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Study on safety and bioavailability of ubiquinol (Kaneka QH™) after single and 4-week multiple oral administration to healthy volunteers

Kazunori Hosoe [a,*], Mitsuaki Kitano [a], Hideyuki Kishida [a], Hiroshi Kubo [a], Kenji Fujii [b], Mikio Kitahara [b]

[a] *Pharmacology and Toxicology Group, Life Science Research Laboratories, Kaneka Corporation, 1-8 Miyamae-machi, Takasa-cho, Takasago-shi, Hyogo 676-8688, Japan*
[b] *Functional Food Ingredients Division, Kaneka Corporation, 3-2-4 Nakanoshima, Kita-Ku, Osaka 530-8288, Japan*

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Abstract

The safety and bioavailability of ubiquinol (the reduced form of coenzyme Q_{10}), a naturally occurring lipid-soluble nutrient, were evaluated for the first time in single-blind, placebo-controlled studies with healthy subjects after administration of a single oral dose of 150 or 300 mg and after oral administration of 90, 150, or 300 mg for 4 weeks. No clinically relevant changes in results of standard laboratory tests, physical examination, vital signs, or ECG induced by ubiquinol were observed in any dosage groups. The C_{max} and $AUC_{0-48\,h}$ derived from the mean plasma ubiquinol concentration-time curves increased non-linearly with dose from 1.88 to 3.19 μg/ml and from 74.61 to 91.76 μg h/ml, respectively, after single administration. Trough concentrations had nearly plateaued at levels of 2.61 μg/ml for 90 mg, 3.66 μg/ml for 150 mg, and 6.53 μg/ml for 300 mg at day 14, and increased non-linearly with dose in the 4-week study. In conclusion, following single or multiple-doses of ubiquinol in healthy volunteers, significant absorption of ubiquinol from the gastrointestinal tract was observed, and no safety concerns were noted on standard laboratory tests for safety or on assessment of adverse events for doses of up to 300 mg for up to 2 weeks after treatment completion.

Keywords: Ubiquinol; Ubiquinone; Coenzyme Q_{10}; Safety; Bioavailability; Healthy volunteer

1. Introduction

Coenzyme Q_{10}, which is also known as ubiquinone, is a lipid-soluble, vitamin-like substance present in nearly all human tissues and involved in essential cellular processes of energy production in mitochondria, where it acts as both an electron carrier and proton translocator during cellular respiration and ATP production (Arroyo et al., 2000; Nohl et al., 2001; Crane, 2001; Kagan et al., 2001; Villalba et al., 2001). In its reduced form (ubiquinol), coenzyme Q_{10} acts as an antioxidant in both mitochondria and lipid membranes by either scavenging free radicals directly or in conjunction with α-tocopherol (Quinn et al., 1999; Lass and Sohal, 2000; Forsmark-Andree et al., 1997; Noack et al., 1994). Because of its important biological roles, coenzyme Q_{10} has been widely used as a dietary supplement by health-conscious individuals and those with ailments including various cardiac disorders (Overvad et al., 1999; Greenberg and Frishman, 1990; Hendler and Rorvik, 2001; Tran et al., 2001; Jones et al., 2002).

Ubiquinol is the most common form of coenzyme Q_{10} in vivo, and accounts for more than 80% of the total ubiquinol + ubiquinone pool in human plasma, intestine, and liver (Okamoto et al., 1989; Frei et al., 1990; Åberg et al., 1992). In addition, Mohr et al. (1992), Stocker and Suarna (1993), Weber et al. (1994) reported that following dietary supplementation with coenzyme Q_{10}, efficient reduction of coenzyme Q_{10} to ubiquinol occurs either during absorption or rapidly after the appearance of coenzyme

* Corresponding author. Fax: +81 794 45 2699.
E-mail address: kazunori.hosoe@kn.kaneka.co.jp (K. Hosoe).

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Q_{10} in the blood. We recently measured the ubiquinol contents of various foods (9 types of meat, 13 types of fish/shellfish, 12 vegetables, and chicken egg) using high performance liquid chromatography (HPLC) with an electrochemical detector. Ubiquinol was identified in most of the foods analyzed, and the mean percentages of ubiquinol in total coenzyme Q_{10} [ubiquinol contents × 100/(ubiquinol + ubiquinone contents)] were 33.5%, 25.8%, 17.2%, and 34.6% in meats, fishes/shellfishes, vegetables, and chicken egg, respectively (Fujii et al., 2006). Cabrini et al. (2001) reported that the percentages of ubiquinol in total coenzyme Q_{10} in extra virgin olive oil, peanut oil, soybean oil, corn oil and sunflower oil were 10.5%, 87.5%, 73.5%, 75.0% and 90.3%, respectively. Furthermore, we found that when rats were orally administered a single-dose (100 mg/kg) of ubiquinol or ubiquinone dissolved in olive oil, there was an approximately 2-fold difference in area under the plasma total coenzyme Q_{10} concentration curve between the two agents, indicating that ubiquinol has higher bioavailability than ubiquinone (Mae et al., 2001).

