Neonatal dexamethasone administration causes progressive renal damage due to induction of an early inflammatory response

Yan Liu,1,5 Harry van Goor,2 Rick Havinga,1 Julius F. W. Baller,1 Vincent W. Bloks,1 Feike R. van der Leij,1,3 Pieter J. J. Sauer,1 Folkert Kuipers,1 Gerjan Navis,4 and Martin H. de Borst2

1Center for Liver, Digestive, and Metabolic Diseases, Laboratory of Pediatrics; 2Department of Pathology and Laboratory Medicine; University Medical Center Groningen, University of Groningen, Groningen; 3Unit Life Sciences, Van Hall University of Applied Sciences, Leeuwarden; 4Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, The Netherlands; and 5Department of Endocrinology, Third Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

Submitted 8 April 2007; accepted in final form 25 January 2008

Address for reprint requests and other correspondence: H. van Goor, Dept. of Pathology and Laboratory Medicine, Univ. Medical Center Groningen, Univ. of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands (e-mail: h.van.goor@path.umcg.nl).

Glucocorticoids (GCs) are widely used in immature newborns to prevent chronic lung disease. However, accumulating evidence from animal studies indicates that overexposure to GCs in early life may have long-term negative effects such as cardiac dilation (4), hyperglycemia (28), increased blood pressure (22), and alterations in social behavior (21).

Renal damage is one consequence of early-life GC overexposure. Data suggest that maternal GC administration, e.g., dexamethasone (DEX) or betamethasone, impairs nephrogenesis and reduces glomeruli numbers, which may contribute to hypertension in late life (17, 33). To the best of our knowledge, effects of neonatal GC administration on kidney function have not been studied in animals. Studies in human infants are also limited; a high incidence of renal calcium deposition in DEX-treated newborns has been described as a clinical observation (11a, 20), but no follow-up study has been reported so far.

In the present study, we sought to establish the effect of neonatal DEX treatment in rats on survival in relation to kidney function. We found that neonatal DEX administration to rat pups causes premature death associated with the unexpected findings of severe progressive renal damage. Thereafter, we carefully evaluated the sequence of events upon postnatal DEX administration leading to fatal kidney disease. Clinical and structural parameters were investigated, especially in the early phase, to elucidate the mechanism involved in the long-term detrimental effect of GCs on the kidney. Our results indicate that an early inflammatory response, i.e., during the first month of life, underlies structural changes in the kidney that leads to severe organ dysfunction in later life.

MATERIALS AND METHODS

Animal model. Experiments were in accord with institutional and legislative regulations and approved by The Local Committee for Animal Experiments. Pregnant Wistar rats (270–300 g) were housed individually and kept under conventional housing conditions with free access to food and water. Pups were born on day 21 or 22 of gestation. On the day of birth (day 0), male pups were selected and randomly divided into treatment and control groups. Room temperature and humidity were kept constant, and the rats had free access to food and water. An artificial 12:12-h light-dark cycle was employed. Rat pups in the treatment group were injected (ip) with dexamethasone 21-phosphate (DEX) on day 1 (0.5 µg/kg body wt), day 2 (0.3 µg/kg body wt), and day 3 (0.1 µg/kg body wt) after birth in the morning between 0900 and 1200, as described previously (4, 22, 28). The dosages of DEX used in the present study were similar to those used in immature human babies with chronic lung disease. The Committee on Fetus and Newborn of the American Academy of Pediatrics reported that, in most cases, DEX is given in a dosage of 0.5 mg·kg−1·day−1 for 3 days, followed by a tapering course of 0.3, 0.1, and 0.05 mg·kg−1·day−1 each for 3 days (1). Controls received equal volumes (10 µl/g body wt) of sterile pyrogen-free saline (SAL).

Experiment 1. This first experiment was designed to establish effects of neonatal DEX administration on survival. On the day of

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Fig. 1. Neonatal dexamethasone (DEX) administration affects survival at older age. Kaplan-Meier survival curves demonstrate that neonatal DEX administration results in significantly increased death rate. Saline (SAL) group, $n = 22$; DEX group, $n = 30$. *$P < 0.05$ by log rank test.

birth (day 0) male pups were selected and randomly divided into treatment and control groups (SAL, $n = 22$; DEX, $n = 30$). Body weight and survival were measured weekly. At 50 wk of age, 24-h urine was collected in a metabolic cage, and urine volume as well as concentrations of creatinine (Cr) and urinary protein were measured. Serum Cr and blood glucose were determined in blood collected at the end of the experiment prior to euthanasia. After the rats were killed, the kidneys were collected without perfusion for periodic acid-Schiff (PAS) staining. Procedure details are described below.

