Renal organic anion transporter 1 is maldistributed and diminishes in proximal tubule cells but increases in vasculature after ischemia and reperfusion

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Some of these organic anions have been suggested to contribute to the uremic syndrome (6–8, 12, 25, 27, 28, 31, 33, 37, 43, 46–49). In the kidney, proximal tubule (PT) cells actively secrete organic anions into the urine via organic anion transporters (OAT), which possess broad substrate specificity (23, 32). A family of organic anion transporters (OAT1–OAT6) has been cloned. OAT1, OAT2, and OAT3 are known to be present in the kidney. OAT1 has the highest affinity for PAH among the renal OAT isoforms and is located on the basolateral membrane of PT cells (2, 11, 21, 32, 38). Recently, our group (16) has reported that, in recipients of cadaveric renal allografts, the transplanted kidneys exhibit maldistribution of OAT1 in PT cells after ischemia and reperfusion, resulting in impairment of PAH clearance. In the present study, we used a rat model of ischemia and reperfusion to characterize renal OAT1 systematically during a severe form of ischemic AKI. For detailed characterization of OAT1, immunohistochemical analysis with the use of confocal microscopy was performed with a three-dimensional reconstruction of serial optical images. In addition, the amount of OAT1 protein and its mRNA expression were also quantified. Our findings provide the basis for this report of detailed characterization of OAT1 after ischemia and reperfusion.

MATERIALS AND METHODS

Animal Model and Surgery

The experimental protocol was previously approved by the Institutional Animal Care and Use Committee of the Penn State University College of Medicine (protocol no. 2005–003).

To characterize the dynamic alterations of OAT1 in ischemic AKI, anesthetized male Sprague-Dawley rats were subjected to renal ischemia by clamping of the left renal pedicle for 5, 30, 45, and 60 min. In other experimental groups, 60 min of bilateral renal arterial clamping was followed by 1, 24, 48, 72, 168, or 240 h of reperfusion. Control kidneys were procured without any preceding operative procedures. Renal function was monitored by measuring serum creatinine and blood urea nitrogen (BUN). Kidney tissues from each experimental condition and control were analyzed by immunohistochemistry, Western blot, and quantitative real-time PCR.

Immunohistochemistry

Antibodies. Anti-OAT1 antibody was prepared as follows: rabbits were immunized with a purified keyhole limpet hemocyanin-conjugated synthesized peptide (CKYMVPLOQASSAEKNGL) corresponding to the COOH terminus of human OAT1 (hOAT1). hOAT1 and rat OAT1 (rOAT1) are 87.6% identical in the peptide sequence. Poly-

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clonal antibodies against this peptide were affinity purified (Covance Research Products) from the serum of immunized rabbits for use at a dilution of 1:1,000. Before immunohistochemical studies, the specificity of this antibody was confirmed by preadsorption of the antibody to rOAT1 peptide. To identify different segments of the nephron by characteristic cellular distribution of filamentous actin (F-actin), tissue sections were dual stained with Texas red-conjugated phalloidin (Molecular Probes, Eugene, OR). In some cases, an antibody against Tamm-Horsfall protein (Accurate Chemical and Scientific, Westbury, NY) was also used at a dilution of 1:200 to identify outer medullary portions of the thick ascending limb of Henle, where the S3 portion of PT may coexist.

**Tissue preparation for immunofluorescence.** The pieces of kidney tissues including cortex and medulla, not thicker than 0.5 cm and not longer than 1.0 cm, were immediately dropped into 10 ml of 2% paraformaldehyde-0.075 M lysine-0.01 M periodate fixative on ice for 30 min. After fixation, the tissue was washed three times with ice-cold PBS consisting of (in mM) 2.7 KCl, 1.5 KH$_2$PO$_4$, 137 NaCl, and 8 NaH$_2$PO$_4$. Each wash was carried out for 10 min on ice. After this step, the tissue was cryoprotected by transferring it to a 50-ml conical tube containing 40 ml of 40% sucrose in PBS. The tissue was allowed to remain in this solution for at least 48 h at 4°C, until the tissue was removed from the 40% sucrose solution, immersed in OCT cryoembedding compound (Miles), frozen in liquid N$_2$, and stored at −80°C.

