Membrane Transport Mechanisms of Quinidine and Procainamide in Renal LLC-PK$_1$ and Intestinal LS180 Cells

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The aim of the present study was to compare the membrane transport mechanisms of procainamide with those of quinidine using renal epithelial LLC-PK$_1$ and intestinal epithelial LS180 cells. In LLC-PK$_1$ cells, the transcellular transport of 10 μM quinidine in the basolateral-to-apical direction was similar to that in the opposite direction, and 1 mM tetraethylammonium (TEA) did not affect the transcellular transport of the drug. On the other hand, the transcellular transport of 10 μM TEA and procainamide in LLC-PK$_1$ cells was directional from the basolateral side to the apical side. In addition, this directional transcellular transport of procainamide was diminished in the presence of 1 mM TEA. In LS180 cells, the temperature-dependent cellular uptake of 100 μM quinidine and procainamide was markedly increased by alkalization of the apical medium, and was inhibited significantly by 1 mM several hydrophobic cationic drugs, but not by TEA. The rank order of the inhibitory effects of hydrophobic cationic drugs on the uptake of procainamide in LS180 cells was imipramine>quinidine>diphenhydramine≈pyrilamine>procainamide, which was consistent with that on the uptake of quinidine. These findings suggested that procainamide (but not quinidine) was transported by cation transport systems in renal epithelial cells, but that both procainamide and quinidine were taken up by another cation transport system in intestinal epithelial cells.

Key words procainamide; quinidine; LLC-PK$_1$ cell; LS180 cell

We previously evaluated the renal excretion mechanism of quinidine, which is a tertiary amine compound, using porcine kidney epithelial LLC-PK$_1$ cells and P-glycoprotein (P-gp)-expressed LLC-GA5-COL150 cells.[1] The transepithelial transport of quinidine in the basolateral-to-apical direction in LLC-PK$_1$ cells was similar to that in the opposite direction. In contrast, quinidine was transported actively in the basolateral-to-apical direction in LLC-GA5-COL150 cells. The results suggested that P-gp is mainly responsible for the tubular secretion of quinidine in the kidney.[1] We also evaluated the intestinal absorption mechanism of quinidine using human intestinal epithelial Caco-2 cells.[2] The temperature-dependent uptake of quinidine in Caco-2 cells grown on a plastic dish was increased by alkalization of the apical medium, and was inhibited by diphenhydramine and imipramine. The results suggested that a cation transport system was involved in the influx of quinidine at the apical membrane in intestinal epithelial cells.[2]

Procainamide, another tertiary amine compound with a pK_a value of 9.23, is classified as a type IA antiarrhythmic drug that works by decreasing conduction velocity, and prolonging tissue refractoriness.[3] More than 80% of orally administered procainamide is absorbed from the intestine in humans.[4] The Kp (octanol/buffer at pH 7.4) value of procainamide is about 0.1, and approximately half of the dose is excreted in the urine as unchanged drug.[3—6] However, the mechanisms responsible for the membrane transport of procainamide in intestinal and renal epithelial cells are still unclear.

In the present study, the transport characteristics of procainamide in LLC-PK$_1$ cells were compared with those of quinidine. In addition, we evaluated whether the transport system for quinidine is present in another intestinal cell line, LS180, as well as Caco-2. We also investigated whether the transport system of procainamide in LS180 cells is the same as that of quinidine.

MATERIALS AND METHODS

Materials [^{14}C]Tetraethylammonium (TEA) bromide (2.04 GBq/mmol), [^{3}H]quinidine (740 GBq/mmol), [^{14}C]procainamide hydrochloride (2.04 GBq/mmol), and [^{3}H]mannitol (740 GBq/mmol) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, U.S.A.). [^{14}C]Mannitol (1.96 GBq/mmol) was purchased from Moravek Biochemicals (Brea, CA, U.S.A.). TEA chloride and procainamide hydrochloride were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Quinidine hydrochloride monohydrate was purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). All other chemicals were of the highest purity available.

