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Angiotensin-Converting Enzyme Inhibition in the Prevention and Treatment of Chronic Renal Damage in the Hypertensive Fawn-Hooded Rat

GAICO H. VERSEPUT,^{*†} ABRAHAM P. PROVOOST,^{*} BRANKO B. BRAAM,[†] JAN J. WEENING,[‡] and HEIN A. KOOMANS[†]

^{*}Department of Pediatric Surgery, Erasmus University Rotterdam, Rotterdam, The Netherlands; [†]Department of Nephrology, Utrecht University, Utrecht, The Netherlands; and [‡]Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

Abstract. The spontaneously hypertensive fawn-hooded rat (FHH) develops accelerated albuminuria and focal glomerular sclerosis (FGS), leading to ESRD and shortening of lifespan. The FHH is characterized by moderate systemic hypertension, a relatively low afferent to efferent arteriolar resistance ratio, and glomerular hypertension. The FHH study presented here was designed to examine the efficacy of early-onset, late-onset, or early-temporary angiotensin I-converting enzyme inhibition (ACE-i) in ameliorating long-term hypertension and FGS, and improving survival, as well as to relate its protective efficacy to preexistent FGS and to reduction of glomerular pressure (P_{GC}). Untreated rats developed hypertension and high P_{GC} , and all ($N = 22$) except one died of ESRD within the 72-wk follow-up period. Early-onset (at 7 wk of age) ACE-i prevented development of systemic and glomerular hypertension, and it largely prevented proteinuria and FGS; all rats survived throughout the follow-up period. Rats treated with late-onset (22 wk) ACE-i were hypertensive and proteinuric at the start of ACE-i, and

they showed beginning FGS. ACE-i corrected the hypertension, albuminuria, and P_{GC} but could not fully prevent some hypertension, albuminuria, and FGS at the later stage. Early-temporary (7 to 22 wk) ACE-i adequately controlled blood pressure and development of FGS during therapy, but after withdrawal of ACE-i, systemic and glomerular hypertension developed as in untreated animals. This regimen postponed but did not control FGS development and early mortality. The results of this study indicate that: (1) early-onset ACE-i very effectively protects against development of renal damage in the FHH; (2) this protection is associated with normalization of the elevated glomerular capillary pressure; (3) ACE-i cannot completely prevent further development of previously established FGS, despite lowering glomerular capillary pressure; (4) early-temporary ACE-i has no long-term controlling effect on arterial and glomerular pressure, and it cannot control development of FGS. (*J Am Soc Nephrol* 8: 249–259, 1997)

The spontaneously hypertensive fawn-hooded rat (FHH) develops albuminuria and renal damage early in life (1–3). This damage consists of focal glomerular sclerosis (FGS), eventually leading to ESRD and shortening of lifespan (2,3). Compared with the spontaneously hypertensive rat (SHR) (4,5), the young FHH has a low afferent arteriolar resistance and displays high capillary hydrostatic pressure and hyperfiltration (6,7). This explains why the kidneys of these rats are very susceptible to the deleterious effects of systemic hypertension. The constellation of hypertension, low afferent arteriolar resistance, and, therefore, high glomerular capillary pressure makes this rat a unique model for the study, prevention, and treatment of spontaneously developing FGS.

Most experience regarding pharmacologic prevention of

FGS in susceptible rodent models concerns angiotensin I-converting enzyme inhibition (ACE-i), which indeed has proven to be quite effective (8–19). In the FHH, this experience is limited to the observation that ACE-i can prevent the acceleration of renal damage induced by uninephrectomy (7,20). Thus, the first question of the study presented here was whether ACE-i, started early in life, can suppress or even prevent spontaneous development of FGS and improve survival in this model. A related question was how beneficial effects of ACE-i were related to changes in glomerular hemodynamics, specifically to a decrease in glomerular pressure.

The effect of late-onset ACE-i in models of hypertension varies from stabilization of renal damage to a lack of renoprotection (11,13,14,21–23). Regarding renal function or survival, protective effects of ACE-i in FHH conceivably diminish if it is started at a later age. If that is true, it is important to know whether the diminishing protective effects of ACE-i are related to glomerular changes already present by the time that ACE-i is initiated or by less effective reduction of glomerular pressure. As far as we know, such detailed studies have not been conducted in any rodent model of FGS.

Finally, it has been found in SHR that temporary ACE-i during a critical early period in the development of hyperten-

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Correspondence to Dr. H.A. Koomans, Department of Nephrology and Hypertension, Room F03.226, University Hospital Utrecht, P.O. Box 85500, 3508 GA The Netherlands.

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sion results in a permanently lower blood pressure and attenuation of vascular damage (24–26). This finding has not been studied in other models of spontaneous hypertension, in particular not in a model prone to develop renal damage. If early-temporary treatment with ACE-i attenuates later development of renal damage, it is important to know whether that damage results from the prevention of early glomerular damage or to permanent lowering of glomerular pressure.

To answer these questions, FHH were treated with ACE-i from 6 wk of age (early onset) or 22 wk of age (late onset) until death, or from 6 to 22 wk of age (early temporary). Long-term study items were hypertension, albuminuria, FGS, and survival. In addition, we examined glomerular hemodynamics, as well as levels of albuminuria and FGS at a critical stage during these interventions; we then related these to the long-term findings.

Materials and Methods

Experimental Design and Rats

Two studies were performed in male FHH. Both study designs are depicted in Figure 1. The long-term study assessed the effects of early-onset ACE-i (7 wk), late-onset ACE-i (22 wk), and early-temporary ACE-i (7–22 wk) on survival and development of hypertension and glomerular disease. In a separate study, micropuncture experiments were performed in four groups of 26-wk-old male FHH, either without treatment, or with early-onset ACE-i, with late-onset ACE-i, or with early-temporary ACE-i.

