Cyclosporin A Administration during Pregnancy Induces a Permanent Nephron Deficit in Young Rabbits

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Abstract. Cyclosporin A (CsA) is an immunosuppressive agent used to prevent graft rejection and to treat autoimmune disorders. Successful pregnancies can be achieved among CsA-treated women, although it is known that CsA is nephrotoxic and crosses the human placenta. The aim of this study was to evaluate the harmlessness of CsA toward the embryonic kidney. Twenty-one pregnant rabbits were divided into four groups. Groups of six and four female animals were subjected to daily injections of 10 mg/kg per d CsA (administered subcutaneously) for 5 d, from day 14 to day 18 of gestation or from day 20 to day 24 of gestation, respectively. In the third group, five female animals received the CsA diluent (Cremophor) from day 14 to day 18 of gestation. The fourth group consisted of six untreated female animals. Pregnancy outcomes among CsA-treated does demonstrated a reduced number of living pups, which were also growth-retarded, with exposure to CsA from day 20 to day 24 of gestation. However, pups exposed to CsA from day 14 to day 18 of gestation exhibited normal fetal growth, and blood concentrations of CsA matched human data. Examinations of kidneys at birth demonstrated vacuolation of proximal and collecting tubules and ureteric bud ends. Increased glomerular volumes and decreased nephron densities suggested nephron mass reduction, which was quantitatively evaluated in 1-mo-old animals. The nephron numbers were reduced by 25 and 33% in day 14 to 18 CsA-treated and day 20 to 24 CsA-treated animals, respectively, which displayed compensatory adaptation of the existing nephrons. However, foci of segmental glomerular sclerosis were already present, which would possibly jeopardize renal function later in life.

For nearly three decades, cyclosporin A (CsA) has been demonstrated to be a powerful immunosuppressive agent; it is commonly used for organ transplantation and the treatment of various autoimmune diseases (for review, see reference 1). Among patients undergoing CsA therapy, increasing numbers of pregnant women have been reported (2–4). However, chronic maternal CsA treatment during pregnancy is not without fetal side effects. Although CsA does not seem to be a major human teratogen, most prenatal exposures to CsA have been associated with some complications, such as abortion, prematurity, or intrauterine growth retardation (4–7). CsA crosses the placenta, and the fetus is exposed to CsA and its metabolites (8–10). The nephrotoxicity of CsA among adults is well documented (11–13), and the harmlessness of CsA toward the embryonic kidney remains to be established.

A few clinical reports indicated that children who were prenatally exposed to CsA exhibited normal renal function at birth (14) and during the first years of life (15,16). Although these short-term clinical data are reassuring, experimental studies are more worrying. Prenatal exposure to CsA induced vacuolation of proximal tubular cells (17,18) and impaired distal nephron differentiation (19) in newborn rats. Moreover, in vitro CsA exposure of embryonic kidneys produced a defect in nephrogenesis (20). Together, these experimental studies indicated that exposure of immature kidneys to CsA interferes with renal differentiation.

The aim of this study was to assess the potential renal side effects of prenatal exposure to CsA in an animal model that is very close to the human situation. The pregnant New Zealand white rabbit model was chosen for several reasons. First, rabbits exhibit a hemochorial type of placentation, as do human subjects, which allows close contact between the maternal circulation and the fetal circulation (21). Second, CsA-induced acute nephrotoxicity in adult rabbits exhibits close similarities to CsA-induced renal side effects among human subjects (22,23). Third, the kidneys of rabbit pups exhibit anatomic and functional characteristics similar to those of human neonatal kidneys (24). Accurate follow-up monitoring of the CsA-treated does, combined with maternal blood CsA determinations, validated our model. Histologic and morphometric approaches then demonstrated that in utero exposure to CsA not only induced a nephron deficit but also produced cellular lesions that were likely to further reduce the nephron endowment in adulthood.
Materials and Methods

