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Short communication

Imidapril inhibits increased transforming growth factor-β1 expression in remnant kidney model

Akinori Hamaguchi [a], Shokei Kim [a,*], Hideki Wanibuchi [b], Hiroshi Iwao [a]

[a] *Department of Pharmacology, Osaka City University Medical School, Osaka 545, Japan*
[b] *First Department of Pathology, Osaka City University Medical School, Osaka 545, Japan*

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Abstract

To elucidate the effect of imidapril, an angiotensin-converting enzyme inhibitor, on molecular events in progressive glomerulosclerosis, we administered imidapril to 5/6 nephrectomized rats and measured the glomerular expression of genes for transforming growth factor (TGF)-β1, fibronectin and collagen IV. Glomerular TGF-β1, fibronectin and collagen IV mRNAs in nephrectomized rats were significantly higher than those in sham-operated rats. Treatment with imidapril for 10 weeks significantly reduced the enhanced glomerular expression of TGF-β1 and collagen IV mRNA in nephrectomized rats, and prevented the associated proteinuria and glomerulosclerosis. Thus, imidapril may arrest progressive glomerulosclerosis by inhibiting the expression of TGF-β1 and collagen IV.

Keywords: Angiotensin-converting enzyme inhibitor; TGF-β1 (transforming growth factor-β1); Extracellular matrix; Glomerulosclerosis; Gene expression

1. Introduction

The rat remnant kidney model is the most studied model of progressive glomerular disease and is characterized by increased glomerular capillary pressure and accumulation of extracellular matrix in remnant glomeruli (Tolins and Raij, 1991). A recent study reported that an angiotensin-converting enzyme inhibitor is highly effective in ameliorating the development of proteinuria and sclerosis in this model (Anderson et al., 1986). However, the effect of an angiotensin-converting enzyme inhibitor on molecular and cellular events in the development of glomerular injury has not been elucidated.

Transforming growth factor-β1 (TGF-β1) is a multifunctional growth factor regulating the production of extracellular matrix proteins (Border and Noble, 1993). The expression of TGF-β1 and extracellular matrix is increased in several models of kidney disease (Border and Noble, 1993) and inhibition of TGF-β1 by neutralizing antibody decreases the accumulation of extracellular matrix in glomerulonephritis (Border et al., 1990). However, the role of TGF-β1 and extracellular matrix expression in chronic glomerular disease remains unclear.

In this study, we sought to examine the glomerular expression of TGF-β1 and extracellular matrix genes in rats with subtotal renal ablation, and also examine the long-term effect of an angiotensin-converting enzyme inhibitor on the expression of these genes.

2. Materials and methods

2.1. Drug

Imidapril, a long-acting angiotensin-converting enzyme inhibitor, was a gift from Tanabe Seiyaku (Osaka, Japan).

2.2. Experimental procedure

Five-week-old male Sprague-Dawley rats were used in this study. Animals were fed on standard rat chow (CE2, Clea, Tokyo, Japan) and given tap water ad libitum.

Rats were subjected to 5/6 renal ablation as previously described (Hamaguchi et al., 1996). At 2 weeks after the surgery, 5/6 nephrectomized rats were orally given (1) vehicle ($n = 10$) or (2) imidapril (5 mg/kg per day, $n = 10$) by gastric gavage once a day. Drug treatment of 5/6 nephrectomized rats was performed for 10 weeks (from 2 to 12 weeks after 5/6 nephrectomy). Concur-

* Corresponding author. Tel.: (81-6) 645-2036; Fax: (81-6) 646-1980.

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rently, the sham-operated rats ($n = 7$) served as control. Systolic blood pressure and 24 h urine protein were measured before and 3, 7, and 10 weeks after start of drug treatment. After 10 weeks of drug treatment, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the kidney was removed. For histological study, a part of the kidney was fixed, as described below. Then, glomeruli were isolated from the rest of the kidney by a sieving method, rapidly frozen in liquid nitrogen and stored at -80°C until use.