In the long-term study, we used male FHH from our colony, bred in the rat facilities at the Erasmus University Rotterdam. From the

twenty-fifth generation of this colony, an additional colony was maintained at the animal facilities of the Utrecht University. Male FHH from this colony were used in the micropuncture study. Rats in both studies were housed in pairs in standard rat cages. They were fed standard rat chow containing 0.40% sodium and 24% protein by weight (Hope Farms, Woerden, The Netherlands) and had free access to tap water. The protocols were approved by the institutional boards for animal studies.

Long-Term Study

Groups. Four groups of FHH were monitored during a preset follow-up period until they reached 72 wk of age (Figure 1). The control group (CON) consisted of 22 untreated rats. The early-onset ACE-i group (7LIS, $N = 10$) received lisinopril (50 mg/L drinking water) from 7 wk of age throughout the further follow-up period. The late-onset ACE-i group (22LIS, $N = 10$) received lisinopril from 22 wk of age throughout the follow-up period. The early-temporary ACE-i rats (7LIS22, $N = 10$) received lisinopril from 7 to 22 wk of age.

Experimental protocol. Every 6 to 8 wk, systolic blood pressure (SBP) was measured in conscious rats with the tail-cuff method (mean of at least four readings; IITC, Woodland Hills, CA), and the rats were placed in macrolon metabolism cages to collect two consecutive 24-h urine samples to assess albumin excretion (UalbV). Because FHH are affected with a bleeding diathesis (27) and we did not want to risk losing rats during the follow-up period because of hemorrhage, plasma creatinine and creatinine clearance were not obtained. Instead, we determined urine osmolality as an indication for kidney function (28). To determine survival, rats in the long-term study were allowed

Figure 1. Design of the studies. Protocols used in the long-term and micropuncture studies. ACE-i is angiotensin I-converting enzyme inhibition with 50 mg/L lisinopril (LIS) dissolved in the drinking water.

to live until natural death and were necropsied within 12 h. For these rats, the final data obtained before death are presented.

At 72 wk of age, surviving rats were anesthetized with 60 mg/kg of sodium pentobarbital. Blood was sampled to measure creatinine, and kidneys were perfusion-fixed for morphologic examination as described below. The non-fixed heart was removed, blotted dry, and weighed.

Micropuncture Study

Groups. Micropuncture was performed at 26 wk of age in four separate groups of FHH equally treated as in the long-term study (Figure 1): an untreated control group (CON, $N = 11$), an early-onset ACE-i group (7LIS, $N = 10$), a late-onset ACE-i group (22LIS, $N = 6$), and an early-temporary ACE-i group (7LIS22, $N = 5$). UalbV and SBP were measured as described above in the week before the micropuncture experiment. In the 22LIS and 7LIS22 rats, these parameters were also determined in the week before the start or withdrawal of lisinopril.

Micropuncture preparations. On the day of the micropuncture experiment, the rats were anesthetized with 60 mg/kg of sodium pentobarbital intraperitoneally and placed on a servo-controlled heated operating table that maintained rectal temperature at 37.5°C. The trachea was intubated with PE-240 tubing to ensure adequate ventilation. The left femoral artery was cannulated with a PE-50 tubing for continuous monitoring of mean arterial pressure (MAP) and blood sampling. Immediately after cannulation, approximately 75 μ L of arterial blood was collected to serve as a baseline value for the p-aminohippurate (PAH) assay.

Femoral arterial pressure was recorded with a Transpac pressure transducer (Abbott, North Chicago, IL) connected to a model 8805B amplifier and model 7758A chart recorder (Hewlett Packard, Waltham, MA). A specially designed double-lumen catheter, consisting of a PE-10 tubing inserted into a PE-60 tubing, was placed in the left femoral vein. The PE-10 tubing was used for the administration of additional anesthetic, and the PE-60 tubing was used for infusion of solutions. The plasma volume of rats prepared for micropuncture was reduced by ~20% (29). To study rats in an euolemic state, infusion was started after insertion of the femoral catheter with a solution of isotonic saline containing 15% inulin (Inutest, Laevosan Gesellschaft, Linz, Austria), 0.5% PAH, and 6% bovine serum albumin (BSA) (Organon, Boxtel, The Netherlands) at a rate of 0.1 mL/min to a total amount equal to 1% of body weight. Thereafter, infusion was continued throughout the experiment with a solution of isotonic saline containing 15% inulin, 0.5% PAH, and 1% BSA at a rate of 30 μ L/min. To assure maintained ACE inhibition during the micropuncture experiment, rats on lisinopril treatment received a continuous infusion of 0.2 mg/kg/h of lisinopril.

The left kidney was approached by a flank incision, freed from connective tissue, and placed in a plastic cup. The left ureter was cannulated with PE-10 tubing for urine collections. A 5% solution of warm agar was dripped around the kidney and on the Lucite cup to form a well, which was filled with saline or mineral oil. Following surgery, a 60-min equilibration period was observed before initiating the micropuncture procedures.

Experimental protocol. To determine whole-kidney GFR and RPF, urine was collected over four 30-min periods, and plasma samples were obtained at the start of the first and after the second and fourth urine collection.

