubscript{UA} increased by 180\% at a dose of 30 mg/kg compared with the control (\textit{P} < 0.01). On the contrary, the effect of benzbro- 
mareone at a dose of 30 mg/kg was modest; the \Delta P\textsubscript{UA} was lower than that of control by 0.46 mg/dl, and the 0- to 4-hour FE\textsubscript{UA} increased by 30\% compared with the control. Figure 3C details the urinary excretion amount of urate in Cebus monkeys 0–8 hours after dotinurad and benzbro- 
mareone 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, benzbro- 
mareone increased the urinary urate excretion to 19.4 mg at a dose of 30 mg/kg. Results of creatinine clearance and urate clearance are shown in Supplemental Table 2.

**Pharmacokinetic Parameters of Dotinurad and 
Benzbro- 
mareone in Cebus Monkeys.** The plasma concentration of dotinurad increased in a dose-dependent manner with C\textsubscript{max} and AUC\textsubscript{0–24h} values of 107 \mu g/ml and 780 \mu g hours/ml, respectively, at a dose of 30 mg/kg (Table 3). On the contrary, C\textsubscript{max} and AUC\textsubscript{0–24h} values of benzbro- 
mareone were 20.9 \mu g/ml and 95.2 \mu g hours/ml, respectively, at a dose of 30 mg/kg. In the plasma obtained from benzbro- 
mareone-treated animals, C\textsubscript{max} and AUC\textsubscript{0–24h} values of 6-hydroxybenzbro- 
mareone were 12.7 \mu g/ml and 80.9 \mu g hours/ml, respectively. Unbound fraction of maximum plasma concentration of dotinurad at doses of 1, 5, and 30 mg/kg and benzbro- 
mareone at a dose of 30 mg/kg were 0.0144, 0.0800, 0.750, and 0.564 \mu g/ml, respectively.

**Effect of Hypouricemic Agents on the Plasma Con- 
centration of Sulfasalazine in Sprague-Dawley Rats.** Effects of febuxostat, benzbro- 
mareone, and dotinurad on the plasma sulfasalazine concentration were assessed in Sprague- 
dawley rats. Febuxostat and benzbro- 
mareone significantly increased the plasma sulfasalazine concentration (Fig. 4). Febuxostat increased the AUC\textsubscript{0–inf} value to 5012 ng hours/ml (\textit{P} < 0.01) from a control value of 713 ng hours/ml and F to 14.1\% from a control value of 2.0\%. Benzbro- 
mareone also increased the AUC\textsubscript{0–inf} value to 2888 ng hours/ml (\textit{P} < 0.01) and F to 8.1\%. Dotinurad did not have any effect on the pharmacokinetic parameters of sulfasalazine (Table 4).

**Effect of Uricosuric Agents on the Plasma Con- 
centration of Adefovir in Sprague-Dawley Rats.** Effects of probenecid, benzbro- 
mareone, lesinurad, and dotinurad on the plasma concentration of adefovir were assessed in

<table>
<thead>
<tr>
<th>Test Article</th>
<th>IC\textsubscript{50} Ratio to URAT1</th>
<th>ABCG2</th>
<th>OAT1</th>
<th>OAT3</th>
</tr>
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<tbody>
<tr>
<td>Dotinurad</td>
<td>1</td>
<td>112</td>
<td>110</td>
<td>35.5</td>
</tr>
</tbody>
</table>
| Benzbro- 
mareone | 1                                | 1.52  | 16.5 | 5.09 |
| Lesinurad    | 1                                | 0.880 | 0.233| 0.0357|
| Probenecid   | 1                                | 2.62  | 0.0661| 0.0144|

**TABLE 2**

IC\textsubscript{50} ratios for ABCG2 and OAT1/3 to URAT1 of dotinurad and 
commercially available uricosuric agents

Each value was calculated as the IC\textsubscript{50} of each compound for ABCG2, OAT1, and 
OAT3/IC\textsubscript{50} for URAT1.
Sprague-Dawley rats. Probencid and lesinurad significantly increased the plasma adefovir concentration (Fig. 5). Probencid increased the plasma concentration of adefovir at 0.083–0.5 hour (P < 0.01). Lesinurad also increased the plasma concentration of adefovir at 0.083–0.5 (P < 0.01) and 1 hour (P < 0.05). 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. In particular, URAT1 in the brush-border membrane and GLUT9 in the basolateral membrane of renal tubules are the main urate reabsorption transporters. Aberrent 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 (Sato et al., 2010; Nigam and Bhatnagar, 2018). SNPs in ABCG2 result in a higher risk of gout caused by dysfunctional urate excretion (extrarenal 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 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–chronic kidney disease cohort (Bhatnagar et al., 2016). In humans, the contribution of other urate secretion transporters to serum urate level has not been reported. Our findings suggested that SURIs had superior hypouricemic effects to those of nonselective urate reabsorption inhibitors.

