Strain differences in the development of hypertension and glomerular lesions induced by deoxycorticosterone acetate salt in mice

Andrea Hartner, Nada Cordasic, Bernd Klanke, Roland Veelken and Karl F. Hilgers

Department of Medicine IV, University of Erlangen-Nürnberg, Erlangen, Germany

Abstract

Background. The genetic background may exert important modifying effects on the course and severity of experimental kidney diseases in mice. We investigated its influence on the development of hypertension and renal injury following treatment with deoxycorticosterone acetate (DOCA) salt in several mouse strains.

Methods. Four mouse strains were used for comparison: 129/Sv, C57BL/6 and F1 and F2 intercrosses of 129/Sv × C57BL/6. Male mice were uninephrectomized and DOCA hypertension was induced for 6 weeks. DOCA animals and controls received 1% NaCl for drinking. Renal damage was evaluated following measurements of blood pressure, urine albumin and renal matrix expansion.

Results. DOCA-induced blood pressure increase, glomerulosclerosis, interstitial fibrosis and albuminuria were markedly higher in 129/Sv than in C57BL/6 mice. F1 and F2 intercrosses displayed intermediate blood pressure, glomerular and interstitial fibrosis comparable to C57BL/6 but albuminuria as high as 129/Sv mice.

Conclusions. 129/Sv mice are more susceptible to the development of DOCA-induced high blood pressure and renal damage than C57BL/6 mice. Intercrosses of both strains show a complex and non-uniform segregation of the susceptibility to DOCA-salt hypertension and nephrosclerosis.

Keywords: albuminuria; blood pressure; deoxycorticosterone acetate; glomerulosclerosis; hypertension; mouse strains

Introduction

Mice with targeted disruption of genes implicated in the development and progression of hypertension and hypertensive lesions are valuable tools for the study of mechanisms leading to hypertensive organ injury. However, there has been growing evidence for an important role for the genetic background in which these models are generated. For example, a targeted deletion mutation in the gene for the angiotensin II type 2 receptor was backcrossed in both FVB/N and C57BL/6 strains. This deletion turned out to have no effect on baseline blood pressure in FVB/N strain [1] but increased blood pressure in the C57BL/6 strain [2], suggesting the presence of one or more genetic modifier loci. These loci seem to have strong impact on the effects of a gene deficiency. To be aware of this variability is particularly important for the choice of wild-type controls, if no wild-type siblings from heterozygote crosses are available, or if the mice with the targeted disruption of a gene are not backcrossed sufficiently into an inbred strain. In the deoxycorticosterone acetate (DOCA) salt model of hypertension and nephrosclerosis, indirect evidence of a role for the genetic background came from conflicting results on the effects of inhibitors of the renin–angiotensin system on blood pressure [3,4]. In these studies, however, the development of hypertension and hypertensive lesions by DOCA-salt treatment were not compared directly between the two mouse strains.

In the present study, we addressed the question of whether or not the development of hypertension and hypertensive renal lesions following DOCA-salt treatment is dependent on the strain in which hypertension is induced. We treated mice from two of the most widely used strains, and their intercrosses, with DOCA salt.
were performed in accordance with the guidelines of the American Physiological Society and were approved by the local government authorities (Regierung von Mittelfranken, AZ # 621-2531.31-1/01).

Four different mouse strains were included in the study: 129/Sv and C57BL/6 (both from Charles River, Sulzdorf, Germany) and F1 and F2 generations of 129/Sv × C57BL/6 intercrosses. The F1 intercross was derived from male 129/Sv and female C57BL/6, and the F2 intercross from brother-sister matings of the F1 offspring.

At an average weight of 16–18 g (corresponding to 6 weeks of age), mice underwent right unilateral nephrectomy. After 2 weeks of recovery, 21-day-release DOCA pellets containing 50 mg DOCA (Innovative Research of America, Sarasota, FL, USA) were implanted subcutaneously by incision of the right flank under light ether anaesthesia. Control animals were sham operated. After 21 days, animals received a replacement pellet. All animals (DOCA and control groups) received isotonic saline (10 g NaCl/litre) for drinking for 6 weeks, starting with the first day of DOCA treatment. The animals were then followed by weekly measurements of weight and conscious systolic blood pressure (Visitech Systems, Apex, NC, USA).

