Angiotensin converting enzyme inhibition prevents development of collapsing focal segmental glomerulosclerosis in Thy-1.1 transgenic mice

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Abstract

Background. Thy-1.1 transgenic mice develop hypercellular focal and segmental glomerulosclerosis (FSGS) lesions that mimic human collapsing FSGS, in 7 days after injection with anti-Thy-1.1 antibodies. These lesions consist of proliferating parietal epithelial cells (PECs). We questioned whether the angiotensin converting enzyme inhibitor (ACE), captopril, could prevent the development of FSGS and if protection is related to the timing of drug administration.

Methods. First, we compared the effect of captopril treatment with angiotensin II-(ANGII) independent antihypertensive therapy (triple therapy). Second, we tested the effects of captopril administered over four different time intervals: days $-7$ to 0 (Ca$^{17<0}$), days $-7$ to 7 (Ca$^{7<7}$), days 0–7 (Ca$^{0<7}$) and days 3–7 (Ca$^{3<7}$) (day 0 being the day of injection of the antibody).

Results. In anti-Thy-1.1 injected control (C) mice we observed dedifferentiation and activation of podocytes, reflected by loss of ASD33 and increased expression of desmin, followed by a marked accumulation of PECs forming hypercellular lesions. PECs showed an increased expression of connective tissue growth factor (CTGF). Triple therapy or captopril pretreatment (Ca$^{17<0}$) had no significant effect on albuminuria or FSGS. In contrast, Ca$^{0<7}$ and Ca$^{3<7}$ treatment significantly lowered albuminuria and attenuated development of FSGS. The latter two treatments attenuated loss of ASD33 expression by podocytes but could not prevent increased desmin expression. In addition, these treatments reduced CTGF expression by PECs and prevented PEC proliferation.

Conclusions. ACE inhibition, but not triple therapy, prevents the development of FSGS, suggesting an important role for ANGII. ACE inhibition has a protective effect even when started 3 days after the initial podocyte insult, which is probably related to the ability of ACE-inhibition to block PEC activation and proliferation.

Keywords: angiotensin converting enzyme; collapsing glomerulopathy; connective tissue growth factor; focal segmental glomerulosclerosis; parietal epithelial cell; podocyte

Introduction

Focal and segmental glomerulosclerosis (FSGS) has become one of the most common patterns of glomerular injury encountered in human renal biopsies. According to the first descriptions, FSGS was characterized by the focal and segmental occurrence of lesions that consisted of (combinations of) mesangial sclerosis, obliteration of glomerular capillaries with hyalinosis and intracapillary foam cells, formation of adhesions between the glomerular tuft and Bowman’s capsule and podocyte hypertrophy. FSGS is not a disease entity, but rather a pattern of injury with quite diverse clinical presentation, morphology, pathogenesis and response to treatment [1].

During the last two decades, the renoprotective effects of angiotensin converting enzyme inhibitors (ACEi) in the treatment of glomerular disease have been extensively studied, and are increasingly appreciated in the treatment of progressive and chronic nephropathies including secondary forms of FSGS associated with hypertension and structural adaptive changes of the glomerulus. Although studied to a lesser extent, the salutary effects of ACEi have also been shown in patients with primary FSGS. Guidelines for the treatment of nephrotic and non-nephrotic patients with primary FSGS include good blood pressure control and the use of ACEi [and/or angiotensin II (ANGII) type I receptor antagonists] [2].
These effects are attributed to the reduction in ANGII formation. Recent studies have provided evidence that the effects of ACE cannot simply be explained by their effects on systemic blood pressure, since other antihypertensive drugs have not been equally effective [3]. It has been suggested that the superiority of ACE may be explained by their specific effects on intraglomerular pressure, and subsequent reduction of proteinuria. In addition, many studies have shown that ANGII is not merely a vasoactive agent but is also a true profibrotic cytokine capable of inducing extracellular matrix (ECM) production, inhibition of ECM degradation and induction of cellular growth by either proliferation or hypertrophy [4]. The profibrotic effects of ANGII are predominantly mediated by the induction of other cytokines or growth factors, like platelet-derived growth factor (PDGF), transforming growth factor (TGF-β) and connective tissue growth factor (CTGF).

