

# The Selective TP Receptor Antagonist, S18886 (Terutroban), Attenuates Renal Damage in the Double Transgenic Rat Model of Hypertension

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## Key Words

Renin-angiotensin system · Hypertension · Thromboxane · Glomerulosclerosis · Tubulointerstitial damage · Proteinuria

## Abstract

**Background/Aims:** Thromboxane receptors play a decisive role in the renovascular actions of angiotensin II. We studied the efficacy of the selective thromboxane receptor antagonist, S18886, in the retardation of renal damage in the double transgenic rats (dTGR), harboring human renin and angiotensinogen genes. **Methods:** dTGR were gavaged daily with either S18886 (30 mg/kg/day, n = 12), or placebo (dTGR-Plac, tap water, n = 14) for 3 weeks. Matched Sprague-Dawley rats (n = 10) served as controls. **Results:** The dTGR-Plac had higher systolic blood pressure (1.7-fold) than controls, and developed profound renal damage with significantly higher proteinuria (6.9-fold), polyuria (2.3-fold), index of glomerulosclerosis (+58%), and tubulointerstitial (+47%) and vascular damage scores (+19%). Creatinine concentration and the mesangiolysis index remained unchanged. In dTGR, S18886 slightly lowered the blood pressure (162 ± 15 vs. 149 ± 13 mm Hg, not significant) and improved proteinuria (558 ± 218 vs. 136 ± 71 mg/μmol creatinine, p < 0.01), polyuria and renal morphology (glomerulosclerosis index: 0.79 ± 0.05 vs. 0.66 ± 0.13, p < 0.01; tubulointerstitial damage index: 1.82 ± 0.22 vs. 1.49 ± 0.27, p < 0.05; mesangiolysis index: 1.31 ± 0.18 vs. 0.36 ± 0.09, p < 0.01). Vascular damage

score and plasma creatinine were not influenced. S18886 did not alter measured markers of oxidative stress. **Conclusion:** The data present the first evidence that thromboxane receptor inhibition ameliorates angiotensin II-induced nephropathy.

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## Introduction

Angiotensin II (Ang-II) activates vasopressor mechanisms and, in subpressor doses, induces oxidative stress in the vessel wall, heart and kidney [1, 2], potentiating its vasoconstrictory effects. It stimulates the synthesis of isoprostanes, which may contribute to the pressor response via induction of vasoconstriction and renal sodium retention [1, 3]. Hence, thromboxane A<sub>2</sub> (TXA<sub>2</sub>) binds to thromboxane receptors (TPr) in renal vascular tissue and thereby aggravates vascular damage [3, 4]. TXA<sub>2</sub> induces contraction of mesangial cells, the glomeruli and the afferent/efferent arterioles [5]. In the central nervous system the dipsogenic action of Ang-II seems to be mediated via the TPr [6].

S18886, a polysubstituted tetrahydronaphthalene derivative, is a new highly selective, long-acting TPr antagonist [7] with potent antiplatelet, antivasoconstrictory and antiproliferative effects. It antagonizes the binding of TXA<sub>2</sub>, and the other arachidonic acid metabolites (i.e. prostaglandins, HETE acids, isoprostanes) to TPr with

different rank order of potency [8]. These actions render the substance beneficial in the prevention of hypertension or atherosclerosis-associated organ damage [7, 9]. S18886 inhibited the development of atherosclerosis in rabbit models [10, 11], and in apolipoprotein E-deficient (apoE<sup>-/-</sup>) mice [12, 13]. It exerted renoprotective and antioxidant actions in diabetic apoE<sup>-/-</sup> mice [14] and in obese Zucker rats [15].

Double transgenic rats (dTGR, harboring human renin and angiotensinogen genes) develop accelerated hypertension leading to marked damage of the kidney, and heart [16]. Ang-II-induced inflammatory response contributes to fatal organ damage [17]. Since TPr play a decisive role in the Ang-II-dependent alterations of renal hemodynamics and oxidative stress, we hypothesized that TPr antagonism with S18886 may retard the renal injury in the dTGR model.

## Animals and Methods

The investigation was conducted according to the guidelines for studies using laboratory animals, after approval of the protocol by the Institutional Ethics Committee for Experimental Animals (Bratislava, Slovakia).

### Rats

Four-week-old male dTGR (RCC Ltd, Füllinsdorf, Switzerland) received a daily gavage of either S18886 (30 mg/kg, n = 12), or placebo (dTGR-Plac, tap water, n = 14) for 3 weeks. Control Sprague-Dawley rats (SD, Charles River, Sulzfeld, Germany, n = 10) received placebo. Animals had free access to drinking water and a standard rat chow. None of the animals died during the study.