Occurrence of ubiquinol in human body and foods, the good bioavailability of ubiquinol, and the fact that ubiquinol is a potent lipophilic antioxidant and that it is the most common form of coenzyme Q_{10} in vivo suggested the possibility of use of ubiquinol as a new novel dietary supplement. However, development of it as a dietary supplement was difficult because it is readily oxidized in air. Recently, however, our chemical research group established a method enabling manufacture of ubiquinol bulk as Kaneka QH™ from our ubiquinone bulk of Kaneka Q10™, as well as stable capsule product containing Kaneka QH™.

In order to assess the safety of Kaneka QH™ (ubiquinol), a series of preclinical safety studies were performed in compliance with relevant Good Laboratory Practice (GLP) Standards with ubiquinol bulk, including an acute toxicity study and subchronic toxicity studies in rats and dogs, as well as in vitro and in vivo genotoxicity studies.

1.1. Review of previously unpublished studies

In the acute toxicity study in rats, groups of 5 male and 5 female rats were given a single oral dose of either 0, 2500, or 5000 mg/kg ubiquinol dissolved in corn oil and then evaluated for morbidity and mortality for the next 14 days. No abnormal clinical signs or significant differences from the control group in body weight were noted in treated animals (Oda, 2003).

In 13-week subchronic toxicity studies in rats, groups of 10 male and 10 female rats were given a daily dose of 0, 300, 600, or 1200 mg/kg of ubiquinol dissolved in corn oil for 13 weeks. No deaths occurred in any group during the study period. No abnormalities in general condition, body weight, food consumption, ophthalmological examination, or urinalysis were observed. Evaluation of hematological parameters revealed statistically significant prolongations of PT and APTT in males of the ubiquinol 1200 mg/kg group. However, these prolongations of PT and APTT were considered of little toxicological significance since they were slight and there were no changes suggestive of hemorrhage in the other examinations performed, including pathologic examination. Blood chemistry examination revealed elevated levels of ASAT (GOT) activity in females of the ubiquinol 300 and 1200 mg/kg groups, elevated levels of ALAT (GPT) activity in females in the ubiquinol 300 mg/kg and higher groups, and elevated LDH activity in females of the ubiquinol 300 and 600 mg/kg groups. Such changes are suggestive of adverse effects on the liver. Pathologic examination revealed test article-related effects on the liver, spleen, and mesenteric lymph nodes in females. In the liver, on histopathologic examination, microgranulomas, focal necrosis, or accumulation of macrophages, as well as fine vacuolation of hepatocytes and vacuolation of Kupffer cells were observed in each ubiquinol group. Liver weight was increased or tended to be increased. However, these changes were considered due to uptake of the administered ubiquinol by the liver, as an adaptive response to a xenobiotic compound, and the microgranulomas and focal necrosis were considered the result of excessive uptake of ubiquinol, which exceeded the capacity for adaptive response. These conclusions were based on the finding of localization to the liver of concentrated coenzyme Q_{10} dissolved in lipoproteins, as suggested by the positive reaction of the liver cells to oil red O staining for lipids on histological examination and by extremely high concentrations of total coenzyme Q_{10} in the liver, more than 8 mg/g detected on HPLC analysis, together with the previous finding by Mohr et al. (1992) and Tomasetti et al. (1999) that ubiquinol is extremely lipophilic and readily distributes into lipoproteins. Furthermore, in females, spleen weight was increased in the ubiquinol 600 mg/kg and higher groups, and accumulation of macrophages was observed in the spleen and mesenteric lymph nodes in each ubiquinol group. As in the liver, all of these effects were considered to be secondary effects following uptake of ubiquinol. Based on these findings, the no-observed-adverse-effect level (NOAEL) under the conditions of this study was estimated to be 600 mg/kg/day for males and less than 300 mg/kg/day for females (Kitano et al., submitted-a).

In light of changes in the clinical chemistry and histopathology observed in females of the 300 mg ubiquinol/kg/day and higher groups in the previous study (Kitano et al., submitted-a), a second 13-week oral toxicity study was conducted in female rats in order to determine NOAEL for ubiquinol in female rats (Kitano et al., submitted-a). Groups of 10 female rats were given a daily dose of 0, 75, 150, 200, or 300 mg/kg of ubiquinol dissolved in corn oil for 13 weeks. No death occurred in any group during the study period. No abnormalities in general condition, body weight, food consumption, ophthalmology examination, urinalysis, or hematology were observed. No dose-related or toxicologically significant changes in absolute or relative organ weights were observed. Evaluation of clinical chemistry parameters revealed somewhat elevated ASAT (GOT) activity in the ubiquinol 300 mg/kg group, which may be

suggestive of effects on the liver. Pathological examination revealed changes in the liver, spleen, and mesenteric lymph nodes similar to those observed in the previous 13-week study (Kitano et al., submitted-a). In the liver, on histopathological examination, fine vacuolation of hepatocytes was observed in the ubiquinol groups at 200 mg/kg and higher, and microgranulomas and focal necrosis at 300 mg/kg. However, these changes were considered by the study director to be secondary effects following uptake of the administered ubiquinol by the liver, as an adaptive response to a xenobiotic compound, and the microgranulomas and focal necrosis were considered due to excessive uptake of ubiquinol, which exceeded the capacity for adaptive response. The microgranulomas, which included foamy macrophages, were judged by the study director to be test article-related, although microgranulomas of this type are occasionally observed as a spontaneous change in this strain of rats. Furthermore, accumulation of macrophages was observed in the spleen and mesenteric lymph nodes in the ubiquinol 300 mg/kg group. As in the liver, each of these effects was considered to be secondary effects following uptake of ubiquinol. Based on these findings, the NOAEL for ubiquinol in female rats was estimated to be 200 mg/kg/day.