Early proximal tubular segments were localized with a 4- to 6- μ m-tip-diameter localization pipette containing artificial tubular fluid (ATF) stained with 0.2% Fast Green (Sigma Chemicals, St. Louis, MO). The composition of the ATF was 135 mmol/L of NaCl, 5 mmol/L of KCl, 10 mmol/L of NaHCO₃, 1 mmol/L of MgSO₄, 1

mmol/L of CaCl₂, 1 mmol/L of Na₂ HPO₄/NaH₂ PO₄, and 4 mmol/L of urea (pH 7.40). Wax blocks were inserted into early proximal tubular segments by means of a 10- to 2- μ m-tip-diameter pipette filled with bone wax (Knochenwachs, Ethicon, Norderstedt, Germany) and connected to a hydraulic wax-blocking device (Research Instruments Manufacturing, Corvallis, OR). Values for stop flow pressure (P_{SF}) were obtained by introducing a 3- to 4- μ m-tip-diameter pressure pipette in a segment upstream of the wax blocks. The pressure pipette was filled with 2 M of NaCl and connected to a continuous recording servo-null pressure system (Model 5A, Instruments for Physiology and Medicine, San Diego, CA). Hydraulic pressure output from the linear motor of the servo-null pressure system was recorded with a Transpac pressure transducer connected to a second channel of the chart recorder. Proximal tubular pressure (P_T) was measured in at least four tubules by introducing the pressure pipette into a proximal tubular segment. Efferent arteriolar pressure (P_E) was assessed from at least four different star vessels by introducing the pressure pipette into a star vessel.

When pressure measurements were completed, the saline in the well was replaced with light mineral oil (Sigma Chemicals). For calculation of single-nephron glomerular filtration rate (SNGFR), three to five exactly-timed fluid samples were collected from randomly selected proximal tubular segments using 10- to 12- μ m-tip-diameter micropipets filled with water-saturated heavy mineral oil, stained with 3% Sudan Black (Sigma Chemicals). After introduction of the pipette, an oil block of 3 to 4 tubular diameters in length was inserted, and tubular fluid was collected for 3 to 5 min by gentle suction on the collection pipet. To assure complete collection, care was taken that the oil block remained situated at the tip of the pipette and that no fluid was leaking from the puncture site. Following termination of the collection, light mineral oil was aspirated into the tip of the pipet to prevent sample loss, and the sample was stored at -20°C until further analysis.

At the end of the experimental protocol, the MAP response to a bolus infusion of 0.1 mL isotonic saline containing 25 ng of angiotensin I was evaluated, and the kidneys were perfusion-fixed for morphologic examination as described below. The non-fixed hearts were removed, blotted dry, and weighed.

Morphology. The left kidney was perfused under pressure via an infrarenal aortic canula with a 0.9% saline solution for 1 min, followed by 1.25% glutaraldehyde in 0.1 M of sodium cacodylate buffer (pH 7.4) for 2 to 3 min. After excision and removal of extrarenal soft tissue elements, both kidneys were weighed. A coronal section of the perfused kidney was immersed in 1.25% glutaraldehyde for 1 day. After fixation, 2- to 3-mm kidney slices were embedded in paraffin and prepared for light microscopy. The extent of glomerular damage was determined on 3- μ m-thick sections stained with hematoxylin and eosin or periodic acid-Schiff reagent. Per rat, 100 glomeruli situated over the entire thickness of the cortex were evaluated for the presence of FGS lesions, i.e., segmental glomerular scarring, obliteration of glomerular capillaries, mesangial matrix expansion, and adhesion formation between tuft and Bowman's capsule. FGS is expressed as the percentage of glomeruli presenting these features.

Biochemical analyses. In 24-h urine collections, albumin concentration was measured with bromocresol green (Merck, Darmstadt, Germany), and creatinine and osmolality were measured with standard methods. In urine and plasma obtained during the micropuncture experiment, inulin was determined photometrically with indoleacetic acid after hydrolyzation to fructose (30). PAH was determined photometrically by a chromogenic aldehyde reaction (31). Hematocrit was determined with a microhematocrit centrifuge (Hettich, Tuttlingen, Germany). Colloid osmotic pressure in arterial samples was measured using a strain gauge micro-oncometer.

Micropuncture samples were transferred to constant-bore 1- μ L glass capillaries for measurement of the sample volumes with a Digimatic micrometer (Mitutoyo, Tokyo, Japan). Inulin concentrations in tubular fluid were measured by microfluorometry (32). Briefly, 8 nl samples were transferred to capillary cuvettes containing 10 mg/mL of dimedone in 85% phosphoric acid and stored in a closed container with silica gel to prevent moist absorption. The fluorophore was formed by heating the cuvettes for 10 min in a microwave oven, and fluorescence was measured with a Kontron SFM 25 fluorometer (Kontron Instruments, Zürich, Switzerland).

Calculations

Values for UalbV in each rat are calculated as the means of 2 consecutive days, and values for SBP were calculated as the means of measurements obtained on 3 consecutive days. UalbV and SBP in the long-term study are given as means of all rats alive at the moment of measurement. Survival was calculated as the percentage of rats in one group alive at any point during the follow-up period. In surviving rats, creatinine clearance was calculated from plasma creatinine measured at autopsy and the final 24-h urine creatinine.

