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Interaction of Coenzyme Q10 with the Intestinal Drug Transporter P-Glycoprotein

SHIROU ITAGAKI,[†] AKIKO OCHIAI,[†] MASAKI KOBAYASHI,[†] MITSURU SUGAWARA,[‡] TAKESHI HIRANO,[†] AND KEN ISEKI*,[†]

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita 12-jo Nishi 6-chome, Kita-ku, Sapporo 060-0812, Japan, and Department of Pharmacy, Hokkaido University Hospital, Kita 14-jo Nishi 5-chome, Kita-ku, Sapporo 060-8648, Japan

In clinical trials, patients usually take many kinds of drugs at the same time. Thus, drug−drug interactions can often directly affect the therapeutic safety and efficacy of many drugs. Oral delivery is the most desirable means of drug administration. Changes in the activity of drug transporters may substantially influence the absorption of administered drugs from the intestine. However, there have been a few studies on food−drug interactions involving transporters. It is important to be aware of the potential of food−drug interactions and to act in order to prevent undesirable and harmful clinical consequences. Coenzyme Q10 (CoQ10) is very widely consumed by humans as a food supplement because of its recognition by the public as an important nutrient in supporting human health. Since intestinal efflux transporter P-glycoprotein (P-gp) is one of the major factors in drug−drug interactions, we focused on this transporter. We report here for the first time that CoQ10, which is widely used as a food supplement, affects the transport activity of P-gp.

KEYWORDS: Coenzyme; P-glycoprotein; intestine; Caco-2; transporter

INTRODUCTION

Coenzyme Q (CoQ, ubiquinone), in addition to serving as an electron and proton carrier in the electron-transport systems of mitochondria and bacteria coupled to ATP synthesis, functions in its reduced form as an antioxidant, protecting biological membranes and serum LDL from lipid peroxidation (*1*–*3*). CoQ, or ubiquinone, has a benzoquinone ring linked to a polyisoprenyl chain of 9 or 10 units in mammalian species. CoQ10 is a ubiquitous compound vital to a number of activities related to energy metabolism. Humans, by nature, have the ability to produce CoQ10. However, this ability starts declining at the age of 20 at a peak, and the amount of CoQ10 in our body decreases more rapidly after the age of 40 (*4*).

CoQ10 is very widely consumed by humans as a food supplement because of its recognition by the public as an important nutrient in supporting human health. The rationale for the use of CoQ10 as a therapeutic agent in cardiovascular and degenerative neurologic and neuromuscular diseases is based on its fundamental role in mitochondrial function and cellular bioenergetics. There are data supporting the therapeutic value of CoQ10 as an adjustment to standard medical therapy in cardiovascular diseases (*5*–*7*), and studies have indicated a beneficial effect of CoQ10 in diabetes and cancer (*8, 9*).

Orally administrated compounds are absorbed in the intestine. The absorption of compounds from the intestine is one of the important determinants for oral bioavailability. It is now recognized that several transporters contribute to the absorption of administrated compounds from the intestine. Numerous CoQ10 products are available on the market in the form of both chewable and nonchewable tablets, powder-filled capsules, and soft gelatin capsules containing an oil suspension of CoQ10. However, the bioavailability of CoQ10 in most of these products is very low. Although the mechanism of uptake of CoQ10 has not been studied, the solubility of compounds is one of the most critical issues. The use of a lipid-based formulation seems promising as a strategy to overcome the problem of poor solubility (*10, 11*). We therefore tried to improve the intestinal absorption of CoQ10 by using an emulsion formulation (*12*). We have demonstrated that a higher plasma concentration of CoQ10 was achieved by using an emulsion formulation and food intake. In addition to solubility, various mechanisms can influence the intestinal absorption and oral bioavailability of compounds. It has been reported that intestinal absorption of some compounds is limited partly because they are preferentially transported in the secretory direction (*13*–*15*). We hypothesized that some secretory transport systems may contribute to the low absorption of CoQ10.

* To whom correspondence should be addressed. Ken Iseki, Ph.D. Laboratory of Clinical Pharmaceutics and Therapeutics, Department of Biopharmaceutical Sciences and Pharmacy, Division of Biopharmaceutical Sciences and Pharmacy, Faculty of Pharmaceutical Sciences, Hokkaido University. Kita 12-jo Nishi 6-chome, Kita-ku, Sapporo 060-0812, Japan. Tel/Fax: +81-11-706-3770. E-mail: ken-i@pharm.hokudai.ac.jp.