In 13-week subchronic toxicity studies in dogs, groups of 3 male and female Beagle dogs were given a daily dose of 0, 150, 300, or 600 mg/kg of ubiquinol dissolved in corn oil for 13 weeks. No death occurred in any group during the study period. While a small number of isolated differences from the control group were noted, no dose-related or toxicologically significant abnormalities in body weight, food consumption, ophthalmology, electrocardiogram, urinalysis, hematology, or clinical chemistry were observed. Likewise, no effects related to the administration of ubiquinol or ubiquinone were revealed on pathological or histopathological examinations. Based on these findings, the NOAEL for ubiquinol in Beagle dogs was estimated to be more than 600 mg/kg/day in both males and females (Kitano et al., submitted-b).

While the NOAEL for ubiquinol was lower in female rats (200 mg/kg/day) than in dogs and male rats (600 mg/kg/day), it was found that female rats exhibited higher sensitivity to accumulation of coenzyme Q10 in the liver, probably as a result of gender-dependent differences in the expression of hepatic enzymes. Shapiro et al. (1995), Mugford and Kedderis (1998) suggested that pronounced gender differences in rat drug metabolism might hinder extrapolation to other species, including humans, in which gender-related differences are generally subtler. Furthermore, in humans and most mammals, including dogs, the predominant form of coenzyme Q is coenzyme Q_{10}, which consists of 10 isoprenoid units in the side chain (Ramasarma, 1985), while in rats and mice the primary form is coenzyme Q_9, which contains 9 isoprenoid units (Battino et al., 1992). Thus, since the female rat appears particularly sensitive to accumulation of coenzyme Q_{10} in the liver, and the dog is more similar to humans, in having endogenous coenzyme Q_{10} as the predominant form of coenzyme Q, the dog was in this case considered the more appropriate animal model for extrapolation of results of animal toxicity studies to humans.

The genotoxic potential of ubiquinol was examined in a reverse mutation assay, chromosomal aberration test, and bone marrow micronucleus test. A reverse mutation assay was conducted on ubiquinol using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, and Escherichia coli WP2uvrA with and without metabolic activation. Both the dose determination test and the main test were performed by the preincubation method, with ubiquinol in acetone at dose levels up to 5000 mg/plate. No increases were observed in the number of revertant colonies with any of the tester strains in either the presence or absence of metabolic activation. Ubiquinol was thus considered to be non-mutagenic in the reverse mutation assay (Kitano et al., submitted-c).

The potential of ubiquinol for inducing chromosomal aberrations was also tested in a Chinese hamster lung fibroblast cell line (CHL/IU). The test doses were established on the basis of the results of a previously performed cell growth inhibition test. Microscopic observation for cells with chromosomal aberrations was performed at 3 doses (1715–3500 μg/ml) for the short-term treatment assay for 6 h without S9 metabolic activation, 4 doses (1201–3500 μg/ml) for the short-term treatment assay for 6hr with S9 metabolic activation, and 3 doses (588–1201 μg/ml) for the continuous treatment assay for 24 h. No clear increase in the incidence of chromosome aberrations was observed with either short-term treatment with or without S9 metabolic activation or continuous treatment in the groups treated with ubiquinol. It was concluded by the study director that ubiquinol did not induce structural chromosomal aberrations in CHL/IU cells (Kitano et al., submitted-c).

The genotoxic potential of ubiquinol was also examined using micronucleus assays on rat bone marrow smears from groups of 6 rats receiving 2 oral doses of degraded ubiquinol dissolved in corn oil (500, 1000, and 2000 mg/kg/day), with a 24-h interval between doses (Kitano et al., submitted-c). Samples were examined to determine the frequency of micronucleated immature erythrocytes (MNIE), and ratios of immature erythrocytes (IE) to total erythrocytes, and of MNIE to total number of IE were calculated. Results indicated that, compared to the negative control group, no significant increase in incidence of MNIE occurred in the groups treated with ubiquinol. In addition, no significant difference from the control group in the ratio of IE to total erythrocytes was observed in the treatment groups. Based on these results, it was concluded by the study director that ubiquinol did not induce micronucleated erythrocytes under the conditions of this assay.

Concerning the safety of ubiquinone (coenzyme Q_{10}), there are many reports of non-clinical toxicity studies demonstrating that coenzyme Q_{10} had no adverse acute, subacute, chronic, or reproductive developmental effects (Williams et al., 1999; Chiba et al., 1972a,b; Notake et al., 1972; Williams, 1995). In addition, there are no reports of

serious adverse effects in humans associated with the use of ubiquinone as a dietary supplement or drug for over 30 years in the United States, European countries, and Japan (Pepping, 1999; Overvad et al., 1999; Hodges et al., 1999; Shults et al., 2002; Tran et al., 2001).

