Similar renoprotection after renin-angiotensin-dependent and -independent antihypertensive therapy in 5/6-nephrectomized Ren-2 transgenic rats: are there blood pressure-independent effects?

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SUMMARY

1. Hypertension plays a critical role in the progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD), but it has also been postulated that antihypertensive drugs that block the renin-angiotensin system (RAS) show class-specific renoprotective actions beyond their blood pressure (BP)-lowering effects.

2. Because this notion has recently been questioned, in the present study we compared the effects of a RAS-dependent antihypertensive therapy (a combination of trandolapril, an angiotensin-converting enzyme inhibitor (ACEI) and losartan, an angiotensin-II (AngII) receptor subtype 1A receptor antagonist) with a ‘RAS-independent’ antihypertensive therapy (a combination of labetalol, an α-and β-adrenoreceptor antagonist with the diuretics, hydrochlorothiazide and furosemide) on the progression of CKD after 5/6 renal ablation (5/6 NX) in Ren-2 renal transgenic rats (TGR), a model of AngII-dependent hypertension. Normotensive transgene-negative Hannover Sprague–Dawley (HanSD) rats after 5/6 NX served as controls.

3. RAS-dependent and -independent antihypertensive therapies normalized BP and survival rate, and prevented the development of cardiac hypertrophy and glomerulosclerosis to the same degree in 5/6 NX HanSD rats and in 5/6 NX TGR. The present findings show that renoprotection, at least in rats after 5/6 NX, is predominantly BP-dependent. When equal lowering of BP was achieved, leading to normotension, cardiac and renoprotective effects were equivalent irrespective of the type of antihypertensive therapy.

4. These findings should be taken into consideration in attempts to develop new therapeutic approaches and strategies aimed to prevent the progression of CKD and to lower the incidence of ESRD.

Key words: alpha and beta adrenergic receptor antagonist, angiotensin-converting enzyme inhibitor, angiotensin-II receptor antagonist type 1, end-organ damage, glomerulosclerosis, hypertension, renin-angiotensin-aldosterone system, renoprotection.

INTRODUCTION

Chronic kidney disease (CKD) represents a serious medical problem of current nephrology, affecting millions of people worldwide and its incidence has been increasing steadily, especially in industrialized countries.1,2 It is well recognized that the natural course of progression of CKD to end-stage renal disease (ESRD) is independent of the initial insult: different renal diseases progress showing common pathomorphological signs, such as tubulointerstitial fibrosis and tubular atrophy, followed by glomerulosclerosis. It has been postulated that, regardless of the primary cause, the mechanisms underlying the progression of CKD are common; extensive investigations of these mechanisms have been carried out over the past 30 years.3–6 To this purpose, models of renal mass reduction have been extensively used; the model most frequently used is that of 5/6 renal ablation (5/6 NX), which results from unilateral nephrectomy combined with removal of 2/3 of the contralateral kidney. Studies using this model showed that hypertension is a major determinant of the rate of progression of CKD and the development of glomerulosclerosis.4–8 In contrast, a large body of experimental evidence has suggested that angiotensin-II (AngII) and aldosterone have a central role in this process.7–14 Based on this evidence showing a consistent renoprotection obtained with the renin-angiotensin system (RAS) blocking agents, the specific renoprotective properties of these drugs cannot be solely explained by their antihypertensive action, as some degree of
renoprotection already occurred with the doses of RAS blocking drugs that did not significantly lower blood pressure (BP). Further studies showed a dissociation between the antihypertensive and the renoprotective dose–response relationships of the RAS blockade in animals after 5/6 renal ablation (5/6 NX).6,12,14–17

However, the aforementioned concept is seriously questioned by more recent studies. These studies showed that if the antihypertensive regimes that did not directly inhibit the activity of the RAS (‘RAS-independent’) did not also impair autoregulation of the renal haemodynamics, (hence any elevations of BP were not transmitted to the glomerular microcirculation), they showed renoprotective effects similar to those obtained with antihypertensive regimes that did directly inhibit the RAS (‘RAS-dependent’).18–21 It was therefore concluded that renoprotective action of the RAS blocking drugs could be entirely attributed to BP reduction, with no evidence of additional BP-independent organ-protective effects.18–21 This conclusion is in direct opposition to the evidence that, at least in the 5/6 NX model, the effects of antihypertensive therapy on the intrarenal activity of the RAS is of crucial importance for renoprotection.22–24 Considering this striking controversy, it is important to thoroughly explore the whole issue in 5/6 NX rats, both in a strain with normal activity of the RAS and, in parallel, in a strain characterized by intrinsic hyperactivity of the RAS. The hypertensive rat transgenic for the mouse Ren-2 renin gene (TGR; strain name TGR(mRen2)27) represents a unique AngII-dependent animal model in which the development of hypertension is attributable to a single gene alteration.25 We and others have found that increased intrarenal activity of the RAS critically contributes to the pathophysiology of hypertension and hypertension-associated end-organ damage in this model.26–29