### Experimental Protocol

Before sacrifice, body weight and systolic blood pressure (SBP, tail plethysmography) were recorded, and 24-hour urine collected. At sacrifice, blood was sampled from the abdominal aorta under anesthesia. Kidneys and heart were removed after retrograde perfusion fixation with glutaraldehyde via the abdominal aorta as previously described [18]. Routine blood and urine chemistry was measured by an autoanalyzer (Vitros 250, J&J, Rochester, N.Y., USA), plasma malondialdehyde (MDA) by HPLC with fluorimetric detection [19], erythrocyte glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity by commercial kits (Randox, Crumlin, UK). Urine osmolarity was determined. Creatinine clearance was calculated.

### Tissue Preparation

Semithin and paraffin-embedded kidney sections stained with methylene blue/basic fuchsin and hematoxylin and eosin (HE) or periodic acid-Schiff (PAS), respectively, were prepared as described previously [15]. Histomorphological evaluations were performed in a blinded manner.

### Semiquantitative Indices of Mesangiolytic (MGI), Glomerulosclerosis (GSI), Tubulointerstitial (TSI) and Vascular Damage (VSI)

Damage of mesangial or endothelial cells and of the mesangial matrix, i.e. the mesangiolytic score (0–4; MGI); mesangial matrix expansion, i.e. the glomerulosclerosis score (0–4; GSI); tubulointerstitial damage (0–4; TSI), i.e. tubular dilatation, tubular atrophy, interstitial inflammation and fibrosis, and vascular damage (0–4; VSI), i.e. wall-thickening and fibrinoid necrosis, were assessed on PAS sections as described [15, 20].

### Glomerular Geometry

Glomerular geometry and area density of glomerular tuft were analyzed at a magnification of 400× on HE sections [18, 20, 21]. The total number of glomeruli was derived from the total volume of the renal cortex and the number of glomeruli per cortex volume, and the mean glomerular tuft volume was calculated [21].

### Glomerular Cells and Capillaries

Semithin sections were qualitatively inspected for glomerular cellular changes, i.e. podocyte enlargement and degeneration, and mesangial or endothelial cell hyperplasia. Glomerular capillarization and cellularity were counted on 5 semithin sections (at least 30 glomeruli per animal) as described previously [18]. The number of cells (mesangial, endothelial, parietal cells, and podocytes) per glomerulus was determined [18, 15].

### Statistics

The data were tested for normality and equality of variance, and appropriate tests were applied to compare the data, i.e. one-way analysis of variance (ANOVA) with post-hoc Scheffé's test, or Kruskal-Wallis with Mann-Whitney U-tests. Results are given as mean ± SD, or as median, mean ± SD (not normally distributed data).  $p < 0.05$  was considered significant.

## Results

### Comparison between dTGR-Plac and SD (table 1)

dTGR-Plac developed hypertension (+63 mm Hg), heart hypertrophy, and renal damage. Kidney to body weight ratio, plasma creatinine concentration and creatinine clearance remained comparable. dTGR developed proteinuria (6.9-fold), polyuria (2.2-fold), had lower urine osmolarity (−60%), and mild glomerulosclerosis (fig. 1, 2a, b), more substantial tubulointerstitial and vascular damage (fig. 1, 2g). Glomerular cell number was significantly higher in the dTGR-Plac, indicating mild glomerular hypercellularity, particularly of mesangial and endothelial cells (fig. 2j, k; table 1). The significantly higher length density (total capillary length per glomerular volume), and lower mean capillary cross-sectional area indicated lengthening and remodeling of glomerular capillaries with narrowing of capillary lumina in dTGR-Plac. No changes in mesangiolytic or mean glomerular volume were observed (fig. 1, 2d, e; table 1).