![Fluorescence microscopy of organic anion transporter 1 (OAT1) in rat control kidney tissues showing the cortex (A, a, B, b) and medulla (C, c). Kidney tissues were dual stained with Texas red-conjugated phalloidin to label F-actin (a, b, c). Proximal tubule (PT) cells are identifiable by the presence of brush border. OAT1 (shown in green) is present mostly in S2 segments and S3 PT segments (a, b). PT cells show basolateral distribution of OAT1 (A, B, a, b). Glomeruli (glomeruli), arterioles (*), and arteries (**) do not express OAT1. In the inner stripe of outer medulla (C, c), vasa rectae (VR) and peritubular cells (arrowheads) weakly express OAT1. Otherwise, OAT1 is not expressed in the medulla. Images were obtained by 3-dimensional reconstruction of serial optical images of kidney tissue sections. Bars = 20 μm. Supplementary material for this article is available on the journal website.](image-url)
Fig. 2. Fluorescence microscopy of OAT1 in renal cortical tissues after ischemia-reperfusion. Control, I5, I30, I45, I60, I60R1, I60R24, I60R48, I60R72, I60R168, and I60R240 indicate the control and experimental conditions: 5, 30, 45, 60 min after ischemia (I) and 1, 24, 48, 72, 168, and 240 h after reperfusion (R) following 60 min of ischemia, respectively. *Arterioles; **artery, ec, Exfoliated tubule cells. Arrows indicate clumps or aggregates of OAT1. Arrowheads indicate peritubular OAT1 staining (I60, I60R72, I60R240). ●, Proximal tubules with discernable lateral membrane OAT1 localization. Images were obtained by 3-dimensional reconstruction of serial optical images of kidney tissue sections. See text for details. Bars = 20 μm.
Immunofluorescence staining. The frozen tissue block was mounted onto chucks and sectioned with a cryotome (CM 1800; Leica, Nussloch, Germany). Sections (8 μm thick) were transferred onto "ProbeOn Plus" glass slides from Fisher Scientific. Frozen sections were extracted for 10 min at room temperature with cytoskeleton buffer consisting of (in mM) 50 NaCl, 300 sucrose, 10 PIPES (pH 6.8), 3 MgCl₂, and 1 PMSF and 0.5% Triton X-100. The slides were then washed twice with PBS at room temperature. Each wash was carried out for 10 min. After this step, slides were incubated in blocking solution for 2 h at room temperature in a humidified chamber. The blocking solution consisted of PBS containing 20% normal goat serum (NGS), 0.2% BSA, 50 mM NH₄ Cl, 25 mM glycine, and 25 mM llysine. After the slides were blocked, they were washed twice with PBS containing 0.2% BSA. The washes were carried out for 10 min at room temperature. The slides were then incubated with an affinity-purified anti-OAT1 antibody solution at a dilution of 1:1,000 for OAT1 overnight at 4°C in a humidified chamber. The primary antibody was diluted in PBS containing 20% NGS and 0.2% BSA. The following day, slides were again washed twice with PBS containing 0.2% BSA. Both washes were conducted for 10 min at room temperature. The slides were then incubated with fluorescein-conjugated anti-rabbit antibody solution and Texas red-conjugated phalloidin for 2 h at room temperature in a humidified chamber for double labeling experiments. Texas red-conjugated phalloidin was used to identify PT and vasculature. Secondary antibodies were diluted 1:200 in PBS containing 20% NGS and 0.2% BSA. After the secondary antibody incubation, slides were washed twice in PBS containing 0.2% BSA as above and then mounted with glass coverslips in PBS containing 16.7% Mowiol (Calbiochem), 33% glycerol, and 0.1% paraphenylene diamine. Slides were viewed using the inverted Leica TCS SP2 AOBS confocal microscope (Exton, PA) with a ×63 oil objective lens at the Penn State Hershey College of Medicine Imaging Core.

