資料 2

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コエンザイムＱ１０の安全性に関する照会について（回答）

「コエンザイムＱ１０の安全性に関する照会について（依頼）」（平成18年4月12日付け府食第286号）により貴委員会から照会があったコエンザイムＱ１０の安全性について、別添のとおり回答を提出します。

なお、別紙４については、協力企業より、非公表の取り扱いを条件として提供を受けていますので、御了承願います。

第 42 号
受 18.5.22 信
食品安全委員会事務局

（別添）

（１）コエンザイムQ10の食品としての安全性を評価する場合には、医薬品と相違して、医療従事者としての関与がなく基礎疾患を持った人等様々な人が摂取することなどを考慮する必要がある。しかし、現在提出されているヒトでの試験成績では、こうした食品という性格を配慮し、かつ摂取上限目安量が判断できる長期摂取試験の成績が不足していると考える。この点について、現在提出されている資料以外に長期摂取の安全性を説明できる情報があれば提出されたい。

(答)

現在提出している資料以外の長期摂取試験の情報は入手していない。

（２）コエンザイムQ10を食品として大量又は長期継続的に摂取した場合の生体内におけるコエンザイムQ10本来の合成・代謝系等に与える影響に関する科学的知見は、安全性評価の上で必須の情報と考えるが、現在提出された資料では欠如している。これに関連する情報があれば提出されたい。

(答)

長期継続的に摂取した場合の合成・代謝系等に与える影響に関する資料は入手していない。なお、短期摂取に関する情報として、現在までに、コエンザイムQ10を投与した後に、内因性コエンザイムQ10の144時間にわたる推移がほぼ一定に保たれ、投与したコエンザイムQ10が内因性コエンザイムQ10にほとんど影響を及ぼさない趣旨の論文（別紙１）を提出しているが、新たに、コエンザイムQ10を4週間経口投与した後の、血中濃度の測定結果から、外因性コエンザイムQ10により内因性コエンザイムQ10の生合成への影響がなかった旨の研究結果を入手したので、併せて提出する（別紙２）。

（３）コエンザイムQ10は、水に溶けにくく体内に吸収されにくい物質であるが、近年これを含むいわゆる健康食品の中には、当該物質の吸収性を改良したと称する製品が流通している。製品によって吸収性が異なるのであれば、体内動態も製品によって異なると考えられるので、当該物質の安全性は、物質としてではなく個別の製品について評価することが適切であると考えられる。吸収性が異ならないとする情報があれば提出されたい。

(答)

吸収性については、製剤設計によって異なり、吸収性が異ならないとする情報はない。もともとコエンザイムQ10は吸収性が悪く、現時点において、吸収性を大きく改善する製品が開発されているとの報告は受けていない。

なお、原材料メーカーのデータによると乳化剤を添加して水溶性にするこ

とにより、吸収率が２倍程度になるとの報告があるのでその資料を提出する（別紙３）。

コエンザイムQ10を含む食品については極めて多数の製品が流通しており、各々の製品について食品健康影響評価を行うことは困難であると考えられることなどから、まずは原体であるコエンザイムQ10そのものの評価を依頼しているところである。

（４）今回の諮問の契機となった２例も含め現在販売されているコエンザイムQ10 製品に関し、問題となるべき健康影響が必ずしも明確でないと考える。現在把握されている副作用事例について、摂取者の背景や摂取状況などに関する情報を提出されたい。

(答)

現在把握されている副作用事例については、第２９回新開発食品専門調査会の資料として提出したとおりである。

なお、ある企業のお客様相談室に寄せられた情報を入手したので、参考として提出する（別紙４）。

（５）医薬品の用量をはるかに超える摂取量となるコエンザイム Q10 が食品として摂取されているが、食薬区分上、医薬品ではなく食品として扱われる理由を説明されたい。

(答)

健康食品の成分が経口摂取の医薬品としても用いられるものについては、原則として医薬品として用いられる量を超えないよう指導しているところである。

コエンザイムＱ１０は、医薬品の成分としても利用されているが、①作用が緩和であること、②生体内で合成され、細胞のエネルギー産生に関わるビタミン類似物質であること、③肉類など通常の食品にも含まれていること、などから、栄養素としての摂取目的もあるとして、従来から「医薬品的効能効果を標榜しない限り医薬品として判断しない成分本質」として取り扱っているものである。

このため、コエンザイム Q10 については、医薬品として承認されている用量を超えている摂取量の製品であっても、医薬品的な形状や効能効果の標榜等をしていない物については、薬事法上の「医薬品」の定義に合致しないことから医薬品としての規制を受けず、当該製品が飲食物であれば食品として扱うこととしている。

なお、この他にも、医薬品としての承認を受けている成分本質が、食品としての扱いもなされることがある事例として、アミノ酸、ビタミン、カフェイン、ショウキョウ（ショウガ）、タイソウ（なつめ）、サンヤク（長いも）

等がある。

参考条文

○薬事法（昭和三十五年法律第百四十五号）

第二条　この法律で「医薬品」とは、次の各号に掲げる物をいう。

一　日本薬局方に収められている物

二　人又は動物の疾病の診断、治療又は予防に使用されることが目的とされている物であつて、機械器具、歯科材料、医療用品及び衛生用品（以下「機械器具等」という。）でないもの（医薬部外品を除く。）

三　人又は動物の身体の構造又は機能に影響を及ぼすことが目的とされている物であつて、機械器具等でないもの（医薬部外品及び化粧品を除く。）

○食品衛生法（昭和２２年法律第二百三十三号）

第四条　この法律で食品とは、すべての飲食物をいう。ただし、薬事法（昭和三十五年法律第百四十五号）に規定する医薬品及び医薬部外品は、これを含まない。

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(別紙１)

Pharmacokinetic study of deuterium-labelled coenzyme Q10 in man

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Abstract. The pharmacokinetics of coenzyme Q10 (CoQ10) in man was studied by utilizing deuterium-labelled coenzyme Q10 (d_5-CoQ10). The absence of an isotope effect in the disposition of d_5-CoQ10 in man was confirmed from the plasma concentration time curves after simultaneous oral dosing of d_5-CoQ10 and unlabelled CoQ10. After oral administration of 100 mg of d_5-CoQ10 to 16 healthy male subjects, the mean plasma CoQ10 level attained a peak of 1.004 ± 0.370 μg/ml at 6.5 ± 1.5 h after administration, and the terminal elimination half-life was 33.19 ± 5.32 h. In most of the subjects, plasma d_5-CoQ10 showed a second peak at 24 h after dosing. This unusual plasma level curve was well described by a newly proposed compartment model based upon the assumption that absorbed CoQ10 is taken up by the liver and then transferred mainly to VLDL and redistributed from the liver to the systemic blood.

Key words: coenzyme Q10 – pharmacokinetics – stable isotope

Introduction

Coenzyme Q10 (CoQ10) was isolated in 1957 from bovine heart muscle, and was found to play an important role in energy production as a constituent of the electron transport system in mitochondria. In subsequent studies, it has been demonstrated that CoQ10 also has many physiological actions, including the ability to improve the condition of ischemic myocardial lesions [Nayler 1978, Mochizuki et al. 1979], maintenance of the integrated function of biological membranes [Baum et al. 1980], and an antioxidative effect against biological peroxides.

Since 1972, CoQ10 has been used clinically for the improvement of cardiac function in patients with mild heart failure in Japan, and clinical studies are now being conducted in the United States and several European countries. However, relatively few pharmacokinetic studies have been carried out in man [Fujita et al. 1972, Kishi et al. 1981, Kishi et al. 1984, Lücker et al. 1984]. Moreover, the disposition of CoQ10 is still unclear, partly because of the difficulty of measuring exogenous CoQ10 separately from endogenous CoQ10.

The present study was carried out to investigate precisely the pharmacokinetics of deuterium-labelled CoQ10 in man after oral administration.

Reprint requests to Dr. Y. Tomono

Materials and methods

Deuterium-labelled CoQ10

Deuterium-labelled CoQ10 (d_5-CoQ10) was kindly supplied by Mr. Hashimoto (Section of Product Chemistry, Eisai Co., Ltd.). The structure is shown in Figure 1. The purity was more than 99.4% when assayed by the UV (275 nm) method, and the thin-layer chromatogram showed a single spot. The content of d_5-CoQ10 determined by the selected-ion monitoring method [Imabayashi et al. 1979] was more than 99% of the total CoQ10.

Human study

Medications

Powder preparations containing 100 mg/g of d_5-CoQ10 and capsules containing 100 mg of CoQ10 were used.

Subjects

Seventeen healthy male volunteers between the ages of 23 and 45 participated in this study. A physical examination and laboratory tests were carried out before and after the study. Each subject had given written informed consent to participation in the study, which was conducted in two parts. Two of the subjects participated in part 1, and sixteen of them in part 2, only one taking part in both.

Study protocol

(Study 1) Three hundred milligrams of d_5-CoQ10 and the same amount of CoQ10 were taken with 180 ml of water within 30 min after breakfast. The subjects abstained from food and alcohol for at least 10 hours before breakfast and for 8 hours after

the administration. Samples of 5 ml of blood were collected at the following times using glass syringes: before administration and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, 72, 216 (10th day) and 336 (15th day) hours after administration. Each sample of heparinized whole blood was immediately centrifuged at 3,000 r.p.m. for 15 min and the plasma was frozen until analysis.