**Table 1.** Animal data and renal morphology

	SD (n = 10)	dTGR-Plac (n = 14)	dTGR-S18886 (n = 12)	ANOVA/K-W	
				F	p
Initial body weight, g	118 ± 10	107 ± 11	110 ± 17	1.34	0.275
Body weight at sacrifice, g	245; 242 ± 14	200; 211 ± 27 <sup>b</sup>	220; 212 ± 17 <sup>b</sup>	10.35	0.006
Weight gain, g	125; 124 ± 8	100; 104 ± 4	103; 102 ± 5	16.60	0.0002
SBP, mm Hg	98; 99 ± 7	163; 162 ± 15 <sup>b</sup>	150; 149 ± 13 <sup>b</sup>	23.56	7.7 × 10 <sup>-6</sup>
Heart weight, g	0.92 ± 0.01	1.21 ± 0.11 <sup>b</sup>	1.16 ± 0.13 <sup>b</sup>	19.77	2.3 × 10 <sup>-6</sup>
Heart/body weight, mg/g	3.8; 3.8 ± 0.3	5.8; 5.8 ± 0.6 <sup>b</sup>	5.4; 5.5 ± 0.4 <sup>b</sup>	22.02	1.7 × 10 <sup>-5</sup>
Kidney/body weight, mg/g	4.5 ± 0.3	4.6 ± 0.3	4.8 ± 0.4	2.16	0.131
Glomerular volume, μm <sup>3</sup>	495 ± 79	510 ± 64	487 ± 61	0.430	0.661
Glomerular cell number	926 ± 120	1,071 ± 88 <sup>b</sup>	1,026 ± 86	6.12	0.006
Podocyte number	278 ± 45	257 ± 32	268 ± 59	0.509	0.607
Mesangial cell number	276 ± 60	351 ± 51 <sup>a</sup>	310 ± 38	5.25	0.012
Endothelial cell number	240 ± 47	346 ± 39 <sup>b</sup>	310 ± 46 <sup>b</sup>	14.10	6.4 × 10 <sup>-5</sup>
Parietal cell number	132 ± 21	137 ± 15	139 ± 14	0.434	0.652
Capillary length density, mm/mm <sup>3</sup>	9,729 ± 434	11,345 ± 640 <sup>b</sup>	11,218 ± 1,243 <sup>b</sup>	10.82	0.0004
Capillary cross-sectional area, μm <sup>2</sup>	36; 36 ± 4	26; 25 ± 2 <sup>b</sup>	28; 28 ± 2 <sup>b, c</sup>	20.05	2.8 × 10 <sup>-5</sup>

Results are means ± SD, or medians with means ± SD for not normally distributed data.

SD = Sprague-Dawley rats; dTGR = double transgenic rats; Plac = placebo; ANOVA = one-way analysis of variance; K-W = Kruskal-Wallis test; SBP = systolic blood pressure.

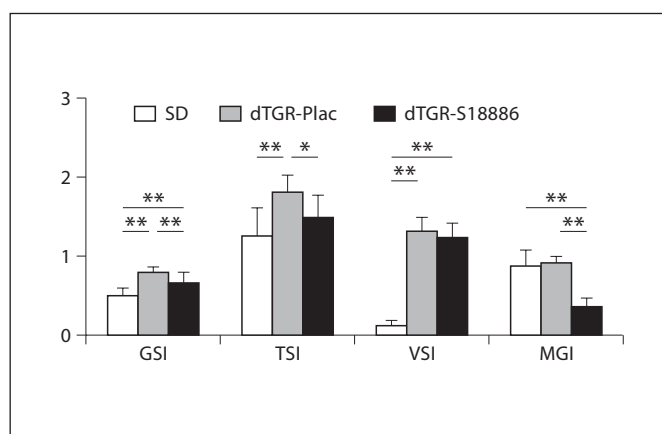
<sup>a</sup> p < 0.05 vs. SD; <sup>b</sup> p < 0.01 vs. SD; <sup>c</sup> p < 0.05 vs. dTGR-Plac.

In the dTGR-Plac, plasma cholesterol levels were higher. Triacylglycerol (TAG) concentration did not differ significantly. Plasma MDA levels were elevated. GPX activity increased. SOD activity remained unaffected (table 2). Body weight (comparable at the initiation of the experiment) was significantly lower in dTGR at sacrifice.

