Short Communication

Concentration-Dependent Inhibitory Effect of Irbesartan on Renal Uric Acid Transporters

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Abstract. Hyperuricemia is currently recognized as a risk factor for cardiovascular diseases. It has been reported that the angiotensin II–receptor blocker (ARB) losartan decreases serum uric acid level. In this study, the effects of another ARB, irbesartan, on [14C]uric acid–transport activity of renal uric acid reabsorptive transporters URAT1 and URATv1 were examined with Xenopus oocytes expressing each transporter. The results showed that irbesartan (100 – 500 μM) inhibited the uptake of uric acid via both transporters. The inhibitory effects of irbesartan exceeded those of losartan and other ARBs, and the results suggest that irbesartan can reduce serum uric acid level.

Keywords: angiotensin II–receptor blocker, transporter, uric acid

Hyperuricemia has been associated with hypertension. Approximately 25% of patients with hypertension have hyperuricemia (1), and approximately 30% of patients with hyperuricemia or gout have hypertension (2). Therefore the effects of antihypertensive drugs on serum uric acid (SUA) level, especially angiotensin II–receptor blockers (ARBs), have been scrutinized in recent years. Losartan has been shown to increase urinary uric acid (UA) excretion and decrease SUA level (3). In contrast, other ARBs such as candesartan and valsartan do not have uricosuric activity (4, 5). Thus, it seems that the ability of each ARB to decrease SUA cannot be predicted until the uricosuric activities of all ARBs are examined. In the present study, we focused on another widely-used ARB, irbesartan. One report showed a tendency for irbesartan to decrease SUA level in hypertensive patients with hyperuricemia (6). Therefore we examined the effect of irbesartan on UA transporters involved in regulating SUA level. The UA transporter URAT1 is involved in lumen-to-cytosol reabsorption of UA along the proximal tubule (7). A sugar transport facilitator family member protein GLUT9 (URATv1) functions as an efflux transporter of UA from tubular cells (8) at the basolateral membrane. Mutation of URAT1 or URATv1 is associated with idiopathic renal hypouricemia (7, 9), indicating URAT1 and URATv1 play a dominant role in UA reabsorption and controlling SUA levels. We used Xenopus oocytes expressing URAT1 or URATv1 to examine the cis-inhibitory effects of irbesartan at various concentrations.

Cloned human URAT1 and URATv1 were expressed in Xenopus oocytes as described previously (7, 8). In brief, defolliculated oocytes were injected with 50 ng of cRNA that was transcribed in vitro using T7 RNA polymerase in the presence of cap analog. After incubation of oocytes in Barth’s buffer at 18°C for 2 – 3 days, uptake studies were performed in ND 96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM HEPES, pH 7.4) containing 20 μM [14C]UA and ARBs at the indicated concentrations; after 1 h incubation, oocytes were lysed and dried at room temperature. Then the amount of the [14C]UA uptake was measured in a scintillation counter and the uptake rate was determined. For determination of the kinetic parameters, the concentrations of urate were varied from 10 to 1500 μM. Kinetic parameters were obtained by using the Eadie-Hofstee equation. The experiments were performed using 8 – 10 oocytes per experiment and repeated three times. All the data are given as the mean ± S.E.M. Student’s t-test was used to determine significant differences. A value of P < 0.05 was considered to be significant.

Figure 1 illustrates the chemical structures of ARBs
examined in this study. In addition to irbesartan, we tested losartan, telmisartan, candesartan, and valsartan. Figure 2A shows that at concentrations of 300 – 500 μM, all ARBs except candesartan significantly inhibited URAT1-mediated [14C]UA uptake. In the present study, valsartan (500 μM) decreased UA uptake via URAT1. Since the administration of a standard dose of valsartan (2 mg/kg) results in a mean plasma concentration of less than 20 μM (10), it seemed unlikely that, clinically, it had any effect on SUA level. Figure 2A also indicates that irbesartan is more inhibitory than losartan. Figure 2B shows the dependence of inhibition on irbesartan concentration. The percentage of UA uptake relative to the control (no inhibitor) level decreased dose-dependently from 30 to 500 μM, indicating the cis-inhibitory effect of irbesartan on the UA uptake via URAT1. Kinetic data (Fig. 2: C and D) showed that the inhibition of irbesartan was non-competitive against URAT1 since irbesartan decreased V_max from 21.54 to 9.84 pmol·oocyte⁻¹·h⁻¹, but did not change the K_m (218.08 to 191.45 μM).

The uptake of URATv1-expressing oocytes is shown in Fig. 3. Both irbesartan and telmisartan inhibited URATv1-mediated UA uptake to a similar extent. The concentration dependence (in the range 30 – 500 μM) of irbesartan-mediated inhibition (Fig. 3B) confirmed its ability to block UA uptake by URATv1. The results shown in Fig. 3, C and D demonstrate that irbesartan effected an increase in the the K_m value (325.1 to 721.43 μM) and a decrease in the V_max (357.66 to 202.52 pmol·oocyte⁻¹·h⁻¹). Those kinetic parameters indicated that irbesartan inhibited URATv1 in both a competitive manner and a non-competitive manner (mixed inhibition).