CTGF is a down-stream mediator of TGF-β, of which the latter is considered to be the most potent profibrotic cytokine in the kidney [4]. Unfortunately, the possibility to study the mechanisms by which ACEi provide renoprotection is limited due to the lack of good animal models for primary FSGS.

Recently, we have described a mouse model of FSGS that resembles primary FSGS. We have used Thy-1.1 transgenic (tg) mice [5]. These mice ectopically express the Thy-1.1 antigen on podocytes and develop massive albuminuria after injection of anti-Thy-1.1 antibodies. The albuminuria peaks at 4–8 h after antibody injection and persists for weeks [6]. The development of the albuminuria is independent of inflammatory cells and complement. We have described the development of FSGS in this mouse model in detail [5]. In brief, affected glomeruli initially show segmental to global collapse of the capillary tuft followed by a marked increase of epithelial cells in Bowman’s space at days 3–7. FSGS lesions with tuft adhesions to Bowman’s capsule are seen at days 6–7. Early lesions resemble the collapsing variant of human FSGS. Previously, we showed that the hypercellular lesions originate from proliferating parietal epithelial cells (PECs) and we concluded that PECs play a crucial role in the development of these lesions [5]. Involvement of PECs in the development of hypercellular FSGS lesions was recently confirmed in two other mouse models of collapsing FSGS [7,8], in a study on a nephrectomy specimen of a patient with recurrence of idiopathic FSGS [9] and in two renal biopsies from patients with human immunodeficiency virus associated nephropathy (HIVAN) [10].

In the present study, we examined whether ACE inhibition by captopril is more effective than ANGII independent anti-hypertensive treatment in preventing the development of collapsing FSGS. In addition, we questioned whether protection was related to the timing of drug administration, and evaluated the role of PECs and podocytes by analysing their phenotype. In addition, we analysed the glomerular expression of CTGF mRNA.

**Methods**

**Animals**

Heterozygous Thy-1.1 tg mice (T6, T-construct mice were generated by injecting a hybrid human–mouse Thy-1.1 gene into pronuclei of zygotes of Thy-1.2 CBA x C57BL/10 mice [11]). These mice express the Thy-1.1 gene abnormally in podocytes, resulting in the presence of the Thy-1.1 antigen on the podocytes. All mice were bred in our animal facility.

**Anti-Thy-1.1 mAb**

For in vivo experiments a mouse anti-mouse Thy-1.1 mAb (19XE5: subclass IgG3) was used. 19XE5 was generated in vitro, by hollow fibre culture, purified by protein-A column affinity chromatography and concentrated (Nematology Department, Agriculture University Wageningen, the Netherlands).

**Animal experiments**

Five-week-old Thy-1.1 tg mice received an intravenous injection with 1 mg anti-Thy-1.1 mAb (19XE5) in 0.1 ml 0.9% saline solution. Transgenic mice, injected with 0.1 ml 0.9% saline solution alone, were used as controls. Mice of different groups received treatment as depicted in the experimental designs (Figures 1 and 2). The Ca<sup>-7>0</sup> and Ca<sup>-7>7</sup> mice were sacrificed at day 8. Urine of these mice was sampled at day 7. The saline, C, Ca<sup>-0>5</sup>, and Ca<sup>-5>3</sup> mice were divided in three groups. The first group was sacrificed at day 6 to examine the effect of captopril treatment on PEC proliferation. Groups 2 and 3 were sacrificed at days 8 and 22, respectively. The latter groups were used to study the albuminuria and FSGS score in either the 1st week or the following 2 weeks. The 18 h urine samples were collected at days 7, 14 and 21 after the anti-Thy-1.1 mAb injection. Tissues were collected at day 8 or day 22. The captopril-treated mice received captopril via drinking water (400 mg/l Capoten® 50, Bristol Myers Squibb b.v. Woerden, The Netherlands). Triple therapy was also administered via drinking water (25 mg/l atenolol, 25 mg/l hydrochlorothiazide and 80 mg/l hydrochlorothiazide, Sigma-Aldrich, St. Louis, MO, USA). The different treatment groups (Figures 1A, 2A) consisted of comparable numbers of female and male mice. There were no differences in albuminuria and FSGS scores between male and female mice within the groups. Throughout the experiments the groups of mice were housed in cages with free access to food and the (drug-containing) drinking water. Urine albumin in 18 h urine was measured by radial immunodiffusion using a goat antiserum against mouse albumin. The 18 h urine samples were collected by placing the animals individually in metabolic cages. During their confinement in the metabolic cages, the mice only had access to tap water. To prevent dehydration during their confinement in the metabolic cages, the mice received 1 ml 0.9% saline by an intra-peritoneal injection.