#### Effects of S18886 in the dTGR (table 1)

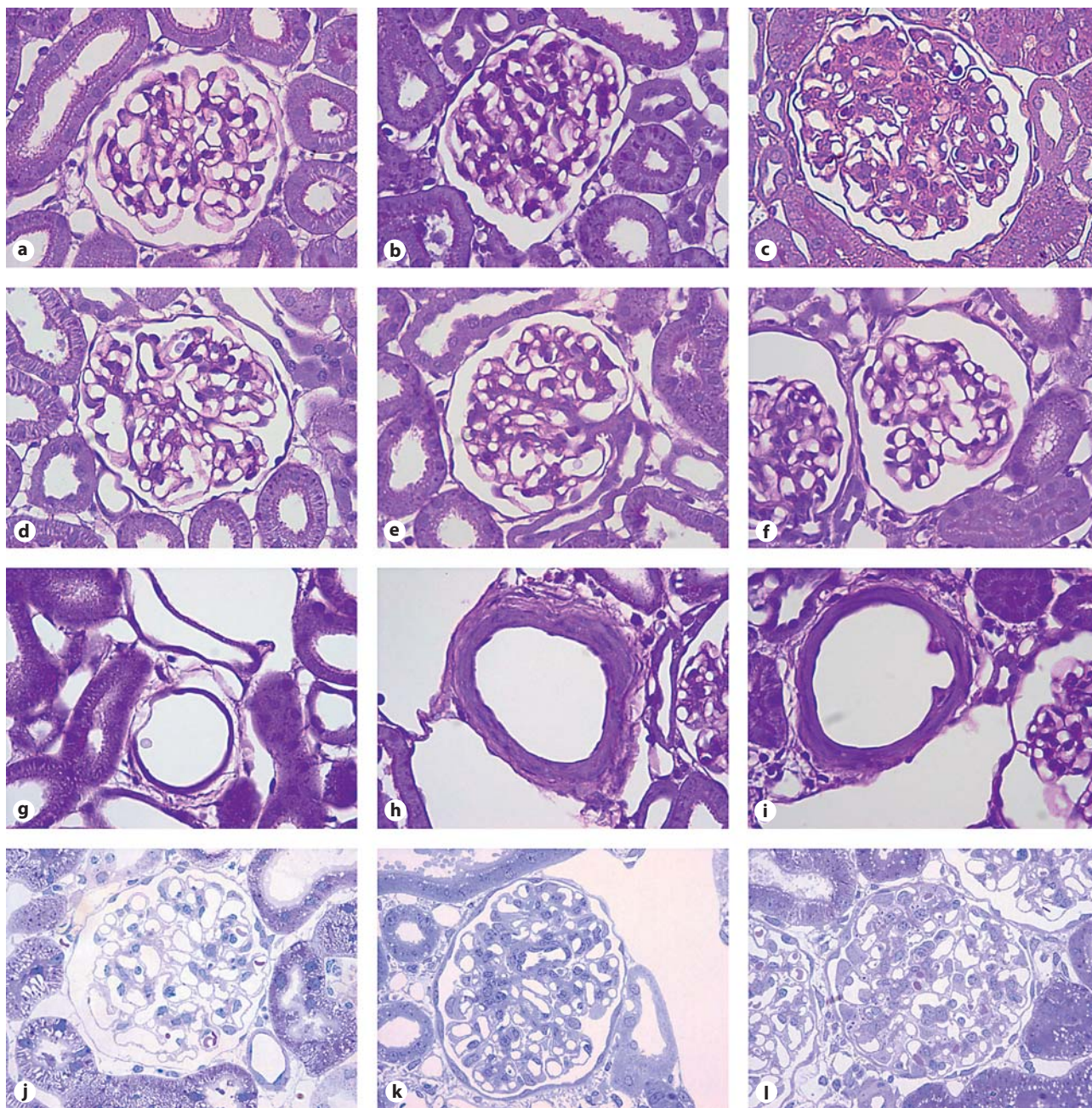
Compared to dTGR-Plac, S18886 decreased SBP by 13 mm Hg, but significance was not reached (p = 0.55). Body weight, heart or kidney to body weight ratio remained unaffected. Plasma creatinine was not influenced. Creatinine clearance decreased. Proteinuria and polyuria were reduced, urine osmolarity increased (to nearly normal levels).

S18886 significantly improved renal morphology: glomerulosclerosis and tubulointerstitial fibrosis scores were lower (fig. 1, 2c, i). The vascular damage remained uninfluenced. Mesangiolytic, albeit not elevated in the dTGR-Plac, was suppressed (fig. 1, 2f). S18886 had no effect on mean glomerular volume (table 1), glomerular cell numbers (apart from slight decrease in mesangial and endothelial hypercellularity (table 1; fig. 2l), and the capillary length density. Mean capillary cross-sectional area



**Fig. 1.** Semiquantitative indices of renal damage. SD = Sprague-Dawley control rats; dTGR = double transgenic rats; Plac = placebo; GSI = index of glomerulosclerosis; TSI = index of tubulointerstitial damage; VSI = index of vascular damage; MGI = mesangiolytic index; \* p < 0.05; \*\* p < 0.01.

was partially restored (table 1). S18886 reduced plasma cholesterol, but not TAG concentration. Plasma MDA concentration, GPX and SOD activities were not influenced (table 2).