[†] Hokkaido University.

[‡] Hokkaido University Hospital.

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Oral delivery is generally the most desirable means of drug administration, mainly because of the patient's acceptance, convenience in administration, and cost-effective manufacturing. Since patients usually take many kinds of drugs at the same time in clinical trials, drug−drug interactions involving transporters can often directly affect the therapeutic safety and efficacy of drugs. Traditionally, a change in metabolic clearance of a drug, particularly via cytochrome P450-mediated metabolism, has been considered as the cause of many clinically important drug interactions. Recently, it is recognized that changes in the activity of drug transporters may also substantially influence the absorption of administered drugs from the intestine, as well as distribution into various organs and secretion into urine, bile, and intestinal lumen. However, there have been a few studies on food−drug interactions involving transporters.

P-glycoprotein (P-gp) is a membrane transporter that actively pumps xenobiotics out of the cells. P-gp is expressed in a broad spectrum of tissues including the adrenals, kidney, liver, lungs, and intestine. Because of the broad substrate specificity of P-gp, P-gp is one of the major factors in drug−drug interactions. Since P-gp mainly transports neutral or cationic drugs (*16*), we focused on this transporter. The human colon adenocarcinoma cell line Caco-2 spontaneously differentiates in culture into polarized cell monolayers with many enterocyte-like properties of transporting epithelia. Since Caco-2 cells retain various transporters expressed in the intestine, Caco-2 cells have been widely used as a model in which to study intestinal absorption or secretion of various drugs and food components (*17–19*). Using this model, a number of studies have been performed to characterize intestinal epithelial transport mechanisms. Caco-2 cells retain polarity of P-gp expression and have been used for the functional analysis of P-gp (*20, 21*). In this study, we therefore used Caco-2 as a model in which to study the interaction of CoQ10 with P-gp.

MATERIALS AND METHODS

Chemicals. CoQ10 powder was kindly supplied by Kougen Co., Ltd. (Shizuoka, Japan: manufactured by Zhejiang Medicine Co.,Ltd. Xinchang Pharmaceutical Factory). CoQ10 was dissolved in DMSO (1% w/v final concentration) because of its poor solubility to water. All other reagents were of the highest grade available and used without further purification.

Cell Culture. Caco-2 cells obtained from American type Culture Collection (Rockville, MD) were maintained in plastic culture flasks (Falcon, Becton Dickinson and Co., Lincoln Park, NJ) as described previously (*22*). These stock cells were subcultivated before reaching confluence. The medium consisted of Dulbecco's Modified Eagle's Medium (Sigma) supplemented with 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH), 1% nonessential amino acid (Gibco), 2 mM L-glutamine (Gibco), and 100 IU/mL penicillin-100 μg/mL streptomycin (Sigma). The monolayer cultures were grown in an atmosphere of 5% CO_2−95% O_2 at 37 °C. The cells were given fresh growth medium every 2 days. When the Caco-2 cells had reached confluence, they were harvested with 0.25 mM trypsin and 0.2% EDTA (0.5−1 min at 37 °C), resuspended, and seeded into a new flask. In the present study, Caco-2 cells were used between passages 37 and 53. For the uptake studies, Caco-2 cells were seeded at a cell density of 1×10^5 cells/cm^2 on 12-well plastic plates (Corning Costar Corp., Cambridge, MA). The cell monolayers were fed fresh growth medium every 2 days and were used at 4 to 6 days for the uptake experiments. For the transport study, Caco-2 cells were seeded at a cell density of 2×10^5 cells/cm^2 on 12-well (3-μm pores, 1.0-cm^2 growth area) Transwell (Corning Costar Corp., Cambridge, MA). The cell monolayers were fed fresh growth medium every 2 days and were used at 16 to 21 days for the transport experiments. TEER was used to monitor the integrity of the monolayers. Monolayers with TEER above 350 Ωcm^2 (after subtracting the back group value of the transwell) were used in the efflux study.

