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Effect of angiotensin-converting enzyme inhibition on growth factor mRNA in chronic renal allograft rejection in the rat

Attila Szabo, Jens Lutz, Karina Schleimer, Balazs Antus, Peter Hamar, Thomas Philipp, and Uwe Heemann

Departments of Nephrology and Surgery, University Hospital Essen, Germany, and Semmelweis Medical University, Budapest, Hungary

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Background. Despite considerable progress in immunosuppression, the incidence of chronic renal allograft rejection has not decreased. Recent studies have revealed that angiotensin-converting enzyme (ACE) inhibition ameliorates graft arteriosclerosis, glomerulosclerosis, and tubular atrophy. Moreover, it decreases systemic and glomerular capillary hydrostatic pressure in a rat kidney allograft model. We evaluated the effects of the ACE inhibitor enalapril on cytokine and growth factor expression in chronically rejecting rat kidney allografts.

Methods. Kidneys of Fisher (F344) rats were orthotopically transplanted into Lewis (Lew) rats. To prevent acute rejection, cyclosporine A (1.5 mg/kg/day) was given to all recipients during the first 10 days after transplantation. Enalapril (60 mg/L) or vehicle was added to the drinking water 10 days after transplantation. Animals were harvested 20 weeks after transplantation for histologic and immunohistologic studies, as well as for evaluation of cytokine and growth factor mRNA by semiquantitative polymerase chain reaction.

Results. Controls developed severe signs of chronic rejection, such as glomerular and vascular lesions, associated with a large number of infiltrating leukocytes. Enalapril-treated animals developed less proteinuria and other signs of chronic rejection. The mRNA levels of transforming growth factor-β_1 (TGF-β_1), platelet-derived growth factor A and B chain (PDGF A and B), insulin-like growth factor-I (IGF-I), interleukin-1 (IL-1), and monocyte chemoattractant protein-1 (MCP-1) were significantly reduced in the enalapril group and were most pronounced for IL-1 and PDGF A. In addition, we found an increased level of renal angiotensinogen mRNA after treatment with enalapril.

Conclusions. Treatment with enalapril attenuated the development of proteinuria, ameliorated morphological damage, decreased leukocyte infiltration, and prevented a rise in renal mRNA levels of growth factors and cytokines in kidney grafts in a rat model of chronic renal allograft rejection.

Key words: ACE inhibition, growth factors, chronic renal rejection, enalapril.

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In recent years, chronic rejection has become a major cause of late renal graft loss after the first year [1]. Although chronic renal allograft rejection has been traditionally regarded as a repeated low-grade response to allogeneic tissue, recent evidence indicates that alloantigen-independent factors may also contribute to its pathogenesis [2–7]. These factors include surgical trauma, ischemia/reperfusion injury, cytomegalovirus infection, and donor age.

Regardless of etiology, these factors contribute to the reduction of nephrons in the single transplanted kidney as compared with an intact organ, consequently inducing an imbalance between allograft nephron supply and the demands of the recipient, thereby stimulating hyperfunction and hyperfiltration in the remaining nephrons [8]. The remaining glomeruli have to adapt to the changed microcirculation, resulting in an increased mean driving force for glomerular capillary blood flow and capillary pressure, and therefore a marked increase in the glomerular filtration rate (GFR) [9, 10]. This hyperfiltration may result in endothelial activation followed by an upregulation of adhesion molecules [such as, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)] and a release of cytokines [interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and monocyte chemoattractant protein-1 (MCP-1)] and growth factors [insulin-like growth factor-I (IGF-I), platelet-derived growth factor (PDGF), and transforming growth factor-β_1 (TGF-β_1)], which in turn induce cell migration and proliferation of leukocytes and smooth muscle cells (SMCs), and play a causative role in extracellular matrix accumulation [11]. Moreover, the local (endothelial) and systemic renin-angiotensin system may be activated, thus causing posttransplant hypertension.

Treatment with angiotensin-converting enzyme (ACE) inhibitors may be able to delay the progression of tubulointerstitial fibrosis and glomerulosclerosis of the kidney in hypertensive patients with chronic renal disease (hy-

pertensive nephropathy) or after renal transplantation [12–14]. The increased efficacy of ACE inhibitors in experimental models and clinical trials as compared with other antihypertensive drugs is probably caused by a reversal of angiotensin II-induced increase in resistance at the efferent arteriole [15–17], and the influence on growth and migration promoting cytokines [18]. The therapeutic effects of ACE inhibitors are independent of their systemic antihypertensive effects since they can be observed in normotensives [19, 20]. Moreover, they reduce proteinuria more effectively in chronic renal disease compared with β blockers or calcium antagonists while having the same blood pressure-lowering effect [21].

Angiotensin II acts as a growth factor for glomerular and tubular cells [22] and may directly induce collagen synthesis, an effect mediated by TGF-β1 and PDGF-A [18]. Additionally, angiotensin II stimulates the adhesion of mononuclear cells to mesangial cells and the chemotaxis of macrophages and lymphocytes [23]. Therefore, in renal transplant recipients, ACE inhibition may not only effectively lower systemic blood pressure, but may also be antiproliferative and antimigrative as it reduces the release of cytokines and growth factors [16–25].

Our present study was designed to determine the effects of the ACE inhibitor enalapril on the occurrence and progression of chronic renal allograft rejection in the Fischer/Lewis model, as a standard transplant model for chronic rejection of the kidney in rats. This model is characterized by tubular atrophy, interstitial fibrosis, intimal thickening of graft arteries, and glomerulosclerosis accompanied by severe proteinuria. Moreover, we investigated the effects of ACE inhibition on the mRNA levels of growth factors and cytokines in rats with chronic kidney allograft rejection.

METHODS

Animals

Experiments were performed with male inbred Fisher (F344) and Lewis (Lew) rats with a body weight between 180 and 250 g. All animals were obtained from Charles River (Sulzfeld, Germany) and housed in standard plastic cages and maintained in a temperature- and humidity-controlled environment. The animals had access to standard chow food and tap water ad libitum. All experiments were approved by a governmental committee on animal welfare.