Animals

Twenty-one New Zealand white rabbit does with known mating dates, weighing between 3.5 and 4.5 kg, were used. The pregnant rabbits were randomly divided into four groups. A group of six does did not receive any treatment and served as control animals. Two groups of pregnant does received daily subcutaneous injections of 10 mg/kg per d Sandimmune (CsA, 50 mg/ml; Cremophor, 650 mg/ml) for 5 d. For one group (six does), the treatment lasted from day 14 to day 18 of gestation (day 14 to 18 CsA group); for the other group (four does), the treatment was from day 20 to day 24 of gestation (day 20 to 24 CsA group). The former treatment overlaps with the onset of metanephros formation, whereas the latter coincides with the beginning of nephron filtration. The final group consisted of five pregnant rabbits that received one subcutaneous injection of Cremophor daily from day 14 to day 18 of gestation. CsA and Cremophor were kindly provided by Novartis (Rueil-Malmaison, France). All rabbits were individually housed and had free access to food and tap water. Experiments were conducted according to the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. At the end of each CsA treatment, maternal blood samples were collected with the procedure described by Levy-Marchal et al. (25), for measurement of CsA concentrations with an AxSYM cyclosporine determination kit (Abbott Laboratories, Abbott Park, IL).

Anatomic and Histologic Studies

Two pregnant female animals from each control group and from the day 14 to 18 CsA group were euthanized on embryonic day 19, for assessment of fetal growth. The remaining 15 rabbit does were allowed to deliver spontaneously. The duration of gestation could be accurately determined, because the mating lasted only 2 h and all deliveries were eyewitnessed. Pups were weighed within 6 h after birth. Two or three pups per litter were randomly chosen and euthanized at birth. The kidneys were removed, weighed, and fixed in Duboscq-Brazil fluid for histologic assessments. At that age, nephrogenesis has ceased for 2 wk and most nephrons are mature. For all newborn and 1-mo-old pups, blood samples were collected for measurement of plasma creatinine levels.

A median slice was cut from each fixed kidney (parallel to the short axis of the kidney), dehydrated, embedded in paraffin, and sectioned. Cross-sections included the full thickness of the cortex and medulla. Sections were stained with periodic acid-Schiff stain, Masson’s trichrome stain, hematoxylin-eosin-saffron, and Maroniizzi silver stain. Observation was performed with a light microscope (Leica, France). Measurements were performed with NIH Image software (National Institutes of Health, Bethesda, MD), after calibration. Cortical heights, positions of glomeruli within the cortical layer, and glomerular volumes of both superficial and juxtamedullary nephron populations were determined as described previously (26).

For nephron counting, kidneys were divided in half and each part was incubated in 50% hydrochloric acid for 90 min at 37°C and then rinsed extensively with tap water. After overnight storage at 4°C, macerated kidneys were placed in 500-ml graduated flasks, gently crushed, and shaken. Suspensions of long tubular structures (up to 3 mm) and intact glomeruli were obtained. Three or four aliquots of 0.5 ml were pipetted and used for counting of glomeruli.

Statistical Analyses

All results are expressed as means ± SEM. Comparisons between groups were performed with ANOVA. Differences were considered to be significant when the p value was <0.05.

Results

Pregnancy Outcomes among CsA-Treated Rabbits

Data on pregnancy outcomes are reported in Table 1. Gestation lasts approximately 1 mo in rabbits, and none of the treatments had an adverse effect on the duration of gestation. Most CsA-treated does transiently ceased feeding during the treatment. This phenomenon was previously reported for chronic CsA exposure (27). However, similar maternal weight gains were observed in the control, Cremophor, and day 14 to 18 CsA groups during the second half of gestation. To ensure that no fetal growth impairment occurred at the end of the treatment (28), two does from those groups were euthanized on embryonic day 19 for fetal and placental weight determinations. Embryonic day 19 fetuses in the control, Cremophor, and

