Pharmacokinetic Analysis of Transcellular Transport of Quinidine across Monolayers of Human Intestinal Epithelial Caco-2 Cells

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To investigate the mechanism responsible for the intestinal absorption of a lipophilic organic cation, quinidine, we performed a pharmacokinetic analysis of transcellular transport across Caco-2 cell monolayers grown on a porous membrane. Basolateral-to-apical transport of the drug was almost constant in the concentration range of 100 nM—100 μM. Transcellular transport was greater in the apical-to-basolateral direction than in the opposite direction. Apical-to-basolateral transport was greater at a concentration of 100 μM than 100 nM. The calculated influx clearance value of the apical membrane was much greater than the other influx/efflux clearance values of cell membranes, and was 5.6-fold the influx clearance value of the basolateral membrane at the drug concentration of 100 μM. We also investigated the uptake of quinidine at the apical membrane of Caco-2 cells grown on plastic dishes. The uptake was markedly increased by alkalization of the apical medium at 37 °C, and was decreased at low temperature (4 °C). In addition, it was inhibited by diphenhydramine and levofloxacin, but not by carvedilol, rifamycin SV, or L-carnitine. These findings indicated that the influx at the apical membrane was the direction-determining step in the transcellular transport of quinidine across Caco-2 cell monolayers, and that some specific transport system was involved in this influx.

Key words quinidine; intestinal absorption; tertiary amine; levofloxacin; Caco-2 cell monolayer

Quinidine, a tertiary amine compound with a site of ionization with a pK_a value of pH 8.8, is classified as a type IA antiarrhythmic drug, and has been used for the management of ventricular arrhythmias.[1,2] The drug is absorbed rapidly and almost entirely after oral administration, and is detected in the plasma within 15 min.[3] Quinidine binds to both albumin and α_1-acid glycoprotein, and the proportion that binds to plasma protein is 70 to 95%.[4] However, its apparent volume of distribution is quite large (2.0 to 3.5 liters/kg), because the drug is highly lipophilic.[4] It is metabolized *via* 3-hydroxylation and *N*-oxygenation by cytochrome P450 3A4, and these metabolites are less electrophysiologically active than the parent drug.[5,6] The hepatic extraction ratio of quinidine is about 30%, and the bioavailability after oral administration is about 70%.[7] In addition, quinidine is partly excreted in the urine (15 to 40% of dose), suggesting that reabsorption at the distal tubules in the nephron is not complete.[4]

The mechanism of intestinal absorption of lipophilic organic cations has been explained as passive diffusion of unionized compounds according to the pH-partition theory. On the other hand, Mizuuchi *et al.* investigated the mechanisms responsible for the transcellular transport of diphenhydramine in Caco-2 cells.[8,9] This cell line forms confluent monolayers of well differentiated enterocyte-like cells with functional properties of transporting epithelia, and is widely used as a model to study the absorption of drugs and other xenobiotics.[10—12] The uptake of diphenhydramine at the apical membrane in Caco-2 cells was pH- and temperature-dependent, but was not inhibited by tetraethylammonium, biological amines, or neurotransmitters.[8] On the other hand, the uptake was inhibited by chlorpheniramine, procainamide, and imipramine, and was *trans*-stimulated by the preloading of chlorpheniramine, dimethylaminochloride, and triethylamine.[8,9] From these results, Mizuuchi *et al.* concluded that the uptake of diphenhydramine at the apical membrane in Caco-2 cells is mediated by a specific transport system, and that this system recognizes the *N*-dimethyl or *N*-diethyl moieties of compounds.[8,9] However, at present, it is unclear whether the transport system for diphenhydramine is involved in the intestinal absorption of other tertiary amine compounds, such as quinidine.