Cell Culture and Preparation of Monolayers LLC-PK$_1$ cells at passage 197 and LS180 cells at passage 38 were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.). These cells were maintained by serial passage in plastic dishes with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Biowest Inc., Nuaille, France) in an atmosphere of 5% CO_2–95% air at 37 °C.

LLC-PK$_1$ cells were seeded at a density of 5×10^5 cells/cm^2 on a 1.12 cm^2 porous membrane (3 μm pore size) in a polyester membrane Transwell®-Clear insert (Costar, Cambridge, MA, U.S.A.) to evaluate the transcellular transport of cationic drugs. The seeded cells were maintained for 6 d to prepare differentiated cell monolayers. The maturity of the monolayer was judged by transepithelial electrical resistance (TEER). TEER was measured using a Millicell-ERS resistance system (Millipore, Bedford, MA, U.S.A.). LLC-PK$_1$ cell monolayers whose TEER was above 60 $\Omega\cdot$cm^2 were used to

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assess the transcellular transport of cationic drugs. All experiments were carried out with the cells between passages 210 and 217. On the other hand, LS180 cells were seeded at a density of 5×10^5 cells/cm^2 on a 3.8 cm^2 plastic dish using a Falcon® multiwell™ plate (BD Bioscience, Franklin Lakes, NJ, U.S.A.), and maintained for 7 d. All experiments were conducted with LS180 cells between passages 63 and 75.

Transcellular Transport of TEA, Quinidine, and Procainamide in LLC-PK$_1$ Cell Monolayers The transcellular transport of [^{14}C]TEA, [^{3}H]quinidine, and [^{14}C]procainamide in LLC-PK$_1$ cell monolayers prepared on a porous membrane was examined as described previously.[1,7] In brief, the monolayer was pre-incubated for 30 min at 37 °C with culture medium (pH 6.3) containing 10 μM unlabeled drug to equilibrate the drug concentration. After the equilibration period, the radio-labeled drug (0.33 μCi/ml) was applied to the apical chamber (0.75 ml) to examine apical-to-basolateral transcellular transport. [^{3}H]Mannitol was used to estimate the paracellular transport and extracellular trapping of [^{14}C]TEA and [^{14}C]procainamide, and [^{14}C]mannitol was used to estimate that of [^{3}H]quinidine.[1,7,8] A volume (50 μl) of medium in the basolateral chamber (1.5 ml) was then collected 30, 60, and 90 min after the radio-labeled drug was applied. Cells on the porous membrane were collected following the last collection of medium. Radioactivity in the medium and cells was determined using a liquid scintillation counter, and normalized against the initially applied doses. The time course of the transport of cationic drugs in the opposite (basolateral-to-apical) direction was examined in a similar manner.

The transcellular transport of [^{14}C]TEA, [^{3}H]quinidine, and [^{14}C]procainamide were analyzed in a model-dependent manner using NONMEM software running on a mainframe UNIX machine at the Kyoto University Data Processing Center, as described previously.[1,7,8] When transcellular drug transport is examined under the condition where the unlabeled drug concentration in the monolayer is equilibrated with that of the incubation medium in the apical and basolateral chambers, transport data for a small amount of radiolabeled drug can be analyzed using the linear pharmacokinetic model. That is, the following mass balance equations were prepared for pharmacokinetic analysis:

$$\frac{dX_A}{dt} = -\frac{CL_{A\to C}}{V_A}\cdot X_A + \frac{CL_{C\to A}}{V_C}\cdot X_C - \frac{CL_{A\leftrightarrow B}}{V_A}\cdot X_A + \frac{CL_{A\leftrightarrow B}}{V_B}\cdot X_B \quad (1)$$

$$\frac{dX_B}{dt} = -\frac{CL_{B\to C}}{V_B}\cdot X_B + \frac{CL_{C\to B}}{V_C}\cdot X_C + \frac{CL_{A\leftrightarrow B}}{V_A}\cdot X_A - \frac{CL_{A\leftrightarrow B}}{V_B}\cdot X_B \quad (2)$$

