Age-associated differences in transporter gene expression in kidneys of male rats

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Abstract. Kidney transporters are involved in the secretion and reabsorption of endogenous and exogenous molecules. Numerous factors may influence their expression and affect drug disposition, efficacy and toxicity. The present study aimed to examine the development- and age-associated variations in primary renal transporters in rats, including 6 uptake transporters: Organic anion transporter (OAT) 1 and 3, organic cation transporter (OCT) 1, 2 and 3 and organic anion-transporting polypeptide (OATP) 4C1, and 6 efflux transporters: Multidrug resistance protein 1 (MDR1), breast cancer resistance protein (BCRP), multidrug resistance-associated protein (MRP) 2 and 4, and multidrug and toxin extrusion protein (MATE) 1 and 2-K. Kidneys from male Sprague Dawley rats during development (-2, 1, 7, 14 and 21 days), maturation (28, 35 and 60 days) and aging (180, 540 and 850 days) were collected and total RNA was extracted, purified and subjected to reverse transcription-quantitative polymerase chain reaction analysis. Total proteins were extracted for western blot analysis. OAT1 and 3, OCT1, BCRP, MRP2 and 4 and MATE2-K expression levels were low in fetal kidneys, increased gradually following birth and markedly increased on maturation and adulthood. High levels were maintained until 850 days. OCT2, OATP4C1, Mdr1b and MATE1 expression levels were low in fetal kidneys, increased gradually following birth, and increased markedly on weaning, maturation and adulthood; however, levels were decreased on aging. OCT3 mRNA expression levels were low in fetal and newborn kidneys, and had two peaks at 35 and 850 days. The selected OAT1 and 3 and MDR1 protein expression levels revealed a similar expression pattern. Thus, kidney transporter expression is affected by ontogeny and aging, which may impact drug and toxicant disposition in children and the elderly.

Introduction

The kidneys are crucial for the elimination of endogenous metabolites and xenobiotics, including drugs and environmental chemicals. At least 37 xenobiotic transporters have been identified in mammalian kidneys (1). Transporter proteins provide organ/tissue defense and are involved in the therapeutic effects and toxicity of numerous drugs and toxicants (2-4). Important basolateral uptake transporters include organic anion transporter (OAT) 1, 2 and 3, organic cation transporter (OCT) 1, 2 and 3 and organic anion transporting polypeptide (OATP4C1). Important efflux transporters on the apical brush-border membrane primarily include multidrug resistance protein [P-glycoprotein, encoded by multidrug resistance protein gene 1b (Mdr1b)], multidrug resistance-associated protein (MRP) 2 and 4, multidrug and toxin extrusion protein (MATE) 1 and 2 and breast cancer resistance protein (BCRP) (2,5,6).

Renal OATs are important in the uptake of common drugs, toxins and nutrients (3,7). For example, OAT1 and 3 are critical for renal mercury absorption and accumulation from inorganic (3,8,9) or organic mercury (10). Deletion of OAT1 and 3 protects against nephrotoxicity induced by mercury chloride (11) and aristolochic acid I (12). Thus, alterations of OAT1 and 3 greatly impact chemical-induced nephrotoxicity (13).

In addition, renal OCTs are important in transporting cationic xeno- and endobiotics across biological membranes. For example, OCT1 and 2 mediate the renal uptake and accumulation of platinum compounds (14) and cadmium (15); modification of OCT expression levels causes altered proximal tubular cell accumulation of cisplatin and cadmium, resulting in decreased or increased toxicity (14-16).

Mdr1 and MRP2 are expressed in proximal tubular epithelial cells and are crucial for protection against toxicant-induced kidney injury. For example, Mdr1 protects against paraquat-induced toxicity in human and murine proximal tubular cells (17), while MRP2 protects against mercury-induced kidney injury (18,19).

MATE1 and 2 mediate cisplatin-induced nephrotoxicity via a reduction in cellular efflux (16). MATE and OCT2 affect

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Abbreviations: BCRP, breast cancer resistance protein; MATE, multidrug and toxin extrusion protein; Mdr1b, multidrug resistance protein 1b; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; RT-qPCR, reverse transcription-quantitative polymerase chain reaction

Key words: kidney, transporter, ontogeny, aging, rats

cisplatin accumulation and toxicity in coordination (20). The efflux transporter BCRP is involved in the elimination of mercury from proximal tubular cells (6).

The expression of renal transporters may be affected by drugs and toxicants, and by physiological variations. For example, females express reduced levels of renal OAT1 and 3, and are therefore less susceptible to mercury-induced kidney injury (21). Older rats are more susceptible to mercury nephrotoxicity compared with younger rats (22), and methylmercury may cross the placenta to the fetus, resulting in accumulation and toxicity (19). Thus, understanding the ontogeny and aging-associated alterations in renal transporter expression is important for the evaluation of drug or toxicants effects in sensitive populations. For this reason, the ontogeny of the expression of renal transporters, including OCTs (23), MATE1 and 2 (24) and OATs (1) has been investigated in mice. However, in rats the majority of studies have examined the expression of OATs (25-27).

