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Effects of imidapril and captopril on streptozotocin-induced diabetic nephropathy in mice

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Abstract

We investigated whether the prevention of the development of diabetic nephropathy by angiotensin-converting enzyme inhibitors is associated with decreases in renal angiotensin-converting enzyme activity and/or blood pressure in diabetic mice. C57Bl/6 mice were injected with streptozotocin (200 mg/kg, i.v.) and randomized to receive either imidapril (1 and 5 mg/kg) or captopril (10 and 50 mg/kg) or vehicle by gavage for 28 days. Each assay was performed on 8–10 mice from each treatment. At 28 days after the start of drug treatment, imidapril and captopril significantly reduced blood pressure of the diabetic mice, and this effect of captopril was stronger than that of imidapril. On the other hand, inhibition of renal angiotensin-converting enzyme activity by imidapril was stronger than that by captopril. Imidapril and captopril dose-dependently inhibited urinary albumin excretion to similar extents, but they failed to inhibit the renal hypertrophy and elevation of creatinine clearance. Total renal angiotensin-converting enzyme activity was significantly reduced in diabetic mice, but immunohistochemical localization of angiotensin-converting enzyme was intensive in the vasculature and glomeruli of the diabetic kidney. In conclusion, both effects on blood pressure and angiotensin-converting enzyme activity may be involved in the prevention of development of diabetic nephropathy by imidapril and captopril in streptozotocin-induced diabetic mice. The data suggest that the degrees of contribution of their effects on blood pressure and renal angiotensin-converting enzyme activity to the inhibition of urinary albumin excretion may be different between the two angiotensin-converting enzyme inhibitors.

Keywords: Angiotensin-converting enzyme inhibitor; Diabetes; Nephropathy; Urinary albumin excretion; Immunohistochemistry

1. Introduction

Angiotensin-converting enzyme inhibitors have been shown to preserve the renal function in diabetic patients with nephropathy (Anderson et al., 1989; Lewis et al., 1993; Rodby et al., 1995; Sowers and Epstein, 1995). In experimental rodent models of diabetes mellitus, advanced lesions were reported to mimic some findings of the early-stage clinical diabetic nephropathy (Rasch and Mogensen, 1980; Sassy-Prigent et al., 1995) and angiotensin-converting enzyme inhibitors have also been shown to attenuate renal diseases (Anderson et al., 1989; Sassy-Prigent et al., 1995). In human (Drury, 1983) and animal (Mogensen and Christensen, 1984; Tikkanen et al., 1998) studies, diabetic nephropathy is commonly associated with hypertension. It is well established that hypertension aggravates diabetic nephropathy (Mogensen, 1982). However, as shown in a meta-analysis of clinical trials, angiotensin-converting enzyme inhibitors reduce proteinuria and attenuate the progression of renal failure in patients with diabetic nephropathy more effectively than treatment with conventional antihypertensive drugs such as Ca^{2+} channel antagonists and β-adrenoceptor antagonists (Bohlen et al., 1994; Gansevoort et al., 1995). Moreover, angiotensin-converting enzyme inhibition seems to be effective in slowing down the progression of human diabetic nephropathy through mechanisms not related to blood pressure (Bjorck et al., 1992; Lewis et al., 1993; Mathiesen et al., 1991). Upregulation of the local tissue renin–angiotensin system in response to injury has been shown in a variety of renal diseases (Anderson et al., 1993; Gilbert et al., 1999; Johnson et al., 1992). These findings leave

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unanswered the question whether the renoprotective effect of an angiotensin-converting enzyme inhibitor was directly related to the inhibition of angiotensin II or to its eventual antihypertensive effect.

In the present study, we investigated the effects of angiotensin-converting enzyme inhibitors imidapril and captopril on renal disease, angiotensin-converting enzyme activity and blood pressure in mice with diabetes induced by streptozotocin. In addition, we applied an immunohistochemical technique to determine the localization of angiotensin-converting enzyme in the kidney of diabetic mice.