2.3. Renal morphology

The kidney was fixed in methyl Carnoy's solution (60% methanol, 30% chloroform, 10% acetic acid), embedded in paraffin and cut into 3 μm thick sections. The sections were stained with periodic acid-Schiff. Histological studies were performed by a pathologist (H.W.) in a blinded fashion. Glomerulosclerosis was assessed in a semiquantitative way with scores (grade 0 to +4), as previously described (Hamaguchi et al., 1996). More than 50 glomeruli were analyzed in the kidney of each rat.

2.4. cDNA probes

cDNA probes used were as follows: rat TGF-β1 cDNA (1.0 kb *Hin*dIII–*Xba*I fragment) (Qian et al., 1990); rat fibronectin cDNA (0.27 kb *Hin*dIII–*Eco*RI fragment) (Schwarzbauer et al., 1983); mouse α1(IV) collagen (0.83 kb *Aba*I–*Pst*I fragment) (Oberbaumer et al., 1985); rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1.3 kb *Pst*I–*Pst*I fragment) (Fort et al., 1985).

2.5. Extraction of glomerular RNA and northern blot analysis

All procedures were performed, as previously described (Kim et al., 1994). In brief, total RNA was extracted from isolated glomeruli by the guanidium thiocyanate–phenol–chloroform method with minor modification. 12 μg of total RNA was electrophoresed on 1% agarose gel and transferred to a nylon membrane (GeneScreen Plus, EI DuPont de Nemours NEN, Wilmington, DE, USA). The membrane was hybridized with the above mentioned ^{32}P-dCTP-labeled cDNA probes, then washed and finally exposed to Kodak XAR-5 film at -70°C. To evaluate tissue mRNA levels, autoradiograms were digitized to measure density, using the public domain NIH image program. The density of an individual mRNA band was divided by that of GAPDH mRNA, to correct for differences in RNA loading and transfer to a nylon membrane.

2.6. Miscellaneous measurements

Systolic blood pressure was measured by the tail-cuff method. Urinary protein was measured by using a protein assay kit (Pierce).

2.7. Statistics

Data are expressed as means ± S.E.M. Statistical significance was determined with an analysis of variance and Duncan's multiple range test. Differences were considered statistically significant at a value of $P < 0.05$.

3. Results

3.1. Effect of imidapril on blood pressure, urinary protein excretion and glomerulosclerosis

As shown in Fig. 1A, at the start of drug treatment, systolic blood pressure of 5/6 nephrectomized rats (131 ± 4 mmHg) was slightly higher than that of sham-operated rats (122 ± 2 mmHg) and there was no difference in

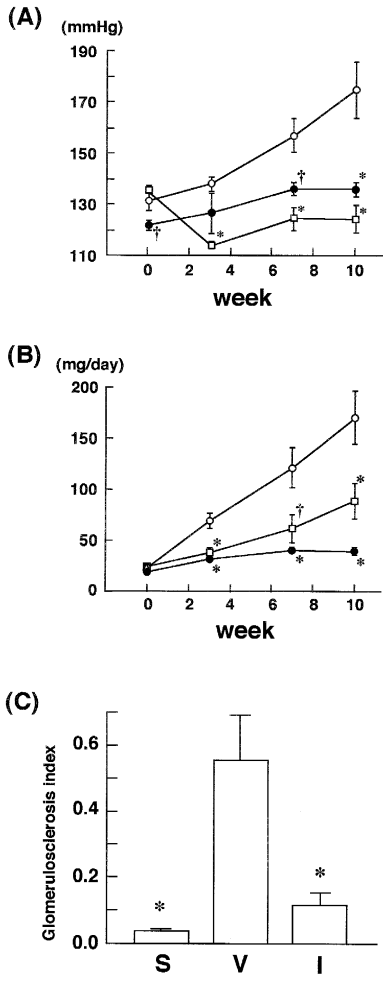

Fig. 1. (A,B) Line graphs showing the time-course of systolic blood pressure (A) and urinary protein excretion (B). (C) Bar graph showing the glomerulosclerosis index of sham-operated rats and nephrectomized rats after 10 weeks of drug treatment. Abbreviations and symbols are: S and ●, sham-operated rats ($n = 7$); V and ○, vehicle-treated nephrectomized rats ($n = 10$); I and □, imidapril-treated nephrectomized rats ($n = 10$). Each value is mean ± S.E.M.; * $P < 0.01$, † $P < 0.05$ compared with the vehicle-treated nephrectomized rats.