Renal transplantation

Fisher (F344) rats served as donors, and Lew rats served as recipients. Under ketamine (100 mg/kg body wt, intraperitoneally; Ketamin 10%; cp-pharma, Burgdorf, Germany), plus xylacine (10 mg/kg body wt, i.p.; Rompun®; Bayer, Leverkusen, Germany) anesthesia, the left kidney of each F344 donor rat was isolated, excised, and perfused with 4°C cold Ringer lactate. The kidneys were then positioned orthotopically in anesthetized, weight-matched Lew recipients whose left renal vessels had been isolated, clamped, and the native kidney removed. Donor and recipient renal artery, vein, and ureter were anastomosed end to end using 10-0 prolene sutures. The contralateral native kidney was removed on the 10th postoperative day.

Experimental protocol

To overcome infectious complications caused by operation and immunosuppression, rats received Cephtriaxone (Rocephin®, 20 mg/kg/day; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) during the first 10 postoperative days. To prevent an initial episode of acute rejection, rats received 1.5 mg/kg/day cyclosporine A (Novartis GmbH, Nürnberg, Germany) for the first 10 postoperative days. Transplanted animals received either normal drinking water (controls, $N = 10$) or 60 mg/L enalapril (MSD, Haar, Germany; enalapril group, $N = 10$). As additional controls, a group of 12 Lew rats was sham operated and treated with cyclosporine A for 10 days.

Body weights and proteinuria were measured once per month. After 20 weeks, rats were anesthetized with diethylether and bled, and the transplanted kidneys were removed. Samples were snap frozen in liquid nitrogen for immunohistological staining and polymerase chain reaction (PCR) or fixed in formalin (4%) for light microscopy.

Functional measurements

Twenty-four–hour urine samples were collected every four weeks. Urinary protein and creatinine excretion, as well as serum creatinine concentrations, was determined photometrically with commercially available test kits, and the creatinine clearance was calculated at the end of the study.

Histology

For histology, kidney tissues were fixed in 4% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The periodic acid-Schiff reaction was performed to evaluate the extent of glomerulosclerosis. It was defined as a collapse of capillaries, adhesion of the obsolescent segment of Bowman's capsule, and the entrapment of hyaline [26]. At least 200 glomeruli were counted in each kidney section, and the proportion of sclerosed to total glomeruli was expressed as a percentage.

Antibodies

Monoclonal antibodies against macrophages (ED1), CD5+ T-lymphocytes (OX19), ICAM-1 (CD-54), and VCAM-1 (CD-106) were purchased from Serotec Ca-

mon Labor-Service GmbH (Wiesbaden, Germany). The secondary rabbit antimouse antibody and the alkaline phosphatase antialkaline phosphatase (APAAP) complex were obtained from Dako A/S (Hamburg, Germany).

Immunohistology

Representative portions of kidney grafts were snap frozen in liquid nitrogen, cut with a cryostat (4 mm), fixed in acetone at 4°C for five minutes, air dried, and stained with the respective antibodies. After incubation with the primary antibody, the sections were incubated with rabbit antimouse IgG and with the APAAP complex. ICAM-1– and VCAM-1–like immunoreactivity were quantitated on a grade 1 to 3 scale (1 = mild, 2 = moderate, 3 = strong). Positive cell counts for macrophages (ED1) and T-lymphocytes (OX19) were expressed as a mean ± SEM of cells per field of view (c/FV); >20 FV/section/specimen were evaluated at ×400.

Total RNA isolation

Total RNA was extracted and used for reverse transcriptase-PCR (RT-PCR). Kidney tissue was stored in 500 mL of cold lysis solution containing 4 mol/L guanidine isothiocyanate (Sigma, St. Louis, MO, USA), 25 mmol/L sodium citrate (pH 7.0), 0.1 mol/L β-mercaptoethanol, 0.5% sarcosyl and frozen in liquid nitrogen. Total RNA was extracted from the kidneys according to the modified guanidine-isothiocyanate preparation [27]. Briefly, frozen tissues were mixed with 4 mL GITC buffer (4 mol/L guanidine isothiocyanate; Sigma) and acid phenolchloroform (pH 4, Roth), and homogenized. The samples were centrifuged at 1500 *g* for 10 minutes at 20°C. The supernatant was added to an equal volume of isopropanol followed by centrifugation. The RNA was purified with the Rneasy, Total RNA Isolations Kit (Qiagen GmbH, Hilden, Germany), and stored at −80°C until further processing. The RNA concentration was measured spectrophotometrically.

Reverse transcription

RNA was amplified by RT with an Oligo$(dT)_{12\text{-}18}$ primer (GIBCO BRL, Karlsruhe, Germany). One milligram of total RNA was added to 0.5 mg of primer. A reaction mixture containing buffer solution [Tris hydrochloride buffer in a concentration of 50 mmol/L (pH 8.3), potassium chloride in a concentration of 75 mmol/L, magnesium dichloride in a concentration of 5 mmol/L, dithiothreitol in a concentration of 5 mmol/L; GIBCO BRL], adenosine triphosphate, thymidine triphosphate, guanosine triphosphate, and cytosine triphosphate each in a concentration of 0.2 mmol/L (deoxynucleoside triphosphates from Boehringer Mannheim GmbH, Mannheim, Germany), 0.5 mL of 40 U/mL of recombinant ribonuclease inhibitor (Promega, Madison, WI, USA), and 0.5 mL of 200 U/mL M-MLV reverse transcriptase (GIBCO BRL) was added, and the first chain reaction was allowed to proceed (36°C, 1 h). The reaction was halted by heating to 95°C for five minutes followed by cooling on ice.