2. Materials and methods

2.1. Drugs

Imidapril was synthesized in Tanabe Seiyaku (Saitama, Japan). Streptozotocin and captopril were purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Animal experiments

All experiments were reviewed and approved by the Committee on Ethics of Animal Experiments, Tanabe Seiyaku, and conducted according to the Guidelines for Animal Experiments of Tanabe Seiyaku. Nine-week-old male C57Bl/6 mice (Japan Clea, Tokyo, Japan) were housed in a specific pathogen-free facility and were maintained on standard mouse chow and tap water ad libitum. Under light ether anesthesia, the mice were injected into the tail vein with a bolus injection of 200 mg/kg of streptozotocin dissolved in citrate buffer (pH 4.8). After 4 days, induction of diabetes was confirmed by measurement of the tail blood glucose level using the glucose oxidase method (New Blood-Sugar test; Boehringer Mannheim, Mannheim, Germany), and hyperglycemic mice with levels >300 mg glucose/dl were used. The diabetic mice were randomly divided into the following five groups (10 animals in each group) treated with vehicle (distilled water), imidapril (1 and 5 mg/kg), and captopril (10 and 50 mg/kg). Ten non-diabetic mice, which had not been injected with streptozotocin, were treated with vehicle. Vehicle or each drug in a volume of 10 ml/kg was given orally to mice by gastric gavage in the morning once a day.

Body weight was measured weekly. The systolic arterial pressure and heart rate of each mouse were measured by the tail-cuff method (UR-5000; Ueda, Tokyo, Japan) before and after the 28-day period of treatment. Blood glucose was measured before treatment and after 14 and 28 days of treatment. On the 29th day, the mice in each group were anesthetized with ether and blood samples were taken from the abdominal aorta. Bilateral kidneys were rapidly removed and weighed. Half the middle portion of the left kidney was immediately fixed for 60 min with methacarn solution for the estimation of glomerular morphometry, or embedded in the Tissue Tek (Miles, Elkhart, IN, USA), and frozen for immunohistochemical analysis of angiotensin-converting enzyme. The rest of the kidney was frozen in liquid nitrogen and stored at -80°C until the measurement of angiotensin-converting enzyme activity.

2.3. Measurements of urinary albumin level and renal function

On Days 14 and 28, the mice were detained in individual metabolic cages for 24 h for urine collection. The urine volume was measured gravimetrically, and urinary albumin concentrations were determined with an enzyme-linked immunosorbent assay using a murine microalbuminuria kit (Albuwell M; Exocell, Philadelphia, PA, USA).

Renal function was evaluated by calculating creatinine clearance (ml/min/100 g body weight). The plasma- and urinary-creatinine levels were measured by an enzymatic method (CRE; Mizuho medy, Saga, Japan) using the autoanalyzer Hitachi 7150 (Hitachi, Tokyo, Japan). The blood (serum) urea nitrogen levels were measured by using the autoanalyzer.

2.4. Morphometric analysis of glomerular

Half middle portion of left kidney was fixed with methacarn solution, and embedded in paraffin. Four-micrometer-thick slices were stained with periodic acid-Schiff. In periodic acid-Schiff stained section of the renal cortex, the glomerular tuft area was determined by the total glomerular area minus the urinary space area and the urinary recesses, as previously described (Sassy-Prigent et al., 1995). More than 30 glomeruli were counted per kidney and the average was used for analysis.

2.5. Measurement of renal angiotensin-converting enzyme activity

Assay of angiotensin-converting enzyme activity was performed on 8–10 mice from each group. Renal angiotensin-converting enzyme activities were measured with tissue samples taken on the 29th day of treatment by using a fluorometric assay described by Cheung and Cushman (1973). The angiotensin-converting enzyme activity was calculated as nanomoles of His–Leu generated per milligram tissue weight/hour.

2.6. Immunohistochemistry for angiotensin-converting enzyme

The frozen renal tissues of five randomly selected mice in the non-diabetic and diabetic groups were cut into 5-μm-thick slices, and mounted on slides. To detect angiotensin-converting enzyme, the slices were fixed in ace-

Table 1
Body weight and blood glucose concentration of streptozotocin-induced diabetic mice before and after treatment with angiotensin-converting enzyme inhibitors
The non-diabetic mice were treated with vehicle. The diabetic mice were randomly divided into the following five groups treated with vehicle (distilled water), imidapril (1 and 5 mg/kg), and captopril (10 and 50 mg/kg). Values are means ± S.E.M.

Group	Dose (mg/kg)	*n*	Body weight (g)	Plasma glucose (mg/dl)
Non-diabetic	–	10		
Day 0			23.2 ± 0.3 *	155.6 ± 4.3 *
Day 28			25.0 ± 0.3 *	148.5 ± 4.9 *
Diabetic	–	10		
Day 0			21.8 ± 0.3	462.2 ± 22.3
Day 28			18.7 ± 0.6	431.2 ± 16.8
Diabetic + imidapril	1	10		
Day 0			21.9 ± 0.3	463.0 ± 17.2
Day 28			18.8 ± 0.7	453.4 ± 18.0
Diabetic + imidapril	5	10		
Day 0			22.0 ± 0.4	463.5 ± 17.2
Day 28			17.6 ± 0.8	413.7 ± 30.1
Diabetic + captopril	10	10		
Day 0			21.9 ± 0.3	462.8 ± 13.8
Day 28			18.6 ± 0.6	449.4 ± 11.5
Diabetic + captopril	50	9		
Day 0			21.9 ± 0.2	464.0 ± 14.2
Day 28			19.0 ± 0.7	493.1 ± 24.2

