MDR1-Mediated Interaction of Digoxin with Antiarrhythmic or Antianginal Drugs

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The multidrug transporter, MDR1-mediated interaction of digoxin with antiarrhythmic or antianginal drugs was examined *in vitro* by using the MDR1-overexpressing LLC-GA5-COL150 cells, which were established by transfection with human *MDR1* cDNA into porcine kidney epithelial LLC-PK₁ cells. Amiodarone, its active metabolite monodesethyl-amiodarone (DEA), and quinidine markedly inhibited the basal-to-apical transport (renal secretion) of [³H]digoxin and increased the apical-to-basal transport (reabsorption), but cibenzoline and lidocaine showed slight inhibition of the transport, and disopyramide and mexiletin had no such effects. The IC₅₀ values for amiodarone, DEA and quinidine on [³H]digoxin transport in LLC-GA5-COL150 cells were 5.48 μ M, 1.27 μ M and 9.52 μ M, respectively. These were comparable to, or only several times the achievable concentration in clinical use, suggesting that MDR1 could be responsible for the drug interaction between digoxin and amiodarone found in clinical reports and that DEA contributes the elevation of digoxin serum concentration. Similarly, dipyridamole altered the transport, but isosorbide showed only slight modification of the transport. The IC₅₀ value for dipyridamole was 40.0 μ M, also only several times the achievable concentration in clinical use, indicating a risk of interaction.

Key words MDR1; P-glycoprotein; digoxin; antiarrhythmic drug; antianginal drug; drug interaction

Digoxin, a cardiac glycoside, is mainly prescribed for patients with congestive heart failure. Cardiac arrhythmia sometimes occurs in patients who are taking digoxin,^{1,2)} and thus antiarrhythmic drugs were often co-administrated with digoxin. In fact, antiarrhythmic or antianginal drugs were also prescribed for 25% and 21% of patients taking digoxin, respectively, in our hospital.³⁾ Digoxin is well known to have only a narrow therapeutic concentration range (trough: 0.5–2.0 ng/ml), and to be prone to drug–drug interaction.⁴⁾ Clinical reports suggested that the serum concentration of digoxin was elevated by amiodarone,^{5–7)} quinidine,^{8–10)} verapamil^{11,12)} and nicardipine,¹³⁾ and the daily dose of digoxin is adjusted based on a sophisticated serial monitoring of serum digoxin concentration.

Digoxin has been found to be mainly excreted by tubular secretion and glomerular filtration,^{4,14)} and tubular secretion has been demonstrated to be mediated *via* MDR1 by using LLC-GA5-COL150 cells, which were established by transfection of *MDR1* cDNA into porcine kidney epithelial LLC-PK₁ cells.^{15,16)}

Herein, the effects of amiodarone, its active metabolite monodesethyl-amiodarone (DEA) and quinidine on the transport of [³H]digoxin was examined using LLC-GA5-COL150 cells to elucidate whether MDR1 was responsible for the drug interaction between digoxin and amiodarone or quinidine found in clinical use. The possibility of interaction with other antiarrhythmic drugs, cibenzoline, lidocaine, disopyramide and mexiletin, and antianginal drugs, dipyridamole and isosorbide, was also examined. These are selected because they are more frequently used with digoxin compared with others, at least in our hospital.

MATERIALS AND METHODS

Chemicals Cibenzoline was a gift from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). DEA was a gift from Taisho Pharmaceutical Co., Ltd. (Tokyo, Japan). Amiodarone, mexiletin and quinidine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Disopyramide, lidocaine, dipyridamole and colchicine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Isosorbide was purchased from Alexis Co. (San Diego, CA, U.S.A.). [³H]Digoxin (595.7 GBq/mmol) was purchased from Du Pont-New England Nuclear (Boston, MA, U.S.A.). Unlabeled digoxin was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). All other chemicals were of the highest purity available.

Culture of LLC-PK₁ and LLC-GA5-COL150 Cells LLC-GA5-COL150 cells were established by transfection of human *MDR1* cDNA into porcine kidney epithelial LLC-PK₁ cells.^{15,16)} LLC-PK₁ and LLC-GA5-COL150 cells were maintained in the culture medium consisting of Medium199 (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) supplemented with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD, U.S.A.), with no antibiotics. Colchicine was also added to a final concentration of 150 ng/ml only for LLC-GA5-COL150 cells. LLC-PK₁ (1.0×10^6 cells/100 mm²) and LLC-GA5-COL150 cells (1.5×10^6 cells/100 mm²) were seeded on plastic culture dishes in 10 ml of the culture medium. Monolayer cultures were grown in a humidified atmosphere of 5% CO₂–95% air at 37°C, and subcultured every 4 and 7 d for LLC-PK₁ and LLC-GA5-COL150 cells, respectively, with 0.02% EDTA–0.05% trypsin (Gibco BRL, Life Technologies, Inc., Grand Island, NY, U.S.A.).

