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Short communication

Grapefruit juice enhance the uptake of coenzyme Q10 in the human intestinal cell-line Caco-2

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ABSTRACT

Coenzyme Q10 (CoQ10) is very widely consumed by humans as a food supplement. However, CoQ10 is taken up from the intestine into the circulation at a low rate. The absorption of compounds from the gastrointestinal tract is one of the important determinants for oral bioavailability. Secretory transport limits the oral bioavailability of compounds. It has been reported that efflux transport of CoQ10 is mediated by P-glycoprotein (P-gp) in Caco-2 cells. We tried to improve intestinal absorption of CoQ10 by modulating P-gp. Since grapefruit juice (GFJ) is reported to inhibit P-gp function, we investigated the effect of GFJ on the transport of CoQ10 by Caco-2 cells. In the presence of GFJ, the basal-to-apical transport of CoQ10 was decreased and the uptake of CoQ10 was increased. These findings suggest that the combined administration of CoQ10 and GFJ could enhance CoQ10 absorption.

1. 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 (Ernster, Forsmark, & Nordenbrand, 1992; Stocker, Bowry, & Frei, 1991). 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 (Kalen, Appelkvist, & Dallner, 1989).

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 (Overvad et al., 1999), and studies have indicated a beneficial effect of CoQ10 in diabetes and cancer (Hodgson, Watts, Playford, Burke, & Croft, 2002; Roffe, Schmidt, & Ernst, 2004).

Numerous CoQ10 products are available on the market in the form of both chewable and non-chewable 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. The absorption of compounds from the gastrointestinal tract is one of the important determinants for oral bioavailability. When the absorption of a drug candidate is poor, various approaches to improve absorption, such as administration prodrugs or analogues or co-administration 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 (Suzuki & Sugiyama, 2000). Among these secretory transport systems, P-glycoprotein (P-gp/ABCB1) has been most extensively investigated (Hunter, Hirst, & Simmons, 1993). We have recently reported that efflux transport of CoQ10 is mediated by P-gp in Caco-2 cells, a model in which to study intestinal absorption or secretion of compounds (Itagaki et al., 2008). It is possible that a strategy combining P-gp substrates with P-gp inhibitors will lead to improvement in the intestinal absorption of P-gp substrates. In this study, we tried to improve the intestinal absorption of CoQ10 by modulating the efflux transporter.

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2. Materials and methods

2.1. Chemicals

CoQ10 powder was kindly supplied by Kougen Co. Ltd. (Shizuoka, Japan: manufactured by Zhejiang Medicine Co. Ltd. Xinchang Pharmaceutical Factory). Grapefruit juice (GFJ) was produced by Dole Food Company Inc. (USA). All other reagents were of the highest grade available and used without further purification.

2.2. Cell culture

Caco-2 cells obtained from RIKEN Cell Bank (Tsukuba, Japan) were maintained in plastic culture flasks (Falcon, Becton Dickinson and Co., Lincoln Park, NJ) as described previously (Kimoto et al., 2007). These stock cells were subcultivated before reaching confluence. The medium consisted of Dulbecco's Modified Eagle's Medium (Sigma, St. Louis, MO) supplemented with 10% foetal bovine serum (ICN Biomedicals Inc., Aurora, OH), 1% non-essential amino acid (Gibco/Invitrogen, Carlsbad, CA), 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 42 and 55. For the uptake studies, Caco-2 cells were seeded at a density of 1×10^5 cells/cm^2 on 12-well plastic plates (Corning Costar Corp., Cambridge, MA). The cell monolayers were fed a fresh growth medium every 2 days and were used at 4–6 days for the uptake experiments. For the transport study, Caco-2 cells were seeded at a density of 2×10^5 cells/cm^2 on 12-well (3-μm pores, 1.0-cm^2 growth area) Transwell™ (Corning Costar Corp.). The cell monolayers were fed a fresh growth medium every 2 days and were used at 16–21 days for the transport experiments. TEER was used to monitor the integrity of the monolayers. Monolayers with TEER above 350 Ω cm^2 (after subtracting the back group value of the transwell) were used in the efflux study.

2.3. Transcellular transport across Caco-2 cell monolayers

Transcellular transport of CoQ10 was measured using monolayer cultures grown in 12-well Transwell™. The incubation medium used for the transcellular transport study was HBSS-HEPES 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 buffer (12-well; 1.5 ml of outside and 0.5 ml of inside). After removal of the medium, incubation medium containing CoQ10 was added outside. In the inhibition study, incubation medium containing GFJ (1%) was added inside. The monolayers were incubated for 120 min at 37 °C. For transport measurements, aliquots of incubation medium were taken from inside at specified times and samples were collected for immediate analysis.