In the present complex project, we used both TGR and control normal rats in the following studies. First, we examined the course of progression of CKD after 5/6 NX in TGR and compared it with that observed in transgene-negative, normotensive, Hannover Sprague–Dawley (HanSD) rats, which is a strain characterized by physiologically normal activity of the RAS.25–29 Then, we examined how the course of CKD after 5/6 NX in TGR and HanSD rats is affected by treatment involving inhibition of the RAS (‘RAS-dependent’) and one devoid of such inhibitory influence (‘RAS-independent’). The former treatment consisted of a combined RAS inhibition when the inhibitor of angiotensin converting enzyme (ACE) and the angiotensin AT1 receptor antagonist were utilized, whereas the latter used a combination of antiadrenergic agent and two diuretics (‘antiadrenergic + diuretic therapy’). Finally, we investigated whether the renoprotective effects of the RAS-dependent or RAS-independent antihypertensive therapies on the progression of CKD after 5/6 NX are associated with systemic or local changes in the RAS. To this purpose, plasma and kidney tissue concentrations of AngII and urinary excretion of aldosterone were assessed in TGR and HanSD rats after 5/6 renal mass reduction.

**METHODS**

**Ethical approval and animals**

The studies were carried out in accordance with guidelines and practices established by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine, Prague, Czech Republic, which are in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All animals used in the present study were bred at the Department of Experimental Medicine of this Institute from stock animals supplied by the Max Delbrück Center for Molecular Medicine, Berlin, Germany, which is accredited by the Czech Association for Accreditation of Laboratory Animal Care. The animals were kept on a 12-h/12-h light/dark cycle. Throughout the experiments, rats were fed a normal salt, normal protein diet (0.45% NaCl, 19–21% protein) produced by SEMED (Prague, Czech Republic) and had free access to tap water.

**Therapeutic regimes**

The activity of the RAS can be pharmacologically altered at various levels. We reasoned that pharmacological intervention at more than one step in the RAS cascade would lead to more effective suppression of the system and, possibly, to better cardio- and renoprotection than interrupting RAS activity at a single level. Indeed, it was reported that a combination of angiotensin-converting enzyme inhibitor (ACEI) and AT1B receptor subtype 1 (AT1A) antagonist in high doses provides greater cardio- and renoprotection than do conventional doses used routinely for the treatment of hypertension.14,30–34 Thus, as the ‘RAS-dependent’ antihypertensive therapy, a combination of the ACE inhibitor trandolapril (Gopent; Abbot, Prague, Czech Republic; at a dose of 6 mg/L drinking water) and of the AT1A inhibitor losartan (Iloptiz, Zentiva, Prague, Czech Republic; at a dose of 100 mg/L drinking water) was used.

As ‘RAS-independent’ antihypertensive therapy, we used a combined alpha- and beta-adrenoceptor antagonist labetalol (Sigma Chemical, Prague, Czech Republic; at a dose of 400 mg/L drinking water) with the diuretic hydrochlorothiazide (Zentiva; at a dose 50 mg/L drinking water) and furosemide (Biotika, Martin, Slovak Republic; at a dose of 125 mg/L drinking water). Because hydrochlorothiazide is poorly soluble in water, we first prepared a stock solution in ethanol and then diluted it in water at pH 7. In order to prevent diuretic-induced hypokalaemia, 750 mg of KCl was added per 1 L drinking water. We have chosen this ‘RAS-independent’ antiadrenergic plus diuretic regime because in our preliminary studies we found it to effectively normalize BP in our TGR strain. Furthermore, this goal could not be readily achieved with the more conventional ‘triple therapy’ (hydralazine-hydrochlorothiazide-reserpine).

The efficiency of these two different antihypertensive regimes was in accordance with the recently published recommendations for BP measurements in experimental animals;25 furthermore, we evaluated this efficiency in preliminary studies employing different groups of TGR using a radiotelemetry system of direct BP measurements (see Fig. S1). Our preliminary studies (n = 5 in each experimental group) showed that both antihypertensive regimens exhibited similar BP-lowering effects in sham-operated (panel A of the Fig. S1) as well as in 5/6 NX TGR (panel B). In addition, the results of these studies showed that sham-operated and 5/6 NX TGR did not show any significant differences in the diurnal variations of the BP.