Recently, the whitepaper of Pediatric Transporter Working Group presented a systematic review of the ontogeny of clinically relevant transporters in intestine, liver and kidney (4). Different developmental patterns for individual transporters exist; however, these remain to be fully elucidated, particularly with regard to elderly populations. The present study aimed to address this. Kidneys were collected from male Sprague Dawley rats at 11 time points: Fetal (-2 days), birth (1 day), nursing (7 and 14 days), weaning (21 days), puberty (28 and 35 days), maturation (60 days), adulthood (180 days) and aging (540 and 850 days). The mRNA expression levels of six primary renal uptake transporters (OAT1 and 3, OATP4C1, and OCT1, 2 and 3) and six primary efflux transporters (Mdr1b, MRP2 and 4, MATE1 and 2, and BCRP) were examined, and three selected transporters (OAT1 and 3, and MDR1) were additionally examined at the protein levels via western blot analysis. The results obtained may be of physiological, pharmacological and toxicological significance.

Materials and methods

Animals. Sprague Dawley rats (weight, 250-300 g; 10 male, 30 female as parents of the experimental mice) were purchased from the Experimental Animal Center of Third Military Medical College (Chongqing, China) and acclimatized for one week prior to mating overnight. Rats were housed in specific pathogen-free-grade animal facilities under a 12-h light/dark cycle, at 22±2°C and 50% humidity, and had access to feed and water *ad libitum*. All animal procedures experiments were performed in accordance with Chinese Guidelines of Animal Care and Welfare, and the present study was approved by the Animal Care and Use Committee of Zunyi Medical College (Zunyi, China).

Tissue collection. If a vaginal plug was present the next morning this was designated as day 0 of gestation. Kidneys were collected from male rats only at developmental days -2, 1, 7, 14, 21, 28, 35 and 60 and ageing days 180, 540 and 850, where day 0 was the day of birth. Rats were anesthetized with 7% chloral hydrate (5 ml/kg), sacrificed by cervical dislocation, and kidneys were frozen in liquid nitrogen and stored at -80°C prior to analysis.

RNA isolation. Kidney tissue (50-100 mg) was homogenized in 1 ml TRIzol® (Takara Biotechnology Co., Ltd., Dalian, China). The quality and quantity of RNA were determined by measuring the absorbance at wavelengths of 260 and 280 nm and calculating the 260/280 ratio, and by gel electrophoresis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was reverse transcribed with the High Capacity Reverse Transcriptase kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). The iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used for qPCR analysis. The primers were designed with Primer3 software (version 4.0) and are listed in Table I. The 15 μl PCR reaction mix contained 3 μl cDNA (10 ng/μl), 7.5 μl iQTM SYBR Green Supermix (Bio-Rad Laboratories, Inc.), 0.5 μl primer mix (10 μM each), and 4 μl ddH2O. The thermocycling conditions were as follows: 5 min denaturation at 95°C; 40 cycles of annealing and extension at 60°C for 45 sec, and denaturation at 95°C for 10 sec. A dissociation curve was performed following the 40 cycles to verify the quality of primers and amplification. Relative expression of genes was calculated by the $2^{-\Delta\Delta Cq}$ method (28), and normalized to the housekeeping gene GAPDH and β-actin of the same sample, and the relative transcript levels were calculated as percentage of -2 days.

Protein extraction and quantification. Kidneys from each group were pooled and ~100 mg was homogenized in 1 ml radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing 1 mM phenylmethanesulfonyl fluoride and proteinase inhibitors. The lysates were centrifuged at 12,000 x g for 10 min at 4°C, and supernatants were collected and stored at -80°C prior to analysis. Protein concentrations were quantified by the Bicinchoninic Acid assay (Beyotime Institute of Biotechnology).

Western blot analysis. Aliquoted proteins were denatured with loading buffer (catalog no. P0015; Beyotime Institute of Biotechnology) at 90°C for 10 min, and ~10 μg protein/lane was separated on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% dry non-fat milk in TBS containing 0.07% Tween-20 (TBST) at room temperature for 2 h, followed by incubation overnight at 4°C with the following primary antibodies, diluted 1:1,000 in 1% bovine serum albumin (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) in TBST. Antibodies were Goat anti-OAT1 (cat.no. sc-161977; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), goat anti-OAT3 (cat. no. sc-107836; Santa Cruz Biotechnology, Inc.), rabbit anti-MDR1 (cat. no. ab170904; Abcam, Cambridge, UK) and mouse anti-β-actin (cat. no. AA128-1; Beyotime Institute of Biotechnology). Following three washes with TBST, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit (cat. no. A0208), anti-mouse (cat. no. A0126), or anti-goat (cat. no. A0181) IgG secondary antibodies, all obtained from Beyotime Institute of Biotechnology) and used at a dilution of 1:5,000 for 1 h at room temperature. Protein-antibody complexes were visualized using an Enhanced Chemiluminescent reagent (catalog no. P0018;

Table I. Primer sequences used in reverse transcription-quantitative polymerase chain reaction analysis.

