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Effects of Angiotensin II Receptor Blockers on Renal Handling of Uric Acid in Rats

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Summary: Elevated serum uric acid level has been associated with increased cardiovascular risk in hypertensive patients. Several angiotensin II receptor blockers exhibit differential effects on regulation of serum uric acid level in humans. We have demonstrated that some angiotensin II receptor blockers trans-stimulate the uptake of uric acid by human URAT1 and others inhibit the transport of uric acid mediated by human URAT1, OAT1, OAT3 and MRP4 in vitro. This study investigated the effects of candesartan, pratosartan and telmisartan on renal handling of uric acid in rats in vivo and in vitro. Candesartan (0.1 mg/kg) significantly decreased the urinary excretion of uric acid and increased the plasma uric acid concentration. The kidney candesartan level after low-dose treatment is close to that required to trans-stimulate uric acid uptake in vitro. Pratosartan exhibited dose-dependent hypouricemic and uricosuric effects, while telmisartan showed no effects on plasma uric acid level. Furthermore, we confirmed the effects of the tested drugs on uric acid transport by rat renal brush border membrane transporter(s) and basolateral Oat1 and Oat3. Effects of angiotensin II receptor blockers in rats may be mainly determined by their intrinsic effects (cis-inhibition and trans-stimulation) on uric acid reabsorption transporter(s) and their pharmacokinetic properties in rats.

Keywords: Angiotensin II Receptor Blockers; Uric Acid; Rat; Transporter

Introduction

Uric acid (UA) is the final product of purine metabolism in humans due to the absence of the function of uricase, which is present in many mammalians, to further oxidize urate to allantoin. In humans, a reduced UA concentration has been implicated into the etiology of multiple sclerosis, Parkinson’s disease and Alzheimer’s disease, whereas increased serum UA level has been suggested to be associated with cardiovascular risk and renal injury, especially in hypertensive patients.1–4) An elevation of 1 mg/dl in serum UA level was suggested to be linked with an elevation of 10 mmHg in systolic blood pressure.5) In addition, risk for development of hypertension increased with increasing serum UA level,6) suggesting that elevated UA level is a pathogenic factor, rather than a secondary result of hypertension. Indeed, accumulating evidence has confirmed the etiological contributions of UA to development of hypertension, including stimulation of vascular smooth muscle cell proliferation, angiotensin II production and oxidative stress.7,8) Given the significant association of serum UA and hypertension, it has been proposed to manipulate serum UA during treatment of hypertension and other associated cardiovascular diseases. Lowering the serum UA appeared to improve cardiac and endothelial functions and reduce myocardial infarct size in hyperuricemic patients with ischemic cardiomyopathy,9,10) or to significantly decrease blood pressure and prevent progression of renal injury in patients with chronic kidney disease and hyperuricemia.11)

It has been reported that losartan, an angiotensin II
receptor blocker (ARB), can lower serum UA level, while the ARB candesartan slightly increases it in hypertensive patients.12) Recently, Ogihara et al. reported hypouricemic effects of pratosartan, a new ARB, in a Japanese population.13) In humans, renal UA excretion accounts for approximately 70% of the daily production of UA, implying its significance in maintaining UA homeostasis. The so-called four-component hypothesis, including glomerular filtration, presecretory reabsorption, secretion, and postsecretory reabsorption, has been proposed to explain the renal handling of UA.14) Enomoto et al. (2002) cloned postsecretory reabsorption, has been proposed to explain the renal handling of UA.14) Enomoto et al. (2002) cloned the URAT1 and proved that it plays a pivotal role in renal reabsorption of UA.15) We have recently used in vitro transporter-expressing cells to show that some ARBs trans-stimulate the uptake of UA by human URAT1, while others inhibit the transport of UA mediated by human URAT1, OAT1, OAT3 and MRP4.16,17) Although Hatch et al. (1996) reported hypouricemic and uricosuric effects of losartan in rats,18) the effects of other ARBs in animal models remain unknown.

It has long been postulated that an anion exchanger is responsible for UA reabsorption on the brush-border membrane of rat proximal tubular epithelial cells (PTCs).19,20) The estimated Km value (about 400 μM) of the rat UA exchanger is close to that of human URAT1 (375 μM).15,20) Chloride or lactate can trans-stimulate, while probenecid and losartan cis-inhibit, the uptake of UA by human URAT1 and in rat renal brush-border membrane vesicles (BBMVs).15,19,20) Recently, the sequence of a rat renal specific transporter (Rst, Slc22a12), the human URAT1 orthologue, has been published (GenBank entry NM_001034943). Accordingly, a human URAT1-like transporter is likely present in rats.

