Effects of Specific Antagonists of Angiotensin II Receptors and Captopril on Diabetic Nephropathy in Mice

Takashi Yotsumoto, Takeshi Naitoh, Ken-ichi Shikada and Sakuya Tanaka

Shiraoka Research Station of Biological Science, Nissan Chemical Industries Ltd., 1470, Shiraoka, Minamisaitama, Saitama 349-02, Japan

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ABSTRACT—We investigated whether angiotensin II was involved with diabetic nephropathy in the mouse model. Twelve days after streptozotocin (STZ) injection, the urinary albumin excretion (UAE) level was increased by 118% of the baseline value. On days 21, 28, 35 and 42 after STZ injection, the UAE levels were significantly increased compared with the level at day 12. A marked elevation of creatinine clearance and diabetic-induced renal hypertrophy were also observed on day 49 after STZ injection. The 35-day treatments of captopril and Dup 753 (angiotensin II type 1 receptor antagonist) significantly attenuated the increment of UAE levels (26.4% on day 14 and 34.6% on day 28). PD123177 (angiotensin II type 2 receptor antagonist) also attenuated the increment of UAE (24.7% on day 14) at the dose of 150 mg/kg. Furthermore, Dup 753 partially prevented diabetic-induced renal hypertrophy. These results suggest that angiotensin II type 2 receptor as well as type 1 receptor may be involved in the development of diabetic nephropathy in the STZ-induced diabetic mice, and these mice are beneficial models of early diabetic nephropathy.

Keywords: Diabetic nephropathy, Urinary albumin excretion, Angiotensin-converting enzyme inhibitor, Angiotensin II receptor antagonist

The long-term hyperglycemia associated with diabetes mellitus results in the slow development of multiple secondary complications (1). Diabetic nephropathy, a major long-term complication, occurs in 30–40% of diabetic patients (2). The early stages of diabetic nephropathy are characterized by an elevation of urinary albumin excretion (UAE), an increment of glomerular filtration rate and renal hypertrophy (3, 4). The UAE progresses to overt proteinuria and finally results in end-stage renal failure in diabetic patients.

In clinical situations, angiotensin-converting enzyme (ACE) inhibitor is effective for diabetic nephropathy (5, 6). Twelve-week treatment with ACE inhibitor in diabetic patients with overt diabetic nephropathy has been proven effective in decreasing the UAE and reducing the rate of deterioration of renal function (7). In diabetic rat studies, ACE inhibition reduced intra-glomerular capillary pressure through vasodilation of the efferent arterioles, decreased albuminuria (8) and prevented renal hypertrophy (9). A recent report suggests that the beneficial effect of ACE inhibitors is primarily due to an inhibition of angiotensin II (Ang II) production (10).

There are two receptor subtypes for Ang II: AT₁ and AT₂. They are distinguished by two structurally different nonpeptide Ang II antagonists, i.e., Dup 753 and PD123177 (11). The well-known pharmacological actions of Ang II, such as various smooth muscle contractions, are predominantly coupled to the AT₁ receptor (12). Although it is reported that PD123177, like Dup 753, has a powerful inhibitory effect on transport in early proximal convoluted tubules (13), the role of the AT₂ receptor is not yet understood.

Various experimental animals have been used to investigate diabetic nephropathy. Streptozotocin (STZ)-induced hyperglycemic rats have been used mainly as a diabetic nephropathy model. STZ-injected rats reproduced some findings of early-stage clinical diabetic nephropathy (9, 14). However, it generally takes at least several months for the features of diabetic nephropathy to appear in the rats (15). It is thus difficult to make a rapid and early evaluation of the effects of various compounds on the nephropathy. Furthermore, if it is possible to use mice rather than rats in this model, the amount of the compound being evaluated will be reduced. In STZ-injected mice, there is presently no report that shows the urinary albumin excretion as an index of diabetic nephropathy and evaluates effects of various compounds, however.

In this study, we investigated whether STZ-injected...
mice manifested the features of diabetic nephropathy, with high levels of UAE and renal hypertrophy. In addition, we investigated whether the high UAE level of STZ-injected mice depended on the hemodynamic factors induced by Ang II.