$$\frac{dX_C}{dt} = \frac{CL_{A\to C}}{V_A}\cdot X_A + \frac{CL_{B\to C}}{V_B}\cdot X_B - \frac{CL_{C\to A}}{V_C}\cdot X_C - \frac{CL_{C\to B}}{V_C}\cdot X_C \quad (3)$$

where X_A, X_B, and X_C are the amounts of radio-labeled drugs in the apical chamber, basolateral chamber, and monolayer determined at time t, respectively. V_A and V_B indicate the volume of the apical chamber (0.75 ml) and the basolateral chamber (1.5 ml), respectively. V_C indicates the cell volume of cell monolayers obtained from sulfanilamide accumulation experiments, as described previously (1.12 μl/cm$^2\times$ 1.12 cm^2=1.25 μl).[1)] The influx and efflux clearance of cationic drugs at the apical membrane of cells was designated as $CL_{A\to C}$ and $CL_{C\to A}$, respectively. The influx and efflux clearance of cationic drugs at the basolateral membrane of cells was designated as $CL_{B\to C}$ and $CL_{C\to B}$, respectively. Paracellular transport clearance ($CL_{A\leftrightarrow B}$) was estimated by analyzing the transport profile of mannitol using the following mass balance equations:

$$\frac{dX_A}{dt} = -\frac{CL_{A\leftrightarrow B}}{V_A}\cdot X_A + \frac{CL_{A\leftrightarrow B}}{V_B}\cdot X_B \quad (4)$$

$$\frac{dX_B}{dt} = \frac{CL_{A\leftrightarrow B}}{V_A}\cdot X_A - \frac{CL_{A\leftrightarrow B}}{V_B}\cdot X_B \quad (5)$$

Cellular Uptake of Quinidine and Procainamide in LS180 Cells The cellular uptake of [^{3}H]quinidine and [^{14}C]procainamide was examined in the presence of 100 μM unlabeled drug using LS180 cells grown on plastic dishes of a multiwell plate. The composition of the incubation medium was as follows: 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-piperazyl]ethanesulfonic acid (HEPES), and the pH of the medium was adjusted with a solution of NaOH. In order to evaluate the effect of the extracellular pH on the cellular uptake of cationic drugs, HEPES (neutral pH) was replaced with 2-(*N*-morpholino) ethanesulfonic acid (pH 5.5). The cells were first pre-incubated for 10 min at 37 or 4 °C with 2 ml incubation medium containing 100 μM unlabeled drug to equilibrate the drug concentration, followed by incubation for 5 min with 1 ml fresh incubation medium. The incubation medium was replaced with 1 ml fresh incubation medium containing radiolabeled drug (0.2 μCi/ml). After the cells were incubated with radio-labeled drug for another 5—30 min at 37 or 4 °C, they were immediately washed with ice-cold phosphate buffer and collected. Radioactivity in the cells was determined as described above. Radio-labeled mannitol was used to estimate the extracellular trapping of cationic drugs.[8—11)]

The cellular uptake of [^{3}H]quinidine and [^{14}C]procainamide was analyzed in a model-dependent manner using NONMEM software, as described previously.[9,10)] The following mass balance equations were prepared for the pharmacokinetic analysis:

$$\frac{dX_M}{dt} = -\frac{CL_{M\to C}}{V_M}\cdot X_M + \frac{CL_{C\to M}}{V_C}\cdot X_C \quad (6)$$

$$\frac{dX_C}{dt} = \frac{CL_{M\to C}}{V_M}\cdot X_M - \frac{CL_{C\to M}}{V_C}\cdot X_C \quad (7)$$

where X_M and X_C are the amount of radio-labeled drugs in the incubation medium and the cells determined at time t, respectively. V_M indicates the volume of incubation medium (1 ml). V_C indicates the cell volume (2.62 μl/cm$^2\times$3.8 cm^2= 9.96 μl), measured with sulfanilamide, as described previously.[8)] The influx and efflux clearances of cationic drugs were designated as $CL_{M\to C}$ and $CL_{C\to M}$, respectively.

