Emulsification Using Highly Hydrophilic Surfactants Improves the Absorption of Orally Administered Coenzyme Q10

Yuki Sato, Hanami Mutoh, Mika Suzuki, Yoh Takekuma, Ken Iseki, and Mitsuru Sugawara*

Faculty of Pharmaceutical Sciences, Hokkaido University; Kita 12, Nishi 6, Kita-ku, Sapporo 060–0812, Japan.
Received June 6, 2013; accepted September 9, 2013

Coenzyme Q10 (CoQ10) is an essential component in the electron-transport systems of mitochondria and bacteria and is often used as a supplementary treatment for some diseases. We previously reported that the bioavailability of CoQ10 powder was less than 10%. In this study, we investigated various preparations to improve the intestinal absorption of CoQ10 with focus on the effect of emulsification. We prepared a suspension and some emulsions with four types of surfactants and investigated the plasma concentration profile after oral administration to rats. The absorption of CoQ10 was improved by an emulsion formulation although there was little absorption of CoQ10 when a suspension was administered. However, little CoQ10 was absorbed in the bile duct-ligated group even when the emulsion formulation was administered (about 50% of the control group). Bile and emulsion formulation are essential for absorption of CoQ10. When the preparations containing Tween20 (polysorbate (20) sorbitan monolaurate) and Tween80 (polyoxyethylene (20) sorbitan monooleate) were administered, plasma concentrations of CoQ10 were higher than those obtained with preparations containing Tween65 (polyoxyethylene (20) sorbitan tristearate) and Span20 (sorbitan monolaurate). Tween20 and Tween80 have higher hydrophile–lipophile balance (HLB) values than those Tween65 and Span20. Our study suggests that highly lipophilic compounds like CoQ10 would diffuse the unstirred water layer and would easily access the intestinal apical membrane by an emulsion containing a surfactant with a high HLB value. Attention must be given to CoQ10 supplementation for patients whose bile is not excreted to the intestine such as patients with cholestasis.

Key words coenzyme Q10; absorption; bile; emulsion; intestine; lutein

Preventive medicine and anti-aging medicine have received much attention recently due to increases in the proportion of elderly people in the population and patients with lifestyle diseases such as hypertension, dislipidemia and diabetes. Oxidative stress is involved in the onset of lifestyle diseases,[1,2] and various antioxidant supplements and antioxidant-fortified functional foods have recently become available. Many epidemiological studies have shown relationships between consumption of polyphenol or carotenoid-rich foods and prevention of these lifestyle diseases.[3,4]

Coenzyme Q (CoQ) has a benzoquinone ring linked to a polyisoprenyl chain of 9 or 10 units in mammalian species. CoQ10, also known as ubiquinone, is a ubiquitous component vital to a number of activities related to energy metabolism. CoQ10 also functions in its reduced form as an antioxidant, protecting biological membranes and serum low density lipoprotein (LDL) from lipid peroxidation.[5,6] The use of CoQ10 as a therapeutic cardiovascular agent was based on its fundamental role in mitochondrial function and cellular bioenergetics.[7,8] Many studies have also indicated a beneficial antioxidant effect of CoQ10 as supplementation, and CoQ10 has received much attention due to its antioxidant activities.[9–12]

The benefits of intake of CoQ10 have recently been recognized. CoQ10 is present in sardines, mackerel, green leafy vegetables such as spinach, soy beans, peanuts and beef liver. However, the absorption of CoQ10 from the gastrointestinal tract is poor due to its large molecular weight and poor water solubility.[12] We previously reported that the bioavailability of CoQ10 was very low, less than 10%.[13] It is therefore important to improve the absorption of CoQ10 and to enhance its physiological and nutritional benefits. The aim of this study was to improve the low bioavailability of CoQ10 with focus on pharmaceutical aspects. We also focused on the effect of bile on CoQ10 intestinal absorption.