Amplification of specific complementary DNA

Specific complementary DNA (cDNA) products corresponding to mRNA for TGF-β_1 [28], PDGF-A chain [29], MCP-1 [30], IGF-I [31], IL-1 [32], angiotensinogen [33], and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [34] were amplified using PCR. One milliliter from the RT reaction was taken for PCR, which was performed in PCR buffer (750 mmol/L Tris hydrochloride, pH 9.0, 200 mmol/L $(NH_4)_2SO_4$, 0.1% (wt/vol) Tween 20, 20 mmol/L magnesium dichloride (Dianova, Hamburg, Germany) using 0.2 mmol/L of each deoxynucleoside triphosphates, 1 mmol/L of both primers (Eurogentec, Köln, Germany), and 2.5 U thermus Aquaticus (Taq) DNA polymerase (Dianova, Hamburg, Germany). A Perkin Elmer Thermal Cycler (Model 2400; Perkin Elmer, Norwalk, CT, USA) was used for amplification with the following sequence profile: initial denaturation at 94°C for three minutes, followed by 30 to 35 cycles of three temperature PCRs (denaturing, 94°C for 30 s; annealing, 55°C for 30 s; and extension, 72°C for 30 s), ending with a final extension at 72°C for seven minutes and cooling to 4°C.

Gel electrophoresis

The amplified PCR product was identified by electrophoresis of 10 mL sample aliquots on 1.5% agarose gel stained with 0.5 mg/mL of ethidium bromide. The sample products were visualized by ultraviolet transillumination, and the gel was photographed. Specific products were identified by size in relationship to a known 1 kb oligonucleotide DNA ladder (GIBCO BRL) run with each gel. Cytokine cDNA was semiquantitated by densitometric comparison with GAPDH (internal control) from the same sample after the positive image was digitized by video for computerized densitometry. The results are given as the ratio of intensity of growth factors or cytokines to GAPDH mRNA ± SEM.

Statistical analysis

Results are expressed as mean ± SEM. For statistical analysis, a paired Student's *t*-test was used. Nonmetric parameters such as the intensity of staining for adhesion molecules were evaluated with Fischer's exact test for ordinal data [35]. A P value of <0.05 was considered to be statistically significant.

Table 1. Urinary protein excretion in controls and enalapril treated rats four to 20 weeks after transplantation

Group	24-Hour protein excretion *mg/dl*				
	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
Controls $N = 10$	23 ± 9.2	34 ± 15.7	37 ± 19.8	37 ± 28.9	71 ± 7.3
Enalapril $N = 10$	24 ± 13.7	20 ± 5.7	26 ± 5.7	24 ± 15.1	27 ± 12.2[a]
Sham-operated $N = 10$	16 ± 4.1	13 ± 2.9	19 ± 4.8	23 ± 3.3	25 ± 8.9

[a] $P < 0.05$ controls vs. enalapril

RESULTS

Functional measurements

While controls developed significant and progressive urinary protein excretion after 16 weeks, enalapril-treated recipients had minimal urinary protein excretion during the entire 20-week follow-up period (at 20 weeks 48 ± 3.6 vs. 23 ± 0.73 mg/24 h, $P < 0.05$; Table 1). Creatinine clearance did not differ significantly between the two groups at the end of the 20-week follow-up period (controls 1.5 ± 0.1 mL/min vs. treated 1.6 ± 0.1 mL/min). Sham-operated animals did not develop any significant proteinuria.

Mean arterial blood pressure

The mean arterial blood pressure was not significantly different between control and enalapril-treated animals (94 ± 10.2 vs. 90 ± 8.9 mm Hg).

Body weight

Body weight tended to be lower in controls as compared with enalapril-treated animals (419 ± 15 vs. 429 ± 11 g).

Histology

All transplants developed changes of chronic rejection such as glomerulosclerosis, interstitial fibrosis, tubular atrophy, and intimal proliferations of graft arteries during the observation period [11, 36, 37]. However, the degree differed between the groups; treatment with enalapril significantly ameliorated signs of chronic rejection, particularly glomerulosclerosis (Table 2 and Fig. 1).

Glomerulosclerosis. Glomerulosclerosis was significantly higher in controls (26 ± 1.7%) than in enalapril-treated animals (19 ± 1.8%, $P < 0.05$).

Interstitial fibrosis. Animals in the control group had significantly more interstitial fibrosis as compared with the enalapril-treated animals ($P < 0.05$). In the control group, one animal had no signs of interstitial fibrosis. Three had grade 1 fibrosis. Six had grade 2, and no animals developed grade 3 fibrosis. In the enalapril-treated group, five animals had no signs of interstitial fibrosis. Five animals had grade 1 fibrosis, and no animal displayed grades 2 or 3 fibrosis (Table 2).

Tubular atrophy. There was a trend toward more severe tubular atrophy in the control group as compared with the enalapril-treated group. In the control group, three animals had no tubular atrophy. Four animals had grade 1, and three animals showed a grade 2 tubular atrophy. In the enalapril-treated group, five animals had no tubular atrophy. Five had grade 1, and no animal displayed a grade 2 or 3 tubular atrophy (Table 2). These results did not differ statistically between the two groups.

Intimal proliferation. There was a trend toward more severe intimal proliferation in controls as compared with enalapril-treated animals. In the control group, two animals did not demonstrate intimal proliferation. Six animals had grade 1, and two grade 2 intimal proliferation. In enalapril-treated animals, grade 1 intimal proliferation was evident in four animals, while all others had no intimal proliferation. These results were not statistically different between the two groups (Table 2).

Immunohistology. At week 20, mononuclear cell infiltration was apparent in both groups. Cellular infiltration was most intense in interstitial areas, particularly around vessels and glomeruli. Whereas some lymphocytes surrounded Bowman's capsule, almost none were observed within the glomeruli. Only macrophages could be detected within the glomerular tuft. The number of macrophages and lymphocytes was significantly reduced in the treated group as compared with controls (Table 3).

The presence of leukocytes was associated with a strong immunoreactive signal of adhesion molecules (ICAM-1, VCAM-1). The ICAM-1–like immunoreactive signal was predominantly higher on endothelial cells of small vessels, mesangial cells, and tubular epithelial cells, whereas VCAM-1–like immunoreactivity was most pronounced on tubular epithelial cells. Treatment with enalapril significantly reduced the immunoreactive signal of both adhesion molecules ($P < 0.01$; Table 3). TGF-β–like immunoreactivity was mainly found on interstitial cells within the grafts. The number of cells with TGF-β–like immunoreactivity was low in treated and control animals with a tendency toward a higher number in animals with moderate fibrosis and was confined to interstitial cells within the graft.