2.4. Uptake study in Caco-2 cell monolayers

The uptake experiment was performed as described previously (Saito et al., 2005). 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. After removal of the medium, 1.0 ml of incubation medium or 1% GFJ containing CoQ10 was added. The monolayers were incubated for the indicated time at 37 °C. Each cell monolayer was washed rapidly twice with 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 determination of the substrate concentration after centrifugation of the mixture (15,000*g*, for 10 min).

2.5. Analytical procedures

CoQ10 was determined by HPLC using an absolute calibration curve method described previously (Ochiai et al., 2007). 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 2000*g* for 5 min at 4 °C. Nine hundred microlitres 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 microlitres of specimens was diluted threefold with methanol. After vortexing, the sample was extracted with 1 ml of *n*-hexane. After shaking the mixture vigorously, the sample was centrifuged at 2000*g* for 5 min at 4 °C. Nine hundred microlitres 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 microlitres of sample was injected into the HPLC system. We used CoQ10 powder for a standard solution. A calibration curve was constructed in the concentration range of 0–2.4 mg/l. The protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) with bovine serum albumin as a standard.

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

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

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. A value of $P < 0.05$ was considered significant.

3. Results and discussion

CoQ10 is a ubiquitous compound vital to a number of activities related to energy metabolism. Because dysfunctional energy metabolism has been cited as a contributing factor for a number of conditions, dietary supplementation of CoQ10 has been used in the treatment of cardiac, neurologic, oncologic and immunologic disorders (Bonakdar & Guarneri, 2005). However, CoQ10 is taken up from the intestine into the circulation at a low rate (Zhang, Aberg, Appelkvist, Dallner, & Ernster, 1995). We hypothesised that some secretory transport systems may contribute to the low absorption of CoQ10 and found that efflux transport of CoQ10 is mediated by P-gp in Caco-2 cells (Itagaki et al., 2008). Moreover, the above-described findings indicated the possibility that the

absorption of CoQ10 can be improved by P-gp inhibition. As a strategy to overcome the problem of low absorption, we focused on this secretory transport system.

There is increasing evidence that intake of GFJ can affect the activity of P-gp in the small intestine (Dahan & Amidon, 2009). GFJ and some of its components have been shown to inhibit transport of P-gp (Honda et al., 2004; Lim & Lim, 2006; Takanaga, Ohnishi, Matsuo, & Sawada, 1998). We therefore investigated the effect of GFJ on the basal-to-apical transport of CoQ10 and uptake of CoQ10 by Caco-2 cells. In the presence of GFJ, the $P_{app\ BL\ to\ AP}$ value of CoQ10 was significantly reduced (Fig. 1 and Table 1). In contrast to efflux transport of CoQ10, GFJ significantly increased the uptake of CoQ10 into Caco-2 cells (Fig. 2).

As shown in Table 1, the inhibitory effect of GFJ on the basal-to-apical transport of CoQ10 is stronger than that of rhodamine 123, a typical inhibitor of P-gp (Itagaki et al., 2008). 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 (Tanigawara et al., 1992). Furthermore, Boyd et al. (2000) reported that verapamil, a well-known P-gp inhibitor, reduced the basolateral-to-apical permeability of digoxin by Caco-2 cells by almost 60%. In the present study, GFJ reduced the basolateral-to-apical permeability of CoQ10 by Caco-2 cells by almost 50%. These results indicate that the combined administration of CoQ10 and GFJ could enhance CoQ10 absorption. Taking these findings into consideration, it is possible that co-administration of CoQ10 with GFJ will be an easily accessible way to improve the pharmacological effects of CoQ10. We have reported that the absorption of CoQ10 was improved by using the emulsion formulation (Ochiai et al., 2007). Moreover, intestinal absorption of CoQ10 after administration of the emulsion formulation was enhanced food intake. The development of appropriate dosing regimens using emulsion formulation of CoQ10 with food supplementation including GFJ may offer further improved intestinal absorption.

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. Many drugs found to have clinically significant interactions with GFJ included the 1,4-dihydropyridine calcium antagonists, immunosuppressants, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, HIV protease inhibitors, antihistamines and benzodiazepines. It is important to be aware of the potential of GFJ–drug interactions and to act in order to prevent undesirable and harmful clinical consequences. It has been reported that GFJ interaction is not limited to just effects on P-gp. Numerous reports have documented drug interactions with GFJ that occur *via* inhibition of CYP3A4 (Dahan & Amidon, 2009). However, to date, there has been no report that CoQ10 is a substrate for CYP3A4.

