Pharmacokinetic Interaction between Nifedipine and Coenzyme Q$_{10}$ in Rats: A New Type of Drug-Supplement Interaction

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We examined pharmacokinetic (PK) and pharmacodynamic (PD) interactions between coenzyme Q$_{10}$ (CoQ$_{10}$) and nifedipine (NFP), which is a popular medicine for treating hypertension, and elucidated possible mechanisms for the interaction between CoQ$_{10}$ and NFP in rats. The mean plasma concentrations of NFP in rats after the oral administration of NFP (1 mg/kg) with CoQ$_{10}$ (75 mg/kg) were increased over the study period and the area under the plasma concentration-time curve (AUC), showed a 1.47-fold increase compared with that of the control. Rats that received NFP with CoQ$_{10}$ showed a continuous decrease in the mean blood pressure over the study period compared with the control. There were no significant changes in the PK parameters of NFP after intravenous administration (1 mg/kg) between with and without oral CoQ$_{10}$ pretreatment, and also no significant changes in the intestinal excretion of rhodamine 123 (Rho123) or NFP between with and without CoQ$_{10}$ were found. In contrast, the portal plasma concentration of NFP after intra loop administration in the presence of CoQ$_{10}$ (75 mg/kg) showed a 1.6-fold increase in the AUC value compared with that of the control. As for physicochemical properties, the partition coefficient of NFP showed a marked increase in the presence of CoQ$_{10}$ over 10 mg/ml in the organic phase (n-hexane). From on an analysis of the absorbance spectrum, CoQ$_{10}$ showed a shift towards a longer wavelength in hydrophobic environments with NFP, suggesting that CoQ$_{10}$ reacts with NFP to form a charge-transfer complex due to a pi-cloud between them. In conclusion, it was found that CoQ$_{10}$ increases the oral bioavailability of NFP and that this interaction between NFP and CoQ$_{10}$ is not caused by metabolism via cycochrome P450 (CYP) 3A in the liver or intestine or by the inhibition of P-glycoprotein function, by the physicochemical interaction between them. Therefore, the solubility of NFP in a hydrophobic environment could be enhanced by forming a charge-transfer complex with CoQ$_{10}$, and it is considered that NFP deviating from a charge-transfer complex may migrate to the blood circulation from the intestinal tract. This mechanism of interaction is considered a new type of drug-supplement interaction.

Key words —— nifedipine, coenzyme Q$_{10}$, oral absorption, drug-drug interaction, pharmacokinetics, pharmacodynamics

INTRODUCTION

There is growing interest in the use of coenzyme Q$_{10}$ (CoQ$_{10}$) as a nutritional supplement. CoQ$_{10}$ is a fat-soluble, vitamin-like benzoquinone compound that functions primarily as an antioxidant, a membrane stabilizer, and a cofactor in the oxidative phosphorylation process that leads to the production of adenosine triphosphate (ATP) in its reduced form.[1—3] CoQ$_{10}$ is widely consumed as a food supplement because of its status as an important nutrient for maintaining human health. The rationale for the use of CoQ$_{10}$ as a medical agent for treating cardiovascular diseases is based on its fundamental role in mitochondrial function and cellular bioenergetics.[4] On the other hand, at present, the dosage of CoQ$_{10}$ as a supplement is often several or many times that in medical use. There are numerous studies on the therapeutic efficacy of CoQ$_{10}$ sup-

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plementation in human subjects.[5−7] However, very little information regarding the interaction between CoQ_{10} and drugs in medical use is available.

CoQ_{10} was originally found to be effective in treating cardiovascular disorders such as cardiomyopathy, angina pectoris, and atherosclerosis.[8−10] It has also been reported that CoQ_{10} has a hypotensive effect.[7,11,12] In addition, a number of people who usually intake CoQ_{10} as a supplement also receive some kinds of drugs to treat high blood pressure. Therefore, on the basis of the health-oriented tendency in the market, where a large number of CoQ_{10} supplements including a relatively high dose of CoQ_{10} are available, the presence of pharmacokinetic (PK) and pharmacodynamic (PD) interactions between CoQ_{10} and drugs for medical use is suspected. However, there is little information regarding the interaction between CoQ_{10} supplement and hypotensive drugs.[13]

In this study, we examined PK and PD interactions between CoQ_{10} and nifedipine (NFP), which is a popular medicine for the treatment of hypertension, and elucidated possible mechanisms for the interaction between CoQ_{10} and NFP in rats.

MATERIALS AND METHODS

Materials —— CoQ_{10} powder was kindly supplied by Morishita-Jintan Co., Ltd. (Osaka, Japan). NFP and midazolam (MDZ) as an internal standard for high-performance liquid chromatography (HPLC) were purchased from Nacalai Tesque (Kyoto, Japan). Rhodamine 123 (Rho123) was purchased from Sigma Chemicals Co. (Steinheim, Germany). All other chemicals were of reagent grade and were used without further purification.

Animals Preparations —— Male Wistar rats (300–350 g) were procured from Nippon SLC Co., Ltd. (Hamamatsu, Japan). Rats had free access to food and water and were housed in a temperature-controlled facility ($22 \pm 2°C$) with a 12 hr light/dark cycle for at least one week prior to the experiment. Rats were fasted for 16–18 hr prior to the experiment, although water was provided *ad libitum*. All rats were anesthetized by the intraperitoneal administration of urethane (1.0 g/kg). All animal experiments were performed in accordance with the guidelines for animal experimentation of Doshisha Women's College of Liberal Arts, Pharmaceutical Division, and the Federal Requirements for Animal Studies.

Preparation of Standard and Test Solutions —— Standard stock solutions of CoQ_{10} were prepared by dissolving in n-hexane at a final concentration of 500 µg/ml and were then stored at −20°C in the dark. Working standards for a calibration curve were prepared by diluting the standard stock solution with methanol at various concentrations. The calibration curve samples were prepared by adding known amounts of the working standards to plasma or perfusate at a volume ratio of 5 : 50. Test solutions of CoQ_{10} for oral or intraloop administration were prepared by dissolving CoQ_{10} in olive oil at a final concentration of 30 mg/ml. NFP for intravenous administration was dissolved in a vehicle composed of 25% polyethylene glycol 400 (PEG 400) and 25% ethanol in saline at a final concentration of 1 mg/ml. NFP for oral and intraloop administration was dissolved in 1% carmellose sodium solution (carboxymethylcellulose-Na) at a final concentration of 0.5 mg/ml. Rho123 solution for intravenous administration was prepared by dissolving Rho123 in 100 µl of dimethyl sulfoxide (DMSO) and diluted with 0.9% saline to give a final concentration of 0.2 mg/ml. A test solution of NFP to determine its partition coefficient was prepared by diluting NFP with methanol at a final concentration of 0.1 µg/ml.