		Sequence (5'-3')	
Gene	GenBank no.	Forward	Reverse
β-actin	NM_007393	catccgtaaagacctctatgccaac	atggagccaccgatccaca
OAT1	NM_017224	cttgtacaccggagagc	aggcatggaggggtagaact
OAT3	NM_031332	gttgacatcccagccaagtt	ctgcatttctgaaggcacaa
OCT1	U17013	agcagctcaccaatcaaagc	gtggagtctgtagtgcctgt
OCT2	NM_031584	ttgtctgctcctccatgtgt	agagccttccctttggtctc
OCT3	NM_031332	gtctctctctggcctggttt	gcacaaagatgagggccaaa
OATP4C1	NM_001002024	tcaagctggcaaaacttccc	ccgcaaagctcgatgtcaat
MRP2	NM_012833	tctcttgcgctcacagaaga	gaaactggaatacgccgcat
MRP4	AY533524	accaggatgccgacatctac	cgtgcaaagtgtggcagata
BCRP	NM_181381	ccagcctcggtattccatct	cagccgaagaatctccgttg
MATE1	NM_001014118	cctgagtggtatccttggca	ggcctggtcaatgtttcctg
MATE2-K	NM_001191920	cacctcccagttcttcctgt	tcccaatctcgaaggtccac
Mdr1b	NM_012623	tgtttgactgcagcatcacc	agctgagtccctttgtctcc

OAT, organic anion transporter; OCT, organic cation transporter; OATP, organic anion-transporting polypeptide; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; MATE, multidrug and toxin extrusion protein; Mdr1b, multidrug resistance protein gene 1b.

Beyotime Institute of Biotechnology), and a ChemiDoc XRS system (Bio-Rad Laboratories, Inc.). Band intensities were semi-quantified by densitometry using Quantity One® software (version 4.6.2, Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analyses were performed in SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as the mean ± standard error. Age-associated differences were analyzed by one-way analysis of variance, followed by the least significant difference *post hoc* test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Basolateral uptake transporter expression levels in kidneys. On the basolateral membrane of the kidneys, the primary anion transporters are OAT1 and 3, and OATP4C1; the primary cation transporters are OCT1, 2 and 3.

OAT1 and 3. OAT1 and OAT3 are involved in the active uptake of chemicals and toxicants. The mRNA expression levels of OAT1 and 3 increased significantly with age from -2 to 850 days (Fig. 1). OAT1 mRNA expression levels were low in fetal kidneys, increased gradually following birth and increased markedly on maturation and adulthood (peaking at 180 days). Compared with OAT1 mRNA expression levels at -2 days, OAT1 levels at 180 days were 35-fold greater, and were maintained at that high level until 850 days (Fig. 1).

OAT3 mRNA expression levels were low in fetal and newborn kidneys; however, these levels increased rapidly following birth and continued to increase during adulthood. Compared with OAT3 mRNA expression levels at birth, OAT3 levels at 180 days were 22-fold greater, and were maintained at that high level and even increased until 850 days (Fig. 1).

The protein expression levels of OAT1 (Fig. 2) and 3 (Fig. 3) followed similar patterns to the mRNA expression levels. OAT1 protein was undetectable at day 1, reached a peak on maturation, and remained high on aging ($P<0.05$ vs. -2 days; Fig. 2). OAT3 protein was undetectable at -2 and 1 days; however, protein expression levels subsequently increased throughout weaning, maturation, adulthood and aging ($P<0.05$ vs. -2 days; Fig. 3).

OCT1, 2 and 3, and OATP4C1. OCTs are responsible for excretion of cationic substances into urine. Tissue OCT expression is important for the disposition and excretion of xenobiotics. On the basolateral membrane of the kidneys, OCT1, 2 and 3 are the primary cation transporters, whereas OATP4C1 is the primary anion transporter. The mRNA expression levels of OCT1, 2 and 3, and OATP4C1 increased significantly with age from -2 to 850 days (Fig. 4).

OCT1 mRNA expression levels were low in fetal kidneys, increased gradually following birth, and increased markedly on weaning, maturation and adulthood (peaking at 540 days). Compared with OCT1 mRNA expression levels at -2 days, OCT1 levels at 420 days were 35-fold greater, and were maintained at that high level until 850 days ($P<0.05$ vs. -2 days; Fig. 4).

Similarly to OCT1, OCT2 mRNA expression levels were low in fetal kidneys, increased gradually following birth, and increased markedly on weaning, maturation and adulthood (peaking at 180 days). Compared with OCT2 mRNA expression levels at -2 days, OCT2 levels at 180 days were 470-fold greater; however, these levels decreased on aging ($P<0.05$ vs. -2 d; Fig. 4).