After 6 weeks of treatment, the mice were put into metabolic cages, and urine was collected for 24 h for measurement of proteinuria and albuminuria. Finally, mice were sacrificed (six DOCA-treated 129/Sv, eight DOCA-treated C57BL/6, five DOCA-treated F1, six DOCA-treated F2, six 129/Sv controls, eight C57BL/6 controls, five F1 controls and six F2 controls). At the day of sacrifice, the mice were equipped with a carotid artery catheter under ketamine/xylazine anaesthesia and intra-arterial blood pressure was measured in conscious mice 2 h after anaesthesia. They were sacrificed by dissecting the abdominal artery and bleeding in deep ketamine/xylazine anaesthesia.

After measuring kidney weight, the organs were decapsulated. Kidneys were put in methyl-Carnoy solution (60% methanol, 30% chloroform and 10% glacial acetic acid) for fixation.

**Urinary protein and albumin content**

Proteinuria was assessed by a Bio-Rad Protein Assay (Bio-Rad Laboratories, Munich, Germany). The urinary albumin content was measured by an EIA kit (CellTrend, Luckenwalde, Germany).

**Renal histology**

After overnight fixation in methyl-Carnoy solution, tissues were dehydrated by bathing in increasing concentrations of methanol, followed by 100% isopropanol. After embedding in paraffin, 2 μm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments, Nussloch, Germany). Before any staining procedure, sections were deparaffinized by bathing in xylol and rehydrated in decreasing concentrations of alcohol. For evaluation of glomerulosclerosis, kidney sections were PAS stained.

To quantify glomerulosclerosis, a score of 0–4 was used as described previously [5]: score 0, normal glomerulus; score 1, mesangial expansion or sclerosis involving <25% of the glomerular tuft; score 2, sclerosis 25–50%; score 3, sclerosis 50–75%, and/or segmental extracapillary fibrosis or proliferation; and score 4, global sclerosis (>75%), or global extracapillary fibrosis or proliferation, or complete collapse of the glomerular tuft. The number of glomeruli per renal cross-section used for evaluation of glomerulosclerosis was 70–170, depending on the size of the cross-section.

**Immunohistochemistry**

In deparaffinized kidney sections, endogenous peroxidase activity was blocked with 3% H₂O₂ in methanol for 20 min at room temperature. Sections were then layered with an antibody to collagen I (1:1000; Biogenesis, Poole, UK) or to collagen IV (1:1000; Southern Biotechnology Associates, Birmingham, AL, USA), and incubated at 4°C overnight. After addition of the secondary antibody (dilution 1:50; biotin-conjugated, goat anti-rabbit immunoglobulin G, Dianova, Hamburg, Germany), the sections were incubated with avidin horseradish peroxidase complex and exposed to 0.1% dianaminobenzidine tetrahydrochloride and 0.02% H₂O₂ as a source of peroxidase substrate. The Vectastain DAB kit (Vector Lab, Burlingame, CA, USA) was used as a chromogen. Each slide was counterstained with haematoxylin. As a negative control, we used equimolar concentrations of pre-immune rabbit immunoglobulin G.

Expansion of interstitial collagen I was measured in a Leitz Aristoplan microscope (Leica Instruments) by Metaview software (Visitron Systems, Puchheim, Germany) in 10 non-overlapping medium-power cortical views per section excluding glomeruli and was expressed as a percentage of stained area per cross-section. Glomerular collagen IV staining was measured by Metaview in every third glomerulus per cross-section, and the stained area was expressed as a percentage of the total area of the glomerular tuft.

**Statistical analysis**

A nested two-way analysis of variance (ANOVA) with the between-subject factors ‘strain’ and ‘treatment’ (DOCA or control) was performed using the general linear model, followed by the Tukey test for *post hoc* comparisons between groups. Pearson correlations were used to assess relationships between parameters. A *P*-value of < 0.05 was considered significant. Procedures were carried out using the SPSS software (release 9.01, SPSS Inc., Chicago, IL, USA). Values are displayed as means ± SEM.

