Diuresis by intravenous administration of xanthurenic acid in rats, and inhibition by probenecid

Yuichi Uwai, Yuta Nakashima, Emi Honjo, Tatsuya Kawasaki, and Tomohiro NabeKura
Department of Pharmaceutics, School of Pharmacy, Aichi Gakuin University, 1-100, Kusumoto, Chikusa, Nagoya 464-8650, Japan
(Received 19 March 2014; and accepted 7 April 2014)

ABSTRACT
The conjugates with sulfate and glucoside of xanthurenic acid, a tryptophan metabolite, were reported to show natriuresis. Sulfotransferase for xanthurenic acid works in the renal proximal tubule to produce the sulfate of xanthurenic acid as well as the liver, and we recently found that xanthurenic acid is a substrate of renal organic anion transporter OAT1. The purpose of this study was to examine relationship between the transport by OAT1 and diuresis related with xanthurenic acid. Drug transport experiment using *Xenopus laevis* oocytes represented that probenecid inhibited xanthurenic acid uptake by rat OAT1 (rOAT1). Although no diuresis was recognized by the intravenous injection of xanthurenic acid as a bolus in rats, the addition of its infusion exhibited natriuresis. Simultaneous administration of probenecid significantly decreased the urine volume and excreted amounts of sodium into urine. These findings showed the diuresis by the xanthurenic acid administration, and it was probenecid-sensitive. The rOAT1-mediated transport of xanthurenic acid might, at least in part, contribute to its diuretic effect.

In the renal proximal tubules, organic anion transporters secrete endogenous metabolites, drugs, and xenobiotics into urine. From the mid-1990s, cDNA of renal organic anion transporters has been isolated, and its function and expression have been characterized. Organic anion transporters OAT1 and OAT3 are thought to be responsible for the renal tubular uptake of organic anions from blood, and multidrug resistance-associated protein MRP4 is the strong candidate mediating their efflux into lumen (2).

Our laboratory has been interested in transport of bioactive substances by OAT1 and OAT3. Recently, we examined interaction of the metabolites of the kynurenine pathway, a main route for the tryptophan metabolism, with OAT1 and OAT3, and represented that kynurenic acid and xanthurenic acid were transported by them (5, 6). Kynurenic acid antagonizes N-methyl d-aspartate (NMDA) receptor in the brain under physiological conditions, and is accepted as the key molecule in several psychiatric disorders (3). Xanthurenic acid is closely structurally related to kynurenic acid (Fig. 1), and its action has remained to be elucidated. However, Cain et al. isolated two metabolites of xanthurenic acid, xanthurenic acid 8-O-sulfate and xanthurenic acid 8-O-β-D-glucoside, from human urine, and showed that their intraarterial infusion stimulated natriuresis in rats (1). In the report, these metabolites were considered to inhibit epithelial sodium channel ENaC, which is expressed in the luminal side of the renal collecting duct, and it was described that the administration of xanthurenic acid did not produce natriuresis (1).

Wikoff et al. reported that the urinary level of xanthurenic acid was lower in OAT1 knockout mice than that in wild-type mice (7). Taken together with the fact that xanthurenic acid is a substrate of OAT1, it is thought that OAT1 contributes to its renal tubular uptake. The purpose of the present study was to examine the relationship between the diuresis by xanthurenic acid and its transport by OAT1. We ad-
ministered xanthurenic acid intravenously as a bolus injection and an infusion to rats, because a detailed method is not described in the report by Cain et al. (1). As a result, we observed the diuresis by the xanthurenic acid infusion. And, effect of simultaneous injection of probenecid, a representative inhibitor for OAT1, was tested.

[3H]Xanthurenic acid (11.2 Ci/mmol) and unlabelled xanthurenic acid were obtained from Moravek Biochemicals (Brea, CA) and MP Biomedicals (Santa Ana, CA, USA), respectively. Kynurenic acid and probenecid were from Enzo Life Sciences (Farmington, NY, USA) and Wako Pure Chemical Industries (Osaka, Japan), respectively. All other chemicals used were of the highest purity available.

To evaluate effect of probenecid on xanthurenic acid transport by rat OAT1 (rOAT1), an uptake experiment using Xenopus laevis oocytes was performed as previously reported (5, 6). Briefly, capped RNA encoding rOAT1 was transcribed from Not I-linearized pBK-CMV containing cDNA of rOAT1 with T3 RNA polymerase. After 50 nL of water or cRNA (25 ng) was injected into defolliculated oocytes, the oocytes were maintained in modified Barth’s medium (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO3)2, 0.4 mM CaCl2, 0.8 mM MgSO4, 2.4 mM NaHCO3, and 5 mM HEPES, pH 7.4) containing 50 mg/L gentamicin at 18°C. Two days after injection, the uptake reaction was initiated by incubating the oocytes in 500 μL uptake buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.4) with [3H]xanthurenic acid at room temperature for 1 h. The uptake reaction was terminated by the addition of 2 mL of ice-cold uptake buffer to each well, and the oocytes were washed 3 times with 2 mL of ice-cold buffer. After washing, each oocyte was transferred to a scintillation counting vial and solubilized in 150 μL of 10% sodium laurel sulfate. Two mL of scintillation cocktail Clear-sol II (Nacalai Tesque, Kyoto, Japan) were added to each solubilized oocyte, and radioactivity was measured with a liquid scintillation counter.