MATERIALS AND METHODS

Chemicals CoQ10 powder was donated by Kougen Co., Ltd. (Shizuoka, Japan: manufactured by Zhejiang Medicine Co., Ltd., Xinchang Pharmaceutical Factory). Lutein was donated by JARD Inc. (Tokyo, Japan) and Koyo Mercantile Co., Ltd. (Kyoto, Japan). Reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise noted. All other reagents were of the highest grade available and used without further purification.

Animals Male Wistar rats, 6 weeks old (160–180 g in weight), were obtained from Jla (Tokyo, Japan). The housing conditions were the same as those described previously.[14] The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals."

Preparation of Suspension and Emulsion Formulations To prepare suspensions of CoQ10, we used METOLOSE® (SHIN-ETSU Chemicals Co., Ltd., Tokyo, Japan), a mixture of hypromellose and methylcellulose. METOLOSE® (concentration: 0.5%, w/v) was added to distilled water warmed to about 80°C and dispersed. Due to the high solubility of methylcellulose at a low temperature, we stopped heating the water and continued stirring with cooling overnight. CoQ10 was then dispersed in 0.5% METOLOSE® and prepared with suspension. For preparation of an emulsion, CoQ10 and lutein was solved in isopropyl myristate, and a surfactant [Tween20 (polyoxyethylene (20) sorbitan monolaurate), Tween80 (polyoxyethylene (20) sorbitan monooleate), Tween65 (polyoxyeth-

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: msuga@pharm.hokudai.ac.jp

ylene (20) sorbitan tristearate) or Span20 (sorbitan monolaurate) (Nacalai Tesque Inc., Kyoto, Japan)] and co-surfactant (propylene glycol) were added and then the mixture was stirred, respectively. Final composition ratio of the emulsion was oil–surfactant–cosurfactant–water=10.8 : 21.5 : 21.5 : 46.2 (wt%). Dose of CoQ10 suspension and emulsion was 25 mg/kg weight (1 mL/kg weight). Doses of lutein suspension and emulsion formulation were 0.5 mg/kg weight (1 mL/kg weight).

Bile Collection and Oral Administration The rats were fasted for 24 h before the experiments. They were anesthetized by an intraperitoneal (i.p.) injection of 50 mg/kg sodium pentobarbital.[14] To determine the effect of bile on absorption after oral administration, the rats were divided into two groups: a sham group (control), in which a small midline incision was made in the abdomen, and a bile duct-ligated group, in which the bile duct was ligated by suturing. Bile was collected from other rats by biliary cannula (PE-10). CoQ10 was orally administered as an emulsion or a suspension. Plasma samples were obtained at a designated time as described previously.[13,15] The samples were kept at −20°C until assay.

Analytical Procedures The concentration of CoQ10 was determined using an HPLC system equipped with an L-4000 pump and an L-7420 UV-VIS detector (HITACHI, Tokyo, Japan). Four hundred microliters of methanol was added to 100 μL of a sample, and the mixture was shaken for 30 s. Then 2 mL of *n*-hexane was added and the mixture was shaken for 10 min. After centrifugation at 800×***g*** for 10 min, 1.5 mL of the organic layer was taken and evaporated to dryness under a nitrogen gas stream. The residue was dissolved in 200 μL of ethanol for HPLC injection. The column for HPLC was an ERC-ODS-1161 (C-18) (Yokohamarika Inc., Kanagawa, Japan). A mobile phase containing ethanol–methanol (65 : 35, v/v) was used. Column temperature and flow rate were 40°C and 0.6 mL/min, respectively. The wavelength for detection was 275 nm. Forty microliters of a sample was injected into the HPLC system. The concentration of lutein was determined using an HPLC system as described previously.[15]

Data Analysis To analyze pharmacokinetics of CoQ10, data sets were fitted by a two-compartment model with Origin 8.1J® (OriginLab Corp., Northampton, MA, U.S.A.).[13] The equation was the same as that reported previously.[15] The area under the curve (*AUC*) was calculated by the trapezoidal rule.