In order to evaluate the effect of extracellular Na^+ on the cellular uptake of [^{3}H]quinidine and [^{14}C]procainamide,

NaCl in incubation medium was replaced with *N*-methyl-D-glucamine hydrochloride, and the pH of the medium was adjusted to 7.4 by the addition of KOH. The effect of various cationic drugs on the uptake of [^{3}H]quinidine and [^{14}C]procainamide in LS180 cells on plastic dishes of a multiwell plate was evaluated at 37 °C. That is, the cells were first preincubated for 10 min with 2 ml incubation medium, followed by 5 min incubation with 1 ml fresh incubation medium supplemented with 1 mM various cationic drugs. The incubation medium was replaced with 1 ml incubation medium containing radio-labeled drug (0.2 μCi/ml). After the cells were incubated with radio-labeled drug for 5 min, the amount of radio-labeled drug in the cells was determined as described above.

Statistical Analysis Values are expressed as the mean±S.E. In all figures, when error bars are not shown, they are smaller than the symbol. Multiple comparisons were performed using Scheffé's test following one-way ANOVA provided that the variances of groups were similar. If this was not the case, Scheffé-type test was applied following Kruskal–Wallis analysis. The statistical significance of differences between two groups was tested using Student's *t*-test provided that the variances of the groups were similar. If this was not the case, the Mann–Whitney *U*-test was applied. $p<0.05$ was considered to be statistically significant.

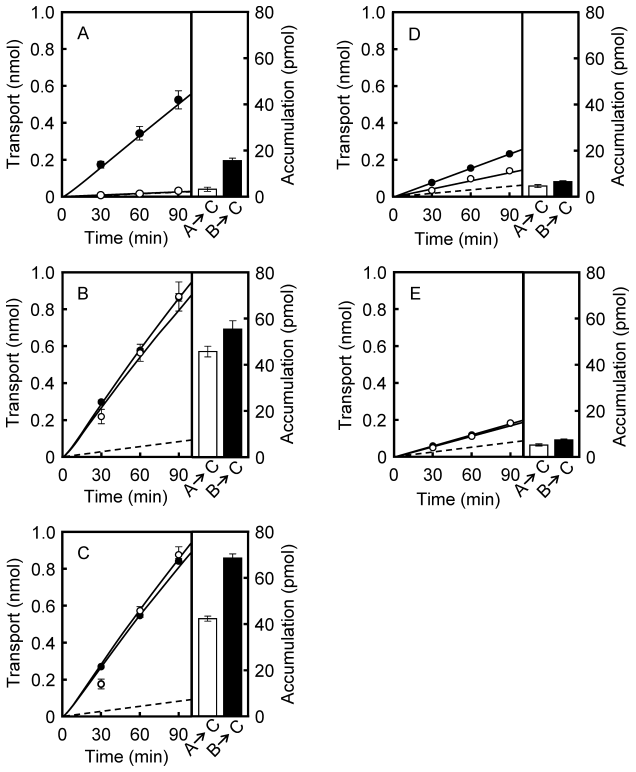

Fig. 1. Transcellular Transport and Cellular Accumulation of Cationic Drugs in LLC-PK$_1$ Cell Monolayers

(A) 10 μM TEA, (B) 10 μM quinidine, (C) 10 μM quinidine with 1 mM TEA, (D) 10 μM procainamide, (E) 10 μM procainamide with 1 mM TEA. Open and closed circles represent apical-to-basolateral (A→B) and basolateral-to-apical (B→A) transport, respectively. Solid and dotted lines are simulation curves obtained by pharmacokinetic analysis for drugs and mannitol transport, respectively. Open and closed columns represent cellular accumulation from the apical (A→C) and basolateral (B→C) sides, respectively. Data are expressed as the mean±S.E. of 6—11 experiments.