The ontogeny of OCT3 differed from OCT1 and 2. OCT3 mRNA expression levels were low in fetal and newborn kidneys, increased rapidly following birth and reached a first peak at 35 days. Compared with OCT3 mRNA expression
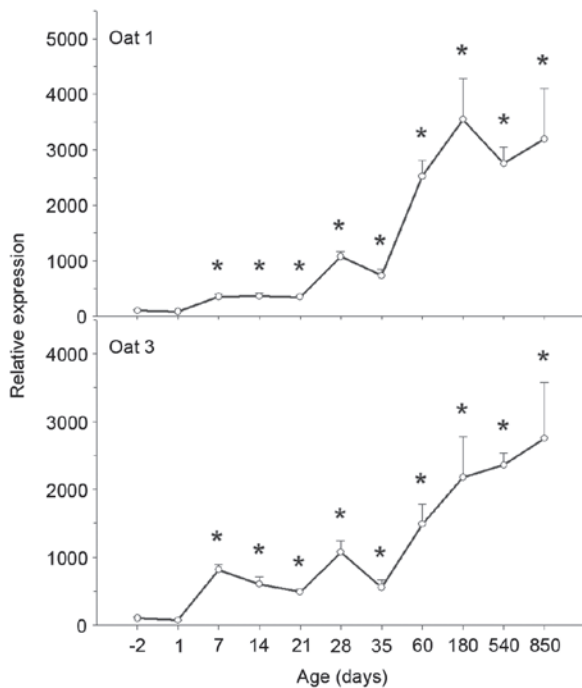
Figure 1. Ontogeny of OAT1 and 3 mRNA expression levels in rat kidney. Kidneys were collected at -2, 1, 7, 14, 21, 28, 35, 60, 180, 540 and 850 days, and subjected to reverse transcription-quantitative polymerase chain reaction analysis using specific primers. mRNA expression levels of OAT1 and 3 increased over time. Data are expressed as the mean ± standard error (n=6). *P<0.05 vs. -2 days. OAT, organic anion transporter.
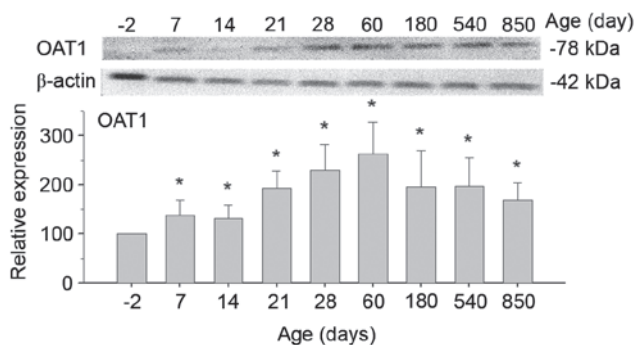

Figure 2. Protein expression levels of OAT1 in rat kidney. Kidneys were collected at -2, 7, 14, 21, 28, 60, 180, 540 and 850 days, and subjected to western blot analysis using specific antibodies. Protein expression levels of OAT1 increased over time, peaking at 60 days. Data are expressed as the mean ± standard error of 3-4 replicates of pooled samples. *P<0.05 vs. -2 days. OAT, organic anion transporter.
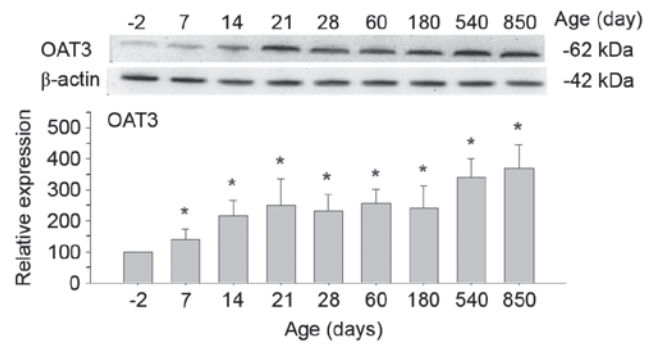

Figure 3. Protein expression levels of OAT3 in rat kidney. Kidneys were collected at -2, 7, 14, 21, 28, 60, 180, 540 and 850 days, and subjected to western blot analysis using specific antibodies. Protein expression levels of OAT1 increased over time. Data are expressed as the mean ± standard error of 3-4 replicates of pooled samples. *P<0.05 vs. -2 days. OAT, organic anion transporter.

levels at birth, OCT3 levels at 35 days were 20-fold greater. Subsequently, OCT3 mRNA expression levels declined, and reached a trough at 180 days. Following this, however, OCT3 mRNA expression levels increased again and reached a second peak at 850 days. Compared with OCT3 mRNA expression levels at birth, OCT3 levels at 850 days were 25-fold greater (P<0.05 vs. -2 days; Fig. 4).

OATP4C1 mRNA expression levels were low in fetal kidneys, increased gradually following birth, and increased markedly after 14 days, peaking at 180 days. Compared with OATP4C1 mRNA expression levels at -2 days, OATP4C1 levels at 180 days were 35-fold greater. Subsequently, OATP4C1 mRNA expression levels decreased (P<0.05 vs. -2 days; Fig. 4).

