Indomethacin administered early in the postnatal period results in reduced glomerular number in the adult rat

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Indomethacin administered early in the postnatal period results in reduced glomerular number in the adult rat. Am J Physiol Renal Physiol 307: F1105–F1110, 2014. First published September 3, 2014; doi:10.1152/ajprenal.00328.2014. —Indomethacin and ibuprofen are administered to close a patent ductus arteriosus (PDA) during active glomerulogenesis. Light and electron microscopic glomerular changes with no change in glomerular number were seen following indomethacin and ibuprofen treatment during glomerulogenesis at 14 days after birth in a neonatal rat model. This present study aimed to determine whether longstanding renal structural changes are present at 30 days and 6 mo (equivalent to human adulthood). Rat pups were administered indomethacin or ibuprofen antenatally on days 18–20 (0.5 mg·kg−1·dose−1 indomethacin; 10 mg·kg−1·dose−1 ibuprofen) or postnatally intraperitoneally from day 1 to 3 or day 1 to 5 (0.2 mg·kg−1·dose−1 indomethacin; 10 mg·kg−1·dose−1 ibuprofen). Control groups received no treatment or normal saline intraperitoneally. Pups were killed at 30 days of age and 6 mo of age. Tissue blocks from right kidneys were prepared for light and electron microscopic examination, while total glomerular number was determined in left kidney. Light microscopic examination with both antenatal and postnatal administration of indomethacin, ibuprofen, and gentamicin to neonatal rats has shown extensive glomerular injury on electron microscopic examination with both antenatal and postnatal administration at day 14 (32). These findings are very different from those found in both adult rat and human renal biopsy findings, which show a predominantly tubular injury (4, 23, 33, 46, 47). Indomethacin was shown to have more significant suppressive effects than ibuprofen on renal COX-2 and vaso-dilator prostanoids in a neonatal rat model (26). Ibufrofen has been shown in premature neonates to result in a low glomerular filtration rate for a month following administration (60). A recent study has shown increased numbers of podocytes in the urine of preterm neonates receiving indomethacin, suggesting drug-induced glomerular injury at the time of ongoing glomerulogenesis (31).

Indomethacin is complete at 34–36 wk gestation. The metanephros begins development at 5 wk gestation, with vesicular glomerular development occurring at 18 wk gestation. Glomerular tubular development occurs from 24 wk gestation and is complete at 36 wk (1, 27, 42). In the rat, glomerulogenesis continues after birth for ~1 wk (36). Accelerated nephrogenesis and abnormal glomerular morphology have been noted in the premature human neonatal kidney (52). A decreased number of glomeruli has been implicated in the development of hypertension and subsequent cardiovascular disease in animal models and human studies (5, 7, 10, 28–30, 37, 49, 61). The implications for long-term renal and cardiovascular health in extremely premature survivors are important.

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Table 1. Control and experimental groups route, timing, and dose of medication

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>Timing</th>
<th>Drug and Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>1 B</td>
<td>Intraperitoneal</td>
<td>Postnatal days 1–5</td>
<td>Normal saline</td>
</tr>
<tr>
<td>2</td>
<td>Oral</td>
<td>Antenatal days 18–20</td>
<td>Indomethacin 0.5 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>Oral</td>
<td>Antenatal days 18–20</td>
<td>Ibuprofen 10 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>Intraperitoneal</td>
<td>Postnatal days 1–3</td>
<td>Indomethacin 0.2 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>Intraperitoneal</td>
<td>Postnatal days 1–5</td>
<td>Indomethacin 0.2 mg/kg</td>
</tr>
<tr>
<td>6</td>
<td>Intraperitoneal</td>
<td>Postnatal days 1–3</td>
<td>Ibuprofen 10 mg/kg</td>
</tr>
<tr>
<td>7</td>
<td>Intraperitoneal</td>
<td>Postnatal days 1–5</td>
<td>Ibuprofen 10 mg/kg</td>
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</table>

At birth, the neonatal rat kidney is similar to that of a 24-wk gestation human fetus allowing administration of indomethacin and ibuprofen to the pregnant dam and the neonate at similar gestations to be administered to the human fetus and extremely premature infant. This study aimed to determine whether administration of antenatal and postnatal indomethacin and ibuprofen associates with prolonged glomerular changes at 30 days and 6 mo of age (equivalent to adulthood).

