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LABORATORY INVESTIGATION

Converting enzyme inhibition and progressive glomerulosclerosis in the rat

JOKE J.B. BEUKERS, ANNEMIEKE VAN DER WAL, PHILIP J. HOEDEMAEKER, and JAN J. WEENING

Department of Pathology, State University of Leiden, Leiden, The Netherlands

Converting enzyme inhibition and progressive glomerulosclerosis in the rat. The effect of converting enzyme inhibition (CEI) by captopril (CAP, 500 mg/liter drinking water) on the development and progression of glomerulosclerosis (GS) was studied in six groups of male uninephrectomized (UN) Wistar rats. In group A, treated with CAP for four to five weeks after UN, a reduction in systolic blood pressure (SBP), filtration fraction and glomerular volumes was found as compared to control group B. Long-term treatment with CAP for eight months after UN (group C) resulted in lowering of SBP with 30 mm Hg, a low level of proteinuria and low incidence of GS (0 to 1.5%) as compared to control rats (group D), with SBP of 131 ± 4 mm Hg, proteinuria up to 103 to 509 mg/day and 9.1 to 29.7% GS at eight months after UN. Groups E and F were followed without therapy up to seven months after UN, at which time a high level of proteinuria was present. CAP therapy then started in group E, did not reduce SBP, proteinuria and GS at 11 months after UN relative to control group F. This study shows that early CEI prevents progressive proteinuria and GS in rats after UN and is associated with a reduction in SBP, filtration fraction and glomerular volume. Once high levels of proteinuria and GS have developed in rats after UN, CEI has no effect on SBP nor on the progression of GS and proteinuria.

Progressive glomerulosclerosis (GS) develops spontaneously with aging in several rat strains [1–5], a process which can be accelerated by a reduction in renal mass [6–11], by high protein feeding [12–14], by streptozotocin-induced diabetes mellitus [15] and by desoxycorticosterone-salt induced hypertension [16]. In addition, progressive GS may develop in chronic aminonucleoside nephrosis [17] and adriamycin nephrosis [18]. Several pathogenetic mechanisms have been shown to be relevant as to the development of the glomerular lesions, which resemble focal segmental glomerulosclerosis in man. Based on a number of recent studies using micropuncture and morphologic techniques, a central role in the pathogenesis of progressive GS has been attributed to elevated intraglomerular pressures and flows [14, 19, 20], resulting in a process of endothelial, epithelial, and mesangial cell damage [21]. Besides these hemodynamic factors, genetic [22], metabolic [23] and coagulation [24, 25] factors have been shown to be important in the development of GS. In several models of progressive GS dietary or pharmacological intervention has been applied to retard or prevent glomerular disease. Protein restriction was found to have a beneficial effect in ablation models [11, 26], in experimental diabetes [27] and hypertension [16]. Anti-coagulatory drugs were also effective in models of ablation [24, 25] and hypertension [28].

In the remnant kidney model linoleic acid, a prostaglandin precursor [29], and lipid lowering agents [30] were found to have an ameliorative effect. Antihypertensive treatment using converting enzyme inhibitors, introduced shortly after the induction of glomerular hyperperfusion was remarkably effective in reducing GS in the remnant kidney model [31] and in streptozotocin-induced diabetes mellitus [32]. Recently, Meyer et al showed that reversal of glomerular hypertension either by converting enzyme inhibition or by protein restriction stabilized established glomerular injury in the remnant kidney model [33].

The remnant kidney model is a model of subtotal ablation, in which one kidney is removed and two thirds of the remaining kidney are infarcted. This model is associated with systemic hypertension, which develops soon after ablation.

The present study was designed to test the efficacy of converting enzyme inhibition on the development and progression of glomerulosclerosis in a less extreme model of renal ablation without development of systemic hypertension. For this purpose, male Wistar rats were subjected to unilateral nephrectomy and captopril treatment was started either immediately or at seven months after nephrectomy, at which time increased levels of proteinuria and glomerulosclerosis had developed.

Methods

Six groups of three months old, male Wistar rats with an initial body weight of 210 to 245 g were subjected to unilateral nephrectomy (UN) under ether anesthesia (Fig. 1). The rats were fed ad libitum with a standard chow (Hope Farms Inc., Woerden, the Netherlands) containing 0.22% sodium and 26% digestible protein.