**Determination of plasma and tissue AngII levels, plasma and urine creatinine, proteinuria, renal glomerular damage, renal cortical tubulointerstitial injury and cardiac hypertrophy**

Rats were killed by decapitation (i.e. without anaesthesia), and plasma and tissue AngII levels were measured by radioimmunoassay. This approach was used because we recently showed that the measured AngII levels are altered by anaesthesia.26 In addition, this standardized approach allowed us to compare the present results with those from our previous studies; this helped evaluate the role of the RAS in the pathophysiology of hypertension on the basis of our long-term research.26,27,36–40 Urinary aldosterone concentrations were measured by a commercially available RIA kit (Immunotech, Prague, Czech Republic) as described previously.40

Plasma and urinary creatinine concentrations were measured by the picric acid colorimetric method using a commercially available kit (Lachema, Brno, Czech Republic). Urinary protein concentration was measured by the Biuret method using a commercially available kit (Lachema). To assess renal glomerular damage, the kidney was quickly removed, fixed in 4% formaldehyde, dehydrated and embedded in paraffin. The sections stained with haematoxylin and eosin and periodic acid–Schiff reaction were
examined and evaluated in a blind-test fashion. A total of 50 glomeruli in each kidney were examined on a semi-quantitative scale as described previously,29,41 grade 0, all glomeruli normal; grade 1, sclerotic area up to 25% (minimal sclerosis); grade 2, sclerotic area 25–50% (moderate sclerosis); grade 3, sclerotic area 50–75% (moderate-to-severe sclerosis); an grade 4, sclerotic area 75–100% (severe sclerosis). The glomerulosclerosis index (GSI) was calculated using the following formula: GSI = [(1 × n₁) + (2 × n₂) + (3 × n₃) + (4 × n₄)] / (n₀ + n₁ + n₂ + n₃ + n₄), where nᵢ is the number of glomeruli in each grade of glomerulosclerosis.

Renal cortical tubulointerstitial injury was evaluated as defined by Nakano et al.,42 and as used in our recent study,29 for inflammatory cell infiltration, tubular dilatation and/or atrophy, or interstitial fibrosis, and was graded semi-quantitatively using the following scale of lesions: grade 0, no abnormal findings; grade 1, mild (< 25% of the cortex); grade 2, moderate (25–50% of the cortex); and grade 3, severe (> 50% of the cortex). The lesions were assessed for at least 30 random and non-overlapping fields in the renal cortex.

Based on our previous experience,29,36,40 the ratio of left ventricle weight (LVW) to tibial length (TL), LVW/TL, was used to evaluate the degree of cardiac hypertrophy.

Experimental protocols

Series 1: Effects of RAS-dependent and RAS-independent antihypertensive therapy on survival rate, systolic BP, proteinuria, endogenous creatinine clearance and urinary aldosterone excretion in HanSD and TGR rats after 5/6 renal ablation

Male HanSD rats and heterozygous TGR (initial body weight 228 ± 11 g) from several litters were randomly assigned to experimental groups to make sure that the animals from a single litter did not prevail in any of the groups. In order to detect intergroup differences in systolic blood pressure (SBP) over time, SBP was measured in accordance with recommendations for BP measurements in conscious animals by tail-plethysmography through a tail-cuff apparatus (MC 4000; Hatteras Instruments, Cary, NC, USA; and RTBP 1007; Kent Scientific, Torrington, CT, USA). At least 3 days before starting measurements, rats were accustomed to the procedure of indirect tail-cuff SBP measurements. This method, regularly used in our laboratory,26,27,20,36,38,40 was previously validated and a close correlation was found between measurements by tail-plethysmography and direct BP measurements using an indwelling catheter in conscious rats. Measurements of SBP were started 7 days before 5/6 NX and carried out every 2 days until the end of the experiment. On day 0, 5/6 NX was carried out under anaesthesia (tiletamine + zolaze-pam; Virbac SA, Carros Cedex, France; 8 mg/kg; and xylasine, Spofa, Czech Republic; 4 mg/kg intramuscularly), as described previously.3,7,13,17,18,20,43,44 Briefly, an abdominal midline incision was carried out to expose the kidneys. The right kidney and both poles of the left kidney were removed surgically in order to remove 5/6 of renal parenchyma, as estimated by kidney weight. The abdominal wall and the skin were sutured. After 48 h recovery, rats received either no treatment or one of the two antihypertensive regimens. Because of the uncertainty regarding the survival rate of rats, especially those of the TGR strain subjected to 5/6 NX, and the expected high variability of the results, high initial n values were used. Thus, it allowed highly convincing data to be achieved, which would enable a reliable comparison of RAS-dependent versus RAS-independent therapy on the long-term survival rate. The following experimental groups were investigated:

1. Sham-operated HanSD rats + regular drinking water (initial n = 11)
2. 5/6 NX HanSD rats + water (initial n = 73)
3. 5/6 NX HanSD rats + trandolapril + losartan (ACEI + AT₁A antagonist) (initial n = 71)
4. 5/6 NX HanSD rats + labetalol + hydrochlorothiazide + furosemide (antiadrenergic + diuretic therapy) (initial n = 72)
5. Sham-operated TGR + water (initial n = 13)
6. 5/6 NX TGR + water (initial n = 77)
7. 5/6 NX TGR + ACEI + AT₁A antagonist (initial n = 71)
8. 5/6 NX TGR + antiadrenergic + diuretic therapy (initial n = 73)

The follow-up period was 16 weeks. At weeks ~2, and 4, 8, 12 and 16, before and after day 0, respectively, the animals were placed in individual metabolic cages, and after appropriate habituation training, their 24-h urine was collected for determination of protein, and for creatinine, to calculate endogenous creatinine clearance (a blood sample for determination of plasma creatinine was taken in the morning of the second day). This approach was previously validated in our laboratory and is regularly used in our studies.27,29,40,43,44

Series 2: Effects of RAS-dependent and RAS-independent antihypertensive therapy on plasma and kidney AngII levels, cardiac hypertrophy, renal glomerular damage and kidney tubulointerstitial injury

Animals were divided into the following experimental groups and were exposed to the same experimental protocol as in series 1:

1. Sham-operated HanSD rats + regular drinking water (initial n = 95)
2. 5/6 NX HanSD rats + water (initial n = 146)
3. 5/6 NX HanSD rats + ACEI + AT₁A antagonist (initial n = 124)
4. 5/6 NX HanSD rats + antiadrenergic + diuretic therapy (initial n = 132)
5. Sham-operated TGR + water (initial n = 103)
6. 5/6 NX TGR + water (initial n = 229)
7. 5/6 NX TGR + ACEI + AT₁A antagonist (initial n = 138)
8. 5/6 NX TGR + antiadrenergic + diuretic therapy (initial n = 142)

In this series, 19 rats from each experimental group were killed in weeks ~2, 4, 8, 12 and 16 before and after 5/6 NX, respectively (in the 5/6 NX TGR + water group, the experimental protocol ended on week 12 after 5/6 NX). Ten remnant kidneys were used for determination of kidney AngII concentrations, and the other nine for evaluation of renal glomerular damage and renal cortical tubulointerstitial injury. The ratio of LVW/TL was assessed in all 19 rats from each experimental group.

The total initial number of animals used in our experimental groups, including preliminary studies, was 1597 HanSD and TGR rats.

Statistical analysis

All values are expressed as mean ± SEM. Using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA), one-way analysis of variance or two-way repeated-measures analysis of variance followed by the Student–Newman–Keuls test were used, as appropriate. Values exceeding the 95% probability limits (P < 0.05) were considered statistically significant.

RESULTS

Series 1: Effects of RAS-dependent and RAS-independent antihypertensive therapies on survival rate, systolic BP, proteinuria, endogenous creatinine clearance and urinary aldosterone excretion in HanSD and TGR rats after 5/6 NX