***In Vivo* PK and PD Studies for NFP after Oral Administration with or without CoQ_{10}** —— After overnight fasting, rats anesthetized with urethane (1.0 g/kg) were placed in a supine position on a surgical table under an incandescent lamp to maintain body temperature at 37°C. The right carotid artery of each rat was cannulated with a polyethylene (PE) tube (0.5 mm internal diameter (i.d.), 0.8 mm i.d.) containing heparinized saline (100 IU/ml) for measuring blood pressure. A small longitudinal incision was made in the skin of each rat over the left jugular vein to collect blood samples over time. One group of rats received CoQ_{10} formulation orally at doses of 75 mg/kg in olive oil, and the other received only olive oil through a stainless-steel needle before oral administration of NFP. After 30 min, NFP test solution was administered orally at a dose of 1 mg/kg. Blood pressure was monitored continually after the oral administration of CoQ_{10}, and 0.12 ml samples of blood were collected from the left jugular vein into heparinized tubes at 30, 60, 120, 180, 240, 300, and 360 min after oral administration of NFP. Blank blood samples were taken 5 min prior to the administration of NFP solution. Plasma was then obtained from whole blood by centrifugation at

$3000 \times g$ for 15 min at 4°C and stored at −80°C until analysis. Arterial blood pressure (BP) was recorded continuously through the PE tube equipped with a transducer (Nihon Kohden, Tokyo, Japan) and mean BP values were recorded every 5 min before (baseline) and up to 6 hr after oral administration using Notocord HEM 3.5 acquisition and analysis system (Primetech Corp., Tokyo, Japan).

***In Vivo* PK Study after Intravenous Administration of NFP with or without CoQ₁₀** —— Rats were prepared before the experiments in the same way as previously described. One group of rats received CoQ₁₀ orally at 75 mg/kg in olive oil and the other received only olive oil through a stainless-steel needle before the intravenous administration of NFP. After 30 min., the NFP solution in a mixture of vehicle containing 25% PEG 400, 25% ethanol, and 50% saline was intravenously administered (1 mg/kg) from the left jugular vein using a syringe. Blood samples of 0.12 ml were collected from the right jugular vein at 3, 7.5, 15, 30, 60, 120, 180, and 240 min. after NFP intravenous administration. Blank blood samples were taken 5 min. prior to the administration of the test solutions. The blood samples were collected in heparinized tubes, and plasma was then obtained from whole blood by centrifugation at $3000 \times g$ for 15 min. at 4°C and stored at −80°C until analysis.

***In Situ* Intestinal Excretion of Rho123 and NFP with or without CoQ₁₀** —— Rats were prepared before the experiments in the same way as previously described. One group of rats received CoQ₁₀ orally at a dose of 75 mg/kg in olive oil and the other received only olive oil through a stainless-steel needle. A midline longitudinal abdominal incision was made and an inlet or outlet silicon tube (4 mm i.d.) was placed at the upper jejunum (15 cm) of the lumen. The lumen was washed 3 times with prewarmed (37°C) phosphate buffered saline containing 25 mM glucose (pH 7.4). After 30 min of perfusion for stabilization, the test solution of Rho123 (0.2 mg/kg) or NFP (1 mg/kg) was injected through the left jugular vein. Simultaneously, the intestinal perfusate was collected every 15 min over 180 min. The volume of perfusate in each fraction and Rho123 and NFP concentrations were measured. All samples were then separated ($3000 \times g$, 15 min, 4°C) and obtained supernatants were stored at −80°C until analysis.

Portal Appearance of NFP after *In Situ* Intraloop Administration with or without CoQ₁₀ —— Rats were prepared before the experiments in the same way as previously described. A midline longitudinal abdominal incision was made and an inlet or outlet silicon tube (4 mm i.d.) was placed at the upper jejunum (15 cm) of the lumen. Then, the lumen was flushed 3 times with prewarmed (37°C) phosphate buffered saline containing 25 mM glucose (pH 7.4). After rats received CoQ₁₀ in olive oil (75 mg/kg) or vehicle for 30 min, the intestinal loop was washed 3 times with the same buffer before the intraloop administration of NFP. Then, NFP solution in 1% CMC-Na was administered to the intestinal loop at a dose of 1 mg/kg. Blood samples of 0.12 ml were collected from the portal vein at 10, 20, 30, 60, 120, 180, 240, and 360 min, then plasma was separated ($3000 \times g$, 15 min, 4°C) and stored at −80°C until analysis.

Tests for Physiochemical Properties —— Five milliliters of n-hexane including CoQ₁₀ at a final concentration ranging from 0.03 to 30 mg/ml was added to 5 ml of NFP test solution (0.1 μg/ml in 50% methanol) in a glass test tube, followed by vigorous agitation for 10 min. After centrifugation at $3000 \times g$ for 20 min, two organic layers (n-hexane and 50% methanol) were separated. NFP concentrations in 50% methanol phase (C_m) and n-hexane phase (C_h) were spectroscopically measured with or without CoQ₁₀, and then a partition coefficient of NFP (K_{NFP}) was calculated by dividing C_h by C_m. Ultraviolet spectra scanning for methanol/n-hexane (49 : 1 v/v) samples including CoQ₁₀ (17.3–69.2 μM) or CoQ₁₀ with NFP (57.8 or 173.4 nM) alone was performed using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan).

Analysis of NFP in Samples —— NFP in plasma, intestinal perfusate, or organic solvents was extracted by placing 50 μl of the samples into 1.5 ml polyoxyethylene centrifuge tubes, adding 5 μl of MDZ methanol solution (1 μg/ml) as an internal standard, and mixing vigorously for 30 sec. Then, 100 μl of 2% (w/v) ZnSO₄ in 50% (v/v) methanol solution was added to precipitate proteins. The mixture was mixed for 10 min and centrifuged at $3000 \times g$ for 15 min. The clear supernatant was transferred into 1.5 ml polyoxyethylene centrifuge tubes and 1 ml of diethyl ether was added, then the mixture was vortexed for 10 min and centrifuged at $3000 \times g$ for 15 min. The supernatant was decanted into a glass test tube and then evaporated until drying in an evaporator for 30 min at 45°C. The obtained residue was reconstituted in 1 ml of mobile phase and then 20 μl was injected into a liquid chromatography-tandem mass spectrometer (LC-MS-MS) system.