Groups A, C, and E were treated with the angiotensin I converting-enzyme inhibitor captopril (Squibb, Rijswijk, the Netherlands) in a dose of 500 mg/liter in the drinking water (Fig. 1). Administration of captopril was started the day after UN in groups A and C and at seven months after UN in group E. Groups B, D, and F received no therapy (control groups). Renal function and glomerular volumes were determined at four to five weeks after UN in group A ($N = 6$) and group B ($N = 8$). Rats in group C ($N = 12$) and group D ($N = 11$) were followed

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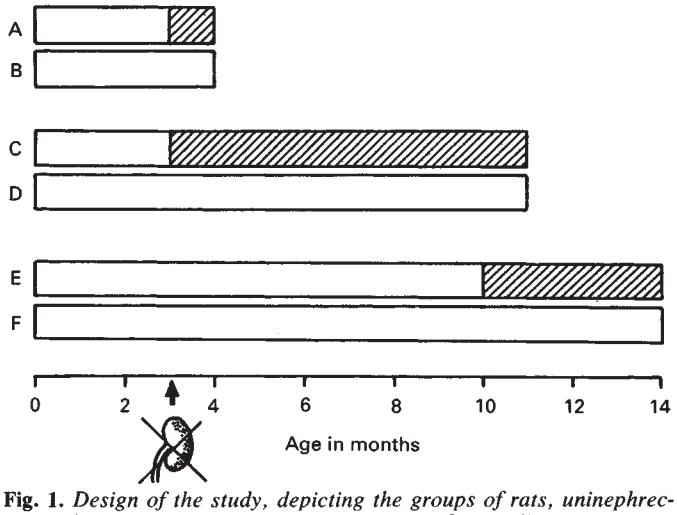

Fig. 1. *Design of the study, depicting the groups of rats, uninephrectomized at 3 months of age.* Symbols are: (▨) Captopril treatment; (□) no treatment.

for eight months after UN during which time body weight, systolic blood pressure and urinary protein excretion were monitored. Systolic blood pressure was measured weekly in awake, trained rats by the tail cuff method [34]. Urinary protein excretion was measured monthly by the biuret method in urine collected by housing the rats for 24 hours in metabolic cages with free access to water and food. Serum levels of cholesterol and triglycerides were measured according to standard methods in blood obtained at the end of the observation period after a 24-hour period of fasting. At sacrifice after perfusion-fixation, kidneys were processed for histology. Rats of group E ($N = 6$) and F ($N = 7$) were followed for seven months after UN without therapeutic intervention. At seven months after UN, proteinuria had increased to 115 to 536 mg/24 hr in group E and to 111 to 407 mg/24 hr in group F. Body weight curves were similar. Captopril treatment (500 mg/liter) was then introduced to group E and both groups were followed for another four months with monthly determination of body weight and urinary protein loss. At 7 and 11 months after UN, creatinine clearances were determined based on creatinine concentration measured in serum and the amount of excreted creatinine in a 24-hour urine collection.

At sacrifice 11 months after UN, systolic blood pressure was recorded by arterial cannulation under Inactin anesthesia (100 mg/kg) and after perfusion-fixation the kidneys were processed for histology.