**Results**

**Development of hypertension**

Systolic blood pressure of all mouse strain increased during the development of disease. There was little effect on blood pressure during weeks 1–3 of DOCA treatment, but systolic blood pressure was consistently elevated during weeks 4–6 (*P < 0.001* for the overall DOCA effect). Figure 1 shows the average systolic blood pressure during week 4–6. There was a significant effect of mouse strain on blood pressure (*P < 0.001*), and the most prominent increase was found in the 129/Sv strain. This was also reflected by intra-arterial blood pressure measurements: in 129/Sv, mean arterial
blood pressure was 154.5 ± 1.4 after DOCA treatment vs 110.0 ± 4.7 in salt-loaded controls (P < 0.05); in C57BL/6, the rise in blood pressure was weaker: 102.3 ± 11.9 after DOCA treatment vs 84.5 ± 1.8 in salt-loaded controls (P < 0.05).

**Body and organ weights**

F2 mice had higher body weights than F1 mice; both intercrosses had higher body weights than the two parental strains (Table 1). DOCA hypertension did not significantly affect body weight. Moreover, body weight did not correlate with blood pressure or any readout parameter of renal injury (glomerulosclerosis, collagen I and IV) in DOCA hypertension. In response to DOCA treatment, relative kidney weight increased to a similar degree in all mouse strains investigated (Table 1), independently of the increase in blood pressure. In contrast, hypertrophy of the left ventricle was strain dependent, being most prominent in 129/Sv and least extensive in F2 (Table 1).

**Diuresis and urinary albumin excretion**

Fluid intake and urine excretion were significantly increased in all DOCA-treated groups (Table 1). In DOCA-treated mice, neither fluid intake nor diuresis correlated with blood pressure, protein or albumin excretion, or any parameter of renal injury. Marked proteinuria and albuminuria was observed in all DOCA-treated strains as compared with control groups after 6 weeks of DOCA administration (Table 1, Figure 2). Remarkably, urinary protein and albumin excretion in DOCA-treated 129/Sv was not higher than in F1 or F2, despite the higher blood pressure observed in 129/Sv. Proteinuria and albuminuria in C57BL/6 were lower than in all other strains (Table 1, Figure 2).

**Interstitial fibrosis**

The expansion of interstitial extracellular matrix was comparable in the control groups, except for F1, where the percentage of collagen stained interstitial area was lower. Interstitial fibrosis was increased in all groups after DOCA treatment (P < 0.001) but differed between strains (P = 0.010), with 129/Sv showing the highest levels in response to DOCA (Figure 3). Interstitial expansion of collagen I in response to DOCA treatment correlated with blood pressure (R = 0.58, P = 0.011, n = 20), albuminuria (R = 0.49, P = 0.011, n = 26), glomerulosclerosis (R = 0.76, P < 0.001, n = 21) and glomerular collagen IV (R = 0.63, P = 0.003, n = 20).

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**Table 1.** Water consumption, urine excretion, body weights, relative weights of kidneys and left ventricles and protein excretion of all experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Water consumption (ml/day)</th>
<th>Urine excretion (ml/day)</th>
<th>Body weight (g)</th>
<th>Relative kidney weight (mg/g)</th>
<th>Relative weight of left ventricle (mg/g)</th>
<th>Protein excretion (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/Sv</td>
<td>5.5 ± 1.0</td>
<td>3.3 ± 0.5</td>
<td>25.5 ± 0.9</td>
<td>10.9 ± 0.9</td>
<td>4.4 ± 0.2</td>
<td>7.8 ± 1.4</td>
</tr>
<tr>
<td>129/Sv/DOCA</td>
<td>12.1 ± 2.0</td>
<td>10.9 ± 1.8</td>
<td>23.9 ± 0.7</td>
<td>14.2 ± 0.9</td>
<td>5.5 ± 0.1</td>
<td>17.2 ± 3.0</td>
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<tr>
<td>C57BL/6</td>
<td>5.2 ± 0.8</td>
<td>2.6 ± 0.7</td>
<td>25.0 ± 0.7</td>
<td>8.7 ± 0.3</td>
<td>3.7 ± 0.1</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>C57BL/6/DOCA</td>
<td>25.6 ± 3.7</td>
<td>21.5 ± 3.1</td>
<td>23.2 ± 1.4</td>
<td>14.1 ± 0.6</td>
<td>4.5 ± 0.7</td>
<td>7.9 ± 2.5</td>
</tr>
<tr>
<td>F1</td>
<td>4.7 ± 1.5</td>
<td>3.4 ± 0.7</td>
<td>27.9 ± 0.8</td>
<td>8.9 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>F1/DOCA</td>
<td>27.2 ± 5.3</td>
<td>24.7 ± 5.4</td>
<td>24.5 ± 0.5</td>
<td>14.2 ± 0.7</td>
<td>5.0 ± 0.3</td>
<td>29.7 ± 14.0</td>
</tr>
<tr>
<td>F2</td>
<td>10.4 ± 1.3</td>
<td>6.0 ± 1.1</td>
<td>28.6 ± 0.8</td>
<td>8.2 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>F2/DOCA</td>
<td>18.1 ± 5.1</td>
<td>14.5 ± 4.6</td>
<td>32.5 ± 0.6</td>
<td>13.2 ± 0.3</td>
<td>4.8 ± 0.2</td>
<td>14.5 ± 3.1</td>
</tr>
<tr>
<td>P (strain)</td>
<td>0.123</td>
<td>0.123</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.026&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>P (DOCA)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.208</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SEM.