The LC-MS-MS analysis was carried out using HPLC system consisting of a LC20AD quaternary pump (Shimadzu) equipped with a vacuum degasser and a SIL 20 A auto sampler with a 100 μl loop (Shimadzu) interfaced with a triple-quadruple tandem mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada). NFP and MDZ were separated on a Chemcopack Quicksorb 5 μm column (2.1 mm i.d.×150 mm, Chemco Scientific Co., Ltd., Oosaka, Japan). The mobile phase, which consisted of 90% (v/v) acetonitrile containing 0.1% formic acid, was degassed before use. The sample was delivered with a flow rate of 0.2 ml/min at a column temperature of 40°C, with each analysis lasting 5.0 min. The MS was operated in the turbo ion spray mode with positive-ion detection. The flow rates of nebulizer gas, curtain gas, and collision gas were set at 8, 8, and 21 l/min, respectively, and the ion spray voltage and temperature were set at 5000 V and 300°C, respectively. The declustering potential, the focusing potential, the entrance potential, the collision energy, and the collision cell exit potential were set at 20, 200, −10, 30, and 6 V, respectively. Multiple-reaction monitoring analysis was performed with the transition m/z 346.9 for NFP and m/z 326.0 for MDZ. All raw data were processed with Analyst Software, version 1.4.1. Taking the peak area ratio of NFP against the internal standard, the calibration curves of NFP were made in plasma or perfusate. The retention times for NFP and the internal standard were 2.24 and 1.78 min, respectively, and all separation was completed within 5.0 min. The calibration curves of NFP were linear and passed through the origin with a correlation coefficient of 0.999 or above. The limit of detection for NFP was 0.005 μg/ml.

Analysis for Rho123 in Perfusate —— The determination of Rho123 concentrations in perfusate was performed immediately after the experiment in accordance with the method of Kageyama *et al.*[14] In brief, after centrifugation of collected perfusate, a 200 μl aliquot of the supernatant was directly added to a 96 well microplate. Then, Rho123 concentrations in the samples were analyzed by fluorimetric detection (Beckman Coulter, DTX 800, Multimode Detector Tokyo, Tokyo, Japan) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The calibration curve for Rho123 was established using the perfusate solution.

PK Data Analysis —— As for PK parameters of NFP, the maximum concentration after oral administration (C_{max}) and the area under the plasma concentration-time curve ($AUC_{po,0-T}$, $AUC_{iv,0-T}$) were calculated using a linear trapezoidal rule. The elimination rate constant for the data of the intravenous study was estimated by analyzing the terminal linear segment of the log plasma concentration-time data, and then the elimination half-life ($t_{1/2}$) was calculated. Oral clearance (CL_{po}), total body clearance (CL_{tot}) and volume of distribution at a steady state (Vd_{ss}) were calculated in accordance with the method we reported previously.[15]

Statistical Analysis —— Statistical analysis was performed using Sigmastat version 3.5. All values are expressed as the mean ± S.E. unless otherwise noted. Statistical differences of the means between two groups were assumed to be significant when $p < 0.05$, as determined by unpaired *t*-test. For more than tree of data groups, statistical differences of the means were assumed when $p < 0.05$ by one-way analysis of variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) tests.

RESULTS

Systemic Plasma Concentration of NFP and Blood Pressure after Oral Administration of NFP with or without CoQ_{10}

Figure 1 shows PK and PD profiles of plasma concentration-time curves of NFP after the oral administration of NFP with or without CoQ_{10} to rats,
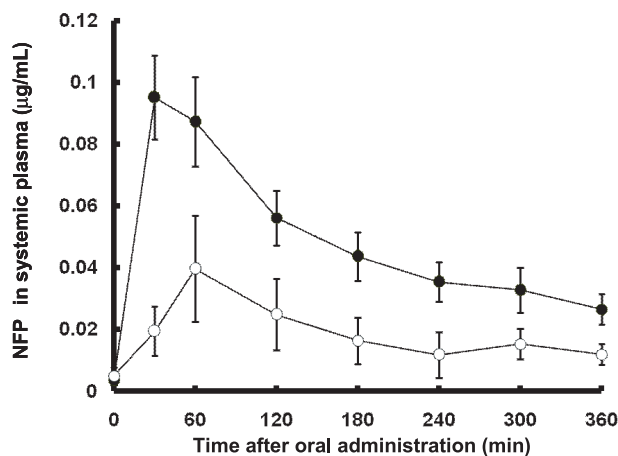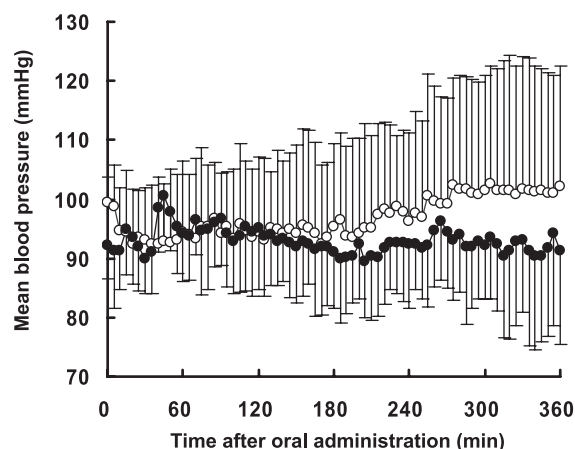

Fig. 1. Systemic Plasma NFP Levels *vs.* Time Profiles after Oral Administration of NFP with and without CoQ_{10} to Rats

Each rat group was pretreated with oral CoQ_{10} (75 mg/kg) or vehicle (olive oil) 30 min prior to oral administration of NFP (1 mg/kg). Each symbol with a bar represents the mean ± S.E. of 4–5 rats. Key: ○, control (without CoQ_{10}); ●, with CoQ_{10}.

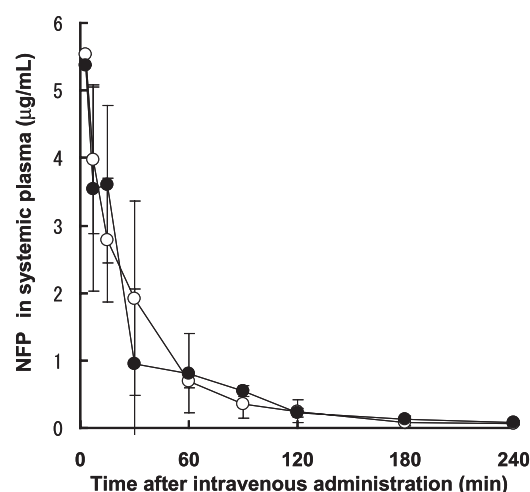**Table 1.** PK Parameters of NFP after Oral Administration with and without CoQ_{10}

		Without CoQ_{10}	With CoQ_{10}
C_{max}	µg/ml	0.040 ± 0.017	0.087 ± 0.014
$AUC_{po,0-T}$	µg·h/ml	0.111 ± 0.036	0.309 ± 0.025
CL_{po}	l/h par kg	2.24 ± 0.72	3.23 ± 0.26

PK analysis after oral administration of NFP (1 mg/kg) to rats pretreated with or without CoQ_{10} (75 mg/kg) was performed using a non-compartment method described in the text. Each value represents the mean ± S.D. of 4–5 rats.