Apical efflux transporter expression levels in kidneys. The brush-border efflux transporters BCRP, MDR1 MRP2 and 4, and MATE1 and 2-K contribute to the secretion of chemicals from the kidneys into the urine.

BCRP and Mdr1b. BCRP and MDR1 transport an extensive range of endogenous and exogenous lipophilic substrates, including lipids, steroids, peptides and xenobiotics. The mRNA expression levels of BCRP and Mdr1b increased significantly with age from -2 to 850 days (Fig. 5).

BCRP mRNA expression levels were low in fetal and newborn kidneys, and increased rapidly following birth, reaching a peak at 180 days and subsequently declining. Compared with BCRP mRNA expression levels at birth, BCRP levels at 180 days were 18-fold greater (P<0.05 vs. -2 days; Fig. 5).

Similarly to BCRP, Mdr1b mRNA expression levels were low in fetal kidneys, increased gradually following birth and increased markedly on maturation, peaking at 540 days. Compared with Mdr1b mRNA expression levels at -2 days, Mdr1b levels at 540 days were 23-fold greater; however, these levels decreased at 850 days (Fig. 5). The protein expression levels of MDR1 followed a similar pattern: Undetectable at 1 day, and increasing on maturation, prior to decreasing at 850 days (P<0.05 vs. -2 days; Fig. 6).

MRP2 and 4. In renal proximal tubules, MRP2 and 4 actively transport numerous organic anions into urine, including drugs and metabolic waste. The mRNA expression levels of MRP2 and 4 increased significantly with age from -2 to 850 days (Fig. 7).

MRP2 mRNA expression levels were reduced 50% in neonatal kidneys (14 days) compared with fetal kidneys, and subsequently increased markedly following weaning, continuing to increase throughout and peaking at 850 days. Compared with MRP2 mRNA expression levels at -2 days, MRP2 levels at 850 days were 3-fold greater (P<0.05 vs. -2 days; Fig. 7).
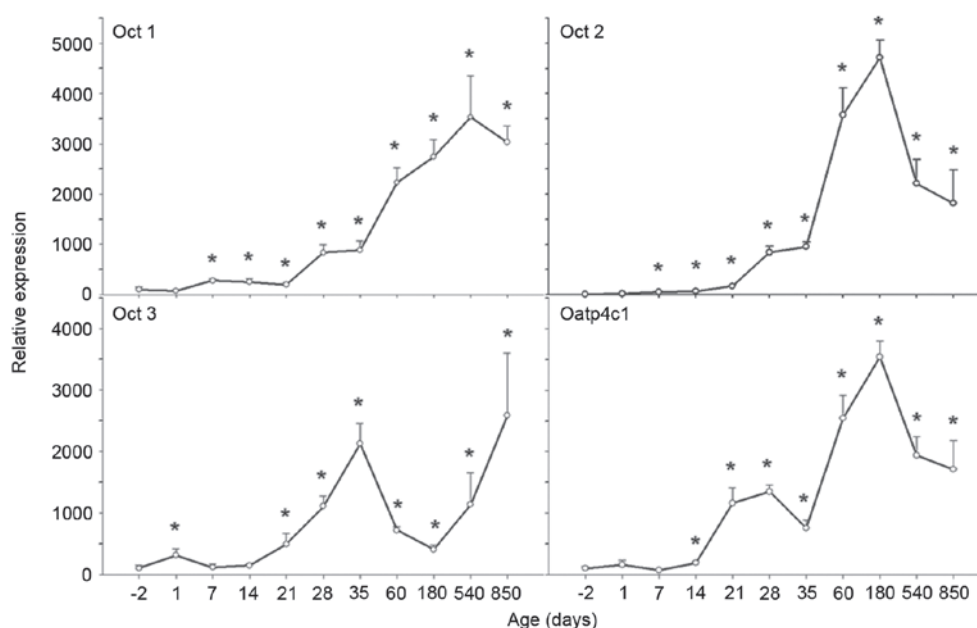
Figure 4. Ontogeny of OCT1, 2 and 3, and OATP4C1 mRNA expression levels in rat kidney. Kidneys were collected at -2, 1, 7, 14, 21, 28, 35, 60, 180, 540 and 850 days, and subjected to reverse transcription-quantitative polymerase chain reaction analysis using specific primers. mRNA expression levels of OCT1 increased over time, levels of OCT2 and OATP4C1 increased over time and subsequently decreased on aging. OCT3 mRNA expression levels exhibited a biphasic pattern. Data are expressed as the mean ± standard error (n=6). *P<0.05 vs. -2 days. OCT, organic cation transporter; OATP, organic anion-transporting polypeptide.
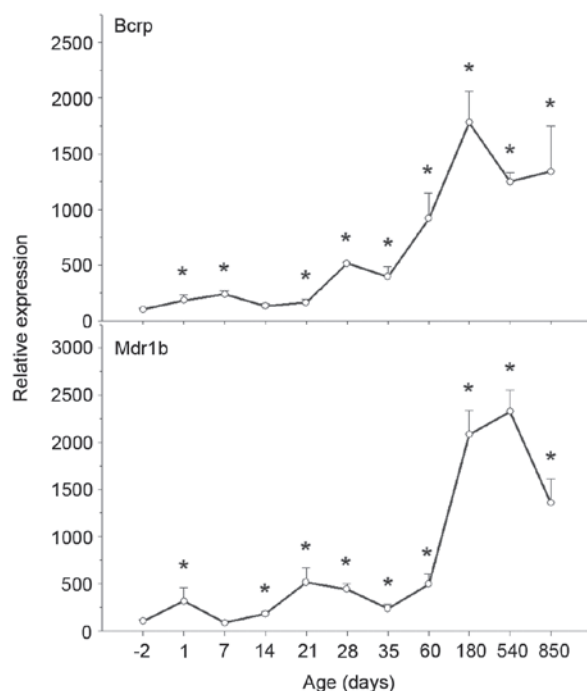