MATERIALS AND METHODS

**Materials**

Streptozotocin (STZ) and captopril were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dup 753 and PD123177 were synthesized at the Central Research Laboratories of Nissan Chemical Industries, Ltd. (Chiba). The drugs were dissolved or suspended in 0.5% methyl cellulose (SM400; Shinetsu Chemical, Tokyo) solution and administered through a gastric tube.

**Preparation of diabetic mice**

Male ddY mice (6-weeks-old; SLC, Shizuoka) were used. The mice were fasted for 16 hr. They were injected intraperitoneally with a bolus injection of 150 mg/kg of STZ dissolved in 3 mM citrate buffer (pH 4.5). After 11 days, plasma glucose levels were determined with a glucose analyzer II (Beckman, Fullerton, CA, USA), and mice with levels > 300 mg/dl were used. On day 12, the diabetic mice were placed in individual metabolism cages for urine collection. After 24 hr, the mice were released from the cages, and blood was collected by capillary pipette from the orbital vein plexus. Plasma glucose levels were determined again with the glucose analyzer. The urinary albumin levels were determined with a microalbumin-HA test kit (Wako Pure Chemical Industries Ltd., Osaka).

**Experimental procedure**

The diabetic mice were divided into 4 groups as follows: 1) 0.5% methyl cellulose (control group), 2) 50 mg/kg captopril group, 3) 30 mg/kg of Dup 753 group, and 4) 150 mg/kg PD123177 group. Fourteen days after the STZ injection, the drug administration commenced: captopril, Dup 753 or vehicle was given orally once a day for 5 weeks; PD123177 was administered for 3 weeks. The mice were placed in individual metabolism cages to collect urine for 24 hr once a week. The plasma glucose levels were determined after the mice were released from the cages. Systolic blood pressures (SBP) were measured by the tail-cuff method (UR-5000; Ueda, Tokyo) on the day before the mice were placed in metabolism cages. Food and water were given ad libitum. After the above experiment, the control, captopril- and Dup 753-treated mice were sacrificed, and kidney tissues and blood samples were collected. The kidney weight was determined gravimetrically. The degree of renal hypertrophy was expressed as the ratio of the two kidneys' weight to total body weight. Renal function was evaluated by measuring creatinine clearance (Ccr, ml/min/100 g body wt.). The plasma- and urinary-creatinine level were determined with a creatinine test kit (Wako). The urine volume was gravimetrically measured, and the urinary albumin level was determined as above.

**Statistical analyses**

All results are expressed as the mean±S.E.M. Statistical analysis was done by Stat View-J 4.5 (Abacus Con-

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### Table 1. Time course of various parameters in non-diabetic and diabetic mice

<table>
<thead>
<tr>
<th>Post-STZ injected day</th>
<th>Condition</th>
<th>Body wt. (g)</th>
<th>Urine vol. (g)</th>
<th>Plasma glucose (mg/dl)</th>
<th>Systolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 days</td>
<td>Non-diabetic</td>
<td>34.3±1.2</td>
<td>1.7±0.2</td>
<td>96.8±6.5</td>
<td>142.5±3.8</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>27.4±1.2</td>
<td>22.1±4.3</td>
<td>460.0±25.8</td>
<td>140.4±4.7</td>
</tr>
<tr>
<td>21 days</td>
<td>Non-diabetic</td>
<td>35.6±0.6</td>
<td>1.6±0.2</td>
<td>92.4±5.0</td>
<td>141.9±3.6</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>28.2±1.5</td>
<td>26.1±4.5</td>
<td>478.9±9.0</td>
<td>149.9±3.0</td>
</tr>
<tr>
<td>28 days</td>
<td>Non-diabetic</td>
<td>36.0±0.6</td>
<td>1.6±0.3</td>
<td>97.2±4.5</td>
<td>148.9±2.9</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>30.2±0.9</td>
<td>32.8±4.1</td>
<td>455.4±12.1</td>
<td>152.3±4.5</td>
</tr>
<tr>
<td>35 days</td>
<td>Non-diabetic</td>
<td>36.6±0.7</td>
<td>1.6±0.4</td>
<td>97.6±5.0</td>
<td>138.8±3.3</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>29.8±1.0</td>
<td>34.7±4.4</td>
<td>433.7±15.4</td>
<td>157.6±5.2</td>
</tr>
<tr>
<td>42 days</td>
<td>Non-diabetic</td>
<td>37.5±0.9</td>
<td>1.4±0.4</td>
<td>112.4±4.8</td>
<td>139.4±5.2</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>31.1±0.9</td>
<td>39.1±3.5</td>
<td>479.4±14.1</td>
<td>157.9±3.5</td>
</tr>
<tr>
<td>49 days</td>
<td>Non-diabetic</td>
<td>37.2±1.0</td>
<td>1.7±0.5</td>
<td>122.8±6.1</td>
<td>143.1±3.9</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>30.1±1.7</td>
<td>34.5±5.7</td>
<td>450.8±31.1</td>
<td>158.1±5.3</td>
</tr>
</tbody>
</table>