Based on the results of pre-clinical toxicity studies with Kaneka QH™ (ubiquinol) and ubiquinone supporting the safety of both reduced and oxidized coenzyme Q_{10}, and substantial clinical experience with ubiquinone indicating its tolerability in humans, clinical evaluation of ubiquinol was begun. As a first step, single-dose and 4 week repeated-dose studies in male and female volunteers were conducted to assess the safety and bioavailability of ubiquinol.

2. Material and methods

The single-dose and 4-week multiple-dose studies were performed at the Kaiyu Clinic, Tokyo, Japan in accordance with Good Clinical Practice guidelines. The study protocols were approved by the Ethics Committee of Kaiyu Clinic, and all subjects provided written informed consent prior to participation. The studies were conducted in accordance with the principles of the Declaration of Helsinki.

2.1. Study design

Fifteen healthy volunteers (5 males and 5 females for the 150 mg dose and 5 males for the 300 mg dose) and 80 healthy volunteers (10 males and 10 females each for placebo, 90 mg, 150 mg, and 300 mg doses of ubiquinol) were enrolled in the single-dose study and 4-week multiple-dose study, respectively. Subjects were screened for eligibility within 4 weeks before administration. Screening included medical history, physical examination, clinical laboratory tests, determination of vital signs, and electrocardiography. The studies were carried out in single-blind fashion within the respective dose groups and in controlled fashion to test the effects of increasing oral doses of ubiquinol.

Soft gelatin capsules containing 30 mg of ubiquinol emulsified with diglycerol monooleate, rapeseed oil, soy lecithin, and beeswax were used as an active formulation (active capsule), while capsules containing all of the ingredients other than ubiquinol were used as a placebo formulation (placebo capsule). The purity of Kaneka QH™ was confirmed by product quality analyses to be above 96%, and the main impurities were ubiquinone and reduced coenzyme Q_9. Kaneka QH™ did not contain the *cis*-isomer of ubiquinol.

In the single-dose study, after breakfast including a 250 g sandwich and a glass of orange juice, subjects received 10 active capsules or the combination of 5 active capsules and 5 placebo capsules with 180 ml of water. In the 4-week multiple-dose study, subjects received 10 capsules daily, 5 capsules each after breakfast and dinner with 180 ml of water for 28 days. The number of capsules (active + placebo) for morning intake and evening intake were 0 + 5 and 0 + 5 for the placebo group, 2 + 3 and 1 + 4 for the ubiquinol 90 mg group, 3 + 2 and 2 + 3 for the ubiquinol 150 mg group, and 5 + 0 and 5 + 0 for the ubiquinol 300 mg group.

Standard laboratory tests for safety including hematology, prothrombin time (PT), activated partial thromboplastin (APTT), urinalysis (pH, protein, glucose, occult blood, bilirubin, urobilinogen), and blood chemistry (aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transaminase, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total bilirubin, glucose, blood urea nitrogen, creatinine, uric acid, albumin, A/G ratio, total protein, sodium, potassium, chloride, hemoglobin A1c, insulin) as well as physical examination, vital signs (blood pressure, heart rate, body temperature) and electrocardiography were performed before administration on the day of treatment (day 0) as baseline and on day 2 in the single-dose study. The same laboratory tests were performed before treatment, 2 and 4 weeks after starting treatment, and 2 weeks after completion of treatment in the 4-week multiple-dose study. Adverse event data were carefully collected by close monitoring and questioning of subjects by study personnel at 1, 2, 4, 6, 8, 12, 24, and 48 h after treatment in the single-dose study. In the 4-week multiple-dose study, adverse events were voluntarily reported by subjects and recorded each day, and were monitored with questioning by study personnel at 2 and 4 weeks after starting treatment, and again 2 weeks after completion. Any untoward changes, whether or not they appeared to be treatment-related, were considered to be adverse events.

In the single-dose study, blood samples were collected in heparinized tubes before as well as 1, 2, 4, 6, 8, 12, 24, and 48 h after treatment to determine plasma levels of ubiquinol and ubiquinone. In the 4-week multiple-dose study, blood samples were collected before administration on the first day of treatment (day 0), day 1, day 14, day 28 (through), and 2 weeks and 6 months after completion of treatment. Plasma was separated and stored at −60 °C or below.

Plasma concentrations of ubiquinol and ubiquinone were determined by a validated HPLC method described by Yamashita and Yamamoto (1997) with a minor modification. Briefly, 920 μl of 2-propanol was added to 100 μl plasma. The resulting solution was vortex-mixed for 3 s and then centrifuged for 4 min at 4 °C in a microfuge tube. Then 20 μl of the supernatant was injected into an HPLC system (FLOM column oven 502, Shimadzu LC10Atvp pump, Uniflows DU-4003 degasser, Shimadzu SIL-10Advp autoinjector, Shimadzu SCL-10Avp system controller, Shimadzu Chromatopac data processor C-R7A plus) with an analytical column (25.0 cm × 0.46 cm, 5-μm; Pegasil-300 ODS) maintained at 20 °C, a reduction column (15.0 mm × 4 mm; Shiseido RC-10), a FLOW electronic six-port valve, and an EICOM ECD-300 amperometric electrochemical detector (ECD). The electronic six-port valve was controlled using the Shimadzu SCL-10Avp system controller so that hydrophilic molecules having a shorter retention time than ubiquinol and ubiquinone could be discharged prior to flowing into the reduction column. The mobile phase consisted of 0.005 M $NaClO_4$ in ethanol/methanol/water (700:300:1.2) at a flow rate of 0.6 ml/min. The oxidation potential for ECD was 430 mV. The concentrations of ubiquinol and ubiquinone were calculated from peak heights using an absolute standard curve method.