The GFR and RPF were assessed from inulin and PAH clearance, respectively. Whole kidney filtration fraction (FF) is calculated from the equation: $FF = GFR/RPF$. Renal blood flow (RBF) was calculated from the equation $RBF = RPF / (1 - Hct)$. Glomerular capillary pressure (P_{GC}) under stop flow condition was calculated from the equation: $P_{GC} = P_{SF} + \pi_A$, in which π_A is the colloid osmotic pressure in femoral artery plasma samples, which is presumed to equal afferent arteriolar colloid osmotic pressure. The glomerular transcapillary pressure gradient (ΔP) was calculated from: $\Delta P = P_{GC} - P_T$. SNGFR was calculated from the equation $SNGFR = \text{tubular flow rate} \times [T/P]_{\text{INULIN}}$, in which $[T/P]_{\text{INULIN}}$ is the tubular fluid-to-plasma inulin ratio. Single nephron plasma flow (SNPF) was estimated from $SNPF = SNGFR/SNFF$ by assuming the single nephron filtration fraction (SNFF) and the FF to be similar (20). Afferent arteriolar single nephron blood flow ($SNBF_A$) was calculated from $SNBF_A = SNPF / (1 - Hct_A)$, in which Hct_A is the afferent arteriolar hematocrit presumed to equal systemic hematocrit. Single nephron efferent arteriolar blood flow ($SNBF_E$) was calculated from $SNBF_E = SNBF_A - SNGFR$. Afferent and efferent arteriolar resistances (R_A and R_E , respectively) were calculated as follows: $R_A = 7.962 \times 10^{10} \times (MAP - P_{GC})/SNBF_A$ and $R_E = 7.962 \times 10^{10} \times (P_{GC} - P_E)/SNBF_E$. Efferent arteriolar plasma protein concentration (C_E) was calculated from $C_E = C_A / (1 - SNFF)$. Using this calculated C_E , the efferent arteriolar colloid osmotic pressure (π_E) was estimated with the equation derived by Landis and Pappenheimer (33): $\pi_E = 1.631C_E + 0.249C_E^2$.

Because all rats were in filtration pressure disequilibrium, with values for $\pi_E/\Delta P < 1$, unique values for the glomerular ultrafiltration coefficient (Kf) could be estimated using the model described by Deen *et al.* (34). The differential equation was solved numerically on a single nephron basis, using the values of SNGFR, SNPF, π_E , and ΔP .

Statistical analyses. Values for each parameter were averaged per rat, and results are expressed as mean $\pm$ SE calculated from these averages. Data were compared with the use of statistical software (SigmaStat, Jandel Scientific, Erkrath, Germany). The tests used, mentioned in the text where appropriate, were considered significant for $P < 0.05$.

Results

Long-Term Study

Hypertension and albuminuria. At 7 wk of age SBP (Figure 2) and UalbV (Figure 3) were normal in all groups, but

with aging, untreated rats rapidly developed hypertension and albuminuria. Early-onset ACE-i prevented hypertension and albuminuria if continued throughout the follow-up period.

In the late-onset ACE-i group, SBP and UalbV had increased to 166 ± 2 mm Hg and 193 ± 18 mg/24 h, respectively, before the start of ACE-i (at 21 wk), and fell to 103 ± 3 mm Hg and 20 ± 3 mg/24 h after 3 wk of ACE-i (both $P < 0.01$, paired *t* test). During follow-up, SBP remained markedly lower than in the untreated group, but at later time points became significantly higher than in the early-onset ACE-i animals. Late-onset ACE-i reduced UalbV initially to levels found in the early-onset ACE-i group. Later, however, UalbV increased progressively from 40 wk of age, *i.e.*, after approximately 18 wk of ACE-i.

Withdrawal of ACE-i after 22 wk was quickly followed by hypertension to levels not different from those of untreated controls. In contrast, UalbV rose gradually. The increase of UalbV was postponed but of similar speed as that observed in untreated rats.

Survival. After 72 wk of follow-up, only one of the 22 untreated rats was alive (Figure 4). The first death occurred at 28 wk of age, and median survival was 55 wk. All rats showed considerable albuminuria (mean UalbV 403 ± 24 mg/24 h) and decreased urine osmolality (661 ± 50 mOsm/kg), indicating death from ESRD. No kidneys were available for light microscopy, but macroscopic examination showed severe fibrosis. The only untreated rat available for autopsy was in a poor condition (SBP, 170 mm Hg; UalbV, 332 mg/24 h; urine osmolality, 756 mOsm/kg), and an 80% FGS incidence indicated imminent ESRD.

In contrast, all rats with continued early-onset ACE-i were alive at 72 wk. With late-onset ACE-i, eight of 10 rats were alive at 72 wk. One death (46 wk) was unrelated to ESRD (final data: SBP, 103 mm Hg; UalbV, 6 mg/24 h; urine osmolality 1390 mOsm/kg), and the other rat death (68 wk) was related to ESRD (SBP, 133 mm Hg; UalbV, 441 mg/24 h; urine osmolality, 520 mOsm/kg). In the ACE-i withdrawal rats, only four out of 10 survived up to 72 wk. Median survival was 68 wk, which was longer than in untreated rats (Mann-Whitney Rank Sum Test, $P < 0.05$). Macroscopically, the kidneys showed severe fibrosis, and final data for UalbV (321 ± 34 mg/24 h) and urine osmolality (553 ± 61 mOsm/kg) similar to those of nonsurviving untreated rats indicate death from ESRD. In the four rats surviving until 72 wk, UalbV was 301 ± 60 mg/24 h, urine osmolality was 1376 ± 134 mOsm/kg, and FGS was present in $25 \pm 1\%$ of the glomeruli. Thus, renal disease in these rats was progressing but had not yet reached ESRD.

Autopsy. Table 1 summarizes final data of the continued early-onset (7LIS) and late-onset (22LIS) ACE-i groups. Rats with early-onset ACE-i were all in excellent condition at 72 wk of age with no hypertension, no signs of renal disease, and virtually no FGS. In surviving rats with late-onset ACE-i, body weight, SBP, creatinine clearance, total kidney weight, and heart weight were not different from those of rats with early-onset ACE-i. However, the higher values for UalbV and FGS incidence indicated progressing renal damage. The FGS incidence ($42.5 \pm 3.4\%$) was higher than in untreated rats at 26 wk

Figure 2. Systolic blood pressure. Serial values for systolic blood pressure (SBP) measured with the tail-cuff method in rats with no treatment (CON, ■), early-onset ACE-i (7LIS, □), late-onset ACE-i (22LIS, ○), and withdrawal of early-onset ACE-i (7LIS22, ●). Numbers in parentheses represent rats alive at that point. Numbering starts at the point prior to the loss of the first rat in that group. At all points after the first, rats with early-onset ACE-i had a significantly lower SBP than untreated rats. After late-onset and withdrawal of ACE-i, SBP was significantly lower and higher, respectively, at all points thereafter. Values are means $\pm$ SE. * $P < 0.05$ versus rats with early-onset ACE-i at the same point. Statistical tests used: two-way analysis of variance followed by a Student Newman Keuls test.