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Duration of Gestation</th>
<th>Weight Gain, 14 to 30 d post coitum (g)</th>
<th>No. of Alive Pups per Litter</th>
<th>No. of Stillborn per Litter</th>
<th>Birthweight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>31 d + 23 h ± 10 h</td>
<td>490 ± 122</td>
<td>9.3 ± 0.5</td>
<td>0.5 ± 0.3</td>
<td>57.0 ± 1.3</td>
</tr>
<tr>
<td>Cremophor</td>
<td>3</td>
<td>31 d + 20 h ± 11 h</td>
<td>481 ± 63</td>
<td>9.0 ± 1.5</td>
<td>0.3 ± 0.3</td>
<td>58.0 ± 2.3</td>
</tr>
<tr>
<td>Day 14 to 18 CsA</td>
<td>4</td>
<td>31 d + 35 h ± 6 h</td>
<td>497 ± 115</td>
<td>5.5 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3 ± 0.3</td>
<td>55.8 ± 2.9</td>
</tr>
<tr>
<td>Day 20 to 24 CsA</td>
<td>4</td>
<td>31 d + 16 h ± 12 h</td>
<td>111 ± 4&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.0 ± 1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.8 ± 1.0&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35.0 ± 3.3</td>
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<sup>a</sup> Cremophor was administered from day 14 post coitum to day 18 post coitum. Gestation duration, maternal weight changes during the treatment and during the second half of gestation, and the total numbers of newborns per litter (alive or stillborn) were assessed. Values are means ± SEM.

<sup>b</sup> For mean birthweight determinations, only alive pups were taken into account.

<sup>c</sup> P < 0.05, compared with control or Cremophor group.

<sup>d</sup> P < 0.05, compared with the other CsA group.

<sup>e</sup> P < 0.01, compared with the other CsA group.
day 14 to 18 CsA groups exhibited similar weights (2.29 ± 0.08 g, n = 18; 2.20 ± 0.08 g, n = 19; and 2.25 ± 0.08 g, n = 14, respectively). No difference in placental weights was observed (control, 1.33 ± 0.05 g; Cremophor, 1.33 ± 0.11 g; day 14 to 18 CsA, 1.32 ± 0.03 g). Therefore, maternal CsA treatment between day 14 and day 18 of gestation had no deleterious effect on fetal growth, as confirmed by normal birthweights (Table 1). However, a trend toward a higher percentage of resorbed fetuses per litter after this treatment was observed; the incidence reached 18%, compared with 5% observed for control animals. These data are consistent with the findings. At high magnification, tubulopathy was observed in animals from all groups (Figure 1, A to D). Only pups from the day 20 to 24 CsA group displayed several pathologic findings. At high magnification, tubulopathy was observed in

<table>
<thead>
<tr>
<th>Table 2. Morphologic and functional data at birth</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Cremophor</td>
</tr>
<tr>
<td>Day 14 to 18 CsA</td>
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<td>Day 20 to 24 CsA</td>
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* p < 0.01, compared with control or Cremophor group.
* p < 0.05, compared with control or Cremophor group.
* p < 0.005, compared with Cremophor group.

In Utero CsA Exposure and Renal Development at Birth

Morphologic data at birth are summarized in Table 2. No difference in kidney weights among pups in the control and day 14 to 18 CsA groups was observed. Only pups in the day 20 to 24 CsA group exhibited reduced kidney weights, consistent with their decreased birthweights. However, similar kidney weight/body weight ratios were measured for all animals. In rabbits, nephrogenesis is initiated near embryonic day 15 and lasts nearly 1 mo and therefore is not completed before the third postnatal week (24). At birth, all stages of nephron development are recapitulated and can be observed in cross-sections. As illustrated in Figure 1, several generations of nephrons have already been induced. At the periphery, the nephrogenic zone contains the nephrogenic mesenchyme, the tips of the growing ureteric tree, and the nephron anlagen up to the renal vesicle stage. The same anatomic organization was observed in animals from all groups (Figure 1, A to D). Only pups from the day 20 to 24 CsA group displayed reduced cortical width. The nephrogenic zone was of the same thickness in all pups (Table 2). However, the neonatal kidneys from pups with CsA-treated mothers displayed several pathologic findings. At high magnification, tubulopathy was observed in