All sham-operated HanSD rats and TGR survived until the end of the experiments. In contrast, untreated 5/6 NX HanSD rats started to die at week 9 after 5/6 NX, and the final survival rate was 57%, as shown in Fig. 1a. Both ACEI + AT₁A blockade and antiadrenergic + diuretic therapy improved the survival rate, to 93% and 97%, respectively (P < 0.05 vs untreated HanSD rats). As shown in Fig. 1b, untreated 5/6 NX TGR began to die at weeks 4–5 after 5/6 NX, and by week 13 after 5/6 NX no more animals survived. Both ACEI + AT₁A blockade and antiadrenergic + diuretic therapy dramatically improved the survival rate: the first mortality occurred at weeks 10 and 11 after 5/6 NX, respectively. The final survival rates were 92% and 97%, respectively.
As shown in Fig. 1c, sham-operated HanSD rats remained normotensive throughout the experiment. At week 4 in untreated HanSD rats, 5/6 NX caused a dramatic increase in SBP to 170 ± 4 mmHg by week 7. Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy not only prevented increases in SBP after 5/6 NX, but resulted in SBP levels significantly lower than that in sham-operated HanSD rats (111 ± 3 and 115 ± 3 vs 132 ± 3 mmHg, \( P < 0.05 \) in both cases) As shown in Fig. 1d, sham-operated TGR were markedly hypertensive throughout the experiment. From the initial SBP of 200 ± 5 mmHg, 5/6 NX caused a further substantial increase in SBP, which reached 232 ± 3 mmHg (\( P < 0.05 \)). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy decreased SBP in 5/6 NX TGR to similar normotensive levels that were not significantly different from that in sham-operated HanSD rats (127 ± 4 and 133 ± 4 vs 132 ± 3 mmHg). One difference between the effects of the two therapeutic regimes on SBP was that with ACEI + AT1A blockade, normotension was achieved 3 weeks earlier than with antiadrenergic + diuretics therapy.

As shown in Fig. 2a, sham-operated HanSD rats showed minimal proteinuria (8.5 ± 1.1 mg/24 h) throughout the experiment. 5/6 NX induced a marked increase in proteinuria, from 8.2 ± 0.6 to 56.4 ± 1.2 mg/24 h, beginning from week 8 after 5/6 NX (\( P < 0.05 \)); subsequently, proteinuria decreased but remained significantly elevated until the end of the experiment. The antiadrenergic + diuretic therapy completely prevented the development of proteinuria after 5/6 NX in HanSD rats. Figure 2b shows that sham-operated TGR showed pronounced proteinuria, more than a double of that observed in sham-operated HanSD rats, throughout the experiment (approximately 20 mg/24 h vs 8.2 ± 0.6 mg/24 h, \( P < 0.05 \)). Untreated 5/6 NX TGR showed a dramatic increase in proteinuria, from 19.1 ± 0.9 to a maximum of 116.2 ± 7.7 mg/24 h observed at the fourth week after 5/6 NX (\( P < 0.05 \)). The antiadrenergic + diuretic therapy also completely prevented the increase in proteinuria that occurred after 5/6 NX in TGR. Remarkably, ACEI + AT1A blockade not only stopped the increase in proteinuria after 5/6 NX, but reduced it below the initial values (4.6 ± 1.7 vs 20.7 ± 1.3 mg/24 h, \( P < 0.05 \)).

As shown in Fig. 2c, untreated 5/6 NX HanSD rats showed a progressive decline in creatinine clearance, from 839 ± 66 to 594 ± 42 mL/min/100 g of BW (\( P < 0.05 \)). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy stopped decreases in creatinine clearance after 5/6 NX in HanSD rats. Figure 2d shows a profound decrease in creatinine clearance in 5/6 NX TGR, to a minimum value observed in week 8 (from 866 ± 46 to
264 ± 46 μL/min/100 g BW; *P < 0.05). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy significantly attenuated these decreases; however, the clearance values remained significantly lower than that observed in sham-operated TGR (649 ± 33 and 632 ± 11 vs 855 ± 56 μL/min/100 g BW; P < 0.05).

Figure 3a shows that urinary aldosterone excretion was stable in sham-operated HanSD rats and increased markedly after 5/6 NX (from 39 ± 4 to 61 ± 3 ng/24 h (P < 0.05). The antiadrenergic + diuretic therapy did not attenuate the increase (from 38 ± 3 to
Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy completely prevented the development of cardiac hypertrophy. In contrast, hypertensive sham-operated TGR showed distinct cardiac hypertrophy when compared with the data for sham-operated HanSD rats (23.5 ± 0.8 vs 15.2 ± 0.7, P < 0.05), the LVW/TL ratio was elevated throughout the experiment (Fig. 5b). 5/6 NX induced a further progressive increase in LVW/TL (from 23.9 ± 0.7 to 29.8 ± 0.6, P < 0.05). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy not only prevented this increase but reduced the LVW/TL ratio below levels observed before 5/6 NX (17.6 ± 0.7 vs 23.8 ± 0.6 and 17.9 ± 0.6 vs 23.6 ± 0.7, P < 0.05).