2.2. Statistical analysis

For analysis of the parameters obtained in the safety examination, the changes from baseline in each group were examined by Student's *t*-test for paired values (two-tailed). For analysis of incidence of adverse events, the χ^2 test was performed between the placebo and treatment groups.

Areas under the plasma ubiquinol concentration-versus-time curve (AUC) were calculated by the trapezoidal rule.

3. Results

A total of 15 healthy volunteers and 78 healthy volunteers (38 females and 40 males) completed the single-dose study and 4-week multiple-dose study, respectively. One female in the placebo group dropped out from the multiple-dose study for personal reasons on day 26, and one female in the ubiquinol 300 mg group stopped taking ubiquinol capsules due to diarrhea (enterocolitis) on day 1. Hence, a total of 78 healthy volunteers (38 females and 40 males) were included in the analysis of safety and bioavailability. The mean age and mean body weight of the subjects are shown in Table 1.

3.1. Safety and tolerability

3.1.1. Single-dose study

No clinically significant changes in vital signs, hematology, PT, APTT, urinalysis, or blood chemistry were

Table 1
Characteristics of the subjects in single-dose and 4-week multiple-dose studies of ubiquinol in healthy volunteers

	Single-dose study ($n = 5$)			4-week multiple-dose study ($n = 10$)							
	150 mg[a]		300 mg[a]	Placebo		90 mg[a]		150 mg[a]		300 mg[a]	
	Male	Female	Male	Male	Female[c]	Male	Female	Male	Female	Male	Female[c]
Age (years)	36.6 ± 5.2[b]	27.2 ± 2.1	34.2 ± 4.6	32.7 ± 4.2	34.0 ± 4.6	31.9 ± 3.4	34.2 ± 4.3	33.7 ± 3.9	34.5 ± 4.2	34.3 ± 3.9	34.4 ± 4.5
Weight (kg)	67.1 ± 3.8	48.1 ± 2.5	66.7 ± 3.7	66.9 ± 2.7	53.1 ± 1.7	64.6 ± 2.4	49.0 ± 1.8	65.2 ± 2.5	50.8 ± 1.2	64.3 ± 1.9	53.8 ± 1.3
Height (m)	166.1 ± 1.2	155.3 ± 3.2	171.5 ± 2.5	173.1 ± 2.0	158.2 ± 1.6	171.5 ± 1.4	157.0 ± 1.6	169.5 ± 2.0	159.7 ± 1.5	170.8 ± 2.4	159.1 ± 1.9

[a] Dose of ubiquinol.
[b] Mean ± SEM.
[c] $n = 9$.

observed in any subjects except one female treated with 150 mg of ubiquinol who exhibited tachycardia on day 2, which was, however, judged unrelated to the treatment by the study director of medical doctor because her symptoms were transient and appeared only on day 2. Although some statistically significant changes were observed in certain items such as uric acid, PT, and basophil count in the ubiquinol 150 mg group, and blood pressure, blood urea nitrogen, sodium, potassium, glucose, hemoglobin A1c, and APTT in the ubiquinol 300 mg group, they were all small, within the respective reference ranges, and judged not to be clinically significant. Soft stool was recorded as an adverse event for one female treated with 150 mg of ubiquinol, but was slight and judged not to be clinically significant.

3.1.2. Four-week multiple-dose study

On hematological examination, slight increases in eosinophil percentage at week 4 of treatment (12%) and 2 weeks after completion of treatment (18%) compared with the initial value (6%) were observed in one male in the ubiquinol 90 mg group. However, these changes were judged not to be clinically important since they were slight and could have had causes other than ubiquinol treatment. On blood chemistry examination, one male in the 150 mg ubiquinol group exhibited slight increase in LDL cholesterol level at week 4 of treatment (162 mg/dl), compared with the initial value (130 mg/dl). However, it also was judged not clinically important since it was slight and the possibility of causes other than ubiquinol treatment such as food intake could not be ruled out. No significant clinical changes in vital signs, hematology, PT, APTT, urinalysis, or blood chemistry were observed in any subjects except those mentioned above. Although there were some statistically significant changes in certain items, including body temperature, GPT, total cholesterol, triglyceride, insulin, PT, and APTT in the placebo group, systolic blood pressure, lactate dehydrogenase, total protein, hematocrit, PT, and segmented leukocyte percentage in the ubiquinol 90 mg group, pulse, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transaminase, A/G ratio, chloride, hemoglobin, insulin, and PT in the ubiquinol 150 mg group, and body temperature, total bilirubin, sodium, potassium, chloride, platelet count, PT, and monocyte percentage in the ubiquinol 300 mg group, they were judged not to be clinically significant because they were small and within the respective reference ranges.