Image analysis. To evaluate OAT1, serial confocal images were taken at 0.325-μm intervals throughout the middle 6-μm depth of tissue sections. A consistent image acquisition setting was used for each batch of immunohistochemical staining. Three-dimensional reconstructions of these images were generated with Metamorph software (Universal Imaging, West Chester, PA). Each three-dimensional rotating video image was evaluated to characterize the cellular and subcellular distribution of OAT1. The degrees of maldistribution of OAT1 in PT cells were scored as follows: 0 = normal basolateral membrane distribution of OAT1 in majority of PT cells, 1 = basal membrane distribution of OAT1 in majority of PT cells, 2 = diffuse cytoplasmic distribution or almost undetectable staining for OAT1 in majority of PT cells, and 3 = cytoplasmic aggregates and/or basal clumps of staining for OAT1 in majority of PT cells.

Western Blot

The rat kidney tissues were homogenized in a buffer containing 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, 1 mM DTT, 1 mM sodium orthovanadate, 1% protease inhibitor cocktail (Sigma; P8340), and 1% protease inhibitor cocktail (Sigma; P2850) and centrifuged to obtain solubilized protein. Aliquots containing 50 μg of protein were analyzed for OAT1 by SDS-PAGE using precast 10% Bis-Tris gel (NuPAGE; NP0321BOX from Invitrogen). Proteins were transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA) in a buffer containing 25 mM bicine, 25 mM Bis-Tris, 0.8 mM EDTA, 20% methanol, and 0.1% antioxidant (NuPAGE antioxidant from Invitrogen). The membrane was blocked for 1 h in a buffer containing 5% nonfat milk, 19 mM Tris base, 80 mM Tris-HCl, 154 mM NaCl, and 0.05% Tween 20. The membrane was then exposed to the affinity-purified anti-OAT1 antibody solution at a dilution of 1:2,000 overnight at 4°C. Secondary antibody was goat anti-rabbit horseradish peroxidase at a dilution of 1:10,000. Washes between steps were done with a wash buffer containing 19 mM Tris base, 80 mM Tris-HCl, 154 mM NaCl, and 0.05% Tween 20. The membrane was detected on Kodak BioMax ML film with enhanced chemiluminescence (Pierce, Rockford, IL). The film was scanned, and each OAT1 was quantified in a densitometer to determine the OAT1 content of each kidney tissue. The data were expressed in ratio to control. At least three batches of rats were analyzed to have a median value for each condition. Rat heart tissue was also processed in parallel for negative tissue control.

Quantitative Real-Time PCR

Total RNA was isolated from snap frozen rat kidney tissues from control and each experimental condition. We then visualized RNA (18S and 28S bands) using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and concentrations were adjusted. First-strand cDNA was then produced from 1.0 μg of total RNA using oligo(dT)₁₂₋₁₈ primers or random hexamer primers (assay ID Rn00568143_m1; Applied Biosystems, Foster City, CA) and the SuperScript III reverse transcription kit (Invitrogen, Carlsbad, CA) by standard methods. The concentration and quality of resulting cDNA were quantified and analyzed with the Agilent 2100 Bioanalyzer or spectroscopically with the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE). Samples were standardized at 20 ng/μl, and 40 ng of cDNA per sample was utilized as a template for real-time PCR using appropriate gene expression assays (assay ID Rn00568143_m1; Applied Biosystems). PCR amplification was performed with the Applied Biosystems sequence detection system 7500 using the absolute quantification with standard curves method. To exclude the possibility of genomic DNA contamination, control PCR reactions with no cDNA template was also performed for gene-specific primer sets. Triplicates of each PCR reaction were performed, and the resultant data were averaged. Amplification data for the gene of interest was normalized to GAPDH gene expression within each individual PCR reaction and calibrated to one control sample to obtain the resulting expression values.

Measurement of BUN and Serum Creatinine

BUN and serum creatinine were measured with assay kits from Ortho-Clinical Diagnostics (VITROS DT60i chemistry slides) and Diazyme Labs (no. DZ072B), respectively, and manufacturers’ instructions were followed for both.