**METHODS**

Sprague-Dawley pregnant dams were obtained from the Australian National University animal house. The animal experiments were approved by the Australian National University Animal Research Ethics Committee and treatment and care of the animals conformed to the National Health and Medical Research Council of Australia Australia Code of Practice for the Care and Use of Animals for Scientific Purposes.

Control animals consisted of pregnant dams and their offspring having no drug treatment during pregnancy or their pups receiving intraperitoneal saline. Seven groups of experimental animals were established and the method of administration, timing, and dose of medication are detailed in Table 1. Animals were killed at 30 days and 6 mo of age. Antenatal indomethacin and ibuprofen were administered during the time period that would be equivalent to indomethacin use as a tocolytic. The doses administered were those of standard oral dosing for these medications. The postnatal doses given intraperitoneally are those that are used for intravenous administration in neonates. The length of timing of the doses was equivalent to two courses of indomethacin or ibuprofen which is a common clinical scenario. Rat litters had 12–14 pups per litter with one to two early deaths in each litter. Four male and female pups were included from each litter.

Immediately after the pups were killed, the abdominal and chest organs were exposed using a midline incision. A butterfly needle was inserted and clamped into the left ventricle and an incision was made. The right kidney was processed for embedding in glycolmethacrylate (Technovit 7100; Heraeus Kulzer), exhaustively sectioned at 15 μm, and stained with PAS. The same technician (LG) performed the glomerular structure and counting assessment and was blinded to treatment groups. An unbiased stereological technique was used known as the physical disector/fractionator combination, previously described (6, 7).

**RESULTS**

<table>
<thead>
<tr>
<th>Weight Difference from Day 1 g</th>
<th>Control</th>
<th>Antenatal Indomethacin</th>
<th>P Value</th>
<th>Antenatal Ibuprofen</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 (SD)</td>
<td>0.9 (0.6)</td>
<td>6.9 (0.6)</td>
<td>0.38</td>
<td>7.8 (0.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>Day 4</td>
<td>3.9 (0.8)</td>
<td>3.8 (0.5)</td>
<td>0.41</td>
<td>4.3 (0.7)</td>
<td>0.16</td>
</tr>
<tr>
<td>Day 7</td>
<td>10.1 (0.7)</td>
<td>9.9 (1.3)</td>
<td>0.62</td>
<td>9.8 (1.1)</td>
<td>0.43</td>
</tr>
<tr>
<td>Day 30</td>
<td>89.1 (5.3)</td>
<td>85.8 (8.2)</td>
<td>0.18</td>
<td>91.4 (6.9)</td>
<td>0.28</td>
</tr>
<tr>
<td>6 mo</td>
<td>437.0 (163.5)</td>
<td>459.0 (148.0)</td>
<td>0.78</td>
<td>461.3 (161.9)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Eight animals per group were killed at 30 days and 6 mo of age with equal numbers of males and females per group.
Weight change of rats from day 1 to day 4, day 7, day 30, and 6 mo. Rat pups receiving ibuprofen antenatally were heavier on day 1 than all other groups (P = 0.003). There was no significant difference with change in weight of treated pups from day 1 to day 4 compared with controls in all groups except for postnatal ibuprofen (P = 0.003). On day 7 rats receiving postnatal indomethacin days 1 to 3 and days 1 to 5 and ibuprofen days 1 to 5 had significantly higher weight change from day 1 (P = 0.006; P < 0.0001; P < 0.0001). On day 30 the weight change from day 1 in all animals receiving both antenatal and postnatal indomethacin and ibuprofen was significantly more than compared with controls (P < 0.0001); however, at 6 mo of age there were no differences in weight in any group compared with control (Table 2).

Weight of kidneys at day 30 and 6 mo. There were no differences in the weight of left kidneys of those rats receiving antenatal indomethacin or ibuprofen compared with controls at 30 days of age; however, left kidneys were heavier in all groups receiving postnatal indomethacin and ibuprofen at day 30 (P < 0.0001). This difference was no longer present at 6 mo of age (Table 3). These findings were similar for the right kidney (data not shown).