**Systemic blood pressure measurements**

The effect of treatment on blood pressure was evaluated in separate experiments. For systemic blood pressure
measurements, mice were anaesthetized with a mixture of isofluorane and oxygen. After anaesthetization, the abdomen was opened. A 19 mm 24-gauge Neoflon™ catheter (Becton Dickinson, Helsingborg, Sweden) was inserted in the aorta just above the aortic bifurcation. The catheter was connected to a pressure transducer (Hewlett Packard, Palo Alto, CA) and after a 3–5 min stabilization period the mean arterial pressure was monitored during a 5 min period in which the mice were only reactive on stimulation of the foot reflex. After the measurement, the mice were sacrificed by cervical dislocation.

The committee of animal experiments of the Radboud University Nijmegen approved all conducted animal experiments.

Light microscopy

For light microscopy, kidney fragments were fixed in Bouin’s solution, dehydrated and embedded in paraplast (Amstelstad, Amsterdam, The Netherlands). Sections of 4 µm were stained with periodic acid-Schiff and 2 µm sections with silver methenamine. To obtain the FSGS-score, at least 60 glomeruli per mouse were evaluated. Only lesions with an adhesion (matrix continuity between the tuft and Bowman’s capsule in the silver stain) were counted.

Immunohistochemistry

Immunohistochemical staining was performed on kidneys, fixed in 4% buffered formaldehyde for 24 h and embedded
in paraffin. Before every immunolabelling with biotinylated secondary antibodies, the tissue sections were treated with an avidin/biotin blocking reagent (Vector Laboratories, Burlingame, CA) to block endogenous biotin. Sections 4 μm were incubated with rabbit anti-mouse Ki-67 (Dianova Immundiagnostic, Hamburg, Germany), goat anti-CD10 (Santa Cruz Biotechnology, Santa Cruz, CA). As secondary antibodies, we used a biotinylated goat anti-rabbit antibody for Ki-67, a horse anti-goat for CD10 (Vector laboratories Inc., Burlingame, CA). Detection of Ki-67 and CD10 was carried out using the vectastain ABC kit (Vector Laboratories, Burlingame, CA), according to manufacturers protocol and by using diaminobenzidine as substrate. For quantification of Ki-67 positive cells, the sections were counterstained with Schiff’s reagent to stain the glycoproteins in the basal membranes. Positive nuclei within Bowman’s space were counted. Per mouse, approximately 50 (at least 40) glomeruli were counted in one cross section. All quantifications were performed by an investigator who was unaware of treatment. To minimize cross-reactivity of the secondary antibodies with endogenous immunoglobulins, preabsorption was performed by adding 4% normal mouse serum to the secondary antibodies. In control sections, primary antibodies were omitted.