* $P < 0.01$ compared with the diabetic group.

tone, and incubated with 1 μg/ml of the anti-human angiotensin-converting enzyme monoclonal antibody (9B9; Chemicon, Temecula, CA, USA) for 60 min at room temperature. The samples were subsequently incubated with rabbit anti-mouse immunoglobulin (Ig) G, and then, with a mouse alkaline phosphatase anti-alkaline phosphatase immune complex. The slide was washed in Tris (pH 7.6)-buffered saline after each step. Bound alkaline phosphatase was visualized by new fuchsin and levamisole to yield a red reaction product. All sections were counterstained with hematoxylin.

2.7. Statistical analysis

Data are expressed as means ± S.E.M. Statistical analysis was done by SuperANOVA (Abacus Concepts, Berkeley, CA, USA) on a MacIntosh computer. Comparison between non-diabetic and diabetic mice was performed by Student's *t*-test. Differences among the diabetic groups were analyzed by using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test for multiple comparison. A P value of 0.05 or less was considered statistically significant.

3. Results

In the diabetic mice, two mice in imidapril (5 mg/kg)-treated group and one mouse in captopril (50 mg/kg)-treated group died spontaneously between the 21st and 29th day of treatment. No mice in the other experimental groups died during the period of treatment.

3.1. Body weight, blood glucose and blood pressure

Diabetes was associated with reduced weight gains and increased plasma glucose levels which were not influenced by the imidapril and captopril treatments (Table 1).

Fig. 1 shows the systolic blood pressure of each group at 28 days after the start of drug treatment. The systolic blood pressure of the diabetic group was slightly, but not significantly higher than that of the non-diabetic group. The systolic blood pressure was significantly reduced by treatment with either imidapril or captopril. The blood pressure lowing by captopril was greater than that by imidapril.

3.2. Renal hypertrophy and function

Urine volume and kidney weight were increased in the diabetic group compared with non-diabetic group (Table 2). Treatment with imidapril and captopril did not influence these parameters. Glomerular tuft areas in diabetic mice were increased (Table 2), but morphometric analysis showed no apparent mesangial sclerosis in diabetic mice

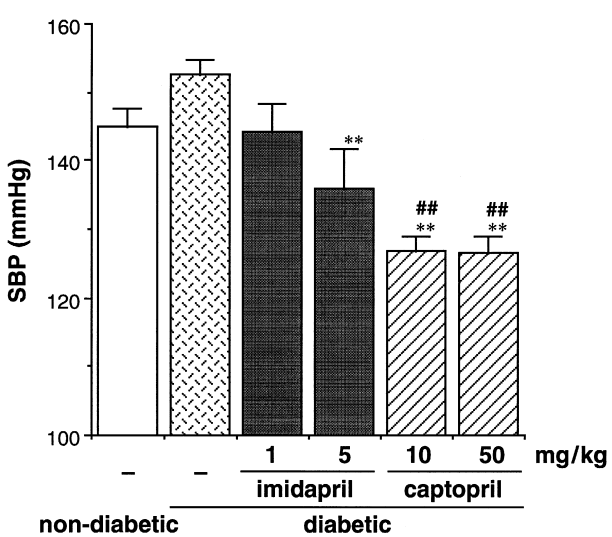

Fig. 1. Systolic arterial pressure in the non-diabetic, diabetic, diabetic + imidapril (1 and 5 mg/kg) and diabetic + captopril (10 and 50 mg/kg) groups after the 28-day treatment in streptozotocin-induced diabetic mice. Values are means ± S.E.M. $n = 8–10$. * * $P < 0.01$ compared with the diabetic group. ## $P < 0.01$ compared with the diabetic + imidapril (1 mg/kg) group.

Table 2
Urine volume, kidney weight and creatinine clearance after the 28-day treatment with angiotensin-converting enzyme inhibitors in streptozotocin-induced diabetic mice
The non-diabetic mice were treated with vehicle. The diabetic mice were randomly divided into the following five groups treated with vehicle (distilled water), imidapril (1 and 5 mg/kg), and captopril (10 and 50 mg/kg). Values are means ± S.E.M. $n = 8–10$. Kidney weight indicates total weight of left and right kidneys.