Statistical Analysis

Results of scoring of PT OAT1, Western blot, quantitative real-time PCR, and measurement of BUN and serum creatinine are presented in box plots illustrating respective median values and quartile distributions of the data. Statistical significance of the differences in the data among the experimental groups and the control was evaluated by one-way ANOVA with post hoc multiple comparisons test. We performed all statistical analyses using SPSS 11.5 software.

RESULTS

OAT1 was expressed in S2 and S3 segments of PT and occasionally in vasculature. Control kidney tissues demonstrated distinct basolateral membrane distribution of OAT1, mostly in S2 segments but also in S3 segments of PT. Basal

Fig. 3. Fluorescence microscopy of OAT1 in renal medullary tissues after ischemia and reperfusion. Bottom right: control tissue section stained with anti-OAT1 antibody preadsorbed to rat OAT1 peptide. Arrowheads indicates peritubular OAT1 staining. Images are 3-dimensional reconstructions of serial optical images of kidney tissue sections. See text for details. Bars = 20 μm.
staining for OAT1 was stronger than lateral staining and heterogeneous in intensity (Fig. 1, A and B). Glomeruli, arterioles, and peritubular cells did not express OAT1. In inner stripe of outer medulla, vasa rectae and peritubular cells weakly expressed OAT1 (Fig. 1C). Otherwise, OAT1 was not expressed in medulla.

**Effects of Ischemia and Ischemia-Reperfusion on Cellular Distribution of OAT1**

After 5 and 30 min of ischemia (Fig. 2, I5 and I30), the lateral membrane staining for OAT1 was diminished but still discernable in superficial cortex. Staining for OAT1 was observed in cytoplasmic aggregates of exfoliated PT cells. In deep cortex, lateral membrane staining was not discernable (not shown). Cytoplasmic staining for OAT1 was observed with heterogeneous basal membrane distribution in PT cells and also visible in exfoliated PT cells. Glomerular mesangial cells were weakly positive for OAT1 after 30 min of ischemia (Fig. 2, I30). In inner medulla, peritubular cells were distinctly positive for OAT1 (Fig. 3, I5 and I30). Scoring of maldistribution of OAT1 in PT cells is shown in Fig. 4.

After 45 and 60 min of ischemia (Fig. 2, I45 and I60), the overall intensity of the tubular staining of OAT1 was remarkably diminished. Subcellular distribution of OAT1 was similar to the findings shown after 30 min of ischemia. However, the lateral membrane staining for OAT1 was hardly discernable in cortex. In deep cortex, occasional cytoplasmic and basal clumps of the staining along the basal membrane distribution of PT were also noted. Arteriolar and arteriolar smooth muscle cells were weakly stained for OAT1. After 60 min of ischemia, peritubular staining of OAT1 was rarely observed. In medulla, peritubular cells and vasa rectae also stained weakly for OAT1 (Fig. 3).

After 1 h of reperfusion following 60 min of ischemia (Figs. 2 and 3, I60R1), the overall intensity of the staining for OAT1 was further diminished. In cortex, only patchy basal membrane and faint cytoplasmic stainings for OAT1 were observed. Cytoplasmic clumps and occasional clumps along the basal membrane were not discernable. A few PT cells showed heterogeneous basal and well discernable lateral membrane staining for OAT1 with rare clumps of staining along the basal membrane (Fig. 2, I60R1-1). In medulla, staining for OAT1 was present in vasa rectae and peritubular area.

After 24, 48, and 72 h of reperfusion following 60 min of ischemia (Figs. 2 and 3, I60R24, I60R24-1, I60R48, and I60R72), the overall intensity of the staining for OAT1 was low and the characteristic patterns of staining for OAT1 was quite variable. In superficial cortex, many PT cells did not express OAT1 (Fig. 2, I60R24-1), whereas some PT cells showed strong basal and faint lateral membrane staining for OAT1 (Figs. 2 and 3, I60R24). Some PT cells were faintly stained for OAT1 in basal and cytoplasmic distribution (Figs. 2 and 3, I60R72). Also, a few clumps of the staining along the basal membrane were noted (Figs. 2 and 3, I60R24 and I60R48). In deep cortex, PT cells, not expressing OAT1, contained intraluminal aggregates stained for OAT1 (Fig. 2, I60R24-1, vertical arrows). Some PT cells demonstrated fine cytoplasmic clumps of staining (Fig. 2, I60R24-1, horizontal arrow). After 48 h of reperfusion following 60 min of ischemia, arteriolar smooth muscle cells were stained for OAT1 and peritubular staining was occasionally observed (Figs. 2 and 3, I60R48). At 72 h of reperfusion, arterial smooth muscle cells and glomerular mesangial cells were strongly stained for OAT1 (Fig. 2, I60R72-1). In medulla, vasa rectae were stained for OAT1 (Fig. 3, I60R72).

