Developmental expression of renal organic anion transporters in rat kidney and its effect on renal secretion of phenolsulfonphthalein

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Nomura M, Motohashi H, Sekine H, Katsura T, Inui K. Developmental expression of renal organic anion transporters in rat kidney and its effect on renal secretion of phenolsulfonphthalein. Am J Physiol Renal Physiol 302: F1640–F1649, 2012. First published March 14, 2012; doi:10.1152/ajprenal.00525.2011.—Organic anion transporters (OAT1 and OAT3) and multidrug resistance-associated proteins (MRP2 and MRP4) play important roles in anionic drug secretion in renal proximal tubules. Changes in the expression of such transporters are considered to affect the tubular secretion of anionic drugs. The purpose of this study was to elucidate the developmental changes in the expression of OAT1, OAT3, MRP2, and MRP4 and their effects on the tubular secretion of drugs. The mRNA level of each transporter was measured by real-time PCR, and the protein expression was evaluated by Western blotting and immunohistochemical analysis. In addition, the tubular secretion of phenolsulfonphthalein (PSP) in infant (postnatal day 14) and adult rats was estimated based on in vivo clearance study. The protein expression of organic anion transporters were very low at postnatal day 0 and gradually increased with age. In postnatal day 14 rats, the expression of OAT1 and OAT3 seemed to be at almost mature levels, while MRP2 and MRP4 seemed to be at immature levels. Immunohistochemical analysis in the kidney of postnatal day 0 rats revealed OATs on the basolateral membrane and MRPs on the brush-border membrane. At postnatal day 0, the distribution of these transporters was restricted to the inner cortical region, while after postnatal day 14, it was identical to that in adult kidney. An in vivo clearance study revealed that the tubular secretion of PSP was significantly lower in postnatal day 14 rats than adult rats. These results indicate that age-dependent changes in organic anion transporter expression affect the tubular secretion of anionic drugs in pediatric patients.

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Developmental changes in the expression of OAT1, OAT3, MRP2, and MRP4 and their effects on the tubular secretion of drugs have not been fully clarified.

In the renal proximal tubules, various kinds of membrane transporters mediate the secretion of a wide range of organic ions (5, 12). Organic anion transporter (OAT) 1 (SLC22A1) and OAT3 (SLC22A8) on the basolateral membrane are involved in the uptake of anionic drugs into epithelial cells (2, 26, 27). Apical types of ATP-dependent primary active transporters, such as multidrug resistance-associated protein (MRP) 2 (ABCC2) and MRP4 (ABCC4), play important roles in the secretion of anionic compounds (29). The transport systems of organic anions in the adult kidney have been investigated extensively. It is important to understand these transporters in the immature kidneys of infants. However, developmental expression levels of these transporter proteins in the infant kidney and their effects on the tubular secretion of drugs have not been fully clarified.

In the present study, we aimed to elucidate the developmental changes in the expression of OAT1, OAT3, MRP2, and MRP4 and their effects on the tubular secretion of drugs. The changes in mRNA and protein expression were evaluated by real-time PCR, Western blotting, and immunohistochemical analysis. In addition, tubular secretion of phenolsulfonphthalein (PSP) during development was examined to compare transporter expression with the tubular secretion of this anionic drug.

MATERIALS AND METHODS

Animals. Animals were treated in accordance with the Guidelines for Animal Experiments of Kyoto University. Wistar/ST rats at various developmental stages [postnatal day 0 (P0)–P28] and adult rats were purchased from SLC Animal Research Laboratories (Shizuoka, Japan). All of the adult rats used for the study were 8-wk-old males (P56).

Real-time PCR. Real-time PCR was performed as described previously (23). Briefly, total RNA was isolated from the whole kidney using an RNeasy minikit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Total RNA was reverse-transcribed to yield cDNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA). For quantification of the amounts of OAT1, OAT3, MRP2, MRP4, and GAPDH, real-time PCR was carried out using an ABI PRISM 7700 sequence detector (Applied Biosystems). The primer-probe sets were purchased from Applied
Biotech (Belgium, WI). GAPDH mRNA was used as an internal control.