Statistical Analysis 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 (ANOVA) followed by the Turkey–Kramer test. And statistical significance was defined as $p<0.05$. Data were expressed as means with standard error (S.E.).

RESULTS AND DISCUSSION

In the first part of this study, we evaluated the efficacy of an emulsion formulation of CoQ10. Figure 1 shows the plasma concentration of CoQ10 after oral administration of an emulsion or suspension formulation. It was found that the absorption of CoQ10 was poor when the suspension was administered. The AUC_{0-24h} values of CoQ10 in the suspension group and emulsion group were 1.4±0.4 and 3.0±0.9 (μg×h/mL), respectively. It was showed that absorption of CoQ10 was significantly improved by emulsification compared with

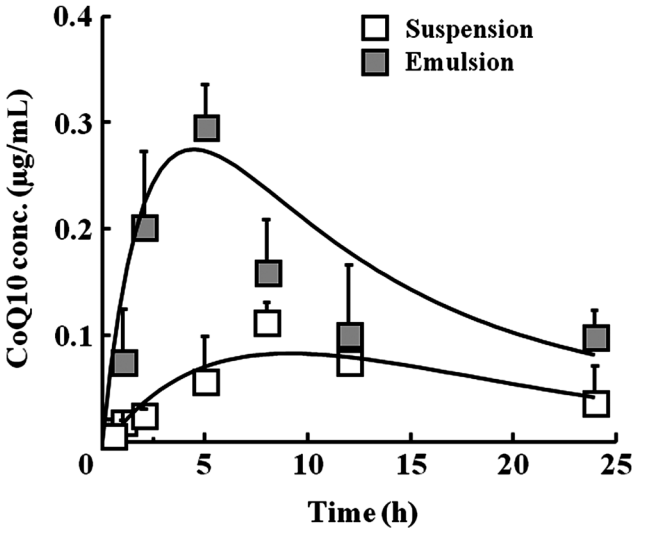

Fig. 1. Plasma Concentration of CoQ10 after Oral Administration of Suspension and Emulsion in Rats

Each point represents the mean with S.E. of 3 measurements. All rats were fasted for 24 h before the experiments. A suspension (open squares) or an emulsion (closed squares) of CoQ10 (25 mg/kg weight) was administered.

suspension. Intestinal absorption of lipophilic compounds such as CoQ10 has been reported to be increased with food rich in fat.[16] We have also reported that the plasma concentration of lutein after oral administration in a food intake group was significantly increased compared with that in a fasting group.[15] Similar to lutein, it has been reported that the plasma concentration of CoQ10 after oral administration in a food intake group was significantly increased compared with that in a fasting group.[13] Foods fed to rats are Rodent laboratory diet® EQ5L37 containing soybean oil, DL-α-tocopherol acetate and cholecalciferol.[15] Regarding the similar lipophilic characteristics of CoQ10 and lutein, we consider that these lipophilic components would increase the intestinal absorption of CoQ10.

In addition to food components, it is well known that micelle formulation and bile are important for intestinal absorption of lipophilic compounds.[17,18] However, it remains unclear how bile influences the intestinal absorption of emulsions of these compounds. We then investigated the plasma concentrations of CoQ10 and calculated *AUC* values for the bile duct-ligated group and control group. The plasma concentration of CoQ10 was higher in the control group than in the bile duct-ligated group (Fig. 2A). On the other hand, plasma concentration and *AUC* of CoQ10 were significantly increased when the bile duct-ligated rats were administered CoQ10 emulsion with bile collected from other rats. AUC_{0-24h} values of CoQ10 in the control, bile duct-ligated and bile duct-ligated +bile groups were 3.0±0.9, 0.2±0.1 and 1.7±0.2 (μg×h/mL), respectively. The *AUC* of CoQ10 in the control group was 10-fold greater than that in the bile duct-ligated group, and little CoQ10 was absorbed without bile even when the emulsion formulation was administered (Fig. 2B). For reference, we confirmed that there was little absorption of CoQ10 after oral administration of CoQ10 suspension to rats in which the bile duct was not ligated (data not shown). These results suggest that both emulsion formulation and bile are essential for intestinal absorption of lipophilic compounds such as CoQ10. We also consider that whole foods (food intake) stimulate the excretion of bile. Since