On the basolateral side of rat PTCs, rat Oat1 and rat Oat3 play pivotal roles in renal secretion of various anions and may act as UA secretion transporters.21,22) Therefore, we hypothesized that rats could be a potential animal model to assess the effects of ARBs on renal handling of UA in vivo. Accordingly, the plasma UA level and fractional excretion of UA (FEUA) were examined in rats treated with ARBs (pratosartan, candesartan and telmisartan), which have been suggested to decrease, to increase and to have no effect on plasma UA level, respectively. In addition, the effects of ARBs on uptake of UA by rat renal BBMVs and rat transporter-expressing cells were studied to investigate the potential mechanisms of ARB effects. These findings have major implications for an understanding of the mechanisms of renal handling of UA and UA homeostasis in humans.

Materials and Methods

Chemicals: [14C]Uric acid (1.96 TBq/mol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). Pratosartan, candesartan and telmisartan were kindly supplied by Koto pharmaceutical (Nagano, Japan). Xanthine oxidase (≥0.5 units/mg protein) and uricase (≥2 units/mg protein) were obtained from Merck (Atsugi, Japan) and Sigma-Aldrich (St. Louis, MO), respectively. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan).

Clearance studies in anesthetized rats: Experiments were approved by Tokyo University of Science Animal Ethics Committee. Male Sprague-Dawley (SD) rats at 10 weeks of age were anesthetized with pentobarbital (45 mg/kg). The jugular vein was cannulated with a polyethylene-10 catheter (OD 0.8 × ID 0.5 mm) for the injection of drugs and for blood sample collection. Through the jugular vein, a solution containing 1% mannitol, 0.52% sodium pentobarbital and NaCl (total NaCl 154 mM) was infused at a rate of 1.9 ml/h. After 1-hr infusion, blood samples were collected into a heparinized tubes at 10 min before and 10, 30 and 50 min after drug administration. The bladder was cannulated and urine samples were collected into pre-weighed tubes containing ice-cold 10% H2SO4 at 0, 20, 40 and 60 min after drug injection. After experiments, the rat kidneys were excised after flushing out residual blood. Plasma and urine uric acid concentrations were determined by a validated HPLC method. Plasma and kidney concentrations of ARBs were quantified with validated LC-MS/MS methods by monitoring transitions of 425.1 to 190.5, 439.0 to 308.1, and 513.1 to 286.8 for pratosartan, candesartan and telmisartan, respectively. Plasma and urine creatinine concentrations were measured by using a commercial kit (Wako Chemicals, Japan).

Uptake studies in rat renal brush-border membrane vesicles: Renal brush-border membrane vesicles (BBMVs) were isolated from the renal cortex of male SD rats (300 g) by the method of Kahn et al. with minor modifications. UA uptake into renal BBMVs was determined at 25°C by the rapid filtration method in the presence of an OH- gradient.23) cis-Inhibitory effects of ARBs were examined by incubating 10 μl of renal BBMVs with UA (40 μM) in uptake buffer (90 μl) containing 120 mM NaCl, 100 mM mannitol and 10 mM Tris (pH 6.0 or 7.4) for 15 sec in the presence or absence of ARBs. Trans-stimulation was initiated by adding 20 ml of UA in uptake buffer (40 μM, pH 7.4) after preincubation of ARBs (0.2 and 2 μM) with renal BBMVs (10 μl) for 1 hr at room temperature. Intra-vesicular UA was extracted into MilliQ water and quantified by using a validated LC-MS/MS method.

Uptake studies in transporter-expressing cells: Rat Oat1-expressing HEK293 cells and rat Oat3-expressing oocytes were prepared as described previously.24) Uptake studies were conducted as described recently.24) Briefly, the cells were incubated with transport buffer containing [14C]Uric acid with or without ARBs at various
concentrations at 25°C for 5 min (Oat1) or 60 min (Oat3). The uptake was terminated by washing the cells three times with ice-cold buffer. The cells were solubilized with 5% sodium dodecyl sulfate solution and the associated radioactivity was counted.