**Western blotting.** Crude membrane fractions were prepared from infant and adult rat kidneys as described previously (21). Crude plasma membrane fractions were separated by 7.5% SDS-PAGE for MR2 and MR4 and 10% SDS-PAGE for OAT1 and OAT3 and transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA) by semidry electroblotting. The blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris and 137 mM NaCl) containing 0.3% Tween 20 (TBS-T) for OAT1 and OAT3, with 1% nonfat dry milk in 0.3% TBS-T for MR2, or with 1% nonfat dry milk in 0.1% TBS-T for MR4. The blots were then incubated overnight at 4°C with primary antibodies specific for rOAT1 (15), rOAT3 (15), rMRP2 (Kamiya Biomedical, Seattle, WA), and rMRP4 (Novus Biologicals, Littleton, CO). The blots were washed three times with TBS-T, and the bound antibodies were detected on X-ray film by enhanced chemiluminescence (ECL) with horseradish peroxidase-conjugated secondary antibodies and cyclic diacylhydrazides (GE Healthcare Bio-Sciences, Piscataway, NJ). The relative amount of the band in each lane was determined densitometrically using NIH Image 1.61 (National Institutes of Health, Bethesda, MD), and densitometric ratios relative to each control (P6 rats for OATs or P28 rats for MRPs) were used as a reference and accorded an arbitrary value of 1.0, respectively.

**Immunohistochemical analysis.** Rats were anesthetized, and kidneys of P21, P28, and P56 animals were perfused with 4% paraformaldehyde (PFA) as described previously (25). The kidneys of P0, P14, P28, and P4, and P14 rats were fixed in 4% PFA at 4°C overnight without perfusion fixation. Fixed tissues were embedded in OCT compound (Sakura Finetec, Tokyo, Japan) and frozen rapidly in liquid nitrogen. Sections (5 μm thick) were cut and covered with the blocking agent Blocking One (Nacalai Tesque, Kyoto, Japan) containing 1 mg/ml RNase A (Nacalai Tesque) for rMRP2 and 5% BSA and 0.3% Triton X-100 in PBS for rOAT1, rOAT3, and rMRP4 at 37°C for 1 h. The covered sections were incubated at 37°C for 2 h with antibodies specific for MR2 (1:100), MR4 (1:100), OAT1 (1:200), or OAT3 (1:200). Following two washes, sections were incubated with Cy3-labeled donkey anti-goat IgG, anti-rabbit IgG (CALTAG Laboratory, San Francisco, CA), or Alexa 546 anti-mouse, and Alexa 488-phalloidin (Molecular Probes, Eugene, OR) at 37°C for 60 min. These sections were examined and captured with a BZ-8000 (Keyence, Osaka, Japan) at ×40, ×100, or ×400 magnification.

**In vivo clearance study.** Adult (P56) and infant (P14) rats were anesthetized with an intraperitoneal administration of pentobarbital sodium at 50 mg/kg for P56 rats and 25 mg/kg for P14 rats. For P14 rats, a digital temperature controller (TC-1000; As One, Osaka, Japan) was used at 37°C throughout the continuous infusion study to maintain body temperature. A catheter was inserted into the left femoral vein with polyethylene tubing (SP-31 [Natsume Seisakusho, Tokyo, Japan] and Intramedic PE-10 (BD Biosciences, San Jose, CA)) for drug administration. Urine was collected from the urinary bladder catheterized with tubing (Intramedic PE-50; BD Biosciences). Thereafter, PSP (Nacalai Tesque) was administered as a bolus via the femoral vein and incorporated into the infused solution as described previously (9, 18). The loading and maintenance doses of PSP containing 5% mannitol were, respectively, 10 mg/kg and 22.7 mg·kg⁻¹·h⁻¹ for adult rats and 10 mg/kg and 22.7 mg·kg⁻¹·h⁻¹ for infant rats. The infusion rate was 8.8 ml·h⁻¹·kg⁻¹ using an automatic infusion pump (Natsume Seisakusho). Mannitol was used to maintain a sufficient and constant urine flow rate. After a 20-min equilibration period, urine samples were collected three times at 20-min intervals, and blood samples were obtained from both jugular veins at the midpoint of urine collection for P56 rats. Urine samples were collected at a 60-min interval after the same equilibration period, and blood samples were obtained from both jugular veins at the same points for P14 rats. The plasma was immediately separated by centrifugation. At the end of the experiment, an adequate volume of blood was collected from the abdominal aorta to examine the plasma protein binding rate, and the kidneys were removed to determine the tissue concentration of PSP. The concentrations of PSP in plasma, urine, and the renal homogenate were determined spectrophotometrically at 560 nm after appropriate dilution with 1 M NaOH. The plasma unbound fraction (fu) of PSP was determined by real-time PCR using an ABI Prism 7700 sequence detector. Values are means ± SE for 5–8 rats. *P < 0.05, significantly different from postnatal day 28 (P28) rats.
was determined by ultrafiltration using a micropartition system (Amicon Ultra-0.5, Millipore, Billerica, MA), as described (30). The free fraction of PSP was expressed as the ratio of the concentration in the ultrafiltrate to that in plasma.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated using standard procedures for each experimental period. Total plasma clearance (CL<sub>total</sub>) was calculated by dividing the infusion rate by the steady-state plasma concentration (C<sub>P,ss</sub>) at the midpoint of urine collection. Renal clearance normalized by the circulating plasma concentration (CL<sub>renal,p</sub>) was obtained by dividing the urinary excretion rate by C<sub>P,ss</sub>. GFR was determined by a separate experiment and assumed to be equal to the renal clearance of creatinine. Tubular secretory clearance (CL<sub>sec</sub>) was calculated by subtracting the clearance associated with filtration (C<sub>P,ss</sub> × fu × GFR) from the urinary excretion rate and dividing by the steady-state plasma concentration (C<sub>P,ss</sub>). Renal clearance normalized by the kidney concentration (CL<sub>renal,k</sub>) was calculated by subtracting the clearance associated with filtration (C<sub>P,ss</sub> × fu × GFR) from the urinary excretion rate and dividing by the steady-state kidney concentration (C<sub>K,ss</sub>). The kidney-to-plasma concentration ratio (K<sub>p,kidney</sub>) was calculated by dividing C<sub>K,ss</sub> at 80 min by C<sub>P,ss</sub>.

