Oxidative Stress Status in Kidney Tissue after Losartan and Atenolol Treatment in Experimental Renal Failure

Ülle Pechter\textsuperscript{a} Marina Aunapuu\textsuperscript{b} Zivile Riispere\textsuperscript{c} Tiiu Vihalemmd
Tiiu Kulissare\textsuperscript{d} Kersti Zilmer\textsuperscript{d} Mihkel Zilmer\textsuperscript{d} Mai Otse\textsuperscript{e}

\textsuperscript{a}Department of Sports Medicine and Rehabilitation, \textsuperscript{b}Institute of Anatomy and Histology, \textsuperscript{c}Institute of Pathology, \textsuperscript{d}Biochemistry Institute and \textsuperscript{e}Department of Internal Medicine, University of Tartu, Tartu, Estonia

\textbf{Key Words}
Angiotensin II receptor antagonist - Chronic renal failure - Losartan - Proteinuria - Oxidative stress

\textbf{Abstract}
\textbf{Background/Aims:} Rats with subtotal nephrectomy (5/6NPX) rapidly develop systemic hypertension and proteinuria. The aim of our study was to evaluate the changes in oxidative stress parameters after 2 and 4 weeks of treatment with renin-angiotensin system (RAS)-blocking agent losartan and beta-blocking agent atenolol in experimental chronic renal failure (CRF).

\textbf{Methods:} After 5/6NPX, rats were immediately treated with losartan or atenolol. The lipid peroxidation (LPO) products malondialdehyde and 4-hydroxyalkenals and oxidized and reduced glutathione values were measured in the renal cortex tissue and in blood; isoprostanes in urine.

\textbf{Results:} There were no differences in the blood pressure values, serum creatinine levels or in daily proteinuria using both antihypertensive treatments. Losartan treatment lowered significantly LPO in kidney tissue after 2 and 4 weeks of treatment compared with untreated and atenolol-treated animals and induced the decrease of excretion of isoprostanes in urine at the end of the study. There was no ameliorating impact of losartan or atenolol observed in the blood status of oxidative stress in this period of time. \textbf{Conclusion:} In the early period of experimental CRF, losartan treatment but not atenolol treatment induces significant decline in LPO grade in the kidney tissue of nephrectomized rats. RAS blockade in the kidney influences local tissue LPO in a much greater extent than in blood.

\textbf{Introduction}
Progression of uraemia is associated with the generation of profound oxidative stress (OxS) [1, 2], leading to rapid development of atherosclerosis and the consequences in the target organs [3]. Vascular changes and end-organ damage are clearly initiated by high blood pressure [4]. It is now becoming increasingly apparent that many signalling events that underlie vascular dysfunction in hypertension are influenced by development of high-grade OxS, whereas, recently, correlations between profound OxS, including glutathione redox status and hypertension have been shown [5–7]. Increased OxS mediates endothelial dysfunction [8], thus, it is possible that OxS
could be playing a supportive role in development of chronic renal failure (CRF).

To investigate the expression of OxS grade in systemic and cellular level we studied different OxS indices in the renal cortex tissue, blood and urine after 5/6 nephrectomy (5/6NPX) in the early stages of experimental CRF, after 2 and 4 weeks of antihypertensive treatment with renin-angiotensin system (RAS)-blocking agent losartan and beta-blocking agent atenolol comparing to untreated controls.

**Materials and Methods**

Animal Studies Ethics Committee of the Tartu University approved the study protocol. Male Wistar rats were purchased from the Laboratory Animal Center, University of Kuopio, Finland. An acclimatization period of 10 days was allowed before any experimental work was undertaken. Rats were kept in a climate-controlled facility at the Faculty of Medicine of the University of Tartu where animals housed under standard conditions on a 12-hour light/dark cycle and fed with standard rodent chow (R 70, Lactamin AB, Sweden) and tap water ad libitum.