After 168 h of reperfusion following 60 min of ischemia (Figs. 2 and 3, I60R168), the intensity of the staining for OAT1 in PT cells was heterogeneous. In cortex, PT cells still showed diffuse cytoplasmic staining for OAT1. However, patchy basal membrane staining with rare basal clumps was also observed. Redistribution of OAT1 to the lateral membrane was also occasionally discernable. Occasionally, arterial and arteriolar smooth muscle cells, peritubular capillaries (not shown), and glomerular mesangial cells were stained for OAT1. In medulla, vasa rectae were strongly stained for OAT1 (Fig. 3, I60R168).

After 240 h of reperfusion following 60 min of ischemia (Figs. 2 and 3, I60R240), the intensity of PT basal staining for OAT1 was stronger than that at I60R168 and fine granular staining in lateral membrane distribution was also noted (Fig. 3, I60R240). Lateral membrane distribution of OAT1 was well discerned in some PT cells (Fig. 2, I60R240-1). Some PT cells only showed diffuse cytoplasmic staining or irregular cytoplasmic granules and clumps of the staining (Figs. 2 and 3, I60R240). Arterial and arteriolar smooth muscle cells and glomerular mesangial cells were weakly stained for OAT1 (Fig. 2, I60R240-2). In medulla, peritubular capillaries and vasa rectae were strongly stained for OAT1 (Fig. 3, I60R240).

**Quantification of OAT1 by SDS-PAGE and Immunoblotting**

OAT1 was present predominantly in cortex of the kidney (Fig. 5, A and B). The amount of renal cortical OAT1 protein tended to increase during ischemia. However, it diminished rapidly after reperfusion in a time-dependent manner until 72 h, when it began to recover. However, the total amount of

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**Fig. 4.** Scoring of maldistribution of OAT1 in proximal tubule cells after ischemia-reperfusion shown in box plots. See text for the detailed description of scoring. Bars within boxes represent respective median values, and numbers on x-axis indicate the number of images in control and each experimental group. *1, P < 0.05 vs. I60, I60R24, I60R48, and I60R72; *2, P < 0.05 vs. control; *3, P < 0.05 vs. control and I5. • Outlier.
OAT1 protein expression did not reach the control level even after 240 h of reperfusion (Fig. 5C).

Quantification of mRNA Expression for OAT1 by Quantitative Real-Time PCR

Real-time RT-PCR of rat kidney tissues tended to show a remarkable (2.4-fold at 5 min of ischemia) but transient increase in OAT1 gene expression after ischemia. However, these expression levels gradually diminished below control levels after reperfusion (Fig. 6). mRNA expression levels for OAT1 were significantly lower than the control level at 24 and 48 h of reperfusion. The mRNA expression tended to increase thereafter.

Assessment of Renal Function by BUN and Serum Creatinine

The concentrations of BUN and serum creatinine rose after reperfusion following 60 min of ischemia (Fig. 7). Concentrations peaked at 24 h after reperfusion. BUN and serum creatinine concentrations gradually decreased thereafter and reached baseline concentrations at 96 and 72 h after reperfusion, respectively.