Statistical analysis. Results are expressed as means ± SE. To calculate the statistical significance of the differences among groups, an unpaired Student’s t-test or one-way ANOVA with Dunnett’s test compared with the group of P28 rats was used.

RESULTS

Real-time PCR. The developmental patterns of OAT1, OAT3, MRP2, and MRP4 mRNA expression in rat kidneys are shown in Fig. 1. The OAT1 mRNA was detected very faintly at P0 (Fig. 1A). Until P6, the OAT1 mRNA level was significantly lower than that in P28 rats. After P6, the expression of OAT1 gradually increased toward the level at P28. OAT3 mRNA was detected very faintly at P0, similar to OAT1 mRNA (Fig. 1B). Until P6, the OAT3 mRNA level was significantly lower than that at P28. After P6, the OAT3 mRNA expression increased toward P14. In contrast, MRP2 and MRP4 mRNA levels did not change significantly during the 4 wk (Fig. 1, C and D).

Western blotting. To examine the developmental changes in transporter protein expression, Western blotting was carried out. As shown in Fig. 2, patterns of developmental expression differed between OATs and MRPs. OAT1 protein was

![Fig. 2. Western blotting of crude plasma membrane fraction from rat kidney at different developmental ages for rOAT1, rOAT3, rMRP2, and rMRP4. A: representative Western blots are shown for each transporter. B: protein levels are expressed in densitometric units. Crude plasma membrane fractions of postnatal days P0–P6 rats and P6–P28 rats were applied to each gel. Data are shown as a ratio to the value at P6 (OAT1 and OAT3). Because MRP2 and MRP4 were not detected until P6, data are shown as a ratio to the value at P28 (MRP2 and MRP4). Values are means ± SE for 4–6 rats. N.D., not detected in Western blotting. *P < 0.05, significantly different from P28 rats.](http://ajprenal.physiology.org/doi/abs/10.1152/ajprenal.00525.2011)
Fig. 3. Immunohistochemical analysis of OAT1 in the neonatal, infant, and adult rat kidney. The kidney was fixed and then embedded. Sections (5 μm) were stained with a specific antibody for rOAT1 (red), phalloidin (A–D, green). A and E (P0): OAT1 staining is already observed clearly in the basolateral membrane. The localization of OAT1 is restricted to the inner cortical region. B and F (P6): OAT1 staining is abundant, but not detected in the peripheral regions of the cortex. C and G (P14): localization of OAT1 is nearly identical to that in the adult kidney. D and H (P56): OAT1 is distributed from the inner cortical region to the peripheral lining. Magnification: E, F, and H, ×40; G, ×100; A–D, ×400.
already detectable at P0 and increased gradually toward P14. The amount of OAT1 did not change significantly from P14 to P28 (Fig. 2B). Similar to OAT1, OAT3 was detected at P0 and increased gradually toward P14, and the level of OAT3 protein was comparable to that at P28 (Fig. 2B). MRP2 and MRP4 were undetectable at P0, and even at P6 (Fig. 2A). MRP2 protein was detected at P14 and gradually increased toward P28 (Fig. 2B). MRP4 was also first de-
ected at \( P14 \), then increased toward \( P21 \) and slightly deceased toward \( P28 \) (Fig. 2A).