Fig. 2. Change in Mean Blood Pressure after Oral Administration of NFP with and without CoQ_{10} to Rats

Each rat group was pretreated with oral CoQ_{10} (75 mg/kg) or vehicle (olive oil) 30 min prior to oral administration of NFP (1 mg/kg). Each symbol with a bar represents the mean ± S.D. of 5 rats. Key: ○, control (without CoQ_{10}); ●, with CoQ_{10}.

Fig. 3. Systemic Plasma NFP Levels *vs.* Time Profiles after Intravenous Administration of NFP with and without CoQ_{10} to Rats

Each rat group was pretreated with oral CoQ_{10} (75 mg/kg) or vehicle (olive oil) 30 min prior to intravenous administration of NFP (1 mg/kg). Each symbol with a bar represents the mean ± S.E. of 5 rats. Key: ○, control (without CoQ_{10}); ●, with CoQ_{10}.

and corresponding parameters for Fig. 1 are listed in Table 1. The mean plasma concentrations of NFP co-administered with CoQ_{10} were increased over the study period compared with those of the control (Fig. 1). The time courses of mean blood pressure after oral administration of NFP with or without CoQ_{10} to rats are shown in Fig. 2. The values of baseline blood pressure for groups with and without CoQ_{10} were 95.2 ±12.3 and 93.5 ±15.8 mmHg, respectively, and there was no statistical significance between them. Blood pressure in both groups showed about a 20–30% decrease at 30 min after oral administration, and rats that received NFP with CoQ_{10} showed a continuous decrease in mean blood pressure over the study period compared with the control rats. On the other hand, the mean level of blood pressure in the control rats recovered to the baseline from 3 to 6 hr after NFP administration. The C_{max} and $AUC_{po,0-T}$ values in the NFP with CoQ_{10} group showed significant increases compared with those of the control. The rate of blood pressure reduction, however, had no one-to-one cor-

respondence with the plasma NFP levels in either group.

Systemic Plasma NFP Concentration after Intravenous Administration of NFP with or without CoQ_{10}

Figure 3 shows the plasma concentration-time curves of NFP after intravenous administration to rats with and without CoQ_{10}. Corresponding PK parameters for Fig. 3 are listed in Table 2. There were no significant changes in the systemic plasma NFP levels and PK parameters ($AUC_{iv,0-T}$, $t_{1/2}$, CL_{tot}, Vd_{ss}) between CoQ_{10} co-administered rats and control rats.
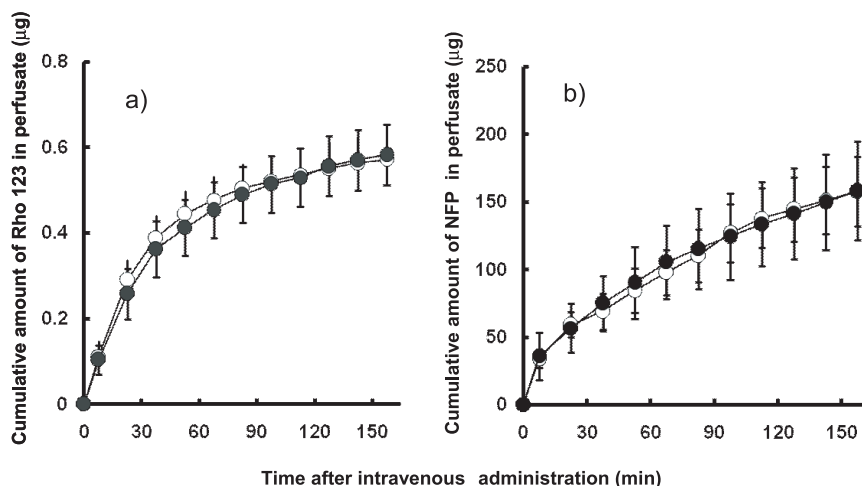
Intestinal Excretion of Rho123 and NFP Using *In Situ* Single Perfusion Method

Figure 4 shows the intestinal excretion of Rho123 and NFP from the blood circulation to the intestinal lumen in CoQ_{10}-treated and control rats. There were no significant changes in the excreted

Table 2. PK Parameters of NFP after Intravenous Administration with and without CoQ_{10}

		Without CoQ_{10}	With CoQ_{10}
$AUC_{iv,0-T}$	µg·h/ml	2.794 ± 1.361	2.754 ± 1.164
$t_{1/2}$	h	0.778 ± 0.252	1.374 ± 0.718
CL_{tot}	l/h par kg	0.446 ± 0.205	0.425 ± 0.251
Vd_{ss}	l/kg	0.396 ± 0.255	0.435 ± 0.295

PK analysis after intravenous administration (1 mg/kg) to rats pretreated with and without CoQ_{10} was performed using a non-compartment method described in the text. Each value represents the mean ± S.D. of 5 rats.

Fig. 4. Effect of Oral CoQ_{10} on Intestinal Excretion of Rho123 (a) and NFP (b)

Each rat group was pretreated with oral CoQ_{10} (75 mg/kg) or vehicle (olive oil) 30 min prior to intravenous administration of Rho123 (0.2 mg/kg) or NFP (1 mg/kg). Each symbol with a bar represents the mean ± S.E. of 6 rats. Key: ○, control (without CoQ_{10}); ●, with CoQ_{10}.

amounts of Rho123 and NFP at any interval between CoQ_{10}-treated and control rats throughout the experimental period.

Portal Plasma Concentration of NFP after *In Situ* Intraloop Administration

Figure 5 shows the portal plasma concentration-time curve of NFP after intraloop administration of NFP to rats with and without CoQ_{10}. The portal plasma concentration of NFP in the presence of CoQ_{10} (75 mg/kg) showed a higher transition compared with that of the control rats. The values of AUC for the control and CoQ_{10} co-administered rats, which were calculated using the portal data in Fig. 5, were 0.528 ± 0.048 and 0.766 ± 0.133 µg·h/ml, respectively, and showed about a 1.6-fold increase in NFP availability in the presence of CoQ_{10}.