Renal function and glomerular volumes

GFR and RPF were determined as described before [35]. Rats of groups A and B were anesthetized with Inactin (Byk Gulden, Konstanz, Germany), 100 mg/kg i.p., and placed on a heated operating table. Body temperature was kept between 37°C and 38°C, monitored by a rectal thermometer (Telethermometer, Yellow Springs, Colorado, USA). After tracheostomy, two polyethylene catheters PE10 (Clay Adams, Parsipanny, New Jersey, USA) were inserted in the left jugular vein for infusion of inulin and serum. The right femoral artery was cannulated with a PE50 polyethylene catheter connected to a pressure transducer (Western Lab, model 91, Colorado, USA) and systolic blood pressure (SBP) was recorded. The left ureter was cannulated with a PE10 catheter. Forty minutes prior to the clearance period, a 4% inulin solution (Sigma Chemical Co., St. Louis, Missouri, USA) was given as a bolus of 0.5 ml, followed by a constant infusion at a rate of 0.034 ml/min. In addition a bolus of 0.2 ml serum was given, followed by infusion at a rate of 0.020 ml/min for replacement of fluid loss. The clearance rate of inulin was determined during two consecutive periods of 20 minutes during which time urine was collected from the left kidney. Arterial blood was obtained by continuous withdrawal from the femoral artery for the coincident 40 minutes (Harvard pump model 4-940). A 0.2 ml sample of renal venous blood was obtained at the end of the second clearance period. Hematocrits were measured in arterial blood samples obtained at the end of the second clearance period. The concentration of inulin in urine and plasma samples was measured by the anthrone colorimetric assay [36]. GFR and RPF were calculated according to standard formulas.

At the end of the second clearance period, the kidney was fixed by perfusion at the measured arterial pressure with 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Renal tissue was postfixed in 4 g% buffered formaldehyde solution and processed for morphometric analysis through methacrylate embedding. Two micron sections were stained with silver methenamine. Fifty systematically sampled glomeruli were traced along the Bowman's capsule and along the glomerular tuft profile over the surface of a graphic tablet morphometric analyzer (MOP-AMO3, Kontron, Echingen, FRG) connected to a Digital PDP11 computer (Digital Equipment Inc., Galway, Ireland). Mean glomerular capsular volume (GV_C) was calculated as described by van Damme and Koudstaal [37] from the mean glomerular radius using the formula $4/3\pi r^3$. The average glomerular tuft volume (GV_T) was calculated as $GV_T = \beta/k \cdot (A^{3/2})$, where A is the mean glomerular random cross-sectional area, β is 1.38, the shape coefficient for spheres (the idealized shape of glomeruli) and k is 1.1, a size distribution coefficient [31, 38].

Histology

The kidneys of rats in groups C, D, E and F were fixed by perfusion and processed for light microscopy as described above. Sections were stained with periodic acid-Schiff (PAS). Glomeruli were counted with overt sclerotic lesions, defined as glomerular lesions consisting of subendothelial and mesangial deposition of hyalin material, increase of mesangial matrix and collapse of glomerular capillaries with adhesions of the tuft to Bowman's capsule. The incidence of GS lesions was determined by scoring at least 100 glomeruli in a coronal section of each kidney.

Statistics

Statistical analysis was performed by Student's *t*-test for comparisons between the means of two groups. The Mann-Whitney test was used to compare levels of urinary protein excretion and the incidence of glomerular lesions between groups C and D and between groups E and F. Statistical significance was defined as two-sided $P < 0.05$.

Table 1. Renal hemodynamic studies, 4 to 5 weeks after UN

Group	N	BW g	SBP mm Hg	GFR ml/min	RPF ml/min	FF %	Hct %
A	6	292 ± 17	105 ± 12[a]	1.92 ± 0.24	8.81 ± 1.81	22.1 ± 2.3[a]	47.0 ± 0.8
B	8	302 ± 13	128 ± 5	1.95 ± 0.23	7.35 ± 1.33	27.1 ± 4.7	47.5 ± 1.9

Values are mean ± SD. Abbreviations used in this table: BW, body weight; SBP, systolic blood pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; Hct, hematocrit.
[a] Student's *t*-test, $P < 0.05$ group A vs. group B

Results

Short term study

Renal function and glomerular volumes. Mean values for body weight (body wt), systolic blood pressure (SBP), glomerular filtration rate (GFR), renal plasma flow (RPF), filtration fraction (FF), and hematocrit (Hct) for groups A and B are summarized in Table 1. Body wt did not differ in the two groups, averaging 292 ± 17 g in group A and 302 ± 13 g in group B. SBP was significantly lower in the captopril-treated animals than in the control animals (105 ± 12 mm Hg vs. 128 ± 5 mm Hg, $P < 0.05$). Mean values for GFR were almost identical in both groups, averaging 1.92 ± 0.24 ml/min in group A and 1.95 ± 0.23 ml/min in group B. Compared to age- and sex-matched, two-kidney control rats studied previously [35], GFR was approximately 1.8-fold higher after UN. In captopril-treated animals, RPF was not significantly higher than in control animals (8.81 ± 1.81 ml/min vs. 7.35 ± 1.33 ml/min, $P > 0.1$). The FF in captopril-treated animals was significantly lower than in untreated animals (22.1 ± 2.3% vs. 27.1 ± 4.7%, $P < 0.05$). Hct did not differ in the two groups.

