Total Coenzyme Q$_{10}$ Concentrations in Asian Men Following Multiple Oral 50-mg Doses Administered as Coenzyme Q$_{10}$ Sustained Release Tablets or Regular Tablets

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Coenzyme Q$_{10}$ (CoQ$_{10}$), a highly lipophilic compound present in the inner mitochondrial membrane, is essential for production of cellular energy in the form of ATP. CoQ$_{10}$ is used as a dietary supplement and for treatment of various cardiovascular disorders. Our goal was to compare the CoQ$_{10}$ levels in Asians following multiple oral doses administered as sustained release or regular tablets. Twenty healthy male volunteers (19—23 years old) were divided into two equal groups. Each subject in Group I received 50 mg oral doses of coenzyme Q$_{10}$ as sustained release tablets once a day for fifteen days, while subject in Group II received 50 mg doses of coenzyme Q$_{10}$ regular tablets. The CoQ$_{10}$ levels were measured by HPLC-UV (reverse phase ODS column, 10 µm, 250×4.6 mm; oven temperature 30 °C). Mobile phase was constituted by methanol–ethanol 9 : 1 v/v. Flow rate was 1.5 ml/min and UV detection was carried out at 275 nm. Coenzyme Q$_{10}$ was used as an internal standard. CoQ$_{10}$ baseline in the morning was 0.88±0.48 µg/ml. Following 1 week 50 mg/d dosing of CoQ$_{10}$, plasma CoQ$_{10}$ concentrations increased to 1.85±1.03 µg/ml for sustained release tablets and up to 1.37±0.74 µg/ml for regular tablets. The net increment proportion in AUC for sustained release and regular tablets were 148.26±176.56%, 102.57±130.00%, respectively. Both preparations significantly increased the systemic exposure when compared to endogenous baseline.

Key words Coenzyme Q$_{10}$; sustained release tablets; HPLC-UV; plasma concentration; Asian human healthy volunteer

MATERIALS AND METHODS

Materials Coenzyme Q$_{10}$, Coenzyme Q$_{6}$, Coenzyme Q$_{10}$ sustained release tablets (50 mg, Q112800), regular tablets (Enzymatic Therapy®, 50 mg, L9300) were supplied by AlphaRX Canada. Methanol (HPLC grade) was purchased commercially. Absolute ethanol and hexane were analytical grade reagents. All the containers were wrapped with aluminium foil to prevent from light exposure.

Subjects and Design Twenty healthy male volunteers (19—23 years old) were selected and a written informed consent agreement was made from every subject. Each subject was determined to be healthy and in good mental status through medical history, physical examination, electrocardiograph examination, and routine laboratory profiles such as hematoloy and blood chemistry. Cigarettes, alcohol, and other drugs were not allowed prior to one week and during the period of experiment.

The specified foods were served before 2 d and during the period of experiment, excluding the food such as animal fat meat and organs. In a randomized no crossover design, twenty subjects were equally divided into two groups. Each subject of Group I received 50 mg dose of coenzyme Q$_{10}$ sustained release tablets every day at 08:00 h for 15 d, and the subject of Group II received 50 mg dose of coenzyme Q$_{10}$ regular tablets every day at 08:00 h for 15 d. After 2 h of the oral administration, breakfast was served. Lunch and dinner were served at 12:00 h and 17:30 h, respectively. Blood samples (3 ml for each) were collected in heparinized tubes at 0, 2, 4, 6, 8, 12, 28, 36, 172, 180, 340, 348, and 412 h. The plasma was separated by centrifugation (3000 r/min for 5
Analytical Procedure  The HPLC system consisted of HP1100 pump, HP1100 variable absorbance detector, HP1100 column heater and HP3395 integrator (Hewlett-Packard Inc., U.S.A.). Column (HyperSIL ODS-2, 10 μm, 250×4.6 mm) was purchased from Dalian Elite Scientific Instrument Co. Ltd. HPLC method and sample processing were modified as described by Kaikonen J. et al. The mobile phase was methanol: ethanol (9:1, v/v). The flow rate was 1.5 ml/min and the column temperature was heated to 30 °C. The attenuation was 1 and the rate of record paper was 0.2 cm/min. UV detector was set at 275 nm. The plasma samples were processed as follows: frozen plasma samples were thawed at room temperature in the dark before analysis. Extractions were performed in 10 ml glass test tubes with stoppers. An aliquot of 50 μl internal standard (CoQ9, 24 mg/l in absolute ethanol) were added into 0.6 ml of plasma, and mixed for 10 s by a vortex mixer, and then the sample was deproteinized with 1.0 ml of methanol and mixed for 10 s by a vortex mixer. To this sample, 5 ml of hexane was added and mixed for 10 s by a vortex mixer and then oscillated for 10 min by an oscillator. The treated sample was statically placed in the dark for 10 min. 4.0 ml of hexane was transferred to a 10 ml glass test tube, and was dried at 25 °C water bath under nitrogen gas stream. The residue was dissolved by 100 μl of absolute ethanol and then 20 μl of the solution was injected onto HPLC system.