Partition Coefficient of NFP with or without CoQ_{10}

Figure 6 shows the effect of CoQ_{10} on K_{NFP} in the biphasic *in vitro* system of n-hexane/50%
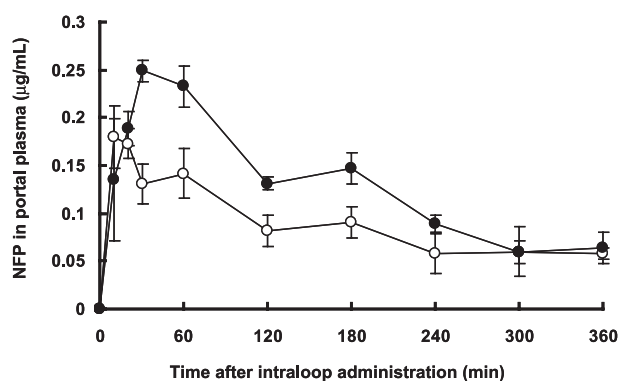
**Fig. 5.** Portal Plasma NFP Levels *vs*. Time Profiles after Intraloop Administration of NFP with and without CoQ_{10} to Rats

Each rat group was pretreated with intraloop CoQ_{10} (75 mg/kg) or vehicle (olive oil). After 30 min the intestinal loop was washed three times with saline, and the NFP (1 mg/kg) was administered. Each symbol with a bar represents the mean ± S.E. of 3 rats. Key: ○, control (without CoQ_{10}); ●, with CoQ_{10}.

methanol. There were no significant changes in the value of K_{NFP} at the range of CoQ_{10} concentrations between 0.03 and 10 mg/ml. However, at higher concentrations of CoQ_{10} ranging from 10 to

Table 3. Effect of NFP on the Shift of CoQ_{10} UV Absorbance

CoQ_{10} concn. in hexane (μM)	Without NFP	With NFP	
		+ 57.8 nM	+ 173.4 nM
17.3	274.8 ± 0.0	275.3 ± 0.3**	276.1 ± 0.3**
34.6	274.5 ± 0.0	275.3 ± 0.3[a)]	276.0 ± 0.1**
69.2	274.8 ± 0.0	275.3 ± 0.2**	275.7 ± 0.3**

Ultraviolet spectra scanning for methanol/n-hexane (49 : 1 v/v) samples including only CoQ_{10} (17.3 μM–69.2 μM) or CoQ_{10} with NFP (57.8 nM or 173.4 nM) was performed. Each value represents λ_{nm} and mean ± S.D. of 3 determinations. a) $p = 0.065$. **, $p < 0.01$ against the appropriate control.
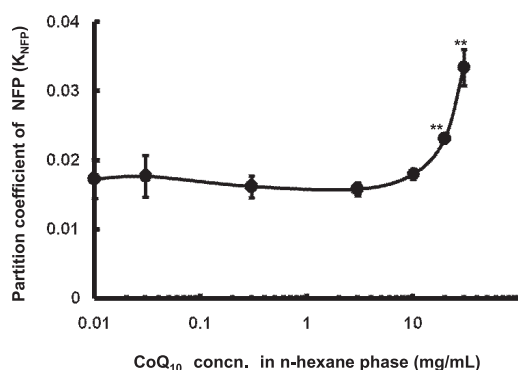
Fig. 6. Relationship between Partition Coefficients of NFP and CoQ_{10} Levels in n-Hexane Phase

Oil-water partition coefficient of NFP was measured in a n-hexane/50% methanol system. 50% methanol was separated from n-hexane completely. Each symbol with a bar represents the mean ± S.E. of 6 experiments. **, $p < 0.01$ compared with the data of CoQ_{10} samples below 10 mg/ml.

30 mg/ml in the n-hexane phase, the values of K_{NFP} increased markedly.

Spectrophotometric Analysis for Interaction between NFP and CoQ_{10}

Table 3 shows results of the spectrophotometric analysis for the molecular interaction between NFP and CoQ_{10}. The solvent, which was used to dissolve NFP (methanol), did not affect the UV absorbance. There were no significant changes in the absorbance wavelength for CoQ_{10} at its three different concentrations without NFP. On the other hand, the UV wavelength of CoQ_{10} showed a slight shift towards a longer wavelength in hydrophobic environments with NFP ($p < 0.01$), suggesting that CoQ_{10} reacts with NFP to form a charge-transfer complex.

DISCUSSION

CoQ_{10} was originally used for the treatment of cardiovascular disease to improve myocardial function.[16)] However, at present, many people take it as a nutritional supplement with the expectation

that it will be effective in anti-aging or preventing metabolic syndrome.[17, 18)] There are a lot of reports stating that CoQ_{10} has high anti-oxidant effects, which are useful in delaying aging of the body. [19, 20)] Awareness of health issues has been growing in Japan after the introduction of a system regulating "Food for Specified Health Use" (Tokuho), under which the use of "health claims" was allowed, in 1991.[21, 22)] Many people worry about "lifestyle-related illnesses" such as heart disease, diabetes, high blood pressure, and obesity. In Japan, medicines to treat hypertension have a large market share, and these kinds of drugs, which are prescribed for the treatment of high blood pressure, include products with fat-soluble properties. NFP is a representative drug for the treatment of hypertension and is well established as a highly extracted typical substrate of cytochrome P450 (CYP) 3A,[23—25)] and is also a lipophilic substance.[26)] Several clinical reports on investigations of the PK interaction between NFP and CoQ_{10} suggested that there may be interactions between them, that is, that the effect of NFP would be enhanced by CoQ_{10} intake.[27, 28)] However, the precise mechanisms underlying the interaction between NFP and CoQ_{10} have yet to be clarified. In this study, we selected olive oil as a vehicle for CoQ_{10} oral administration. Many CoQ_{10} products are available on the market in the form of soft gelatin capsules containing olive oil, vitamin E oil and/or medium-chain triglyceride oil to achieve several formulations at emulsion or suspension. In this study, however, it was required to dissolve both CoQ_{10} and NFP together to investigate drug-supplement interaction. Converting an applied-dose of CoQ_{10} or NFP to rats into case of man, they are equal to the amounts used in the market at present. On the other hand, the amount of olive oil used as a vehicle is much larger than general use, and there is a possible that the absorption of NFP is enhanced by the olive oil because the olive oil acts as an absorption enhancer. Therefore, the

results in this study show relative ones because control groups of rats received the same amount of olive oil without CoQ_{10}.