**Immunofluorescence microscopy**

Kidney fragments were snap-frozen in liquid nitrogen and 2μm acetone-fixed serial cryostat sections were used. A double-immunolabelling technique was performed to determine the co-localization of Thy-1.1 and desmin. Thy-1.1 was detected using a rat polyclonal anti-Thy-1 antibody (59AD2.2; IgG2a). As secondary antibody, we used a Fluorescein isothiocyanate (FITC)-labelled sheep anti-rat antibody (Sero Tec, Oxford, UK). After washing with phosphate buffered saline, the sections were incubated with rabbit anti-desmin [12], and detected with a goat anti-rabbit AlexaTM 568 antibody (Molecular probes Inc., Leiden, The Netherlands). Thy-1.1 was detected using a rat polyclonal anti-Thy-1.1 mAb (Ca0730, Denmark). For detection of the podocyte we have also used ASD33, a rat monoclonal antibody, generated in our laboratory, which only reacts with the podocyte cell membrane [13]. The identity of the antigen is unknown. For the detection of ASD33, we used a FITC-labelled rabbit anti-rat antibody (Dako A/S, Glostrup, Denmark). To minimize cross-reactivity of the secondary antibodies with endogenous immunoglobulins, preabsorption was performed by adding 4% normal mouse serum to the secondary antibodies. In control sections, primary antibodies were omitted.

**In situ hybridization**

The in situ hybridization was performed on 4μm paraffin section of kidneys fixed in 4% buffered formaldehyde for 24 h. A 542 bp cDNA fragment of rat CTGF (Genbank gii5070343 496-1037) was amplified by PCR (PCR2.1-TOPO vector (invitrogen, breda, The Netherlands) [14]. In vitro transcription of the purified insert was performed using SP6 or T7 RNA polymerases and digoxigenin (DIG)-conjugated uridine 5’-triphosphate (UTP) (Roche, Almere, The Netherlands) to produce DIG-labelled sense or antisense riboprobes, respectively. Hybridization of the probe was detected by an alkaline phosphatase-labelled sheep anti-DIG antibody, and visualized with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate. The prevalence of PECs and podocytes expressing CTGF mRNA was assessed in a semi-quantitative way. For each mouse, a single cross section of both kidneys was judged for the presence of CTGF expressing PECs and podocytes by an investigator who was unaware of treatment, and a score was assigned. The following scale was used: 1 = low, 2 = moderate, 3 = high prevalence of cells expressing CTGF.

**Statistical analysis**

For multiple comparisons, ANOVA was used and post hoc analyses were done with Tukey’s test. Significance of differences between the mean values of the semi-quantitative CTGF scores was evaluated using the non-parametric Kruskal–Wallis test. A value of P < 0.05 was considered statistically significant. All values are expressed as means ± SEM.

**Results**

**Animal data**

We have assessed the antihypertensive effects of captopril and triple therapy by measuring blood pressures in a separate set of anti-Thy-1.1-injected tg mice after 3, 4 and 7 days of treatment. In control (C) mice mean blood pressure (MBP) was 97 ± 2 mmHg at day 7 after the anti-Thy-1.1 mAb injection. This value was not different from blood pressures in saline-injected Thy-1.1 tg mice (data not shown). The MBP was 89 ± 1 mmHg (P < 0.01 vs C), 82 ± 1 mmHg and 73 ± 2 mmHg (P < 0.001 vs C), at days 3, 4 and 7 after start of captopril, respectively. Triple therapy resulted in even lower blood pressures with an MBP of 69 ± 1, 61 ± 3 and 63 ± 5 mmHg (all days: P < 0.001 vs C) at days 3, 4 and 7 respectively (Figure 1B). As expected, injection of anti-Thy-1.1 mAb induced a substantial albuminuria. FSGS lesions were observed in 24% of the glomeruli at day 8 (Figure 1C). Almost complete protection was observed in mice treated with captopril throughout the study period (day 0 until day 7, Ca0730-C07). Therefore, we tested the effect of captopril administered at different time intervals during the development of FSGS, we questioned whether protection was dependent on the timing of drug administration. Therefore, we tested the effect of captopril administered at different time intervals during the development of FSGS lesions, as depicted in Figure 2A. Results are depicted in Figure 2B and C. Captopril pre-treatment from day −7 until the day of injection of the anti-Thy-1.1 mAb (Ca0730−7) had no effect on the level of albuminuria or on the number of glomeruli with FSGS lesions (Figure 2B). In contrast, if treatment was continued for an additional 7 days (Ca0730−7), albuminuria and FSGS were markedly
and significantly reduced. A similar effect was obtained with captopril treatment from 4 h after the anti-Thy-1.1 mAb injection until day 7 (Ca0-7). Remarkably, even a delayed start of captopril treatment, resulting in a short period of treatment, from days 3 to 7 (Ca3-7), significantly reduced albuminuria and FSGS. We have further evaluated the persistence of the protective effects of captopril by studying additional groups of mice, treated with captopril from days 0 to 7 or from days 3 to 7, and sacrificed at day 22. In these mice, albuminuria was measured at days 7, 14 and 21 and FSGS lesions were scored at day 22. As illustrated in Figure 3, albuminuria gradually decreased in C mice, without further increase of FSGS score. In captopril-treated mice albuminuria remained unchanged. Notably, in these mice there was no progression of FSGS after stopping captopril treatment (Figure 3). Thus, the temporary treatment with captopril in the active phase of FSGS development resulted in a permanent reduction in the number of FSGS lesions.
Renal histology