An aliquot of 50 μl internal standard (CoQ9, 24 mg/l in absolute ethanol) and 100 μl of CoQ10 solution (0.0, 1.2, 3.0, 6.0, 12.0, 18.0, 24.0 mg/l in absolute ethanol, respectively) were added to 0.6 ml of fresh plasma to prepare the calibration curves; same amount internal standard and 100 μl of CoQ10 solution (1.2, 6.0, 24.0 mg/l in absolute ethanol, respectively) were added to 0.6 ml of fresh plasma to measure absolute recovery and inter-day relative standard deviation (R.S.D.). Calibrators and quality control extraction and other procedures were as described above. Calibration curve was constructed in the concentration range of 0.2—4.0 mg/l. The peak-area ratio increments of CoQ10/CoQ9 were plotted as a function of the concentration of CoQ10. Where the peak-area ratio increment was the difference value of "peak-area ratio of total CoQ10/CoQ9" minus "peak-area ratio of endogenous CoQ10/CoQ9". The absolute recoveries of CoQ10 were estimated by comparison of the peak-area ratio increments (CoQ10/CoQ9) after extraction from plasma to the peak-area ratios (CoQ10/CoQ9) obtained after direct injection of a solution contained CoQ9 and CoQ10 in ethanol: hexane (1:6, v/v). The analysis was repeated five times at each level. Inter-day assay precision was determined by the analysis of the same concentration solutions on different days.

Pharmacokinetic Calculations  Partial area under concentration–time curve (AUCp) was calculated by trapezoidal rule. AUCp was divided into two parts: AUCp was estimated from time-point 0 to 36 h, and AUCp that was from 36 h to 412 h. AUCp (baseline AUC) was the product of concentration at zero time point multiplied by total sampling time, and AUCp (increment AUC) was the difference between AUCp and AUCp. AUC net increment proportion (fnet) was obtained from the quotient between AUCp and AUCp. Relative bioavailability (Fp) between two formulations of CoQ10 was obtained from quotient of mean AUCp between two products. Oral clearance including endogenous CoQ10 (Cl/F, Cl denoted the total clearance of CoQ10, from human body and F was absolute bioavailability) was estimated using the formula: Cl/F=Dose(T×Cp), where T is the dose interval and Cp was the steady state concentration at 348 h.

Statistic Analysis  Single factor ANOVA (analysis of variance) was used for analysis of AUC1, AUC2, AUC3, concentration dataset (Ci=CoQ10/CoQ9) after extraction from plasma to the peak-area ratio increment was the difference value of "peak-area ratio of endogenous CoQ10/CoQ9" minus "peak-area ratio of endogenous CoQ10/CoQ9". The paired t-test was used to compare AUCp with AUCp for increase in systemic exposure after oral administration of the two pharmaceutical preparations.

RESULTS

HPLC Method

Typical HPLC-UV chromatograms showed that the endogenous CoQ10 peak in fresh plasma appeared around at 7.9 min (Fig. 1A) and the retention time of internal standard CoQ9 was about at 6.4 min (Fig. 1B). Chromatograms in Figs. 1A and B were obtained from fresh plasma without or with supplement of CoQ9. Chromatogram in Fig. 1C was obtained from subject plasma that was supplemented with internal standard CoQ9.