Table 2 presents all the adverse events that occurred in the 4-week multiple-dose study. Subjects reporting at least one adverse event included 9 of 19 in the placebo group, 13 of 20 in the ubiquinol 90 mg group, 9 of 20 in the ubiquinol 150 mg group, and 10 of 20 in the ubiquinol 300 mg group. There were no statistically significant differences between placebo and active treatment with regard to incidence of adverse events. The vast majority of adverse events were mild or moderate in severity and of no importance clinically. Only one adverse event, diarrhea (enterocolitis), which occurred in one subject on day 1 in the ubiquinol 300 mg group, was serious, and the subject withdrew from the study on day 1. This adverse event was considered unrelated to ubiquinol treatment since hematology examinations for the subject on screening and day 1 revealed leukocytosis, indicating clinically inapparent infection by microorganisms. The leukocyte counts on screening, day 1, and 2 weeks after discontinuation of treatment were 8100, 10200, and 5600 per microliter, respectively.

3.2. Bioavailability

The mean plasma concentration-time curves for ubiquinol after single oral administrations of 150 and 300 mg of ubiquinol are shown in Fig. 1. Following its administration, ubiquinol was gradually absorbed, and mean ubiquinol concentration reached maxima of 1.88 μg/ml for 150 mg and 3.19 μg/ml for 300 mg at 6 h after administration, and thereafter exhibited a shoulder peak of 1.76 μg/ml for 150 mg and 2.20 μg/ml for 300 mg at 24 and 12 h after administration, respectively. At all time points, the ratios of ubiquinol concentration to total coenzyme Q_{10} concentration ranged from 96.8% to 98.2%. The maximum concentration (C_{max}) and $AUC_{0-48\,h}$ derived from the mean plasma ubiquinol concentration-time curves at doses of 150 and 300 mg were 1.88 and 3.19 μg/ml, and 74.61 and 91.76 μgh/ml, respectively. These parameters thus increased dose-dependently, though at a rate less than proportional to that of dose.

The mean plasma concentrations of ubiquinol before administration on the first day of treatment (day 0), day 1, day 14, day 28 (trough), and 2 weeks after completion of treatment in the 4-week multiple-dose study at daily doses

Table 2
Summary of adverse events after 4-week multiple oral administration of ubiquinol in healthy volunteers

Adverse event		Placebo	Ubiquinol		
			90 mg	150 mg	300 mg
Respiratory system	Common cold syndrome	0[a]	2	3	1
	Sore throat	0	1	0	0
	Total	0	3	3	1
Central and peripheral nervous systems	Dizziness on standing up	0	0	0	1
	Stiff shoulder	0	0	1	0
	Total	0	0	1	1
Psychiatric	Sleepiness	0	0	0	1
Body as a whole	Headache	1	1	0	0
	Heat stroke	0	1	0	0
	Total	1	2	0	0
Skin and appendages	Exanthema	0	0	0	1
Gastrointestinal system	Abdominal pain	2	1	1	2
	Stomachache	0	0	0	1
	Stomach heaviness	0	0	1	0
	Enterocolitis	0	0	0	1
	Loose stool	0	3	0	0
	Diarrhea	3	5	1	3
	Constipation	0	1	0	0
	Nausea	1	0	0	0
	Stomatitis	0	1	0	1
	Gingival pain	1	0	0	0
	Total	7	11	3	8
Musculo-skeltal system	Arthralgia	0	0	1	0
	Joint stiffness	0	0	1	0
	Lumbar pain	0	0	0	2
	Whiplash injury	0	0	1	0
	Total	0	0	3	2
Reproductive system, female	Painful menses	2	3	1	1
	Shortened menstrual cycle	0	0	1	0
	Total	2	3	2	1

[a] Number of adverse events.

of 90, 150, and 300 mg are shown in Fig. 2 and Table 3. Baseline plasma ubiquinol levels prior to each administration ranged from 0.57 to 0.66 μg/ml. Mean trough concentration increased dose-dependently at all sampling points, i.e., day 1, day 14, and day 28, though the increase was less than proportional to that in dose. The mean trough concentrations at day 14 and day 28 were 2.61 and 2.84 μg/ml for 90 mg, 3.66 and 3.84 μg/ml for 150 mg, and 6.53 and 7.28 μg/ml for 300 mg, respectively and the ratios of the mean concentrations on day 28 and day 14 ranged from 1.09 to 1.11, indicating that plasma ubiquinol concentration had nearly reached steady-state by 2 weeks after the start of treatment. Compared with concentrations following the first dose, those at steady-state were 2.1- to 2.8-fold increased. During the two-week period following completion of treatment, the concentrations declined to 0.74 μg/ml for 90 mg, 0.89 μg/ml for 150 mg, and 1.15 μg/ml for 300 mg, which were 1.3 to 1.8-fold those before administration. At all time points, the ratios of ubiquinol concentration to total coenzyme Q_{10} concentration ranged from 96.0% to 98.5%.

As a follow-up study, plasma ubiquinol levels 6 months after completion of treatment were measured for 4 males and 7 females in the 300 mg group. The plasma concentration of ubiquinol (mean ± SEM) was 0.72 ± 0.05 μg/ml and equivalent to that before administration (0.71 ± 0.07 μg/ml).