**Fig. 2.** Representative changes in renal morphology (GSI, MSI, vascular damage) in SD controls (first column), placebo-treated dTGR (second column) and S18886-treated dTGR (third column). **a–c** Mesangial matrix expansion and sclerosis were higher in dTGR-Plac (**b**) than in SD (**a**) and in dTGR-S188886 (**c**). Paraffin section, PAS stain, orig. magnif.  $\times 20$ . **d–f** The index of mesangiolysis, i.e. dissolution of the mesangium with capillary widening, was comparable in controls (**d**) and dTGR-Plac (**e**); it was

significantly lower in dTGR-S18886 (**f**). Paraffin section, PAS stain, orig. magnif.  $\times 20$ . **g–i** Vascular damage, i.e. thickening of the vascular wall, was significantly higher in dTGR-Plac (**h**) and dTGR-S18886 (**i**) than in SD (**g**). Paraffin section, PAS stain, orig. magnif.  $\times 20$ . **j–l** Representative semithin sections demonstrating glomerular hypercellularity in dTGR-Plac (**k**) and dTGR-S18886 (**l**) compared to SD (**j**). Semithin section, methylene blue and basic fuchsin stain, orig. magnif.  $\times 20$ .

**Table 2.** Blood and urine chemistry

	SD (n = 10)	dTGR-Plac (n = 14)	dTGR-S18886 (n = 12)	ANOVA/K-W	
				F	p
Plasma creatinine, $\mu\text{mol/l}$	23; 25 $\pm$ 6	24; 23 $\pm$ 3	26; 27 $\pm$ 5	3.39	0.180
Creatinine clearance, ml/min	0.90 $\pm$ 0.28	0.85 $\pm$ 0.18	0.58 $\pm$ 0.12 <sup>a, c</sup>	5.62	0.010
Diuresis, ml/24 h	9; 10 $\pm$ 3	22; 22 $\pm$ 8 <sup>b</sup>	7; 7 $\pm$ 4 <sup>d</sup>	24.03	6.0 $\times$ 10 <sup>-6</sup>
Proteinuria, mg/ $\mu\text{mol}$ creatinine	74; 81 $\pm$ 34	532; 558 $\pm$ 218 <sup>b</sup>	133; 136 $\pm$ 71 <sup>d</sup>	25.82	2.5 $\times$ 10 <sup>-6</sup>
Urine osmolarity, mosm/kg H <sub>2</sub> O	1,249; 1,386 $\pm$ 527	533; 556 $\pm$ 211 <sup>b</sup>	992; 1,091 $\pm$ 531 <sup>c</sup>	16.89	0.0002
Cholesterol, mmol/l	1.47 $\pm$ 0.18	1.84 $\pm$ 0.12 <sup>b</sup>	1.69 $\pm$ 0.17 <sup>a, c</sup>	15.70	1.95 $\times$ 10 <sup>-5</sup>
TAG, mmol/l	0.76 $\pm$ 0.15	0.83 $\pm$ 0.19	0.92 $\pm$ 0.22	1.94	0.161
MDA, $\mu\text{mol/l}$	1.90 $\pm$ 0.21	2.40 $\pm$ 0.30 <sup>b</sup>	2.38 $\pm$ 0.49 <sup>a</sup>	6.30	0.006
SOD, U/g Hb	2,423; 2,554 $\pm$ 415	2,387; 2,404 $\pm$ 203	2,386; 2,326 $\pm$ 417	0.70	0.706
GPX, U/g Hb	700 $\pm$ 112	917 $\pm$ 71 <sup>b</sup>	828 $\pm$ 152	10.15	0.0004

Results are means  $\pm$  SD, or medians with means  $\pm$  SD for not normally distributed data.

SD = Sprague-Dawley rats; dTGR = double transgenic rats; Plac = placebo; ANOVA = one-way analysis of variance; K-W = Kruskal-Wallis test; TAG = triacylglycerols; MDA = malondialdehyde; SOD = superoxide dismutase activity; GPX = glutathione peroxidase activity.

<sup>a</sup> p < 0.05 vs. SD; <sup>b</sup> p < 0.01 vs. SD; <sup>c</sup> p < 0.05 vs. dTGR-Plac; <sup>d</sup> p < 0.01 vs. dTGR-Plac.