Our previous study showed that in rats treated with topotoxostat, a urate production inhibitor, dotinurad decreases plasma urate levels and increases FEUA in a dose-dependent manner without inhibiting xanthine oxidase or uricase (Matsumoto et al., 2011; Taniguchi et al., 2016). 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 IC50 value of 0.0372 μM. The effect of dotinurad was approximately 5.11, 806, and 4440 times more potent than that of benzbromarone, lesinurad, and probencid, respectively, because the IC50 values of these compounds were 0.190, 30.0, and 165 μM, respectively (Table 1). Dotinurad modestly inhibited urate

### Table 3: Pharmacokinetic parameters of dotinurad and benzbromarone in Cebus monkeys

<table>
<thead>
<tr>
<th>Administered Article</th>
<th>Dose</th>
<th>Measured Article</th>
<th>Pharmacokinetic Parameters</th>
<th>Cmax</th>
<th>Fu x Cmax</th>
<th>Tmax</th>
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<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td></td>
<td></td>
<td>μg/ml</td>
<td>μg/ml</td>
<td>hours</td>
<td>μg·h/ml</td>
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</table>

- **Cmax**: maximum plasma concentration.
- **Fu x Cmax**: unbound maximum plasma concentration.
- **Tmax**: time to reach maximum plasma concentration.

**S.D. of five animals.**

**P < 0.01, significant differences from the 0.5% MC control group after Dunnett’s multiple comparison test.**
TABLE 4
Pharmacokinetic parameters of sulfasalazine and effects of concomitant treatment with hypouricemic agents in Sprague-Dawley rats

Pharmacokinetic parameters were calculated using Phoenix WinNonlin. F values were calculated using the following equation: $F = \frac{(AUC_{inf})_{control}}{(AUC_{inf})_{test}} \times 100$. Values are reported as the mean ± S.D. of three to four animals.

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Dose</th>
<th>$C_{max}$</th>
<th>$T_{1/2}$</th>
<th>$AUC_{0-\infty}$</th>
<th>$AUC_{inf}$</th>
<th>CLT</th>
<th>F</th>
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<tr>
<td></td>
<td>mg/kg</td>
<td>ng/ml</td>
<td>hours</td>
<td>ng h/ml</td>
<td>ng h/ml</td>
<td>ml/hours/kg</td>
<td>%</td>
</tr>
<tr>
<td>0.5% MC</td>
<td>--</td>
<td>$422 \pm 182$</td>
<td>$1.49 \pm 0.28$</td>
<td>$665 \pm 98$</td>
<td>$713 \pm 90$</td>
<td>$28,395 \pm 3800$</td>
<td>2.0</td>
</tr>
<tr>
<td>Febuxostat</td>
<td>20</td>
<td>$3138^{++} \pm 272$</td>
<td>$1.30 \pm 0.13$</td>
<td>$5094^{++} \pm 851$</td>
<td>$5012^{++} \pm 848$</td>
<td>$4067^{++} \pm 607$</td>
<td>14.1</td>
</tr>
<tr>
<td>Benzbromarone</td>
<td>50</td>
<td>$1520^{++} \pm 202$</td>
<td>$1.24 \pm 0.46$</td>
<td>$2858^{++} \pm 750$</td>
<td>$2888^{++} \pm 729$</td>
<td>$729^{++} \pm 1909$</td>
<td>8.1</td>
</tr>
<tr>
<td>Dotinurad</td>
<td>1.3</td>
<td>$415 \pm 68$</td>
<td>$1.88 \pm 1.56$</td>
<td>$708 \pm 166$</td>
<td>$885 \pm 264$</td>
<td>$24,126 \pm 7777$</td>
<td>2.5</td>
</tr>
</tbody>
</table>

$AUC_{inf}$, area under the plasma concentration-time curve from zero to infinity; $AUC_{0-\infty}$, $AUC$ at 0–12 hours; CLT, total clearance; F, bioavailability; MC, methylcellulose; $T_{1/2}$, elimination half-life.