**Immunohistochemical analysis.** In the immunohistochemical analysis, specific immunostaining for OAT1 and OAT3 was observed in the basolateral membrane of the proximal tubular cells of adult rat kidney (Figs. 3D and 4D). In the kidney of \( P0 \) rats, OAT1 and OAT3 signals were clearly observed in the basolateral membrane of the tubular epithelial cells (Figs. 3A and 4A). At \( P6 \) and \( P14 \), OAT1 and OAT3 signals were observed in the basolateral membrane, similar to in the adult kidney (Figs. 3, B and C, and 4, B and C). In the adult kidney, OAT1 and OAT3 were detected exclusively in the cortex, and these transporters distributed from the inner cortical region to the extreme peripheral lining, under the renal capsule (Figs. 3H and 4H). The distribution of OAT1 and OAT3 in the neonatal kidney differed from that in the adult kidney. In \( P0 \) rats, OAT1 and OAT3 were restricted to the inner cortical region (Figs. 3E and 4E). OAT1 and OAT3

![Figure 5](http://ajprenal.physiology.org/Downloadedfrom/10.220.32.247)
signals were not detected in the peripheral regions of the cortex until P6 (Figs. 3F and 4F). At P14, the distribution of OAT1 and OAT3 was nearly identical to that in the adult kidney (Figs. 3G and 4G).

Specific immunostaining for MRP2 and MRP4 was observed in the brush-border membrane of the proximal tubular cells of adult rat kidney (Figs. 5C and 6C). At P0, MRP2 and MRP4 signals were observed in the brush-border membrane of tubular cells (Figs. 5A and 6A). Consistent with the results of OAT immunostaining, MRP2 and MRP4 signals were not detected in the peripheral regions of the cortex until P6 (Figs. 5, E and G, and 6, E and G). After P14, MRP2 and MRP4 signals were observed in the extreme peripheral lining of the cortex, and the distribution of MRP2 and MRP4 was nearly identical to that in the adult kidney (Figs. 5, I, K, and M, and 6, I, K, and M).

Pharmacokinetics of PSP in infant and adult rats. We compared the pharmacokinetics of PSP in P14 rats and adult rats (P56). First, equal PSP dosage (22.7 mg·h⁻¹·kg⁻¹) was infused into both rats. The plasma concentration of PSP was significantly higher in P14 rats than adult rats (Fig. 7A). The kidney concentration after the intravenous infusion of PSP was also significantly greater in the P14 rats (Fig. 7B). Table 1 shows the pharmacokinetic parameters of PSP in adult rats and P14 rats. The CL_total and CL_renal, p of PSP and GFR in adult rats were comparable to values in a previous report (9). The CL_total of PSP was significantly lower in P14 rats than adult rats. The CL_renal, p of PSP in P14 rats was ~43% of that in adult rats. There was no significant difference in the kidney-to-plasma concentration ratio (K_p, kidney) between P14 rats and adult rats. The GFR of P14 rats was comparable to that in a previous report (7). To evaluate the excretion of PSP in P14 rats and adult rats more precisely, the dosage of PSP for P14 rats was decreased to 7.57 mg·h⁻¹·kg⁻¹. This dosage made the plasma concentration of PSP in P14 rats after intravenous infusion similar to the plasma concentration of preceding adult rats (Fig. 7A). There was no significant difference in the renal concen-