First, we examined the PK and PD profiles of NFP with or without CoQ_{10} after oral administration to rats (Figs. 1 and 2, Table 1). With administration of CoQ_{10}, the plasma NFP concentration increased compared with that of the control. In addition, with CoQ_{10}, the mean blood pressure showed a continuous reduction over 6 hr compared with that of the control (Fig. 2). These observations clearly indicate that CoQ_{10} enhanced NFP absorption from the intestinal tract and reduced the blood pressure. The dosages of NFP and CoQ_{10} used in our experiments were 1 and 75 mg/kg, respectively, and these dosage ranges are appropriate for several clinical settings. Since CoQ_{10} is taken as a nutritional supplement at relatively high doses ranging from 30 to 3000 mg/day,[29, 30] it is of concern that unrestricted use of CoQ_{10} as a supplement during NFP therapy may cause side effects of NFP owing to PK and/or PD interaction between them.

Next, we investigated the underlying mechanism for the interaction between NFP and CoQ_{10} utilizing some biopharmaceutical techniques. As shown in Fig. 3, there are no significant changes in systemic NFP after intravenous administration with and without CoQ_{10}. These results suggest that CYP3A, which is located in the liver cells, is not affected by CoQ_{10} administration. There are many points which are still unclear about the PK profiles of CoQ_{10}, and there are many contradictions in reports about the characteristics of tissue distribution or plasma CoQ_{10} levels. In our previous report, we demonstrated that the PK profiles of CoQ_{10} after oral administration showed a biphasic pattern, that is, it has a first peak within 6 hr and a second peak at 24 hr after administration.[15] In addition, plasma CoQ_{10} levels were within 20–300 ng/ml, and the absorption availability at olive formulation was below 0.03%.[15] Therefore, in the present acute oral administration study, it is suspected that CYP3A in the liver may not be affected by CoQ_{10} treatment because of much lower CoQ_{10} plasma levels and absorption availability. Moreover, the percentage of remaining CoQ_{10} in the upper jejunum after intraloop administration was around 75% of dose (75 mg/kg). Hence, it is considered that markedly large amount of CoQ_{10} remains and/or adsorbs on the intestinal tissues. However, if anything, when a plasma CoQ_{10} level is increased, its total body clearance would be increased because CoQ_{10} pro-

mote an electron transport chain at mitochondria. Nevertheless, further study at chronic administration phase should be required to confirm whether a higher plasma CoQ_{10} levels affect liver CYP3A.

As another factor that may alter the PK profiles of lipophilic drugs, the role of P-glycoprotein (P-gp), which is located in normal tissues including gastrointestinal epithelium, canalicular membrane of the liver, kidney,[31] and capillary endothelial cells in the central nervous system,[32] is considered. Because of its tissue localization and its broad substrate specificity, P-gp appears to play a key role in the absorption, distribution, and elimination of many drugs.[33] The modulation of P-gp through its inhibition or induction by various drugs and/or herbs can lead to significant drug-drug interaction and/or drug-herb interaction by affecting various PK parameters of the drug. Particularly, the role of P-gp as an efflux transporter in determining the permeability and overall bioavailability of drugs has attracted considerable attention.[34] NFP is a well known substrate for CYP3A; however, it is unclear whether NFP is a substrate for P-gp. In our previous study utilizing an *in situ* intestinal efflux method, we revealed that NFP belongs to the group of P-gp substrates. Under treatment with cyclosporine, marked inhibition of NFP and/or Rho123 (a specific substrate of P-gp) efflux from blood circulation to the intestinal tract was observed.[35] On the basis of this finding, we examined the intestinal efflux of NFP and/or Rho123 after intravenous administration in the absence or presence of CoQ_{10}. As shown in Fig. 4, there were no significant changes in the cumulative amounts of Rho123 (Fig. 4a) and NFP (Fig. 4b) in the intestinal perfusate. These observations clearly indicate that CoQ_{10} has no effect on the function of P-gp. Recently, an *in vitro* study was reported that showed that the efflux transport of CoQ_{10} is mediated by P-gp in Caco-2 cells, and that Rho123 and quinidine significantly decreased the transepitherial flux of CoQ_{10} from the basal to the apical side in Caco-2 cell monolayers.[36] On the other hand, several *in vivo* studies on humans or rats demonstrated that CoQ_{10} has biphasic and nonlinear kinetics with double peaks after oral administration.[15, 37, 38] In detail, the secondary peaks of CoQ_{10} appear at around 24 hr after oral administration with 20- to 100-fold higher peaks than the primary ones at around 6 hr.[15, 37] These observations clearly suggest that the absorption kinetics of CoQ_{10} has a marked absorption lag because CoQ_{10} in the intestinal tract adsorbs to the surface of gut cells

to remain in the intestine for a long time.[37, 38] Although there are conflicting results between *in vitro* and *in vivo* studies, it is considered that differences in experimental conditions between the living body and cultured cells complicate these issue. Further precise examinations are required to elucidate the disposition of CoQ_{10}.

From these biopharmaceutical results, it can be concluded that CoQ_{10} has no effect on the function of CYP3A or P-gp. As shown in Fig. 1, however, it is indisputable that plasma NFP concentrations increased with CoQ_{10} compared with those of the control. If CoQ_{10} inhibits the function of P-gp or CYP3A, which is located in the intestine or liver, these factors may reasonably explain the interaction between NFP and CoQ_{10}. From our results, at least, it could be considered that there are some mechanisms of interaction besides biopharmaceutical factors in the intestinal tract. As a next step, we focused on the physicochemical interaction between NFP and CoQ_{10}. As shown in Fig. 6, K_{NFP} had a biphasic pattern, and were increased with increasing the concentration of CoQ_{10} in the concentration range of CoQ_{10} in n-hexane from 10 to 30 mg/ml. This phenomenon indicates that NFP in the 50% methanol phase was transferred to the n-hexane phase as CoQ_{10} concentration in the n-hexane phase increased above 10 mg/ml. In addition, these results demonstrate that highly concentrated CoQ_{10} in the n-hexane phase promoted dissolution of NFP in the n-hexane phase, suggesting that the physicochemical properties of NFP would be altered in the presence of highly concentrated CoQ_{10}. As such, we assumed that these two compounds can form a charge-transfer complex, resulting in increasing solubility of NFP in the n-hexane phase, because CoQ_{10} has a benzoquinone molecular structure, and benzoquinone is electron-lucent. In contrast, NFP is an electron-dense material because it has a 1,4-dihydropyirdine structure containing an unshared electron pair. NFP and CoQ_{10}, therefore, would interact with each other owing the interaction between pi-electrons in both derived from the double-linkage of CoQ_{10} and the unshared electron pair of NFP. These considerations are supported by the shift in UV-scanning profile as listed in Table 3. CoQ_{10} showed a shift towards a longer wavelength in hydrophobic environments with NFP, suggesting that CoQ_{10} reacts with NFP to form a charge-transfer complex due to a pi-cloud between them. Bangal *et al*. reported that a molec-