**Experimental Design**

Rats were subjected to subtotal (5/6) NPX as previously described [9] at week 0. At approximately 8 weeks of age, rats weighing 262–280 g were anesthetized with intraperitoneal methohexital sodium, 5 mg per 100 g body weight. Renal ablation was then accomplished by right nephrectomy and selective ligation of extrarenal branches of the left renal artery in such a way that approximately 2/3 of the left kidney was infarcted. Fifty-five rats were randomized after surgery and divided into six groups matched for age and body weight at week 0 and studied during 2 and 4 weeks: NPX 2 weeks (n = 8), NPX 4 weeks (n = 7), NPX + losartan 2 weeks (n = 10), NPX + losartan 4 weeks (n = 10), NPX + atenolol 2 weeks (n = 10), NPX + atenolol 4 weeks (n = 10). Body weight was measured every week for the duration of the study. Systolic blood pressure (SBP) was measured weekly by a tail-cuff manometer (Harvard Apparatus, USA) in awake prewarmed rats. The urine was collected for 24 h using metabolic cages at weeks 2 and 4, for determination of urine creatinine and proteinuria that were measured with a Hitachi 912 Analyser.

**Oxidative Stress Methods**

The procedures met the criteria and principles described previously [10]. The lipid peroxidation (LPO) products malondialdehyde and 4-hydroxynonenal were measured in renal cortex tissue homogenate and in serum by colorimetric assay for LPO using the methods described [10]. The lipid peroxidation (LPO) products malondialdehyde (MDA) and 4-hydroxynonenal were measured in renal cortex tissue homogenate and in serum by colorimetric assay for LPO using the methods described [10].

**Statistical Analysis**

Data were collected at baseline and after 5/6NPX in rat groups at weeks 2 and 4. Data are presented as mean values ± SD. Data were analyzed by one-time ANOVA with the Tukey-Kramer test for comparisons significant at the 0.05 level or repeated ANOVA measures with post-hoc testing as appropriate using the commercially available statistical package SAS. The null hypothesis was rejected at p < 0.05.

**Results**

There were no significant differences in body weight among the studied groups at any time point. Average levels of SBP in all treated groups were significantly lower than in untreated animals (p < 0.05). There were no differences in mean SBP among losartan- and atenolol-treated groups. Therapy significantly reduced urine protein excretion rate (UprotV) both in losartan and atenolol groups compared with untreated animals. At week 2, the mean UprotV was significantly lower in the losartan-treated group than in the atenolol-treated group, but there were no significant differences among the losartan- and atenolol-treated groups by week 4. Mean S-Creat was significantly lower in the treated groups at week 4 compared with untreated animals (p < 0.05). There were no differences in mean S-Creat among losartan- and atenolol-treated groups (table 1).
Table 1. Body weight, urine protein excretion rate (UprotV), systolic blood pressure (SBP) and serum creatinine (S-Creat) after 2 and 4 weeks of antihypertensive treatment

<table>
<thead>
<tr>
<th></th>
<th>Untreated 2 weeks</th>
<th>Losartan-treated 2 weeks</th>
<th>Atenolol-treated 2 weeks</th>
<th>Untreated 4 weeks</th>
<th>Losartan-treated 4 weeks</th>
<th>Atenolol-treated 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>283 ± 3</td>
<td>305 ± 5</td>
<td>311 ± 7</td>
<td>284 ± 6</td>
<td>303 ± 9</td>
<td>329 ± 6</td>
</tr>
<tr>
<td>UprotV, mg/24 h</td>
<td>34.1 ± 6.4</td>
<td>17.4 ± 9.0* **</td>
<td>23.2 ± 2.4#</td>
<td>47.2 ± 10.4</td>
<td>19.4 ± 1.8*</td>
<td>22.3 ± 2.4#</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>143.0 ± 4.3</td>
<td>100.0 ± 1.0*</td>
<td>92.5 ± 3.1*</td>
<td>154.0 ± 4.0</td>
<td>90.0 ± 2.7*</td>
<td>86.9 ± 5.8*</td>
</tr>
<tr>
<td>S-Creat, µM</td>
<td>105.3 ± 5.6</td>
<td>107.6 ± 5.2</td>
<td>99.5 ± 4.7</td>
<td>104.7 ± 4.6</td>
<td>93.3 ± 2.9*</td>
<td>92.9 ± 2.8*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *p < 0.05, losartan-treated vs. untreated at corresponding week; **p < 0.05, losartan-treated vs. atenolol-treated at corresponding week; #p < 0.05, losartan-treated vs. atenolol-treated at corresponding week.