Uptake Study. The uptake experiment was performed as described previously (*23*). The uptake of CoQ10 was measured using monolayer cultures grown in 12-well plastic plates. The incubation medium used for the uptake study was HBSS-HEPES (pH 7.4) buffer (25 mM D-glucose, 137 mM NaCl, 5.37 mM KCl, 0.3 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 10 mM HEPES). After removal of the growth medium, cells were preincubated at 37 °C for 10 min with 1.0 mL of HBSS-HEPES buffer (pH 7.4). After removal of the medium, 1.0 mL of incubation medium containing a substrate was added. The monolayers were incubated for the indicated time at 37 °C. Each cell monolayer was rapidly washed twice with a 1.0 mL ice-cold incubation medium at the end of the incubation period. The cells were suspended in 0.5 mL of an extraction solution (0.03 M phosphate buffer (pH 7.0)/methanol = 50/50) for 1 h at room temperature. The extraction solution was used for the determination of the substrate concentration after centrifugation of the mixture (15,000 × *g*, for 10 min). In the energy-dependency studies, the buffer was preincubated at 37 °C for 10 min in the presence of 10 mM sodium fluoride (NaF) and 10 mM sodium azide (NaN_3).

Transcellular Transport Study. Transcellular transport of CoQ10 was measured using monolayer cultures grown in 12-well Transwell. The incubation medium used for the transcellular transport study was the HBSS-HEPES (pH 7.4) buffer. After removal of the growth medium from both sides of monolayers, the cells were preincubated at 37 °C for 10 min with HBSS-HEPES (pH 7.4) buffer (12-well; 1.5 mL of basolateral and 0.5 mL of apical). After removal of the medium, incubation medium containing CoQ10 was added to the basolateral side. The monolayers were incubated for 120 min at 37 °C. For transport measurements, aliquots of incubation medium on the other side were taken at specified times and samples were collected for immediate analysis.

Analytical Procedures. CoQ10 was determined by HPLC using an absolute calibration curve method described previously (*12*). For the analysis of samples obtained from studies using Caco-2 cells, 100 μL of specimens was extracted with 1 mL of *n*-hexane. After shaking the mixture vigorously, the sample was centrifuged at 2,000 × *g* for 5 min at 4 °C. Nine hundred microliters of the organic layer was evaporated to dryness under a gas stream. The residue was redissolved in 100 μL of mobile phase for HPLC injection. One hundred microliters of specimens was diluted 3-fold with methanol. After vortexing, the sample was extracted with 1 mL of *n*-hexane. After shaking the mixture vigorously, the sample was centrifuged at 2,000 × *g* for 5 min at 4 °C. Nine hundred microliters of the organic layer was evaporated to dryness under a gas stream. The residue was redissolved in 100 μL of mobile phase for HPLC injection. The concentration of CoQ10 was determined using an HPLC system equipped with a JASCO 880-PU pump and a 870-UV UV−vis detector. The column was a GL Science ODS-2 (5 μm in particle size, 4.6 mm in inside diameter × 250 mm). A mobile phase containing 2-propanol/methanol/tetrahydrofuran (55/39/6) was used. The column temperature and flow rate were 40 °C and 1.0 mL/min, respectively. The wavelength for detection was 275 nm. Forty microliters of sample was injected into the HPLC system. We used CoQ10 powder for a standard solution. The calibration curve was constructed in the concentration range of 0−2.4 mg/L. The measurement of rhodamine (Rho) 123 was carried out in a multilabel counter Wallac 1420 ARVOse (Perkin-Elmer, Wellesley, MA) with an excitation wavelength of 485 nm and emission wavelength of 535 nm. Protein was measured by the method of Lowry et al. with bovine serum albumin as a standard (*24*).

The apparent permeability coefficient (P_{app}) was calculated using the following equation:

$$P_{app} = dQ/dt \cdot 1/(A \cdot C_0) \quad (1)$$

where dQ/dt is the linear appearance rate of mass in the receiver solution, A is the filter/cell surface area (1.00 cm^2), and C_0 is the initial concentration of substrate (10 μM).

Student's *t*-test was used to determine the significance of differences between two group means. Statistical significance among means of more than two groups was determined by one-way analysis of variance. A value of $P < 0.05$ was considered significant.

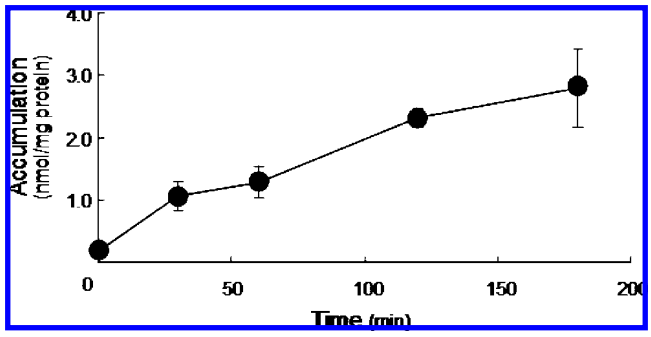

Figure 1. Time course of the uptake of CoQ10 by Caco-2 cells. Cells were incubated in a medium at pH 7.4 for indicated periods with CoQ10 (10 μM). Each point represents the mean with SD of three measurements.