Group	Dose (mg/kg)	Urine volume (ml/24 h)	Kidney weight/ body weight (mg/g)	Glomerular tuft area (μm^2)	Creatinine clearance (ml/min/100 g body weight)
Non-diabetic	–	1.7 ± 0.1*	11.86 ± 0.11*	2257 ± 31*	1.53 ± 0.06*
Diabetic	–	26.7 ± 1.7	19.84 ± 0.72	3055 ± 69	2.57 ± 0.09
Diabetic + imidapril	1	26.1 ± 2.8	18.37 ± 0.53	3034 ± 55	2.63 ± 0.25
Diabetic + imidapril	5	22.9 ± 2.8	19.62 ± 0.64	3040 ± 58	3.08 ± 0.22
Diabetic + captopril	10	25.3 ± 1.2	20.12 ± 0.55	2985 ± 45	2.46 ± 0.16
Diabetic + captopril	50	25.7 ± 2.5	18.58 ± 0.61	2922 ± 31	2.22 ± 0.16

* $P < 0.01$ compared with the diabetic group.

(data not shown). Blood urea nitrogen (mg/dl) and serum creatinine (mg/dl) were not affected in the diabetic group (31 ± 1 and 0.09 ± 0.01, respectively) compared with non-diabetic group (30 ± 1 and 0.10 ± 0.00, respectively). Creatinine clearance increased clearly in the diabetic group, but showed no further change in the mice treated with either imidapril or captopril (Table 2).

3.3. Effects on urinary albumin excretion

The urinary albumin excretion level of each group on Day 28 is shown in Fig. 2. The urinary albumin excretion level of the diabetic group was higher than that of the non-diabetic group. The urinary albumin excretion level was dose-dependently reduced by treatment with either imidapril or captopril. This urinary albumin excretion-reducing effect was not different between the treatments with the two angiotensin-converting enzyme inhibitors. There were no significant differences in urinary albumin excretion levels on Day 14 among the diabetic mice treated with drug (data not shown).

3.4. Activity and immunolocalization of angiotensin-converting enzyme

Renal angiotensin-converting enzyme activity was decreased significantly in the diabetic group compared with the non-diabetic group, and was further decreased by treatment with imidapril and captopril. The angiotensin-converting enzyme activity of the imidapril-treated groups was much lower than that of the captopril-treated groups (Fig. 3).

In the non-diabetic mice, immunoreactivity for angiotensin-converting enzyme was slightly present in the endothelium of the renal vein and glomeruli (Fig. 4A and

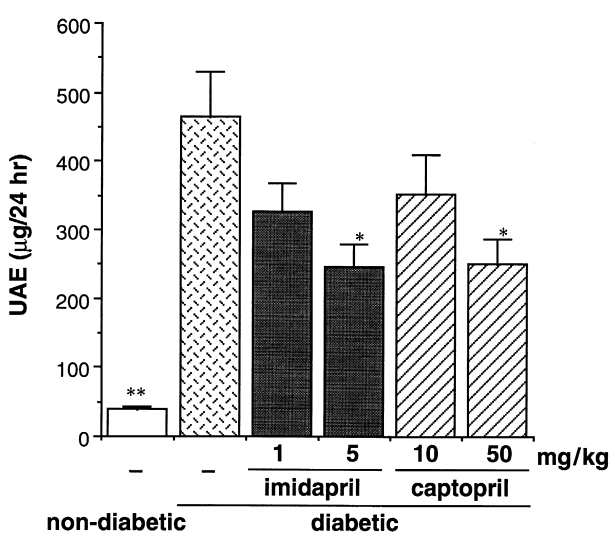

Fig. 2. Urinary albumin excretion in the non-diabetic, diabetic, diabetic + imidapril (1 and 5 mg/kg) and diabetic + captopril (10 and 50 mg/kg) groups after the 28-day treatment in streptozotocin-induced diabetic mice. Values are means ± S.E.M. $n = 9–10$. * $P < 0.05$ compared with the diabetic group.