Each value is a mean±S.E.M. STZ was intraperitoneally injected at 150 mg/kg (n = 7) or not injected (n = 10) in ddY mice. Systolic blood pressures were measured on the day before the mice were placed in metabolism cages. *P<0.05, significantly different from the 12th day value (Student’s t-test); **P<0.05, significantly different from the day matched non-diabetic mice (Student’s t-test).
RESULTS

Effect of STZ on various parameters in mice

Table 1 summarizes the effects of STZ on various parameters in mice. Twelve days after the injection of STZ, body weight was significantly decreased, and then the values were lower than those of the non-diabetic group throughout the experiment period. Urine volume and plasma glucose levels were significantly increased, compared with the non-diabetic mice. The urine volume tended to increase depending on the period after STZ administration, except for on day 49 after STZ administration. On days 28, 35 and 42 after STZ administration, the urine volume was significantly increased, compared with the level at day 12. The diabetic group maintained marked hyperglycemia (>430 mg/dl) throughout the experimental period. SBP were significantly increased depending on the period after STZ administration, and the values at days 34, 41 and 48 were significantly increased, compared with the non-diabetic group.

Effect of STZ on UAE in mice

The UAE levels of the STZ-injected and non-injected mice are shown in Fig. 1. Twelve days after the injection of STZ, the UAE levels were significantly increased, and the values were more than twofold higher than that of the non-diabetic mice (478±55 vs 1042±126 µg/24 hr, respectively). The UAE levels tended to increase depending on the period after STZ administration, except for at day 49 after STZ administration. On days 21, 28, 35 and 42 after STZ administration, the UAE levels were significantly increased, compared with the level at day 12.

Effects of captopril, Dup 753 and PD123177 on UAE in diabetic mice

Table 2 summarizes the effects of various drugs on the UAE levels in diabetic mice. The oral consecutive administration of captopril significantly reduced the UAE levels on days 14 and 28 after starting the drug administration, compared with the control group (maximally 26.8% on day 14). Although the UAE levels at days 21 and 35 were lower than those of the diabetic control, the