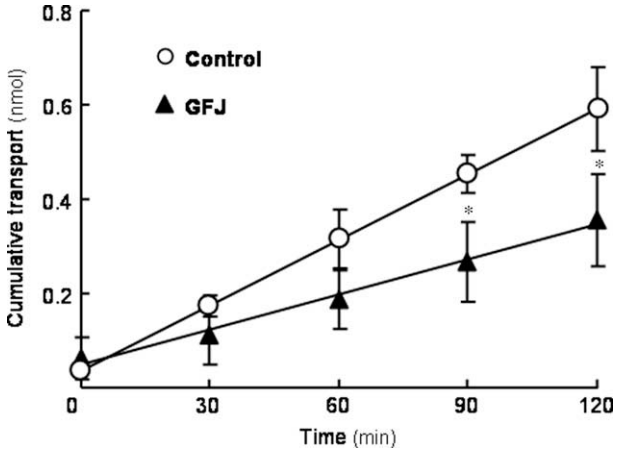

Fig. 1. Effect of GFJ on the transepithelial flux of CoQ10 by Caco-2 cell monolayers. Incubation medium at pH 7.4 containing CoQ10 (10 μM) was added to the basal compartment, while a drug-free buffer (control) or 1% GFJ was added to the apical compartment. The monolayers were incubated for 120 min. *Abbreviation:* GFJ, grapefruit juice. $^{*}P < 0.05$, significantly different from the control.

Table 1
IC_{50} values of EGCG and rutin on xanthine oxidase-induced light emission and superoxide anion scavenging activity.

	$P_{app\ BL\ to\ AP}$ (10^{-6} cm/s)
Control	6.86 ± 0.97
GFJ	3.68 ± 0.67*
Rhodamine123[a]	4.78 ± 0.02*

Abbreviation: GFJ, grapefruit juice.
* $P < 0.05$, significantly different from the control.
[a] Itagaki et al. (2008).

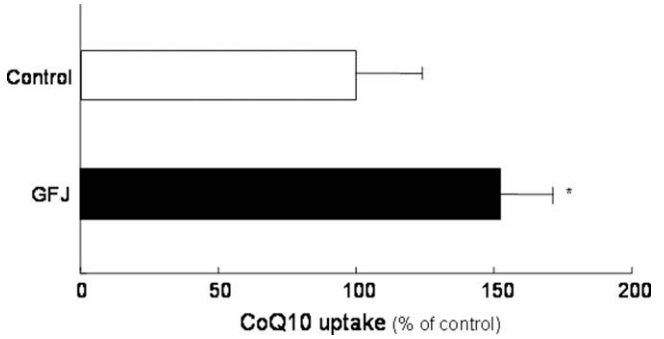

Fig. 2. Effect of GFJ on the uptake of CoQ10 by Caco-2 cells. Cells were incubated in a medium at pH 7.4 (control) or 1% GFJ containing CoQ10 (10 μM) for 120 min. Each value represents the mean with SD of 3–4 measurements. *Abbreviation:* GFJ, grapefruit juice. $^{*}P < 0.05$, significantly different from the control.

Numerous approaches to improve the solubility of CoQ10 have been explored. Many of these attempts have used various forms of lecithin, liposomes, aqueous dispersions, co-precipitate, and co-melt preparations. Formulators have also tried complexation with a soluble adjuvant, or the co-administration of a thermonutrient such as piperine. It has been reported that P-gp inhibitors can significantly increase the absorption of lecithin vesicles of cyclosporine A in the rat small intestine (Chen, Ping, Guo, Lv, & Gao, 2003). Thus, intestinal P-gp inhibition by GFJ may offer further improved absorption of lecithin vesicles of CoQ10. On the other hand, it has been reported that piperine inhibits transport of P-gp substrates digoxin and cyclosporine A (Bhardwaj et al., 2002). Moreover, Sharma, Varma, Chawla, and Panchagnula (2005) reported that intestinal permeability of cyclosporine A was significantly increased in the presence of piperine (Sharma et al., 2005). They have hypothesised that the increase in intestinal permeability of cyclosporine A can be attributed for the P-gp inhibitory activity of piperine. Thus, it is possible that improved intestinal absorption and bioavailability of CoQ by co-administration of piperine is, at least in part, attributed for the P-gp inhibitory activity of piperine. Further studies are needed to elucidate the effect of GFJ on the improved CoQ10 absorption by these approaches.

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. CoQ10 and GFJ co-administration may offer improved

pharmacological effects. Further study is needed to determine whether co-administration of CoQ10 with GFJ can prevent diseases.

In summary, we have demonstrated that a higher cellular uptake of CoQ10 was achieved in the presence of GFJ. It is possible that co-administration of CoQ10 with GFJ constitutes an easily accessible way to improve the intestinal absorption of CoQ10. Further studies are needed to assess the pharmacological effects of CoQ10 in the case of combined administration of GFJ. Such investigations will provide important information for improving the pharmacological effects of CoQ10.

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