Sham-operated animals developed no signs of chronic rejection, neither histologically nor immunohistologically.

Table 2. Glomerulosclerosis, interstitial fibrosis, vasculopathy, and tubular atrophy according to the Banff '97 classification

Group	Glomerulosclerosis %	Interstitial fibrosis	Tubular atrophy	Intimal proliferation	Glomerulopathy
		grade = *N*			
Controls *N* = 10	26 ± 1.7	grade 0 = 1	grade 0 = 3	grade 0 = 2	grade 0 = 4
		grade 1 = 3	grade 1 = 4	grade 1 = 6	grade 1 = 4
		grade 2 = 6	grade 2 = 3	grade 2 = 2	grade 2 = 2
		grade 3 = 0	grade 3 = 0	grade 3 = 0	grade 3 = 0
Enalapril *N* = 10	19 ± 1.8[a]	grade 0 = 5	grade 0 = 5	grade 0 = 6	grade 0 = 5
		grade 1 = 5	grade 1 = 5	grade 1 = 4	grade 1 = 5
		grade 2 = 0	grade 2 = 0	grade 2 = 0	grade 2 = 0
		grade 3 = 0	grade 3 = 0	grade 3 = 0	grade 3 = 0
Sham-operated *N* = 10	5 ± 0.4[a]	grade 0 = 9	grade 0 = 10	grade 0 = 10	grade 0 = 9
		grade 1 = 1	grade 1 = 0	grade 1 = 0	grade 1 = 1
		grade 2 = 0	grade 2 = 0	grade 2 = 0	grade 2 = 0
		grade 3 = 0	grade 3 = 0	grade 3 = 0	grade 3 = 0

[a] $P < 0.05$ controls vs. enalapril

Growth factor mRNA levels

Growth factor mRNA levels paralleled the development and progression of interstitial fibrosis and glomerulosclerosis [3, 37, 38] 20 weeks after transplantation. PDGF-A chain mRNA levels were fourfold higher in controls than in enalapril-treated animals. TGF-β_1 mRNA levels in the enalapril-treated group were 1.6-fold lower than in controls. Additionally, treatment with enalapril significantly reduced the mRNA levels of PDGF-B chain and IGF-I (1.5-fold; Fig. 2).

The mRNA levels of MCP-1, a potent chemotactic factor specific for monocytes/macrophages and a contributor of chronic inflammatory responses [39], was also significantly lower in the enalapril-treated group (Fig. 2). MCP-1 mRNA levels paralleled the number of mononuclear cells infiltrating the graft.

The expression of the monocyte/macrophage product IL-1β mRNA, also implicated in the development of sclerosis and fibrosis, was significantly reduced (2.5-fold) in the enalapril-treated group as compared with controls (Fig. 2). As with MCP-1, the decreased level of IL-1β mRNA correlated to a decreased number of macrophages. Angiotensinogen mRNA levels were significantly higher in enalapril-treated animals as compared with controls ($P < 0.05$; Fig. 2).

DISCUSSION

The beneficial effects of ACE inhibition on the progression of renal disease and chronic renal allograft rejection have been demonstrated in different animal models [25, 40–42]. However, the precise mechanisms of action remained unknown. Our present study demonstrated decreased levels of growth factor mRNA in transplanted rat kidneys following ACE inhibition, associated with attenuation of chronic renal allograft rejection. The very short time and low dose of cyclosporine A used in our model render the possibility that the observed histologic changes are due to cyclosporine A nephrotoxicity highly unlikely. Thus, it came as no surprise that sham-operated animals did not develop any signs of chronic rejection. This confirms the results of our previously published experiments [43, 44].

In our study, controls developed massive 24-hour protein excretion 20 weeks after transplantation. The administration of enalapril resulted in a significantly reduced 24-hour protein excretion. The mechanisms involved are hypothesized to be an intraglomerular and/or a direct ACE inhibitory effect on glomerular cells. In rats, intraglomerular hypertension is thought to induce protein loss by the formation of large, nonselective pores in the glomerular wall, prior to the development of glomerulosclerosis [45]. Micropuncture studies suggest that lowering the glomerular capillary hydraulic pressure by ACE inhibition reduces proteinuria by a reduction of the number of large pores in the glomerular wall [14, 46]. Therefore, this effect might protect the kidney from further damage and thus prevent the progression of renal disease. In addition, Amann et al demonstrated in a subtotal nephrectomy model in rats that ACE inhibitors, but not other antihypertensive drugs, prevented podocyte injury closely associated with the reduction of proteinuria [47]. Lewis et al demonstrated a more pronounced reduction of proteinuria in patients with chronic renal disease with ACE inhibitors as compared with patients treated with calcium antagonists or β blockers, although the blood pressure-lowering effect was similar in all groups [21].

In controls of the present experiment, progressive glomerulosclerosis and severe glomerular as well as interstitial infiltration of macrophages and lymphocytes were observed, whereas these changes were markedly reduced in the enalapril group. These findings could be related to a modification of proliferative and immunomodulatory signals mediated by angiotensin II. Angiotensin II has been reported to have immunoregulating effects, such