During drug absorption in the intestine, therapeutic compounds or nutrients first enter intestinal epithelial cells from their apical side, then pass through the epithelia to the basolateral side, and finally appear in the blood stream. Therefore, to investigate intestinal drug absorption, it is important to separately assess these sequential processes. However, in many cases, drug transport on the apical and basolateral sides of the monolayer in Caco-2 cells was not separately examined, and the influx and efflux clearance rates were not evaluated.[13] For the characterization of transcellular drug transport, a pharmacokinetic approach is useful. Transcellular drug transport in Caco-2 cell monolayers can be analyzed in detail in a model-dependent manner, where their drug concentration–time profiles on both sides of the monolayer can be assessed by curve fitting calculations and the influx and efflux clearance of the monolayer separately evaluated. In addition, 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, the transport data for a small amount of radio-labeled drug can be analyzed using a linear pharmacokinetic model.[13]

In the present study, to characterize the mechanism responsible for the intestinal absorption of a lipophilic organic cation, we performed a pharmacokinetic analysis of the transcellular transport of quinidine across Caco-2 cell monolayers. This analysis indicated that the influx clearance of quinidine at the apical membrane was much greater than any other clearance values of cell membranes. Therefore, we also investigated the uptake mechanism of quinidine at the apical membrane of Caco-2 cells grown on plastic dishes.

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MATERIALS AND METHODS

Materials Quinidine hydrochloride monohydrate and quinine hydrochloride were purchased from Sigma (St. Louis, MO, U.S.A.). Digitonin, rifamycin SV sodium salt, L-carnitine hydrochloride, imipramine hydrochloride, and levofloxacin hydrochloride were obtained from Nacalai Tesuque Inc. (Kyoto, Japan). Diphenhydramine hydrochloride was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Carvedilol was supplied by Daiichi Pharmaceutical (Tokyo, Japan). [^{3}H]quinidine (740 GBq/mmol) was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, U.S.A.). All other chemicals were of the highest purity available.

Cell Culture and Preparation of Monolayers Caco-2 cells at passage 40 were obtained from the Riken Bioresource Center (Tsukuba, Japan), and all experiments were carried out with the cells between passages 47 to 65. The cells were maintained by serial passage in plastic dishes with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Valley Biochemical Inc., Winchester, VA, U.S.A.) in an atmosphere of 5% CO_2–95% air at 37 °C. The medium was changed every second or third day, and when the cells reached 80—90% confluence, they were subcultured using a 0.02% EDTA/0.05% trypsin solution.[13)]

The cells were seeded at a density 5×10^5 cells/cm^2 on a 0.9 cm^2 porous membrane (0.4 μm pore size) in a Falcon® cell culture insert (BD Bioscience, Bedford, MA, U.S.A.) in order to evaluate transcellular transport of quinidine. The seeded cells were maintained for 21 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.). Caco-2 cell monolayers whose TEER was above 900 $\Omega\cdot$cm^2 were used for experiments of transcellular transport of quinidine. On the other hand, the cells were seeded at a density 5×10^5 cells/cm^2 on a 3.8 cm^2 plastic dish using a Falcon® multiwell™ plate (BD Bioscience), and were maintained for 21 d to investigate the transport mechanism of quinidine at the apical membrane.

Pharmacokinetic Analysis of Transcellular Transport of Quinidine The transcellular transport of quinidine in Caco-2 cell monolayers prepared on a porous membrane was examined as described previously.[13)] In brief, the monolayer was pre-incubated for 60 min at 37 °C with culture medium containing unlabeled quinidine (100 nM—100 μM) to equilibrate the drug concentration. After the 60-min equilibration period, [^{3}H]quinidine (1-2.3 μCi/well) was applied to the apical chamber (1 ml) to examine the apical-to-basolateral transcellular transport of quinidine. A volume (50 μl) of medium in the basolateral chamber (2.3 ml) was then collected after 60, 120, and 180 min. Following the last collection, the Caco-2 cell monolayers on the porous membrane were immediately washed three times with ice-cold phosphate buffer, and the cells were collected. The amounts of [^{3}H]quinidine in the medium and cells were determined using a liquid scintillation counter, and normalized against the initially applied doses. The time course of the transport of quinidine in the opposite direction (basolateral-to-apical) was examined in the same manner.