Table 1. Pharmacokinetic parameters of PSP in adult and postnatal day 14 rats

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<th>CL_total, ml·min⁻¹·kg⁻¹</th>
<th>CL_renal, p, ml·min⁻¹·kg⁻¹</th>
<th>K_p, kidney</th>
<th>GFR, ml·min⁻¹·kg⁻¹</th>
<th>CL_sec, ml·min⁻¹·kg⁻¹</th>
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<tr>
<td>Adult (n = 6)</td>
<td>3.60 ± 0.19</td>
<td>1.17 ± 0.14</td>
<td>0.26 ± 0.033</td>
<td>2.17 ± 0.13</td>
<td>0.042 ± 0.009</td>
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<tr>
<td>P14 (22.7 mg·h⁻¹·kg⁻¹)</td>
<td>3.83 ± 0.27</td>
<td>1.21 ± 0.14</td>
<td>0.35 ± 0.05</td>
<td>2.17 ± 0.13</td>
<td>0.042 ± 0.009</td>
</tr>
<tr>
<td>P14 (7.57 mg·h⁻¹·kg⁻¹)</td>
<td>3.01 ± 0.15</td>
<td>1.17 ± 0.14</td>
<td>0.35 ± 0.05</td>
<td>2.17 ± 0.13</td>
<td>0.042 ± 0.009</td>
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Values are means ± SE. Data are taken from Fig. 7. P: postnatal day; PSP, phenolsulfonphthalein; CL_total, total clearance; CL_renal, p, renal clearance with respect to the circulating plasma concentration; K_p, kidney, kidney tissue-to-plasma ratio; fu, plasma unbound fraction; CL_sec, tubular secretory clearance; CL_renal, k, renal clearance with respect to the kidney concentration; GFR, glomerular filtration rate [determined by the renal clearance of creatinine in adult (P56) and infant (P14) rats not infused with PSP]. *P < 0.05. †P < 0.001, significantly different from adult rats.

Fig. 7. Time profiles of plasma (A) and kidney (B) concentrations of phenolsulfonphthalein (PSP) in adult and infant rats. A: PSP was infused at 22.7 mg·h⁻¹·kg⁻¹ in adult (c) and P14 (●) rats and 7.57 mg·h⁻¹·kg⁻¹ in P14 (●) rats. B: open bar, adult rats; filled bar, P14 rats infused with 22.7 mg·h⁻¹·kg⁻¹ PSP; shaded bar, P14 rats infused with 7.57 mg·h⁻¹·kg⁻¹ PSP. Values are means ± SE for 6–8 rats. *P < 0.05, ***P < 0.001, significantly different from adult rats.
tration between infant rats and preceding adult rats (Fig. 7B). The CL\text{total} of PSP was significantly lower in P14 rats than in preceding adult rats. Although there was no significant difference in the kidney-to-plasma concentration ratio ($K_p$, kidney) between P14 rats and preceding adult rats, the CL\text{renal,p} of PSP in P14 rats was $\sim$33% of that in preceding adult rats. The $fu$ of PSP was significantly higher in P14 rats than adult rats. The CL\text{sec} of PSP in P14 rats was $\sim$26% of that in preceding adult rats (Table 1). To evaluate the secretion of PSP in P14 rats and adult rats more precisely, secretory clearance with respect to the kidney concentration (CL\text{renal,k}), showing the efflux clearance at the brush-border membranes, was calculated. The CL\text{renal,k} of PSP in P14 rats was $\sim$30% of that in preceding adult rats (Table 1).

DISCUSSION
Numerous physiological studies have focused on the tubular secretion of drugs and renal drug transporters in adults, but information on developmental changes in renal drug transporters is limited and the effect of these changes on the tubular secretion of drugs remains unclear. The mRNA levels of these transporters in postnatal development have been measured in mice and rats and shown to increase with age (3, 4, 20, 24). In the present study, similar patterns of mRNA expression for renal organic anion transporters OAT1, OAT3, MRP2, and MRP4 in the developing rat kidney were observed (Fig. 1). The expression of these transporter proteins was also shown to be upregulated with age (Fig. 2). It was revealed for the first time that maturation patterns of OAT proteins were different from those of MRP proteins (Fig. 2).