($16.4 \pm 1.5\%$, $P < 0.05$, unpaired t test), *i.e.*, around the start of late-onset ACE-i, indicating that FGS had increased despite treatment.

Micropuncture Study

Hypertension and albuminuria. In all groups, both SBP and UalbV prior to the micropuncture experiment (Figure 5) were similar as found at comparable ages in the long-term study (unpaired t tests). Early-onset ACE-i significantly lowered SBP (106 ± 4 mm Hg) and virtually prevented proteinuria (10 ± 1 mg/24 h). Rats with late-onset ACE-i had developed hypertension (SBP 164 ± 3 mm Hg) and albuminuria (118 ± 33 mg/24 h) at 21 wk of age. ACE-i lowered SBP ($103 \pm$ mm Hg, $P < 0.01$, paired t test) and UalbV (23 ± 3 mg/24 h, $P < 0.01$). Compared with early-onset ACE-i, SBP was similar, but UalbV remained slightly elevated. Withdrawal of ACE-i (7LIS22 rats) increased SBP from 106 ± 4 to 168 ± 3 mm Hg ($P < 0.01$)—thus, to a level similar to that in untreated controls. The UalbV increased from 8 ± 1 to 72 ± 8 mg/24 h ($P < 0.01$), but remained significantly lower than in untreated rats of the same age.

Systemic and whole-kidney data during the micropuncture experiment. Early-onset ACE-i rats had somewhat decreased body weight, reduced arterial pressure, GFR and FF,

and unchanged RBF (Table 2). Late-onset ACE-i animals showed equally reduced blood pressure and GFR, and somewhat more decreased FF. In the ACE-i withdrawal rats, all of these data were comparable with those in the untreated controls.

Glomerular hemodynamics. Compared with untreated controls, SNGFR was numerically lower in the early-onset ACE-i rats and significantly decreased in the late-onset ACE-i rats (Table 3). Both groups showed markedly reduced P_{GC} . In the ACE-i withdrawal rats, SNGFR was similar, and P_{GC} was somewhat elevated, compared with the untreated controls. Both early and late-onset ACE-i reduced R_A and R_E to a similar extent, and the R_A/R_E ratio was maintained roughly around 1. The Kf was significantly increased only in the early-onset ACE-i rats. The ACE-i withdrawal rats showed significantly increased R_A and R_E , and similar Kf compared with those in the untreated control rats.

Pressor responses to angiotensin I infusion following the micropuncture protocol indicated adequate ACE-i in both groups receiving lisinopril. MAP increased by 17 ± 1 mm Hg in the untreated rats, by 16 ± 2 mm Hg in the ACE-i withdrawal group, by 3 ± 1 mm Hg in the early-onset ACE-i rats, and by 3 ± 2 mm Hg in the late-onset ACE-i rats. Both latter

Figure 3. Albuminuria. Serial values for 24-h urinary albumin excretion (UalbV). Symbols and number of rats are the same as in Figure 2. After late-onset and withdrawal of ACE-i, UalbV was significantly lower and higher, respectively, at all points thereafter. Values are means $\pm$ SE. * $P < 0.05$ versus rats with early-onset ACE-i at the same point (two-way analysis of variance and Student Newman Keuls test).

responses were significantly less than in the untreated controls ($P < 0.01$, one-way analysis of variance and Student Newman Keuls test).

Pathology. Figure 6 depicts heart weight and FGS in rats that had micropuncture. Heart weights of rats with early- and late-onset ACE-i were significantly lower compared with heart weights of untreated rats. Four weeks of ACE-i withdrawal abolished this decrease in heart weight. Untreated rats had developed FGS at 26 wk of age ($16.4 \pm 1.5\%$). However, rats with early-onset ACE-i had developed virtually no FGS ($0.7 \pm 0.2\%$). In rats with late-onset ACE-i, FGS ($22.3 \pm 4.6\%$) was not significantly different from FGS in untreated rats. Four weeks of ACE-i withdrawal resulted in a FGS incidence of $5.0 \pm 0.8\%$, which was significantly higher than in rats with continued early-onset ACE-i.

Discussion

This study in FHH rats shows that ACE-i, if started early in life and given continuously, very effectively prevents hypertension and renal damage, and markedly prolongs life span. If started at later in life, ACE-i also effectively decreases blood pressure, reduces albuminuria, and prolongs life span; however, established FGS appears to progress in these rats as indicated by the increase in FGS incidence. Temporary treat-

ment with ACE-i early in life can only postpone development of hypertension, albuminuria, and renal damage.