Figure 5. Ontogeny of BCRP and Mdr1b mRNA expression levels in rat kidney. Kidneys were collected at -2, 1, 7, 14, 21, 28, 35, 60, 180, 540 and 850 days, and subjected to reverse transcription-quantitative polymerase chain reaction analysis using specific primers. mRNA expression levels of BCRP increased over time, whereas levels of Mdr1b1 increased over time and subsequently decreased on aging. Data are expressed as the mean ± standard error (n=6). *P<0.05 vs. -2 days. BCRP, breast cancer resistance protein; Mdr1b, multidrug resistance protein gene 1b.
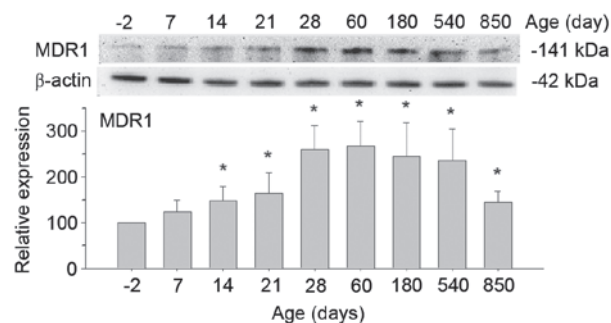

Figure 6. Protein expression levels of MDR1 in rat kidney. Kidneys were collected at -2, 7, 14, 21, 28, 60, 180, 540 and 850 days, and subjected to western blot analysis using specific antibodies. Protein expression levels of MDR1 increased over time and subsequently decreased on aging. Data are expressed as the mean ± standard error of 3-4 replicates of pooled samples. *P<0.05 vs. -2 days. MDR, multidrug resistance.

peaking at 28 days. Compared with the MRP4 mRNA expression levels at -2 days, MRP4 levels at 28 days were 6-fold greater (P<0.05 vs. -2 days; Fig. 7).

MATE1 and 2-K. The MATE transporters mediate cellular efflux of a variety of organic cations, including numerous drugs. The mRNA expression levels of MATE1 and 2-K increased significantly with age from -2 to 850 days (Fig. 8).

MATE1 mRNA expression levels were low in fetal kidneys, increased gradually following birth, and increased markedly following weaning, peaking at 180 days. Compared with MATE1 mRNA expression levels at -2 days, MATE1 levels at 180 days were 18-fold greater. MATE1 mRNA expression levels subsequently decreased (P<0.05 vs. -2 days; Fig. 8).

MATE2-K mRNA expression levels were low in fetal kidneys, increased gradually following birth, and increased