(Study 2) One hundred milligrams of d_5-CoQ10 was taken 30 min after breakfast (8:30 am) with 180 ml of water. The subjects abstained from taking food and alcohol for at least 10 hours before breakfast. They were given 180 ml of orange juice and two cookies at 11 am, lunch at 1 pm, 180 ml of chilled coffee and two cookies at 3 pm and dinner at 5 pm.

Blood collections were made at the following times using glass syringes: before administration and 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72 and 144 hours after administration.

Analytical procedure

The amounts of CoQ10 and d_5-CoQ10 in plasma were measured by direct-inlet selected-ion monitoring method (DI-SIM) [Imabayashi et al. 1979].

Extraction procedure

Plasma (1 ml) was mixed with 3 μg of internal standard (2,3-dimethoxy-5-ethyl-6-decaprenyl benzoquinone) in 0.2 ml of isopropanol, 20 mg of pancreatin in 2 ml of tris-HCl buffer solution and 8 mg of taurocholate in 0.2 ml of aqueous solution. The mixture was gently shaken for 2 h at 37° C, then extracted twice with 5 ml of a mixture of n-hexane-isopropanol (3:1). The combined organic phase was evaporated at 45° C under a nitrogen gas stream, and the residue was dissolved in 0.4 ml of ethyl acetate. Two microliters of the solution was placed in a capillary and heated with a drier to evaporate the solvent. Then the glass capillary was inserted into the ionization chamber of a JEOL JMS-DX 300 mass spectrometer.

Mass spectrometry

The conditions of mass spectrometry were as follows: enricher temperature 250° C, probe temperature 200–285° C (32°/min), ionization voltage 30 V, ionization current 300 μA, multi gain 200. For the DI-SIM analysis, m/z 864 $[M+2]^+$, m/z 869 $[M+2]^+$ and m/z 876 $[M+2]^+$ were used for the determination of CoQ10, d_5-CoQ10 and internal standard, respectively.

Calibration curve

The selected-ion monitoring chromatograms and the calibration curves are shown in Figures 2 and 3. The CV% of the analysis of d_5-CoQ10 in plasma at the concentrations of 0.1 μg/ml and 5.0 μg/ml were 5.24% and 2.93%, respectively.

Data analysis

The compartment model shown in Figure 4 was used for the pharmacokinetic analysis of plasma CoQ10. Nonlinear regression analysis was performed by using the simplex method [Nelder et al. 1965] on a Hewlett-Packard system, Model 9836 computer.

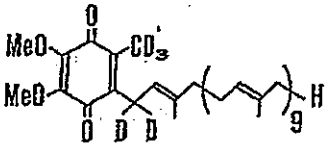
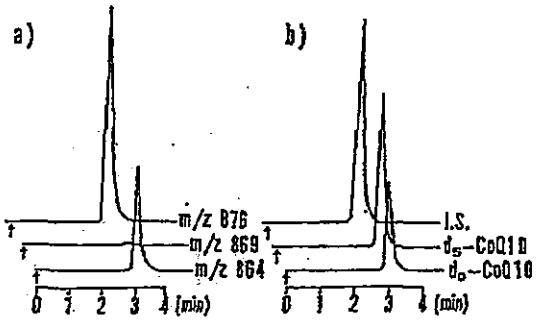


Fig. 1 Chemical structure of deuterium-labelled CoQ10.



† : Sample insertion time
a) Plasma blank
b) 1 μg of d_5-CoQ10 added to plasma

Fig. 2 Chromatograms of human plasma with or without addition of d_5-CoQ10.

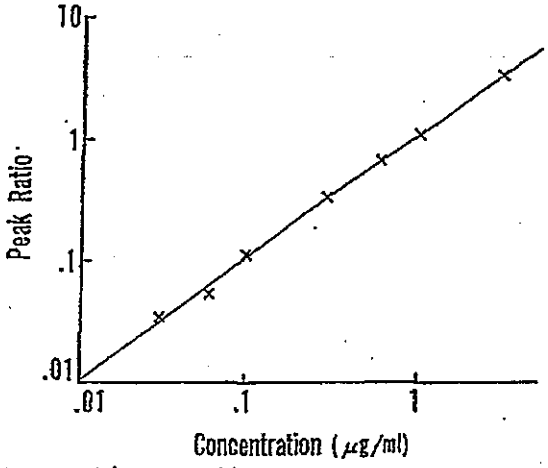


Fig. 3 Calibration curve of d_5-CoQ10.

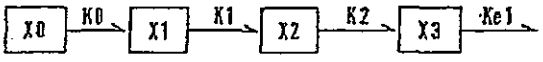


Fig. 4 Compartment model of the disposition of CoQ10. X0 is the gastro-intestinal compartment; X1 is the compartment which represents the plasma chylomicrons and the tissues to which rapid distribution can take place; X2 is the liver; X3 is the compartment which represents VLDL and other tissues which are rapidly accessible to the CoQ10 in these proteins; and K0, K1, K2 and Kel are the rate constants in the respective steps. Plasma concentration of CoQ10 represents the sum of the concentrations in compartments X1 and X3.

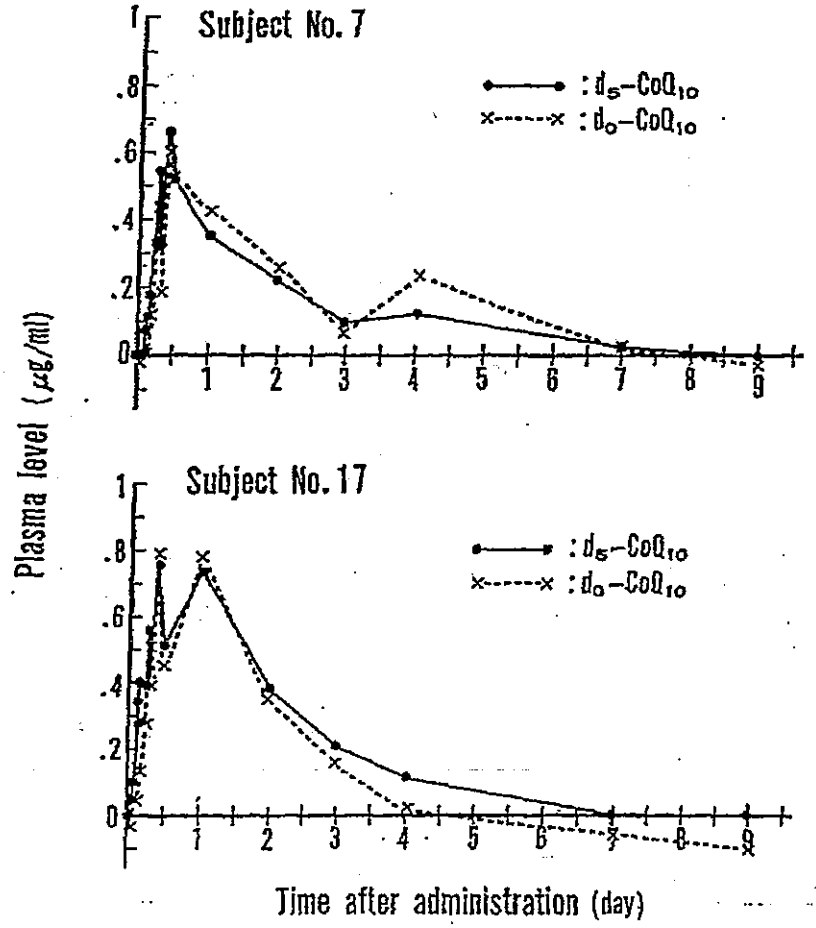


Fig. 5 Time course of plasma concentrations of d_5-CoQ10 (solid line) and d_0-CoQ10 (dotted line) after oral administration of 3 g of powder preparation containing 100 mg/g of d_5-CoQ10 and three CoQ10 capsules containing 100 mg/capsule of d_0-CoQ10.

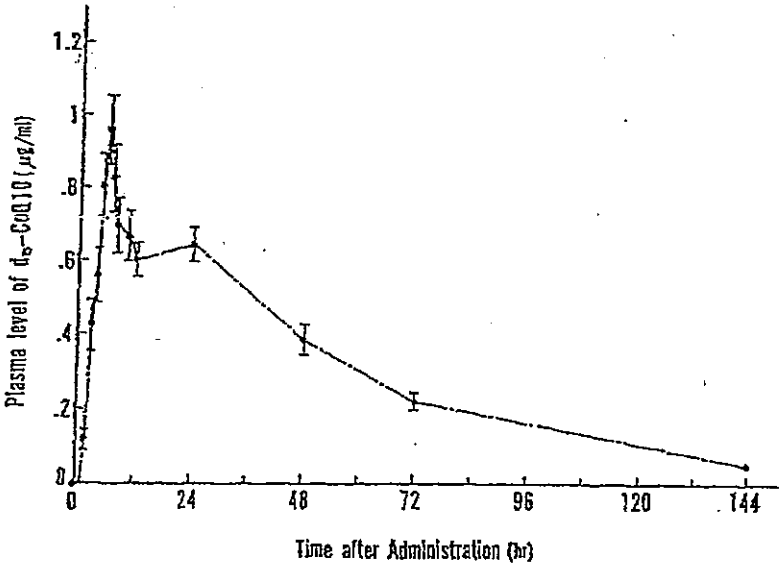


Fig. 6 Time course of plasma concentration of d_5-CoQ10 after oral administration of 100 mg of d_5-CoQ10. Each point represents the mean ± SE of 16 subjects.

Table 1 Bioavailability parameters of d_5-CoQ10.

