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

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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.

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 iso-prostanates, which may contribute to the pressor response via induction of vasoconstriction and renal sodium retention [1, 3]. Hence, thromboxane A2 (TXA2) binds to thromboxane receptors (TPr) in renal vascular tissue and thereby aggravates vascular damage [3, 4]. TXA2 induces contraction of mesangial cells, the glomeruli and the afferent/erferent 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 TXA2, and the other arachidonic acid metabolites (i.e. prostaglandins, HETE acids, isoprostanates) 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
(aopE–/–) mice [12,13]. It exerted renoprotective and an-
tioxidant actions in diabetic apoE–/– mice [14] and in
obese Zucker rats [15].

Double transgenic rats (dTGR, harboring human renin
and angiotensinogen genes) develop accelerated hy-
pertension leading to marked damage of the kidney, and
heart [16]. Ang-II-induced inflammatory response con-
tributes to fatal organ damage [17]. Since TPr play a deci-
50
sive role in the Ang-II-dependent alterations of renal he-
modynamics 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 proto-
col by the Institutional Ethics Committee for Experimental Ani-
mals (Bratislava, Slovakia).

Rats

Four-week-old male dTGR (RCC Ltd, Füllinsdorf, Switzer-
land) 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 collect-
ed. At sacrifice, blood was sampled from the abdominal aorta un-
der 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 fluorimet-
ric detection [19], erythrocyte glutathione peroxidase (GPX) and
superoxide dismutase (SOD) activity by commercial kits (Ran-
dox, Crumlin, UK). Urine osmolarity was determined. Creati-
nine clearance was calculated.

Tissue Preparation

Semithin sections were qualitatively inspected for glomerular
and mesangial or endothelial cell hyperplasia. Glomerular capil-
larization 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 podo-
cytes) 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 Scheffe’s test, or
Kruskal-Wallis tests 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 creati-
nine 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 tubulo-intertstitial and vascular
damage (fig. 1, 2g). Glomerular cell number was signifi-
cantly higher in the dTGR-Plac, indicating mild glomer-
ular hypercellularity, particularly of mesangial and en-
dothelial cells (fig. 2j, k; table 1). The significantly higher
length density (total capillary length per glomerular vol-
ume), and lower mean capillary cross-sectional area in-
dicated lengthening and remodeling of glomerular capil-
laries with narrowing of capillary lumina in dTGR-Plac.
No changes in mesangiolysis or mean glomerular volume were observed (fig. 1, 2d, e; table 1).
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. Mesangiolysis, 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 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).

### Table 1. Animal data and renal morphology

<table>
<thead>
<tr>
<th></th>
<th>SD (n = 10)</th>
<th>dTGR-Plac (n = 14)</th>
<th>dTGR-S18886 (n = 12)</th>
<th>ANOVA/K-W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>118 ± 10</td>
<td>107 ± 11</td>
<td>110 ± 17</td>
<td>1.34</td>
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<tr>
<td>Body weight at sacrifice, g</td>
<td>245; 242 ± 14</td>
<td>200; 211 ± 27b</td>
<td>220; 212 ± 17b</td>
<td>10.35</td>
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<td>Weight gain, g</td>
<td>125; 124 ± 8</td>
<td>100; 104 ± 4</td>
<td>103; 102 ± 5</td>
<td>16.60</td>
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<td>SBP, mm Hg</td>
<td>98; 99 ± 7</td>
<td>163; 162 ± 15b</td>
<td>150; 149 ± 13b</td>
<td>23.56</td>
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<td>Heart weight, g</td>
<td>0.92 ± 0.01</td>
<td>1.21 ± 0.11b</td>
<td>1.16 ± 0.13b</td>
<td>19.77</td>
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<tr>
<td>Heart/body weight, mg/g</td>
<td>3.8 ± 0.3</td>
<td>5.8 ± 0.6b</td>
<td>5.4; 5.5 ± 0.4b</td>
<td>22.02</td>
</tr>
<tr>
<td>Kidney/body weight, mg/g</td>
<td>4.5 ± 0.3</td>
<td>4.6 ± 0.3</td>
<td>4.8 ± 0.4</td>
<td>2.16</td>
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<tr>
<td>Glomerular volume, μm³</td>
<td>495 ± 79</td>
<td>510 ± 64</td>
<td>487 ± 61</td>
<td>0.430</td>
</tr>
<tr>
<td>Glomerular cell number</td>
<td>926 ± 120</td>
<td>1,071 ± 88b</td>
<td>1,026 ± 86</td>
<td>6.12</td>
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<tr>
<td>Podocyte number</td>
<td>278 ± 45</td>
<td>257 ± 32</td>
<td>268 ± 59</td>
<td>0.509</td>
</tr>
<tr>
<td>Mesangial cell number</td>
<td>276 ± 60</td>
<td>351 ± 51a</td>
<td>310 ± 38</td>
<td>5.25</td>
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<tr>
<td>Endothelial cell number</td>
<td>240 ± 47</td>
<td>346 ± 39b</td>
<td>310 ± 46b</td>
<td>14.10</td>
</tr>
<tr>
<td>Parietal cell number</td>
<td>132 ± 21</td>
<td>137 ± 15</td>
<td>139 ± 14</td>
<td>0.434</td>
</tr>
<tr>
<td>Capillary length density, mm/mm³</td>
<td>9,729 ± 434</td>
<td>11,345 ± 640b</td>
<td>11,218 ± 1,243b</td>
<td>10.82</td>
</tr>
<tr>
<td>Capillary cross-sectional area, μm²</td>
<td>36; 36 ± 4</td>
<td>26; 25 ± 2b</td>
<td>28; 28 ± 2b, c</td>
<td>20.05</td>
</tr>
</tbody>
</table>