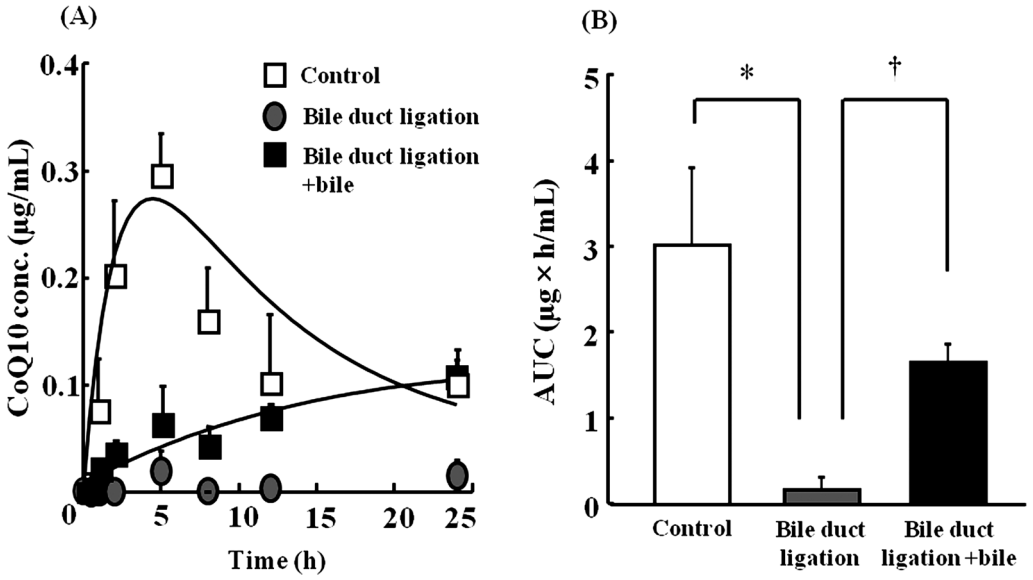

Fig. 2. Effects of Bile Acid on Plasma Concentration (A) and *AUC* (B) of CoQ10 after Oral Administration of Emulsion in Rats

(A) Each point represents the mean with S.E. of 3 measurements. All rats were fasted for 24 h before the experiments. An emulsion of CoQ10 (25 mg/kg weight) was administered. Open symbols (squares) show the control group and closed symbols (circles) show the bile duct-ligated group. Closed symbols (squares) show the bile duct-ligated group with bile collected from other rats. (B) Each column represents the mean with S.E. of 3 measurements. The open column shows the *AUC* of the control group and the closed column shows that of rats with the bile duct ligated before the experiments. ∗ Significantly different from the control group at $p<0.05$. † Significantly different from the bile duct-ligated group at $p<0.05$.