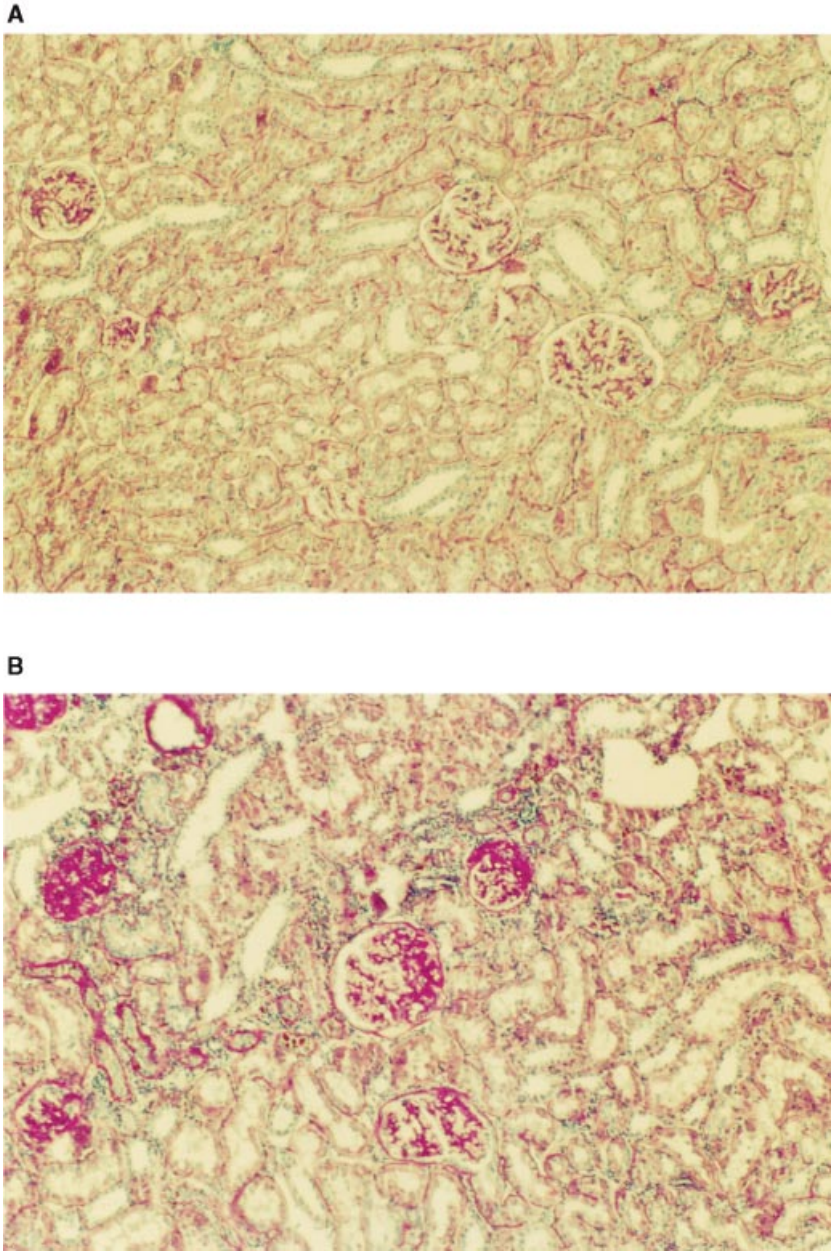

Fig. 1. Effect of enalapril on development of glomerulosclerosis and cell infiltration. Representative sections of kidney allografts from enalapril-treated (*A*) and untreated (*B*) animals (PAS reaction; ×100).

Table 3. Lymphocyte and macrophage infiltration, and quantification of extracellular matrix deposition for adhesion molecules (ICAM-1 and VCAM-1) in transplanted kidney

	Lymphocytes	Macrophages	ICAM-1	VCAM-1
Group	*cells/FV*		*grade = N*	
Controls $N = 10$	58 ± 2.5	67 ± 3.4	grade 1 = 1 grade 2 = 2 grade 3 = 7	grade 1 = 1 grade 2 = 2 grade 3 = 7
Enalapril $N = 10$	46 ± 1.9[a]	32 ± 2.2[a]	grade 1 = 4 grade 2 = 4 grade 3 = 2	grade 1 = 4 grade 2 = 5 grade 3 = 1
Sham-operated $N = 10$	12 ± 3.8	15 ± 4.3	grade 1 = 8 grade 2 = 2 grade 3 = 0	grade 1 = 7 grade 2 = 3 grade 3 = 0

Abbreviation FV is field of view.
[a] $P < 0.01$ controls vs. enalapril; differences in ICAM-1 and VCAM-1 were statistically different ($P < 0.01$)