As described above, the kidneys of rats of groups A and B were fixed by perfusion at the measured SBP. To compare data on glomerular volumes with previous data in the same strain [35] and with data from other studies, both average glomerular capsular and tuft volumes were determined. As shown in Table 2, the average glomerular capsular and tuft volumes were significantly higher in the untreated rats (GV_C: 2.14 ± 0.39 × 10^6 μm^3; GV_T: 1.80 ± 0.24 × 10^6 μm^3) than in the captopril-treated animals (GV_C: 1.43 ± 0.18 × 10^6 μm^3; GV_T: 1.15 ± 0.14 × 10^6 μm^3). In untreated rats of group B, GV_C was close to the value documented in a previous study [35]. To determine whether the difference in perfusion pressure could account for the disparity in mean glomerular volume, kidneys of additional captopril-treated animals were perfused at 100 mm Hg ($N = 3$) or at 130 mm Hg ($N = 3$). The difference in perfusion pressure was found to have no effect on the mean glomerular capsular and tuft volume.

Long-term studies: Early captopril treatment

As measured by the tailcuff method, untreated control rats of group D remained normotensive throughout the eight months observation period (129 ± 5 mm Hg at 5 months after UN; 131 ± 4 mm Hg at 8 months after UN). SBP in captopril-treated rats of group C was significantly lower than SBP in the control group D (101 ± 3 mm Hg at 5 months after UN; 102 ± 3 mm Hg at 8 months after UN) (group C vs. group D, $P < 0.05$).

Table 2. Glomerular capsular (GV_C) and tuft (GV_T) volumes, 4 to 5 weeks after UN

Group	N	GV_C $\times 10^6$ μm^3	GV_T $\times 10^6$ μm^3
A	6	1.43 ± 0.18[a]	1.15 ± 0.14[a]
B	8	2.14 ± 0.39	1.80 ± 0.24

Values are mean ± SD
[a] $P < 0.05$, group A vs. group B

During the eight-month observation period, captopril treatment did not affect the growth of the animals. Captopril-treated rats of group C and control rats of group D showed comparable weight gain (Table 3). At sacrifice rats of group C had a body wt of 400 ± 42 g, whereas rats of group D weighed 420 ± 33 g ($P > 0.2$).

The untreated control rats of group D developed progressive proteinuria reaching values of 103 to 509 mg/24 hr at eight months after UN, whereas significantly less proteinuria was found in the captopril-treated rats of group C with levels of 30 to 49 mg/24 hr (Table 3).

At sacrifice, cholesterol levels were significantly higher in control animals of group D than in captopril-treated animals of group C (2.56 ± 0.59 mmol/liter vs. 1.90 ± 0.17 mmol/liter, $P < 0.05$). However, there was no statistical significant difference in levels of triglycerides in the two groups (control rats: 0.85 ± 0.26 mmol/liter; captopril-treated rats: 0.66 ± 0.24 mmol/liter, $0.05 < P < 0$ 10).

In the kidneys of proteinuric untreated rats of group D, glomeruli were present displaying a spectrum of morphologic abnormalities as described by Rennke [21]. Mesangial expansion and hypercellularity, epithelial cell swelling and segmental lesions defined as areas of collapsed capillaries with accumulation of condensed hyalin material and adhesions to Bowman's capsule were observed (Fig. 2). Tubulointerstitial changes varied in severity and consisted of tubular dilation as well as atrophy, protein casts, interstitial edema and patchy mononuclear infiltrates. The incidence of segmental glomerulosclerotic lesions ranged from 9.1 to 29.7% (median: 13.0%) in untreated control rats of group D, whereas in captopril-treated rats of group C lesions were found in 0 to 1.5% (median: 1.0%) of the glomeruli ($P < 0.05$) (Fig. 3).