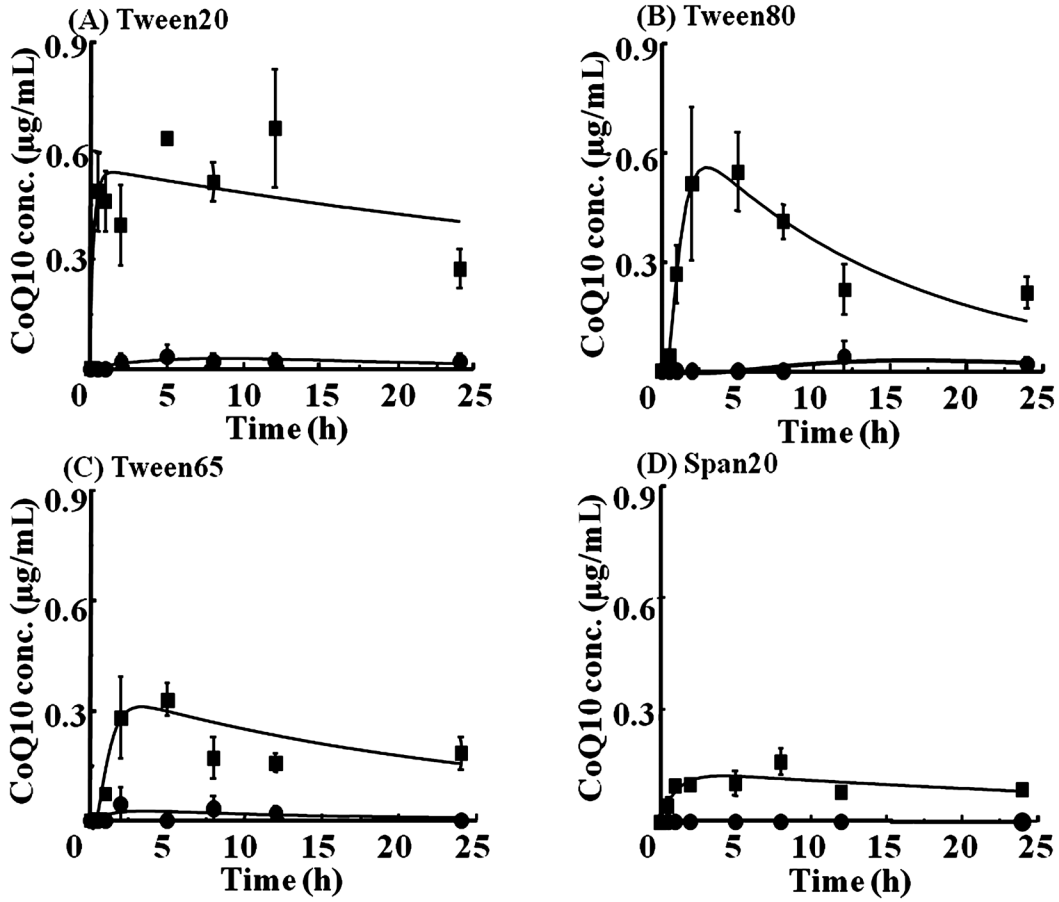

Fig. 3. Plasma Concentrations of CoQ10 in Rats after Oral Administration of Emulsions Prepared with Tween20 (A), Tween80 (B), Tween65 (C) and Span20 (D) as Surfactants

Each point represents the mean with S.E. of 3 measurements. All rats were fasted for 24 h before the experiments. CoQ10 (25 mg/kg weight) was administered as an emulsion with the surfactant Tween20 (A), Tween80 (B), Tween65 (C) and Span20 (D) to rats. Squares show sham-operated rats (control group) and circles show rats with the bile duct ligated before the experiments.

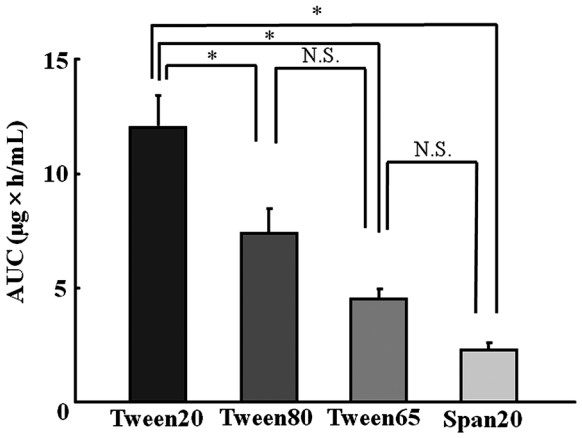

Fig. 4. Effects of Various Surfactants of the Emulsion on *AUC* of CoQ10 after Oral Administration in Rats

Each column represents the mean with S.E. of 3 measurements. *AUC* was calculated by the trapezoidal rule. * Significantly different from the group of CoQ10 emulsion prepared with Tween20 at $p<0.05$. N.S., not significant.