Figure 6a shows that GSI was low and remained unchanged in sham-operated HanSD rats throughout the experiment. 5/6 NX resulted in a progressive rise in GSI, which reached its maximum 16 weeks after the operation (0.91 ± 0.07 vs 0.09 ± 0.02; P < 0.05). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy abolished the increases in GSI after 5/6 NX. As shown in Fig. 6b, sham-operated TGR showed a GSI that was substantially higher than in sham-operated HanSD (0.43 ± 0.03 vs 0.07 ± 0.015; P < 0.05). 5/6 NX caused marked increases in GSI, which reached its maximum in the 16th week after the operation (2.29 ± 0.09 vs 0.42 ± 0.02, P < 0.05). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy completely prevented the increases in GSI after 5/6 NX.

As shown in Fig. 6c, sham-operated HanSD rats showed a minimal degree of kidney tubulointerstitial injury. 5/6 NX resulted in a significant increase in renal tubulointerstitial injury, from 0.06 ± 0.015 to 1.12 ± 0.11 (P < 0.05). Both ACEI + AT1A blockade and antiadrenergic + diuretic therapy completely prevented the increases in renal tubulointerstitial injury after 5/6 NX. Figure 6d shows that in sham-operated TGR, the tubulointerstitial injury score was significantly higher than in sham-operated HanSD rats (0.19 ± 0.02 vs 0.06 ± 0.015, P < 0.05). 5/6 NX induced distinct increases in renal tubulointerstitial injury (from 0.19 ± 0.02 to 2.36 ± 0.09; P < 0.05). The progression of renal tubulointerstitial injury was significantly attenuated by both ACEI + AT1A blockade and antiadrenergic + diuretic therapy, but these protective effects were more pronounced during RAS inhibition (0.51 ± 0.04 vs 0.89 ± 0.05; P < 0.05).

**DISCUSSION**

The results of the present studies confirm that the progression of CKD after 5/6 NX in normal HanSD rats (development of proteinuria, impairment of renal function, progressive renal glomerular and cortical tubulointerstitial injury) and the development of cardiac hypertrophy are associated with the development of hypertension and an increase in the intrarenal activity of the RAS in the remnant kidney.9–14 Remarkably, in hypertensive TGR, a unique rat strain with intrinsic hyperactivity of the RAS, renal mass reduction (5/6 NX) caused a dramatic decrease of survival rate, and a further increase in BP and cardiac hypertrophy. The associated post-5/6 NX increases in proteinuria, glomerulosclerosis, and kidney tubulointerstitial injury, as well as a decrease in creatinine clearance, were more pronounced in TGR than observed in their HanSD counterparts. As expected, TGR showed elevated plasma and whole kidney AngII levels and urinary aldosterone excretion, but these values increased further after 5/6 NX in these animals. Taken together, these findings strongly support the notion that hypertension and increased intrarenal aldosterone excretion compared with sham-operated HanSD rats (64 ± 3 vs 37 ± 4 ng/24 h; P < 0.05). After 5/6 NX, TGR showed a progressive dramatic increase in urinary aldosterone excretion, from 63 ± 3 to 209 ± 11 ng/24 h (P < 0.05), and this change was not altered by antiadrenergic + diuretic therapy (persisting increase from 62 ± 4 to 231 ± 14 ng/24 h; P < 0.05). Similarly, as with HanSD rats, ACEI + AT1A blockade not only abolished the post-5/6 NX increase in urinary aldosterone excretion in TGR, but decreased it below levels observed in sham-operated TGR (16 ± 4 vs 64 ± 3 ng/24 h, P < 0.05).
activity of the RAS are two major determinants of the rate of progression of CKD, and suggest that antihypertensive therapy involving inhibition of the RAS ('RAS-dependent') could prevent the progression of CKD more effectively than can be obtained with 'RAS-independent' therapy. Thus, the preventive action of the former therapy might extend beyond its BP-lowering effects.7,9,10,14–17
The notion favouring BP-independent renoprotection, as suggested by superior effectiveness of RAS blockade, is consistent with two major theories describing the mechanisms underlying the progression of CKD. Renal micropuncture studies have shown that hyperfiltration in remnant nephrons that occurs as a part of the compensatory response to renal mass reduction ($5/6$ NX) is mediated by an increase in glomerular capillary pressure ($P_{GC}$). This increase is mainly the result of a relatively greater vasoconstriction of the glomerular efferent as compared with the afferent arteriole, an effect that is known to be mediated by AngII. Another hypothesis explaining the mechanism underlying the development of CKD, the so called ‘hypertrophy’ theory, is based on studies showing a strong correlation between the glomerular size (i.e. ‘growth’) and the degree of glomerulosclerosis. It is noteworthy that also in this theory a central role for AngII has been postulated.