Experiment 2. This second experiment was designed to explore the mechanism underlying renal damage induced by DEX. We performed a time course study in rats aged from 2 days to 32 wk. On day 0, male pups were selected and randomly divided into treatment and control groups ($n = 6$ per group). Rats pups received the same treatment described in experiment 1. Rats were killed at indicated ages from 2 days to 32 wk in the morning between 0900 and 1200.

Systolic blood pressure measurement. It has been suggested that early-life GC overexposure may cause increased blood pressure in adult life (15, 22, 28). Therefore, systolic blood pressure (SBP) was measured in these animals. SBP was measured from age 4 wk to 32 wk after 2 wk of daily training before the first measurement. An automated multichannel system was used with tail cuffs and photoelectric sensors to detect the tail pulse (Apollo 179; IITC Life Science, Woodland Hills, CA) as described previously (37). The rats were maintained at 27–29°C. For each rat, the value was calculated from the mean of three or four consecutive measurements.

Urinary protein, blood glucose, and Cr measurements. Twenty-four-hour urine was collected in a metabolic cage from 4- to 50-wk-old rats. Urinary Pr and Cr concentrations were measured by use of an autoanalyzer (Merck Mega, Darmstadt, Germany). Blood samples were collected from abdominal artery under anesthesia. Blood glucose was measured with a Medisense Precision glucose meter (Abbott, Chicago, CO), or a mouse monoclonal antibody against α-SMA (clone 1A4; Sigma Chemical, St. Louis, MO), a polyclonal rabbit antibody against pJNK (Cell Signaling Technology, Denver, CO), or a mouse monoclonal antibody against ED1 (Sertotec, Kidlington, UK). Antibodies were incubated for 60 min at room temperature except for the pJNK antibody, which was incubated for 120 min. Binding of the antibody was detected using sequential incubations with peroxidase-labeled (PO-) secondary and tertiary antibodies in accordance with the species in which the primary antibody was raised (Dako Cytomation, Glostrup, Denmark) for 30 min. Antibody dilutions were made in PBS supplemented with 1% BSA and 1% normal rat serum. Peroxidase activity was developed by using 3,3’-diaminobenzidine tetrachloride for 10 min. Sections were counterstained with hematoxylin. For all immunohistochemical staining, controls in which the primary antibody was replaced by PBS and appropriate isotype controls were included; these were consistently negative.

For glomerular measurement of ED1, the number of positive cells per glomerulus was counted (average of 50 glomeruli). For interstitial measurement of ED1, the number of positive cells per square millimeter was counted (average of 30 fields per kidney): vessels and glomeruli were excluded from measurements. For measurement of pJNK, the number of positive cells per square millimeter was counted by a blinded observer (average of 30 fields per kidney).

RNA isolation and real-time quantitative PCR. A number of proinflammatory factors that may trigger inflammatory response and fibrosis have been measured by real-time quantitative PCR. For RNA isolation, kidneys tissues from 2-day- to 32-wk-old animals were rapidly excised and immediately frozen in liquid nitrogen following the procedure mentioned above. RNA was extracted from kidney samples (including cortex and medulla) by using the Tri Reagent method (Sigma). The integrity of total RNA was assessed using a Lab-on-a-Chip 2100 Bioanalyzer (Agilent). Total RNA was reverse transcribed as described previously (30). Real-time quantitative PCR was performed using an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, CA) in a final volume of 25 µl consisting of 2.5 µl of cDNA, 200 nM of each primer, and 12.5 µl of the 2x SYBR Green PCR Master Mix (Applied Biosystems). Reaction conditions were as follows: 1 cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Primers were designed for each target using Primer Express Software version 2.0 (Applied Biosystems). The expression level of each target was normalized to the expression levels of the housekeeping gene cyclophilin A. Differences in mRNA expression were calculated by the 2−ΔΔCT method.