The maturation of the organic anion transport system in the infant rat kidney was evaluated using an anionic drug, PSP, a diagnostic agent used to test renal function and a known fluorescent tracer. PSP was significantly higher in neonates and young children than in adults (Table 1). GFR was lower in P14 rats and preceding adult rats, the CL\text{renal,p} of PSP in P14 rats was $\sim$33% of that in preceding adult rats (Table 1). When we evaluated the precise function of tubular secretion of PSP, it was revealed that secretory clearance with respect to the kidney concentration (CL\text{renal,k}), showing the efflux clearance at the brush-border membranes, in P14 rats was $\sim$30% of that in adults (Table 1). The level of MRP2 protein in P14 rat kidneys was $\sim$30% of the value in adults and that of MRP4, $\sim$60% (Fig. 2, C and D), which paralleled the functional maturation shown in PSP efflux clearance. On the other hand, the expression of basolateral membrane transporter proteins (OAT1 and OAT3) in P14 rats seemed to be at mature levels (Fig. 2, A and B), and the kidney-to-plasma concentration ratio ($K_p$, kidney) in P14 rats was comparable to that in adult rats (Table 1). It was expected that changes in the expression of the apical transporters without changes in the expression of the basolateral transporters increased the $K_p$ value. However, the $K_p$ values were not different between P14 rats and adult rats. There are two possibilities to cause this phenomenon. First, the efflux of PSP to the blood may be greater than that to the lumen and therefore the $K_p$ value in the kidney of P14 rats was not different from that in the adult rat kidney. In fact, MRP1 and MRP5 mRNAs were reported to be expressed at adult levels at birth or be highest at birth and gradually decrease over the first month in mouse kidney (20). Second, $\alpha$-ketoglutarate (KG) concentrations in the proximal tubular epithelial cells may be lower in P14 than adult rats. Transport by OAT1 and OAT3 is driven by intracellular $\alpha$-KG. In the present study, the concentration of $\alpha$-KG in the kidney could not be measured simultaneously with other experiments because of sample limitations. Further studies are needed to clarify these possibilities. Taken together, it was suggested that low levels of brush-border membrane transporter proteins (MRP2 and/or MRP4) affect the tubular secretion of PSP in P14 rats.

Immunohistochemical analysis showed that OATs were present on the basolateral membrane and MRPs on the brush-border membrane in proximal tubules in P0 rat kidney, identical to those in adult rat kidney (Figs. 3A, 4A, 5A, and 6A). However, the distribution of these transporters in the rat kidney changed with development. These transporters were observed in the inner cortical region at P0 (Figs. 3E, 4E, 5E, and 6E) and had reached the peripheral regions of the cortex by P14 (Figs. 3G, 4G, 5I, and 6I). This result was consistent with a previous report in which the distribution of OAT1 was examined under development (24). At the early stages of kidney development, nephrons are induced in the inner cortical region and finally in the most peripheral region. At birth, the rat kidney is morphologically immature and nephrogenesis continues for $\sim$11 days after birth. Then, it was shown that the distribution of organic anion transporters with kidney development was accompanied by development of the drug transporters during nephrogenesis. It was revealed that not only expression levels but also the distribution of OAT and MRP proteins changed in the developing kidney. To clarify the effect of differences in the distribution of transporters on the transport system of drugs, further investigation is required.

The current results demonstrated age-dependent changes in the expression of organic anion transporters in the rat kidney. To extrapolate pharmacokinetic data from rats to humans, it is necessary to consider species differences in kidney development and transporter maturation. In humans, nephrogenesis is completed by 35-wk gestation, while in rats nephrogenesis is completed by 11 days after birth (16). In light of this, P14 in rats is considered to coincide with the perinatal period in humans. In children, immaturity in the renal processing of drugs may be reflected as drug-induced renal toxicity. In fact, in clinical case studies, renal failure associated with NSAIDs in infants and children was reported (8, 22, 28). There is a critical need to predict effect and disposition in newborns and infants (11). These age-dependent physiological data will be useful for predicting the pharmacokinetics of drugs in children.

In the present study, we investigated the developmental expression of OAT1, OAT3, MRP2, and MRP4 proteins in the rat kidney and their effects on tubular secretion of PSP. It was suggested that the expression of MRPs on the brush-border membrane affects tubular secretion of PSP. These results indicate that age-dependent changes in renal transporter expression affect the tubular secretion of drugs. Therefore, information about drug transporter expression during developing stages could be used to predict the pharmacokinetics of drugs in children and contribute to pharmacotherapy for pediatric patients.
GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: M.N., H.M., and H.S. provided conception and design of research; M.N., H.M., and H.S. performed experiments; M.N., H.M., and H.S. analyzed data; M.N., H.M., H.S., T.K., and K.I. interpreted results of experiments; M.N. and H.S. prepared figures; M.N. and H.S. drafted manuscript; H.M., T.K., and K.I. edited and revised manuscript; H.M. approved final version of manuscript.

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