Table 2. Indices of oxidative stress in kidney tissue: lipid peroxidation products (LPO), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione redox ratio (GSSG/GSH) and isoprostanes in urine after 2 and 4 weeks of antihypertensive treatment

<table>
<thead>
<tr>
<th></th>
<th>Untreated 2 weeks</th>
<th>Losartan-treated 2 weeks</th>
<th>Atenolol-treated 2 weeks</th>
<th>Untreated 4 weeks</th>
<th>Losartan-treated 4 weeks</th>
<th>Atenolol-treated 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO, pmol/mg protein</td>
<td>0.202 ± 0.01</td>
<td>0.151 ± 0.0*</td>
<td>0.178 ± 0.03</td>
<td>0.185 ± 0.02</td>
<td>0.134 ± 0.02* **</td>
<td>0.191 ± 0.09</td>
</tr>
<tr>
<td>GSSG, µM</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>2.8 ± 1.0</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>GSH, µM</td>
<td>11.0 ± 2.9</td>
<td>13.3 ± 2.6</td>
<td>6.2 ± 1.5</td>
<td>7.1 ± 2.4</td>
<td>14.4 ± 4.4**</td>
<td>5.5 ± 1.0</td>
</tr>
<tr>
<td>GSSG/GSH</td>
<td>0.322 ± 0.117</td>
<td>0.149 ± 0.031*</td>
<td>0.246 ± 0.115</td>
<td>0.399 ± 0.133</td>
<td>0.354 ± 0.080</td>
<td>0.220 ± 0.031</td>
</tr>
<tr>
<td>Isoprostanes, ng/mg creat</td>
<td>2.95 ± 0.58</td>
<td>3.74 ± 1.25</td>
<td>3.41 ± 1.35</td>
<td>2.72 ± 1.19</td>
<td>1.81 ± 0.55* **</td>
<td>6.37 ± 2.9*</td>
</tr>
</tbody>
</table>

Data are mean ± SD. *p < 0.05, losartan-treated vs. untreated at corresponding week; **p < 0.05, losartan-treated vs. atenolol-treated at corresponding week; #p < 0.05, losartan-treated vs. atenolol-treated at corresponding week.

Table 3. Indices of oxidative stress in blood: lipid peroxidation products (LPO), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione redox ratio (GSSG/GSH) after 2 and 4 weeks of antihypertensive treatment

<table>
<thead>
<tr>
<th></th>
<th>Untreated 2 weeks</th>
<th>Losartan-treated 2 weeks</th>
<th>Atenolol-treated 2 weeks</th>
<th>Untreated 4 weeks</th>
<th>Losartan-treated 4 weeks</th>
<th>Atenolol-treated 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO, nmol/ml</td>
<td>0.586 ± 0.04</td>
<td>0.910 ± 0.03*</td>
<td>0.997 ± 0.11*</td>
<td>0.954 ± 0.06</td>
<td>1.205 ± 0.05</td>
<td>1.080 ± 0.14</td>
</tr>
<tr>
<td>GSSG, µM</td>
<td>159.0 ± 18.5</td>
<td>159.0 ± 17.8</td>
<td>159.0 ± 12.7</td>
<td>172.3 ± 17.4</td>
<td>159.0 ± 17.8</td>
<td>159.0 ± 9.4</td>
</tr>
<tr>
<td>GSH, µM</td>
<td>622.1 ± 70.3</td>
<td>475.0 ± 40.6*</td>
<td>555.7 ± 101.0</td>
<td>823.2 ± 80.3</td>
<td>398.2 ± 47.9* **</td>
<td>182.3 ± 14.4</td>
</tr>
<tr>
<td>GSSG/GSH</td>
<td>0.322 ± 0.051</td>
<td>0.441 ± 0.064</td>
<td>0.325 ± 0.066</td>
<td>0.208 ± 0.034</td>
<td>0.452 ± 0.06* **</td>
<td>0.600 ± 0.040</td>
</tr>
</tbody>
</table>

Data are mean ± SD. *p < 0.05, losartan-treated vs. untreated at corresponding week; **p < 0.05, losartan-treated vs. atenolol-treated at corresponding week; #p < 0.05, losartan-treated vs. atenolol-treated at corresponding week.

Oxidative stress indices in the kidney tissue and in the urine and in blood are presented in table 2 and 3, respectively. Losartan treatment significantly suppresses LPO in the renal cortex tissue in 2 weeks. At week 4 the level of LPO remains significantly lowered in losartan-treated rats compared with the atenolol-treated or untreated animals. In addition, the glutathione redox ratio in the kidney tissue remains significantly lower in losartan-treated rats by week 2 compared with untreated rats (table 2). The level of the reduced glutathione (a cellular principal...
antioxidant) in losartan-treated rats at week 2 was significantly higher compared with atenolol-treated animals. Losartan treatment diminished the excretion of isoprostanes by week 4, whereas with atenolol treatment a further increase occurred.