ular of benzoquinone and two molecular of tetrahydrofuran interacted strongly each other by forming a charge-transfer complex, and the wavelength of benzoquinone showed 5 nm shift towards a long wavelength.[39] Therefore, a 1.5 nm shift found between CoQ_{10} and NFP indicates to form a weaker bound complex between them rather than in the case of between benzoquinone and tetrahydrofuran. Moreover, in our previous report, we demonstrated that CoQ_{10} could not be absorbed from the intestinal tract because of high lipophilicity.[15] Therefore, adsorbed and/or remaining CoQ_{10} in concentrated amounts in the neighborhood of the intestinal wall after oral administration can form an unstirred lipid layer to cover the intestinal apical side, and the CoQ_{10} lipid layer can draw increased NFP by forming a charge-transfer complex with NFP at the near-gut wall. Then, the charge state of NFP changes NFP to have a convenient lipophilicity that allows it to be absorbed through membrane, resulting in enhanced absorption of NFP from the intestinal tract as shown in Fig. 5. However, since the charge-transfer complex between CoQ_{10} and NFP has a weak adhesive power judging by the slight shift towards a longer wavelength compared with the case of only CoQ_{10} in hydrophobic environments (Table 3), it is considered that NFP deviating from a charge-transfer complex may migrate to blood circulation from the intestinal tract. In addition, considering a very few absorbed-fraction, a very low plasma level of CoQ_{10}[15] and a very slow tissue distribution property,[40] the increase in the portal plasma levels of NFP is not due to the inhibition of CYP3A in the intestine as same as in the liver at present acute experiments. Moreover, the fact that increase in the AUC values from CoQ_{10}-NFP interaction was relatively small compared with those in the cases of NFP-ketoconazole interaction,[41] where ketoconazole are exclusive CYP3A inhibitors for NFP, is also one basis that the intestinal CYA3A is not influenced by acute CoQ_{10} treatment. Nevertheless, further study at chronic administration phase should be required to confirm whether CoQ_{10} treatment affect intestinal CYP3A.

In conclusion, it was found that oral NFP with oral CoQ_{10} increased the bioavailability of NFP, and this interaction between NFP and CoQ_{10} is caused not by the inhibition of metabolism via CYP3A in the liver or intestine or by the inhibition of P-gp function, but by physicochemical interaction between them through the formation of a charge-transfer complex that has a weak adhesive power.

This mechanism of interaction is considered a new type in drug-supplement interaction. Given today's health-oriented market, the usual dose of CoQ$_{10}$ as a supplement is very high in comparison with that in a clinical setting; therefore, this type of interaction between NFP and CoQ$_{10}$ would lead to concomitant lowering of blood pressure in patients who receive NFP therapy. These observations suggest caution in the use of other antihypertensive drugs that are physicochemically similar to NFP.

REFERENCES

1) Ernster, L., Forsmark, P. and Nordenbrand, K. (1992) The mode of action of lipid-soluble antioxidants in biological membranes: relationship between the effects of ubiquinol and vitamin E as inhibitors of lipid peroxidation in submitochondrial particles. *Biofactors*, **3**, 241–248.

2) Forsmark, P., Aberg, F., Norling, B., Nordenbrand, K., Dallner, G. and Ernster, L. (1991) Inhibition of lipid peroxidation by ubiquinol in submitochondrial particles in the absence of vitamin E. *FEBS Lett.*, **285**, 39–43.

3) Stocker, R., Bowry, V. W. and Frei, B. (1991) Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 1646–1650.

4) Ochiai, A., Itagaki, S., Kurokawa, T., Kobayashi, M., Hirano, T. and Iseki, K. (2007) Improvement in intestinal coenzyme Q10 absorption by food intake. *Yakugaku Zasshi*, **127**, 1251–1254.

5) Zheng, A. and Moritani, T. (2008) Influence of CoQ10 on autonomic nervous activity and energy metabolism during exercise in healthy subjects. *J. Nutr. Sci. Vitaminol.* (Tokyo), **54**, 286–290.

6) Tiano, L., Belardinelli, R., Carnevali, P., Principi, F., Seddaiu, G. and Littarru, G. P. (2007) Effect of coenzyme Q10 administration on endothelial function and extracellular superoxide dismutase in patients with ischaemic heart disease: a double-blind, randomized controlled study. *Eur. Heart J.*, **28**, 2249–2255.

7) Hodgson, J. M., Watts, G. F., Playford, D. A., Burke, V. and Croft, K. D. (2002) Coenzyme Q10 improves blood pressure and glycaemic control: a controlled trial in subjects with type 2 diabetes. *Eur. J. Clin. Nutr.*, **56**, 1137–1142.

8) Singh, R. B. and Niaz, M. A. (1999) Serum concentration of lipoprotein(a) decreases on treatment with hydrosoluble coenzyme Q10 in patients with coronary artery disease: discovery of a new role. *Int. J. Cardiol.*, **68**, 23–29.

9) Singh, R. B., Neki, N. S., Kartikey, K., Pella, D., Kumar, A., Niaz, M. A. and Thakur, A. S. (2003) Effect of coenzyme Q10 on risk of atherosclerosis in patients with recent myocardial infarction. *Mol. Cell. Biochem.*, **246**, 75–82.

10) Kocharian, A., Shabanian, R., Rafiei-Khorgami, M., Kiani, A. and Heidari-Bateni, G. (2009) Coenzyme Q10 improves diastolic function in children with idiopathic dilated cardiomyopathy. *Cardiol. Young*, **19**, 501–506.

11) Rosenfeldt, F. L., Haas, S. J., Krum, H., Hadj, A., Ng, K., Leong, J. Y. and Watts, G. F. (2007) Coenzyme Q10 in the treatment of hypertension: a meta-analysis of the clinical trials. *J. Hum. Hypertens.*, **21**, 297–306.

12) Kumar, A., Kaur, H., Devi, P. and Mohan, V. (2009) Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. *Pharmacol. Ther.*, **124**, 259–268.

13) Danysz, A., Oledzka, K. and Bukowska-Kiliszek, M. (1994) Influence of coenzyme Q-10 on the hypotensive effects of enalapril and nitrendipine in spontaneously hypertensive rats. *Pol. J. Pharmacol.*, **46**, 457–461.

14) Kageyama, M., Fukushima, K., Togawa, T., Fujimoto, K., Taki, M., Nishimura, A., Ito, Y., Sugioka, N., Shibata, N. and Takada, K. (2006) Relationship between excretion clearance of rhodamine 123 and P-glycoprotein (Pgp) expression induced by representative Pgp inducers. *Biol. Pharm. Bull.*, **29**, 779–784.

15) Nishimura, A., Yanagawa, H., Fujikawa, N., Kiriyama, A. and Shibata, N. (2009) Pharmacokinetic profiles of coenzyme Q10: absorption of three different oral formulations in rats. *J. Health Sci.*, **55**, 1–9.