Transcellular Transport of [³H]Digoxin across LLC-

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PK₁ and LLC-GA5-COL150 Cell Monolayers Transcellular transport of [³H]digoxin across LLC-PK₁ and LLC-GA5-COL150 cell monolayers was examined as described previously.^{3,15,17–19} Cells were seeded on Transwell™ (Cat. No. 3414, Costar, Cambridge, MA) at a density of 2.0×10^6 and 2.4×10^6 cells/well, respectively, and cultured under a humidified atmosphere of 5% CO₂–95% air at 37 °C for 3 d to form the monolayers. The MDR1 was highly expressed on the apical membrane in LLC-GA5-COL150 cell monolayers which accelerated the basal-to-apical transport (renal secretion) and inhibited the apical-to-basal transport (reabsorption). Basal-to-apical and apical-to-basal were examined independently. Three hours prior to the start of transport experiments, the culture medium was replaced with fresh culture medium. Colchicine was not included even for LLC-GA5-COL150 cells. The transport study was initiated by replacement of the culture medium on the donor side (basal or apical side) with 2 ml fresh culture medium containing [³H]digoxin (100 nM, 18.5 kBq/ml) together with [methoxy-¹⁴C]inulin (6.0 μM, 1.85 kBq/ml), used as a cell monolayer integrity marker, and that on the receiver side (apical or basal, respectively) with 2 ml fresh culture medium. The monolayers were incubated at 37 °C, and 25 μl aliquots of the medium were sampled from the receiver side at three points (1, 2, 3 h). The data was accepted when the appearance of [methoxy-¹⁴C]inulin on the receiver side was less than 0.4% per h of the initial amount on the donor side.

The effects of antiarrhythmic and antianginal drugs on the transcellular transport of [³H]digoxin were evaluated by their addition to both sides of the monolayers at 1 h before the start of the transport experiments, and at the start of the transport experiments. The cells were exposed to them for 4 h total. The concentration of antiarrhythmic and antianginal drugs was 50 μM, which was used in the previous reports.^{3,17–19} Although this is high, it is convenient to compare with the results on the previous reports.^{3,17–19}

The radioactivity associated with the collected media was counted in 3 ml of ACS II (Amersham International) by liquid scintillation counting (LSC-5100, Aloka Co., Ltd., Tokyo). Data are presented as the percentage fraction of the initial amount of total radioactivity added to the donor side.

Data Analysis Statistical analysis of the data was performed by one-way analysis of variance followed by Scheffé's post-hoc test (two-tailed), with $p < 0.05$ considered significant.

The IC₅₀ values for amiodarone, DEA, quinidine and dipyridamole on [³H]digoxin transport were estimated as described previously.^{20,21} The net basal-to-apical transport of [³H]digoxin at 3 h was calculated by subtracting the apical-to-basal transport from the basal-to-apical transport at 3 h. At several concentrations, amiodarone, DEA, quinidine and dipyridamole caused inhibition of net basal-to-apical transport at 3 h, shown as the percentage of reduction as compared with net basal-to-apical transport in the absence of drug. The percentage inhibition of net basal-to-apical transport at 3 h was plotted against the concentration of amiodarone, DEA, quinidine and dipyridamole, and fitted to the sigmoid $E_{\max}$ model by nonlinear least-squares fitting method (Solver, Microsoft® Excel 98).

$$E = E_{\max} \times C^{\gamma} / (C^{\gamma} + IC_{50}^{\gamma})$$

Where E is the percentage inhibition (%) by amiodarone, DEA and dipyridamole on net basal-to-apical transport of [³H]digoxin, $E_{\max}$ is the maximum inhibition rate (that is, 100%), C is the concentration of amiodarone, DEA, quinidine and dipyridamole (μM) in the transport medium, IC_{50} is the concentration (μM) producing 50% of $E_{\max}$, and γ is the sigmoidicity factor.

RESULTS

Transcellular Transport of [³H]Digoxin across LLC-PK₁ and LLC-GA5-COL150 Monolayers and Its Inhibition by Antiarrhythmic Drugs The basal-to-apical transport of [³H]digoxin was increased, and the apical-to-basal transport was decreased in LLC-GA5-COL150 cells when compared with parent LLC-PK₁ cells. The basal-to-apical transport of [³H]digoxin was higher than the apical-to-basal transport in LLC-GA5-COL150 cells, indicating that [³H]digoxin was a substrate for MDR1 (Table 1).