RESULTS

Transcellular Transport and Membrane Transport Characteristics of TEA, Quinidine, and Procainamide in Renal Epithelial LLC-PK$_1$ Cells Previously, we investigated the transcellular transport of 100 μM and 0.1 μM quinidine in LLC-PK$_1$ cells, and reported that quinidine is not significantly transported *via* the transport systems involved in the directional transport of TEA.[1)] In the present study, we first evaluated the transcellular transport of 10 μM TEA and quinidine across LLC-PK$_1$ cell monolayers (Figs. 1A—C), and performed a pharmacokinetic analysis of the data on the transcellular transport and cellular accumulation of radiolabeled drug using a 3-compartment (apical and basolateral chambers, and cell) model (Table 1). The transcellular transport of 10 μM TEA in the basolateral-to-apical direction was much greater than that in the opposite direction (Fig. 1A). The $CL_{B\to C}$ value was greater than the $CL_{A\to C}$ value, and the $CL_{C\to A}$ value was greater than the $CL_{C\to B}$ value (Table 1). On the other hand, the transcellular transport of 10 μM quinidine in the basolateral-to-apical direction was similar to that in the opposite direction (Fig. 1B). The $CL_{B\to C}$ value of quinidine was similar to the $CL_{A\to C}$ value, and the $CL_{C\to B}$ value was also similar to the $CL_{C\to A}$ value. In addition, the transcellular transport and membrane transport clearance of quinidine were not affected by 1 mM TEA (Fig. 1C). According to these findings, it was reconfirmed that the transport systems for TEA contribute little to the transcellular transport of quinidine in renal LLC-PK$_1$ cells.

We next evaluated the transcellular transport and membrane transport clearance of 10 μM procainamide in LLC-PK$_1$ cell monolayers (Figs. 1D, E). Procainamide was transported directionally from the basolateral side to the apical side (Fig. 1D). The estimated $CL_{C\to A}$ value was greater than any other clearance values (Table 1). Moreover, directional transport of procainamide was diminished in the presence of 1 mM TEA (Fig. 1E). The $CL_{B\to C}$ and $CL_{C\to A}$ values were decreased to 82.7% and 51.4% of the control values, respec-

Table 1. Influx and Efflux Clearance (μl/min/cm^2) of Cationic Drugs in LLC-PK$_1$ Cell Monolayers

Drug	Conc. (μM)	$CL_{B\to C}$	$CL_{C\to B}$	$CL_{A\leftrightarrow B}$	$CL_{C\to A}$	$CL_{A\to C}$
TEA	10	0.515±0.015	0.013±0.001	0.024±0.002	0.401±0.009	0.094±0.026
Quinidine	10	1.633±0.056	0.196±0.012	0.084±0.004	0.228±0.010	1.740±0.004
+1 mM TEA		1.673±0.009	0.188±0.004	0.083±0.002	0.206±0.003	1.729±0.002
Procainamide	10	0.284±0.005	0.201±0.002	0.055±0.007	0.348±0.004	0.212±0.011
+1 mM TEA		0.235±0.004*	0.224±0.002*	0.078±0.002	0.179±0.005*	0.172±0.004*

Values are expressed as the mean±S.E. for 6—11 experiments. $CL_{B\to C}$ and $CL_{C\to B}$ represent the influx and efflux clearance at the basolateral membrane, respectively. $CL_{A\to C}$ and $CL_{C\to A}$ represent the influx and efflux clearance at the apical membrane, respectively. $CL_{A\leftrightarrow B}$ represents the paracellular transport clearance. * $p<0.05$; significantly different from each control.

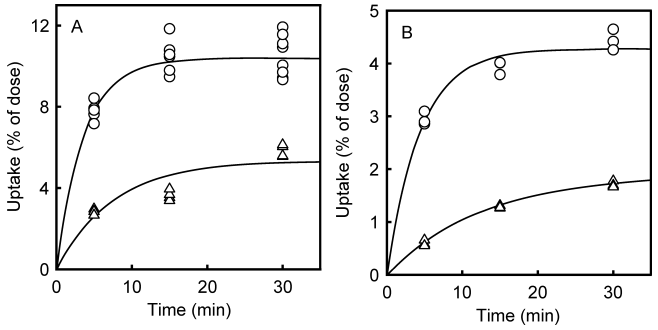

Fig. 2 Time Course of the Uptake of Quinidine and Procainamide in LS180 Cells

(A) 100 μM quinidine, (B) 100 μM procainamide. Circles and triangles represent cellular uptake of drugs at 37 °C and 4 °C, respectively. Solid lines represent simulation curves obtained by pharmacokinetic analysis of the drug accumulation.