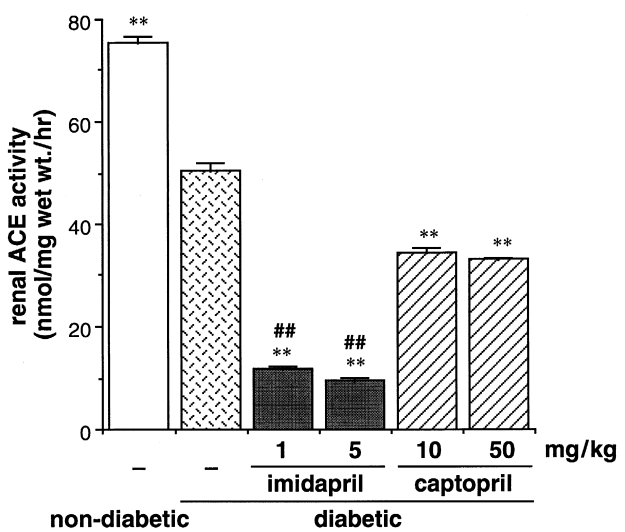

Fig. 3. Renal angiotensin-converting enzyme activity in the non-diabetic, diabetic, diabetic + imidapril (1 and 5 mg/kg) and diabetic + captopril (10 and 50 mg/kg) groups after the 28-day treatment in streptozotocin-induced diabetic mice. Values are means ± S.E.M. $n = 8–10$. ** $P < 0.01$ compared with the diabetic group. ## $P < 0.01$ compared with the diabetic + captopril (50 mg/kg) group.

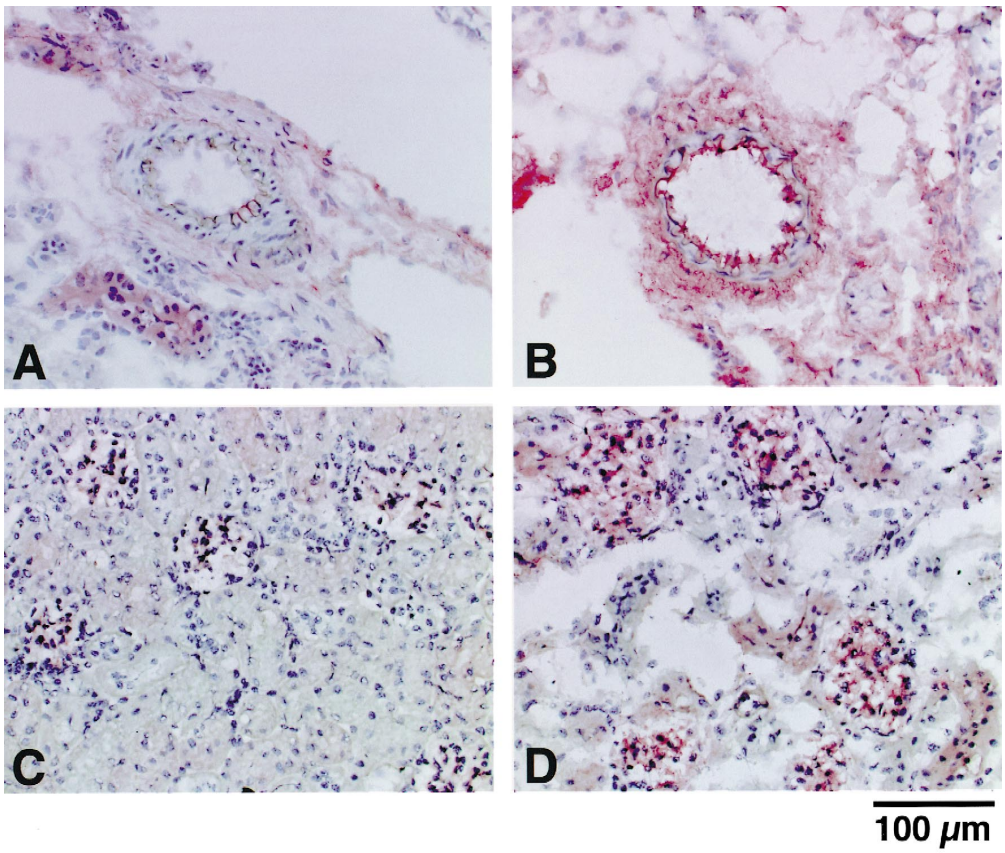

Fig. 4. Immunohistochemical localization of angiotensin-converting enzyme in the renal arteries (A and B) and glomeruli (C and D) sacrificed on the 33rd day after streptozotocin treatment. (A and C) Sections from the kidney of the non-diabetic group. (B and D) Sections from the kidney of the diabetic group. Bar indicates 100 μm.

C). On Day 29, both the endothelium of renal arteries and the glomeruli in the diabetic group were intensely immunoreactive to the angiotensin-converting enzyme antibody (Fig. 4B and D), but the intensity of staining for angiotensin-converting enzyme in the tubules was slightly decreased. No immunoreactivity was noted when the angiotensin-converting enzyme antibody was replaced with the non-immune IgG (negative control).