<sup>a</sup>The last two rows show the P-values for the factors 'strain' and 'DOCA treatment', respectively, obtained by the nested two-way ANOVA.

<sup>b</sup>F2 > F1 (P < 0.001), F1 and F2 > C57BL/6 and 129/Sv (P < 0.01), C57BL/6 vs 129/Sv P = 0.22.

<sup>c</sup>129/Sv > F2 (P = 0.003), no other significant differences between strains.

<sup>d</sup>F1 > C57BL/6 (P = 0.03), no other significant differences between strains.

<sup>e</sup>129/Sv > C57BL/6 (P = 0.006), no other significant differences between strains; see also albumin excretion (Figure 2).
Glomerular lesions

The extent of glomerulosclerosis was assessed by a semi-quantitative score (1–4; Figure 4A) or by quantifying glomerular collagen IV staining (Figure 4B). Glomerulosclerosis scores of uninephrectomized, salt-loaded controls did not differ significantly between the control groups. DOCA treatment resulted in pronounced glomerular matrix expansion in all mouse strains ($P < 0.001$), with significant interstrain differences ($P < 0.01$). The most prominent alterations occurred in 129/Sv (Figure 4). In DOCA-treated 129/Sv, glomerular scarring had resulted in a massive loss of glomerular cells and capillaries (Figure 5C). In DOCA-treated 129/Sv, F1 generation of 129/Sv × C57BL/6 intercrosses; F2, F2 generation of 129/Sv × C57BL/6 intercrosses. There were significant overall effects of DOCA treatment ($P < 0.001$) and strain ($P = 0.003$). Data are means ± SEM. *$P < 0.05$ vs 129/Sv strain of respective group; **$P < 0.01$ vs C57BL/6 strain of respective group.

Discussion

Our results show that the degree of hypertension and hypertensive renal lesions is markedly different between various mouse strains commonly used in research. All strains investigated displayed an increase in blood pressure and developed hypertrophy of the left ventricle as well as glomerulosclerosis, but those changes were most prominent in 129/Sv. Thus, 129/Sv seem to be more susceptible to hypertension and hypertensive injury than the other strains investigated. There were also differences between strains in factors such as body weight and relative kidney weight, and there was some trend towards differences in fluid intake and diuresis. However, none of those differences could readily account for the different extent of hypertension and renal injury. These observations leave the genetic
background of the different mouse strains as the most likely explanation.

One reason for the different susceptibility to DOCA-salt hypertension could be the differences in the number of renin genes, as suggested by a recent study by Wang et al. [6]: 129/Sv mice harbour two renin genes (Ren-1\textsuperscript{a} and Ren-2, resulting in four renin gene copies), while C57BL/6 mice only have one renin gene (Ren-1\textsuperscript{c}, resulting in two renin gene copies). Consequently, F1 mice will be heterozygous at the renin gene locus (three renin gene copies), while F2 mice may vary between two and four renin gene copies. Ren-1 is produced mainly by cells of the juxtaglomerular apparatus, while Ren-2 expression is predominantly found in the submaxillary gland [7]. The presence of two renin genes could therefore result in increased blood pressure in 129/Sv. However, data obtained from experiments targeting the gene for the angiotensin II receptor 2 argue against this assumption. The deletion mutation was induced in 129/Sv and backcrossed into different mouse strains. When backcrossed into FVB/N, baseline blood pressure was normal [1], while in C57BL/6 mice the lack of the angiotensin II receptor 2 resulted in a significant increase in blood pressure [2], although C57BL/6 mice harbour only one renin gene. Studies in 129/Sv mice with a targeted inactivation of the Ren-2 gene suggested that this gene is not essential for the regulation of blood pressure [8].