The transcellular transport of quinidine was analyzed in a model-dependent manner using the NONMEM software running on a mainframe UNIX machine at the Kyoto University Data Processing Center, as described previously.[13)] In brief, the following mass balance equations were prepared for the pharmacokinetic analysis:

$$\frac{dX_A}{dt} = -\frac{CL_{AC}}{V_A}\cdot X_A + \frac{CL_{CA}}{V_C}\cdot X_C - \frac{CL_{PARA}}{V_A}\cdot X_A + \frac{CL_{PARA}}{V_B}\cdot X_B \quad (1)$$

$$\frac{dX_B}{dt} = -\frac{CL_{BC}}{V_B}\cdot X_B + \frac{CL_{CB}}{V_C}\cdot X_C + \frac{CL_{PARA}}{V_A}\cdot X_A - \frac{CL_{PARA}}{V_B}\cdot X_B \quad (2)$$

$$\frac{dX_C}{dt} = \frac{CL_{AC}}{V_A}\cdot X_A + \frac{CL_{BC}}{V_B}\cdot X_B - \frac{(CL_{CB}+CL_{CA})}{V_C}\cdot X_C \quad (3)$$

where X_A, X_B, and X_C are the amount of quinidine in the apical chamber, the basolateral chamber, and the monolayer determined at time t, respectively. V_A and V_B indicate the volume of the apical chamber (1 ml) and the basolateral chamber (2.3 ml), respectively. V_C indicates the cell volume (2.25 μl), which was previously measured with sulfanilamide in our laboratory.[13)] The influx and efflux clearance of quinidine on the apical membrane of the cells was designated as CL_{AC} and CL_{CA}, respectively. The influx and efflux clearance of quinidine on the basolateral membrane of the cells was designated as CL_{BC} and CL_{CB}, respectively. Paracellular transport clearance (CL_{PARA}) was fixed to 0.0141 μl/min (0.0157 μl/min/cm^2), which was obtained from the transport profile of mannitol.[13)]

Cellular Uptake of Quinidine The cellular uptake of [^{3}H]quinidine was examined in the presence of 100 μM quinidine using Caco-2 cell monolayers prepared 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 $MgSO_4\cdot7H_2O$, 100 μM quinidine, and 25 mM HEPES (pH 7.4). In order to evaluate the effect of the medium's pH on the cellular uptake of [^{3}H]quinidine, 25 mM HEPES (pH 7.4 or 6.5) was replaced with 2-(*N*-morpholino)ethanesulfonic acid (pH 5.5), or Tris (pH 8.0). The monolayers were first pre-incubated for 60 min at 37 °C or 4 °C with 2 ml of incubation medium. To maintain the pH of the incubation medium, the apical medium was replaced with fresh incubation medium at 10 min before the addition of [^{3}H]quinidine (1 μCi/well). After the cells were incubated with [^{3}H]quinidine for another 30 min at 37 °C or 4 °C, they were immediately washed three times with ice-cold phosphate buffer, and collected. The amounts of [^{3}H]quinidine in the cells were determined using a liquid scintillation counter, and normalized against the initially applied doses.

The effect of digitonin, carvedilol, rifamycin SV, and L-carnitine on the apical uptake of [^{3}H]quinidine in Caco-2 cells was evaluated at 37 °C in the presence of 100 μM quinidine. The incubation medium was replaced with fresh medium containing 40 μM digitonin, 50 μM carvedilol, 20 μM rifamycin SV, or 50 μM L-carnitine, at 10 min before the addition of [^{3}H]quinidine.[14—17)] The cells were incubated with [^{3}H]quinidine for 30 min at 37 °C, and the amounts of [^{3}H]quinidine in the cells were determined, as described above.

The effect of 5—20 mM tertiary amines and levofloxacin on the apical uptake of [^{3}H]quinidine in Caco-2 cells was

also evaluated at 37 °C in the presence of 100 μM quinidine.[8,9,18] The incubation medium was replaced with fresh medium containing 5 mM quinidine, 5 mM quinine, 20 mM diphenhydramine, 20 mM imipramine, or 20 mM levofloxacin, at 5 min before the addition of [^{3}H]quinidine. The cells were incubated with [^{3}H]quinidine for 15 min at 37 °C, and the amounts of [^{3}H]quinidine in the cells were 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 a one-way ANOVA provided that the variances of groups were similar. If this was not the case, a Scheffé-type test was applied following Kruskal–Wallis analysis. $p<0.05$ was considered to be statistically significant.