4. Discussion

In the studies reported here, the safety, tolerability, and bioavailability of various single-dose and multiple-dose

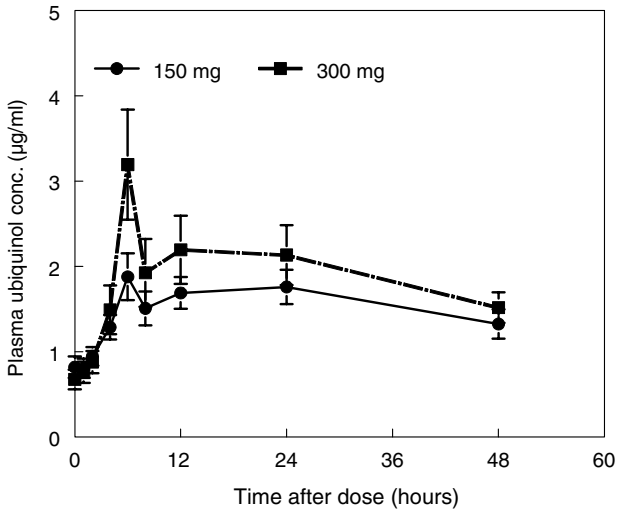

Fig. 1. Mean plasma ubiquinol concentration-time curves after single oral administration of ubiquinol to healthy volunteers. Each point represents the mean ± SEM of 10 subjects (150 mg) or 5 subjects (300 mg).

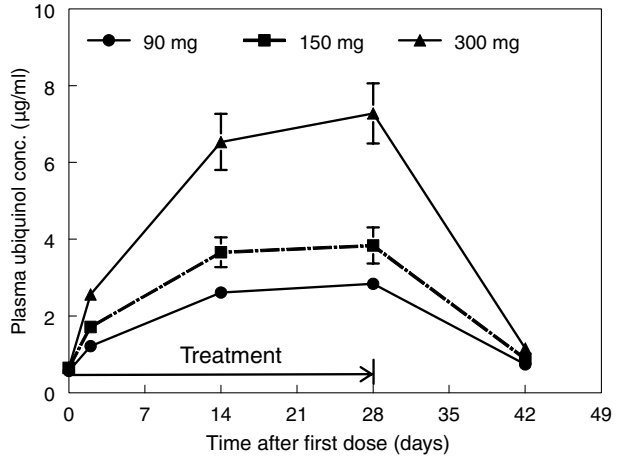

Fig. 2. Morning trough concentrations of ubiquinol in plasma during 4-week multiple oral administration of ubiquinol and 2 weeks after completion of the treatment in healthy volunteers. Each point represents the mean ± SEM of 20 subjects (90 mg and 150 mg) or 19 subjects (300 mg).

oral administrations of ubiquinol were examined for the first time in humans. No significant safety concerns were revealed in the studies, and no serious adverse events were observed. Ubiquinol thus exhibited an acceptable safety profile as a dietary supplement up to multiple daily doses of 300 mg for up to 4 weeks. Although some statistically significant changes were observed on routine laboratory tests, they were slight intensity or considered due to factors other than ubiquinol treatment, and were thus not clinically significant. The most frequent adverse events were gastrointestinal symptoms. Since these symptoms were slight, not dose-related, and occurred in both the ubiquinol and placebo groups, relationship of them to ubiquinol treatment was excluded.

It has been reported that natural coenzyme Q_{10} is readily reduced after dietary uptake (Mohr et al., 1992; Stocker and Suarna, 1993; Weber et al., 1994), and that a high ratio of ubiquinol to total coenzyme Q_{10} (sum of ubiquinol and oxidized coenzyme Q_{10}, approximately 85%) was maintained even when serum concentrations of total coenzyme Q_{10} were enhanced through oral supplementation with the oxidized form of coenzyme Q_{10} (ubiquinone) (Okamoto et al., 1989). Furthermore, Mohr et al. (1992) reported that dietary supplementation with ubiquinone (100–300 mg/day) increased concentrations of ubiquinol-10 in plasma and in all of its lipoproteins without altering the ratio of ubiquinol-10 to ubiquinone-10 in LDL or plasma. Although in most clinical trials the safety of ubiquinone supplementation has not been the focus, the large number of clinical studies that have been conducted with ubiquinone and have demonstrated its safety (Lansjoen et al., 1990; Baggio et al., 1994; Hofman-Bang et al., 1995; Singh et al., 1998; Tran et al., 2001; Shults et al., 2002; Feigin et al., 1996; Shults et al., 2004; Shults et al., 1998; Huntington Study Group, 2001; Ferrante et al., 2005) support the findings obtained in the current study regarding the safety of ubiquinol. Since ubiquinol has approximately twice the bioavailability of ubiquinone, the results of the clinical studies conducted with ubiquinone at relatively high-doses ranging from 600 to 3000 mg/day for at least 2 weeks and up to 16 months (Shults et al., 2002; Feigin et al., 1996; Shults et al., 2004; Shults et al., 1998; Huntington Study Group, 2001; Ferrante et al., 2005) are especially important in confirming the safety of ubiquinol.