Table 2. Influx and Efflux Clearance (μl/min/cm^2) of Quinidine and Procainamide in LS180 Cells

Drug	Temperature (°C)	$CL_{M\to C}$	$CL_{C\to M}$
Quinidine	37	7.542±0.140	0.647±0.029
	4	1.897±0.025*	0.335±0.003*
Procainamide	37	2.575±0.040	0.573±0.006
	4	0.396±0.020*	0.202±0.009*

Values are expressed as the mean±S.E. for 3—8 experiments. $CL_{M\to C}$ and $CL_{C\to M}$ represent the influx and efflux clearance in LS180 cells grown on plastic dishes, respectively. * $p<0.05$; significantly different from 37 °C.

tively, by 1 mM TEA (Table 1). These findings indicated that the transport systems for TEA significantly contribute to the transcellular transport of procainamide in renal LLC-PK$_1$ cells.

Cellular Uptake of Quinidine and Procainamide in Intestinal Epithelial LS180 Cells We have previously reported that a cation transport system is involved in the apical uptake of 100 μM quinidine in human intestinal epithelial Caco-2 cells.[2,7] In the present study, we investigated whether the cation transport system for quinidine is present in another human intestinal epithelial cell line, LS180. Figure 2A shows the time course of the uptake of 100 μM quinidine at 37 °C and 4 °C in LS180 cells grown on plastic dishes. The cellular uptake of quinidine was temperature-dependent, and reached approximately 10% of the dose for 30 min at 37 °C (Fig. 2A). We performed a pharmacokinetic analysis of the data on the cellular accumulation of quinidine using a 2-compartment (cell and medium) model (Table 2). The $CL_{M\to C}$ and $CL_{C\to M}$ values of quinidine at 37 °C were much greater than those at 4 °C (Table 2). We next investigated the effect of extracellular Na^+ and pH on the initial (5-min) uptake of quinidine in LS180 cells (Figs. 3A, 4A). No marked difference was observed in the cellular uptake of quinidine in the absence of extracellular Na^+ (Fig. 3A), whereas the cellular uptake of quinidine was markedly greater at pH 7.5 than at pH 5.5 (Fig. 4A). We also evaluated the effect of 1 mM various cationic drugs on the cellular uptake of quinidine, which was significantly inhibited by hydrophobic organic cations, but not by typical hydrophilic cations, TEA and choline (Fig. 5A). The rank order of the inhibitory effects of hydrophobic cationic drugs on the uptake of quinidine was imipramine> quinidine>diphenhydramine≈pyrilamine>procainamide

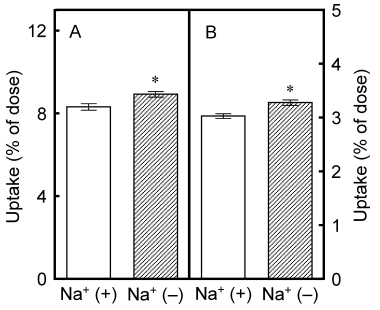

Fig. 3 Effect of Extracellular Na^+ on the Uptake of Quinidine and Procainamide in LS180 Cells

(A) 100 μM quinidine, (B) 100 μM procainamide. The cells were incubated with the radio-labeled drug for 5 min. Each column represents the mean±S.E. for 6 measurements. * $p<0.05$; significantly different from Na^+ (+).