Subject No.	C_{max} (μg/ml)	T_{max} (h)	Kel (1/h)	$t_{1/2}$ (h)	AUC (μg/h/ml)	CL[a] (l/h)
1	0.609	6.0	0.022	31.68	29.57	3.38
2	1.178	7.0	0.019	37.45	70.67	1.42
3	0.798 (0.827)[b]	6.0 (24.0)[b]	0.025	28.22	50.35	1.99
4	0.787	6.0	0.029	23.84	42.53	2.35
5	0.561	5.0	0.021	33.12	18.99	5.27
6	1.195	6.0	0.020	34.05	49.10	2.04
7	1.379	10.0	0.017	40.18	60.84	1.64
8	1.742	6.0	0.026	27.07	55.66	1.80
9	1.106	10.0	0.017	40.67	51.60	1.94
10	0.794	5.0	0.020	34.94	24.22	4.13
11	0.796	6.0	0.022	32.12	37.98	2.63
12	0.583 (0.650)[b]	8.0 (24.0)[b]	0.019	36.16	52.75	1.90
13	0.566 (0.566)[b]	6.0 (24.0)[b]	0.021	33.62	66.08	1.51
14	1.554	6.0	0.021	33.79	44.52	2.25
15	1.150	5.0	0.029	23.95	32.53	3.07
16	1.270	6.0	0.017	40.24	47.29	2.11
Mean	1.004	6.5	0.021	33.19	45.92	2.46
SD	0.370	1.5	0.004	5.32	14.53	1.04

[a] Apparent clearances obtained by assuming 100% absorption. [b] The concentration and time of the second peak.

Results

Study 1

The plasma levels of exogenous CoQ10 and d_5-CoQ10 are shown in Figure 5. The increase in plasma concentration of CoQ10 due to absorption of exogenous CoQ10 was calculated by subtracting the plasma level of CoQ10 before administration from the plasma concentrations after administration. As is clear from this figure, the time course of plasma concentration of d_5-CoQ10 was the same as that of exogenous CoQ10. This result makes the existence of an isotope effect in the disposition of d_5-CoQ10 unlikely.

Study 2

Plasma levels of d_5-CoQ10 and bioavailability parameters

The mean plasma concentration of d_5-CoQ10 in 16 subjects and the bioavailability parameter obtained in each subject are shown in Figure 6 and Table 1, respectively. Each subject showed a sharp peak at 5 to

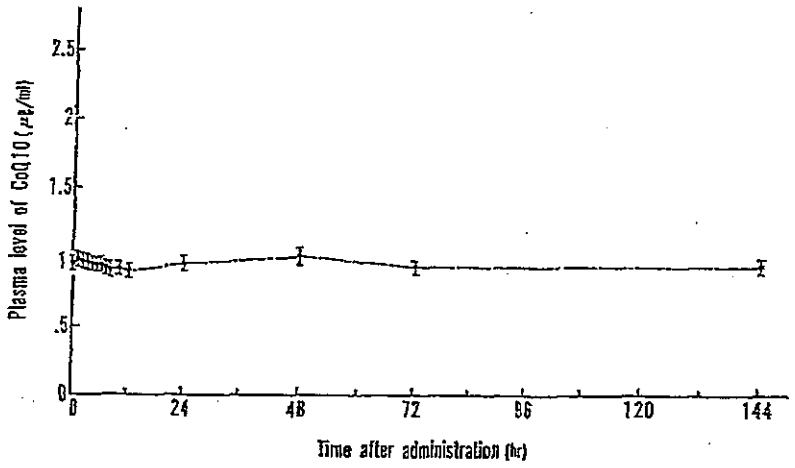


Fig. 7 Effect of administered d_5-CoQ10 on the plasma level of endogenous CoQ10. Each point represents the mean ± SE of 16 subjects.

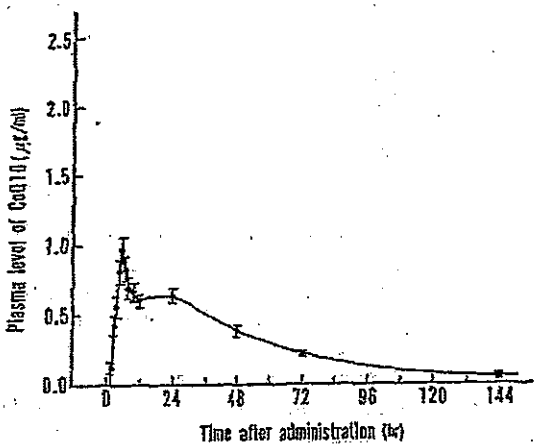


Fig. 8 Computer-fitted curve for the plasma concentration of d_5-CoQ10. Each point represents the mean ± SE of 16 subjects. The solid curve represents the nonlinear least-squares regression fit to the experimental data.

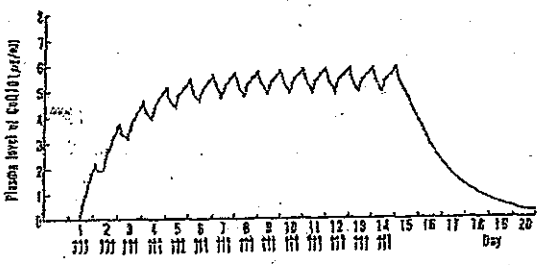


Fig. 9 Predicted plasma concentration of CoQ10 in subjects on a multiple dosing schedule (100 mg t.i.d. at 9:00, 14:00 and 19:00). The pharmacokinetic parameters shown in Table 2 were used for the simulation.

Table 2 Pharmacokinetic parameters of d_5-CoQ10.

Parameter		Obtained value
T	(h)	6.213
K1	(1/h)	0.338
K2	(1/h)	0.199
Kel	(1/h)	0.023
K0/V1	(g/ml h)	0.365
K0/V3	(g/ml h)	0.193
Lag	(h)	1.654
$t_{1/2}$ K3	(h)	2.05
$t_{1/2}$ K2	(h)	3.47
$t_{1/2}$ Kel	(h)	30.15

T: time after administration when absorption ceases; Lag: lag time of absorption; V1: volume of distribution of compartment one; V3: volume of distribution of compartment three; These parameters were obtained by computer fitting of the mean concentration of 16 subjects using the simplex method.

10 h after dosing, and the mean values of C_{max} and T_{max} were 1.004 ± 0.37 μg/ml and 6.5 ± 1.5 h, respectively. However, most of the subjects also gave a wide second peak at 24 h after dosing, and in three of them, the second peak was higher than the first peak. The C_{max} and T_{max} of second peak in these subjects are also presented in Table 1. Because of lack of information about the extent of absorbed CoQ10 after oral dosing, true plasma clearance cannot be calculated and the clearance values shown in Table 1 are the apparent clearances which were obtained by assuming 100% absorption. Since actual plasma clearance should be equal to or less than these values, it can be concluded that CoQ10 is a drug with low clearance. Due to this low clearance, the plasma half-life was fairly long (mean ± SD, 33.19 ± 5.32 h).

Effect of exogenous d_5-CoQ10 on plasma level of endogenous CoQ10

Figure 7 shows the mean plasma levels of endogenous CoQ10 after oral administration of d_5-CoQ10. There was little effect of the administered dose on the plasma levels of endogenous CoQ10, and the mean value was nearly constant.

Computer analysis

Because of the marked hump 24 h after administration, simple compartment analysis was considered inapplicable in this case. We carried out the fitting of the data to a newly derived equation based upon the compartment model shown in Figure 4. The computer-fitted curve for the mean data after administration of 100 mg of d_5-CoQ10 is shown in Figure 8. The entire plasma concentration curve was well described by the proposed model.

Discussion

Although several reports on the pharmacokinetics of CoQ10 in man have appeared in the past decade, its pharmacokinetic profile is still not well understood. Since CoQ10 is an endogenous compound and about 0.5 to 1.0 μg/ml of CoQ10 is present in the plasma, it is very difficult to estimate precisely the pharmacokinetic parameters. In addition, CoQ10 shows an unusual plasma level curve after oral dosing, and this makes it difficult to analyze the data based on a simple compartment model.

In this study, d_5-CoQ10 was used to minimize the interference of endogenous CoQ10 with the study of the disposition of the CoQ10 absorbed from the gastro-intestinal tract. Although no appreciable change of endogenous plasma CoQ10 level was observed in the mean data, individual data showed diurnal variation to some extent. This observation

makes it feasible to utilize d_5-CoQ10 for the pharmacokinetic study of CoQ10 in man, especially in small doses. In order to extrapolate the results of a pharmacokinetic study carried out with a stable-isotope-labelled compound to an unlabelled compound, it is necessary to confirm the absence of isotope effects. Since the absence of any significant isotope effect was indeed confirmed, the pharmacokinetic profile of d_5-CoQ10 obtained in this study can be considered to represent that of CoQ10.

The mean T_{max} value of 6.5 h indicates slow absorption of CoQ10 from the gastro-intestinal tract; this value is similar to the previously reported value (5.7 h) [Lücker et al. 1984]. The slow absorption of CoQ10 can be understood as being due to its low water solubility and high molecular weight. The plasma half-life obtained (33.19 ± 5.32 h) was slightly shorter than that previously reported (50.57 ± 19.74 h) [Lücker et al. 1984].

It was suggested that the existence of enterohepatic recycling caused the increase in plasma CoQ10 levels 24 h after oral administration [Lücker et al. 1984]. However, enterohepatic recycling cannot explain why the second peak is sometimes higher than the first peak, as observed in the present study. Recently, Yuzuriha et al. [1984] showed that CoQ10 intravenously administered to guinea pigs was trapped by liver, then incorporated in VLDL, and finally secreted into the blood from the liver. They also observed a second peak of CoQ10 in plasma even in guinea pigs with a bile fistula. From these results, they suggested that the elevation of blood level from 2 to 8 h after administration resulted from the redistribution of administered CoQ10 from the liver to the blood.