## Discussion

Protection from Ang-II-induced alterations in dTGR has been demonstrated by administration of Ang-II type 1 receptor blockers (ARB) [22], endothelin antagonists [23] and compounds with antioxidant/anti-inflammatory properties [24, 25]. Our study presents first evidence that treatment with the TPr antagonist, S18886, results in a striking improvement of functional and morphological parameters in the Ang-II-induced nephropathy.

Although S18886 did not influence the systolic blood pressure significantly, reduction by 13 mm Hg in mean (-8%) might not be excluded as a renoprotective mechanism. The profound decline in proteinuria (-76%) is impressive with regard to the persistent hypertension. Decline in elevated cholesterol levels probably reflects the forestalled proteinuria, and might not be attributed to a direct S18886 action. A comparable decrease in albuminuria, without blood pressure-lowering effect, was observed in diabetic apoE<sup>-/-</sup> mice administered S18886 [14]. Thromboxane, via its receptors, has been implicated in mediating glomerular permeability to albumin [26].

Despite the glomerulosclerosis and tubulointerstitial fibrosis, the creatinine clearance of the dTGR-Plac remained unaffected. This is most likely a sign of hyperfiltration possibly caused by increased plasma thromboxane with subsequent constriction of the vas efferens. Inhibition of TPr significantly reduced creatinine clearance,

which might be explained by lower tonus of the vas efferens, followed by a functional decline of creatinine clearance. Thromboxane/thromboxane mimetics exerted contrasting renovascular effects in different experimental models: a predominant vasoconstriction of the vas afferens [27], the vas efferens [28], or of both arterioles [29]. However, the effects of endogenous thromboxane may differ from those of exogenously administered thromboxane agonists. The decrease of creatinine clearance resembles the well-known effects of angiotensin-converting enzyme inhibitors and ARBs, which are particularly pronounced in the presence of an activated renin-angiotensin system. Since we did not determine renal plasma flow, an explanation for the altered intrarenal hemodynamics is precluded.

Polyuria and a lower urinary osmolarity in the dTGR-Plac were forestalled under S18886. Whether inhibition of TPr in the brainstem influenced the Ang-II-induced thirst [6] cannot be excluded, since the rats had free access to drinking water.

Amelioration of renal morphology in dTGR after inhibition of TPr was similar to that in diabetic apoE<sup>-/-</sup> mice. In these animals, S18886 reduced matrix deposition in the glomeruli and renal interstitium and the degenerative changes in tubules, in part via attenuation of various parameters of oxidative stress and inflammation [14]. Renal injury in the dTGR model was improved by administration of compounds with antioxidant/anti-in-

flammatory properties [24, 25]. S18886 did not significantly affect the altered parameters of oxidative status in dTGR-Plac. This might be due to limited effects of S18886 on hypertension, a prominent pro-oxidant condition. Persistent hypertension might also explain the insignificant attenuation of renal vasculopathy. Interestingly, S18886 profoundly reduced mesangiolysis, albeit the values in dTGR-Plac were within the normal range. In obese Zucker rats, a model with pathologically increased mesangiolytic score, S18886 significantly ameliorated mesangial damage, independent of blood pressure [15]. TXA<sub>2</sub> delayed the clearance of macromolecules in the rat glomeruli and mesangial cells, while a TPr antagonist normalized these effects [30]. TXA<sub>2</sub> also stimulated the production of plasminogen activator inhibitor-1 and plasminogen activators by mesangial cells through a TPr-dependent mechanism [31]. Thus, the mesangium may represent an important target for S18886.

In apoE<sup>-/-</sup> mice or the obese Zucker rats, S18886 does not lower blood pressure significantly [14, 15]. Thus, in dTGR its effects on blood pressure need to be analyzed further, perhaps using telemetry. We assume that the insignificant blood pressure reduction in S18886-treated dTGR might not be the single mechanism for the marked improvement of renal damage. In rats, malignant hyper-

tension retards weight gain. Both dTGR groups gained comparably less weight during the study than the control SD group. Persisting cardiac hypertrophy may be a consequence of the continued hypertension. Moreover, the striking antimesangiolytic effects of S18886 might not solely be attributed to the blood pressure decline.

We present here the first data that the administration of the TP receptor antagonist, S18886, markedly improves the renal damage in the model of transgenic rats harboring human renin and angiotensinogen genes. These findings suggest the fundamental role of activated TP receptors in the pathogenesis of Ang-II-induced renal injury, one of the major contributors to renal morbidity.

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