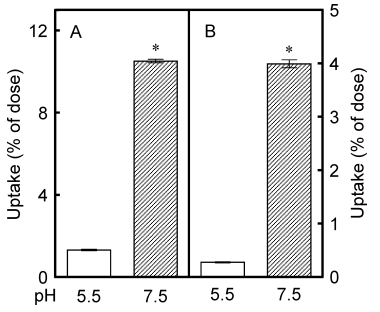

Fig. 4 Effect of Extracellular pH on the Uptake of Quinidine and Procainamide in LS180 Cells

(A) 100 μM quinidine, (B) 100 μM procainamide. Open and hatched columns represent the uptake of drugs at pH 5.5 and 7.5, respectively. The cells were incubated with the radio-labeled drug for 5 min. Each column represents the mean±S.E. for 6 measurements. * $p<0.05$; significantly different from pH 5.5.

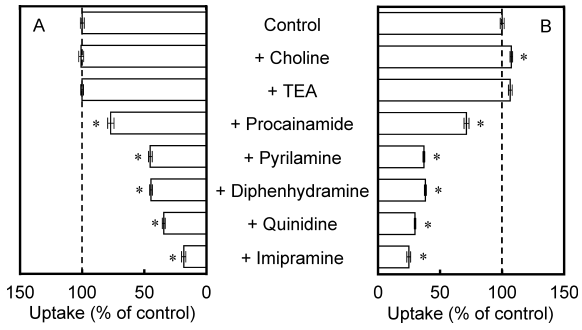

Fig. 5 Effect of Organic Cations on the Uptake of Quinidine and Procainamide in LS180 Cells

(A) 100 μM quinidine, (B) 100 μM procainamide. The cells were incubated with radio-labeled drug for 5 min in the presence of 1 mM organic cations. Each column represents the mean±S.E. for 4—12 measurements. * $p<0.05$; significantly different from the control.

(Fig. 5A). These findings suggested that quinidine was transported by the cation transport system in intestinal LS180 cells, as well as Caco-2 cells.

To compare the membrane transport characteristics of procainamide with those of quinidine in intestinal epithelial cells, we also evaluated the uptake of 100 μM procainamide using LS180 cells. The cellular uptake of procainamide was temperature-dependent, and reached approximately 4.4% of the dose for 30 min at 37 °C (Fig. 2B). The $CL_{M\to C}$ and $CL_{C\to M}$ values of procainamide at 37 °C were much greater than those at 4 °C (Table 2). As shown in Figs. 3B and 4B,

the initial (5-min) cellular uptake of procainamide was essentially Na^+-independent, but was significantly increased at pH 7.5 as compared with pH 5.5. In addition, the cellular uptake of procainamide was also inhibited by hydrophobic organic cations, but not by TEA and choline (Fig. 5B). The rank order of the inhibitory effects of hydrophobic cationic drugs on the uptake of procainamide was imipramine>quinidine> diphenhydramine≈pyrilamine>procainamide, which was consistent with that on the uptake of quinidine (Figs. 5A, B). These findings suggested that the cation transport system for procainamide in intestinal LS180 cells is similar/identical to that for quinidine.

DISCUSSION

The present study had two major findings. First, procainamide (but not quinidine) was transported by the cation transport systems in renal epithelial LLC-PK_1 cells. Second, both procainamide and quinidine were transported by another cation transport system in intestinal epithelial LS180 cells.

During the secretion of organic cations in the renal proximal tubules, cations are translocated across the basolateral membrane, and then are released across the luminal membrane.[12] The porcine kidney epithelial cell line LLC-PK_1 possesses the morphological structure and function similar to those of renal proximal tubular cells, and has been widely used to elucidate the transport properties of cationic drugs.[1,13—16] The membrane transport characteristics of TEA, a prototypical substrate of organic cation transport systems in the kidney, have been well evaluated using LLC-PK_1 cells.[1,14,17] TEA was transported directionally across LLC-PK_1 cell monolayers from the basolateral side to the apical side.[1,14] The unidirectional transport of TEA was temperature-dependent and saturable, and was inhibited by organic cations, such as cimetidine and choline.[14] The basolateral uptake of TEA in LLC-PK_1 cells was dependent on the inside negative potential, and was decreased by lowering basolateral medium pH.[15,18] On the other hand, the apical efflux of TEA in LLC-PK_1 cells was markedly stimulated by acidification of the apical medium.[14,17] Recent studies have demonstrated that organic cation transporter 2 (OCT2) is expressed on the basolateral membrane of epithelial cells in renal proximal tubules, and that it translocates TEA in an electrogenic manner.[12,19] In addition, multidrug and toxin extrusion 1 (MATE1) is localized to the brush-border membrane of renal proximal tubules, and is a TEA/proton antiporter.[12,19]