Affected glomeruli in kidneys of C mice showed segmental or global collapse of the capillary tuft and a marked increase of epithelial cells in Bowman’s space (Figure 4). Adhesions (continuity of ECM between the capillary tuft and Bowman’s capsule) were formed by thin strands of ECM that were located in between the epithelial cells in Bowman’s space. Captopril treatment (either from day 0 or day 3 onwards) markedly reduced the severity of the lesions, with fewer ECM adhesions and only minor collapse of the glomerular tuft (Figure 4, Ca3−7). In particular the hypercellular lesions, characteristically observed in Bowman’s space of affected glomeruli in C mice, were virtually absent after captopril treatment (Figure 4, Ca3−7). The few affected glomeruli in the captopril-treated mice were primarily situated in the juxtamedullary segment of the kidney. The lesions observed in mice sacrificed at day 22 were more advanced, with a high degree of ECM accumulation, and the formation of segmental- or even global-scars (data not shown).

Podocyte phenotype

In our model, albuminuria is induced by injection of mAbs directed against the Thy-1.1 antigen present on podocytes. We therefore questioned if the initial podocyte injury could be the decisive step towards development of FSGS. Pre-treatment with captopril might then be protective. However, this approach failed. We next evaluated the changes in podocyte phenotype during development of FSGS in C mice and in mice treated with captopril from day 0 or day 3. To this end we studied the expression of desmin (associated with podocyte dedifferentiation or injury) and the podocyte specific marker ASD33 (a unidentified cell membrane antigen, strongly expressed on mouse podocytes in Thy-1.1 mice and a sensitive marker of podocyte dedifferentiation) at days 3 and 8 after the Thy-1.1 mAb injection. In a previous study, we had shown that at day 7 after the Thy-1.1 injection, activated or injured podocytes had lost expression of ASD33 and synaptopodin but could still be identified by their expression of WT-1 and the Thy-1.1 antigen [5].

In C mice we observed increased desmin staining at day 3 after mAb injection, compatible with podocyte activation or injury (Figure 5). Captopril treatment, started at day 0 or day 3, did not decrease desmin staining (Figure 5). The desmin staining was not exclusively present in glomeruli showing collapsed segments or adhesions but was also observed in morphologically normal glomeruli. In C mice we observed a decreased staining of ASD33 at day 8, in particular in glomerular segments that contained a tuft adhesion or tuft collapse. The percentage of glomeruli with segmental loss of ASD33 staining was equal to the percentage of FSGS lesions. In both captopril-treated groups we observed significantly less glomeruli with decreased ASD33 expression, in agreement with the reduced number of glomeruli with FSGS lesions (data not shown).