In opposition to the views on a partly BP-independent beneficial role of RAS inhibition, the crucial finding of the present study is that the long-term RAS-dependent and the RAS-independent antihypertensive therapies provide a similar degree of cardio- and renoprotection in animals subjected to a major renal mass reduction. This is observed both in normal (HanSD) rats and in rats with genetically determined hyperactivity of RAS (TGR). This clearly shows that when BP is effectively restored to the normotensive range, the cardio- and renoprotective effects are independent of the type of antihypertensive therapy and no additional benefit is obtained related to the inhibition of the RAS.

Of special interest are our results showing that, also in the case of TGR, the RAS-independent antihypertensive therapy applied in the animals subjected to $5/6$ NX normalized the survival rate, proteinuria and glomerulosclerosis, restoring them to levels observed in sham-operated TGR. Furthermore, the therapy markedly attenuated the decrease in creatinine clearance and the development of kidney tubulointerstitial injury. It is important to emphasize that none of these organ-protective actions of the RAS-independent therapy was associated with any decrease in the intrarenal activity of the RAS. In contrast, whole kidney AngII concentration and urinary aldosterone excretion tended to be higher in $5/6$ NX TGR under antihypertensive therapy than in the untreated rats, even though the difference did not reach the level of significance. It is also noteworthy that antihypertensive therapy elicited significant increases in plasma AngII levels, both in $5/6$ NX HanSD rats and $5/6$ NX TGR. This was probably the result of the well-known intrarenal baroreceptor- and macula densa-mediated stimulatory effects of diuretics on renin release. The same therapy was also recently...

Fig. 6 (a,b) Glomerulosclerosis index and (c,d) kidney tubulointerstitial injury score in sham-operated Hannover Sprague–Dawley (HanSD; transgene-negative) rats and in heterozygous Ren-2 renin transgenic rats (TGR), and in $5/6$ nephrectomized ($5/6$ NX) HanSD and TGR rats, untreated or receiving either a combination of trandolapril, an angiotensin converting enzyme inhibitor (ACEI), and losartan, an antagonist of angiotensin-II AT$_{1A}$ receptor, or a combination of labetalol, an adrenergic receptor antagonist and diuretics, hydrochlorothiazide and furosemide (antihypertensive + diuretic therapy). *$P < 0.05$ compared with basal values. **$P < 0.05$ compared with treated groups at the same time point.
found to increase plasma AngII in normotensive rats and in the 
Cyp1a1-Ren-2 transgenic rats, another AngII-dependent model of 
hypertension.36

In this context, our results regarding the effects of the RAS-depen-
tant antihypertensive therapy on AngII levels and urinary aldo-
sterone excretion are of special interest. First, the finding that plasma 
AngII levels in 5/6 NX HanSD rats and 5/6 NX TGR under ACE 
inhibition + AT1 receptor blockade were the highest among all our 
experimental groups is in good agreement with previous stud-
iess36,37,51,52 and is consistent with the concept that interruption of 
the short-loop negative feedback effect of AT1 receptor activation results in 
increased renin secretion and circulating plasma AngII levels.52

Second, we showed that ACEI + AT1 blockade not only prevented 
the rise in kidney AngII concentrations and in urinary aldosterone 
excretion in 5/6 NX HanSD rats and in 5/6 NX TGR, but decreased 
both these parameters substantially below the values observed in 
sham-operated normotensive HanSD rats. These results show that 
besides the ability of the remnant kidney to produce AngII from 
enogenous intrarenal components, which occurs mostly through the 
ACE-dependent pathway, the augmentation of AngII levels in the 
remnant kidney is also the result of the uptake of circulating AngII 
by intrarenal AT1 receptors. These findings are in good agreement 
with previous studies aimed at evaluating the role of intrarenal RAS 
in the pathophysiology of hypertension and hypertension-associated 
end-organ damage.10,11,14,36,37,53

Considering the finding that our RAS-dependent antihypertensive 
therapy normalized BP to the same degree as did the RAS-indepen-
dent therapy and, in addition, it reduced intrarenal activity of the 
RAS even below levels observed in normotensive animals, one 
would expect a more effective renoprotection against the progression 
of CKD with the former therapy variant. This should be the case at 
least in 5/6 NX TGR that are characterized by marked activation of 
the intrarenal RAS and by accelerated progression of CKD. How-
ever, our present findings show that the long-term RAS-independent 
and RAS-dependent antihypertensive therapies show almost identical 
cardio- and renoprotective effects, both in 5/6 NX HanSD rats and 
in 5/6 NX TGR. At the most, with respect to the prevention of 
kidney tubulointerstitial injury, in the latter strain the ACEI + AT1 
blockade was slightly more effective than the antidiuretic + 
blockade. 