systolic blood pressure between vehicle and imidapril-treated groups. Systolic blood pressure in 5/6 nephrectomized rats progressively increased (137 ± 3, 157 ± 6 and 174 ± 11 mmHg at 3, 5, and 10 weeks, respectively). However, this increased systolic blood pressure of 5/6 nephrectomized rats was significantly lowered by treatment with imidapril. As shown in Fig. 1B, imidapril also significantly decreased proteinuria in 5/6 nephrectomized rats. As shown in Fig. 1C, glomerulosclerosis was observed in 5/6 nephrectomized rats. The glomerulosclerosis score in vehicle-treated 5/6 nephrectomized rats was 15-fold greater than that in sham-operated rats at 10 weeks after start of treatment. Treatment with imidaril for 10 weeks significantly reduced the sclerosis index in 5/6 nephrectomized rats.

3.2. Effect of imidapril on glomerular mRNA expression

At the start of drug treatment, glomerular TGF-β1, fibronectin and collagen type IV mRNA levels in 5/6 nephrectomized rats were already 3.5, 6.2 and 3.2-fold, respectively, higher than those in sham-operated rats (data not shown). These mRNA levels in 5/6 nephrectomized rats also remained higher than those in sham-operated rats at 10 weeks after treatment (Fig. 2). Administration of imidapril for 10 weeks significantly reduced glomerular mRNA levels for TGF-β1 and collagen type IV but not for fibronectin in 5/6 nephrectomized rats (Fig. 2).

4. Discussion

One of the most effective therapies in glomerular disease is inhibition of the renin–angiotensin system (RAS) (Tolins and Raij, 1991). In the rat remnant kidney model, angiotensin-converting enzyme inhibitors have been shown to reduce proteinuria and prevent sclerosis of the remnant glomeruli: the effects are believed to be mediated by the hemodynamic actions of angiotensin-converting enzyme inhibitors which include normalization of systemic and glomerular hypertension (Anderson et al., 1986; Tolins and Raij, 1991). Our present study was focused on molecular events in progressive glomerular injury induced by renal ablation and we demonstrated for the first time the effect of an angiotensin-converting enzyme inhibitor on glomerular gene expression in this model.

In this study, we found that glomerular TGF-β1 was already significantly increased in 5/6 nephrectomized rats at 2 weeks after renal ablation without severe hypertension. This increase was associated with enhanced fibronectin and collagen IV expression. Interestingly, long-term treatment with imidapril prevented enhanced

