Children’s toxicology from bench to bed - Drug-induced Renal Injury (4): Effects of nephrotoxic compounds on fetal and developing kidney

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ABSTRACT — The kidney is a major target of toxic compounds. Susceptibility of the kidney to toxic effects of compounds is attributed to the unique morphologic and physiologic features of this organ. Renal development and maturation begins in the fetal period and continuous throughout the postnatal period. The effects of noxious compounds on the kidney are influenced by the state of renal development and maturation. Therefore, to assess the renal toxic potential of a compound in fetuses and infants, the development and maturation of the kidney should be taken into account. Renal development and maturation involves both morphological and functional aspects. Renal development begins in the fetus and proceeds to partial functional capacity before birth. After birth, the kidney continues morphologic and functional maturation during the postnatal period of infancy. The fetal or infant kidney is vulnerable to the renal toxicity of certain compounds due to its morphological and functional characteristics. On the other hand, the infant kidney is sometimes more tolerant to nephrotoxic compounds compared to the adult kidney because of its immature glomerular filtration, concentrating capability and active transportation. Design and interpretation of studies in fetuses and infants regarding renal toxicity should include careful consideration of the state of renal development and maturation and of the mechanisms of renal injury.

Key words: Renal toxicity, Nephrotoxicity, Renal development and maturation, Fetus, Infant, Juvenile animal

INTRODUCTION

The kidney is a major target of noxious compounds. Susceptibility of the kidney to toxic effects of noxious compounds is attributed to the unique morphologic and physiologic features of this organ. The kidney receives a high amount (about 20-25%) of the resting cardiac output. The processes involved in forming concentrated urine serve to concentrate potential toxicants in the tubular fluid. The tubule cells have several types of transporters, such as organic anion transporters, organic cation transporters, p-glycoproteins and oligopeptide transporters. Consequently, any compound will be delivered to the tubular cells by active transport pathway in addition to passive diffusion. Cytochrome P450 activity in tubular cells is also a contributing factor to the enhanced susceptibility of the kidney to toxic injury. In addition, tubular cells appear to be more susceptible to ischemic injury.

Finally, progressive concentration of urine also results in intraluminal precipitation of relatively insoluble compounds.

The unique morphologic and physiologic features of the kidney are considered when assessing the renal toxic potential of a compound. Renal development and maturation begins in the fetal period and continues through the postnatal period of infancy, the state of which influences the effects of noxious compounds on the kidney. Therefore, for assessment of the renal toxic potential of a compound in fetuses and infants, not only the unique morphologic and physiologic features of the kidney but also the development and maturation of the kidney should be taken into account. In this paper, renal development and maturation is briefly described. Several non-clinical study results concerning the effects of noxious compounds on the developing and maturing kidney are also presented as the basis for future assessment of the renal toxic potential.
of a compound in fetuses and infants.

Renal development and maturation
Considering renal development, both morphological and functional changes should be considered. Morphological renal development begins in the fetal stage proceeding acquirement of some of the functional capacity before birth. After birth, the kidney continues morphologic and functional maturation throughout the postnatal period of infancy. A brief description of morphologic development and maturation is presented herein, followed by a brief description of functional development and maturation with emphasis on the major renal functions of glomerular filtration, concentrating capability and active transport by transporters.

Morphology
The development of the vertebrate kidney consists of 3 stages: pronephros, mesonephros and metanephros. The metanephros is the adult kidney and requires nephrogenesis for development. The branching uretic epithelia interact with loose metanephric mesenchyme, resulting in condensation of the mesenchyme. This process is followed by the infolding of the primitive glomerular epithelium to form comma- and S-shaped bodies. Elongation of the proximal and distal tubular elements subsequently occurs along with further infolding of the glomerular epithelium and vascular structures to form the mature glomerular capillary network (Zoetis and Hurtt, 2003). Fig. 1 shows the histological nature of the metanephros in mouse. At day 14.5 of gestation, undifferentiated cell clusters and mesenchymal cells with tubular, aggregated or loose form with multiple mitotic figures were observed in metanephros. Immature tubules also formed. However, the cortex and medulla are undistinguishable.