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the most mature proximal tubules, near the cortex-medulla boundary (Figure 1F). Tubular segments were filled with numerous vacuoles within the basal area of epithelial cells. Some areas of moderate interstitial fibrosis were also observed in the deep cortex (Figure 1F), a region in which the first nephrons appeared, concomitant with CsA exposure. Tubular vacuolization was observed in cortical collecting tubules up to the tips of the ureteric bud, as well as within the tubular part of S-shaped bodies (Figure 1E). This was more severe in pups exposed to CsA in utero, reduced densities of nephron anlagen (renal vesicles and S-shaped bodies) were observed (C and D). At higher magnification, cellular lesions such as tubular swelling (arrows) and interstitial fibrosis (arrowheads) were present in the immature renal parenchyma of CsA-exposed pups (E and F). In two newborns, very few nephrons, with tubular lesions (arrows) and enormous glomeruli, were observed (G). In those pups, the nephrogenic zone was absent. Photographs were taken from silver-stained sections. Bars represent 100 μm.

Figure 1. Histologic features of the renal cortex in newborn rabbits. Pups were born to untreated mothers (A), mothers treated with Cremophor (B) or cyclosporin A (CsA) (C, E, F, and G) on gestational days 14 to 18, or mothers treated with CsA on days 20 to 24 (D). At low magnification, specimens of renal cortex from the control (A) and Cremophor (B) groups illustrate the centrifugal pattern of renal organogenesis, with the nephrogenic zone lying beneath the renal capsule. In pups exposed to CsA in utero, reduced densities of nephron anlagen (renal vesicles and S-shaped bodies) were observed (C and D). At higher magnification, cellular lesions such as tubular swelling (arrows) and interstitial fibrosis (arrowheads) were present in the immature renal parenchyma of CsA-exposed pups (E and F). In two newborns, very few nephrons, with tubular lesions (arrows) and enormous glomeruli, were observed (G). In those pups, the nephrogenic zone was absent. Photographs were taken from silver-stained sections. Bars represent 100 μm.

In Utero CsA Exposure and Renal Morphologic Features in Young Rabbits

Further examinations were performed with 1-mo-old pups, in which nephrogenesis was fully completed. Morphologic data are presented in Table 3. At that age, body weights were not different among the groups. The slightly increased weight observed for the day 14 to 18 CsA group was likely secondary to the reduced number of feeding pups during the weaning period. For the same reason, pups in the day 20 to 24 CsA group achieved normal weights. The kidney weight in young rabbits exposed to CsA in utero on days 14 to 18 of gestation was significantly greater than values for the control groups, but the kidney weight/body weight ratios were not different among the groups. However, in all pups born to CsA-treated mothers, the numbers of nephrons were significantly reduced, compared with control animals. The numbers were reduced by one-fourth and one-third for pups in the day 14 to 18 CsA and day 20 to 24 CsA groups, respectively. Assessment of the number of