Taken together, our findings show that, at least in rats after 5/6 
NX, cardio- and renoprotection are predominantly or almost exclu-
sively BP-dependent. When equal BP reduction down to the normo-
tensive range is achieved, cardio- and renoprotective effects are 
equivalent irrespective of the type of antihypertensive therapy. 
In our present study, we cannot offer a fully satisfactory explanation 
for the lack of any evidence on BP-independent organ-protective 
action of the RAS-dependent antihypertensive therapy. This finding 
is in contrast to some previous studies evaluating the effects of the 
RAS blockade on the development of end-organ damage associated 
with hypertension, which showed that the protection provided by 
the blockade was to some extent BP-independent.15,9,12,14-17 How-
ever, our present results are in very good agreement with the recent 
findings of Mori and Cowley,54 who tried to evaluate the relative 
contribution of BP- versus AngII-dependent effects to the develop-
ment of renal injury in AngII-infused hypertensive rats. In this 
elegant study using a servo-control technique that maintains renal 
perfusion pressure in hypertensive rats within the normotensive 
range, they showed that the major portion of renal injury is 
BP-dependent and only 20-25% of the interstitial fibrosis could be 
accounted for by a direct effect of AngII. In this context, it is also 
important to note that Mori and Cowley,54 and Ihara et al.55 
reported that juxtamедullary glomeruli are more sensitive to BP-
dependent and proteinuria-induced injury than are superficial cortical 
glomeruli. Therefore, it was concluded that RAS-dependent anti-
hypertensive therapies could show better protective efficiency in 
juxtamедullary glomeruli. However, the results of the present study 
do not allow us to address this issue, because we did not determine 
the injury in juxtamедullary glomeruli. It should be also mentioned 
that because in our preliminary studies we did not observe any sig-
nificant differences in diurnal variations of BP between individual 
groups of TGR, it is unlikely that such variations appreciably 
contributed to the pattern and extent of renal injury in TGR after 
5/6 reduction of renal mass.

The studies by the group of Griffin and Bidani18-20 suggest that 
the reason for the discrepancy between studies fostering and rejecting 
BP-independent protective action might be of methodological nature. 
The former studies solely used the conventional BP measurements 
by the tail-cuff method. This is a serious methodological limitation; 
as reported recently, this method might not be suitable to determine 
minimal but still significant BP changes.18-21,35 In order to obviate 
this problem, in our preliminary study, we used radiotelemetric BP 
measurements in TGR and confirmed that BP reductions are equal 
with the two antihypertensive regimes used in the present study. 
Thus, any differences in the cardio- and renoprotective effects could 
not be ascribed to superior BP reduction obtained with either anti-
hypertensive treatment protocol.

In summary, the present results show that: (i) in hypertensive TGR 
subjected to 5/6 renal mass reduction, the progression of CKD was 
dramatically accelerated compared with that observed in initially nor-
matotensive HanSD rats, which indicates that hypertension is a major 
determinant of the rate of progression of CKD in this AngII-depen-
dent hypertensive model after 5/6 NX; and (ii) RAS-independent 
and RAS-dependent antihypertensive therapies induced equal reduc-
tions of BP and almost identical cardio- and renoprotective effects on 
the progression of CKD after 5/6 NX, in both HanSD rats and TGR. 
This shows that cardio- and renoprotection after 5/6 NX are 
predominantly BP-dependent. Taken together, our findings strongly 
suggest that normalization of BP, irrespective of the type of antihy-
pertensive therapy applied, is the best approach to achieve renoprot-
ecction in subjects with severe reduction of renal mass and function. 
This should be considered in attempts to develop new therapeutic 
approaches and strategies for the prevention of progression of CKD 
and reduction of the incidence of ESRD.
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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Fig. S1** Mean arterial pressure measured by radiotelemetric method in (a) sham-operated and (b) 5/6 nephrectomized (5/6 NX) conscious heterozygous Ren-2 renin transgenic rats (TGR), untreated or receiving either a combination of trandolapril, an angiotensin converting enzyme inhibitor (ACEI), and losartan, an antagonist of angiotensin-II AT1A receptor, or a combination of labetalol, an adrenergic receptor antagonist and diuretics hydrochlorothiazide and furosemide (adrenergic + diuretic therapy). *P < 0.05* compared with basal values.

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