Long-term studies: Late captopril treatment

At seven months after UN, groups E and F had similar body weights, and body weight curves showed no significant changes during the following four months when captopril was given to rats of group E (Table 3).

Table 3. Body weights and urinary protein excretion in groups C, D, E, and F

Group	*N*	Body weight *g*			Urinary protein excretion *mg/24 hr*		
		at UN	at 7 mo after UN	at sacrifice[a,b]	at UN	at 7 mo after UN	at sacrifice[a,b]
C	12	224 ± 11	397 ± 43	400 ± 42	r:20–49 m:28	r:32–50[c] m:41	r:30–49[c] m:41
D	11	224 ± 13	415 ± 37	420 ± 33	r:18–46 m:26	r:100–409 m:193	r:103–509 m:222
E	7	231 ± 6	410 ± 37	410 ± 22	r:21–46 m:37	r:115–536 m:265	r:273–883 m:355
F	6	235 ± 11	407 ± 38	419 ± 48	r:20–45 m:35	r:111–407 m:223	r:191–721 m:484

Values are mean ± SD or range (r) with median (m).
[a] Groups C and D at 8 months after UN
[b] Groups E and F at 11 months after UN
[c] Mann-Whitney test, $P < 0.05$ group C vs. group D

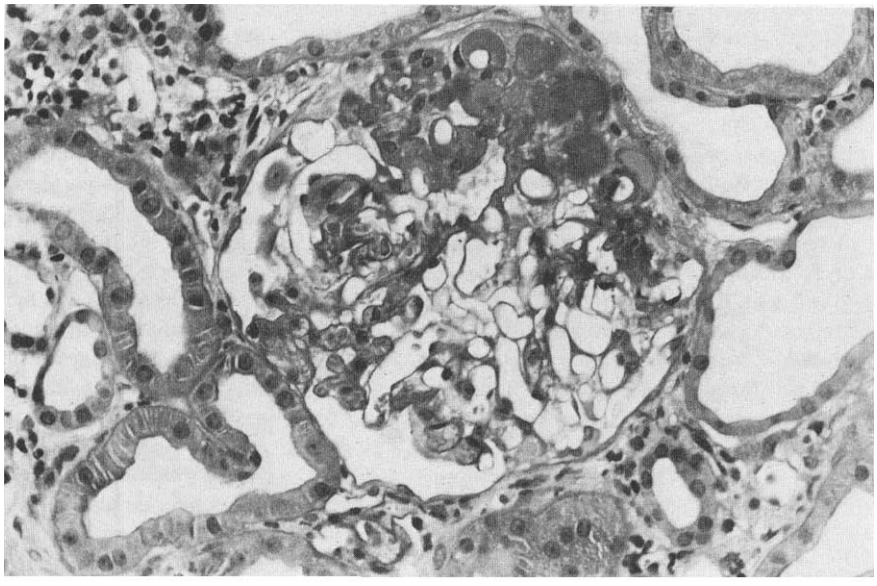

Fig. 2. *Light micrograph of renal cortex illustrating a representative glomerulus from an untreated control rat from group F showing a glomerular lesion consisting of a segmental area of collapsed capillaries with accumulation of condensed hyalin material and adhesions to the Bowman's capsule (PAS, × 400).*

In animals of group E, urinary protein loss increased from 21 to 46 mg/24 hr at UN to 115 to 536 mg/24 hr seven months after UN and increased further during the four months of captopril treatment to 273 to 883 mg/24 hr at 11 months after UN (Table 3). Untreated rats of group F developed similar levels of proteinuria with a range of 20 to 45, 111 to 407 and 191 to 721 mg/24 hr at UN, at seven months and at 11 months after UN, respectively. There was no statistically-significant difference between groups E and F at all intervals.

At seven months after UN, creatinine clearances were similar in both groups: 0.47 ± 0.10 ml/min/100g body wt in group E and 0.46 ± 0.05 ml/min/100g body wt in group F. As compared to seven months after UN, creatinine clearances determined at 11 month after UN had significantly decreased in both groups, reaching values of 0.31 ± 0.12 ml/min/100 g body wt and 0.30 ± 0.08 ml/min/100 g body wt in groups E and F, respectively ($P < 0.05$). Creatinine clearances at 11 months after UN in the two groups were not statistically different.