The study presented here confirms that FHH spontaneously develops FGS and severe proteinuria, resulting in a short life span (1–3,6,7). The cause of the accelerated glomerular damage is not entirely clear, but it may well be related to alterations in glomerular hemodynamics. In agreement with earlier findings, we observed that the FHH has an afferent-to-efferent arteriolar resistance ratio of ~ 1 , indicating a relatively low afferent arteriolar resistance (6,7). This characteristic, combined with the elevated systemic blood pressure, leads to a relatively high glomerular capillary hydrostatic pressure and hyperfiltration (6,7). Wistar rats (WAG), a normotensive control strain, have a slightly lower systemic blood pressure but afferent arteriolar resistance twice as high as that found in FHH (6). The WAG does not develop accelerated FGS. Similarly, the SHR, which has a much higher blood pressure than that found in the FHH, has a much higher afferent-to-efferent arteriolar resistance ratio (4,5) and does not develop FGS. On the other hand, a relatively low afferent arteriolar resistance is observed after renal ablation (8,13) and in experimental diabetic nephropathy (10,18), both well-known models of progressive FGS.

One of our aims was to assess whether ACE-i, started early

Figure 4. Survival. Plot showing survival rate of rats with no treatment (CON), early-onset ACE-i (7LIS), late-onset ACE-i (22LIS), and withdrawal of early-onset ACE-i (7LIS22).

in life, provides prolonged renal protection in the FHH. Considering the characteristic glomerular hemodynamic effects of ACE-i, this was presumable. The data indicate that the protection offered by ACE-i was complete for the entire follow-up period until 72 wk of age. Survival was 100%, whereas it was 5% in untreated rats. In fact, as albuminuria and FGS were virtually absent at 72 wk of age, one might expect that these rats would have lived much beyond the time of sacrifice. Early-onset ACE-i has been studied in other models of FGS (8,11–17,19), but the follow-up was often relatively short.

Table 1. Data obtained at 72 wk of age^a

Parameter	7LIS Early Onset	22LIS Late Onset
Number	10	8
Body weight (g)	466 ± 15	454 ± 12
SBP (mm Hg)	112 ± 7	114 ± 4
UalbV (mg/24 h)	17 ± 7	77 ± 17 ^b
Plasma creatinine (mmol/L)	62 ± 1	63 ± 2
Creatinine clearance (mL/min)	1.42 ± 0.07	1.28 ± 0.08
Urine osmolality (mOsm/kg H ₂ O)	1566 ± 72	1478 ± 59
Heart weight (g)	1.26 ± 0.04	1.29 ± 0.02
Total kidney weight (g)	2.94 ± 0.09	2.96 ± 0.09
FGS (%)	1.7 ± 0.4	42.5 ± 3.4 ^b

^a Values are means ± SE, unpaired *t* test. SBP, systolic blood pressure (tail-cuff); UalbV, 24-h urinary albumin excretion rate; FGS, focal glomerular sclerosis.

^b *P* < 0.01 versus 7LIS.

Long-term follow-up is available for rats with experimental diabetes (10,18) and phase II aminonucleoside glomerulopathy (9). In these models, early-onset ACE-i largely or completely prevented FGS (9,10,18). In Munich Wistar (MW)-Fromter rats, 6 months of ACE-i was shown to largely prevent FGS, although some proteinuria developed (16,17). Early-onset ACE-i was also demonstrated to prevent renal damage in aging MW rats, but these are normotensive and develop FGS very slowly (11). Importantly, acceleration of FGS in each of these models is much less than in the FHH of the study presented here; this is also apparent from the fact that none of these studies mentions significant mortality. In this context, the efficacy of ACE-i to prolong life span in the FHH is a unique and most convincing observation.

The renoprotective effect of ACE-i is usually attributed to hemodynamic changes, leading to a reduction in P_{GC} (8–13,16,18). Indeed, ACE-i caused a consistent fall in P_{GC} in the FHH, along with a substantial fall in arterial pressure. Remarkably, the fall in blood pressure, whether measured in conscious rats or during anesthesia, was larger than in other models of FGS, such as in MW-Fromter rats (16,17) or rats with experimental diabetic nephropathy (10,18). Apparently, the FHH is very sensitive to the antihypertensive effects of ACE-i. The associated relaxation of the glomerular vasculature was distributed over afferent and efferent arterioles. This distribution is commonly found in rats during chronic ACE-i (5,8,9,12,13,16,20), except in rats with experimental diabetes (10,18), where ACE-i preferentially lowers R_E , or in aging rats (11), where R_E is unaffected. Therefore, the fall in P_{GC} was the consequence of the reduction in arterial pressure rather than of preferential efferent arteriolar relaxation. The other hemodynamic effects, *i.e.*, maintained SNPF and elevated K_f , are also well-known effects of chronic ACE-i (5,8–11,13,16,18). However, the reduction in GFR and SNGFR after ACE-i in the FHH is not compatible with our earlier observations in uninephrectomized FHH (20). This difference might be explained by the very strong fall in blood pressure with lisinopril used in this study when compared with the effect of enalapril (20).

Thus, we conclude that in the FHH, ACE-i reduces filtration pressure and filtration rate in the presence of maintained plasma flow. Clearly, our data are compatible with—but cannot prove a causal role for—the reduced P_{GC} and SNGFR in glomerular protection. Besides, it has been suggested that ACE-i may not only protect the kidney through renal hemodynamic changes (21,35,36). Alternative action concerns reduction of angiotensin II-mediated stimulation of growth of various renal cell types and reduction of angiotensin II-mediated stimulation of collagen production, both involved in the process of FGS (37,38).