Table 1. Characteristics of 50-wk-old rats upon neonatal treatment with SAL or DEX

<table>
<thead>
<tr>
<th></th>
<th>SAL</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW), g</td>
<td>625 ± 39</td>
<td>559 ± 61*</td>
</tr>
<tr>
<td>Kidney weight (KW), g</td>
<td>1.69 ± 0.13</td>
<td>1.60 ± 0.26</td>
</tr>
<tr>
<td>KW/BW ratio, mg/g</td>
<td>2.72 ± 0.22</td>
<td>2.76 ± 0.79</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>6.71 ± 1.32</td>
<td>5.35 ± 0.31*</td>
</tr>
<tr>
<td>Serum Cr, µmol/l</td>
<td>67.0 ± 5.8</td>
<td>102.0 ± 30.0*</td>
</tr>
<tr>
<td>Volume of 24-h urine, ml</td>
<td>15.0 ± 3.5</td>
<td>39.6 ± 20.0*</td>
</tr>
<tr>
<td>Urinary Pr/Cr, g/mmol</td>
<td>0.22 ± 0.06</td>
<td>0.55 ± 0.23*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; $n = 8$ per treatment group. SAL, saline treated; DEX, dexamethasone treated; Cr, creatinine; Pr, protein. *$P < 0.05$ DEX vs. SAL by Mann-Whitney U-test.
Biosystems, Foster City, CA) according to the manufacturer’s instructions. Primers were obtained from Invitrogen (Carlsbad, CA). Fluorescent probes, labeled with 6-carboxyfluorescein and 6-carboxytetramethyl-rhodamine, were made by Eurogentec (Seraing, Belgium). The following primers and probes were used, in addition to 36B4, which has been published before (36): TNF-α (NM_012675, forward GCC CAC GTC GTA GCA AAC, reverse AGT TGG TTG TCT TTG AGA TCC ATG, probe CGC TGG CTC AGC CAC TCC AGC); TGF-β (X52498, forward GGG CTA CCA TGC CAA CTT CTG, reverse GAG GGC AAG GAC CTT GCT GTA, probe CCT GCC CCT ACA TTT GGA GCC TGG A); MCP-1 (CCL2, NM_031530 forward TGT CTC AGC CAG ATG CAG TTA AT, reverse CCG ACT CAT TGG GAT CAT CT, probe CCC CAC TCA CCT GCT GCT ACT CAT TCA). Gene expression data were subsequently standardized for 36B4 mRNA, which was quantified in separate runs.

**Statistics.** Data are expressed as means ± SD. Differences between two age-matched groups were assessed by Mann-Whitney U-test. Survival analysis was investigated by the Kaplan-Meier method, and the difference between two groups was examined by the log-rank test. The level of significance was set at $P < 0.05$. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL).

**RESULTS**

*Neonatal DEX administration leads to reduced life span and severe kidney disease in surviving rats at 50 wk of age.* Rats neonatally exposed to DEX showed a significantly increased death rate, with a survival percentage at 50 wk of only 83%, and all deceased rats had severe kidney disease. No single death was observed in the SAL-treated group at 50 wk (Fig. 1).

Compared with the SAL group, DEX-treated rats that survived up to 50 wk of age showed a significantly reduced blood glucose concentration. Serum Cr concentration, volume of 24-hr urine, and urinary protein/Cr ratio were all significantly elevated in 50-wk-old rats treated with DEX (Table 1).
All surviving 50-wk-old rats that had received DEX treatment presented with renal disease, and PAS staining indicated severe nephropathy with extensive scarring, glomerulosclerosis, and dilation of the tubular system. Accumulation of lymphocytes and macrophages indicated inflammation (Fig. 2, B and D). No overt renal pathological alterations were found in the SAL control group.

**Neonatal DEX administration leads to body and kidney growth retardation.** Subsequently, a time course study was initiated to allow identification of primary events in the cascade leading to kidney failure. Growth retardation was persistent during the entire experiment in DEX-treated animals compared with age-matched controls. No catch-up growth was observed in this study up to 32 wk of age (Fig. 3A).

Compared with SAL-treated rats, a significantly higher ratio of average kidney weight vs. body weight was found in 2-day- and 7-day-old rats upon DEX treatment ($P < 0.05$). Thereafter, this ratio was either similar or significantly lower in DEX-treated rats (Fig. 3B).

**Blood pressure increases after neonatal DEX administration.** SBP of SAL- and DEX-treated rats slowly increased from 8 wk to 32 wk of age. Compared with controls, a significantly increased SBP was observed in DEX-treated rats from 8 wk onwards (Fig. 4).

**Serum Cr and proteinuria.** No differences in serum Cr concentrations between SAL- and DEX-treated rats were noted up to 32 wk (Table 2). Compared with the SAL group, a slight increase in 24-h urine volume was noted from 12 wk onward upon DEX treatment, which was statistically significant at 20 wk (data not shown). Urinary protein/Cr ratio is a sensitive marker for protein loss in urine (10). Compared with SAL treatment, neonatal DEX treatment led to a highly significant increase in urine protein/Cr ratio from 8 wk of age onward, reaching a fourfold difference between the groups at week 32 (Fig. 5).