Evaluation of proliferation

We have examined the expression of the proliferation marker Ki-67 and the PEC marker CD10 in kidneys of mature control and captopril-treated mice at day 6 after anti-Thy-1.1 mAb injection. In the C mice we observed the anticipated increase of Ki-67 cells in Bowman’s space, which was absent in the
captopril-treated mice (Figure 6A, 6B). Notably, even captopril started at day 3 resulted in reduced proliferation. We previously demonstrated that in our model the proliferating epithelial cells in Bowman’s space are PECs- and CD10-positive (Figure 6C) [5]. In C mice we observed accumulation of CD10-positive cells in Bowman’s space (Figure 6D), whereas in the captopril-treated mice we could no longer detect CD10 positive hypercellular lesions.

**CTGF expression**

We have analysed the effect of captopril treatment on expression of CTGF during development of FSGS (Figure 7). Studying the expression of CTGF mRNA, instead of the (secreted) protein, allows us to identify the cell type producing CTGF. In all groups we observed a comparable prevalence of podocytes expressing CTGF. CTGF expression by PECs was negligible in the saline treated mice. In contrast, in C mice we observed an increased number of PECs expressing CTGF. The expression of CTGF by PECs in these mice was observed in glomeruli with FSGS lesions as well as in morphologically normal glomeruli. In the Ca0.7 mice the number of PECs expressing CTGF was reduced. In the group Ca3.7 the majority of the mice showed a moderate reduction in PECs expressing CTGF compared with the C mice.

**Discussion**

The ACEi captopril reduced albuminuria, and almost completely prevented the development of FSGS in our anti-Thy-1.1 mouse model. Evidently, captopril was more effective than triple therapy. Notably, triple therapy did not lower albuminuria, despite a considerable reduction of the systemic blood pressure. This finding may be explained by the possibility that lowering of the systemic blood pressure by triple therapy does not result in an equivalent reduction of the intraglomerular blood pressure. Our data add more evidence to the observations in animal models and humans that the beneficial effects of ACEi cannot
be explained merely by a decrease in systemic blood pressure [3].

The present data also provide additional evidence for the role of the PECs in FSGS. In our previous study, we have discussed the role of podocyte and PEC in the development of FSGS. In our Thy-1.1 mouse model, we have shown that administration of anti-Thy-1.1 antibody caused podocyte activation with increased expression of desmin; activated podocytes interacted with PECs; PECs started to proliferate from day 3 onwards and proliferation peaked at day 7. The PECs produced ECM that leads to scarring; podocytes did not proliferate, and from day 3 onwards lost podocyte specific markers such as ASD33 and synaptopodin but remained WT-1 and Thy-1.1 positive; in advanced sclerotic glomeruli WT-1 positive cells were lost [5]. In the present study we observed that captopril prevented development of FSGS, despite identical initial podocyte activation and desmin staining, which is a marker of podocyte injury or activation [15,16]. Thus, these findings indicate that captopril does not offer protection through interference with the initial activation of podocytes. This conclusion is particularly strengthened by the observation that captopril administered from day 3 onwards also afforded protection. Renoprotection was associated with inhibition of PEC proliferation and reduced loss of ASD33, which is indicative of maintenance of podocyte integrity. Both mechanisms may be relevant in the renoprotective effects of captopril. Since the proliferative, hypercellular variants of FSGS are the most aggressive, we feel that inhibition of PEC proliferation may be most important.

It remains to be established how ACEi prevents PEC proliferation, either indirectly via lowering of intraglomerular blood pressure, reduction of proteinuria and maintenance of podocyte differentiation or directly by blocking ANGII-mediated cellular proliferation. It is well-known that ANGII has proliferative and profibrotic effects, likely mediated by growth factors such as PDGF, plasminogen activator inhibitor type 1, TGF-β [4] and CTGF [17]. In our model, induction of proteinuria was followed by an increased expression of CTGF mRNA in PECs, which was largely diminished by captopril treatment. Thus, PECs may be pivotal in the process of fibrosis via the production of CTGF. Recent studies have indeed shown that CTGF induces ECM production (collagen I, III, IV, fibronectin) in mesangial cells and PECs in vitro. In addition, studies have proposed that CTGF expressing proliferating PECs participate in glomerular scarring in human and experimental crescentic glomerulonephritis [14,18].