Amiodarone and quinidine at 50 μM extensively inhibited the basal-to-apical transport of [³H]digoxin, and increased the apical-to-basal transport in LLC-GA5-COL150 cells (Table 1, Figs. 1, 2), showing these drugs to be inhibitors of [³H]digoxin transport *via* MDR1, however disopyramide and mexiletin had no such effects even at 50 μM (Table 1). Cibenzoline and lidocaine showed slight inhibition of the transport (Table 1). Similar to amiodarone, DEA (10 μM) was demonstrated to be an inhibitor of MDR1-mediated transport of [³H]digoxin (Table 1). Inhibitory effects of amiodarone, DEA and quinidine on [³H]digoxin transport were concentration-dependent with an estimated IC₅₀ of 5.48 μM, 1.27 μM and 9.52 μM, respectively (Figs. 1, 2, Table 2).

Transcellular Transport of [³H]Digoxin across LLC-GA5-COL150 Monolayers and Its Inhibition by Antianginal Drugs Dipyridamole also significantly suppressed the basal-to-apical transport, and increased the apical-to-basal transport in LLC-GA5-COL150 cells, and its effect was concentration-dependent with an estimated IC₅₀ of 40.0 μM (Ta-

Table 1. Effects of Antiarrhythmic and Antianginal Drugs (10–50 μM) on Transcellular Transport of [³H]Digoxin (100 nM) in LLC-GA5-COL150 Cells

Treatment	Digoxin transport (% of dose after 3 h)	
	Basal-to-apical	Apical-to-basal
LLC-PK ₁ Cells	10.4 ± 1.0 ^{a)}	4.40 ± 0.33
LLC-GA5-COL150 Cells	21.9 ± 0.2	0.71 ± 0.03
Antiarrhythmic drugs		
+ Amiodarone	6.66 ± 0.12*	6.60 ± 0.33*
+ DEA	7.80 ± 0.24*	5.30 ± 0.02*
+ Cibenzoline	19.3 ± 0.7*	1.07 ± 0.02
+ Disopyramide	21.4 ± 0.5	1.77 ± 0.28
+ Lidocaine	16.9 ± 0.2*	1.77 ± 0.08
+ Mexiletin	21.8 ± 0.2	1.85 ± 0.29
+ Quinidine	7.89 ± 0.05*	6.31 ± 0.20*
Antianginal drugs		
+ Dipyridamole	11.1 ± 0.2*	5.23 ± 0.14*
+ Isosorbide	19.6 ± 0.4*	1.94 ± 0.08*

DEA: monodesethyl-amiodarone, an active metabolite of amiodarone. a) Values are means ± S.E.M. from three independent experiments, except for basal-to-apical and apical-to-basal transport across LLC-PK₁ cell monolayers ($n=9$ and 8, respectively). Concentration of test drugs was 50 μM except for DEA at 10 μM. * $p < 0.05$ significantly different from the respective groups of LLC-GA5-COL-150 cells.

Fig. 1. Transcellular Transport of [³H]Digoxin across LLC-GA5-COL150 Cell Monolayers and Its Inhibition by Amiodarone (A) and DEA (B)

The concentrations of amiodarone and DEA were 0 μM (control) (●), 1 μM (○), 5 μM (△), 10 μM (□), 25 μM (◇) and 50 μM (▽). Each point represents the mean ± S.E.M. of results from 3–6 independent experiments. In most cases, error bars were hidden in symbols.

Fig. 2. Transcellular Transport of [³H]Digoxin across LLC-GA5-COL150 Cell Monolayers and Its Inhibition by Quinidine

The concentrations of quinidine were 0 μM (control) (●), 1 μM (○), 5 μM (△), 10 μM (□), 25 μM (◇) and 50 μM (▽). Each point represents the mean ± S.E.M. of results from three independent experiments. In most cases, error bars were hidden in symbols.

bles 1, 2, Fig. 3). In contrast, isosorbide showed only slight alteration of the transport (Table 1).