Since it is likely that CoQ10 absorbed after oral administration to man is handled in a manner similar to that found in the guinea pig after intravenous administration, the compartment model shown in Figure 4 was used for the pharmacokinetic analysis of plasma CoQ10 in this study. The model could successfully describe the entire plasma concentration curve of CoQ10 (Figure 8). Although concrete experimental evidence in man is not available yet, the results of the compartment analysis support the proposed mechanism of occurrence of the second peak in man. The pharmacokinetic parameters obtained are presented in Table 2. The absorption rate constant (K0) was found to be better expressed by assuming a zero-order rate constant than by assuming a first-order kinetics, as was suggested by Gibaldi et al. 1982.

Figure 9 shows the simulated curve for multiple oral administration of CoQ10 based on the obtained pharmacokinetic parameters. Plasma concentration of CoQ10 can be expected to attain 90% of the steady-state level within 4 days after the commencement of administration. The mean steady-state level was estimated to be 5.4 μg/ml after 100 mg t.i.d. dosing, and this is about 4 to 7 times the level of endogenous CoQ10.

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Safety assessment of coenzyme Q10 (Kaneka Q10) in healthy subjects: A double-blind, randomized, placebo-controlled trial

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Abstract

The safety profile of Coenzyme Q10 (Kaneka Q10) at high doses for healthy subjects was assessed in a double-blind, randomized, placebo-controlled study. Kaneka Q10 in capsule form was taken for 4 weeks at doses of 300, 600, and 900 mg/day by a total of eighty-eight adult volunteers. No serious adverse events were observed in any group. Adverse events were reported in 16 volunteers with placebo, in 12 volunteers with the 300 mg dose, in 20 volunteers with the 600 mg, dose and in 16 volunteers with the 900 mg dose. The most commonly reported events included common cold symptoms and gastrointestinal effects such as abdominal pain and soft feces. These events exhibited no dose-dependency and were judged to have no relationship to Kaneka Q10. Changes observed in hematology, blood biochemistry, and urinalysis were not dose-related and were judged not to be clinically significant. The plasma CoQ10 concentration after 8-month withdrawal was almost the same as that before administration. These findings showed that Kaneka Q10 was well-tolerated and safe for healthy adults at intake of up to 900 mg/day.

Keywords: Coenzyme Q10; Ubiquinone; Safety; Clinical trial; Toxicity; Semi-acute toxicity

1. Introduction

Coenzyme Q10 (CoQ10) is a biological quinone compound that is widely found in living organisms including yeasts, plants, and animals. Many plants and animals, including humans, have CoQ10 mainly with 10 isoprenoid chains (Turunen et al., 2004).

CoQ10 is widely used as a dietary supplement. Two major physiological activities of it have been reported (Turunen et al., 2004). One is enhancement of mitochondrial activity related to the synthesis of ATP, and the other is antioxidant activity. The antioxidant activity appears with the reduced form (ubiquinol) only. The oxidized form (ubiquinone) may be reduced to ubiquinol enzymatically after absorption (Mohr et al., 1992).

CoQ10 is biosynthesized and concentrated in the heart, kidneys, liver, muscle, pancreas, and thyroid gland. The content of CoQ10 in organs decreases with age (Kalen et al., 1989). Although CoQ10 can be obtained from foods such as meat and fish, its content in them is very low (Weber et al., 1996). Therefore, some nutritionists have considered CoQ10 suitable as a dietary supplement.

CoQ10 has come to be widely used as a dietary supplement, and daily intake of it has increased in recent years. Safety assessment of dietary supplements is very important since consumers use them without doctor's advice. In such assessment, the impurity profiles of test ingredients should be determined. Minor components sometimes cause health problems. The impurity profiles of CoQ10 ingredients differ because of differences between ingredients in methods of

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production. For example, there are two major methods for production of CoQ10. One is fermentation using yeast or bacteria, while the other is chemical synthesis. The all-*trans* form of CoQ10 is the natural one and the *cis* form is not observed in nature. The fermentation method produces all-*trans* CoQ10 while chemical synthesis produces mixed *cis* and *trans* product. No information is available on the safety or biological activity of the *cis*-isomer. It should be noted these differences in impurity profile might affect the safety of ingredients.

Because Kaneka Q10 is produced by fermentation with yeast, it does not contain *cis*-isomer (all-*trans* type CoQ10). The content of CoQ10 in Kaneka Q10 is over 98%, with CoQ9 and CoQ11 as major impurities.

The safety of CoQ10 (Kaneka Q10) has been examined in several studies. Acute, single-dose toxicity tests showed the LD50 of Kaneka Q10 to be over 5000 mg/kg. Sub-acute toxicity (4 weeks) and chronic toxicity (52 weeks) tests were performed with rats. In particular, a 52-week study revealed no toxicity even at a dose of 1200 mg/kg/day (Williams et al., 1999), from which the acceptable daily intake (ADI) for adults weighing 50 kg was estimated to be 600 mg/day. It was also reported in clinical studies of patients with early Parkinson's disease (300, 600, and 1200 mg/day for 16 months) (Shults et al., 2002), Huntington's disease (600 mg/day for 30 months) (The Huntington Study Group, 2001), and heart diseases (50–150 mg/day for 3 months) (Baggio et al., 1994) that the frequency of side effects was almost equal to that in the control groups, indicating that the dosage levels examined were within the limits of tolerable intake. However, the safety of CoQ10 for healthy individuals has not been reported.

In this study, we assessed the safety and measured blood concentrations resulting from administration of large amounts of Kaneka Q10, up to 900 mg/day, to healthy adults for 4 weeks.

2. Subjects and methods

2.1. Subjects

A total of 55 healthy male volunteers (age: 20–60, weight: 50 kg or over) and 33 healthy female volunteers (age: 24–55, weight: 40 kg or over) were enrolled. Volunteers in the following categories were excluded: (i) Those who took medicines or supplements that contained CoQ10. (ii) Those who had serious diseases such as diabetes, liver disease, kidney disease, heart disease, etc. (iii) Those allergic to food or alcoholic. (iv) Those who had participated in other clinical tests in the past 3 months, or who were participating in other tests at the start of our clinical trial. (v) Those who were pregnant or likely to become pregnant. The procedures were approved by the Ethics Committee of Hara-Doi Hospital and carried out in accordance with the Helsinki Declaration of 1975 as revised in 1983.

2.2. Protocol

This study was performed in a double-blind, placebo-controlled manner. The subjects were randomized into four groups, by number, as follows: a placebo group (11 males and 11 females), a 300 mg/day group (11 males and 11 females), a 600 mg/day group (11 males and 11 females), and a 900 mg/day group (22 males and no females). Capsules contained 150 mg of Kaneka Q10. Each subject took three capsules (placebo and/or Kaneka Q10 capsules) twice a day, in the morning and in the evening after meals. A physical examination, hematological tests, serum chemistry examination, and urinalysis were performed before, after 4 weeks of administration, and at 2 weeks after withdrawal. The tested items are listed in Table 1.

Table 1
List of test items

Test	Items
Physical examination	Body weight, blood pressure, pulse, electrocardiogram, body temperature, medical examination by interview
Hematology	Leucocytes, basophils, eosinophils, lymphocytes, monocytes, neutrophils, erythrocytes, hemoglobin, hematocrit, platelets
Blood biochemistry	Total protein, albumin, A/G, AST(GOT), ALT(GPT), LDH, ALP, γ-GTP, total bilirubin, creatinine, urea nitrogen, uric acid, CPK, TC, HPL-C, LDL-C, TG, Na, K, Cl, glucose, HbA1c, fructosamine, lactic acid, insulin, glyco-albumin
Urinalysis	pH, protein, glucose, occult blood, bilirubin, urobilinogen

Adverse events were defined as unfavorable medical events (subjective symptoms, objective symptoms, or unfavorable abnormal clinical data) that the volunteers commented on after taking the prescribed material, regardless of whether they appeared related to the test material. In the case of an adverse event, the volunteer was treated as required, and the symptoms and findings were recorded. The degree of severity was judged by physicians to be mild, moderate, or severe.

For each event, a relevance rating (plausibly related, possibly related, uncertain relationship, unrelated) was assigned according to the following criteria by physicians—plausibly related: the source of the adverse event was probably the test material, as judged by the recognition of a clear temporal relationship to the administration of the test material. Possibly related: although a clear temporal relationship to administration was recognized, the possibility that the source of the adverse event could have been something other than the test material remained. Uncertain relationship: no clear temporal relationship to administration of the test material was found, and it appeared likely that the source of the adverse event was something other than the test material. Unrelated: a source of the adverse event other than the test material could be clearly identified.

2.3. Composition of Kaneka Q10 capsules

The capsule used in this study was provided by Kaneka Corporation. Each capsule contained Kaneka Q10 150 mg, lecithin SLP-PasteNGS (Tsuji Oil Mill) 0.6 mg, safflower oil (Rinoru Oil Mills) 202 mg, Poem S-100 V (Riken Vitamin) 0.9 mg, and yellow beeswax (Miki Chemical Industry) 6.5 mg. The placebo capsule contained safflower oil instead of CoQ10. The purity of Kaneka Q10 was over 98%, and the main impurities were CoQ9 and CoQ11. Kaneka Q10 did not contain *cis*-isomer of CoQ10.