$^{++}P < 0.01$, significant differences from the 0.5% MC control group, as analyzed by Dunnett’s multiple comparison test.

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transport by ABCG2 and OAT1/3 with IC$_{50}$ values of 4.16, 4.08, and 1.32 μM, which were 112, 110, and 35.5 times higher than its IC$_{50}$ values for URAT1, respectively (Tables 1 and 2). By contrast, the IC$_{50}$ ratios of benzbromarone, lesinurad, and probenecid for ABCG2 and OAT1/3 to URAT1 were 1.52–16.5, 0.0357–0.88, and 1.24, respectively. These findings showed that dotinurad inhibited URAT1 more selectively than the other uricotic agents. Several lines of evidence showed the inhibitory effect of benzomaron, lesinurad, and probenecid on urate transport by OAT3 (Ichida et al., 2003; Miner et al., 2016; Miyata et al., 2016), although the inhibitory effects of benzomaron 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 of benzbromarone, lesinurad, and probenecid on urate transport by URAT1, ABCG2, OAT1, and OAT3 (Ichida et al., 2003; Miner et al., 2016; Miyata et al., 2016), although the inhibitory effects of benzomaron 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 of benzbromarone, lesinurad, and probenecid on urate transport by URAT1, ABCG2, OAT1, and OAT3 are 3.53 and 13.23 μM, 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). (US Food and Drug Administration, 2017; Pharmaceuticals, 2019).

Subsequently, we compared the hypouricemic efficacy of dotinurad with that of benzomaron in Cebus monkeys. Dotinurad at doses of 1, 5, and 30 mg/kg decreased plasma urate levels and increased FEUA in a dose-dependent manner, and its effect at 1 mg/kg was approximately equal to that of 30 mg/kg benzomaron (Fig. 3, A and B). However, the stringency of URAT1 inhibition (the IC$_{50}$ values of dotinurad and benzbromarone were 0.0372 and 0.190 μM, 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 $AUC_{0-\infty}$ values than those of benzomaron (Table 3). The unbound fractions of maximal plasma concentration of dotinurad at doses of 1, 5, and 30 mg/kg and that of benzomaron 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, 0.080, 0.75, and 0.564 μM/ml, respectively (Table 3). 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 dotinurad and 30 mg/kg benzomaron (Supplemental Table 3; Table 3). Considering that 6-hydroxybenzomaron, a major metabolite of benzomaron, also inhibited URAT1 and contributed to the hypouricemic effect in addition to benzomaron 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 nonselective inhibitor.

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, benzomaron and the positive control agent febuxostat markedly increased the plasma concentration and F of sulfasalazine (Fig. 4; Table 4), and these increases were assumed to be caused by inhibition of intestinal ABCG2, which appeared to be important for...
into account. The AUC0

The AUC0

uricemia because of its more effective hypouricemic action than that shown by benz bromarone (Ahn et al., 2016). In this report, authors discussed that URAT1 selectivity, defined as potent URAT1 inhibition without an effect on OAT1/3, is important for producing a 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 benz溴arone (Jun et al., 2017).

Figure 6 shows a scheme of urate pool reduction by SURIs or nonselective inhibitors. SURIs effectively reduce the urate pool by increasing renal excretion of urate. On the contrary, nonselective inhibitors modestly reduce the urate pool because of their inhibitory effect on urate secretion transporters. For nonselective inhibitors to reduce the 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 poses a risk factor for urolithiasis (Yu and Gutman, 1967). In the present study, benz bromarone 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 dotinurad (Fig. 3C). The phenomenon appeared to be mediated by inhibition of urate secretion transporters, especially ABCG2 in the intestine. These findings suggested that nonselective inhibitors can only reduce the 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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**Authorship Contributions**

*Participated in research design:* Taniguchi, Ashizawa, Matsumoto, Motoki, Iwanaga

*Conducted experiments:* Taniguchi, Ashizawa, Matsumoto, Motoki

*Wrote or contributed to the writing of the manuscript:* Taniguchi, Ashizawa, Matsumoto, Saito, Motoki, Hashiba, Iwanaga.

**References**