In the study of Wang et al. [6], mice with one renin gene did not develop high blood pressure in response to DOCA-salt treatment, while renal hypertrophy was readily detected. Our intra-arterial recordings, however, revealed significant increases of blood pressure in response to DOCA-salt treatment in both mice with one renin gene and mice with two renin genes. This is in keeping with data from Peng et al. [3], who detected a DOCA-induced rise in blood pressure in mice with one renin gene. A recent study in adenosine 1 receptor-deficient mice bred on a mixed background revealed that plasma renin activity and concentration was not different in litters harbouring one or two renin genes [9]. Taken together with the differential interstrain pattern of albuminuria and renal injury, these data do not support the notion of a simple relationship between the number of renin genes and elevations in blood pressure or development of renal lesions.

A strain variability in the extent of kidney damage was also observed in other models of kidney disease and in mice with targeted deletion of genes. Nakao et al. [10] backcrossed mice with a deficiency for tenascin C into three congenic strains: C57BL/6, BALB/c and GRS/A. The three different knockout strains developed normally and no phenotype was detected. Induction of a reversible model of glomerulonephritis in those strains, however, revealed a strain-dependent severity of the disease. The kidney damage was mild in the tenascin C knockouts backcrossed into C57BL/6, moderate in the BALB/c backcrosses and severe in GRS/A backcrosses. In the latter, the course of disease was progressive and the animals died some weeks after the induction of the disease due to renal failure. Similarly, deletion mutations for the angiotensin II receptor 1 were backcrossed into 129/Sv and C57BL/6 [11]. On the C57BL/6 background, the absence of angiotensin II receptor 1 resulted in an abnormal renal phenotype, while the kidneys were normal in 129/Sv deficient in angiotensin II receptor 1.

Surprisingly, the amount of albumin excretion did not correspond to the degree of glomerular damage. Although DOCA-treated F1 and F2 displayed significantly lower glomerulosclerosis scores and less glomerular collagen IV accumulation than DOCA-treated 129/Sv, albuminuria was increased to comparable levels in all three groups. The amount of albuminuria in this model does not seem to be a good predictor for the degree of glomerular scarring. This finding has obvious implications for the screening of mouse models for a renal phenotype—albumin excretion, though attractive in principle for high-throughput analysis, may not be a reliable indicator for glomerular damage. Although F1 and F2 displayed intermediate blood pressure levels, as would be expected if blood pressure was dependent on the amount of renin genes, albuminuria in F1 and F2 was at least as high as in 129/Sv. In contrast, glomerulosclerosis was less pronounced in F1 than in 129/Sv and C57BL/6. These findings suggest that putative loci for hypertensive renal disease may segregate independently from loci for hypertension in mice, as in rats [12]. Somewhat unexpectedly, albumin excretion did correlate with interstitial fibrosis. We cannot fully explain this finding, but we speculate that the recently discussed contribution of filtered protein load to tubulointerstitial damage [13] may contribute.
Our findings underscore the importance of the genetic background for the development of hypertension and target organ damage. An identical background is crucial for the evaluation of the role of targeted mutations in single genes for the development of hypertension and target organ injury. Moreover, our findings also indicate that cosegregation studies in backcrosses of 129/Sv and C57BL/6 could be useful to identify genes which modify the development of hypertension and glomerulosclerosis. For that purpose, a large number of F2 backcrosses ($n > 100$) might undergo DOCA-salt treatment, followed by phenotyping for blood pressure, albumin excretion and renal damage. A whole-genome scan for markers cosegregating with the phenotypes [14] may identify gene loci for hypertension and nephrosclerosis, as was previously described in several rat models, for example in Fawn-hooded rats [12].

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