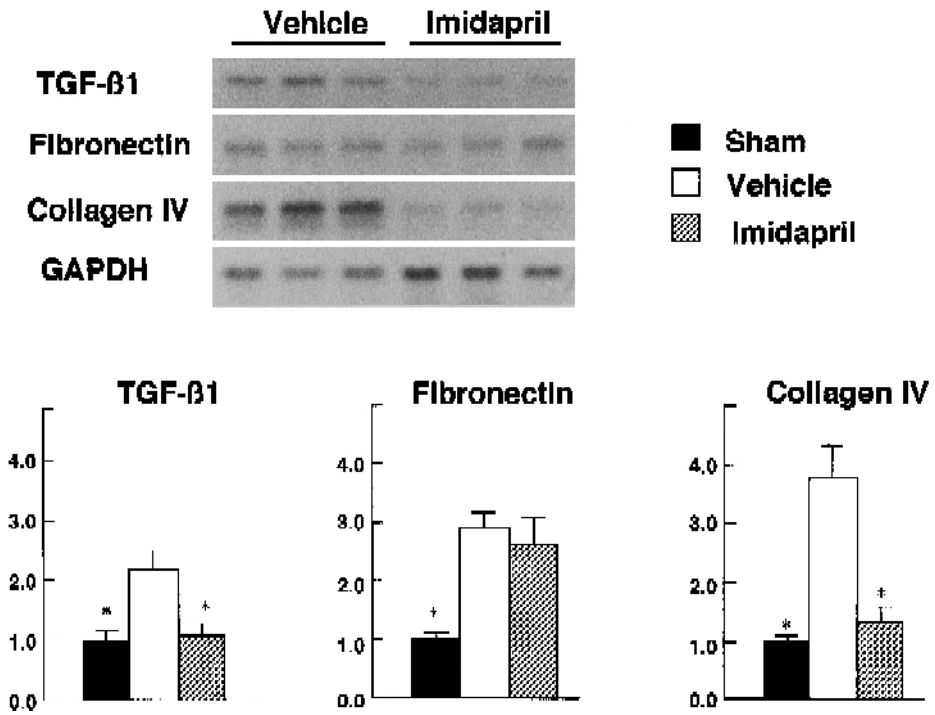

Fig. 2. Effects of imidapril on glomerular mRNA levels in 5/6 nephrectomized rats. Upper panel shows typical autoradiograms of glomerular mRNA for transforming growth factor-β1 (TGF-β1, 2.5 kb), fibronectin (7.9 kb), collagen IV (6.8 kb) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.4 kb) in each group. Bar graphs show glomerular TGF-β1, fibronectin and collagen IV mRNA levels, corrected for GAPDH mRNA levels, in each group. Abbreviations are: Sham, sham-operated rats ($n = 7$); Vehicle, vehicle-treated nephrectomized rats ($n = 10$); Imidapril, imidapril-treated nephrectomized rats ($n = 10$). The mean value in Sham is represented as 1. Each value is mean ± S.E.M.; * $P < 0.01$ compared with the vehicle-treated nephrectomized rats.

glomerular TGF-β1 and collagen IV expression in 5/6 nephrectomized rats. Furthermore, these inhibitory effects were associated with a reduction of urinary protein excretion and histological improvement of sclerosis. Thus, our results suggest that blockade of TGF-β1 and collagen IV gene expression may contribute to limiting glomerular injury. The fibronectin mRNA level in 5/6 nephrectomized rats was not affected by imidapril. Our previous data indicate that the increase in fibronectin precedes the accumulation of other extracellular matrix components in kidney disease (Hamaguchi et al., 1995). In vitro study has shown that fibronectin acts as a scaffold for collagen deposition (Vaheri et al., 1985). Thus, the role of fibronectin in glomerular injury differs from that of other extracellular matrix components, and it may play a role in the accumulation of extracellular matrix in glomerular injury.

The present study did not allow us to elucidate the mechanism of inhibition of TGF-β1 expression by imidapril. It is possible that the inhibition of TGF-β1 might be secondary to the hypotensive effect of imidapril. However, our present study showed that glomerular TGF-β1 was already increased in 5/6 nephrectomized rats without severe hypertension (at 2 weeks after 5/6 nephrectomy). Furthermore, we have previously shown that angiotensin-converting enzyme inhibitor decreases renal TGF-β1 gene expression in deoxycorticosterone acetate-salt hypertensive rats, despite there being no decrease in blood pressure, and that this inhibition of TGF-β1 is associated with a reduction of urinary protein excretion and histological improvement of renal injury (Kim et al., 1994). Accumulating evidence indicates that RAS exists not only in the circulation but also within the kidney and that intrarenal angiotensin II concentrations are over 100-fold higher than plasma levels (Seikaly et al., 1990; Ingelfinger and Dzau, 1991), thereby supporting the notion that intrarenal RAS plays a critical role in renal disease. Furthermore, in vitro studies, with mesangial cells, have shown that angiotensin II has a direct action as a growth factor, stimulates TGF-β1 and collagen gene expression, and causes glomerular cell proliferation and hypertrophy (Wolf and Neilson, 1993; Kagami et al., 1994). These findings suggest that the inhibition of glomerular TGF-β1 by imidapril may be mediated mainly by the suppression of intrarenal RAS rather than by its hypotensive effect. However, further study is needed to elucidate our proposal.

In conclusion, we found that imidapril attenuates the increase in glomerular TGF-β1 and collagen IV mRNA expression in the remnant kidney model. These inhibitory effects were associated with a reduction of urinary protein excretion and an improvement of sclerosis. Therefore, the mechanism by which imidapril inhibits glomerulosclerosis may be in part through a decrease in the expression of TGF-β1 and collagen IV mRNA.

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