2.4. Determination of plasma CoQ10 concentration

A 5 ml blood sample was collected the day before the start of the test, at 2 days, 2 weeks, and 4 weeks after the start, and at 2 weeks after the termination of intake, in fasted (overnight) condition. To estimate the long-term effects of oral intake on CoQ10 biosynthesis, plasma CoQ10 concentration was determined for 11 male subjects in the 900 mg/day group and for 7 females in the 600 mg/day group at 8 months after withdrawal following 4-week administration. Other subjects were rejected because of they took CoQ10 supplement in this periods.

The collected blood samples were immediately centrifuged at 2500–3000 rpm for 10 min. The plasma was immediately frozen with dry ice and stored at −70 °C until testing. The concentration of plasma CoQ10

was calculated by summing the contents of CoQ10 in reduced form (ubiquinol) and oxidized form (ubiquinone), each of which was measured by HPLC. The method used for determination was as described in Yamashita and Yamamoto (1997) with minor modifications. A 0.2 ml portion of plasma was added to 0.8 ml of isopropyl alcohol (iPA) to extract CoQ10. After centrifugation (12,000 rpm × 3 min), 0.04 ml of iPA phase was injected into HPLC. The HPLC system was a Nanospace SI-2 (Shiseido) including an electrochemical detector. The mobile phase was methanol:isopropyl alcohol (90:10) containing 50 mM sodium perchlorate. The retention times of ubiquinol and ubiquinone were 15 and 21 min, respectively.

2.5. Statistical analysis

For statistical analysis of data, we performed Student's t test for test items before starting administration in each group, and the χ^2 test for subjective symptoms in the placebo group.

3. Results

3.1. Subjects

A total of 88 volunteers were enrolled in the test: 11 males and 11 females in the placebo group, 300 mg group, and 600 mg group, and 22 male volunteers in the 900 mg group. The following subjects were excluded from analysis: one male in the placebo group with exanthema, one male in the placebo group with fever and headache, and one female in the 300 mg group with urticaria. One male in the placebo group had acute colitis. Although his rate of test substance intake was 76.8%, his data were included in the analysis. The compliance rate of the other subjects exceeded 80%. The data for 20 subjects in the placebo group, 21 in the 300 mg group, 22 in the 600 mg group, and 22 in the 900 mg group were used for analysis.

3.2. Physical examination

Body weight and diastolic blood pressure are summarized in Table 2. Significant but small changes were observed in weight in the 300 mg group and diastolic pressure in the placebo group, but were not clinically significant. No significant change in systolic pressure, pulse, or body temperature was observed. No electrocardiographic abnormalities were observed. A statistically significant difference in systolic pressure was noted in the placebo group, but was considered a random event.

3.3. Laboratory tests

The results of hematology test are summarized in Table 3. On between-group analyses of number of erythrocytes, hemoglobin, and hematocrit, significant but small changes were observed, but were deemed not to be of clinical importance. No significant change in white blood cell count or platelet count was observed in any group.

The results of serum chemistry examination are summarized in Table 4. No significant differences were observed among groups in the 13 items, but small changes within the ranges of standard values were noted.

The results of urinalysis are summarized in Table 5. Only in urine pH was a significant, though small, change within the standard value range observed, but it was deemed not to be of clinical importance. No significant changes were observed in any of the other items.

3.4. Subjective symptoms

Numbers of subjective symptoms are listed by classification in Table 6 and by relevance rating in Table 7. The following subjective symptoms were reported in the placebo group (10 subject, 7 males and 3 females): common cold, loose stool, stomachache, nausea, headache, fever, slight fever, exanthema, and enterocolitis. Subjective symptoms and their relationship to the test material were: 1 case possibly related (enterocolitis), 8 cases with uncertain relationship, and 7 cases unrelated.

The following subjective symptoms were reported in the 300 mg group (8 subjects, 3 males and 5 females): common cold, pharyngitis, fever, light-headed feeling, headache, fatigue, lumbar pain, loose stool, diarrhea, vomiting, urticaria, and gingival pain. Relevances of symptoms to the test material were: 10 cases with uncertain relationship and 2 cases unrelated.

The following subjective symptoms were reported in the 600 mg group (11 subjects, 5 males and 6 females):

Table 2
Summary of physical examination

Items	Dose (mg/day)	n	Before intake	4 weeks after intake	2 weeks after termination
Weight (kg)	Placebo	20	61.9 ± 2.7	62.0 ± 2.8	61.9 ± 2.8
	300	21	61.1 ± 2.6	61.1 ± 2.6	60.6 ± 2.5[a]
	600	22	59.7 ± 2.0	59.4 ± 2.1	59.4 ± 2.0
	900	22	68.1 ± 2.2	68.0 ± 2.2	67.9 ± 2.1
Diastolic pressure (mmHg)	Placebo	20	83.7 ± 2.1	79.4 ± 1.9[a]	80.6 ± 1.9
	300	21	81.7 ± 1.8	79.6 ± 1.2	78.3 ± 1.7
	600	22	83.6 ± 1.4	82.3 ± 1.0	83.5 ± 1.9
	900	22	84.6 ± 2.0	84.6 ± 1.3	82.6 ± 1.9

[a] $p < 0.05$ vs. before intake (Student t test).

Table 3
Summary of hematology

Items	Dose (mg/day)	n	Before intake	4 weeks after intake	2 weeks after termination
Hematocrit (%)	Placebo	20	43.02 ± 0.96	42.79 ± 1.12	42.84 ± 1.12
	300	21	43.84 ± 1.04	42.83 ± 1.02[a]	43.16 ± 0.92
	600	22	43.91 ± 1.03	42.80 ± 0.86[b]	42.40 ± 0.94[a]
	900	22	45.83 ± 0.56	45.18 ± 0.48	45.15 ± 0.45
Eosinophils (%)	Placebo	20	3.29 ± 0.38	3.29 ± 0.45	3.91 ± 0.46
	300	21	3.03 ± 0.45	3.40 ± 0.37	3.31 ± 0.49
	600	22	3.55 ± 0.38	3.14 ± 0.37	3.32 ± 0.36
	900	22	2.85 ± 0.38	3.53 ± 0.55[a]	3.17 ± 0.48
Erythrocytes ($10^4/\mu L$)	Placebo	20	468.3 ± 9.2	468.7 ± 10.6	469.5 ± 10.1
	300	21	467.3 ± 8.9	459.5 ± 8.2	463.7 ± 7.3
	600	22	470.6 ± 9.1	464.2 ± 8.2	460.4 ± 8.8[a]
	900	22	484.8 ± 9.7	481.3 ± 8.6	482.4 ± 7.9
Hemoglobin (g/dL)	Placebo	20	14.14 ± 0.39	14.16 ± 0.42	14.17 ± 0.42
	300	21	14.20 ± 0.42	13.95 ± 0.41	14.14 ± 0.39
	600	22	14.30 ± 0.40	14.10 ± 0.34	14.00 ± 0.37[a]
	900	22	15.23 ± 0.20	15.15 ± 0.17	15.16 ± 0.15

[a] $p < 0.05$ vs. before intake (Student t test).
[b] $p < 0.01$ vs. before intake (Student t test).

cold, common cold, cough, stomatitis, pharyngitis, slight fever, stomachache, spasms, diarrhea, anorexia, urticaria, edema of the lower extremities, and chalazion on the right eyelid. Relevances of symptoms to the test material were: 9 cases with uncertain relationship and 11 cases unrelated.

The following subjective symptoms were reported in the 900 mg group (13 subjects): cold, common cold, sniffling, sore throat, lumbar pain, erosive gastritis, stomachache, headache, feeling of abdominal heaviness, and vomiting. Relevances of symptoms to the test material were: 4 cases with uncertain relationship and 12 cases unrelated.

There were no significant differences in frequency between the placebo group and Kaneka Q10 intake groups in subjective symptoms. There were no differences by sex in any subjective symptom, either. Many subjective symptoms were reported by multiple subjects, though only a few subjects had multiple subjective symptoms (Table 7).

3.5. Plasma concentrations of CoQ10

The graph in Fig. 1 represents plasma concentrations of CoQ10 throughout the test period. In the Kaneka Q10 intake groups, total plasma CoQ10 concentration reached a maximum in 2 weeks, after which it remained stable to 4 weeks. By 2 weeks after withdrawal, the plasma CoQ10 concentration had decreased to basal level. The increase in concentration depended on the dose of Kaneka Q10. In a follow-up study of 11 males in the 900 mg group and 7 females in the 600 mg group at 8 months after withdrawal, the plasma CoQ10 concentration was almost the same as before administration (Table 8), suggesting that high doses of Kaneka Q10 have little effect on the biosynthesis of CoQ10 after withdrawal.

4. Discussion

In this clinical safety study, Kaneka Q10, the most widely used CoQ10 ingredient in the world, was used to examine the safety of high doses in a double-blind, controlled manner.

No clinically severe problems were observed on physical examination, hematology, serum chemistry examination, or urinalysis in this study. The most commonly found event was respiratory system disorder, in 24 cases. However, this may be associated with seasonal factors, as the study was performed in the winter season, when the subjects were prone to catch cold independently of ingestion of the test material. The finding of no significant difference in frequency of this event among the groups appears consistent with this.

Gastrointestinal system disorders were found in 24 cases, and may be attributed to the large content of oil in the test capsules. Since commercial capsules use oil as a base content because of the hydrophobicity of CoQ10, attention should be paid to the effects of component on the gastrointestinal system, especially when high doses of it are taken over a short period of time. No significant difference in number of subjects who had subjective symptoms was found among the groups. There were no differences by sex in subjective symptoms, either. Many subjective symptoms were reported in multiple subjects, though only a few subjects had multiple subjective symptoms. It may therefore be concluded that CoQ10 causes no medically problematic subjective symptoms.