Late-onset ACE-i was beneficial in that it prolonged life span, and limited the albuminuria found at the end of follow-up. Indeed, late-onset ACE-i effectively lowered the increased blood pressure and glomerular capillary pressure. Albuminuria dropped sharply. However, the renal protection was incomplete, since one of the 10 rats died of ESRD, and at the end of follow-up, albuminuria was again increasing in all rats. Furthermore, the incidence of FGS already present at the start of ACE-i was further increased. This result differs from a previ-

Table 2. Systemic and whole-kidney parameters at 26 wk of age^a

Parameter	CON Untreated	7LIS Early Onset	22LIS Late Onset	7LIS22 Withdrawal
Number	11	10	6	5
Body weight (g)	391 ± 6	345 ± 13 ^b	373 ± 4	394 ± 16
MAP (mm Hg)	121 ± 3	85 ± 2 ^b	76 ± 1 ^{b,c}	127 ± 2 ^c
GFR (mL/min)	1.52 ± 0.06	1.18 ± 0.08 ^b	1.09 ± 0.03 ^a	1.54 ± 0.08 ^c
FF	0.29 ± 0.02	0.21 ± 0.01 ^b	0.16 ± 0.01 ^{b,c}	0.37 ± 0.02 ^{b,c}
RBF (mL/min)	9.89 ± 0.45	9.80 ± 0.67	11.08 ± 0.63	8.45 ± 1.02

^a MAP, mean arterial pressure; FF, whole kidney filtration fraction. Values are means ± SE, one-way analysis of variance and Student Newman Keuls test.

^b $P < 0.05$ versus CON.

^c $P < 0.05$ versus 7LIS.

Table 3. Glomerular hemodynamics at 26 wk of age^a

Parameter	CON Untreated	7LIS Early Onset	22LIS Late Onset	7LIS22 Withdrawal
Number	11	10	6	5
SNGFR (nL/min)	58.6 ± 3.3	47.6 ± 3.8	38.9 ± 3.6 ^b	57.3 ± 4.9
SNBF (nL/min)	373 ± 20	422 ± 40	393 ± 39	317 ± 46
P _T (mm Hg)	12.2 ± 0.3	9.6 ± 0.2 ^b	11.1 ± 0.3 ^c	11.1 ± 0.06 ^c
P _E (mm Hg)	9.4 ± 0.5	7.7 ± 0.4	9.2 ± 0.5	10.6 ± 0.6 ^c
C _A (g/dL)	5.1 ± 0.1	4.6 ± 0.2	5.1 ± 0.1	4.8 ± 0.1
C _E (g/dL)	7.2 ± 0.2	5.9 ± 0.3 ^b	6.1 ± 0.1 ^b	7.7 ± 0.3 ^c
π _A (mm Hg)	15.6 ± 0.3	14.5 ± 0.5	16.3 ± 0.2 ^c	15.5 ± 0.6
π _E (mm Hg)	27.0 ± 1.1	20.1 ± 1.3 ^b	20.8 ± 0.4 ^b	30.2 ± 1.7 ^c
P _{SF} (mm Hg)	39.3 ± 0.8	25.6 ± 1.0 ^b	25.7 ± 1.1 ^b	44.8 ± 1.7 ^{b,c}
P _{GC} (mm Hg)	55.0 ± 0.9	40.1 ± 1.1 ^b	42.1 ± 1.2 ^b	60.3 ± 1.7 ^{b,c}
ΔP (mm Hg)	42.7 ± 1.1	30.5 ± 1.0 ^b	31.0 ± 1.1 ^b	49.3 ± 1.3 ^{b,c}
R _A (10 ¹⁰ dyne · sec · cm ⁻⁵)	1.44 ± 0.09	0.89 ± 0.06 ^b	0.73 ± 0.08 ^b	1.85 ± 0.32 ^{b,c}
R _E (10 ¹⁰ dyne · sec · cm ⁻⁵)	1.19 ± 0.07	0.77 ± 0.09 ^b	0.78 ± 0.11 ^b	1.70 ± 0.32 ^{b,c}
K _f (nl · s ⁻¹ · mm Hg ⁻¹)	0.046 ± 0.003	0.064 ± 0.006 ^b	0.053 ± 0.005	0.035 ± 0.003 ^c

^a SNGFR, single-nephron glomerular filtration rate; SNBF, single-nephron blood flow; P_T, proximal tubular pressure; P_E, efferent arteriolar pressure; C_A and C_E, afferent and efferent arteriolar plasma protein concentrations, respectively; π_A and π_E, afferent and efferent arteriolar colloid osmotic pressures, respectively; P_{SF}, stop flow pressure; P_{GC}, glomerular capillary pressure; ΔP, glomerular transcapillary pressure gradient; R_A and R_E, afferent and efferent arteriolar resistances, respectively; K_f, glomerular capillary ultrafiltration coefficient. Values are means ± SE, one-way analysis of variance and Student Newman Keuls test.

^b $P < 0.05$ versus CON.

^c $P < 0.05$ versus 7LIS.

ous finding in outbred FHH, in which similar late-onset ACE-i fully prevented the progression of FGS (39). However, this outbred strain developed FGS much slower than the current inbred FHH strain.

The study presented here allows comparison of early-onset and late-onset treatment with ACE-i. Such a comparison has been made in two other rat models, *i.e.*, unilateral (22) or 5/6 nephrectomy (13) and experimental diabetes (14). It appeared that, started amply before the development of proteinuria, ACE-i offered complete protection against the development of FGS; however, started once proteinuria had developed, ACE-i did not influence (14,22) or limited only partially (13) the degree of FGS found at the study end point. Recently, it was reported that late-onset ACE-i in aging MW rats was equally successful in reducing glomerular hydrostatic pressure as was

early-onset ACE-i, but much less effective in ameliorating proteinuria and FGS (11). An exceptional finding concerns diabetic rats, in which ACE-i, started at 20–24 wk of diabetes and modest proteinuria, prevented development of FGS, whereas later start of ACE-i did not, despite effective blood pressure control (14).