MRP4 mRNA expression levels were low in fetal kidneys and increased gradually following birth and through weaning,
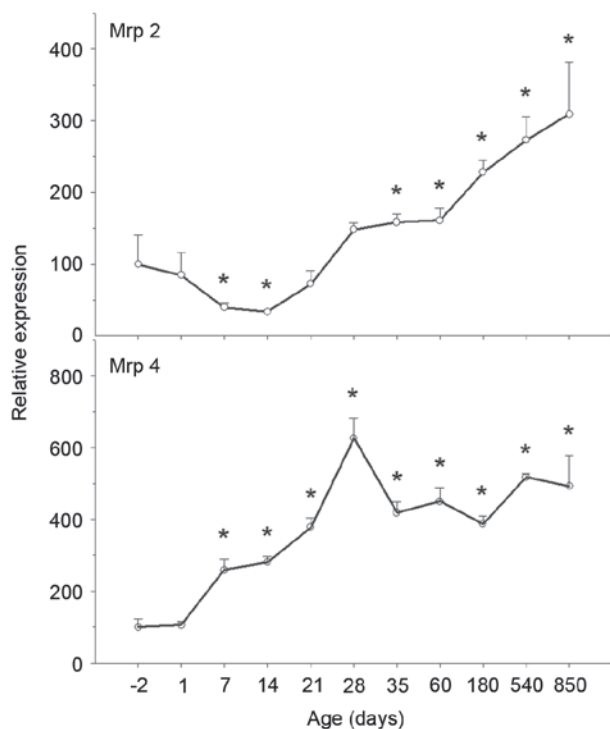
Figure 7. Ontogeny of MRP2 and 4 mRNA expression levels in rat kidney. Kidneys were collected at -2, 1, 7, 14, 21, 28, 35, 60, 180, 540 and 850 days, and subjected to reverse transcription-quantitative polymerase chain reaction analysis using specific primers. mRNA expression levels of MRP2 and 4 increased over time. Data are expressed as the mean ± standard error (n=6). *P<0.05 vs. -2 days. MRP, multidrug resistance-associated protein.
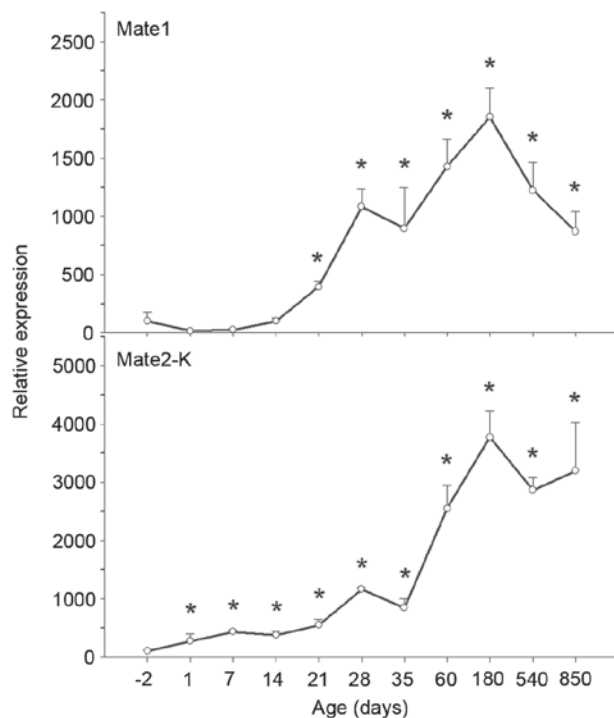

Figure 8. Ontogeny of MATE1 and 2-K mRNA expression levels in rat kidney. Kidneys were collected at -2, 1, 7, 14, 21, 28, 35, 60, 180, 540 and 850 days, and subjected to reverse transcription-quantitative polymerase chain reaction analysis using specific primers. mRNA expression levels of MATE2-K increased over time, whereas levels of MATE1 increased over time and subsequently decreased on aging. Data are expressed as the mean ± standard error (n=6). *P<0.05 vs. -2 days. MATE, multidrug and toxin extrusion protein.

markedly following maturation, peaking at 180 days. Compared with MATE2-K mRNA expression levels at -2 days, MATE2-K levels at 180 days were 37-fold greater, and were maintained at that high level on aging (P<0.05 vs. -2 days; Fig. 8).

Discussion

The present study demonstrated the ontogeny and age-associated variations in 12 primary kidney transporters in rats. These transporters include the transporters responsible for renal uptake of xenobiotics (OAT1 and 3, OCT1, 2 and 3, and OATP4C1) and transporters associated with kidney efflux and excretion of xenobiotics (MDR1, MRP2, MRP4, BCRP and MATE1 and 2-K). Typically, the mRNA expression levels of these transporters were low in fetal kidneys, increased gradually following birth, and increased markedly on maturation and adulthood, maintaining high expression levels on aging. However, the mRNA expression levels of certain transporters, OCT2, OATP4C1, Mdr1b and MATE1 were decreased on aging. The patterns of mRNA and protein expression levels for the three transporters that underwent western blot analysis were similar. The profile of the ontogeny and age-associated expression of these transporters may provide useful information for the disposition of drugs and toxicants in the kidney, similar to our previous study on liver (29). The present study systematically profiled the ontogeny of 12 primary kidney transporters, and to the best of our knowledge, is among the first to profile age-associated variations in transporter expression.

The OAT family has been extensively studied due to its role in the transport of drugs including cisplatin, aristolochic acid and tanshinol (12,30), toxicants including mercury and indoxyl sulfate (13), and nutrients (3,7). OAT expression is markedly altered during renal failure (31). The importance of OAT1 and 3 in HgCl$_2$ and MeHg-induced renal injury has been documented (8,10,11,32). In addition, our recent studies revealed the alterations of OAT1 and 3 in HgCl$_2$-induced acute and subacute renal injury, but not following exposure to HgS (33,34). The pharmacological modulation of the expression and/or function of OAT1 and 3 may be a potential therapeutic strategy for reducing the nephrotoxicity of HgCl$_2$ (9). The expression of OAT1 and 3 is additionally influenced by gender (21), and the increased expression of OAT1 and 3 in males has been revealed to be regulated by the transcription factor B-cell lymphoma 6 (35). In the kidney, OAT1 transcripts appeared at mid-gestation, alongside proximal tubule differentiation, and increased as nephrons matured (36). The present study demonstrated the ontogeny of OAT expression in the kidney, including during the aging process.