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dose (mg/kg/day)</th>
<th>n</th>
<th>Days</th>
<th>Pre</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>7</td>
<td></td>
<td>1042±126</td>
<td>1470±84*</td>
<td>1472±109*</td>
<td>1481±164*</td>
<td>1452±103*</td>
<td>1228±145</td>
</tr>
<tr>
<td>Captopril</td>
<td>50</td>
<td>8</td>
<td></td>
<td>1019±70</td>
<td>1513±136*</td>
<td>1078±134*</td>
<td>1240±74</td>
<td>1129±90</td>
<td>1177±128</td>
</tr>
<tr>
<td>Dup 753</td>
<td>30</td>
<td>8</td>
<td></td>
<td>1028±70</td>
<td>1319±98</td>
<td>1084±36*</td>
<td>1212±128</td>
<td>950±68</td>
<td>981±122</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>12</td>
<td></td>
<td>1077±80</td>
<td>1387±76*</td>
<td>1359±79*</td>
<td>1400±171</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>PD123177</td>
<td>150</td>
<td>15</td>
<td></td>
<td>1181±66</td>
<td>1246±92</td>
<td>1023±98*</td>
<td>1297±92</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Each value is a mean ± S.E.M. The drug was administered from the 14th day after STZ injection. The "pre" value indicates the urinary albumin excretion on the 12th day after STZ injection. *P<0.05, significantly different from each pre-value (Student's t-test); †P<0.05, significantly different from each control value (Fisher's PLSD test); N.D., not determined.
differences were not significant. Dup 753, the AT₁-receptor antagonist, also significantly attenuated the UAE level on days 14 and 28. Although the UAE levels in the Dup 753 group on days 21 and 35 tended to be less than those of the control group, the differences were not significant. PD123177, the AT₂-receptor antagonist, significantly decreased the UAE level only on day 14, compared with the control. All drugs significantly inhibited the time-dependent UAE level increase, except for the value on day 7 in the captopril group. On only day 27, captopril significantly decreased the SBP compared with the diabetic control (141.3±5.0 vs 157.9±3.5 mmHg, respectively). Dup 753 also significantly decreased the SBP on days 27 and 34, compared with the diabetic control (139.6±4.5 vs 157.9±3.5 mmHg on day 27 and 136.3±2.8 vs 158.1±5.3 mmHg on day 34, respectively). However, they were not significantly different from those of normal mice (139.4±5.2 on day 27 and 143.1±3.9 mmHg on day 34, respectively). None of the drugs affected the plasma glucose levels (data not shown).

**Effect of captopril and Dup 753 on Ccr in diabetic mice**

We determined Ccr as an index of renal function. In the diabetic control group, the Ccr was significantly increased, and the value was about threefold higher than that of the non-diabetic group (1.24±0.15 vs 0.40±0.05 ml/min/100 g body wt., respectively) (Fig. 2). The administration of captopril and Dup 753 for 35 days did not cause a significant change in this diabetes-induced increase in Ccr, although the captopril tended to increase the Ccr (1.61±0.14 and 1.09±0.10 ml/min/100 g body wt., respectively). The Ccr level of PD123177 administration (n=5) for 21 days was lower than that of the day matched diabetic control (n=5), but not significantly (1.03±0.10 vs 1.80±0.37 ml/min/100 g body wt., respectively).

**Effects of captopril and Dup 753 on renal hypertrophy in diabetic mice**

We calculated the ratio of two kidney-weight to body weight in the various groups of mice to assess renal hypertrophy (Table 3). The diabetic control group mice had a significantly increased ratio compared with the non-diabetic mice. The net two kidney-weight of the diabetic control group was higher than that of the non-diabetic group, although the difference was not significant. This renal hypertrophy was partially prevented by the administration of Dup 753 for 35 consecutive days. Captopril significantly decreased the net two kidney-weight compared with the control. The ratio of two kidney-weight to body weight tended to be decreased by captopril compared with the diabetic control, but not significantly. The administration of PD123177 (n=5) for 21 days sig-

![Figure 2](https://via.placeholder.com/150)

**Table 3.** Effects of captopril and Dup 753 on renal hypertrophy in diabetic mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
<th>Dose (mg/kg/day)</th>
<th>n</th>
<th>Two-kidney wt. (g)</th>
<th>Two-kidney wt./10 g body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic</td>
<td>Control</td>
<td>10</td>
<td>0.497 ± 0.025</td>
<td>0.133 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>Captopril</td>
<td>7</td>
<td>0.567 ± 0.025</td>
<td>0.189 ± 0.008*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dup 753</td>
<td>8</td>
<td>0.468 ± 0.030f</td>
<td>0.178 ± 0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>0.483 ± 0.025f</td>
<td>0.163 ± 0.008f</td>
<td></td>
</tr>
</tbody>
</table>

Each value is a mean ± S.E.M. Test drugs were given p.o. daily from the 14th day to the 49th day after STZ injection. *P < 0.05, significantly different from non-diabetic mice (Student's t-test); †P < 0.05, significantly different from diabetic control mice (Fisher's PLSD test).
nificantly increased the net two kidney-weight compared with the diabetic control (n=5, 0.616±0.033 vs 0.518±0.024 g, respectively), but did not affect the ratio of two kidney-weight to body weight (0.183±0.008 vs 0.162±0.008, respectively).