In this safety study, we also measured plasma CoQ10 concentrations. The pharmacokinetics of CoQ10 has been previously reported (Bentinger et al., 2003; Tomono et al., 1986). Plasma CoQ10 level decreased rapidly and recovered to basal level within 1 week. Tomono et al. reported that the half-life

Table 4
Summary of serum chemistry examination

Items	Dose (mg/day)	n	Before intake	4 weeks after intake	2 weeks after termination
Total protein (g/dL)	Placebo	20	7.28±0.09	7.29±0.08	7.22±0.10
	300	21	7.18±0.07	7.00±0.08[a]	7.12±0.09
	600	22	7.31±0.06	7.25±0.08	7.14±0.08
	900	22	7.32±0.09	7.21±0.08	7.23±0.08[b]
Albumin (g/dL)	Placebo	20	4.56±0.04	4.53±0.05	4.49±0.06
	300	21	4.62±0.05	4.50±0.06[a]	4.55±0.05
	600	22	4.60±0.06	4.55±0.05	4.49±0.06[b]
	900	22	4.66±0.05	4.53±0.04[b]	4.55±0.05
A/G (g/dL)	Placebo	20	1.700±0.050	1.660±0.041	1.669±0.049
	300	21	1.822±0.049	1.821±0.052	1.801±0.051
	600	22	1.710±0.043	1.698±0.037	1.700±0.036
	900	22	1.770±0.042	1.701±0.036[b]	1.712±0.037
Urea nitrogen (mg/dL)	Placebo	20	12.46±0.61	11.32±0.48[a]	11.17±0.57[a]
	300	21	12.22±0.65	12.90±0.72	13.34±0.65[b]
	600	22	13.21±0.82	12.49±0.58	12.85±0.62
	900	22	13.17±0.58	13.06±0.54	13.57±0.57
Total cholesterol (mg/dL)	Placebo	20	200.4±6.7	207.3±7.7	205.5±8.1
	300	21	191.0±8.6	184.3±7.4	188.8±7.0
	600	22	191.3±7.1	188.1±6.3	187.0±5.8
	900	22	202.7±9.2	194.6±8.9[a]	200.5±9.5
HDL-cholesterol (mg/dL)	Placebo	20	65.2±4.00	64.3±3.5	62.9±3.6
	300	21	68.1±4.30	64.7±3.7	62.5±2.9[a]
	600	22	63.3±2.80	65.5±2.8	63.9±3.0
	900	22	60.5±2.70	60.4±2.9	60.1±3.1
Triglyceride (mg/dL)	Placebo	20	103.9±20.1	118.4±25.3	126.6±24.6
	300	21	83.1±8.8	141.0±56.0	142.9±43.7
	600	22	91.8±10.5	69.5±5.8[b]	80.6±8.5
	900	22	105.2±7.1	102.8±14.1	101.0±11.9
Na (mEq/L)	Placebo	20	141.9±0.4	141.2±0.4	141.0±0.4
	300	21	142.0±0.4	142.3±0.4	141.2±0.3
	600	22	142.0±0.4	142.2±0.4	141.6±0.5
	900	22	142.3±0.4	141.6±0.4	141.6±0.3[a]
Cl (mEq/L)	Placebo	20	104.8±0.5	104.1±0.5	103.7±0.4[a]
	300	21	104.7±0.4	105.1±0.5	103.7±0.5[a]
	600	22	103.9±0.5	104.9±0.5[a]	104.6±0.6
	900	22	103.9±0.5	104.4±0.5	104.0±0.4
Glucose (mg/dL)	Placebo	20	88.5±2.0	88.4±1.8	88.4±1.8
	300	21	88.7±1.8	89.3±1.4	87.5±2.1
	600	22	87.2±1.7	92.0±2.4[a]	88.5±1.8
	900	22	89.0±1.7	90.9±1.6	90.0±2.0
Fructosamine (μmol/L)	Placebo	20	243.2±2.6	244.2±4.1	242.4±5.0
	300	21	245.3±3.7	239.0±4.4[a]	243.0±4.6
	600	22	246.8±2.9	246.8±3.4	243.8±4.0
	900	22	248.5±3.5	243.1±2.7[b]	245.4±3.8
Lactic acid (mg/dL)	Placebo	20	8.87±0.89	10.45±1.13	10.93±0.90
	300	21	11.28±1.37	9.38±0.97	11.07±1.26
	600	22	10.96±1.08	11.43±1.23	11.46±0.79
	900	22	8.82±0.70	8.65±0.50	10.70±0.82[a]
Glyco-albumin (%)	Placebo	20	13.61±0.23	13.85±0.27[a]	13.90±0.23[b]
	300	21	13.90±0.20	14.08±0.21[a]	14.18±0.22[b]
	600	22	13.98±0.19	14.39±0.20[b]	14.41±0.20[b]
	900	22	13.89±0.18	14.06±0.20[a]	14.22±0.19[b]

[a] $p < 0.05$ vs. before administration (Student t test).
[b] $p < 0.01$ vs. before administration (Student t test).

Table 5
Summary of urinalysis

Items	Dose (mg/day)	n	Before intake	4 weeks after intake	2 weeks after termination
pH	Placebo	20	5.73 ± 0.12	5.80 ± 0.17	6.23 ± 0.16
	300	21	5.74 ± 0.18	5.76 ± 0.13	5.64 ± 0.12
	600	22	5.80 ± 0.13	5.41 ± 0.10[a]	5.57 ± 0.13
	900	22	5.84 ± 0.13	5.64 ± 0.11	5.75 ± 0.11

[a] $p < 0.01$ vs. before administration (Student t test).

Table 6
Numbers of subjective symptoms

	Number of subjective symptoms					
	Plausibly related	Possibly related	Total	Uncertain relationship	Unrelated	Total
Placebo	0	1	(1)	8	7	(15)
300 mg	0	0	(0)	10	2	(12)
600 mg	0	0	(0)	9	11	(20)
900 mg	0	0	(0)	4	12	(16)

Plausibly related: the source of the adverse event was probably the test material, as judged by the recognition of a clear temporal relationship to the administration of the test material. Possibly related: although a clear temporal relationship to administration was recognized, the possibility that the source of the adverse event could have been something other than the test material remained. Uncertain relationship: no clear temporal relationship to administration of the test material was found, and it appeared likely that the source of the adverse event was something other than the test material.

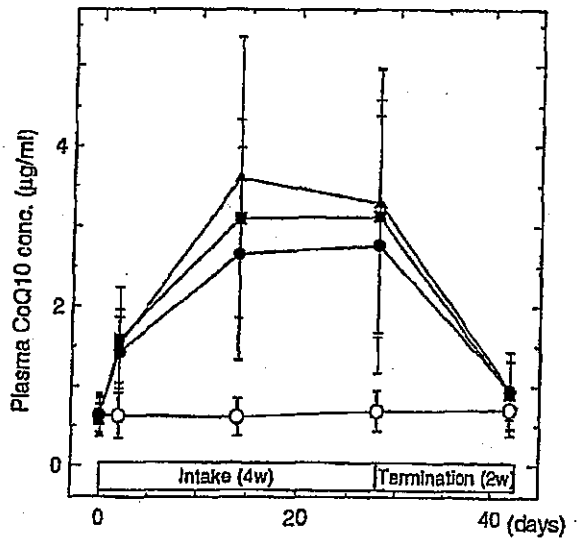

Fig. 1. Changes in plasma CoQ10 concentration during test periods. Values are mean ± SD in placebo (open circles), 300 mg/day (closed circles), 600 mg/day (closed squares), and 900 mg/day (closed triangles) groups.

Table 8
Comparison of plasma CoQ10 concentrations

	Plasma CoQ10 conc. (μg/ml)	
	Before intake	8 months after withdrawal
900 mg (male, $n = 11$)	0.59 ± 0.21 (100)	0.66 ± 0.16 (112)
600 mg (female, $n = 7$)	0.66 ± 0.27 (100)	0.62 ± 0.26 (94)

Values are means ± SD. Parentheses show percentages of plasma CoQ10 concentration before intake.

of CoQ10 in human plasma was 35 h. Bentinger et al. reported that the amounts of CoQ10 in organs decreased in time-dependent fashion, suggesting that CoQ10 does not accumulate in organ and plasma. However, the dose of CoQ10 in our study was much higher than in these pharmacokinetic studies. We therefore determined plasma concentrations of CoQ10. Plasma concentration increased dose-dependently and reached a plateau 2 weeks after initiation of intake. Plasma CoQ10 level remained stable during administration, but then decreased after withdrawal. These findings clearly show that CoQ10 did not accumulate in plasma. One of the attributes of the safety of Kaneka Q10 is this lack of accumulation.