The present study has demonstrated, for the first time, that irbesartan inhibits renal UA transporters in vitro. Two UA transporters, URAT1 and URATv1 involved in the transport of UA at the apical and basolateral membrane, respectively, were examined (7, 8), and the inhibitory effects of several ARBs were compared with that of irbesartan. As shown in Fig. 1, ARBs with a single anionic group were the most effective UA transporter inhibitors; losartan, irbesartan, and telmisartan with one anionic group but not candesartan and valsartan with two anionic groups markedly decreased UA-uptake. Although further examination is needed, the blocking ability of irbesartan on UA transporters provides important information about the substrate specificity of UA transporters.

Moreover, the inhibitory effect of irbesartan exceeded that of losartan. A losartan concentration of 500 μM seemed to be too small to inhibit URATv1 (Fig. 3A), which is in accordance with previous reports (1 mM) (8). In contrast, irbesartan significantly decreased the UA-uptake by URAT1 and URATv1 at concentrations of 100 – 500 and 300 – 500 μM, respectively (Figs. 2B and 3B). Since the clinically achievable mean plasma concentrations of losartan and irbesartan are 3 and 6 μM (11, 12), respectively, neither drug can have any effect on SUA level from the basolateral (blood) side of the renal proximal tubules. Nevertheless, the urinary concentration

Fig. 1. Chemical structures of angiotensin-receptor blockers (ARBs). Dotted grey circles indicate anionic groups as described in the text.
Irbesartan Blocks Uric Acid Transporters

Fig. 2. The \textit{cis}-inhibitory effect of irbesartan on URAT1-mediated uric acid uptake by URAT1-expressing oocytes. A: Uptake of \[^{14}\text{C}\]uric acid (20 \(\mu\text{M}\)) by URAT1-cRNA injected oocytes was measured in the presence or absence (no inhibitor) of ARBs [500 \(\mu\text{M}\), except for telmisartan (300 \(\mu\text{M}\))] for 1 h. B: Concentration-dependence of inhibition of urate uptake via URAT1 by irbesartan (1 – 500 \(\mu\text{M}\)). The URAT1-mediated uptake is expressed as percentage of the no-inhibitor control (absence of ARBs). C: The kinetic curve of urate uptake with 0.2 mM irbesartan. D: Eadie-Hofstee plot of URAT1-mediated urate uptake with 0.2 mM irbesartan. V/S, velocity per concentration of substrate; V, pmol·oocyte\(^{-1}\)·h\(^{-1}\). Each data point is the mean ± S.E.M. from 3 independent experiments using 8 – 10 oocytes. *** \(p < 0.001\), compared to the sample with no inhibitor. NS, not significant.

Fig. 3. The \textit{cis}-inhibitory effect of irbesartan on URATv1-mediated uric acid uptake by URATv1-expressing oocytes. A: Uptake of \[^{14}\text{C}\]uric acid (20 \(\mu\text{M}\)) by URATv1-cRNA injected oocytes was measured in the presence or absence (no inhibitor) of ARBs [500 \(\mu\text{M}\), except for telmisartan (300 \(\mu\text{M}\))] for 1 h. B: Concentration-dependence of inhibition of urate uptake via URATv1 by irbesartan (1 – 500 \(\mu\text{M}\)). The URATv1-mediated uptake is expressed as percentage of the no-inhibitor control (absence of ARBs). C: The kinetic curve of urate uptake with 0.5 mM irbesartan. D: Eadie-Hofstee plot of URATv1-mediated urate uptake with 0.5 mM irbesartan. V/S, velocity per concentration of substrate; V, pmol·oocyte\(^{-1}\)·h\(^{-1}\). Each data point is the mean ± S.E.M. from 3 independent experiments using 8 – 10 oocytes. ** \(p < 0.01\), *** \(p < 0.001\), compared to the sample with no inhibitor. NS, not significant.
of irbesartan was almost 2 μM and it was lower than that of losartan (7 μM) (11, 12). This indicated that irbesartan may decrease SUA level from the luminal (apical) side because the drugs secreted into the tubular lumen will accumulate within the renal tubular system reaching a concentration exceeding that in plasma (13). Telmisartan, the other positive control, inhibited URAT1 and URATv1 to the same extent as irbesartan did. However, there is no indication that telmisartan enters the kidney (14) and therefore inhibition of renal UA reabsorption from the luminal side would seem to be unlikely. In addition, clinical data has shown no significant change in SUA in telmisartan-administered hypertensive patients (15), indicating that the inhibitory effect observed in vitro may not be relevant to in vivo UA transport in the kidney. Therefore irbesartan is likely to be a clinically more effective blocker of the renal UA transporter than telmisartan. In conclusion, our in vitro experiment demonstrated strong interaction of irbesartan with UA transporters, which exceeded that of losartan. Irbesartan could be used in hyperuricemic patients to decrease SUA level.

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References