After single doses of 150 mg and 300 mg of ubiquinol, mean plasma levels reached peaks associated with 2.3- and 4.7-fold increases over baseline plasma levels of ubiquinol after 6 h, respectively, indicating that significant amounts of ubiquinol were absorbed from the gastrointestinal tract. The time to reach peak concentration of 6 h after a single dose indicates slow absolrption of ubiquinol from the

Table 3
Morning trough concentrations of ubiquinol in plasma during 4-week multiple oral administration of ubiquinol and 2 weeks after completion of the treatment in healthy volunteers

Dose (mg)	No. of subjects	Plasma ubiquinol concentration (μg/ml)					Ratio of mean values	
		Day 0	Day 1	Day 14	Day 28	Post 2 weeks[b]	(Day 28/day 14)	(Post 2 weeks/day 0)
90	20	0.57 ± 0.04[a]	1.21 ± 0.07	2.61 ± 0.17	2.84 ± 0.20	0.74 ± 0.05	1.09	1.30
150	20	0.65 ± 0.04	1.71 ± 0.10	3.66 ± 0.39	3.84 ± 0.47	0.89 ± 0.07	1.05	1.37
300	19	0.66 ± 0.04	2.56 ± 0.18	6.53 ± 0.73	7.28 ± 0.78	1.15 ± 0.09	1.11	1.74

[a] Mean ± SEM.
[b] Two weeks after completion of treatment.

gastrointestinal tract, attributable to its low water solubility (less than 0.1 mg/ml) and high molecular weight of 865, as in the case of ubiquinone (Tomono et al., 1986; Lücker et al., 1984). The finding of a shoulder peak at 12 or 24 h after administration also agrees with the findings for ubiquinone (Tomono et al., 1986; Bogentoft et al., 1991; Weis et al., 1994), and may be explained in similar fashion, i. e., that absorbed ubiquinone is taken up by the liver and then redistributed from it to the systemic blood circulation (Tomono et al., 1986; Yuzuriha et al., 1983). Although the plasma half-life of ubiquinol could not be accurately calculated due to insufficient number of sampling points in this study, this half-life appears to be about 48 h, since plasma ubiquinol levels 1.6–2.2 times higher than baseline were observed at 48 h after administration. This estimate is comparable to the half-life of ubiquinone in blood, between 33 and 50 h (Bogentoft et al., 1991; Lücker et al., 1984; Tomono et al., 1986). Determination of the pharmacokinetics of ubiquinol will require further studies with sufficient numbers of sample points, especially around the second peak and in the elimination phase. Although many published papers are available regarding the bioavailability of ubiquinone in humans, such as Miles et al. (2002), Joshi et al. (2003), and Ullmann et al. (2005), comparison of the bioavailability of ubiquinol determined in this study with those of ubiquinone reported with different formulations in these papers is difficult, since it has been reported that the bioavailability of ubiquinone strongly depends on the formulation used (Chopra et al., 1998; Kurowska et al., 2003). Furthermore, since there is also significant inter-individual variability in absorption of ubiquinone from supplements (Kurowska et al., 2003), crossover studies with the same formulation and the same subjects will be needed to accurately compare the bioavailabilities of ubiquinol and ubiquinone in human.

In the 4-week multiple-dose study, daily administration of 90, 150, and 300 mg of ubiquinol resulted in 2.1-, 4.6-, and 5.0-fold increases, 2.6-, 5.7-, and 5.9-fold increases, and 3.9-, 9.9-, and 11.1-fold increases above baseline plasma levels of ubiquinol on day 1, day 14, and day 28, respectively. These nonlinear changes in plasma ubiquinol levels may also be ascribed to physicochemical properties of ubiquinol including its relatively large molecular weight and poor water solubility, which result in a long half-life of elimination. However, the finding that plasma ubiquinol concentration reached steady-state within 2 weeks of treatment and returned to near baseline level 2 week after completion of treatment suggests that no accumulation of ubiquinol occurs over 4 weeks of treatment.

Since ubiquinol is biosynthesized, exogenous administration of it may down-regulate its endogenous synthesis. The finding that plasma ubiquinol level 6 months after completion of treatment in the 300 mg group was equivalent to that before administration suggests that multiple-dose treatment did not affect endogenous synthesis of ubiquinol. Similarly, Ikematsu et al. (2006) reported that plasma coenzyme Q_{10} concentration was after 8-month withdrawal almost the same as that before intake of 150, 300, or 900 mg of coenzyme Q_{10} (ubiquinone) for 4 weeks in healthy subjects, suggesting that exogenous administration of coenzyme Q_{10} did not affect coenzyme Q_{10} biosynthesis. Turunen et al. (1999) also reported that the uptake of dietary coenzyme Q_{10} into liver and spleen did not down-regulate endogenous synthesis of coenzyme Q_9, the predominant form in rats. Tomono et al. (1986) reported that there was little effect of oral administration of deuterium-labeled coenzyme Q_{10} on plasma levels of endogenous coenzyme Q_{10}.

In conclusion, following single- or multiple-doses of Kaneka QH™ (ubiquinol) in healthy volunteers, significant absorption of ubiquinol from the gastrointestinal tract was observed, and no safety concerns were noted on standard laboratory tests for safety or on assessment of adverse events for doses of up to 300 mg for up to 2 weeks after treatment completion.

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