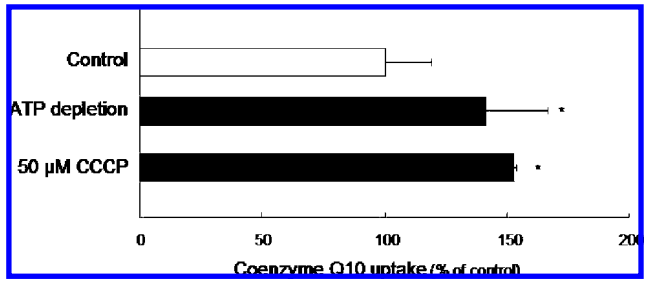

Figure 2. Effects of metabolic inhibitors on the uptake of CoQ10 by Caco-2 cells. Cells were incubated in a medium at pH 7.4 containing CoQ10 (10 μM) for 120 min with or without (control) inhibitors. Each column represents the mean with SD of four measurements. The control value for the uptake of CoQ 10 was 2.64 $\pm$ 0.36 nmol/mg protein/60 min. *, $P < 0.05$, significantly different from the control.

RESULTS AND DISCUSSION

In this study, we focused on the efflux mechanisms of CoQ10 involving transporters in the apical membrane. Since the Caco-2 cells were grown in wells, basolateral transporters make a minor contribution to the transport of CoQ10 (*25*) In order to characterize the efflux transport of CoQ10 from Caco-2 cells, an uptake study was carried out. Since the uptake of CoQ10 was almost linear at 120 min after the start of incubation (**Figure 1**), the efflux of CoQ10 was characterized by the amount of CoQ10 remaining in Caco-2 cells for 120 min in the presence of inhibitors. It has been reported that several intestinal secretory transport systems require metabolic energy. Therefore, to clarify the possible contribution of energy-dependent transporters to CoQ10 transport in the intestine, the effect of metabolic inhibitors on the uptake of CoQ10 by Caco-2 cells was investigated. The uptake of CoQ10 was increased in an ATP-depleted condition (**Figure 2**). Moreover, typical ATP-depleting compounds, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), also increased the uptake of CoQ10 (**Figure 2**). These findings suggest that CoQ10 is secreted by a carrier-mediated system. Among the transporters involved in secretion, P-gp has been the most extensively investigated. Furthermore, P-gp mainly transports neutral or cationic drugs. We then investigated the effects of various compounds that are known to be inhibitors of P-gp on the uptake of CoQ10 by Caco-2 cells. Rho123, digoxin, and quinidine, which have been reported to be substrates of P-gp (*26, 27*), significantly increased the uptake of CoQ10 (**Figure 3**). The results suggest that P-gp plays a role in the efflux of CoQ10 across the apical membrane in Caco-2 cells. We examined the effect of Rho123 and quinidine on the transcellular transport of CoQ10. Rho123 and quinidine

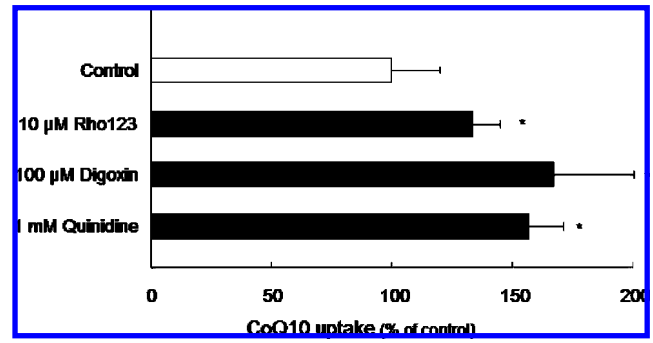

Figure 3. Effects of Rho123, digoxin and quinidine on the uptake of CoQ10 by Caco-2 cells. Cells were incubated in a medium at pH 7.4 containing CoQ10 (10 μM) for 120 min with or without (control) inhibitors. Each column represents the mean with SD of four measurements. *, $P < 0.05$, significantly different from the control.