Takano *et al.* investigated the transport of procainamide in LLC-PK_1 cell monolayers.[16] They reported that the uptake of procainamide in LLC-PK_1 cells grown on plastic dishes was temperature-dependent and saturable, and was inhibited by cimetidine. The apical uptake of procainamide was greater at alkaline pH than acidic pH, and increased when intracellular pH was decreased, indicating the involvement of a H^+/procainamide antiport system in the apical membrane of LLC-PK_1 cells. In addition, the basolateral-to-apical transport of procainamide across LLC-PK_1 cell monolayers was greater than transport in the opposite direction, and was inhibited by cimetidine.[16] To our knowledge, the present study is the first report which provides apical and basolateral membrane transport clearance values of procainamide in renal epithelial LLC-PK_1 cells. The directional transport of procainamide across LLC-PK_1 cell monolayers was less than that of TEA (Figs. 1A, D), and the $CL_{B\to C}$ and $CL_{C\to A}$ values of procainamide were smaller than those of TEA (Table 1). The findings suggested that procainamide is transported by cation-specific transport systems in renal LLC-PK_1 cells, but that the intrinsic activity and/or affinity are low as compared with TEA.

We previously evaluated the cellular uptake of 100 μM quinidine in intestinal epithelial Caco-2 cells, which are widely used as an experimental model to study the intestinal absorption of various drugs.[2,7] The cellular uptake of quinidine in Caco-2 cells grown on plastic dishes was temperature-dependent, and markedly increased by alkalization of the apical medium at 37 °C.[2] In addition, the uptake of quinidine was inhibited by hydrophobic organic cations, and the rank order of the inhibitory effects of hydrophobic cationic drugs was imipramine>quinidine>diphenhydramine.[2,7] In the present study, to confirm the involvement of the cation transport system in intestinal absorption of quinidine, we investigated the membrane transport mechanism of quinidine using another intestinal epithelial cell line, LS180. This cell line possesses characteristics of the small intestine (*e.g.*, expression of microvillus), and has also been used as an *in vitro* model of the intestine.[8—11,20] The cellular uptake of quinidine in LS180 cells was temperature- and pH-dependent, and was inhibited by hydrophobic cationic drugs with the following rank order of inhibitory effect: imipramine>quinidine> diphenhydramine≈pyrilamine>procainamide (Figs. 2—5). These findings indicated that the cation transport system is present not only in Caco-2 cells, but also in LS180 cells.

In the present study, we demonstrated that procainamide is transported by the TEA-insensitive cation transport system in human intestinal epithelial LS180 cells. Procainamide may be actively taken up by the cation transport system not only in the human intestine, but also in the rabbit intestine. Katsura *et al.* investigated the transport mechanism of procainamide using rabbit intestinal brush-border membrane vesicles.[21] The uptake of procainamide in the vesicles was stimulated by an outward H^+ gradient, and the stimulation was reduced by the addition of carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), a protonophore. In addition, the initial uptake of procainamide was inhibited by tertiary amines, such as diphenhydramine and triethylamine, but not by TEA. They thought that the transport of procainamide at the intestinal brush-border membrane is mediated by the H^+/tertiary amine antiport system.[21] Further studies will be needed to identify the specific transport system for cationic drugs in the rabbit and/or human intestine.

In conclusion, cation transport systems in renal epithelial LLC-PK_1 cells transport procainamide, but not quinidine. In contrast, another cation transport system in intestinal epithelial LS180 cells takes up both quinidine and procainamide.

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