Mammalian kidneys follow a similar developmental pathway among species; however, the time frame with regard to birth varies (Table 1). In human and mouse, Table 1. Onset of metanephros development and timing of nephrogenesis completion

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total gestation period (days)</td>
<td>267</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Onset of metanephros development</td>
<td>35-37 days gestation</td>
<td>11 days gestation</td>
<td>12.5 days gestation</td>
</tr>
<tr>
<td>Timing of nephrogenesis completion</td>
<td>35 weeks gestation</td>
<td>before birth</td>
<td>postnatal week 4-6</td>
</tr>
</tbody>
</table>

Data from Zoetis and Hurtt (2003)
nephrogenesis begins during gestation and is completed at birth. In rat, nephrogenesis occurs prenatally, continues at a rapid pace between days 0 and 8 after birth and is virtually complete by 4 to 6 weeks of age (Kavlock and Gray, 1982; Zoets and Hurtt, 2003).

Nephrons complete their differentiation during the early postnatal/infancy stage and continue to increase in size until adulthood. In rats, the absolute weight of kidneys quickly increases from birth to 10 weeks old. The postnatal morphological maturation has been described as centrifugal, that is, maturation proceeds from the inner toward the outer cortex. The first nephrons form in the juxtamedullary area and the last to form are the superficial ones. Historically, immature kidneys of infants have small, densely cellular glomeruli and the cortical tubules are small with crowded nuclei, limited amounts of cytoplasm and inconspicuous tubular lumens. In the outer cortex are the histologically undifferentiated or immature cells (Gibson, 1976).

**Function**

A well-recognized feature of the newborn kidney is its relatively low level of functioning. Compared to the adult, the infant has less glomerular filtration and renal blood flow. The renal functions continue to increase and reach adult levels after birth. In rats, the glomerular filtration rate (GFR) sharply increases during the first 6 weeks of postnatal life (Horster, 1977). The newborn infant is incapable of excreting concentrated urine at birth but the concentrating ability develops dramatically with age. Neonatal rats also do not excrete concentrated urine at birth but this function reaches maturity by 2 to 3 weeks of age. (Kavlock and Gray, 1982; Kleinman, 1982).

Many kinds of transporters are expressed in the proximal tubular cells and the levels of expression change with maturation. For example, P-glycoprotein and multidrug resistance-related proteins (Mdr and Mrp), which are members of the ATP-binding cassette superfamily of transporters, are located on the cellular membrane and actively pump a wide range of substrates out of the cell. The mRNA expression levels of Mdr1a, Mdr1b and Mrp2 transcripts are relatively low in fetal and neonatal rats but, after birth, increase dramatically, and reach adult levels at 3 to 5 weeks old. However, the expression level of Mrp1 transcript does not show any modulation (Rosati et al., 2003).

Organic anion transporter 1 (Oat1) is located on the basolateral membrane of the middle proximal tubule and mediates the accumulative transport of organic anions from the peritubular fluid. The level of expression of mRNA and protein of Oat1 is relatively low at the fetal stage. Immediately after birth, Oat1 expression increases remarkably, followed by a decrease at day 6 and the immature excretory capacity of the proximal tubules of the neonatal kidney can be attributed to the low expression level (Nakajima et al., 2000).

Organic cation transporters (Octs) play an important role in transporting cationic xenobiotics across biological membranes. Oct1, 2 and 3 are referred to as the carnitine/organic cation transporters and have a low degree of similarity to the Oct1-3 genes. The mRNA expression of Oct1 in mice is very low at the fetal stage. After birth, the expression gradually increases during the first 3 weeks, reaching a plateau around day 22 after birth. Oct2 mRNA levels in mice are also essentially undetectable 2 days before birth but are approximately one-fourth the adult level at birth. Oct1 and Oct2 mRNA expression in mouse gradually increase from 2 days before birth until about 2 weeks of age, when the levels reach a plateau (Alnouti et al., 2006).

In addition, the antioxidative potential and the generation of reactive oxygen species also changes in the rat kidney during the early postnatal/infancy period. Superoxide anion generation is low in the kidney during the early postnatal period, whereas hydrogen peroxide levels remain unaltered during maturation. Of the enzymic antioxidants, the level of glutathione peroxidase in the maturing kidney is high compared to adults. However, superoxide dismutase, catalase and glutathione reductase are significantly low from early postnatal up to 16 days of age and attain the maturational level by 4 weeks of age. The levels of antioxidant enzymes in the maturing kidney are low but subsequently increase with age (Gupta et al., 1999).