RESULTS

Transcellular Transport and Cellular Accumulation of Quinidine in Caco-2 Cell Monolayers We first examined the transcellular transport and cellular uptake of quinidine in Caco-2 cell monolayers. Figure 1 shows the transcellular transport profiles of [^{3}H]quinidine at concentrations of 100 nM, 10 μM, and 100 μM. Quinidine was transported in a direction-specific manner at the concentration of 100 nM, where the amount of quinidine transported in the apical-to-basolateral direction within 180 min was greater than that transported in the opposite direction (Fig. 1A). Basolateral-to-apical transport of quinidine was almost constant in the concentration range of 100 nM—100 μM. In constant, apical-to-basolateral transport of the drug was increased at 10 μM and 100 μM as compared with 100 nM (Fig. 1).

Table 1 shows the cellular accumulation of [^{3}H]quinidine after the 180-min transcellular transport. The amount of quinidine accumulated into cells from the apical medium was much greater than that from the basolateral medium (Table 1). The cellular concentration of [^{3}H]quinidine at a drug concentration in the medium of 100 nM (2.72%/2.25 μl) was 13.4-fold higher than that in the apical medium (89.9%/1000 μl) after 180 min of apical-to-basolateral transport. The accumulation of [^{3}H]quinidine at the concentration of 10 μM was slightly higher than that at 100 nM and 100 μM, though the difference was not statistically significant (Table 1).

Influx and Efflux Clearance of Quinidine in Caco-2 Cell Monolayers To characterize the transcellular transport of quinidine in Caco-2 cell monolayers, we performed a pharmacokinetic analysis using the data on the transcellular transport and cellular accumulation of [^{3}H]quinidine. As shown in Table 2, the mean efflux clearance of the apical membrane (CL_{CA}) was 6.1-fold greater than that of the basolateral membrane (CL_{CB}) at a drug concentration in the medium of 100 nM (0.238 *vs.* 0.039 μl/min/cm^2). CL_{CA} at 10 μM was decreased by 32% as compared with that at 100 nM, indicating that the apical efflux of quinidine was partially saturated at the concentration of 10 μM. This finding was consistent with the result that the apical-to-basolateral transport of [^{3}H]quinidine was greater at 10 μM than 100 nM (Figs. 1A, B). It was reported that P-glycoprotein (P-gp) is expressed on the apical membrane of Caco-2 cells, and that quinidine is a substrate of P-gp.[19,20] Therefore, P-gp may be at least partially responsible for the CL_{CA} value, and plays a role in the intestinal absorption of quinidine. On the other hand, CL_{CA} and CL_{CB} were increased significantly at a drug concentration of 100 μM as compared with 100 nM, which suggested that [^{3}H]quinidine bound to cellular components was displaced by unlabeled quinidine, and that the increased unbound fraction of quinidine in the cells may contribute to the increase in the efflux clearance of [^{3}H]quinidine. In addition, this finding was consistent with the result that the cellu-

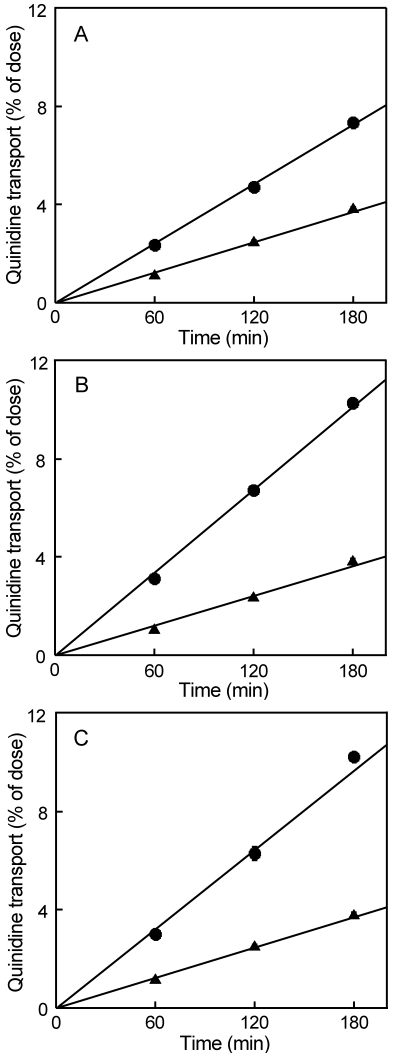

Fig. 1. Transcellular Transport Profiles of 100 nM (A), 10 μM (B) and 100 μM (C) Quinidine in Caco-2 Cell Monolayers

