Drug Metabolism and Pharmacokinetics xxx (2017) 1–7

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular Article

Functional characteristics of a renal H^+/lipophilic cation antiport system in porcine LLC-PK_1 cells and rats

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ARTICLE INFO

Article history:
Received 4 October 2017
Received in revised form
15 November 2017
Accepted 28 November 2017
Available online xxx

Keywords:
Quinidine
Bisoprolol
H^+/lipophilic cation antiport system
LLC-PK_1 cells
Rat
Renal excretion

ABSTRACT

We have recently found an H^+/quinidine (a lipophilic cation, QND) antiport system in Madin-Darby canine kidney (MDCK) cells. The primary aim of the present study was to evaluate whether the H^+/lipophilic cation antiport system is expressed in porcine LLC-PK_1 cells. That is, we investigated uptake and/or efflux of QND and another cation, bisoprolol, in LLC-PK_1 cells. In addition, we studied the renal clearance of bisoprolol in rats. Uptake of QND into LLC-PK_1 cells was decreased by acidification of the extracellular pH or alkalization of the intracellular pH. Cellular uptake of QND from the apical side was much greater than from the basolateral side. In addition, apical efflux of QND from LLC-PK_1 cells was increased by acidification of the extracellular pH. Furthermore, lipophilic cationic drugs significantly reduced uptake of bisoprolol in LLC-PK_1 cells. Renal clearance of bisoprolol in rats was approximately 7-fold higher than that of creatinine, and was markedly decreased by alkalization of the urine pH. The present study suggests that the H^+/lipophilic cation antiport system is expressed in the apical membrane of LLC-PK_1 cells. Moreover, the H^+/lipophilic cation antiport system may be responsible for renal tubular secretion of bisoprolol in rats.

1. Introduction

Renal tubular epithelial transport of hydrophilic organic cations, tetraethylammonium (TEA) and metformin, is mediated by an organic cation transporter (OCT) on the basolateral membrane, and by multidrug and toxin extrusion (MATE) on the apical membrane [1,2]. Cimetidine and procainamide are also the substrates of OCT and MATE; however, most lipophilic organic cations, such as quinidine (QND), are not very good substrates of OCT and MATE [3–6]. We previously reported the directional transcellular transport of lipophilic organic cations (QND, bisoprolol, and flecainide) in P-glycoprotein (P-gp)-expressing LLC-GA5-COL150 cells [3,7]. The findings suggested that the renal tubular secretion of lipophilic organic cations is mediated partly by P-gp (Fig. 1). However, the extent of contribution of P-gp on actual renal excretion of lipophilic organic cations is still unclear.

Recently, we have reported that a new membrane transport system for QND is expressed in Madin-Darby canine kidney (MDCK) cells [8]. Uptake of QND was saturable, temperature dependent, extracellular Na^+-independent, and membrane potential independent. Moreover, uptake of QND was increased by alkalization of extracellular medium and by intracellular acidification with pre-treatment of NH_4Cl, and was decreased by intracellular alkalization with acute treatment of NH_4Cl [8]. In addition, efflux of QND from MDCK cells at extracellular medium pH 5.4 was greater than at pH 8.4. These findings suggested that an H^+/lipophilic cation antiport system is involved in pH-dependent renal reabsorption and/or secretion of some drugs in renal tubular cells (Fig. 1) [8].

In the present study, we evaluated whether an H^+/QND antiport system is expressed in porcine kidney LLC-PK_1 cells as well as canine kidney MDCK cells. In addition, we evaluated whether the system in LLC-PK_1 cells recognizes a secondary amine, bisoprolol, which is widely used for the treatment of patients with cardiovascular diseases [9]. Moreover, we also investigated whether the H^+/lipophilic cation antiport system is involved in the renal excretion of bisoprolol in rats.

2. Materials and methods

2.1. Materials

[^{3}H]QND (740 GBq/mmol) and [^{14}C]mannitol (2.04 GBq/mmol) were obtained from American Radiolabeled Chemical Inc. (St Louis,

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https://doi.org/10.1016/j.dmpk.2017.11.313
1347-4367/

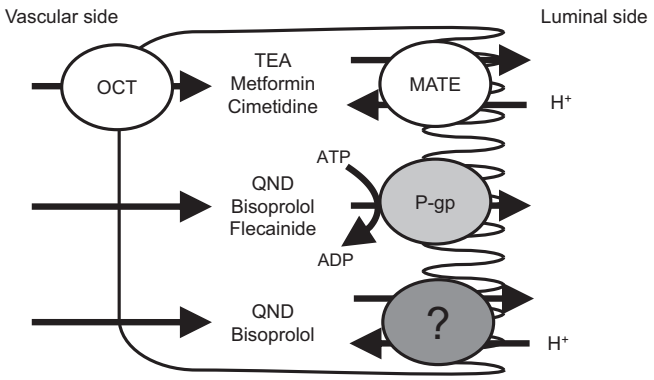

Fig. 1. Secretion mechanisms of hydrophilic and lipophilic organic cations in renal tubular epithelial cells.

MO, USA). QND hydrochloride, metformin, and pyrilamine maleate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Diphenhydramine (DPH) hydrochloride was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). TEA chloride and cimetidine were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Bisoprolol hemifumarate was obtained from Mitsubishi Tanabe Pharma Co. (Osaka, Japan).