**In vitro xanthine oxidase and uricase activity measurements:** The enzyme activities were determined by incubation of xanthine (1 μM) with xanthine oxidase (0.5 mU/ml in phosphate buffer, pH 7.8) or UA (10 μM) with uricase (25 mU/ml in phosphate buffer, pH 7.8) in the presence and absence of ARBs and specific inhibitors (allopurinol and oxonate for xanthine oxidase and uricase, respectively). UA and its catabolite allantoin were determined by HPLC with ultraviolet detection at 292 and 220 nm, respectively.

**Statistical analysis:** All results are presented as means ± S.E.M. The significance of differences in mean kinetic parameters was tested by one-way analysis of variance (ANOVA) with a Dunnett’s multiple comparison test. Student’s paired t test was conducted for comparison of values before and after treatment, and the criterion of significance was set at the level of P < 0.05.

**Results**

**In vitro effects of ARBs on xanthine oxidase and uricase activities:** Xanthine oxidase and uricase activities were decreased in the presence of the known inhibitors allopurinol and oxonate, respectively. However, there was no change of xanthine oxidase or uricase activity in the presence of any of the ARBs tested (Fig. 1).

**In vitro uptake studies**

**UA uptake by rat renal BBMV’s:** Figure 2 shows the concentration dependence of the inhibitory effects of various ARBs, including pratosartan, losartan, candesartan and telmisartan, on UA uptake by rat renal BBMVs at 15 sec. The estimated IC50 values are 152, 431, 10 and 76 nM for pratosartan, losartan, candesartan and telmisartan, respectively. Figure 3 shows the concentration dependence of the trans-stimulatory effect of candesartan after preincubation. Candesartan resulted in up to 28% greater UA uptake by the BBMVs compared with the control, while telmisartan had no significant effect.

**UA uptake by cells expressing rat Oat1 and Oat3:** Figures 4-B and -C show the inhibitory effects of pratosartan and telmisartan on UA uptake by rat Oat1; the IC50 values were 6,473 and 316 nM, respectively. Candesartan (Fig. 4-A) had no effect on rat Oat1-mediated UA uptake at concentrations up to 10 μM. Figures 4-D, -E and -F show the concentration dependence of the inhibitory effects of the tested ARBs on UA uptake by rat Oat3; the IC50 values were 264, 1,285 and 723 nM for candesartan, pratosartan and telmisartan, respectively.

**In vivo clearance studies:** Candesartan at low dose (0.1 mg/kg) significantly decreased the fractional excretion of UA (FEUA) and increased the plasma UA concentration, while treatment with higher doses (1 and 10 mg/kg) had no apparent effect (Fig. 5A). There was no apparent change of plasma UA level after treatment with telmisartan at 0.1, 2 or 10 mg/kg. Unfortunately, higher doses of telmisartan could not be tested due to the limited solubility of the drug. In contrast, pratosartan at high dose (20 mg/kg) significantly decreased plasma UA concentration and increased the value of FEUA, whereas low-dose (10 mg/kg) pratosartan had no significant effect on the UA concentration.
Fig. 2. Cis-inhibitory effects of ARBs on UA uptake by rat renal BBMVs. Uptake of UA (40 μM) was measured in the presence or absence (control) of ARBs (1–1000 nM), including pratosartan (A), candesartan (B), losartan (C) and telmisartan (D) in uptake buffer (pH 6.0 or 7.4) at 15 sec. UA uptake through the UA transporter was determined as the difference between the uptake amount in the presence and absence of a pH gradient. All experiments were standardized by setting the control to 100%. Data are means ± SEM of three replicates.

Fig. 3. Trans-stimulatory effect of candesartan on UA uptake by rat renal BBMVs. Uptake of UA (40 μM) at 15 sec was measured after preincubation of ARBs (0.2 μM, hatched bar, and 2 μM, closed bar) or blank uptake buffer (control, open bar). Trans-stimulatory effects were expressed as percentage of the control. Data are means ± SEM of three replicates. *P < 0.05.

(Fig. 6). Interestingly, the plasma unbound pratosartan concentrations were above the IC_{50} value determined for the inhibition of UA uptake in rat BBMV studies for over 50 min after dosing of 20 mg/kg.

