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Transcellular transport of organic cations in double-transfected MDCK cells expressing human organic cation transporters hOCT1/hMATE1 and hOCT2/hMATE1

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ABSTRACT

To clarify the transcellular transport of organic cations via basolateral and apical transporters, we established double-transfected Madin–Darby canine kidney (MDCK) cells expressing both human organic cation transporter hOCT1 and hMATE1 (MDCK-hOCT1/hMATE1), and hOCT2 and hMATE1 (MDCK-hOCT2/hMATE1) as models of human hepatocytes and renal epithelial cells, respectively. Using the specific antibodies, hOCT1 and hMATE1 or hOCT2 and hMATE1 were found to be localized in the basolateral and apical membranes of MDCK-hOCT1/hMATE1 or MDCK-hOCT2/hMATE1 cells, respectively. A representative substrate, [^{14}C]tetraethylammonium, was transported unidirectionally from the basolateral to apical side in these double transfectants. The optimal pH was showed to be 6.5 for the transcellular transport of [^{14}C]tetraethylammonium, when the pH of the incubation medium on the apical side was varied from 5.5 to 8.5. The basolateral-to-apical transport also decreased in the presence of 10 mM 1-methyl-4-phenylpyridinium or 1 mM levofloxacin on the basolateral side of both double transfectants. In MDCK-hOCT2/hMATE1 cell monolayers, but not in MDCK-hOCT1/hMATE1 cell monolayers, the accumulation of [^{14}C]tetraethylammonium was decreased in the presence of 10 mM 1-methyl-4-phenylpyridinium, but significantly increased in the presence of 1 mM levofloxacin. The uptake of [^{14}C]tetraethylammonium, [^{3}H]1-methyl-4-phenylpyridinium, [^{14}C]metformin and [^{3}H]cimetidine, but not of [^{14}C]procainamide and [^{3}H]quinidine, by HEK293 cells was stimulated by expression of the hOCT1, hOCT2 or hMATE1 compared to control cells. However, transcellular transport of [^{14}C]procainamide and [^{3}H]quinidine was clearly observed in both double-transfectants. These cells could be useful for examining the routes by which compounds are eliminated, or predicting transporter-mediated drug interaction.

1. Introduction

Renal tubular secretion of drugs, toxins and endogenous metabolites is one of the most important functions in the kidney. The characteristics of the transport of tetraethylammonium (TEA), a representative substrate of the organic cation transport system, by the basolateral and brush-border membranes revealed that transcellular transport across the renal epithelial cells was mediated by basolateral uptake from blood and subsequent extrusion from the cells into the lumen. The mechanisms of renal secretion of cationic drugs were examined using isolated membrane vesicles from rat kidney

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[1]. It was reported that the TEA transport across basolateral membranes was stimulated by an inside-negative membrane potential, and that across brush-border membranes was driven by an H^+ gradient.

Human organic cation transporter hOCT1 (SLC22A1) and hOCT2 (SLC22A2), which act as membrane potential dependent organic cation transporters, are expressed in the basolateral membranes of the liver and kidney, respectively [2]. The basolateral entry of cationic drugs is mediated mainly by hepatic hOCT1 and renal hOCT2 in humans, and hepatic Oct1 and renal Oct1 and Oct2 in mice, depending on the membrane potential [3–5]. In 2005, human multidrug and toxin extrusion 1 (hMATE1/SLC47A1) was isolated, and hMATE1 is expressed in the liver, kidney and skeletal muscle [6]. Thereafter, we identified a kidney-specific hMATE2-K (SLC47A2) [7]. Both mediated oppositely directed H^+ gradient dependent transported cationic compounds, called as H^+/organic cation antiporter, and were located in the brush-border membranes of the renal proximal tubules. Considering their characteristics, the hMATE family mediated the excretion of cationic drugs from the epithelial cells to luminal side.

Their substrate specificity, membrane localization and driving force suggested that hOCT2 and hMATE1 mediated tubular secretion of cationic drugs from blood to urine [3,7,8]. However, an in vitro model that reflects the vectorial transport of cationic drugs across human epithelial cells has not been established. Consequently, the porcine kidney epithelial cell line LLC-PK_1 has been employed to analyze transcellular transport [9,10]. These studies indicated that cationic drugs were transported unidirectionally from the basolateral to apical side in LLC-PK_1 cell monolayers. Previously, MDCK cells expressing both human organic anion transporter 8 (OATP8/SLCO1B3) and multidrug resistance protein 2 (MRP2/ABCC2) or both human organic anion-transporting polypeptide (OATP-C/SLCO1B1) and MRP2 were constructed to determine the transcellular transport of organic anions, and the vectorial transport of double-transfectants suggested their usefulness as in vitro hepatocyte models with an anion transport system [11,12].