OCTs are important for the disposition and excretion of xenobiotics, including platinum compounds (14,20), cadmium (15,37), metformin (38) and mercury (34). OCT2 and MATEs coordinate to eliminate cationic drugs, including cisplatin, from the kidney (14,16). OCT1 and 2 expression has been demonstrated to be altered during renal failure (31), and OCT mRNA expression levels in kidneys is influenced by age (23). In the present study, OCT1 and 2 revealed a typical ontogeny pattern, whereas OCT3 exhibited a biphasic pattern with peaks at 35 and 850 days, which is in agreement with

a previous study (25). The age-associated variations in OCT expression may affect the ability of the kidney to process heavy metals and toxicants.

OATP4C1 was identified as a novel uptake transporter primarily expressed at the basolateral membrane of rat kidney proximal tubules. It was hypothesized to act as a vectorial transport partner of an apically-expressed efflux transporter, to enable the translocation of substrates, including uremic toxins (39,40), digoxin and estrone 3-sulfate (41), and sitagliptin, a therapeutic agent for type 2 diabetes (42), into urine. Numerous factors, including age-associated variation, may influence the expression of OATP4C1, thereby altering drug disposition, efficacy and toxicity (1). Our recent study revealed the ontogeny and age-associated alterations of OATP expression in the liver (29), and in the present study, the ontogeny of OATP4C1, the primary OATP in the kidney, was characterized.

The transporters BCRP and MDR1 belong to the multidrug resistance protein family. BCRP has recently been identified as an additional potential transporter in the elimination of mercury from proximal tubular cells (6), and aristolochic acid I is an additional substrate that is excreted by BCRP (43). The organ- and age-specific expression patterns of these transporters have been demonstrated in adult organs (44). The ontogeny of BCRP in the kidney resembles that of the liver with greater expression in adults (5), and increased expression of BCRP in male rat kidneys was revealed (45). In the present study, the expression levels of BCRP increased with age, and were maintained at high levels until 850 days. Kidney MDR1 is similar to BCRP during development (44). Mdr1a and Mdr1b in kidney exhibit increased expression in females due to their inhibition by androgens (46), and Mdr1a is important for removing paraquat from kidneys and protecting against its subsequent toxicity (17). MDR1 expression is low at birth, and gradually increases to mature levels at ~30 days of age (46). In the present study, MDR1 shared a similar development pattern with BCRP; however, MDR1 expression levels were decreased during aging, implying decreased renal excretion function in elderly.

MRP2 and 4 are localized in proximal tubular epithelial cells and actively transport numerous organic anions into urine (47), including $HgCl_2$ (18,19,48) and the immunosuppressant mycophenolic acid (49). In our recent studies, HgS and HgS-containing traditional medicines differed from $HgCl_2$ and MeHg as they were unable to increase renal expression of MRP2 and 4 (33,34). The renal toxicity of Hg(2+) differs in young adult and aged Wistar rats (22), and this may be partially due to the potential aging-associated expression of MRPs. In kidneys, MRP1 and 5 were expressed at adult levels at birth, whereas MRP2, 3, 4 and 6 expression typically increased with time (50); however, little is known about their expression during the aging process. The present study profiled the ontogeny and aging-associated expression of MRP2 and 4 to address this issue.

In the kidney, MATEs coordinate with OCTs to eliminate organic cations. More than 40 therapeutic agents and various endogenous compounds are known to be substrates or inhibitors of MATEs, including cisplatin, metformin and lamivudine (51,52). The inhibitory potencies of ondansetron on MATE1 and 2-K caused increases in tubular cell accumulation of metformin and cisplatin, with increased renal toxicity (16,20). In rats treated repeatedly with $HgCl_2$, MATE2-K expression was increased in an attempt to eliminate cellular Hg (34). The mRNA expression levels of MATE1 in the kidneys of males and females were similar, with levels increasing gradually from prenatal day -2 to 45 days of age. A gender difference appeared at day 30 (24); however, little is known regarding expression during the aging process. In the present study, MATE1 and 2-K exhibited similar ontogeny patterns, and the levels of mRNA expression remained high throughout adulthood, although MATE1 levels decreased during aging.

In conclusion, the results of the present study characterized the ontogeny and age-associated alterations in six primary renal uptake transporters (OAT1 and 3, OCT1, 2 and 3, and OATP4C1) and six primary renal efflux transporters (MDR1, BCRP, MRP2 and 4, and MATE1 and 2-K) at the mRNA level, and at the protein level of selected transporters (OAT1 and 3, and MDR1). The results confirmed the ontogeny pattern of certain transporters described in the literature, and is among the first to demonstrate the pattern of their expression during development in fetal (-2 days), neonatal (1, 7, 14, 21 and 28 days), mature (35 and 60 days) and older (180, 540 and 850 days) rat kidneys. These data may further understanding of age-dependent variations of drug-drug interactions, and drug efficacy and toxicity.

Acknowledgements

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