EM findings. At 30 days, the glomerular measurements were significantly smaller (measuring 90–120 μm) in those receiving postnatal indomethacin and ibuprofen (P = 0.001), but at 6 mo this difference was no longer evident (P = 0.08). At 30 days, there was no significant difference in foot process effacement (P = 0.4), but at 6 mo those receiving antenatal indomethacin and postnatal indomethacin had more foot process effacement (20–40%; P < 0.0001; Fig. 1). At 30 days, there was no difference in deposits present (P = 0.05), while at 6 mo there were significantly more deposits in those receiving antenatal indomethacin and ibuprofen and postnatal ibuprofen (P < 0.0001). At 30 days, there was more moderate to severe mesangial expansion in those receiving postnatal indomethacin and at 6 mo those who had received antenatal indomethacin, postnatal indomethacin, and postnatal ibuprofen (P < 0.0001). At 30 days, there were significantly more glomerular basement membrane humps, bumps, and splits after antenatal indomethacin and ibuprofen and postnatal indomethacin and ibuprofen had been received (P < 0.0001), and this persisted at 6 mo of age for those exposed to antenatal indomethacin and postnatal indomethacin and ibuprofen (Fig. 2). Chi square test was used for all analyses.

Light microscopy findings. Glomerular appearance on light microscopy was normal in all groups at 30 days and 6 mo of age. There was no difference in the degree of proximal tubular lumen differentiation at 30 days and at 6 mo of age in any group. Proximal tubular mitoses were more common in control rats at 30 days than treated animals (P = 0.01), but this difference was no longer present at 6 mo of age. There was no difference in the extent of proximal tubular epithelial cell drop out in any treated group at 30 days or 6 mo of age. There was no difference in medullary calcifications, medullary inflammation, medullary edema, or papillary edema in the treated groups at 30 days or 6 mo of age.

Glomerular number. There was no significant difference between gender of animals and number of glomeruli at 30 days or 6 mo of age in either control groups (P = 0.86 and 0.16, respectively) or those receiving indomethacin (P = 0.06 and 0.57, respectively) or ibuprofen (P = 0.33 and 0.77, respectively). There was no significant difference between total glomerular volume at 30 days and 6 mo between any of the groups (P = 0.4 and 0.5, respectively) and no significant

Table 3. Weight of left kidneys in grams at day 30 and 6 mo of age

<table>
<thead>
<tr>
<th>Weight Left Kidney g</th>
<th>Control</th>
<th>Antenatal Indomethacin P Value</th>
<th>Antenatal Ibuprofen P Value</th>
<th>Postnatal Indomethacin Days 1–5 P Value</th>
<th>Postnatal Indomethacin Days 1–5 P Value</th>
<th>Postnatal Ibuprofen Days 1–5 P Value</th>
<th>Postnatal Ibuprofen Days 1–5 P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 30 (SD)</td>
<td>0.66 (0.052)</td>
<td>0.64 (0.055) 0.52</td>
<td>0.65 (0.052) 0.89</td>
<td>0.82 (0.055) &lt;0.0001</td>
<td>0.75 (0.053) 0.001</td>
<td>0.88 (0.04) &lt;0.0001</td>
<td>0.78 (0.034) &lt;0.0001</td>
</tr>
<tr>
<td>6 mo (SD)</td>
<td>1.97 (0.75)</td>
<td>2.03 (0.7) 0.87</td>
<td>1.85 (0.64) 0.74</td>
<td>2.2 (0.63) 0.51</td>
<td>1.96 (0.7) 0.98</td>
<td>2.31 (0.83) &lt;0.0001</td>
<td>2.18 (0.7) 0.57</td>
</tr>
</tbody>
</table>

Fig. 1. Electron microscopy of glomeruli from control animal (left) and postnatal indomethacin (right) at 6 mo (adult equivalent) showing normal appearance (red arrows) and foot process effacement (green arrows).
difference between total corpuscle volume at 30 days and 6 mo

(P = 0.6 and 0.8, respectively). There was no significant
difference in the number of glomeruli at 30 days between those
rats receiving postnatal indomethacin or ibuprofen (P = 0.5).
There were no differences in glomerular number between
controls and those receiving indomethacin postnatally in each
individual group. However, when using a mixed generalized
linear model using a Tweedie model type with log link and the
Chi square statistics based on a likelihood ratio method, at 6
mo of age those rats having received postnatal indomethacin
had significantly less glomeruli compared with all other groups
(P = 0.003; Table 4). Response variables used were total
number of glomeruli at 30 days and at 6 mo. Predictors
included the experimental groups examined by main effects
and interaction effects with confounding factors sex, weight (g)
at day 1, and weight of kidney (g) at death.