Circles and triangles indicate apical-to-basolateral and basolateral-to-apical transport, respectively. Solid lines are the simulation curves obtained by the pharmacokinetic analysis of the apical-to-basolateral and basolateral-to-apical transport. Each point expresses the mean±S.E. for 5 experiments.

Table 1. Quinidine Accumulation Measured at 180 min in Caco-2 Cell Monolayers

Concentration	% of dose	
	A→C	B→C
100 nM	2.72±0.35	0.338±0.013
10 μM	3.82±0.41	0.508±0.038
100 μM	2.13±0.12	0.260±0.011

Values are expressed as the mean±S.E. for 5 experiments.

Table 2. Influx and Efflux Clearance of Quinidine in Caco-2 Cell Monolayers

Concentration	Clearance (μl/min/cm^2)			
	CL_{CA}	CL_{CB}	CL_{AC}	CL_{BC}
100 nM	0.238±0.014	0.039±0.003	3.75±0.48	0.689±0.021
10 μM	0.161±0.007*	0.039±0.003	3.88±0.25	0.759±0.019
100 μM	0.312±0.021*	0.063±0.002*	3.98±0.35	0.706±0.015

Values are expressed as the mean±S.E. for 5 experiments. *$p<0.05$; significantly different from 100 nM quinidine.

lar accumulation of quinidine after the apical-to-basolateral and basolateral-to-apical transport study at the drug concentration of 100 μM tended to be less than that at 10 μM (Table 1).

As shown in Table 2, the mean influx clearance of the apical membrane (CL_{AC}) was 5.4-fold greater than that of the basolateral membrane (CL_{BC}) at the drug concentration of 100 nM (3.75 *vs.* 0.689 μl/min/cm^2), and was much greater than any other clearance values including the CL_{PARA} value (0.0157 μl/min/cm^2). In addition, CL_{AC} was constant in the concentration range of 100 nM—100 μM, suggesting that the influx clearance at the apical membrane has the characteristics of low affinity and high capacity. The findings indicated that the influx of the apical membrane (CL_{AC}) was the primary factor, which was responsible for the greater apical-to-basolateral transport of quinidine across the intestinal epithelial cells (Fig. 1).

Effect of pH and Temperature on Apical Uptake of Quinidine in Caco-2 Cell Monolayers To characterize the transport of quinidine at the apical membrane of Caco-2 cells, the uptake of [^{3}H]quinidine was investigated using Caco-2 cell monolayers prepared on plastic dishes of a multiwell plate. As shown in Fig. 2, the uptake of quinidine at 4 °C was increased by the alkalization of the apical medium, but the profile was similar to that of the unionized fraction of quinidine calculated with the Henderson–Hasselbalch equation (Fig. 2). The results indicated that passive diffusion of unionized quinidine is responsible for the uptake at the apical membrane at 4 °C. On the other hand, the apical uptake was markedly increased by alkalization of the apical medium at 37 °C. In addition, the uptake at 37 °C was much greater than that at 4 °C, indicating that some transport system was involved in the uptake of quinidine at the apical membrane of Caco-2 cells.