DISCUSSION

In previous study using this experimental system, the estimated IC₅₀ values for nifedipine, verapamil, diltiazem and nifedipine on [³H]digoxin transport were obtained to be 4.54 μM, 13.2 μM, 77.7 μM and 472 μM, respectively.¹⁹⁾ In clinical reports, nifedipine (60–90 mg/d)¹³⁾ and verapamil

Table 2. Estimated IC₅₀ Values for Amiodarone, Monodesethyl-amiodarone, Quinidine and Dipyridamole on [³H]Digoxin Transport in LLC-GA5-COL150 Cells

Drugs	IC ₅₀ values (μM)
Amiodarone	5.48 ^{a)}
DEA	1.27
Quinidine	9.52
Dipyridamole	40.0

DEA: monodesethyl-amiodarone, an active metabolite of amiodarone. a) Each value represents the IC₅₀ values for various drugs on the net basal-to-apical transport of [³H]digoxin at 3 h.

Fig. 3. Transcellular Transport of [³H]Digoxin across LLC-GA5-COL150 Cell Monolayers and Its Inhibition by Dipyridamole

The concentrations of dipyridamole were 0 μM (control) (●), 5 μM (△), 10 μM (□), 25 μM (◇) and 50 μM (▽). Each point represents the mean ± S.E.M. of results from 3–9 independent experiments. In most cases, error bars were hidden in symbols.

(240 mg/d)^{11,12)} are known to increase digoxin serum concentrations in human, but diltiazem (90–180 mg/d)^{22,23)} and nifedipine (55–60 mg/d)^{24,25)} show no or only slight increase of serum concentrations. The total body clearance (l/h/kg) of these calcium antagonists are about 0.50, 0.80, 0.70 and 0.50, respectively, and the absolute bioavailability are about 40%, 20%, 40% and 60%, respectively,^{26,27)} therefore the steady-state concentrations are calculated around 0.07–0.15 μM. The estimated IC₅₀ values are about 40–100 times these concentrations for nifedipine and verapamil, and more than 500–3000 times for diltiazem and nifedipine. Relatively higher concentration of [³H]digoxin, 100 nM might be responsible for the discrepancies between the estimated IC₅₀ values and steady-state concentrations, and relatively higher expression of MDR1 compared with human renal epithelial cells presumably contribute. It is impossible to estimate the possibility of drug interaction with digoxin by direct comparison of the estimated IC₅₀ values and steady-state concentrations, however, the rank order of interaction might be estimated.

In this study, it has been indicated that amiodarone and DEA inhibited the transport of [³H]digoxin transport with estimated IC₅₀ values of 5.48 and 1.27 μM, respectively (Table 2). These are comparable to the steady-state plasma concentrations of 1.06 μg/ml (1.55 μM) and 1.04 μg/ml (1.59 μM) at a dose of 200 mg/d, respectively,²⁸⁾ and MDR1 was suggested to be responsible for drug interaction with amiodarone, and that DEA contributed to the elevation of digoxin serum concentrations, although the comparison of concentrations of

protein unbound form should be necessary. The half-lives of amiodarone and DEA after a single administration are 7.0–67.6 h and 40.1–113.4 h, and are 14.9–30.2 d and 16.8–39.3 d after repetitive administration, respectively.²⁹⁾ These findings suggest that unexpected high serum concentrations of digoxin might occur several months following the discontinuation of amiodarone use. Quinidine also inhibited the transport of [³H]digoxin transport with the estimated IC₅₀ value of 9.52 μ M, being similar to the plasma concentration at a dose of 300 mg three times a day, 3.9–8.0 μ M.³⁰⁾ Thus, MDR1-based mechanism seems to be applied even for the interaction between digoxin and quinidine. Cibenzoline and lidocaine showed slight inhibition of the transport, and disopyramide and mexiletin had no such effects, suggesting their interaction with digoxin, if exists, is not due to MDR1.

Of the antianginal drugs, dipyridamole inhibited the transcellular transport of [³H]digoxin in LLC-GA5-COL150 cells with the IC₅₀ of 40.0 μ M (Table 2). The maximum serum concentration of dipyridamole was around 2.0 μ g/ml (4.0 μ M) following a single oral administration of 100 mg,³¹⁾ and the initial whole blood concentration after intravenous administration of 20 mg was around 8.0 μ g/ml (16.0 μ M).³²⁾ The estimated IC₅₀ value is also only several times the achievable concentration in clinical use. Dipyridamole has been reported to increase the cytotoxicity of vinblastine and etoposide in drug-resistant tumor cells expressing MDR1.^{33,34)} These findings indicate a risk of interactions.

In conclusion, it has been demonstrated that MDR1 is responsible for the drug interaction between digoxin and amiodarone or quinidine reported in clinical use, and that active metabolite, DEA also contributes the elevation of digoxin serum concentrations in the case of amiodarone.

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