DISCUSSION

Some reports indicate that the AT1 receptor predominantly affects mesangial hypertrophy and hyperplasia, whereas the functional relevance of the AT2 receptor remains open to debate (12, 16). Our present results show that Dup 753, an AT1 receptor-selective antagonist, attenuated the increase in the UAE level. We also observed that PD123177, an AT2 receptor-selective antagonist, attenuated the increase in the UAE level. Several reports have indicated that increased kinin played a role in the antihypertensive and antiproteinuric effects of ACEI (17, 18). Our results, however, suggest that the increase in UAE in this model depends mostly on the Ang II, independent of the kinin. This study is the first report that the AT2 receptor is involved in the development of diabetic nephropathy.

Systemic hypertension is a well-known cause of progressive renal injury in both humans (19) and experimental animals (3). Several studies have reported that captopril attenuated the increase in the UAE level in diabetic patients (20) and an experimental diabetes model (8). In contrast, treatment with the calcium antagonists nifedipine and bendipine did not reduce urinary protein excretion in diabetic spontaneously hypertensive rats (SHR), despite the comparable blood pressure reduction (21). These findings suggest that intra-glomerulus hypertension may be dissociated from systemic hypertension itself in diabetic nephropathy. We demonstrated in the present study that captopril and Dup 753 reduced albuminuria with an antihypertensive effect, but not with the change of Ccr. The blood pressure and renal hemodynamic changes after three months of ACE inhibition in proteinuric patients are completely reversed by acute exogenous Ang II, whereas the antiproteinuric response is not affected (22). This suggests that both blood pressure reduction and the improvement of renal hemodynamic factors play a minor role in the antiproteinuric effect. Captopril suppressed the UAE level and Ccr in STZ-treated SHR (23). Our present results suggest that captopril affects factors other than the intra-glomerulus pressure for the suppression of the UAE level.

The permeability of glomerular basement membrane (GBM) is dependent on both size-selectivity and charge-selectivity (24). Some reports indicate that the abnormality of charge selectivity depends on the reduced content of negative-charged materials, such as heparan sulfate proteoglycan, on the GBM (23, 25). Four-week captopril treatment protected against the reduction of negative-charged materials on GBM in STZ-injected SHR (23). Some reports suggest that glomerular endothelial and/or epithelial cells also play a role as the permeability barrier against high-molecular materials (26, 27). Our present results suggest that the effect of captopril depends on the protection against the alteration of GBM charge rather than the improvement of renal hemodynamics.

Because captopril prevented diabetic-induced renal hypertrophy without any change of Ccr in this study, we speculated that the Ang II predominantly acts through two mechanisms in renal and glomerular hypertension: Ang II 1) deteriorates the intra-glomerulus hemodynamics, and 2) has a direct effect on mesangial cells. This direct effect on mesangial cells is possible given the presence of Ang II receptors on this cell type (16). Ang II has been shown to mediate trophic effects on mesangial cells (28), including extracellular matrix production (29). We speculated that the effect of captopril on renal hypertrophy may be due to a direct cellular effect on mesangial cells.

This study showed that STZ-injected mice had significantly increased UAE levels compared with normal mice, in a manner similar to clinical reports and other experimental models. This increase in UAE levels was accompanied by the development of hypertension, increased Ccr and renal hypertrophy. Clinical and experimental diabetic studies have suggested that the development of albuminuria is involved with the increase in intra-glomerulus pressure (14, 30). We used the Ccr level as an index of intra-glomerulus pressure in the present study. Our results revealed that STZ-injected mice had markedly increased Ccr compared with normal mice. Our observation supports that the development of hypertension and the increase in intra-glomerulus pressure contribute to the increased UAE level in rat models and in diabetic patients.

In conclusion, the STZ-injected mice had increased UAE levels with rising Ccr and renal hypertrophy 49 days after STZ injection. The increase in UAE levels and the renal hypertrophy were attenuated by captopril. These findings are similar to those in clinical situations, and they were produced in a short period, i.e., several weeks. The attenuation of the UAE appeared after the captopril, Dup 753 and PD123177 administration. These results suggest that STZ-injected mice could be a beneficial model of diabetic nephropathy. We propose that this model makes possible the rapid evaluation of the effects of various compounds.
REFERENCES