4. Discussion

The present study demonstrates two major findings. First, the angiotensin-converting enzyme inhibitors imidapril and captopril equally prevented the elevation of urinary albumin excretion levels in streptozotocin-induced diabetic mice with high blood glucose, but showed differences in the potency of reducing blood pressure and angiotensin-converting enzyme activity. Second, renal angiotensin-converting enzyme activity was significantly decreased in the diabetic group compared with the non-diabetic group, but on immunohistochemical analysis, both the endothelium and the glomeruli of the diabetic kidney were intensely immunoreactive to the angiotensin-converting enzyme antibody.

Imidapril (1 and 5 mg/kg) showed a dose-dependent inhibitory effect on the elevation of urinary albumin excretion levels in a pattern identical to the effect seen with captopril (10 and 50 mg/kg) in the diabetic mice. Systemic hypertension is a well-known cause of progressive renal injury in both humans (Drury, 1983) and experimental animals (Mogensen and Christensen, 1984; Tikkanen et al., 1998). In the present study using the diabetic mice, the antihypertensive efficacy of captopril was stronger than that of imidapril. Therefore, the hypotension did not only account for the inhibitory effects of these angiotensin-converting enzyme inhibitors on the urinary albumin excretion level in diabetic mice. On the other hand, inhibition of renal angiotensin-converting enzyme activity by imidapril was greater than that by captopril in the diabetic mice. The reason for this difference was not clear, but some angiotensin-converting enzyme inhibitors, which equally inhibit circulating angiotensin-converting enzyme, have shown major differences in affinity for tissue angiotensin-converting enzyme (Cushman et al., 1989; Nakajima et al., 1992; Ruzicka et al., 1995). Thus, our observations suggest that the renoprotective effects of the angiotensin-converting enzyme inhibitors may be attributable to both hypotension and inhibition of renal angiotensin-converting enzyme activity.

Another explanation for the effects of the angiotensin-converting enzyme inhibitors on the urinary albumin excretion level is the amelioration of permeability properties of the glomerular basement membrane. The permeability of glomerular basement membrane is dependent on both size-selectivity and charge-selectivity. Morelli et al. (1990) reported that angiotensin-converting enzyme inhibition diminished glomerular permeability to proteins by enhancing barrier size-selectivity measuring by the dextran fractional clearance in humans with diabetic glomerulopathy. Cartwright and Jaenke (1988) demonstrated that captopril prevented the loss of glomerular basement membrane anionic binding sites using cationic ferritin tracer probe method in rats with unilateral nephrectomy. Therefore, the protection against the alteration of glomerular basement membrane may be related to the mechanisms by which imidapril and captopril exert their beneficial effects in the present study.

Angiotensin II has a stimulatory effect on transforming growth factor-ß production (Gibbons et al., 1992; Kagami et al., 1994) and there is evidence that angiotensin II increases extracellular matrix production in the diabetic kidney (Gilbert et al., 1998; Rumble et al., 1998). These results suggest that one of these effects may be involved in the therapeutic effect of angiotensin-converting enzyme inhibitors in diabetic nephropathy. Angiotensin-converting enzyme was shown to be the rate-limiting step for the biological action of the renin–angiotensin system (Cambien et al., 1992; Nyström et al., 1997). In addition, tissue angiotensin-converting enzyme activity correlates positively with the tissue angiotensin II levels (Müller et al., 1997). Therefore, to explore the role of angiotensin-converting enzyme, we compared the activity and immunolocalization of renal angiotensin-converting enzyme in normal and diabetic mice. As a result, renal angiotensin-converting enzyme activity in the diabetic mice was significantly lower than that in normal mice. However, angiotensin-converting enzyme immunostaining intensity was enhanced in the glomeruli and renal vasculature of the diabetic kidney. Thus, we speculate that in these sites in diabetes, the increase in local tissue angiotensin-converting enzyme leads to increased local angiotensin II. These results, which coincide with the results of a study on streptozotocin-induced nephropathy in rats (Anderson et al., 1993), suggest that the findings of reduced whole kidney angiotensin-converting enzyme activity do not necessarily imply a uniform reduction of tissue angiotensin-converting enzyme activity in those sites (e.g. glomeruli and vasculature), and that the intrarenal angiotensin-converting enzyme may be involved in the development of nephropathy in streptozotocin-induced diabetic mice.