**Pathological changes in kidneys induced by neonatal DEX exposure.** PAS staining indicated no pathological alterations in kidneys of 2 day-, 14 day-, and 8-wk-old animals of SAL- and DEX-treated groups. Glomerular sclerosis was found in 32-wk-old rats upon DEX treatment (Fig. 6F). In contrast to SAL-treated rats, increased tubulointerstitial staining of α-SMA was observed in rats treated with DEX at 32 wk, indicating myofibroblast transformation, an early indication of kidney fibrosis (Fig. 7, A and B).

Nephron numbers were found slightly but significantly reduced in rats both at 8 and 24 wk with DEX treatment compared with age-matched controls (Fig. 8, A and B).

Numbers of macrophages in glomeruli were increased at 8 wks and 32 wk of age in rats treated with DEX (see Fig. 10A), which matched the time course of increased urinary protein loss. Compared with SAL-treated rats, the total number of macrophages in the tubulointerstitium was significantly reduced in DEX-treated 2-day-old rats (Fig. 9, A and B and Fig. 10B). Significant and persistent increases in the numbers of tubulointerstitial ED1-positive macrophages were observed from day onward in DEX-treated rats compared with age-matched controls (Fig. 9, C–F and Fig. 10B).

**Time-dependent changes in renal gene expression upon neonatal DEX treatment.** Compared with SAL-treated animals, renal gene expression of TNF-α was significantly suppressed in DEX-treated 2-day-old rats, with a subsequent increase at 7 days and 4 wks of age (Fig. 11A). Significantly increased renal gene expression levels of MCP-1 were noted in DEX-treated animals at 7 days, 14 days, and 32 wk of age compared with the age-matched controls (Fig. 11B).

Compared with SAL-treated rats, TGF-β gene expression was significantly suppressed in the DEX group at 2 and 7 days of age. Thereafter, a slight but significantly increased TGF-β gene expression were noted from 4 wk to 32 wk of age in rats upon DEX treatment, which may promote progressive kidney fibrosis (Fig. 11C).

Table 2. Concentration of serum Cr in rats neonatally treated with SAL or DEX

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>SAL</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>10.4±1.7</td>
<td>13.7±1.9</td>
</tr>
<tr>
<td>8</td>
<td>11.9±2.0</td>
<td>15.4±2.3</td>
</tr>
<tr>
<td>16</td>
<td>14.2±3.2</td>
<td>18.3±3.6</td>
</tr>
<tr>
<td>24</td>
<td>17.1±4.5</td>
<td>21.5±5.0</td>
</tr>
<tr>
<td>32</td>
<td>19.4±6.0</td>
<td>24.7±6.5</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; n = 6 per treatment group every age. *$P < 0.05$ DEX vs. SAL by Mann-Whitney-U test.

![Fig. 4. Neonatal DEX administration results in increased systolic blood pressure (SBP).](image)

![Fig. 5. Neonatal DEX administration increases urinary protein-to-creatinine (Pr/Cr) ratio from 8 wk of age.](image)
Transient changes in renal pJNK immunostaining upon neonatal DEX treatment. Activated JNK (pJNK) was found in a number of tubular epithelial cells of both SAL- and DEX-treated rats. In 2-day-old rats, neonatal DEX administration resulted in significantly decreased pJNK-positive nuclei numbers compared with SAL-treated animals (Fig. 12, A–C). At 7 days of age, on the other hand, significantly increased numbers of pJNK-positive nuclei were noted in rats treated with DEX compared with same-age controls. At 14 days, there was no longer any difference between the two groups (Fig. 12C).

Fig. 6. Renal histology at 2 days, 8 wk, and 32 wk. Representative images of PAS staining show normal kidney structure of SAL-treated rats at age of 2 days, 8 wk, and 32 wk (A, C, and E). DEX treatment does not result in apparent structural renal damage in rats aged 2 days or 8 wk (B and D). However, glomerular sclerosis was observed at 32 wk in DEX-treated rats (F); n = 6 per group every age. Magnification: A–F, ×400. Arrow, glomerular sclerosis.

Fig. 7. Renal α-SMA staining of 32 wk of age. Increased interstitial α-smooth muscle cell actin (α-SMA) staining (brown precipitate) was observed at 32 wk of age with DEX treatment (B) in contrast to age-matched controls (A, SAL group); n = 6 per group. Magnification, ×400.