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Table 3. Morphologic and functional data for 1-mo-old animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g)</th>
<th>Kidney Weight (g)</th>
<th>Kidney Weight/Body Weight (%)</th>
<th>Cortical Width (μm)</th>
<th>Superficial Glomerular Volume (mm$^3 \times 10^6$)</th>
<th>Deep Juxtamedullary Glomerular Volume (mm$^3 \times 10^6$)</th>
<th>Creatinine Level (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>563 ± 22</td>
<td>2.966 ± 0.11</td>
<td>0.91 ± 0.02</td>
<td>0.63 ± 0.11</td>
<td>2.33 ± 0.12</td>
<td>2.00 ± 0.11</td>
<td>44.8 ± 1.5</td>
</tr>
<tr>
<td>Cremophor 14</td>
<td>560 ± 23</td>
<td>2.74 ± 0.11</td>
<td>0.98 ± 0.03</td>
<td>2.41 ± 0.20</td>
<td>1.93 ± 0.03</td>
<td>2.15 ± 0.11</td>
<td>42.5 ± 1.2</td>
</tr>
<tr>
<td>Day 14 to 18 Csa</td>
<td>630 ± 53</td>
<td>3.21 ± 0.09</td>
<td>1.03 ± 0.09</td>
<td>2.49 ± 0.20</td>
<td>1.49 ± 0.03</td>
<td>2.00 ± 0.07</td>
<td>47.5 ± 1.3</td>
</tr>
<tr>
<td>Day 20 to 24 Csa</td>
<td>581 ± 37</td>
<td>2.85 ± 0.18</td>
<td>1.00 ± 0.07</td>
<td>1.34 ± 0.12</td>
<td>1.34 ± 0.12</td>
<td>2.00 ± 0.07</td>
<td>48.6 ± 1.4</td>
</tr>
</tbody>
</table>

* P < 0.005, compared with control or Cremophor group. ** P < 0.001, compared with control or Cremophor group. 

**Discussion**

In this study, the fetal nephrotoxicity of CsA was investigated. The dose used and the maternal blood concentrations of CsA were identical to those reported for female subjects of childbearing age and transplant-treated pregnant women undergoing CsA therapy. The nephron deficit we observed, in the absence of impaired fetal growth, for the day 14 to 18 CsA group. As expected, data for the control and Cremophor groups never differed. In addition, no gender differences with respect to any of the parameters were noted. Light-microscopic examinations and morphologic studies revealed histologic damage and provided data on the origin of the nephron deficit (Figure 2). Cross-sections of kidneys exposed to CsA in utero revealed several foci of tubular dilation surrounded by interstitial inflammation, in the cortex (Figure 2B) and the outer medulla (data not shown). As illustrated in Figure 2, the extent of these lesions was greater in some animals that also exhibited sclerotic glomeruli. As evident at higher magnification, early signs of tubular and glomerular sclerosis, such as thickened tubular membranes and glomerular tuft adhesion to Bowman’s capsule, respectively, were present (Figure 2, D and E). These lesions were never observed in control rabbits at 1 mo of age (Figure 2C). Significant correlations between the final numbers of nephrons and the birthweights were observed for the control and Cremophor groups ($r = 0.713, n = 14, P < 0.01$; and $r = 0.789, n = 14, P < 0.001$, respectively), with the same slopes. Data were pooled and compared with those for the day 14 to 18 CsA group (Figure 3A). Nephron numbers were also correlated with birthweights in that group ($r = 0.770, n = 12, P < 0.01$). In contrast, no such relationship was observed for the day 20 to 24 CsA group. Measurements of the positions of the glomeruli within the cortex provided additional information regarding the defect of nephrogenesis with CsA exposure. First, the renal cortex was significantly thinner in pups exposed to CsA in utero, compared with control animals (Table 3), and this was true for both CsA groups. Second, the histogram of the distribution of glomeruli within the cortex, as presented in Figure 3B, indicated that the nephron deficit was not uniformly distributed within the cortex. In the deep cortex, where the first nephrons were induced at the onset of CsA exposure, the nephron population was reduced by $>50\%$, compared with control animals. In the superficial layers of the cortex, the number of nephrons was significantly reduced by approximately 30% in the CsA group. Consistent with these findings, the glomerular volumes of both superficial and deep nephrons were significantly enlarged (Table 3). Despite the reduced nephron mass, 1-mo-old pups born to CsA-treated mothers presented no signs of overt renal functional changes. Indeed, the plasma creatinine levels in that group were similar compared with control animals and Cremophor groups. These findings support the concept that renal development is more sensitive to CsA exposure during the earlier stages of nephron induction.
group demonstrated that *in vivo* CsA treatment had deleterious effects on the newly formed metanephros. In a recent meta-analysis of data on CsA therapy during pregnancy, it was suggested that CsA did not seem to be a major human teratogen, although it might be associated with increased rates of prematurity (4,7). However, that analysis revealed a trend toward an increased risk of congenital malformations among infants born to transplant recipients who had received CsA throughout their pregnancies. Our data indicate that a short *in utero* exposure to CsA is harmful for embryonic kidneys, even at therapeutic doses. Administration of halved CsA concentrations to the mothers still induced nephron mass reductions in the progeny, although to a lesser extent (data not shown). No adverse effect of Cremophor was noted, indicating that CsA alone is responsible for the impaired nephrogenesis.