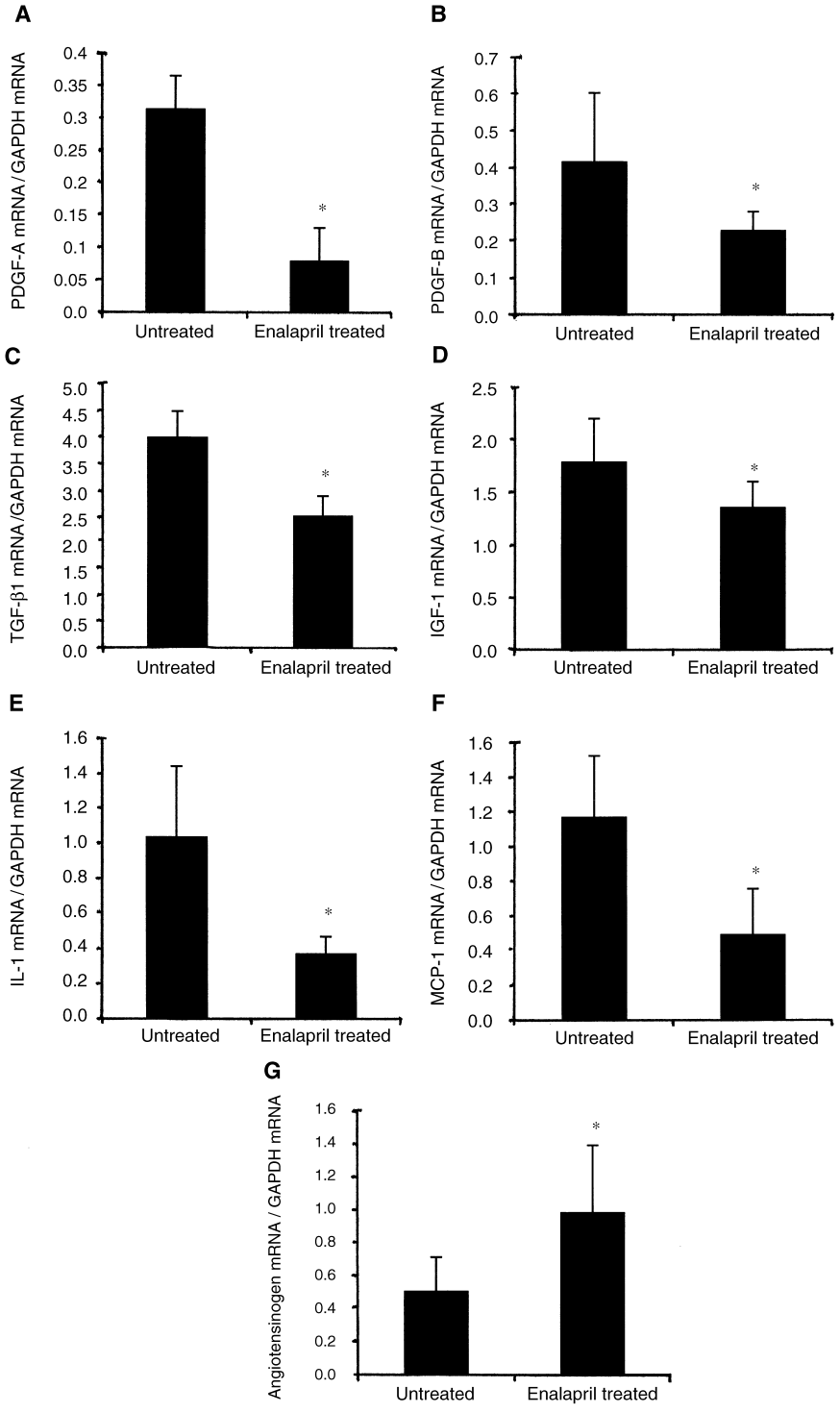

Fig. 2. Growth factor and cytokine mRNA levels in renal allografts 20 weeks after transplantation ($N = 10$). Panels are: (*A*) platelet-derived growth factor (PDGF)-A chain; (*B*) PDGF-B chain; (*C*) transforming growth factor-β_1 (TGF-β_1); (*D*) insulin-like growth factor-I (IGF-I); (*E*) interleukin-1 (IL-1); (*F*) monocyte chemoattractant protein-1 (MCP-1); (*G*) angiotensinogen (*$P < 0.05$).

as leukocyte adhesion and chemotaxis [48, 49]. Ruiz-Ortega et al observed a significant reduction in glomerular and interstitial infiltration in rats with immune complex nephritis treated with the ACE inhibitor quinalapril [42]. However, how ACE inhibition interferes with the adhesion promoting and chemoattractant effects of angiotensin II remains unclear thus far.

Chemoattractants for monocytes/macrophages include MCP-1 and TGF-β_1. Both play a major role in chronic renal allograft rejection [39] and chronic in-

flammatory responses, as observed in transplant vasculopathy [39]. Moreover, MCP-1 may also activate or increase the expression of adhesion molecules and thus facilitate monocyte adhesion. In this experiment, similar to the observations of Nadeau, Azuma, and Tilney [39], a progressive increase in MCP-1 mRNA levels was detected in kidney allografts, preceding macrophage infiltration. In the present study, long-term enalapril treatment significantly decreased the expression of MCP-1 and TGF-β_1 levels. TGF-β–like immunoreactivity was mainly localized to interstitial cells within the graft. These results were paralleled by a significantly reduced expression of ICAM-1 and VCAM-1 in the enalapril group as compared with controls. These results could also be explained by the modification of angiotensin II effects on adhesion, migration, and proliferation of mononuclear cells [42, 50].

Theoretically, many effects of ACE inhibitors in vivo can be attributed to an inhibition of the angiotensin II production [51–53]. In our experiment, angiotensinogen was up-regulated when compared with controls. This observation supports the report of Jonsson, Frewin, and Head, who also demonstrated an up-regulation of angiotensinogen following continuous administration of an ACE inhibitor [54]. Therefore, we can assume that the conversion from angiotensin I to angiotensin II was blocked by enalapril in our experiment. The role of angiotensin II in SMC proliferation has been well established. Growth and migration of SMCs are important events in the development of intimal hyperplasia and early atherosclerosis. IGF-I, PDGF, TGF-β_1 and IL-1 are important growth factors for SMCs. Angiotensin II has been reported to elevate mRNA encoding PDGF-A chain and TGF-β_1 in cultured vascular SMCs [55]. Angiotensin II blockade prevented all of these changes, and coincubation with TGF-β_1–neutralizing antibodies prevented extracellular matrix synthesis. Angiotensin II increased the conversion of latent to active TGF-β_1 [46] and increased TGF-β_1 mRNA levels in glomeruli [46]. Moreover, chronic infusion of angiotensin II elevated glomerular capillary pressure and induced glomerulosclerosis in naive rats [56]. In the context of these studies, it is possible that glomerular capillary hypertension and therefore endothelial cell activation in transplanted rats increases glomerular angiotensin II activity and thus TGF-β_1 expression and bioactivity. In another study, angiotensin II increased the level of PDGF-A mRNA in vascular SMCs [57]. As PDGF-A and TGF-β_1 independently induce the expression of other factors such as IL-1, these studies suggest that PDGF-A chain, TGF-β_1, and IL-1 in rat glomeruli are related to the activity of angiotensin II induced by glomerular capillary pressure. The reduction of mRNA levels of growth factors in chronically rejecting kidney induced by enalapril treatment in our experiment is consistent with this hypothesis.

Furthermore, we demonstrated that enalapril also reduced the expression of IGF-I mRNA, a chemoattractant for SMCs with fibrotic activity. Recently, angiotensin II has been shown to exert proapoptotic and antiapoptotic effects in various cell types, including SMCs [55]. In addition, ACE inhibitors have been reported to enhance apoptosis that occurred in association with regression of vascular growth, as a part of their antihypertensive and/or antihypertrophic effect [58].

Apart from a reduced angiotensin II formation, ACE inhibition also increases bradykinin concentrations, with the subsequent generation of nitric oxide and prostaglandins. Thus, the beneficial effects of ACE inhibitors may not be mediated only within the kidney.

In summary, we demonstrated that the administration of the ACE inhibitor enalapril attenuates the development of severe proteinuria, ameliorates morphological lesions, and decreases leukocyte infiltration and the mRNA levels of growth factors and cytokines in the process of chronic rejection. In the future, this class of drugs could prove efficient in clinical practice to reduce the pace of chronic renal allograft rejection, thus increasing long-term renal allograft survival.