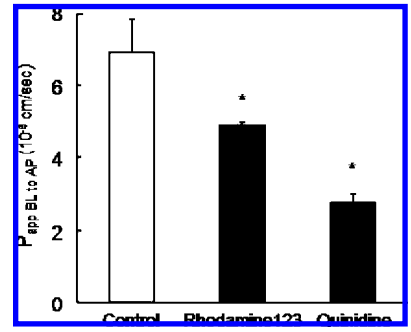

Figure 4. Effects of Rho123 and quinidine on the transepithelial flux of CoQ10 by Caco-2 cell monolayers. The monolayers were incubated in a medium at pH 7.4 containing CoQ10 (10 μM) for 60 min with or without (control) inhibitors. Each column represents the mean with SD of three measurements. *, $P < 0.05$, significantly different from the control.

significantly decreased CoQ10 transport (**Figure 4**). The $P_{app BL\ to\ AP}$ value of CoQ10 across Caco-2 cell monolayers was smaller than those of Rho123, digoxin, and quinidine (*26, 27*). In addition to P-gp, several transporters contribute to the intestinal absorption or secretion of various compounds. However, the contribution of P-gp and other intestinal efflux transporter(s) to the intestinal efflux of CoQ10 has not been elucidated yet. Further studies are needed to elucidate the mechanism of intestinal efflux of CoQ10.

Rho123 is widely used as a typical substrate for P-gp (*26*). In order to confirm the contribution of P-gp to the transport of CoQ10, the opposite inhibitory effect of CoQ10 on the uptake of Rho123 was investigated. The efflux of Rho123 was characterized by the amount of Rho123 remaining in Caco-2 cells for 60 min in the presence of inhibitors. The uptake of Rho123 was increased in the presence of CoQ10 (**Figure 5**). This result suggests that efflux transport of CoQ10 is mediated by P-gp in Caco-2 cells. Our conclusion was further supported by this finding.

In a clinical setting, patients usually take many kinds of drugs at the same time. Drug−drug interactions involving drugs having a narrow therapeutic range might have serious adverse consequences. Food−drug interactions also increase the risks of adverse events. It is well known that digoxin is a substrate for P-gp and that inhibition of P-gp by concurrently prescribed drugs or endogenous substances can cause a clinically significant alteration in pharmacokinetics of digoxin (*28*). It is important to be aware of the potential of CoQ10−drug interactions and to act in order to prevent undesirable and harmful clinical consequences.

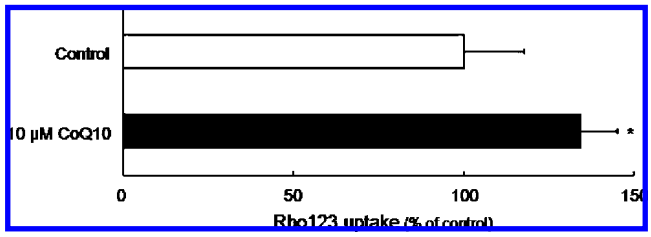

Figure 5. Effect of CoQ10 on the uptake of Rho123 by Caco-2 cells. Cells were incubated in a medium at pH 7.4 containing Rho123 (10 μM) for 60 min with or without (control) inhibitors. The control value for the uptake of Rho123 was 0.46 $\pm$ 0.03 nmol/mg protein/60 min. Each column represents the mean with SD of four measurements. *, $P < 0.05$, significantly different from the control.

The importance of CoQ10 in the life of living organisms is illuminated most clearly by the number of reports describing the genetic disorders in which CoQ10 synthesis is impaired. Lowered lipid content in organs causes serious metabolic disturbances, but CoQ10 supplementation reestablishes mitochondrial and other functions. However, CoQ10 is taken up from the intestine at a low rate. When the absorption of a drug candidate is poor, various approaches to improve absorption, such as administration of prodrugs or analogues, or coadministration of absorption enhancers, are often undertaken. Studies on the mechanisms of intestinal absorption of various compounds have revealed that secretory transport limits the oral bioavailability of certain drugs (*15*). The above-described findings indicated the possibility that the absorption of CoQ10 can be improved by P-gp inhibition. CoQ10 and P-gp inhibitor coadministration may offer improved pharmacological effects.

In summary, we have demonstrated that CoQ10 affects the transport activity of P-gp and that efflux transport of CoQ10 is mediated by P-gp in Caco-2 cells. In a clinical setting, patients usually take many kinds of drugs at the same time. It is important to be aware of the potential of CoQ10−drug interactions and to act in order to prevent undesirable and harmful clinical consequences.

ABBREVIATIONS USED

CoQ, coenzyme; P-gp, P-glycoprotein; NaF, sodium fluoride; NaN_3, sodium azide; Rho, rhodamine; P_{app}, apparent permeability coefficient; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

ACKNOWLEDGMENT

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