DISCUSSION

AKI occurs in ~2–5% of all hospitalized patients and is associated with 30 to over 90% of mortality in patients (1, 3, 4, 13, 17, 18, 41, 44, 50). It is caused by an ischemic insult in the majority of cases. The high morbidity and mortality are due to systemic accumulation of uremic solutes from sustained AKI. In AKI, tubular secretion of organic solutes is markedly depressed and may contribute to uremic state (5, 22, 34). Numerous organic anions, for example, indoxyl sulfate, 3-carboxy-4-methyl-5-propyl-2-furanpropionate, indoleacetate, hippurate, and p-cresol, have been proposed to cause uremic syndrome (6–8, 12, 25, 27, 28, 31, 33, 37, 43, 46, 47). In this study, we aimed to elucidate pathophysiological and cellular mechanisms...
responsible for reduction in solute clearance via renal tubules after ischemia and recovery from it. Recently, our group (16) reported that, in cadaveric renal allografts, OAT1 was remarkably maldistributed after ischemia and reperfusion and PAH clearances were depressed. The values of net tubular PAH secretion were quite variable even at the same level of glomerular filtration rate (16). In the present study, we focused on renal OAT1 as a major mediator of the tubular secretory function. In humans, OAT1 is the major prototypical OAT with the highest affinity for PAH among the OAT isoforms; transport affinity ($K_m$) of hOAT1 for PAH is 5–9.3 $\mu$M, whereas $K_m$ of hOAT3 for PAH is 87 $\mu$M, although it has been reported that human kidneys contain approximately twofold more hOAT3 than hOAT1 in mRNA expression and protein amount (24). In the mammalian kidney, two organic anion transporters, OAT1 and OAT3, which belong to a larger OAT family, have been cloned, immunocytochemically and functionally characterized, and found to transport PAH in addition to a variety of other substrates (35, 39, 45). OAT2 was also demonstrated in the kidney (14, 19, 36), but its PAH-transporting capability is negligible compared with that shown in OAT1 and OAT3. OAT1, with its 5–10 times higher affinity for PAH than that of OAT3, may be the major player in the secretion of PAH and some other organic anions in the mammalian kidney (2, 35, 39). It is a classical PAH/dicarboxylate exchanger mediating high-affinity uptake of PAH by an active sodium-dependent tertiary process in the basolateral membrane of PT cells (2, 11, 21, 32, 38, 39).

Previously, a confocal microscopic analysis of normal male Sprague-Dawley rat kidneys showed that immunoreactivity for OAT1 was detected exclusively in the PT (S1, S2, S3) in the cortex with basolateral membrane staining. Recently, Matsu- zaki et al. (22) reported that levels of mRNA and protein of both rOAT1 and rOAT3 were markedly depressed in the ischemic kidneys after 6–48 h of reperfusion following 30-min bilateral renal vascular clamp and correlated with the uptake of PAH and estrone sulfate by renal slices of the ischemic rats. Schneider et al. (34) also reported that, using a rat model of AKI, after 6- to 336-h reperfusion following bilateral clamping of renal arteries for 45 min, inulin and PAH clearance along with PAH net secretion were initially diminished after ischemia-reperfusion injury and gradually recovered during follow-up, and this initial impairment after AKI was accompanied by decreased

![Fig. 6. Box plots of quantitative real-time PCR analysis of mRNA expression for OAT1 in rat kidney tissue. Values were standardized to controls. Bars within boxes represent respective median values, and numbers on x-axis indicate the number of animals in control and each experimental group. See text for details. *P < 0.05 vs. control.](http://ajprenal.physiology.org/)