We showed in this study that imidapril and captopril reduced the increase in the urinary albumin excretion level, but did not affect creatinine clearance and renal hypertrophy. These results are consistent with previous reports (Tikkanen et al., 1998; Yotsumoto et al., 1997), which investigated the effects of angiotensin-converting enzyme inhibitors administered for 4 or 8 weeks in streptozotocin-induced diabetic models. However, some authors (Gilbert et al., 1998; Sassy-Prigent et al., 1995), who treated the animals with angiotensin-converting enzyme inhibitors for 12 and 24 weeks, found that the angiotensin-converting enzyme inhibitors inhibited glomerular hyperfiltration and/or renal hypertrophy. Especially, Sassy-Prigent et al. (1995) showed that the angiotensin-converting enzyme inhibitors inhibited the increase in urinary albumin excretion but not the renal hypertrophy at 4 weeks in streptozotocin-induced diabetic rats. Thus, we speculate that the lack of effects of the angiotensin-converting enzyme inhibitors on increased creatinine clearance and renal hypertrophy may be due to the duration of treatment in our study. Furthermore, using morphometric techniques, we found that the glomerular tuft areas were increased, but mesangial sclerosis did not occur in diabetic mice. We showed that the blood urea nitrogen and serum creatinine levels were not increased in diabetic mice. These results suggest that mice, 4 weeks after the streptozotocin injection, may be in early stage of diabetic nephropathy. Further studies are needed to clarify the influence of other variables known to modulate the angiotensin-converting enzyme.

In conclusion, we found that the angiotensin-converting enzyme inhibitors in doses equipotent for reduction of the increase in urinary albumin excretion level showed different effects on blood pressure and renal angiotensin-converting enzyme activity. Our results in the present study support the concept that the prevention by angiotensin-converting enzyme inhibitors of the development of nephropathy in experimental diabetes may involve both the decreased blood pressure and inhibition of renal angiotensin-converting enzyme activity. In addition, the data suggest that the angiotensin-converting enzyme inhibitors imidapril and captopril may exhibit major differences in their mechanisms of urinary albumin excretion inhibition in diabetic mice.

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References

Anderson, S., Rennke, H.G., Garcia, D.L., Brenner, B.M., 1989. Short and long term effects of antihypertensive therapy in the diabetic rat. Kidney Int. 36, 526–536.

Anderson, S., Jung, F.F., Ingelfinger, J.R., 1993. Renal renin–angiotensin system in diabetes: functional, immunohistochemical, and molecular biological correlations. Am. J. Physiol. 265, F477–F486.

Bjorck, S., Mulec, H., Johnsen, S.A., Norden, G., Aurell, M., 1992. Renal protective effect of enalapril in diabetic nephropathy. Br. Med. J. 304, 339–343.

Bohlen, L., de Courten, M., Weidmann, P., 1994. Comparative study of the effect of ACE-inhibitors and other antihypertensive agents on proteinuria in diabetic patients. Am. J. Hypertens. 7, 84s–92s.

Cambien, F., Poirier, O., Lecerf, L., Evans, A., Cambou, J.P., Arveiler, D., Luc, G., Bard, J.M., Bara, L., Richard, S., Tiret, L., Amouyel, P., Alhenc-Gelas, F., Soubrier, F., 1992. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 359, 641–644.

Cartwright, M.E., Jaenke, R.S., 1988. Effects of dietary protein and captopril on glomerular permselectivity in rats with unilateral nephrectomy. Lab. Invest. 59, 492–499.

Cheung, H.S., Cushman, D.W., 1973. Inhibition of homogenous angiotensin-converting enzyme of rabbit lung by synthetic venom peptides of *Bothrops jararaca*. Biochim. Biophys. Acta 293, 451–463.

Cushman, D.W., Wang, F.L., Fung, W.C., Harvey, C.M., DeForrest, J.M., 1989. Differentiation of angiotensin-converting enzyme (ACE) inhibitors by their selective inhibition of ACE in physiologically important target organs. Am. J. Hypertens. 2, 294–306.

Drury, P.L., 1983. Diabetes and arterial hypertension. Diabetologia 4, 1–9.

Gansevoort, R.T., Sluiter, W.J., Hemmelder, M.H., de Zeeuw, D., de Jong, P.E., 1995. Antiproteinuric effect of blood-pressure-lowering agents: a meta-analysis of comparative trials. Nephrol., Dial., Transplant. 10, 1963–1974.

Gibbons, G.H., Pratt, R.E., Dzau, V.J., 1992. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. J. Clin. Invest. 90, 456–461.

Gilbert, R.E., Cox, A., Wu, L.L., Allen, T.J., Hulthen, U.L., Jerums, G., Cooper, M.E., 1998. Expression of transforming growth factor-beta1 and type IV collagen in the renal tubulointerstitium in experimental diabetes: effects of ACE inhibition. Diabetes 47, 414–422.