CoQ10 is not absorbed without bile, attention must be given to patients whose bile is not excreted to the intestine such as patients with cholestasis or cholelithiasis.

An emulsion is composed chiefly of water, oil and surfactants, and solubilizing agents and co-surfactants are also used. We next focused on components of the emulsion formulation and investigated the mechanism of improvement in intestinal absorption by emulsification in order to obtain new insights for pharmaceutical design. Various surfactants are classified by their hydrophile–lipophile balance (HLB) value. The HLB value is one of the most widely used indicators for characteristics of surfactants.[19] The value is expressed as a number, with a larger number indicating higher hydrophilicity, and it is used to understand its characteristics predicting tendency between oil and water.[20] We examined the effects of surfactants on the intestinal absorption of emulsion formulations of CoQ10. The HLB values of the surfactants we used in this study were 16.7 for Tween20, 15.0 for Tween80, 10.5 for Tween65 (all of which are nonionic surfactants) and 8.6 for Span20, a non-ionic surfactant.[20] High plasma concentration profiles of CoQ10 were obtained in the group of high HLB values (Tween20 and Tween80) compared with those in the group of low HLB values (Tween65 and Span20) (Figs. 3A–D). The peak plasma concentrations (C_{max}) after emulsion prepared with Tween20, Tween80, Tween65 and Span20 are 0.542, 0.558, 0.283 and 0.122 μg/mL, respectively. We then investigated the effect of bile duct ligation on the plasma concentration of orally administration of CoQ10 emulsion. In case of bile duct ligation, little CoQ10 was absorbed even when various emulsion formulation was administered and the C_{max} was quite little or not be calculated (Table 1). We regard that this result shows the evidence of our consideration from the result of Fig. 2.