Fig. 8. Nephron numbers. Estimation of nephron numbers were performed in PAS-stained slides from rats of 8 wk (A) and 24 wk old (B). Total numbers of glomeruli were counted under ×100 magnification, and 20 fields per animal were scored. Open bars, SAL groups; filled bars, DEX groups. Data are expressed as means ± SD; n = 5–6 rats per group every age. *P < 0.05 DEX vs. SAL, by Mann-Whitney U-test.
DISCUSSION

In the present study, neonatal DEX administration was shown to result in severe and progressive kidney disease in rats, likely contributing to the reduced life span observed under these conditions. A time course study demonstrated that neonatal DEX treatment led to persistent growth retardation and reduced kidney weight. Increased blood pressure and proteinuria were observed from 8 wk of age onward, leading to extensive renal fibrosis and glomerular sclerosis at 32 wk of age. An early renal inflammatory response was observed in rat pups, after withdrawal of DEX, that may have triggered the subsequent persistent and progressive renal fibrotic process that ultimately led to progressive renal impairment.

While this paper was being prepared, a study in a similar animal model of neonatal DEX exposure was published showing that neonatal DEX administration is associated with premature death and kidney failure (22), which is consistent with our findings described in the present paper. Furthermore, Ortiz et al. (29) show that there are specific times of DEX administration that seem to have an adverse effect. We found that DEX administration shortly after birth might lead to reduced glomerular numbers later in life. The study by Ortiz et al. was on the maternal situation, the dosage of DEX was relatively lower, especially the real dose of DEX that could reach the fetus. Yet our data could be in line with their finding that apparently there are time windows critical for nephrogenesis, which makes the fetus more sensitive to stimuli or inhibitors in that particular period. The first week after birth might be a crucial time period for renal nephrogenesis.

As described previously (26), postnatal DEX administration in rats results in persistent growth retardation, which might be due to the suppression of growth hormones and of the insulin-like growth factor axis (18, 34). A significantly increased ratio of kidney vs. body weight was observed with DEX at 2 and 7 days, which might be largely due to water retention since this ratio was increased acutely and no morphological hypertrophy was observed at those ages. The subsequent persistently lower relative kidney weight may indicate that neonatal DEX administration permanently inhibited kidney growth or may reflect the process of fibrotic scarring.

DEX administration led to a significantly increased systolic blood pressure from 8 wk of age onward. This is in accord with a recent study using the same model showing that increased blood pressure was present at 3 and 11 mo of age (22). The molecular mechanism underlying the “programmed” hypertension induced by early GC exposure is still obscure. It has been speculated that altered renal angiotensin system activity, reduced nephron numbers, and an overactive brain angiotension system might all participate in the pathogenesis of hypertension induced by prenatal DEX exposure (16). In rats, the kidney is still immature at birth, and nephrogenesis is not complete until at least 1 wk after birth (25). Although the method of counting nephron numbers in the present study was limited compared with previous methods described (5, 12), it is likely that neonatal DEX administration in the first 3 days after birth leads to reduced nephron numbers in adult life by inhibition of nephrogenesis, which has also been found in prenatal studies (14, 17). In addition, in our study, DEX-treated rats had increased urinary protein loss from 8 wk onward. We speculate that reduced nephron number and/or renal damage may both be involved in increased blood pressure in adulthood. It should be noted that SAL-treated rats in the current study had higher blood pressure levels compared with data from the literature (11), which might be due to increased endogenous GC levels at the neonatal stage induced by stress of handling.

In the early phase of renal disease, in general there is an influx of inflammatory cells, i.e., macrophages, into the kidney.

Fig. 9. Renal macrophage accumulation. Representative images of renal immunohistochemistry for macrophage marker ED1. A and B show less interstitial ED1+ cells in 2-day-old rats with DEX treatment vs. age-matched SAL group: A, SAL group 2 days; B, DEX group 2 days. Increased numbers of interstitial ED1+ cells were observed in DEX group at 14 days in contrast to control: C, SAL group, 14 days; D, DEX group, 14 days. ED1+ cells were scarce in 32-wk-old SAL-treated rats (E). In contrast, large interstitial amounts of ED1+ macrophages were found in rats with DEX treatment at 32 wk (F). Magnification A–F, ×400. Arrows, ED1+ cells (group size n = 6 at every age).
irrespective the initial insult (27). This process can be stimulated by proinflammatory factors such as TNF-α and chemokines like MCP-1. Recent data suggest that TNF-α plays a crucial role in development of kidney disease (23) by inducing macrophage accumulation and fibrogenesis. In our study, we observed suppression of TNF-α gene expression at 2 days of age, i.e., during the DEX administration, which is most likely due to the anti-inflammatory effects of GCs. Remarkably, after withdrawal of GCs, we observed markedly increased renal TNF-α gene expression at 7 days and 4 wks of age. This is consistent with an inflammatory response that could potentially be involved as a trigger for the onset of renal damage that we observed subsequently.