![Fig. 7. Concentrations of blood urea nitrogen (BUN, mg/dl; top) and serum creatinine (S-Cr, mg/dl; bottom) after ischemia-reperfusion, shown in box plots. Bars within boxes represent respective median values; numbers on x-axes indicate the number of animals at each time point. *1, P < 0.05 vs. I60R24 and I60R48; *2, P < 0.05 vs. control, I60, I60R1, I60R96, I60R120, I60R144, I60R168, and I60R240; *3, P < 0.05 vs. control, I60, I60R1, I60R120, I60R144, I60R168, and I60R240; *4, P < 0.05 vs. I60R24, *P < 0.05 vs. control, I60, I60R1, I60R96, I60R120, I60R144, I60R168, and I60R240.](http://ajprenal.physiology.org/)
mRNA and protein levels of OAT1 and OAT3 in clamped animals compared with sham-operated controls. In correlation with the improvement of kidney function, both mRNA and protein levels of OAT1 and OAT3 were upregulated during follow up (34). However, the previous reports did not elucidate alterations in the cellular and subcellular localization of OAT1. In the present study, using confocal microscopy, detailed immunohistochemical analysis of OAT1 was performed with a three-dimensional reconstruction of serial optical sections. Ischemia was induced by clamping the left renal artery for 5, 30, 45, and 60 min to elucidate time-dependent alterations of OAT1 after ischemia. To clarify dynamic alterations of renal OAT1 in a severe form of ischemic renal injury, ischemia was induced by clamping bilateral renal pedicles for 60 min before reperfusion. The reperfusion experiments were performed at various timed intervals: 1, 24, 48, 72, 168, or 240 h after the bilateral renal arterial clamp was released. Our findings demonstrated that OAT1 did not decrease in protein amount until after 1 h of reperfusion. However, its distribution was markedly altered as early as 5 min of ischemia. The transient increases in OAT1 protein during ischemia may suggest that the increased amount of the protein is present in the form of intraluminal aggregates of exfoliated PT cells. The diminished and maldistributed OAT1 protein did not completely recover to the control level even after 240 h of reperfusion. Of note, OAT1 expression remarkably increased in smooth muscle cells of vasculature (arteries, arterioles, and vasa rectae) and mesangial cells after ischemia-reperfusion. Recently, Price et al. (30) reported that human vascular smooth muscle cells express an urate transporter (URAT1). Organic anion transporting polypeptide 3 (Oatp3) immunoreactivity is known to be detected in mouse brain capillary endothelial cells (29). The potential contributory role of vascular OAT1 in ischemic AKI warrants further research.

Our findings showing maldistribution of OAT1 resemble a demonstration of similar maldistribution of Na\(^{+}\)-K\(^{-}\)-ATPase from the normal basolateral membrane to the cytoplasm after ischemia (15). However, OAT1 staining in the present study differs from that of Na\(^{+}\)-K\(^{-}\)-ATPase mainly in that cytoplasmic aggregates, especially along the basal membrane, are more prominent from ischemia-reperfusion injury. Most nonselective protein degradation takes place in lysosomes. Protein degradation can also occur through ubiquitination. Ubiquitination serves to target proteins for proteasomal destruction (10). The mechanism(s) by which OAT1 protein is degraded in PT cells after ischemia-reperfusion remains to be elucidated. Whether protein sorting pathways are cell type specific or protein specific during recovery is not entirely clear. Nonetheless, three sites of protein sorting have been identified: the trans-Golgi network, endosomes, and the plasma membrane (26). How the newly formed OAT1 is sorted out in renal PT cells will require future research. Of note, mRNA expression for OAT1 was transiently increased after 5 min of ischemia and not correlated to the protein expression.

Serum creatinine concentrations have been most commonly used as a marker representing “renal function.” Our findings confirm that it may not be a surrogate marker representing total renal solute clearance in ischemic AKI since serum creatinine values are already normalized even when renal OAT1 remains drastically deranged.

In summary, we have shown that OAT1 was present predominantly in some PT cells of renal cortex. OAT1 was distributed to basolateral membranes of PT cells in controls. After ischemia-reperfusion, OAT1 was maldistributed to the cytoplasm, often forming aggregates. The protein amount of OAT1 diminished after reperfusion following 60 min of ischemia until 72 h after reperfusion. However, even at 240 h, the amount of OAT1 did not reach control levels. Quantitative real-time PCR of the kidney tissues tended to show a remarkable but transient increase in mRNA expression for OAT1 at 5 min of ischemia. Interestingly, a distinct increase in OAT1 expression was noted in vasculature early after ischemia and after reperfusion. This may suggest a contributory role of vascular OAT1 when PT cell OAT1 diminishes in ischemic AKI. These findings may provide insights into dynamic alterations of renal OAT1 during ischemic AKI and recovery from it, especially when therapeutic intervention is attempted to increase solute clearances through the kidney.

GRANTS

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ALTERATION OF RENAL OAT1 AFTER ISCHEMIA-REPERFUSION