Fig. 5. Desmin expression: in saline injected mice there is a faint staining for desmin within the mesangium of the glomerulus (saline). Anti-Thy-1.1 mAb-injected mice already showed at day 3 after the anti-Thy-1.1 mAb injection increased desmin staining within the glomerulus and also in peritubular capillaries (C day 3). At day 8 there was comparable desmin expression in the glomerular tuft. But in affected glomeruli there was also desmin expression surrounding Bowman’s capsule (C day 8). Ca²⁺⁺/Ca³⁺⁺ mice showed no decrease in desmin expression compared with the non-treated anti-Thy-1.1 mAb-injected mice (Ca²⁺⁺, Ca³⁺⁺) Original magnification: 200×. To examine the podocyte specific desmin expression the desmin staining (red) was merged with the Thy-1.1 transgene expression (green); co-expression results in yellow staining (merge) Original magnification: 400×.
Admittedly, it remains to be elucidated whether the increased CTGF production observed in the present study induces PEC proliferation, migration and/or ECM production, and if the effect of captopril on CTGF expression is mediated via the depletion of ANGII.

As discussed previously, our mouse model of FSGS resembles human primary FSGS [5]. The early phase is characterized by collapse and proliferation of epithelial cells and resembles collapsing FSGS, whereas the late phase is characterized by the development of segmental scars [5]. Thus far, the salutary effects of ACEi and/or ANGII antagonism has been established mostly in experimental models of secondary FSGS, in which FSGS lesions develop secondary to glomerular hyperfiltration and/or increased glomerular pressure. The present study suggests that the efficacy of ACEi is not limited to secondary forms of FSGS. ACEi may even be beneficial in collapsing FSGS, the most severe form of primary FSGS.

How to reconcile our findings with clinical data? Up till now, few studies in humans have evaluated ACEi in patients with primary FSGS. Some authors have suggested that ACEi may slow deterioration of renal function, however efficacy seems limited compared with the success of ACEi in secondary forms of FSGS [2]. The unimpressive results of ACEi in human primary FSGS may be explained by the fact that ACEi are often administered long after onset of the disease. Many patients are diagnosed with FSGS long after the first symptoms. Renal biopsy seldom discloses active hypercellular lesions. Administration of ACEi in advanced disease may indeed be too late. In animal studies, Yoshida et al. [19] showed that
ACEi prevented progression of disease when glomeruli were in the early stages of sclerosis, but were unable to halt progression in glomeruli with advanced sclerosis. Our finding suggests that the efficacy of ACEi is strikingly high in early FSGS lesions with prominent proliferation. Treatment with captopril from day 0 until day 7 attenuated the proliferation of the epithelial cells and prevented the development of segmental scars at days 8 and 22. Since the FSGS lesions in our mouse model resemble those seen in human collapsing FSGS, we would predict ACEi to be beneficial in human collapsing FSGS. Indeed, several cohort studies have demonstrated significant benefits of ACEi, with a 3- to 5-fold longer renal survival, in HIV nephropathy, the best known example of the collapsing variant of FSGS in humans [20].

In conclusion, captopril prevented the development of FSGS in a mouse model of FSGS that closely resembles human collapsing FSGS. A similar effect was not observed with ANGII independent antihypertensive treatment, pointing to a specific role of ANGII. Our data suggest that inhibition of PEC proliferation is the most important mechanism of action.

Acknowledgments. We thank Roy Wetzels for performing in situ hybridizations. This work was supported by a grant from the Dutch Kidney Foundation (Nierstichting Nederland, grant: C 99.1844).

Conflict of interest statement. None declared.

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Received for publication: 31.5.06
Accepted in revised form: 24.7.06