**DISCUSSION**

This study shows that postnatal administration of indometh-
acin during the period of ongoing glomerulogenesis in the
neonatal rat results in decreased glomerular number in adult-
hood. The indomethacin-treated rats contain up to 12% fewer
nephrons than rats in other groups. Any differences in body
and kidney weights at 30 days were no longer evident at 6 mo.
The stage of glomerular development in this neonatal rat model
is consistent with that of a premature neonate at 24 –26 wk
gestation, at which time indomethacin or ibuprofen would be
given to treat a PDA. With reduced glomerular number as an
adult, there is increased risk for hypertension, cardiovascular
and renal disease (5, 7, 10, 28 –30, 37, 49, 61).

The difference in glomerular number at 6 mo compared with
30 days is consistent with damaged, dysfunctional glomeruli
resulting in glomerular loss in adulthood. The EM findings
showing smaller glomeruli at 30 days and ongoing foot process
effacement both at 30 days and 6 mo support this model.
Urinary podocytes have been found in preterm neonates re-
ceiving indomethacin supporting the EM findings at 14, 30
days, and 6 mo, of significant glomerular injury (31, 32).

Prematurity itself may be implicated in reduced glomerular
number. Histomorphological studies of postmortem renal tis-
sue have shown accelerated postnatal maturation, reduced
nephrogenic zone width, reduced renal vesicle formation, and
increased glomerular generations compared with age-matched
controls (52). Mice delivered 1–2 days preterm have a signif-
icant reduction in nephron number (51). Reduced nephrogenic
zone width has also been seen in the premature baboon model
treated with ibuprofen (49). Rodriguez et al. (48) reported a
reduction in the number of glomerular generations at the
completion of nephrogenesis, suggesting a nephron deficit.
Along with the changes in nephrogenic zone width and glomerular generations, up to 13% of glomeruli in the preterm kidney were noted to have enlarged Bowman’s space and shrunken glomerular tufts (52). With reduced capillarization and impaired vascular development/injury, these glomeruli may be nonfunctional (24, 25).

Indomethacin may often be prescribed at the same time as ampicillin and gentamicin in the first few days of life, which have both been shown to affect nephron development and function (13, 17–20, 35, 38, 40, 43, 57). Minimizing the coadministration of nephrotoxic medications during this active period of nephrogenesis in the preterm infant would now appear to be an important consideration in neonatal intensive care. Alternative options to indomethacin and ibuprofen should also be considered. Paracetamol has been reported in a number of case series and small randomized controlled trials to be as effective as oral ibuprofen to close a patent ductus arteriosus (14, 39, 44, 55). Paracetamol works as an inhibitor of the peroxidase component of the prostaglandin-H2 synthetase pathway, whereas indomethacin and ibuprofen work on the cyclooxygenase pathway.

The ductus arteriosus is kept patent in the fetus due to low partial pressure of oxygen and circulating or locally produced prostaglandins and nitric oxide. Inhibition of prostaglandin synthesis will result in closure of the duct and this is brought about by inhibition of cyclooxygenase (22). Inhibition of cyclooxygenase by selective COX-1 and COX-2 inhibitors, including indomethacin, has been shown in rats, mice, and lambs to constrict the patent ductus arteriosus (9, 12, 50, 56). This study used slightly higher doses of both indomethacin and ibuprofen compared with that in the clinical setting. Usual doses of indomethacin would be 0.2 mg/kg on the first day followed by 0.1 mg/kg daily for 5 days or 0.2 mg/kg 12 hourly for 3 doses and for ibuprofen 10 mg/kg for one dose followed by 5 mg/kg for 2 daily doses or 20 mg/kg for one dose followed by 10 mg/kg for 2 daily doses. However, the doses used in this animal study are relatively comparable and neonates may receive two courses of either medication to try and successfully close a PDA, and as such the doses used in this animal study are similar to the additive nephrotoxic exposure to these medications neonates would receive.

In conclusion, early postnatal administration of indomethacin results in a reduced glomerular number in the adult rat. This effect was not seen with ibuprofen. This study will not be replicable in the human preterm neonate, and as such the use of indomethacin for a PDA should be used judiciously, and perhaps ibuprofen (oral or intravenously) should be considered the first line treatment for a PDA until potential other treatments, such as paracetamol, become available.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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TRANSLATIONAL PHYSIOLOGY

Reduced glomeruli following postnatal indomethacin

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