Discussion

The present rat studies confirm the previously reported differential effects of the tested ARBs on renal handling of UA in humans.\(^{12,25}\) Our in vitro metabolism studies exclude the possibility of a reduction in UA synthesis by a pharmacological dose of any of the ARBs tested. However, UA production was significantly inhibited by allopurinol, a known inhibitor of xanthine oxidase. In addition, ARBs had no effects on uricase, whose activity was inhibited by oxonate (Fig. 1B). Similarly, it has been reported that the hypouricemic effects of losartan in vivo in rats could not be explained by changes of xanthine oxidase or uricase activity.\(^{18}\)

On the other hand, our in vitro transport studies suggest that the tested ARBs interfere with renal transport of UA in rats. In rat renal BBMVs, all of the tested ARBs exhibited concentration-dependent inhibition of UA uptake, with IC_{50} values of 10, 76, 152 and 431 nM for candesartan, telmisartan, pratosartan and losartan, respectively. Candesartan appears to be the most potent inhibitor in rat renal BBMVs, while it has no effect on human URAT1 or OAT4-mediated UA uptake at clinically relevant concentrations,\(^{16,17}\) suggesting some species differences in the inhibition of UA reabsorption by candesartan between rat and human. Based on the estimated plasma unbound concentration, candesartan at a dose higher than 1 mg/kg may efficiently inhibit UA reabsorption in rats (Table 1). In addition, candesartan exhibited trans-stimulation in rat renal BBMVs at a concentration (2 μM) similar to the estimated total kidney concentration in vivo after administration of a low dose (0.1 mg/kg) (Fig. 3, Table 1). This is similar to our recent results, in which candesartan trans-stimulates human URAT1.\(^{16}\)
Fig. 4. Cis-inhibitory effects of candesartan (A, D), pratosartan (B, E) and telmisartan (C, F) on uptake of UA by rat Oat1-expressing HEK293 cells (A, B, C) and rat Oat3-expressing Xenopus oocytes (D, E, F). The cells were incubated with transport buffer containing [14C]uric acid with or without ARBs at various concentrations at 25°C for 5 min (Oat1) or 60 min (Oat3). UA uptake through the Oat1 or Oat3 was determined as the difference between uptakes in transporter-expressing cells and mock cells or water-injected oocytes. All experiments were standardized by setting the control to 100%. Data are presented as means ± SEM of three to four replicates of 10 oocytes each.

Fig. 5. Effects of candesartan (A) and telmisartan (B) on plasma UA level (open bars) and FEUA value (closed bars) in rats at 50 min after IV administration. All experiments were standardized by setting the control to 100%. Data are presented as means ± SEM of six to eight rats. *P<0.05.
Candesartan had no effect on rat Oat1 (Table 1), while it reduced the transport of UA via rat Oat3 with about 26 times lower affinity compared with that for reabsorptive transporter(s) observed in the BBMVs (Table 1).

Taken together, the above observations indicate that the hyperuricemic and antiuricosuric effects of low-dose (0.1 mg/kg) candesartan in rats may be best explained by trans-stimulation of rat UA reabsorption transporter(s), while the apparent lack of effect of candesartan at high doses (1 and 10 mg/kg) in rats could be due to the compensatory effect of inhibition of the UA reabsorptive transporter(s) by candesartan (Fig. 5 and 7, Table 1). However, the hyperuricemia caused by candesartan in humans may be mainly due to its trans-stimulation effect on UA reabsorption since it does not inhibit URAT1 or OAT4-mediated UA uptake.\textsuperscript{16,17} We have previously reported that pratosartan and losartan exhibited trans-stimulation effects on UA uptake only after preloading at low concentrations, but not high concentrations, in human URAT1-expressing oocytes, due to their concomitant cis-inhibitory effect at high concentrations.\textsuperscript{16} Similar results have been reported for torasemide in a re-
Fig. 7. Proposed mechanisms of differential effects of ARBs on renal handling of UA in rats. ARBs with affinity for reabsorption transporter(s) will exhibit uricosuric effects when the lumen ARB concentration is sufficiently high (i.e. > IC_{50}), whereas some ARBs will be antiuricosuric if they stimulate UA influx from intracellular spaces and/or inhibit UA secretion mediated by basolateral transporters. Consequently, the serum UA concentration will be differentially modulated by different ARBs. Cis, Cis-inhibition; Trans, Trans-stimulation.

In conclusion, the clinically observed differential effects of ARBs on plasma UA level and renal UA clearance were well reproduced in the rat model. The effects of ARBs in rats may be mainly dependent on both their intrinsic effects (cis-inhibition and trans-stimulation) on UA reabsorption transporter(s) (Fig. 7) and their pharmacokinetic properties, including plasma concentration and kidney accumulation. The in vivo rat model in combination with in vitro transporter studies should yield a better understanding of the mechanisms of renal handling of UA, and may be useful in the development of drugs to modulate plasma UA level in high-risk populations.

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