**Effects of noxious compounds on developing and maturing kidney**

The fetal or infant kidney is vulnerable to the renal toxicity of certain compounds due to its morphological and functional characteristics. On the other hand, the infant kidney is sometimes more tolerant to nephrotoxic compounds compared to the adult kidney because of its immature glomerular filtration, concentrating ability and active transportation. Described herein are the results from several nonclinical studies on the effects of noxious compounds on the immature kidney.

*In utero exposure of cyclosporin A to fetal rabbits*

Renal toxicity of cyclosporin A (CsA) in the embryonic kidney was evaluated using pregnant rabbits (Tendron et al., 2003). Female rabbits were subjected to daily injec-
tions of 10 mg/kg/day CsA subcutaneously for 5 days from days 14 to 18 of gestation or from days 20 to 24 of gestation. At 1-month-old, infants were necropsied and the kidneys histopathologically evaluated. Histologically, nephron density showed a decrease with nephron numbers reduced by 25% and 33% at days 14 to 18 and 20 to 24 of CsA treatment, respectively, indicating that CsA administration during pregnancy suppresses the generation of nephrons.

**In utero exposure of gentamicin to fetal rats**

In utero gentamicin-induced nephrotoxicity in rat neonates was studied using pregnant rats (Mallié *et al.*, 1988). Fifty or 75 mg/kg of gentamicin was administered by subcutaneous injection on days 7 to 11 and on days 14 to 18. Functional effects were evaluated in rat neonates on day 1 after birth. In neonates treated with gentamicin, plasma creatinine was higher with a lower creatinine clearance and body weight decreased. Gentamicin concentrations revealed that gentamicin was present in the neonatal kidney of the treated groups and the morphological changes in differentiated nephrons in the proximal tubules were comparable to those observed in adults. It was concluded that treating pregnant mothers with gentamicin affects the developing kidney.

**Exposure of gentamicin to infant rats**

A multiage rat model was used to identify potential age-related differences of renal injury following exposure to gentamicin (Espandiari *et al.*, 2007). In this study, 10-, 25-, 40-, and 80-day-old rats received a subcutaneous injection of gentamicin at a dose level of 50 or 100 mg/kg once a day for 6 or 14 days. The maximum tolerated dose was lower in 10-day-old rats than for other ages (none survived 11 days of treatment). Eighty-day-old rats given the highest dose showed a diminished rate of growth and an increase in serum creatinine, blood urea nitrogen, urinary kidney injury molecule-1, and pathological changes. Ten- and 40-day-old rats given 100 mg/kg/day of gentamicin for 6 or 14 days also had increased levels of serum blood urea nitrogen and creatinine and renal pathological changes whereas only mild renal changes were found in 25-day-old rats. These findings indicate that clear age-related differences exist in gentamicin-induced renal toxicities and that the neonatal kidney (25-day-old immature kidney) is more tolerant to the nephrotoxic effects of gentamicin.

**Exposure of p-cumylphenol to infant rats**

Renal toxicity of p-cumylphenol (PCP) against the immature kidney and its critical period were evaluated in an infant rat study (Nakazawa *et al.*, 2008). The rats were given daily oral administration of PCP at the dose level of 300 mg/kg for 14 days starting at the age of 2-, 3-, 4- or 5-weeks-old, with necropsy at the end of the administration period. In the 2-week-old group, significant dilatation of the collecting ducts, mainly in the outer medulla, was observed (Fig. 2). The same change was observed in the 3-week-old group but at lower levels and no changes were observed in the 4- and 5-week-old groups. The results clearly indicate that PCP induces cystic changes in the kidney and that the critical period was from the day of birth to 3 weeks old and that the pathogenesis of PCP is closely related to the maturation process of the kidney.

In conclusion, the effects of compounds on the fetal and infant kidney are influenced by the state of renal development and maturation. Morphological renal development begins at the fetal stage and proceeds to acquire some of its functional capacity before birth. After birth, the kidney continues morphologic and functional maturation during the postnatal/infancy period. The fetal and infant kidney present vulnerabilities and tolerance to nephrotoxicity unlike the adult kidney. The design and interpretation of renal toxicity studies in mammalian fetuses and infants requires careful consideration of the state of renal development and maturation and of the mechanisms of renal injury.

![Kidney section of rat treated with PCP for 14 days starting at the age of 2-weeks-old. Dilatation of the tubules, mainly in the outer medulla, is observed. Hematoxylin and eosin staining. Data from Nakazawa *et al.* (2008).](image-url)
ACKNOWLEDGEMENTS

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REFERENCES