These and other data suggest that the protection against renal damage offered by ACE-i is absent or limited if some glomerular dysfunction and sclerosis have already developed (11,13,14,22). Remarkably, accurate data on glomerular function at the start of ACE-i has not been provided in any of these studies. In the study presented here, glomerular damage at the start of late-onset ACE-i was confirmed by significant pathological changes and also was functionally apparent from a low K_f and albuminuria. The level of FGS at the start of late-onset

Figure 5. Systolic blood pressure and albuminuria. Systolic blood pressure (SBP, left panel) and 24-h urinary albumin excretion (UalbV, right panel) measured 1 wk prior to the micropuncture experiment in rats with no treatment (CON), early-onset ACE-i (7LIS), late-onset ACE-i (22LIS), and withdrawal of early-onset ACE-i (7LIS22). * $P < 0.01$ versus CON. # $P < 0.01$ versus 7LIS (one-way analysis of variance and Student Newman Keuls test).

Figure 6. Pathology at micropuncture. Heart weight (left panel) and focal glomerular sclerosis (FGS, right panel) in rats with no treatment (CON), early-onset ACE-i (7LIS), late-onset ACE-i (22LIS) and withdrawal of early-onset ACE-i (7LIS22), after they had underwent micropuncture. * $P < 0.01$ versus CON. # $P < 0.01$ versus 7LIS (one-way analysis of variance and Student Newman Keuls test).

ACE-i was high compared with levels found in other models of renal disease (11,16,17), yet much of the albuminuria at this stage can result from a high P_{GC} and, thus, may not reflect glomerular damage *per se*. Indeed, the rapid fall in albuminuria after institution of ACE-i is unlikely due to glomerular repair but reflects the decrease in blood pressure. Nonetheless, at 4 wk after initiation of ACE-i, a subtle albuminuria remained, despite the fact that the micropuncture study showed a markedly lowered P_{GC} , not different from that in the early-onset ACE-i treated rats. Thus, it is likely that at this stage, an incipient functional lesion existed.

Because late-onset treatment with ACE-i could not prevent

development of albuminuria and a substantial increase in FGS later in life, one may conclude that this was related to the damage already present at the start of ACE-i and not to decreased ability of ACE-i to lower P_{GC} . This is a critical conclusion, but we have to recall the inevitable limitation that glomerular hemodynamic measurements were made during anesthesia. As it is, anesthesia may increase the dependency of systemic and glomerular hemodynamics from the renin-angiotensin system (40). Although late-onset ACE-i and early-onset lowered blood pressure similarly in conscious animals prior to the micropuncture experiments, we cannot exclude that the efficacy of late-onset ACE-i in lowering P_{GC} was not as high

in conscious animals. Moreover, at later stages, the blood-pressure-lowering effect of ACE-i appeared less than found after early-onset ACE-i. Decreased sensitivity of the blood pressure to ACE-i may reflect the effect of renal damage already present at the start of ACE-i. Conversely, less effective lowering of blood pressure may have contributed to the less effective renal protection.

Successful mitigation of FGS in proteinuric rats has been associated with profound decrease in proteinuria within weeks after the start of ACE-i (14), whereas failure in mitigation of FGS has been associated with no or only modest short-term drops in proteinuria after ACE-i (13,14,22). This closely resembles observations in humans with nondiabetic renal disease, where a high initial fall in proteinuria with ACE-i was associated with a reduced decline in long-term GFR (41). Although the findings of this study after late-onset ACE-i are not incompatible with this notion, it is also clear that even minute proteinuria persisting after the start of ACE-i may reflect the presence of FGS, of which the further evolution cannot be prevented, perhaps by the sustained loss of serum proteins (42).

Early-temporary ACE-i only postponed the development of renal damage. This part of our study was based on the finding in the SHR that a period of ACE-i in an early, critical phase of blood pressure rise prevented full expression of hypertension later in life (24–26). Clearly, this was not the case in the FHH: both systemic and glomerular hypertension developed fully after ACE-i withdrawal. Still, one could expect some protection against later development of renal damage, assuming that the temporary treatment with ACE-i had prevented FGS in a critical phase of development. The latter is likely, given the absence of FGS at 26 wk in early-onset ACE-i treated rats, compared with extensive FGS found in untreated rats of similar age. Nonetheless, glomerular preservation until this stage could not prevent, after withdrawal of ACE-i, the progression of albuminuria at a rate similar to that found in untreated rats. Since mortality in this group was delayed by an interval approximately as long as the duration of ACE-i treatment, it is likely that FGS indeed progressed at the same relentless speed as in the untreated rats. Moreover, the strong susceptibility of the FHH to develop FGS appears most striking because of the finding that only 4 wk of interruption of ACE-i was sufficient to show FGS more than observed in the continuously treated rats.

Consistent with findings in the SHR, heart weight was reduced with ACE-i and increased after withdrawal of ACE-i (43,44). This shows that hypertension-associated cardiac hypertrophy can be reversed with ACE-i, whereas high P_{GC} or high angiotensin II-mediated FGS cannot. However, the diminishing of cardiac hypertrophy could also be a direct effect of ACE-i independent of the effect on blood pressure, since Linz *et al.* (45) reported that a nonantihypertensive dose of ramipril completely reversed cardiac hypertrophy in hypertensive rats.

In summary, both early- and late-onset ACE-i effectively reduced systemic hypertension and albuminuria, and prolonged life span in the FHH. The key problem of the FHH is insufficient afferent arteriolar constriction, which, in association with modest systemic hypertension, results in glomerular hyperten-

sion. The resultant tendency to develop extreme FGS was effectively controlled by early-onset ACE-i, which normalized glomerular capillary pressure. However, when ACE-i was started after FGS was established, glomerular capillary pressure was still lowered, but only partial long-term protection was observed. Temporary treatment with ACE-i early in life only postponed the development of systemic and glomerular hypertension, albuminuria, and glomerular injury.

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