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.

* p < 0.05 vs. SD; ** p < 0.01 vs. SD; *** p < 0.05 vs. dTGR-Plac.

**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 = mesangiolysis index; * p < 0.05; ** p < 0.01.
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-S18886 (c). Paraffin section, PAS stain, orig. magnif. ×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. ×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. ×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. ×20.
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−/− 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−/− 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-

Table 2. Blood and urine chemistry

<table>
<thead>
<tr>
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<th>SD (n = 10)</th>
<th>dTGR-Plac (n = 14)</th>
<th>dTGR-S18886 (n = 12)</th>
<th>ANOVA/K-W</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td>Plasma creatinine, µmol/l</td>
<td>23; 25 ± 6</td>
<td>24; 23 ± 3</td>
<td>26; 27 ± 5</td>
<td>3.39</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>0.90 ± 0.28</td>
<td>0.85 ± 0.18</td>
<td>0.58 ± 0.12abc</td>
<td>5.62</td>
</tr>
<tr>
<td>Diuresis, ml/24 h</td>
<td>9; 10 ± 3</td>
<td>22; 22 ± 8b</td>
<td>7; 7 ± 4d</td>
<td>24.03</td>
</tr>
<tr>
<td>Proteinuria, mg/µmol creatinine</td>
<td>74; 81 ± 34</td>
<td>532; 558 ± 218b</td>
<td>133; 136 ± 71d</td>
<td>25.82</td>
</tr>
<tr>
<td>Urine osmolarity, mosm/kg H2O</td>
<td>1,249; 1,386 ± 527</td>
<td>533; 556 ± 211b</td>
<td>992; 1,091 ± 531c</td>
<td>16.89</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>1.47 ± 0.18</td>
<td>1.84 ± 0.12b</td>
<td>1.69 ± 0.17abc</td>
<td>15.70</td>
</tr>
<tr>
<td>TAG, mmol/l</td>
<td>0.76 ± 0.15</td>
<td>0.83 ± 0.19</td>
<td>0.92 ± 0.22</td>
<td>1.94</td>
</tr>
<tr>
<td>MDA, µmol/l</td>
<td>1.90 ± 0.21</td>
<td>2.40 ± 0.30b</td>
<td>2.38 ± 0.49a</td>
<td>6.30</td>
</tr>
<tr>
<td>SOD, U/g Hb</td>
<td>2,423; 2,554 ± 415</td>
<td>2,387; 2,404 ± 203</td>
<td>2,386; 2,326 ± 417</td>
<td>0.70</td>
</tr>
<tr>
<td>GPX, U/g Hb</td>
<td>700 ± 112</td>
<td>917 ± 71b</td>
<td>828 ± 152</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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; TAG = triacylglycerols; MDA = malondialdehyde; SOD = superoxide dismutase activity; GPX = glutathione peroxidase activity.

a p < 0.05 vs. SD; b p < 0.01 vs. SD; c p < 0.05 vs. dTGR-Plac; d p < 0.01 vs. dTGR-Plac.
flamatory properties [24, 25]. S18886 did not significa-
cantly 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 insignifi-
cant attenuation of renal vasculopathy. Interestingly,
S18886 profoundly reduced mesangiosis, albeit the val-
ues in dTGR-Plac were within the normal range. In obese
Zucker rats, a model with pathologically increased me-
sangioytic score, S18886 significantly ameliorated me-
sangial damage, independent of blood pressure [15].
TXA2 delayed the clearance of macromolecules in the rat
glomeruli and mesangial cells, while a TPr antagonist
normalized these effects [30]. TXA2 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−/− 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 in-
significant 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 con-
sequence 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 harbor-
ing human renin and angiotensinogen genes. These find-
ings suggest the fundamental role of activated TP recep-
tors in the pathogenesis of Ang-II-induced renal injury,
one of the major contributors to renal morbidity.

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