The end products of enzyme reactions may have negative feedback effects. It is possible that administration of a large amount of CoQ10 decreased its endogenous biosynthesis. Correspondingly, had CoQ10 biosynthesis been affected by high-dose intake, plasma CoQ10 level would have been decreased compared with that before administration. Endogenous CoQ10 concentration was not affected by one time of d5-CoQ10 intake at dosing 100 mg (Tomono et al., 1986). It may be suggested that down regulation of CoQ10 biosynthesis was not occurred in short term of intake. In this study, it was shown that plasma CoQ10 concentration after 8-month withdrawal was almost the same as that before intake. This

Table 7
Classification of subjective symptoms

Symptoms	Number of subjective symptoms								
	Placebo		300mg		600mg		900mg		Total
	M	F	M	F	M	F	M	F	
Respiratory system	5	2	1	2	3	4	7	—	24
Gastrointestinal system	5	1	2	2	2	7	5	—	24
Body as whole-general	2	0	1	2	0	1	3	—	9
Skin and appendages	1	0	0	1	0	1	0	—	3
Central and peripheral nervous system	0	0	0	1	0	1	0	—	2
Musclo-skeletal system	0	0	0	0	0	0	1	—	1
Vision	0	0	0	0	1	0	0	—	1
Number of subjects	7	3	3	5	5	6	13	—	42

There was no significant difference in the frequency between placebo group and CoQ10-intake group (χ^2 test).

finding suggests that intake of Kaneka Q10 up to 900 mg/day did not affect CoQ10 biosynthesis over the long term.

Serum chemistry examination was performed to check the efficacy of Kaneka CoQ10. It was observed that plasma cholesterol level in the 900 mg group and triglyceride level in the 600 mg group were significantly decreased within normal range. However, these decreases did not exhibit clear dose-dependency. A significant decrease in fructosamine in the 300 and 900 mg groups was also observed, suggesting that blood glucose level was not maintained high after meals. The efficacy of CoQ10 in treating diabetes was reported by Kihara et al., who noted that the mechanism of efficacy of CoQ10 might be increase in both glucose metabolism and insulin secretion (Kihara et al., 1978). This hypothesis is supported by our observations.

In conclusion, these findings showed that Kaneka Q10 was well-tolerated and safe for healthy adults at intake of up to 900 mg/day. Intake of Kaneka Q10 for 4 weeks had no long-term effect on CoQ10 biosynthesis. These findings suggest that Kaneka Q10 can be used safely at dosages above.

Acknowledgments

We are grateful to Mr. Iizuka and Dr. Yamamoto (TTC Co., Ltd) for supporting this study.

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●Absorption improvement of CoQ10 by fine particles design

微粒子設計によるCoQ10の吸収改善

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はじめに

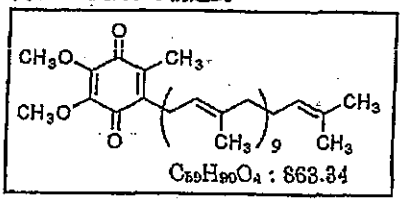
コエンザイムQ10（以下CoQ10と略記）（図1）は、日清製粉㈱（現・日清ファルマ㈱）が1960年代より工業化研究を行い、世界に先駆けて大量生産が可能となる工業化技術を確立した。CoQ10は、2001年の食薬区分改正により食品として、また、2004年に一般名ユビデカレノンが化粧品原料として認可され、機能性食品素材や化粧品原料として注目、期待されている[1-5]。

CoQ10の二大作用として、生体内での強力な「抗酸化作用」と、細胞の「エネルギー産生」を促進する作用が知られ、生体にとって必要不可欠な物質である。現在CoQ10の市場拡大が期待されているが、商品化にあたっては、CoQ10の物理化学的性質を考慮した製品設計が要望されている[6]。

CoQ10の吸収と微粒子設計

CoQ10は、融点が約48℃、黄色～だいだい色の結晶性の粉末である。脂溶性物質であり、水にはほとんど溶けない。また、光によって徐々に分解する。脂溶性であるCoQ10は、腸管で胆汁酸などにより乳化、ミセル化された後、リンパ管を経て肝臓でリポ蛋白に結合して細胞に運ばれる。従ってCoQ10の吸収は、胆汁酸によって乳化され吸収されるので食後に服用したほうが吸収されやすく、空腹時にはほとんど吸収されない。このことから、腸管で容易にミセル化されることが吸収改善の大きなポイントとなる。CoQ10に新しい機能性を付加し、空腹時など何時何処で摂取しても確実に吸収されるCoQ10素材を開発するため、微粒子設計技術を応用した水溶化CoQ10の開発を行った。

図1　CoQ10の構造式



次に脂溶性物質や難吸収性物質について、吸収メカニズムや吸収改善技術について述べる。

我々が摂取する動植物油脂の長鎖脂肪酸トリグリセリド（LCT）は、胃において脂肪乳を形成し、少しずつ十二指腸に移行する。腸に移行したLCTは膵リパーゼにより加水分解を受け、その後胆汁酸によって非常に小さいミセルを形成し、絨毛を通じて腸粘膜細胞に吸収される。その後トリグリセライドに再合成されてカイロミクロンを形成し、リンパ系に転送された後、血液に入ると考えられており複雑な消化吸収経路をもつ。中鎖脂肪酸トリグリセライド（MCT）は、LCTと異なり極めて単純である。膵リパーゼによってLCTよりも簡単に加水分解され、絨毛を経て腸粘膜に吸収される。このように油脂によって消化吸収が異なることが報告されている[7]。一般的に薬物の吸収は、薬物の投与前後に摂取される食事や、さらには製剤中の添加物によって影響される場合が多く、これらの摂取物と薬物との物理化学的相互作用や、摂取物の消化管の生理状態に影響を与えることも考えられるので十分注意、考察する必要がある。油に溶けた状態で投与されると、薬物は油から水溶液中へ分配し、水溶液から粘膜へと吸収されるものと考えられる。そのため親油性が高い油から水溶液に分配しにくく吸収が遅くなる。したがって脂溶性であるCoQ10も、微粒子設計技術を応用し、水溶化することにより生体内吸収性が向上できるのではないかと推察した[8]。例えば合成ホルモン剤ethynylestradiolは、水性懸濁液として投与した方が、ごま油溶液として投与したときよりも吸収速度が大きいことが確認されている。

医薬品の文献では、ラット（W、オス）に界面活性剤により可溶化した^{3}H-ubidecarenone 0.6mg/kgを経口投与したときの血中放射能濃度は、投与1時間後に未変化体換算として約60ng/mlの最高値を示した[9]。経口投与におけるubidecarenoneの吸収は、主としてリンパ系を介して行われる[10]。また、CoQ10製剤の投与剤形の違いによる人血漿CoQ10値におよぼす影響について、経口摂取されたCoQ10は、消化管内で胆汁酸塩などにより乳化され、小腸壁より吸収されてカイロミクロンに取り込まれリンパ系などを経て、全身循環に入る[11]。

一般論として、脂肪の吸収に関し、脂肪を精乳化し粒子径を0.1～0.2μmと静注用脂肪乳剤レベルまで微細とすることにより、消化管からの吸収が迅速となり消化吸収上有意義であることが判明したとの報告がある[12]。さらに、難水溶性薬物の製剤化には物理化学的性質の改善、特に溶解性の改善が強く望まれる。難水溶性薬物の溶解性改善には結晶多形、溶媒和物の利用、非晶質化、混合粉砕による微細化、表面改質による濡れ性改善、シクロデキストリンによる包接化、易溶性の塩形成など種々検討されている。難溶性薬物の吸収性改善に関し、一般的に粒子径を小さくすることが行われており、みかけ溶解速度は、Noyes-Whitneyの式によれば表面積に比例する。粒子径の細かいほうが表面積は大きく、溶解速度も速い。表面積は粒子径が小さくなればなるほど増大するので、見かけ溶解速度は粒子径によって大きく変わる。表面積が6倍にな

図2 「Aqua Q_{10} L10」の水分散時粒度分布

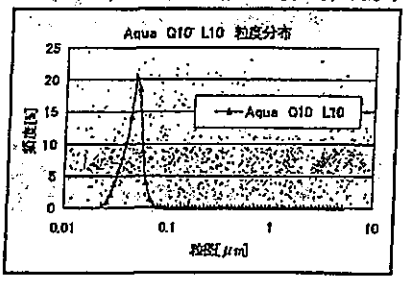
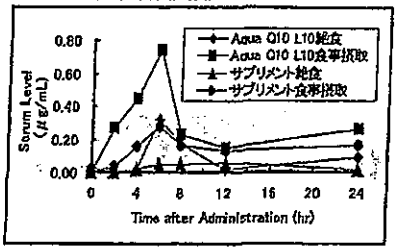


図3 「Aqua Q_{10} L10」摂取後の血漿中濃度変化



ると吸収は2.5倍になるといわれているが、老人や病人などのように胆汁やリパーゼの分泌が少なくかつ、胃及び腸の撹拌機能が弱くても、吸収を向上させるためには消化管中において微粒子とすることが必要である。

近年、難水溶化薬物の粒子径をナノメーター領域の粒子にまで微粒子化することにより、製剤の機能向上を狙ったナノ粒子化が注目されている。ナノメーターサイズの粒子はマイクロメーターサイズの粒子に比べてより消化管粘膜深部に侵入すると報告されている。難水溶性薬物はナノ粒子化することによって薬物粒子自身の溶解性が著しく向上するだけでなく、これら薬物を界面活性剤や高分子などの皮膜で被覆した微粒子キャリアに封入することによっても消化管の粘膜層に深く侵入でき、その部分で薬物を放出するので、吸収されにくい薬物の吸収改善が期待できると考えられる[13]。

CoQ10の水溶化技術と製品特性

CoQ10の水溶化を目的に、生物学的性質、物理化学的性質等のデータを基に、添加物の選定、製造条件などの検討を行ったが、特に粉末粒子の濡れ性改善と水分散時粒度の微細化が重要な課題であった。それらの課題に対し配合、製法などの研究を行い3種類の「水溶化CoQ_{10}素材」を開発した。新規開発した水溶化CoQ10素材は、多くの利点を有しておりこの特徴を生かした食品への展開が可能であり、飲料、タブレット、顆粒剤及び、各種食品にそのまま添加することができる。