Based on these backgrounds, it is necessary to clarify whether the basolateral hOCT1 or hOCT2 and the apical hMATE1 mediated the transcellular transport of cationic compounds. In the present study, we established MDCK cells stably expressing both hOCT2 and hMATE1 as an in vitro model of human renal epithelial cells. Human hepatocyte model expressing both hOCT1 and hMATE1 was also constructed. Moreover, the availability of these double-transfectants was evaluated to examine the transcellular transport of several cationic drugs.

2. Materials and methods

2.1. Materials

[^{14}C]Tetraethylammonium (TEA; 2.035 GBq/mmol), [^{14}C]creatinine (2.035 GBq/mmol), [^{14}C]procainamide (2.035 GBq/mmol), and [9-^{3}H]quinidine (740 GBq/mmol) were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). [^{14}C]Metformin (962 MBq/mmol), [^{14}C]guanidine hydrochloride (1.961 GBq/mmol) and [1-^{14}C]-D-mannitol were purchased from Moravek Biochemicals Inc. (Brea, CA). [^{3}H]1-Methyl-4-phenylpyridinium acetate (MPP; 2.7 TBq/mmol) and D-[1-^{3}H(N)]-mannitol were from PerkinElmer Life Analytical Science (Boston, MA). [*N*-Methly-^{3}H]cimetidine (451 GBq/mmol) was from GE Healthcare (Buckinghamshire, UK). All other chemicals used were of the highest purity available.

2.2. Cell culture and transfection

The parental MDCK cells (ATCC CCL-34) obtained from American Type Culture Collection were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (Sigma–Aldrich, St. Louis, MO) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) in an atmosphere of 5% CO_2 and 95% air at 37 °C. The hOCT1 or hOCT2 cDNA was subcloned into the Not I-cut mammalian expression vector pcDNA3.1(+) (Invitrogen). The hMATE1 cDNA was subcloned into the XbaI- and Kpn I-cut mammalian expression vector pcDNA3.1(+)/Hygro (Invitrogen). MDCK cells were cotransfected with either pcDNA3.1(+) containing hOCT1 or hOCT2 cDNA and pcDNA3.1(+)/Hygro containing hMATE1 cDNA using LipofectAMINE 2000 Reagent (Invitrogen) according to the manufacturer's instructions. Forty-eight hours later, the cells split between 1:25 and 1:100 were cultured in complete medium containing G418 (0.5 mg/ml: Nacalai Tesque Inc., Kyoto, Japan) and Hygromycin B (0.2 mg/ml; Invitrogen). Seven to fourteen days after the transfection, single colonies appeared and several G418- and Hygromycin B-resistant colonies were picked out based on the growth rate and morphology of the cells. These MDCK cells were selected on the basis of the cellular uptake of [^{14}C]TEA and named MDCK-vector (MDCK cells cotransfected with pcDNA3.1(+) empty vector and pcDNA3.1(+)/Hygro empty vector), MDCK-hOCT1/hMATE1 (MDCK cells cotransfected with pcDNA3.1(+) containing hOCT1 cDNA and pcDNA3.1(+)/Hygro containing hMATE1 cDNA) and MDCK-hOCT2/hMATE1 (MDCK cells cotransfected with pcDNA3.1(+) containing hOCT2 cDNA and pcDNA3.1(+)/Hygro containing hMATE1 cDNA). For the transcellular transport experiments, cells were seeded on microporous membrane filters [3.0-μm pores, 4.7 (or 1.0) cm^2 growth area] inside a Transwell cell culture chamber (Costar, Cambridge, MA) at a density of 5×10^5 cells/cm^2 with complete medium, as described above. In this study, MDCK cells were used between the 80th and 86th passages. HEK293 cells (American Type Culture Collection CRL-1573) were cultured as well as MDCK cells. pCMV6-XL4 plasmid vector DNA (OriGene Technologies, Rockvill, MD) that contained hOCT1 cDNA or hOCT2 cDNA or pcDNA3.1 (+)/Hygro vector DNA that contained hMATE1 cDNA was introduced into HEK293 cells using LipofectAMINE 2000 Reagent. At 48 h after the transfection, the cells were used for uptake experiment.

2.3. Polyclonal antibodies and immunofluorescence microscopy

Polyclonal antibodies were raised against the candidate peptide as described, previously [7,13]. For immunostaining, the double-transfected MDCK cells were grown 4 days in Matsunami Micro Cover Glass (Matsunami Glass Ind., Ltd., Osaka, Japan). The double-transfected MDCK cells were fixed