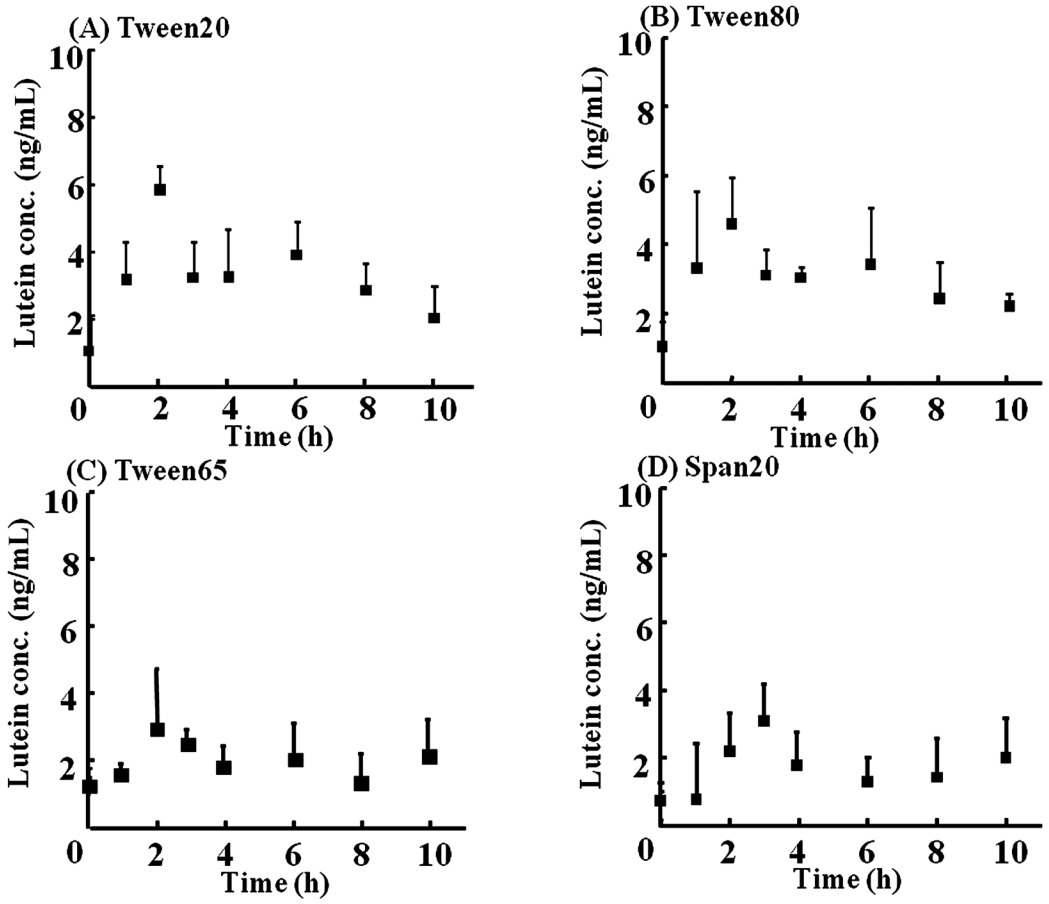

Fig. 5. Plasma Concentrations of Lutein in Rats after Oral Administration of Emulsions Prepared with Tween20 (A), Tween80 (B), Tween65 (C) and Span20 (D) as Surfactants

Each point represents the mean with S.D. of 3 measurements. All rats were fasted for 24 h before the experiments. Lutein (0.5 mg/kg weight) was administered as an emulsion with the surfactant Tween20 (A), Tween80 (B), Tween65 (C) and Span20 (D) to rats.

On the other hand, the time to reach maximum (T_{max}) was calculated to be about 2–9 h. There would be no relationship of T_{max} in emulsion prepared with each surfactant. High *AUC*s based on the plasma concentration profiles were also obtained (Fig. 4). These results suggest that the effect of these components on intestinal absorption of lipophilic compounds such as CoQ10 is greater in the case of higher HLB surfactants. We found that a similar tendency of plasma concentration profile and high *AUC*s based on the plasma concentration profiles after oral administration of lutein was also obtained (Figs. 5, 6). Generally, the process of intestinal absorption of lipophilic compounds is the diffusion of the unstirred water layer. Regarding the absorption of highly lipophilic compounds, a moderate hydrophilicity would be needed to permeate the intestinal unstirred water layer. These emulsions would form mixed micelles with bile acid in the intestinal lumen. We do consider that highly hydrophile surfactant (high HLB value) in the emulsion would miniaturize the lipophilic compound like CoQ10 well and they would promote the permeation of the unstirred water layer compared with lowly hydrophile surfactant (low HLB value), thus facilitating permeation of the apical membrane. It is not to say that only Tween20 showed a specific effect of improvement of intestinal absorption of CoQ10. It is needed to study the better composition ratio and the better components of the emulsion.

Components of the mixed micelles are thought to be bile and phospholipids. It has been reported that greater intracellular uptake and higher plasma concentration of carotenoids were obtained by an emulsion with lysophosphatidylcholine, a more hydrophilic surfactant, than by an emulsion with phosphatidylcholine.[21,22] As in the case of carotenoids, a high plasma concentration profile of CoQ10 using the self-emulsifying drug delivery systems (SEDDS) formulation with a co-surfactant having a high HLB value was obtained.[23] In addition, combinations of a high HLB surfactant and lower HLB surfactants have often been used for preparation of emulsions of lipophilic compounds.[24,25] Based on results of those studies, an emulsion composed of appropriate components and an appropriate combination of surfactants would improve intestinal absorption of such highly lipophilic compounds. Taking into account the safety of these components, further investigations to establish evidence of pharmaceutical design for theoretical improvement of intestinal absorption of these compounds are in progress.