「Aqua Q_{10} L10」は、CoQ10を10%含有する水溶化液で主に飲料などに配合が可能であり、水分散時の平均粒子径は約50nmとナノ粒子設計された可溶化液である。成人男子にCoQ10 60mgを単回経口投与後の血漿中濃度変化の推移を確認した結果、空腹時でも優れた吸収を示した。水分散時の粒度分布を図2に示し、ヒトでの吸収を確認した結果を図3に示す。

「Aqua Q_{10} P5」は、CoQ10を5%含有する水溶化粉末で、様々なサプリメントにそのまま添加可能で、水分散時の平均粒子径は約0.8μmとナノ粒子設計された粉末である。製法に関して特許第3549197号として登録されており、米国でも特許が成立している。この粉末は、安定性、水への分散性及び、吸収が良いことが特徴である。この粉末の水分散時の粒度分布を図4に示し、粉末の電子顕微鏡写真(写真1)及び、ヒトでの吸収を確認した結果を図5に示す。成人男子にてCoQ10 60mgを単回経口投与後の血漿中濃度変化の推移を確認し、CoQ10の吸収性を確認した結果、空腹時でも優れた吸収を示した。

この水溶化粉末は、基剤としてアラビアガムを用いているが、これは天然の水溶性ガムであり、分子量20万～50万で、ポリペプチドと多糖類部分のアラビノガラクタンが結合した構造をとっており、多糖類部分は複合多糖で塩類や塩基性のアミノ酸なども含まれている[14]。このためCoQ10の安定性を向上させるため有機酸を添加することにより安定性を高めることができた。

「Aqua Q_{10} P40」は、CoQ10を40%含有する高濃度水溶化粉末で様々なサプリメントにそのまま添加可能であり、水分散時の平均粒子径は約0.19μmとナノ粒子設計された粉末である。この水溶化粉末は、CoQ10の物理化学的性質の改質による高付加価値化を目指した粉末で、ナノ粒子化(粒子設計)、表面改質(濡れ性改善)及び、非晶質化により吸収性を改善した。いわゆる消化管粘膜層浸入及び、消化管内分散性を高め、ミセル化促進を改善した水溶化粉末である。また、粉末X線回折測定結果から、結晶形が非晶質化されており吸収性の改善が示唆された。粉体特性は、打錠時のトラブルいわゆるスティッキング低減や、タブレット表面の原末由来斑点低減も可能となり、流動性の良い打錠粉末が得られ、ハードカプセルの充填性にも優れている。タブレット表面の写真を写真2に示し、粉末X線回折測定結果を図6に示す。

この高濃度水溶化粉末を用いて、例えばCoQ10を高含有する小型タブレットの製品設計も可能となり、その他多くの一般食品への応用も可能である。また、優れた安定性と水への分散性及び、良好な吸収性が特徴である。特にタブレットを製造する場合、加工しやすい粉体物性であり又、空腹時でも優れた吸収を示すことが示唆されている。この粉末の水分散時粒度分布を図7、粉体物性値を表1に示し、粉末の電子顕微鏡写真を写真3に示す。成人男子にてCoQ10 60mgを単回経口投与後の血漿中濃度変化の推移を確認し、CoQ10の吸収を確認した結果、空腹時でも優れた吸収を示した。「Aqua Q_{10} P40」を用いた「水溶化CoQ_{10}(タブレッ

写真1 「Aqua Q_{10} P5」の電子顕微鏡写真

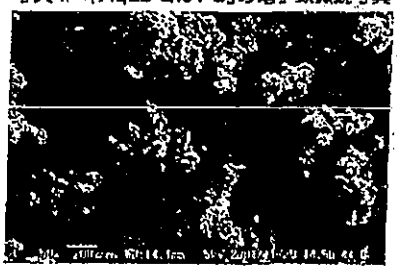


図4 「Aqua Q_{10} P5」の水分散時粒度分布

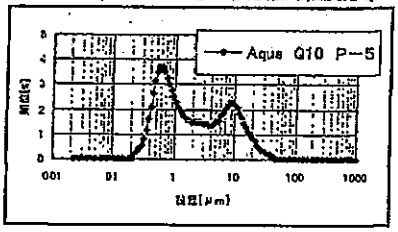


図5 「Aqua Q_{10} P5」摂取後の血漿中濃度変化

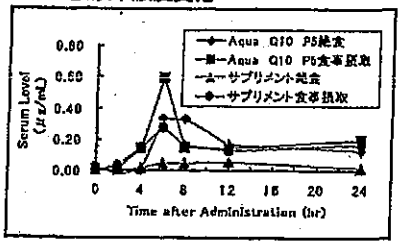


写真2　タブレット試作品写真

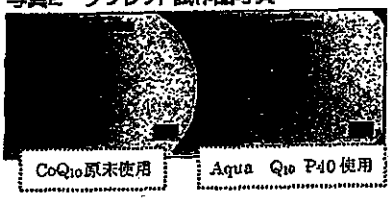
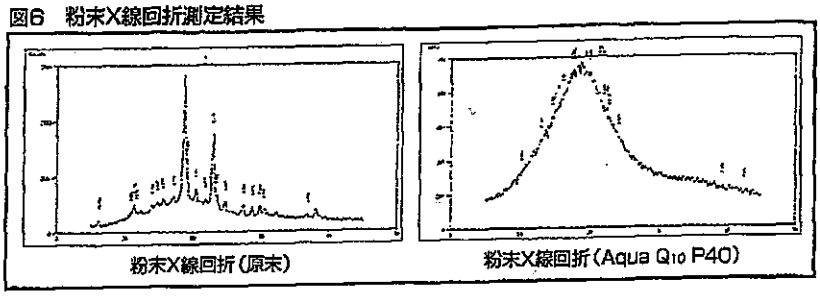


図6　粉末X線回折測定結果



ト）」と一般的な「脂溶性CoQ10（ソフトカプセル）」を用いてヒトでの吸収性を確認した結果を図8、図9に示す。

CoQ10水溶化技術に関しては、素材の生産技術や商品化技術に関して多くの特許出願を行っている。例えば、特許第3549197号で水溶化粉末の製造方法、また、特許第3618336号でCoQ10の安定化技術に関し特許査定となっている。この他、タブレット製造方法など、加工技術に関し特許出願を行っている。

まとめ

水溶化CoQ10を、水に分散させた場合直ぐに微粒子状態まで分散し、容易に腸管で胆汁酸などにより乳化、ミセル化されリンパ管を経て吸収される。微粒子設計により難吸収性であるCoQ10を空腹時でも確実に吸収させることが可能となった。生体内吸収性に関しては「Aqua Q10 L10」は、可溶化された乳化液の粒子径がナノオーダーであるため消化管粘膜に相溶しやすく、直ぐに胆汁酸などでミセル化し吸収されると考えられる。「Aqua Q10 P5」及び、「Aqua Q10 P40」は、水への溶解性・分散性が高い粉末を基剤として用いているため、水への分散・溶解性がよく、胆汁酸などで容易にミセル化され消化管粘膜層に深く進入し吸収しやすいと考えられる。高脂肪食と一緒に摂取しなくても吸収が良いため、食事の量が少ない方、低脂肪の食事をとられている方、カロリーコントロールをされている方、運動前に食事をコントロールされる方、仕事などで忙しい方及び、高齢者や高齢者で食事を制限されている方などに最適である。

「エネルギー産生の促進」「強力な抗酸化作用」の二大作用を十分発揮するため、水溶化CoQ10を利用した新しい機能性食品の開発が期待される。特にナノ粒子設計された水溶化CoQ10は、生体内への吸収が良いことから、いつ飲んでも吸収が確実に得られる新しい食品を提供することが可能である。バイオファクターとしての基礎研究も盛んに行われており、今後の新しい展開が期待されている[15,16]。

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表1　「Aqua Q10 P40」の粉体物性データ（実測値）

平均粒子径[μm]		見掛比重	見掛比重	圧縮度	安息角
水分散	粉末	(ゆるみ) [g/mL]	(固め) [g/mL]	[%]	[度]
0.19	59	0.32	0.50	36.3	50.1

図7　「Aqua Q10 P40」の水分散時粒度分布

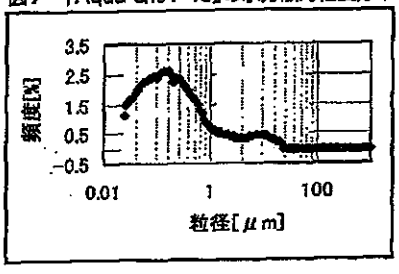

写真3　「Aqua Q10 P40」の電子顕微鏡写真

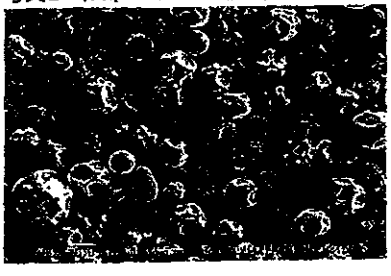

図8　脂溶性CoQ10と水溶化CoQ10の吸収性比較（血中濃度曲線0−12hr）

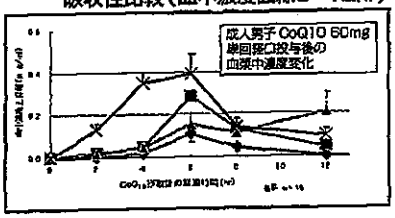

図9　脂溶性CoQ10と水溶化CoQ10の吸収性比較（AUC 0−12hr）