Precise Comparison of Protein Localization Among OCT, OAT, and MATE in Human Kidney

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ABSTRACT: Organic anion transporters (OATs) and organic cation transporters (OCT) play pivotal roles in the uptake of drugs into epithelial cells at the basolateral membranes, and multidrug and toxin extrusion (MATE) mediates drug secretion into urine at the brush-border membranes. In this study, the expression and distribution of apical MATE1 and MATE2-K, and basolateral OAT1, OAT3, and OCT2 were compared using serial sections of human kidney cortex. First, mRNA expression in the proximal tubules was evaluated using laser microdissection. Levels of OAT, OCT2, and MATE mRNA in the proximal tubules were greatly higher compared with glomerulus. The results quantitatively indicated that these transporters were localized to proximal tubules in the renal cortex. Second, MATE1 and MATE2-K protein were detected in proximal epithelial cells in which OCT2 protein was expressed at the basolateral membranes. In addition, MATE1 was expressed at the brush-border membranes of tubular epithelial cells in which OAT1 and OAT3 were expressed. The results confirmed that OAT1, OAT3, OCT2, MATE1, and MATE2-K were coexpressed in tubular epithelial cells. The cooperation among OAT, OCT, and MATE in renal drug secretion was consistent with their distribution. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3302–3308, 2013

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INTRODUCTION

The kidney plays an important role in the excretion of endogenous and exogenous xenobiotics, such as drugs and toxins. In renal proximal tubules, membrane transport systems are responsible for the renal tubular secretion or reabsorption of drugs.[1] Various transporters in the basolateral membrane have been identified including organic anion transporters (OATs)[2–4] and organic cation transporters (OCTs),[5,6] which mediate the uptake of drugs from the blood into tubular cells. In previous studies,[7] we have revealed the expression profile of drug transporters in human kidney. Importantly, the mRNA levels of OAT1 and OAT3 were higher than those of other transporters, and OAT1 and OAT3 proteins were expressed in the basolateral membranes of proximal convoluted tubules (S1 and S2 segments). In addition, OCT2 was expressed in proximal convoluted tubules and proximal straight tubules (S1–S3 segments), and its mRNA level was the highest of any organic cation transporter in the kidney. OAT1, OAT3, and OCT2 are assumed to be major transporters in the basolateral membranes of proximal tubules.

In the brush-border membrane, H^+/organic cation antiport systems have been proposed to be responsible for the secretion of cationic substrates. Recently, human multidrug and toxin extrusion (MATE) 1 and MATE2-K have been identified as H^+/organic cation antiporters.[8,9] Functional analyses using cultured cells expressing MATE1 and MATE2-K suggested that MATE1 and MATE2-K

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Table 1. The Profiles of Seven Surgically Nephrectomized Patients with Renal Cell Carcinoma

Sex (Male/Female)	Age (Years)	Serum Creatinine (mg/dL)	Blood Urea Nitrogen (mg/dL)
4/3	54–76	0.70–0.90	11.0–19.0

transport typical cationic compounds, such as tetraethylammonium, neurotoxin 1-methyl-4-phenylpyridinium (MPP), N^1-methylnicotinamide, and creatinine.[10–13] Various cationic drugs including the antiarrhythmic agent procainamide, H_2-blocker cimetidine, and antidiabetic metformin are also transported by these proteins. In the human kidney, MATE1 and MATE2-K may play an important role in the secretion of cationic xenobiotics. MATE1 and MATE2-K have a similar substrate specificity with some exceptions. Zwitterionic β-lactam antibiotics such as cephalexin and cephradine were shown to be transported by MATE1 but not by MATE2-K.[13] Furthermore, zwitterionic drugs such as fexofenadine and fluoroquinolones were transported by MATE1.[14,15] In addition, MATE1 was found to be responsible for the renal tubular secretion of a zwitterionic substrate, cephalexin, *in vivo* using MATE1 knockout mice.[16]

Apical and basolateral transporters may function cooperatively at the respective membranes of proximal epithelia. However, their precise localization is not compared in the human and rodent kidney. Previously, we precisely compared the localization of OAT1, OAT3, and OCT2 in the human kidney using serial sections.[7] In this study, the distribution of apical MATE1 and MATE2-K, and basolateral OCT2, OAT1, and OAT3 were compared using serial sections of human kidney cortex.

MATERIALS AND METHODS

Patient Profiles

Normal parts of renal tissues were obtained from seven surgically nephrectomized patients with renal cell carcinoma at Kyoto University Hospital (four men and three women) from 2008 to 2009 (Table 1). The patients ranged in age from 54 to 76 with average age of 67 years. The results of clinical laboratory tests for renal function were in the normal range. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Kyoto University. All patients gave written informed consent.

Isolation of Total RNA from Specimens Dissected by Laser Microdissection

Total mRNA was isolated from tissue samples dissected by a laser microdissection system.[17] The renal samples were embedded in OCT compound (Sakura Finetek, Tokyo, Japan) without any fixation and rapidly frozen in liquid nitrogen. Sections (6 μm thick) were cut serially using a microtome, stained with a 0.01% toluidine blue solution, and washed with ice-cold RNase-free water. Under visual control, selected tissue compartments (glomerulus and proximal tubules) were cut from surrounding tissue by laser microdissection (Leica CTR MIC, Fig. 1). More than 50 pieces of glomerulus and proximal tubules were collected for each patient. Collected samples were placed in the lysis buffer of an RNeasy Plus Micro Kit (Qiagen, Hilden, Germany).

Levels of mRNA for transporters were evaluated by real-time PCR as described previously.[7,9] Briefly, total RNA was extracted from renal specimens using the RNeasy Plus Micro Kit (Qiagen) according to the manufacturer's procedure. Isolated total RNA was reverse-transcribed to cDNA, and the reaction mixtures were used for real-time PCR.

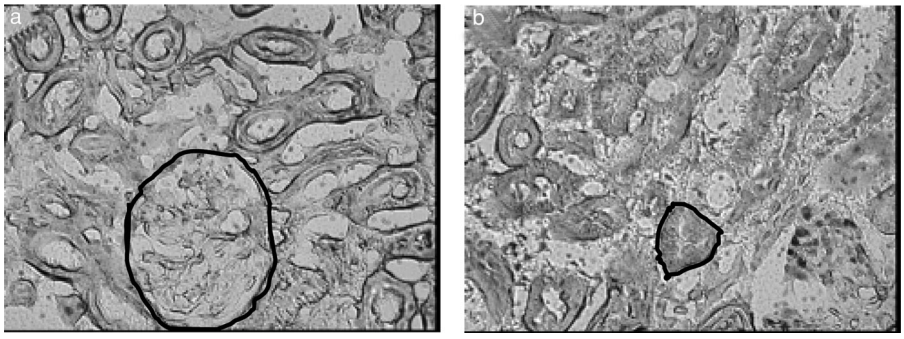

Figure 1. The collection of renal glomerulus (a) and proximal tubules (b) using the Leica laser microdissection (LMD) system. Frozen sections of kidney cortex were stained with a 0.01% toluidine blue solution and glomerulus and proximal tubules were exactly cut precisely as marked by the black lines.