Seven-week old male Wistar/ST rats were treated in accordance with regulations of the Institutional Animal Use and Care Committee of School of Pharmacy, Aichi Gakuin University, and were from Chubu Kagaku Shizai (Nagoya, Japan). Under the anesthesia with ethyl carbamate and α-chloralose, catheters were inserted into the femoral vein with polyethylene tubes (SP-31; Natsume Seisakusho, Tokyo, Japan) filled with heparin solution (50 IU/mL) for drug administration. Urine was collected from urinary bladder catheterized with SP-31 polyethylene tubes. Xanthurenic acid, kynurenic acid and probenecid were dissolved at 5 mM in saline. They were injected to the vein instantaneously at 0.4 mL/100 g body weight and infused continuously at 2.2 mL/h. The urine volume was calculated with urine density at 1 g/mL. The sodium concentration in urine was determined using compact ion meter (Horiba, Kyoto, Japan), and the amounts of sodium excreted into urine were calculated.

Obtained data were represented as mean ± SD. Data were analyzed by one-way analysis of variance followed by Dunnett’s test using KaleidaGraph (Synergy Software, Reading, PA, USA). Differences were considered significant at P < 0.05.

Fig. 2 represents effect of probenecid on the transport of xanthurenic acid by rOAT1. As previously reported (5), this study also illustrated the stimulation of the xanthurenic acid uptake by the injection of rOAT1 cRNA into the oocytes. And, probenecid reduced the transport dose-dependently. Accordingly, it was shown that probenecid has the inhibitory effect on the uptake of xanthurenic acid by rOAT1.

Fig. 3 shows the diuretic effect of the xanthurenic acid administration in rats. The intravenous injection of xanthurenic acid as a bolus did not facilitate the diuresis (Fig. 3A). However, the addition of xanth-
Diuresis by xanthurenic acid

Fig. 2  Effect of probenecid on xanthurenic acid uptake by rOAT1. Water-injected oocytes were incubated with 89 nM [3H]xanthurenic acid for 1 h. rOAT1 cRNA-injected oocytes were incubated with 89 nM [3H]xanthurenic acid in the absence (control) or presence of probenecid at the indicated concentrations for 1 h. The uptake amounts of [3H]xanthurenic acid in each oocyte were determined. Each column represents the mean ± SD of 20 oocytes from two experiments. *** P < 0.001, significantly different from control.

Fig. 3  Urine volume in rats intravenously administered with xanthurenic acid as a bolus (A) and a following infusion (B). (A) After rats were injected with saline (open circle) or 5 mM xanthurenic acid (closed circle) at 0.4 mL/100 g body weight as a bolus, urine was collected and urine volume was determined. Each point represents the mean ± SD of 3 rats. (B) After saline (open column), 5 mM xanthurenic acid (closed column) or 5 mM kynurenic acid (shaded column) were injected to rats at 0.4 mL/100 g body weight as a bolus, the solutions were infused at 2.2 mL/h for 120 min. During the infusion, urine was collected and urine volume was determined. Each column represents the mean ± SD of 4 to 9 rats. ** P < 0.01, significantly different from saline-administered rats.

turenic acid infusion enhanced the urine volume (Fig. 3B). Although no significant effect of the xanthurenic acid infusion was observed first 30 min, the urine volumes were significantly greater in rats receiving xanthurenic acid than those in rats receiving saline during other periods. On kynurenic acid, diuretic effect was not recognized. Cain et al. failed to obtain the diuresis by the xanthurenic acid administration in rats (1), and this is the first report showing the diuresis in rats administered with xanthurenic acid. They did not describe the dosage in the report (1), and their dosage might be lower than our dosage. In addition, their infusion was via the artery, but our administration was from the vein. This difference may influence the results. There are no reports describing the diuresis by the xanthurenic acid administration in other species.

Fig. 4A shows effect of probenecid on the diuresis by the xanthurenic acid administration. Probenecid significantly reduced the urine volume stimulated by xanthurenic acid. Fig. 4B represents the excreted amounts of sodium into urine. Xanthurenic acid accelerated the urinary excretion of sodium, and this was also decreased by probenecid. The diuresis induced by the xanthurenic acid infusion should be natriuresis. Because 8-O-sulfate and 8-O-β-D-glucoside of xanthurenic acid induced natriuresis (1), it was considered that the metabolites were involved in the diuresis observed in this study. Unfortunately, these conjugates are not commercially available at present, and we could not investigate their effect. In Fig. 3B, the effect of xanthurenic acid on the urine volume was not significantly recognized first 30 min. It may take time so that the metabolites form and they get to the action sites.
tubular uptake may contribute to the diuresis observed in this study. The liver and gastrointestinal tract as well as kidney were shown to conjugate xanthurenic acid with sulfate, and another metabolite 8-\(\text{O}\)-\(\beta\)-D-glucoside of xanthurenic acid works as a natriuretic hormone (1, 4). At present, we cannot exclude possibility that probenecid inhibited these metabolisms of xanthurenic acid.

In conclusion, this study shows that the xanthurenic acid administration stimulated the natriuresis in rats, and that probenecid repressed them. This information is useful for elucidating the physiological roles of xanthurenic acid.

Acknowledgements

We thank Ken-ichi Inui, Professor Emeritus of Kyoto University (Kyoto, Japan), for kindly providing pBK-CMV plasmid vectors containing cDNA of rOAT1. This work was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant no. 24790176) and the research grant from Institute of Pharmaceutical Life Sciences, Aichi Gakuin University.

REFERENCES