MCP-1 is expressed at sites of injury and inflammation to direct macrophage recruitment. It has been suggested that MCP-1 is predominantly expressed by tubular epithelial cells and not by glomeruli and promotes tubular epithelial cells and not glomerular damage (31, 35). We found increased kidney gene expression of MCP-1 in 7- and 14-day-old rats after DEX treatment. In the present study, stress MCP-1 gene expression might be a crucial factor in the development of progressive kidney damage in later life by its stimulation of inflammatory pathways during a pivotal stage of kidney development.

To further address the cellular mechanisms involved in this inflammatory response, we evaluated the activation state of the JNK pathway, which has been suggested to modulate MCP-1 expression (2, 13, 38, 39). In the present experiment, we found that the extent of renal JNK activation (as determined by expression of pJNK) was significantly reduced at 2 days of age upon DEX treatment. This seems also reasonable since previous studies have indicated that GCs may inhibit activation of the JNK pathway (8, 9). Remarkably, we found significantly increased pJNK in 7-day-old rats after DEX withdrawal, consistent with rebound inflammatory effects. Our studies thus suggest involvement of the JNK pathway in DEX-induced renal injury, although further studies including interventions...
with specific JNK inhibitors are required to further investigate its role.

GCs are widely used in immunosuppressive therapy in inflammatory kidney diseases with well-established inhibitory effects on macrophages recruitment (19). This is consistent with our finding of reduced numbers of macrophages in the tubulointerstitium at 2 days of age during DEX treatment. Surprisingly, we found persistently increased macrophage numbers in tubulointerstitium from 14 days of age onward, which strongly suggests the existence of life-long renal inflammatory reactions after postnatal DEX treatment. Our data also suggest that macrophage accumulation in tubulointerstitium and not in glomeruli participates predominantly in development of early kidney damage. In recent years, it has become widely accepted that interstitial inflammation plays a central role in progression to end-stage renal failure. In this process, interstitial macrophages are involved in both the initiation and continuation of the inflammatory response (32).

Fibrosis is characteristic of progressive renal damage. Elevated α-SMA expression has been regarded as an important early marker for renal fibrosis (3). Neonatal DEX treatment resulted in increased α-SMA protein levels at 32 wk of age, i.e., when renal damage is extensive. TGF-β is a key regulatory molecule in the control of the activity of fibroblasts and has been implicated in several disease states characterized by excessive fibrosis (24). In the present study DEX administration suppressed TGF-β gene expression in 2-day-old rats, which is consistent with previous findings in rat hepatic stellate cells in vitro (7). Although reduced TGF-β expression slowly restored during the first 2 wk of life in DEX-treated animals, it is highly expressed in the rest of life after weaning, contributing to a persistent profibrotic environment in the kidney.

GCs are widely used for their anti-inflammatory effects for many different disease conditions. However, our data indicate that the transient inhibition of renal inflammatory parameters during DEX in neonatal rats is followed by a rebound of proinflammatory factors, leading to a permanent increase in interstitial macrophage accumulation after withdrawal, followed by progressive renal damage in later life. Therefore, early lifetime GC administration, especially during the crucial period of nephrogenesis, should not be taken lightly.

So far, unfortunately, human data are lacking. Since a huge number of newborns have been treated with DEX and other GCs from the 1980s on, however, one might consider assessing blood pressure, proteinuria, and kidney function in this population to establish whether the GCs might have elicited deleterious long-term effects.

In conclusion, data provided in this study suggest that neonatal DEX administration leads to end-stage renal disease in rats in later life. The accumulation of inflammatory factors in the tubulointerstitium induced by DEX at early-life age might participate in kidney function impairment, fibrosis, and kidney failure. Furthermore, although it is dangerous to extrapolate from animal experiment to human situations, we propose that follow-up studies on kidney functions in humans that received neonatal DEX are necessary.

ACKNOWLEDGMENTS

We thank Fjodor van der Sluijs for primer design and Marian Bulthuis for technical assistance.
REFERENCES