**CsA Therapy and Pregnancy Outcomes**

The effects of a short CsA administration were investigated at two different periods of pregnancy, one corresponding to the onset of renal organogenesis, when no nephrons have been induced, and the other concomitant with the beginning of renal function in the fetus. In both cases, CsA proved to be toxic to the developing kidneys, leading to deficits in the numbers of nephrons generated. In the day 14 to 18 CsA group, fetal growth was not impaired despite a transient weight loss by the treated mothers. In the day 20 to 24 CsA group, the does suffered significantly during the second half of gestation and their weight gain was negligible. This indicated that physiologic changes occurring during the last one-third of gestation in rabbits were very sensitive to CsA (33), whereas CsA administration was demonstrated to have no effect on food intake and total weight gain among nonpregnant rabbits, even at twice the dose (23,34). This factor produced fetal growth retardation in the day 20 to 24 CsA group only. Reduced birth weight by itself is known to be linked with nephron deficits in rats, mice, rabbits, sheep, and human subjects (28,35), as verified here for rabbits. However, this relationship was lost in the day 20 to 24 CsA group, which was likely attributable to the cumulative

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**Figure 2.** Light-microscopic examinations of the kidneys of pups born to mothers treated with Cremophor (A and C) or CsA (B, D, and E) on gestational days 14 to 18. Examinations of the cortex revealed several foci of tubular dilation and retracted glomerular tufts (arrows) in pups exposed to CsA *in utero* (B). At high magnification, thickening of the tubular membrane (arrows) and of Bowman’s capsule (arrowheads) was observed (D). Early signs of glomerular sclerosis, such as adhesion of the glomerular tuft to Bowman’s capsule (arrows), were present in the upper glomerulus (E). Photographs were taken from periodic acid-Schiff-stained sections. Bars represent 200 μm in A and B and 50 μm in C to E.
the first 150 measurements were performed. No glomeruli were observed within the number of pups analyzed in the CsA group. A total of 2228 control group distribution of glomeruli was calculated for 12 pups, to control and 14 Cremophor-treated animals studied at that age. The renal capsule. Data for the control group were recorded for the 14 pups died. Of the 20 day 14 to 18 CsA-treated rabbits included, indicating that 16% of the newborn pups from the day 20 to 24 CsA group. The numbers of glomeruli were examined in cortical layers of 200-μm thickness, starting from the renal capsule. Data for the control group were recorded for the 14 control and 14 Cremophor-treated animals studied at that age. The control group distribution of glomeruli was calculated for 12 pups, to match the number of pups analyzed in the CsA group. A total of 2228 measurements were performed. No glomeruli were observed within the first 150 μm below the renal capsule.

effects of CsA and severe growth retardation. The duration of pregnancy was not altered with either period of CsA treatment, but the numbers of resorbed fetuses and stillborn animals were greater with CsA exposure, confirming clinical data (6,7,36). The deaths within the first 2 wk after birth were not expected. The findings were confirmed with additional CsA-treated does not included in this study, indicating that 16% of the newborn pups died. Of the 20 day 14 to 18 CsA-treated rabbits included in other studies, one doe delivered malformed pups. Those pups exhibited shortened lower limbs and skeletal alterations. Even if rare, such developmental abnormalities might have occurred among human subjects after in utero exposure to CsA. At least two cases have been reported; one infant exposed to CsA in utero displayed leg and foot malformations (37) and another child was born with multiple anomalies, including bilateral clubfeet (7).