Treatment with both drugs was not able to have improving effects considering systemic oxidative stress, as showed data of blood samples (table 3); it even noticeably aggravated systemic LPO compared with untreated animals. A small further increase of LPO was noticed in both losartan-treated and atenolol-treated groups by week 4. There was significant difference in GSH level in blood between losartan-treated and atenolol-treated groups at week 4. The glutathione redox ratio was significantly lower in losartan-treated animals at week 4, compared with atenolol-treated, but the smallest redox ratio was still found in untreated animals.

Less FSGS was found in remnant kidneys of losartan-treated animals (2.3 ± 2.3) compared with atenolol-treated (4.0 ± 1.3), although not significantly, and compared to untreated controls (6.4 ± 5.5) (p < 0.05) at week 4. The interstitial fibrosis score was significantly lower in both losartan- (1.0 ± 0.1) and atenolol-treated (0.9 ± 0.3) animals compared with untreated controls (2.0 ± 0.0) (p < 0.05) at week 4.

Discussion

After renal mass reduction, the remaining nephrons undergo functional as well as structural hypertrophy; glomerular and systemic hypertension develops [12]. The segmental sclerotic lesions which develop in remnant glomeruli of rats after subtotal nephrectomy resemble those seen in a variety of human chronic renal diseases. This remnant kidney model is used in our study to evaluate the dynamics of local and generalized OxS parameters in the early period of experimental CRF. Ang-II is undoubtedly related to OxS induction [13]. Studies have shown the presence of systemic OxS in hypertensive individuals [6, 7]. RAS blockade modulates the OxS status as previously studied [14, 15].

Both RAS-blocking agent (losartan) and a beta-blocker (atenolol) lowered blood pressure and proteinuria to a similar extent during the study period. However, the oxidative status in losartan-treated animals was different from atenolol-treated animals.

The LPO and reduced glutathione levels in the renal tissue of the losartan treatment group were significantly better than in the atenolol treatment group, suggesting the local antioxidant action with blocking the very high activity of tissue RAS in the injured kidney. We found that the RAS blockade with losartan significantly diminishes LPO in renal tissue level already in the early stages of experimental CRF, at week 2, having declining tendency further at week 4, when the difference between the atenolol-treated and the losartan-treated group was found to be highly significant. These results confirm the previous ones which have demonstrated that in blunting Ang-II action there is a decrease in OxS [16, 17].

GSH values showed that its synthesis was higher during losartan treatment, but depressed in atenolol-treated rats. The use of RAS-blocking agent in our study ameliorates the antioxidant enzyme activity in renal tissue of 5/6NPX rats, probably providing local antioxidative impact, which was not achieved with atenolol treatment. In condition of elevated oxygen radical production, losartan was able to blunt the deleterious effect of metabolic changes in the kidney tissue.

Despite a similar blood pressure-lowering effect, both the LPO level and GSH in the renal tissue of the losartan-treated group were significantly better than in the atenolol-treated group.

In blood samples LPO was elevated in all groups without blunting action of antihypertensive drugs in our study. It is of particular interest that losartan treatment lowered renal tissue but not systemic LPO at weeks 2 and 4. Moreover, in both treatment groups the LPO value in plasma was significantly higher than in untreated controls at week 2, and remained higher, although not significantly, at week 4 where the atenolol group showed even lower level than the losartan group. The discrepancies between tissue and plasma LPO values in early period of CRF are difficult to discuss. It is evident that the RAS blockade in the kidney influences local tissue LPO in a much greater extent than in blood, at least in the studied period of experimental CRF.

The GSH level in blood at week 4 was significantly higher in losartan-treated animals, compared with the atenolol treatment, which could be interpreted as better antioxidative properties of losartan in systemic level. Urinary isoprostanes decreased in losartan-treated rats from week 2 to week 4, in atenolol-treated rats a further increase occurred. It could be related to effects of antihypertensive agents on LPO process in serum and probably in filtered load. The level of urinary isoprostanes is characterized by further increase in atenolol-treated rats and the difference between atenolol treatment and losartan treatment was found to be significant.
Although there were no major changes concerning the physiological and morphological parameters between the animals treated with losartan or atenolol in the follow-up of our study, the lipid peroxidation decline presumably due to local tissue RAS diminished activity, showed the antioxidative properties of losartan in the observed period of experimental CRF.

References