Systolic blood pressures, measured at sacrifice under Inactin anesthesia, were not significantly different between both groups: 129 ± 27 mm Hg in group E and 141 ± 20 mm Hg in group F ($P > 0.10$).

At sacrifice at 11 months after UN, glomerular changes in both group E and group F were more severe than observed in group D. Both segmental and global glomerulosclerotic lesions were present, associated with severe tubulointerstitial changes. No statistically significant differences were observed in the incidence of glomerulosclerotic lesions reaching values of 15.4 to 87.2% in group E and 12.6 to 68.6% in group F, respectively (Fig. 3).

Discussion

The present study shows that converting-enzyme inhibition by captopril prevents the development of progressive GS and proteinuria in aging uninephrectomized rats when treatment is started early after UN. When given at a later stage to uninephrectomized animals suffering from established GS and proteinuria, captopril neither diminishes nor retards the progression of proteinuria and GS.

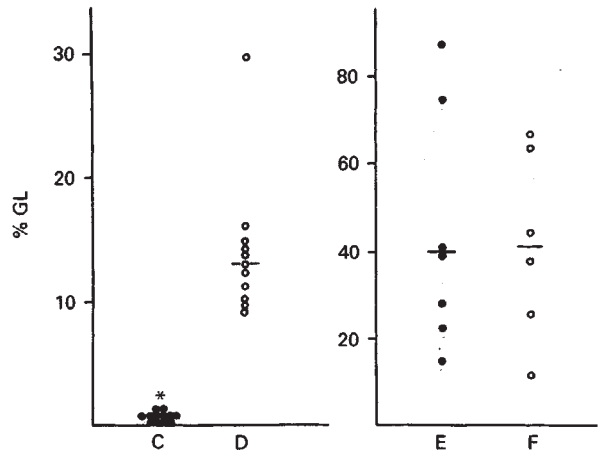

Fig. 3. *Incidence of glomerular lesions (GL) observed at sacrifice in the captopril-treated groups C and E (●) and control groups D and F (○).* (*) Mann-Whitney U-test, $P < 0.05$ group C vs. group D; (—) median value.

The study was performed in male rats of a Wistar strain known to be susceptible to develop progressive GS spontaneously with aging [2, 8]. Unilateral nephrectomy has been shown to accelerate this process [8, 35]. In the current study, the remaining kidney of the uninephrectomized untreated rats of group B showed a compensatory increase in GFR and RPF at one month after UN and a marked expansion of glomerular volumes as compared to what we found earlier in two-kidney animals of this strain [35]. Early treatment with captopril (group A) markedly reduced systemic blood pressure and the filtration fraction without affecting GFR, and prevented the increase in glomerular volume expansion after UN. These findings suggest a captopril-induced reduction of glomerular transcapillary pressure, similar to what has been found during converting-enzyme inhibition by enalapril in the remnant kidney model [31, 33] and in experimental diabetes mellitus [32], two models associated with elevated intraglomerular flows and pressures. Micropuncture studies in these two models revealed that converting enzyme inhibition protected the glomerular capillary network from intracapillary hypertension by reducing systemic blood pressure and total arteriolar resistance without affecting the glomerular flow and filtration rate.

Morphometric analysis in those studies documented an increase in glomerular volumes in the untreated state of hyperfiltration and a reduction in glomerular volumes during converting enzyme inhibition [31, 32]. Long-term treatment with enalapril markedly ameliorated the development of GS in the remnant kidney model and in experimental diabetes mellitus. However, a comparison between the different models of glomerular hyperfiltration and GS is difficult because of obvious differences in systemic and intrarenal hemodynamic and metabolic determinants, and in genetic factors which may play a role in the susceptibility for progressive GS [22].