Effect of Various Compounds on Apical Uptake of Quinidine in Caco-2 Cell Monolayers To elucidate the characteristics of quinidine's transport across the apical membrane of Caco-2 cells, the effect of digitonin, carvedilol, rifamycin SV, and L-carnitine on the apical uptake of quinidine was evaluated. Digitonin, a mild nonionic detergent that permeabilizes cellular membranes,[14] significantly decreased the uptake to 20% of the control value (Fig. 3). The P-gp inhibitor carvedilol[15] did not affect the apical uptake of quinidine (Fig. 3). In addition, rifamycin SV and L-carnitine, which were used as the organic anion-transporting polypeptide (OATP) inhibitor[16] and as an organic cation transporter (OCTN2) substrate,[17] respectively, had no effect on the uptake (Fig. 3). These results suggested that some transport system contributes to the transport of quinidine into the cells, but that P-gp, OATPs, and OCTN2 are not involved in the apical uptake of quinidine in Caco-2 cells.

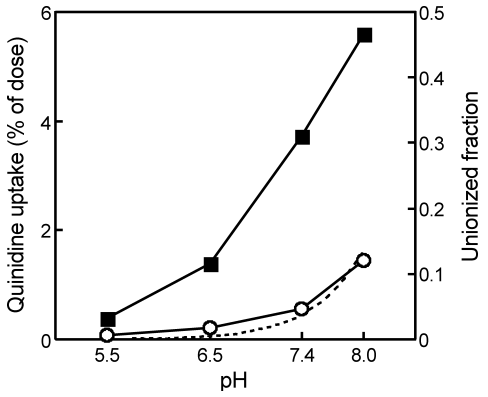

Fig. 2. Effect of pH and Temperature on Cellular Uptake of Quinidine in Caco-2 Cell Monolayers

The cells were incubated with [^{3}H]quinidine for 30 min at 37 °C (closed squares) or 4 °C (open circles). The pH of the incubation medium was between 5.5 and 8.0. The dotted line expresses the unionized fraction calculated with the Henderson–Hasselbalch equation. Each point expresses the mean±S.E. for 4 experiments.

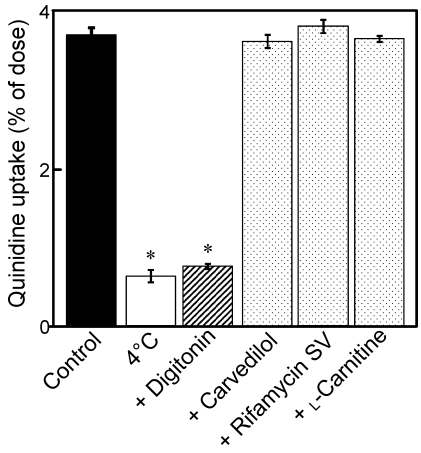

Fig. 3. Effect of Digitonin, Carvedilol, Rifamycin SV, and L-Carnitine on Cellular Uptake of Quinidine in Caco-2 Cell Monolayers

The cells were incubated with [^{3}H]quinidine for 30 min following pre-incubation for 10 min with incubation medium containing digitonin (40 μM), carvedilol (50 μM), rifamycin SV (20 μM), or L-carnitine (50 μM). Each column expresses the mean±S.E. for 5—10 experiments. *$p<0.05$; significantly different from control.

Furthermore, we examined the effect of tertiary amines and levofloxacin on the uptake of quinidine. As shown in Fig. 4, diphenhydramine and levofloxacin significantly decreased the uptake to 5.2% and 6.4% of the control value, respectively. Quinidine, quinine, and imipramine also reduced uptake, though the difference was not statistically significant (Fig. 4). These results suggested that the transport system for the apical uptake of quinidine is similar to that responsible for the transport of tertiary amines at the apical membrane of Caco-2 cells.[8,9,18]

DISCUSSION

The present study had two major findings. First, the transcellular transport of quinidine across monolayers of human intestinal epithelial Caco-2 cells was greater in the apical-to-basolateral direction than the opposite direction, mainly due to the large influx of the drug at the apical membrane of the cells. Second, the uptake of quinidine at the apical membrane was mediated by a temperature-sensitive specific transport