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Reprint requests to Dr. Uwe Heemann, Department of Nephrology, University Hospital Essen, D-45122 Essen, Germany.
E-mail: uwe.heemann@uni-essen.de

REFERENCES

1. Schweitzer EJ, Matas AJ, Gillingham KJ: Causes of renal allograft loss: Progress in the 1980s, challenges for the 1990s. *Ann Surg* 214:679–688, 1991
2. Tilney NL, Whitley WD, Diamond JR, Kupiec-Weglinski JW, Adams DH: Chronic rejection—An undefined conundrum. *Transplantation* 52:389–398, 1991
3. Paul LC: Chronic renal transplant loss. *Kidney Int* 47:1491–1499, 1995
4. Conolly JK, Dyer PA, Martin S: Importance of minimizing HLA-DR mismatch and cold preservation time in cadaveric renal transplantation. *Transplantation* 61:709–713, 1996
5. Heemann UW, Azuma H, Tullius SG, Schmid C, Philipp T, Tilney NL: Infections and reduced functioning mass induce chronic rejection in rat kidney allografts. *Clin Nephrol* 46:34–38, 1996
6. Burke JF, Pirsch JD, Ramos EL: Long-term efficacy and safety of cyclosporine in renal transplant patients. *N Engl J Med* 331:358–363, 1994
7. Paul LC, Benediktsson H: Post-transplant hypertension and chronic renal allograft failure. *Kidney Int* 48(Suppl 52):34–37, 1995
8. Brenner BM: Hemodynamically mediated glomerular injury and the progressive nature of glomerular disease. *Kidney Int* 23:647–652, 1983
9. Hostetter TH, Olson JL, Rennke HG, Venkatachalan MA, Brenner BM: Hyperfiltration in remnant nephrons: A potentially

adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
10. Heemann UW, Azuma H, Tullius SG, Mackenzie HS, Brenner BM, Tilney NL: The contribution of reduced functioning mass to chronic kidney allograft dysfunction in rats. *Transplantation* 58:1317–1322, 1994
11. Hancock WH, Whitley WD, Tullius SG, Heemann UW, Wasowska B, Baldwin WM III, Tilney NL: Cytokines, adhesion molecules, and the pathogenesis of chronic rejection of rat renal allografts. *Transplantation* 56:643–650, 1993
12. Ritz E, Nowacek R: Detrimental and beneficial effects of converting enzyme inhibitors on the kidney. *J Cardiovasc Pharmacol* 16(Suppl 14):S70–S75, 1990
13. Opsahl JA, Abraham PA, Keane WF: Angiotensin converting enzyme inhibitors in chronic renal failure. *Drugs* 39(Suppl 2):23–32, 1990
14. Traindl O, Falger S, Reading S, Banyai M, Liebisch B, Gisinger J, Templ E, Mayer G, Kovarik J: The effects of lisinopril on renal function in proteinuric renal transplant patient. *Transplantation* 55:1309–1313, 1993
15. Brunner FP, Thiel G, Hermle M: Long-term enalapril and verapamil in rats with reduced renal mass. *Kidney Int* 36:969–977, 1989
16. Curtis JJ, Luke RG, Whelchel JD: Inhibition of angiotensin converting enzyme in renal transplant recipients with hypertension. *N Engl J Med* 308:377–381, 1983
17. Anderson S, Jung FF, Ingelfinger JR: Renal renin-angiotensin system in diabetes: Functional, immunohistochemical, and molecular biological correlations. *Am J Physiol* 265:F477–F486, 1993
18. Itoh H, Mukoyama M, Pratt RE, Gibbons GH, Dzau VJ: Multiple autocrine growth factors modulate vascular smooth muscle growth response to angiotensin II. *J Clin Invest* 91:2268–2274, 1993
19. Müller GA, Schettler V, Müller CA, Strutz F: Prevention of progression of renal fibrosis: How far are we? *Kidney Int* 49(Suppl 54):75–82, 1996
20. Remuzzi A, Puntorieri S, Battaglia C: Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. *J Clin Invest* 85:541–549, 1990
21. Lewis EJ, Hunsicker LG, Bain RP, Rhode RD: The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Engl J Med* 329:1456–1462, 1993
22. Wolf G, Neilson EG: Angiotensin II as a renal growth factor. *J Am Soc Nephrol* 3:1531–1540, 1993
23. Hilgers KF, Mann JFE: Role of angiotensin II in glomerular injury: Lessons from experimental and clinical studies. *Kidney Blood Press* 19:254–262, 1996
24. Mimran A, Ribstein J: Angiotensin converting enzyme inhibitors and renal function. *J Hypertens* 7(Suppl 5):S3–S9, 1989
25. Benediktsson H, Chea R, Davidoff A, Paul LC: Antihypertensive drug treatment in chronic renal allograft rejection in the rat. *Transplantation* 62:1634–1642, 1996
26. Rennke HG: Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. *Am J Kidney Dis* 13:443–456, 1989
27. Chomczinsky P, Sacchi N: Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Ann Biochem* 162:156–161, 1987
28. Ando T, Okuda S, Tamaki K, Yoshitomi K, Fujishima M: Localization of transforming growth factor-β and latent transforming growth factor-β binding protein in rat kidney. *Kidney Int* 47:733–739, 1995
29. Feng L, Xia Y, Tang WW, Wilson CB: Cloning a novel form of rat PDGF A-chain with a unique 5′-UT: Regulation during development and in glomerulonephritis. *Biochem Biophys Res Commun* 194:1453–1459, 1993
30. Russel ME, Adams DH, Wyner LR, Yamashita Y, Halnon NJ, Karnovsky MJ: Early and persistent induction of monocyte chemoattractant protein-1 in rat cardiac allografts. *Proc Natl Acad Sci USA* 90:6086–6092, 1993
31. Cheng W, Reiss K, Kajstura J, Kowal K, Quaini F, Anversa P: Down-regulation of the IGF-I system parallels the attenuation in the proliferative capacity of rat ventricular myocytes during postnatal development. *Lab Invest* 72:646–655, 1995
32. Siegling A, Lehmann M, Platzer C, Emmrich F, Volk HD: A novel multispecific competitor fragment for quantitative PCR analysis of cytokine gene expression in rats. *J Immunol Methods* 177:23–28, 1994
33. Pierzchalski P, Reiss K, Cheng W, Cirielli C, Kajstura J, Nitahara JA, Rizk M, Capogrossi MC, Anversa P: p53 induces myocyte apoptosis via the activation of the renin-angiotensin system. *Exp Cell Res* 234:57–65, 1997
34. Tso JY, Sun XH, Kao TH, Reece KS, Wu R: Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. *Nucleic Acid Res* 13:2485–2497, 1985
35. Hartung J: *Lehr und Handbuch der angewandten Statistik* (6th ed). München, Wien, R. Oldenburg Verlag, 1987
36. Häyry P, Isoniemi H, Yilmaz S, Mennander A, Lemström K, Räisänen-Sokolowski A, Koskinen P, Ustinov J, Lautenschlager I, Taskinen E, Krogerus L, Aho P, Paavonen T: Chronic allograft rejection. *Immunol Rev* 134:33–81, 1993
37. Fellström BC, Larsson E: Pathogenesis and treatment perspectives of chronic graft rejection (CVR). *Immunol Rev* 134:83–98, 1993
38. Shihab FS, Tanner AM, Shao Y, Weffer MI: Expression of TGF-β1 and matrix proteins is elevated in rats with chronic rejection. *Kidney Int* 50:1904–1913, 1996
39. Nadeau KC, Azuma H, Tilney NL: Sequential cytokine dynamics in chronic rejection of rat renal allografts: Roles for cytokines RANTES and MCP-1. *Proc Natl Acad Sci USA* 92:8729–8733, 1995
40. Brunner HR: ACE inhibitors in renal disease. *Kidney Int* 42:463–479, 1992
41. Savage S, Schrier RW: Progressive renal insufficiency: The role of angiotensin converting enzyme inhibitors. *Adv Intern Med* 37:85–101, 1991
42. Ruiz-Ortega M, Gonzalez S, Seron D, Condom E, Bustos C, Largo R, Gonzalez E, Ortiz A, Egido J: ACE inhibition reduces proteinuria, glomerular lesions and extracellular matrix production in a normotensive rat model of immune complex nephritis. *Kidney Int* 48:1778–1791, 1995
43. Mackenzie HS, Tullius SG, Heemann UW, Azuma H, Rennke HG, Brenner BM, Tilney NL: Nephron supply is a major determinant of long-term renal allograft outcome in rats. *J Clin Invest* 94:2148–2152, 1994
44. Tullius SG, Heemann U, Wayne WH, Azuma H, Tilney NL: Long-term kidney isografts develop functional and morphologic changes that mimic those of chronic allograft rejection. *Ann Surg* 220:425–435, 1994
45. Yoshioko T, Shiraga H, Yoshida Y: "Intact nephrons" as the primary origin of proteinuria in chronic renal disease: Study in the rat model of subtotal nephrectomy. *J Clin Invest* 82:1614–1623, 1988
46. Kagami S, Border WA, Miller DE, Noble NA: Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-β expression in rat mesangial cells. *J Clin Invest* 93:2431–2437, 1994
47. Amann K, Nichols J, Törnig U, Schwarz M, Zeiger G, Mall G, Ritz E: Effect of ramipril, nifedipin, and monoxidin on glomerular morphology and podocyte structure in experimental renal failure. *Nephrol Dial Transplant* 11:1003–1011, 1996
48. Defraissy JF, Galanaud P, Balavoine JF, Wallon C, Dormont J: Captopril and immune regulation. *Kidney Int* 25:925–929, 1984
49. Weinstock JV, Blum AM, Kassab JT: Angiotensin II is chemotactic for a T-cell subset which can express migration factor activity in murine *Schistosomiasis mansoni. Cell Immunol* 107:180–187, 1987
50. Ichikawa I, Harris RC: Angiotensin actions in the kidney: Renewed insight into the old hormone. *Kidney Int* 40:583–596, 1991
51. Janiak P, Pillon A, Prost JF, Wilaine JP: Role of angiotensin subtype-2 receptor in neointima formation after vascular injury. *Hypertension* 20:737–745, 1992
52. Daemen MJAP, Lombardi DM, Bosman FT, Schwartz SM: Angiotensin II induces smooth muscle cell proliferation in the normal and injured arterial wall. *Circ Res* 68:450–456, 1991
53. Wong J, Rauhöft C, Dilley RJ, Agrotis A, Jennings GL, Bobik

A: Angiotensin-converting enzyme inhibition abolishes medial smooth muscle PDGF-AB biosynthesis and attenuates cell proliferation in injured carotid arteries: Relationships to neointima formation. *Circulation* 96:1631–1640, 1997

54. Jonsson JR, Frewin DB, Head RJ: The effect of captopril treatment and its withdrawal on the gene expression of the renin-angiotensin system. *Blood Press* 3:97–105, 1994
55. Yamada T, Akishita M, Pollman MJ, Gibbons GH, Dzau VJ, Horiuchi M: Angiotensin II type 2 receptor mediates vascular smooth muscle cell apoptosis and antagonizes angiotensin II type I receptor action: An in vitro gene transfer study. *Life Sci* 63:PL289–PL295, 1998
56. Miller PL, Rennke HG, Meyer TW: Glomerular hypertrophy accelerates glomerular injury in rats. *Am J Physiol* 261:459–465, 1991
57. Naftalin AJ, Pratt RE, Dzau VJ: Induction of platelet derived growth factor A chain and c-myc gene expressions by angiotensin II in cultured rat vascular smooth muscle cells. *J Clin Invest* 83:1419–1424, 1989
58. Sharifi AM, Schiffrin EL: Apoptosis in vasculature of spontaneously hypertensive rats: Effect of an angiotensin converting enzyme inhibitor and a calcium antagonist. *Am J Hypertens* 11:1108–1116, 1998