A reduction in SBP by approximately 30 mm Hg was observed in the captopril-treated animals of group C of the present study. The relatively high, systemic blood pressure in untreated rats of group D could be an important factor in the pathogenesis of GS, depending on the afferent arteriolar resistance. This level of SBP by itself does not inevitably cause GS, since rats of the WKY strain with similar levels of SBP are remarkably resistant to the development of GS [39]. Moreover, Brandis et al [40] recently described a relative resistance to GS in the spontaneously hypertensive Milan strain, in contrast to a high incidence of GS in the normotensive Milan strain, suggesting that systemic hypertension per se does not have to increase intraglomerular pressure or induce progressive GS.As stated earlier by Baldwin [41] and Brenner [42], afferent vasoconstriction is crucial in protecting the glomerulus from the systemic blood pressure. Elevation of intracapillary pressure can be caused by a disproportional dilatation of the afferent arteriole, which can occur after high protein intake [10, 14] or renal ablation [10, 31], and in experimental diabetes mellitus [15, 27] and hypertension [16, 43]. Other factors possibly involved in the pathogenesis of progressive GS, such as metabolic changes or coagulation factors, were not studied by us. Therefore we can not rule out an effect of converting enzyme inhibition along one of these lines. Serum cholesterol levels were higher in the proteinuric animals which may have had an additional effect on the progression of GS, since lipids are known to accumulate in the glomerular lesions [44], and lipid lowering agents have been shown to have a beneficial effect on the development of progressive GS in obese Zucker rats [45].

The introduction of captopril in proteinuric animals of group E at seven months after UN did not affect the progression of proteinuria and glomerular injury when compared to non-treated control animals. Moreover, a reduction in systemic blood pressure as observed after early treatment with captopril was not found in rats of group E. These findings are in contrast to the study recently published by Meyer et al [33]. They showed that the introduction of the converting enzyme inhibitor enalapril eight weeks after 5/6 renal ablation reversed systemic and glomerular hypertension, prevented a further rise in proteinuria and limited glomerular lesions at 18 weeks relative to untreated control rats. A similar effect was obtained by dietary protein restriction. The discrepancy between the study performed by Meyer et al and our study could be due to a number of differences in (a) the model: the remnant kidney model with systemic hypertension versus the milder model of unilateral nephrectomy without hypertension; (b) the time interval between ablation and intervention; (c) the converting enzyme inhibitor: enalapril versus captopril; and (d) the strain of rats: Munich-Wistar strain versus Wistar strain.

The lack of effect of captopril on systemic blood pressure and glomerular pathology in aging and already proteinuric rats in the present study may have been caused by several factors. Structural glomerular changes caused by hyalin deposition, sclerosis, mesangial cell proliferation and hypertrophy, may have resulted in a less sensitive target for pharmacological intervention, similar to what has been described for vascular and renal changes in advanced stages of systemic hypertension [46]. A lack of effect of CEI on proteinuria in animals of group E was not unexpected, since CEI generally leaves GFR and RPF intact as shown in animals of group A and in studies of the remnant kidney model [31]. With preservation of GFR and RPF, proteins will be filtered across the severely damaged capillary wall to a similar extent as in untreated animals. Recently, studies on the effect of converting enzyme inhibition in Adriamycin nephrosis showed that the introduction of a

converting-enzyme inhibitor, while lowering systemic [47, 48] and intraglomerular [47] pressures did not decrease protein excretion [47, 48]. However, protein excretion could be reduced in this model by dietary protein restriction [48, 49].

A recent study in patients with advanced diabetic nephropathy described a beneficial effect of CEI on urinary protein loss [50]. In man, protein restriction has already been shown to be effective in retarding the progression of several forms of chronic renal failure [51, 52]. A number of prospective clinical trials are now being carried out to study the effect of CEI on the course of chronic renal failure in man. Although it is hazardous to extrapolate findings of experimental models to the clinical setting, the results of the current study seem to caution for too optimistic expectations of CEI treatment in chronic renal failure.

In summary, captopril was found to prevent the development of GS in uninephrectomized male Wistar rats only when CEI was started early after UN. Under these circumstances captopril had a profound effect on SBP and renal vascular resistance as measured one month after UN. Initiation of captopril treatment at a later stage, when GS and proteinuria had already developed, proved to be ineffective with regard to SBP and the progression of proteinuria and glomerular lesions.

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Reprint requests to Jan J. Weening, M.D., Department of Pathology, P. O. Box 9603, 2300 RC Leiden, The Netherlands.

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