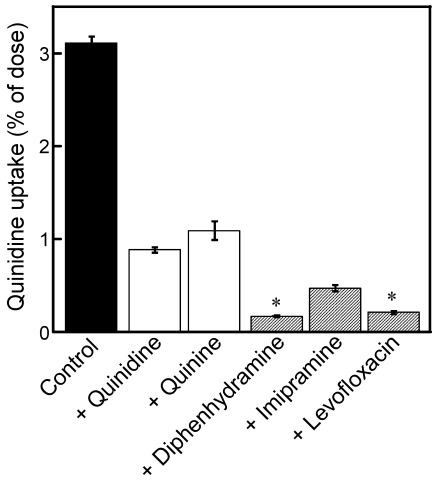

Fig. 4. Effect of Tertiary Amines on Cellular Uptake of Quinidine in Caco-2 Cell Monolayers

The cells were incubated with [^{3}H]quinidine for 15 min following pre-incubation for 5 min with incubation medium containing quinidine (5 mM), quinine (5 mM), diphenhydramine (20 mM), imipramine (20 mM), or levofloxacin (20 mM). Each column expresses the mean±S.E. for 5—10 experiments. * $p<0.05$; significantly different from control.

system.

We previously investigated the transcellular transport of digoxin across Caco-2 cell monolayers, and found that the efflux of the drug at the apical membrane was the direction- and rate-determining step in the large basolateral-to-apical transport of the drug as compared with the apical-to-basolateral transport.[13] P-gp is expressed at the apical membrane of Caco-2 cells, and the basolateral-to-apical transport of digoxin across cell monolayers is inhibited by typical inhibitors of P-gp, quinidine and carvedilol.[13,19] We, therefore, thought that the efflux of digoxin at the apical membrane was mediated by P-gp. The present finding that the transcellular transport of quinidine was greater in the apical-to-basolateral direction than the opposite direction (Fig. 1), suggests that the transport activity of P-gp for quinidine is weaker than that for digoxin although quinidine is a substrate for P-gp.[7] In addition, the calculated efflux clearance of the apical membrane (CL_{CA}) of quinidine at a concentration of 100 nM was 77% lower than that of digoxin at a concentration of 10 nM (0.238 *vs.* 1.02 μl/min/cm^2).[13] The mean CL_{CA} value of quinidine was further decreased at 10 μM probably because of partial saturation of the P-gp activity, although the CL_{CA} value was marginally increased at 100 μM probably because of the decreased intracellular binding of the drug (Table 2). On the other hand, the influx clearance of the apical membrane (CL_{AC}) of quinidine was much greater than the other influx/efflux clearance values of cell membranes, suggesting that the influx at the apical membrane was the direction-determining step in the intestinal absorption (Table 2). In addition, the mean CL_{AC} value of quinidine at 100 μM was 9.0-fold that of digoxin at 10 nM (3.98 *vs.* 0.44 μl/min/cm^2),[13] which was consistent with the observation that the intestinal absorption of quinidine was very rapid and almost complete.[3,4]

In the present study, we evaluated the uptake of quinidine at the apical membrane in Caco-2 cell monolayers grown on plastic dishes, and found that this uptake was temperature-dependent and was decreased significantly in the presence of 20 mM diphenhydramine and 20 mM levofloxacin (Figs. 2, 4). These results suggested that the specific transport system for quinidine at the apical membrane of Caco-2 cells was similar to that of new quinolones as well as diphenhydramine.[8,18] That is, the uptake of diphenhydramine and new quinolones at the apical membrane of Caco-2 cells was temperature-dependent.[8,18] The uptake of diphenhydramine was significantly inhibited by chlorpheniramine (1—20 mM) and imipramine (5 mM), but not by biological amines or neurotransmitters.[8] On the other hand, the uptake of [^{14}C]levofloxacin was inhibited by grepafloxacin (10 mM), levofloxacin (20 mM), and quinidine (5 mM), but not by tetraethylammonium, *p*-aminohippurate, probenecid, amino acids, β-lactam antibiotic or monocarboxylates at a concentration of 10 mM.[18] However, in the present study, it is unclear whether the specific transport system for quinidine is identical to that for diphenhydramine and levofloxacin, and further investigation may be required to clarify the transport system for lipophilic organic cations in the human intestine.

In conclusion, our findings indicated that some specific transport system was involved in the influx of quinidine at the apical membrane of Caco-2 cells. This transport system may contribute to the rapid and almost complete absorption of quinidine from the human intestine.

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