2.2. Cell culture

LLC-PK_1 cells at passage 197 were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were seeded at a density of 5×10^5 cells/cm^2 on a 2- or 3.8-cm^2 plastic dish using a Falcon multiwell plate (BD Bioscience, Bedford, MA, USA). In addition, the cells were seeded at the same density on a 1.12-cm^2 porous membrane (0.4-μm pore size) in a polyester membrane Transwell®-Clear insert (Costar, Cambridge, MA, USA). Cells were maintained with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France) in an atmosphere of 5% CO_2-95% air at 37 °C for 7 days. All experiments with LLC-PK_1 cells were carried out between passages 210 and 217.

2.3. Apical uptake of [^{3}H]QND in LLC-PK_1 cells

The effect of the extracellular pH and concomitant DPH on the apical uptake of [^{3}H]QND in LLC-PK_1 cells was investigated as reported previously with a minor modification [8,10]. First, the cells were pre-incubated with 500 μL of incubation medium (125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, and 25 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES), pH 7.4) containing 30 μM unlabeled QND for 30 min at 37 °C. Then, the cells were incubated with 500 μL of incubation medium containing 30 μM QND, of which 10 nM was [^{3}H]QND (0.1 μCi/well), for 5 min. After the incubation, the cells were immediately washed with ice-cold phosphate buffer. They were solubilized by 1.0 mL of 2% sodium dodecyl sulfate. The amount of [^{14}C]mannitol was determined to estimate the extracellular trapping of QND. The amount of radiolabeled compounds and protein content of the cells were determined as described previously [8].

The effect of intracellular pH on uptake of [^{3}H]QND in LLC-PK_1 cells was evaluated using 30 mM NH_4Cl, as reported previously [8].

2.4. Basolateral uptake of [^{3}H]QND in LLC-PK_1 cells

Cellular uptake of QND from the apical and basolateral side was investigated using LLC-PK_1 cells seeded on a porous membrane. One mL and 2.3 mL incubation medium (pH 7.4 or 6.4) containing 30 μM unlabeled QND were applied to the apical chamber and the basolateral chamber, respectively. The cells were pre-incubated for 5 min at 37 °C, and incubation medium in the apical chamber was replaced with 1 mL incubation medium containing 30 μM QND, of which 10 nM was [^{3}H]QND, in order to examine the uptake from apical side. In the case of the uptake study from the basolateral side, incubation medium in the basolateral chamber was replaced with 2.3 mL incubation medium containing 30 μM QND, of which 10 nM was [^{3}H]QND. The cells were incubated for 5 min at 37 °C, and were washed with ice-cold phosphate buffer. Radioactivity and protein content were determined as described above.

2.5. Apical efflux of [^{3}H]QND from LLC-PK_1 cells

The cells were pre-incubated for 45 min at 37 °C with 500 μL of incubation medium (pH 7.4) containing 30 μM QND, of which 10 nM was [^{3}H]QND. Then, the cells were incubated with 500 μL of incubation medium (pH 5.4 or 8.4) containing 30 μM unlabeled QND for 1 min, and then washed with ice-cold phosphate buffer. The radioactivity and protein content were determined as described above.

2.6. Effect of cationic compounds on apical uptake of cimetidine and bisoprolol in LLC-PK_1 cells

The effect of several compounds on uptake of cimetidine or bisoprolol was investigated using LLC-PK_1 cells cultured on a multiwell plate. First, the cells were pre-incubated with 500 μL of fresh incubation medium (pH 7.4) containing an inhibitor for 5 min at 37 °C. After pre-incubation, the cells were incubated with 500 μL of incubation medium containing the inhibitor and 100 μM cimetidine or bisoprolol for 5 min. The cells were immediately washed with ice-cold phosphate buffer. The amount of cimetidine or bisoprolol was measured with an HPLC method, as described below.

2.7. Renal excretion of bisoprolol and cimetidine in rats

Male Wistar rats (228–301 g) were obtained from Japan SLC Inc. (Hamamatsu, Japan). All animal experiments were performed in accordance with the Guidelines for Animal Experiments of University of Toyama.

The jugular vein was cannulated with a polyethylene tube (SP-31, Natsume Seisakusho, Tokyo, Japan) for intravenous drug infusion, and the femoral artery was cannulated for blood collection. The bladder was cannulated with a polyethylene tube (PE-50, Becton Dickinson and Co., Franklin Lakes, NJ, USA) for urine collections. The 2% mannitol-saline solution was intravenously infused at a rate of 6 mL/h throughout the experiment, including the 40-min stabilizing period. Then, bisoprolol hemifumarate or cimetidine was intravenously infused at a 2-fold higher rate than the specified infusion rate from time 0–10 min and, thereafter, it was infused at the specified infusion rate from time 10–70 min. The urine was collected from 60 to 70 min, and the blood collection was carried out at 70 min.

The effect of the urine pH on renal excretion of bisoprolol was investigated using $NaHCO_3$. That is, intravenous $NaHCO_3$ infusion at a rate of 40 mg/min/kg was performed to induce the alkalization of urine.

2.8. Analytical methods

The amount of bisoprolol in the sample was determined by a reversed-phase HPLC method as described previously [9]. The column was COSMOSIL 5C18-AR-II (15 cm × 4.6 mm, i.d. 4.5 μm particle size, Nacalai Tesque). The mobile phase consisted of 10 mM

Please cite this article in press as: Matsui R, et al., Functional characteristics of a renal H^+/lipophilic cation antiport system in porcine LLC-PK_1 cells and rats, Drug Metabolism and Pharmacokinetics (2017), https://doi.org/10.1016/j.dmpk.2017.11.313