Humans, by nature, have the ability to produce CoQ10. However, this ability starts declining after reaching a peak at the age of about 20 years and the amount of CoQ10 in the body decreases with aging. The amounts of CoQ10 in various tissues including the heart and kidney were shown to be about 50% smaller in an age group of 77–81 years than in an age group of 19–21 years.[26] These daily intake of CoQ10 in diet is about 5–10 mg whereas a recommended daily intake is about 100 mg. We therefore need more adequate intake of CoQ10 from foods or in the form of a supplement. In a clinical setting, CoQ10 is used as Neuquinon®. According to its interview form, the exogenous peak plasma concentration of 10 mg CoQ10 after single oral administration to healthy men is about 0.5 μg/mL[27] while healthy adults contain 0.75–1.0 μg/mL CoQ10 endogenously.[7,28] It would take 2–4 weeks to have clinical effects of Neuquinon®. It is difficult to compare the clinical effective concentrations of CoQ10 simply because of species difference and the difference of study designs since this study was focused on the improvement of CoQ10 absorption by emulsification in rats. Further investigations are needed to establish of the evidence of more efficient and effective intake of CoQ10.

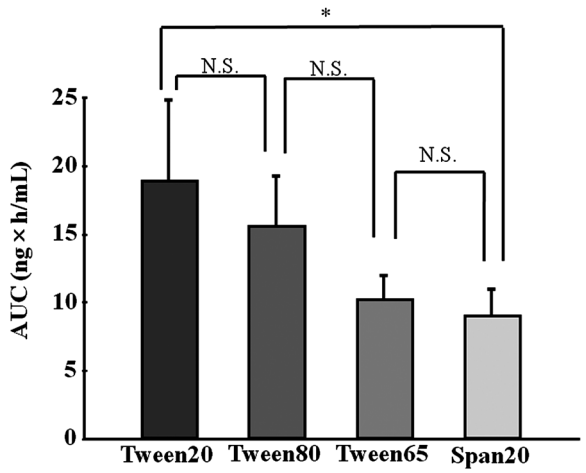

Fig. 6. Effects of Various Surfactants of the Emulsion on *AUC* of Lutein after Oral Administration in Rats

Each column represents the mean with S.E. of 3–4 measurements. *AUC* was calculated by the trapezoidal rule. * Significantly different from the group of lutein emulsion prepared with Tween20 at $p<0.05$. N.S., not significant.

Table 1. Pharmacokinetic Parameters of Orally Administered Each CoQ10 Emulsion

Surfactant		C_{max} (μg/mL)	T_{max} (h)
Tween20	Sham	0.542	1.538
	Bile duct ligation	0.028	8.745
Tween80	Sham	0.558	2.955
	Bile duct ligation	N.D.	1.417
Tween65	Sham	0.283	3.363
	Bile duct ligation	0.029	2.667
Span20	Sham	0.122	4.180
	Bile duct ligation	N.D.	N.D.

N.D., not detected (not calculated) Each parameter was showed as the average calculated by Origin 8.1J® from the data of Fig. 3.

In summary, our study showed that intestinal absorption of CoQ10 can be improved by emulsification. Little CoQ10 was absorbed without bile even when the emulsion formulation was administered, indicating that bile and emulsion formulation are essential for the intestinal absorption of lipophilic compounds including CoQ10. Improvement in intestinal absorption of lipophilic compounds such as CoQ10 and lutein was greater when surfactants with higher HLB were used. Highly lipophilic compounds would diffuse the unstirred water layer and would easily access the intestinal apical membrane by an emulsion containing surfactants with high HLB values. Some nutritional attention must be given to CoQ10 supplementation for patients whose bile is not excreted to the intestine such as cholestasis and cholelithiasis.

Acknowledgments This work was supported in part by Urakami Foundation for Food and Food Culture Promotion, OTC Self-Medication Promotion Foundation and Grant-in-Aid for Young Scientists (B) (Grant number 24790140) from the Japan Society for the Promotion of Science (JSPS).

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