CsA and the Developing Kidney

The concentration of CsA used in this study is clinically relevant, as indicated by the CsA residual blood concentrations measured in pregnant rabbits, which were identical to clinical data (31–33). During CsA therapy after transplantation or in autoimmune diseases, CsA dosages must be adjusted to maintain residual blood trough levels in the range of 200 to 400 ng/ml (5,38), as observed here for both CsA groups. At the beginning of the day 14 to 18 CsA exposure, the metanephros was composed of a ureteric bud that had just started to branch within the surrounding metanephrogenic mesenchyme. During the 5-day treatment, the ureteric bud branched extensively and the first nephron anlagen emerged within the metanephric blastema, in contact with ureteric bud ends. Probably low levels of CsA reached the embryonic kidney, but they were sufficient to significantly interfere with nephrogenesis. It must be noted that, unless the nephron number was determined and the renal architecture was carefully analyzed with light microscopy, no sign of impaired renal development was apparent. To our surprise, the nephron deficit not only was localized in the deep cortical zone that contained the nephrons formed first but also was observed in the superficial layers of the cortex, an area where the last generations of nephrons appeared weeks after the CsA treatment ceased. This clearly indicates that disturbance of the early steps in renal organogenesis may impair the last generations of nephrons. The adverse effects of CsA on the early stages of kidney development likely indicate a direct effect of CsA on nephron induction, because the kidney is a major site for drug accumulation (39,40), as already described for other drugs administered in utero (41,42). Numerous mechanisms of CsA nephrotoxicity toward the developing kidney can be proposed. CsA can enhance the synthesis of type I and III collagens (43–45), which could interfere with the necessary shift in matrix composition from an interstitial mesenchymal type to the differentiated epithelial type (46) required for nephron formation. The impaired nephrogenesis at the end of renal organogenesis, which occurred postnatally in rabbits, is likely to be secondary to reduced proliferation of the metanephrogenic mesenchyme at the onset of CsA exposure, because of either increased rates of apoptosis or stimulation of TGF-β expression. Indeed, CsA can accelerate apoptosis (47) and promote TGF-β expression (48). Further investigations are required to identify the molecular origin of the CsA-induced nephron deficit.

In conclusion, harmlessness of CsA toward the embryonic kidney is very unlikely. The induction of a nephron deficit in vivo after in utero exposure to CsA raises the possibility of similar effects among human subjects. Of course, we use caution in extrapolating experimental findings to clinical situations. However, it must be remembered that infants born to CsA-treated mothers generally exhibit growth retardation and therefore already have a reduced nephron mass. A more severe inborn nephron deficit, as we observed in pups from the day 20

Figure 3. (A) Relationship between the number of glomeruli and birthweight in rabbits. ○, control groups. Data were pooled for the two control groups because they displayed the same relationship. ●, day 14 to 18 CsA group. (B) Distribution of glomeruli within the renal cortex in 1-mo-old rabbits. □, control group; ■, day 14 to 18 CsA group; ◊, day 20 to 24 CsA group. The numbers of glomeruli were analyzed in cortical layers of 200-μm thickness, starting from the renal capsule. Data for the control group were recorded for the 14 control and 14 Cremophor-treated animals studied at that age. The control group distribution of glomeruli was calculated for 12 pups, to match the number of pups analyzed in the CsA group. A total of 2228 measurements were performed. No glomeruli were observed within the first 150 μm below the renal capsule.
to 24 CsA group, compared with pups from the day 14 to 18 CsA group, is thus to be feared. This may increase the risk of long-term glomerular damage and impaired renal function early in adulthood.

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