16) Langsjoen, P., Langsjoen, P., Willis, R. and Folkers, K. (1994) Treatment of essential hypertension with coenzyme Q10. *Mol. Aspects Med.*, **15**, 265–272.

17) Janson, M. (2006) Orthomolecular medicine: the therapeutic use of dietary supplements for anti-aging. *Clinical Interventions in Aging*, **1**, 261–265.

18) Kunitomo, M., Yamaguchi, Y., Kagota, S. and Otsubo, K. (2008) Beneficial effect of coenzyme Q10 on increased oxidative and nitrative stress and inflammation and individual metabolic components developing in a rat model of metabolic syndrome. *J. Pharmacol. Sci.*, **107**, 128–137.

19) Ratnam, D. V., Ankola, D. D., Bhardwaj, V., Sahana, D. K. and Kumar, M. N. (2006) Role of antioxidants in prophylaxis and therapy: A pharma-

ceutical perspective. *J. Control. Release*, **20**, 189–207.

20) Kaliora, A. C., Dedoussis, G. V. and Schmidt, H. (2006) Dietary antioxidants in preventing atherogenesis. *Atherosclerosis*, **187**, 1–17.

21) Saito, M. (2007) Role of FOSHU (food for specified health uses) for healthier life. *Yakugaku Zasshi*, **127**, 407–416 (in Japanese).

22) Yamada, K., Sato-Mito, N., Nagata, J. and Umegaki, K. (2008) Health claim evidence requirements in Japan. *J. Nutr.*, **138**, 1192–1198.

23) Lin, J. H., Chiba, M. and Baillie, T. A. (1999) Is the role of the small intestine in first-pass metabolism overemphasized? *Pharmacol. Rev.*, **51**, 135–158.

24) Dresser, G. K., Spence, J. D. and Bailey, D. G. (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin. Pharmacokinet.*, **38**, 41–57.

25) Doherty, M. M. and Charman, W. N. (2002) The mucosa of the small intestine: how clinically relevant as an organ of drug metabolism? *Clin. Pharmacokinet.*, **41**, 235–253.

26) Based on "The Interview Form of the Adalt" ⟨http://www.bayer-hv.jp/hv/products/if/ac.pdf⟩ p. 4 by Bayer HealthCare (cited 22 January, 2010).

27) Hodgson, J. M. and Watts, G. F. (2003) Can coenzyme Q10 improve vascular function and blood pressure? Potential for effective therapeutic reduction in vascular oxidative stress. *Biofactors*, **18**, 129–136.

28) Rosenfeldt, F., Hilton, D., Pepe, S. and Krum, H. (2003) Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure. *Biofactors*, **18**, 91–100.

29) Shults, C. W., Oakes, D., Kieburtz, K., Beal, M. F., Haas, R., Plumb, S., Juncos, J. L., Nutt, J., Shoulson, I., Carter, J., Kompoliti, K., Perlmutter, J. S., Reich, S., Stern, M., Watts, R. L., Kurlan, R., Molho, E., Harrison, M. and Lew, M.; Parkinson Study Group (2002) Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. *Arch. Neurol.*, **59**, 1541–1550.

30) Ferrante, K. L., Shefner, J., Zhang, H., Betensky, R., O'Brien, M., Yu, H., Fantasia, M., Taft, J., Beal, M. F., Traynor, B., Newhall, K., Donofrio, P., Caress, J., Ashburn, C., Freiberg, B., O'Neill, C., Paladenech, C., Walker, T., Pestronk, A., Abrams, B., Florence, J., Renna, R., Schierbecker, J., Malkus, B. and Cudkowicz, M. (2005) Tolerance of high-dose (3,000 mg/day) coenzyme Q10 in ALS. *Neurology*, **65**, 1834–1836.

31) Wang, Y., Hao, D., Stein, W. D. and Yang, L. (2006) A kinetic study of Rhodamine123 pumping by P-glycoprotein. *Biochim. Biophys. Acta.*, **1758**, 1671–1676.

32) Daood, M., Tsai, C., Ahdab-Barmada, M. and Watchko, J. F. (2008) ABC transporter (P-gp/ABCB1, MRP1/ABCC1, BCRP/ABCG2) expression in the developing human CNS. *Neuropediatrics*, **39**, 211–218.

33) Ayrton, A. and Morgan, P. (2001) Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica.*, **31**, 469–497.

34) Hennessy, M., Kelleher, D., Spiers, J. P., Barry, M., Kavanagh, P., Back, D., Mulcahy, F. and Feely, J. (2002) St. Johns wort increases expression of P-glycoprotein: implications for drug interactions. *Br. J. Clin. Pharmacol.*, **53**, 75–82.

35) Dorababu, M., Nishimura, A., Prabha, T., Naruhashi, K., Sugioka, N., Takada, K. and Shibata, N. (2009) Effect of cyclosporine on drug transport and pharmacokinetics of nifedipine. *Biomed. Pharmacother.*, **63**, 697–702.

36) Itagaki, S., Ochiai, A., Kobayashi, M., Sugawara, M., Hirano, T. and Iseki, K. (2008) Interaction of coenzyme Q10 with the intestinal drug transporter P-glycoprotein. *J. Agric. Food Chem.*, **56**, 6923–6927.

37) Shimomura, M. and Miyamoto, Y. (2006) Comparative bioavailability between "coenzyme Q 10 emulsion powder" and "Coenzyme Q 10 in bulk" in humans. *J. Nutr. Food*, **9**, 43–51 (in Japanese).

38) Tomono, Y., Hasegawa, J., Seki, T., Motegi, K. and Morishita, N. (1986) Pharmacokinetic study of deuterium-labelled coenzyme Q 10 in man. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **24**, 536–541.

39) Bangal, P. R., (2007) Density functional studies on the effects of hydrogen bonding on the formation of a charge-transfer complex between p-benzoquinone and 2,6-dimethoxyphenol. *J. Phys. Chem. A*, **111**, 5536–5543.

40) Takahashi, T., Okamoto, T. and Kishi, T. (2004) Recent Progress in Coenzyme Q research. *Vitamins*, **78**, 587–598 (in Japanese).

41) Nagatomo, A., Nishida, N., Kozaki, T., Asada, M., Nishimura, A., Naruhashi, K., Onishi, N., Shibata, N. (2009) Interaction between health foods and drugs (3): effects of Agaricus blazei Murill on the pharmacokinetics of nifedipine. *Proc. 129th Annu. Meet. Pharm. Soc. Jpn.*, Kyoto, Japan, March 2009, 27Q–pm207.