Gilbert, R.E., Wu, L.L., Kelly, D.J., Cox, A., Wilkinson-Berka, J.L., Cooper, M.E., 1999. Pathological expression of renin and angiotensin II in the renal tuble after subtotal nephrectomy. Implications for the pathogenesis of tubulointerstitial fibrosis. Am. J. Pathol. 155, 429–440.

Johnson, R.J., Alpers, C.E., Yoshimura, A., Lombardi, D., Pritzl, P., Floege, J., Schwartz, S.M., 1992. Renal injury from angiotensin II-mediated hypertension. Hypertension 19, 464–474.

Kagami, S., Border, W.A., Miller, D.E., Noble, N.A., 1994. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. J. Clin. Invest. 93, 2431–2437.

Lewis, E.J., Hunsicker, L.G., Bain, R.P., Rohde, R.D., 1993. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N. Engl. J. Med. 329, 1456–1462.

Mathiesen, E.R., Hommel, E., Giese, J., Parving, H.H., 1991. Efficacy of captopril in postponing nephropathy in normotensive insulin dependent diabetic patients with microalbuminuria. Br. Med. J. 303, 81–87.

Mogensen, C.E., 1982. Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. Br. Med. J. 285, 685–688.

Mogensen, C.E., Christensen, C.K., 1984. Predicting diabetic nephropathy in insulin-dependent patient. N. Engl. J. Med. 311, 89–93.

Morelli, E., Loon, N., Meyer, T., Peters, W., Myers, B.D., 1990. Effects of converting-enzyme inhibition on barrier function in diabetic glomerulopathy. Diabetes 39, 76–82.

Müller, D.N., Bohlender, J., Hilgers, K.F., Dragun, D., Costerousse, O., Ménard, J., Luft, F.C., 1997. Vascular angiotensin-converting enzyme expression regulates local angiotensin II. Hypertension 29, 98–104.

Nakajima, T., Yamada, T., Setoguchi, M., 1992. Prolonged inhibition of local angiotensin-converting enzyme after single or repeated treatment with quinapril in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 19, 102–107.

Nyström, F., Karlberg, B.E., Öhman, K.P., 1997. Serum angiotensin-converting enzyme activity correlates positively with plasma angiotensin II: a population-based study of ambulatory blood pressure and the renin–angiotensin system. J. Hum. Hypertens. 11, 301–306.

Rasch, R., Mogensen, C.E., 1980. Urinary excretion of albumin and total protein in normal and streptozotocin diabetic rats. Acta Endocrinol. 95, 376–381.

Rodby, R.A., Rohde, R., Evans, J., Bain, R.P., Mulcahy, W.S., Lewis, E.J., 1995. The study of the effect of intensity of blood pressure management on the progression of type 1 diabetic nephropathy: study design and baseline patient characteristics, Collaborative Study Group. J. Am. Soc. Nephrol. 5, 1775–1781.

Rumble, J.R., Gilbert, R.E., Cox, A., Wu, L., Cooper, M.E., 1998. Angiotensin converting enzyme inhibition reduces the expression of transforming growth factor-beta1 and type IV collagen in diabetic vasculopathy. J. Hypertens. 16, 1603–1609.

Ruzicka, M., Skarda, V., Leenen, F.H., 1995. Effects of ACE inhibitors on circulating versus cardiac angiotensin II in volume overload-induced cardiac hypertrophy in rats. Circulation 92, 3568–3573.

Sassy-Prigent, C., Heudes, D., Jouquey, S., Auberval, D., Belair, M.F., Michel, O., Hamon, G., Bariety, J., Bruneval, P., 1995. Morphometric detection of incipient glomerular lesions in diabetic nephropathy in rats. Protective effects of ACE inhibition. Lab. Invest. 73, 64–71.

Sowers, J.R., Epstein, M., 1995. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. An update. Hypertension 26, 869–879.

Tikkanen, T., Tikkanen, I., Rockell, M.D., Allen, T.J., Johnston, C.I., Cooper, M.E., Burrell, L.M., 1998. Dual inhibition of neutral endopeptidase and angiotensin-converting enzyme in rats with hypertension and diabetes mellitus. Hypertension 32, 778–785.

Yotsumoto, T., Naitoh, T., Shikada, K., Tanaka, S., 1997. Effects of specific antagonists of angiotensin II receptors and captopril on diabetic nephropathy in mice. Jpn. J. Pharmacol. 75, 59–64.