The minimum concentration of CoQ10 that could be detected in plasma was 0.02 mg/l. This was determined based on signal to noise ratio of approximately 3. The calibration curve was ΔY=1.6115C−0.2414, where ΔY=the peak-area ratio increment and C=sample concentration (r=0.9986). The absolute recoveries of CoQ10 ranged from 94.0% to 3.2% to 97.8±6.2% as shown in Table 1, and the precision of intra-day and inter-day were shown Table 2. The R.S.D. values ranged from 4.2% to 10.5% for intra-day assay, and from 4.3% to 12.1% for inter-day assay.

Plasma Concentrations and Pharmacokinetic Parameters

The total coenzyme Q10 concentrations in the human plasma following multiple oral 50 mg doses administered as coenzyme Q10 sustained release tablets or regular ones were described in Table 3. The mean concentrations of CoQ10 (from 36 h to 412 h) following multiple oral administration sustained release tablets are slightly higher but not significantly (p>0.05), compared to those administered with regular tablets, as shown in Fig. 2. The pharmacokinetic parameters for both pharmaceutical preparations were listed as Table 4, consisting of AUCp, AUCp, AUCp, fnet, Cl/F and Fp. Results showed that the AUCp (42.5±17.35 h·mg/l) of sustained release tablet was close to that (417.8±7.14 h·mg/l) of regular tablet. From 36 h, mean AUCp (613.46±194.26 h·mg/l) was larger but not significantly (p>0.05) than that of regular tablets (477.68±94.39 h·mg/l), leading to an increase in the partial AUCp (655.96±208.78 vs. 519.45±97.50 h·mg/l). AUCp was significantly different from AUCp following oral administration of both products (p<0.01 for sustained release tablets, p<0.01 for regular tablets), and the results showed that both pharmaceutical preparations improved the CoQ10 levels. Both preparations increased the Net increment in AUC following oral administration of CoQ10 sustained release tablets (fnet=148.26±176.56%) or CoQ10 regular tablets (fnet =102.57±130.00%). Although the AUCp for sustained release tablets were larger than those of regular tablets, the difference was not statistically significant (p>0.05). The oral
clearances (Cl/F) between two pharmaceutical preparations were not significantly different (0.2284±0.3854 for sustained release tablets vs. 0.1506±0.0737 for regular tablets, p>0.05). The relative bioavailability (F_r) of CoQ10 sustained release tablets was 143.0%, compared with CoQ10 regular tablets.

DISSCUSSION

Coenzyme Q10 is almost insoluble in water, soluble in hot
ethanol and freely soluble in n-hexane. The determination method was established by improving the extraction recoveries.\(^5\) The uses of methanol to precipitate protein in plasma and n-hexane to extract the CoQ\(_{10}\) and CoQ\(_9\) improved the extraction efficiency. CoQ\(_{10}\) and the internal standard CoQ\(_9\) are unstable under the light, thus all the samples were wrapped with aluminium foil and treatments were carried out under the illumination of yellow light or in the dark. In addition, CoQ\(_{10}\) is also unstable at the high temperature. It is found that there are peaks of decomposed CoQ\(_{10}\) in water bath at 40°C, but stable at 25°C or at 30°C under nitrogen gas stream for drying. Temperature of analytical column can influence the chromatogram peak shape, column set at 30°C produced chromatograms of good resolution rate and excellent peak shape for CoQ\(_{10}\) and CoQ$_9$.

CoQ\(_{10}\) baseline in the morning was 0.88±0.48 mg/l (mean value, calculated from the both groups, range 0.21—1.81 mg/l). This is similar to the reported baseline range in European population (0.97±0.25, range 0.47—1.99 mg/l in men, and 0.84±0.22 range 0.24—1.92 mg/l in women\(^6\)). CoQ\(_{10}\) levels reach up to 1.85±1.03 mg/l following 1 week dosing (50 mg/d) of CoQ\(_{10}\) sustained release tablets and up to 1.37±0.74 mg/l following 1 week dosing (50 mg/d) of CoQ\(_{10}\) regular tablets. CoQ\(_{10}\) levels in the Europeans ranged from 0.5 to 2 μmol/l (0.4—1.7 mg/l) following single dosing of 100 mg, as described by Kaikonen J.\(^7\) and Weis M.\(^9\) It is already well known that plasma CoQ\(_{10}\) concentrations are highly dependent on serum lipoproteins (cholesterol levels), which are the carriers of CoQ\(_{10}\) in the circulation. CoQ\(_{10}\) concentrations include both endogenous and exogenous levels. Diet can influence the endogenous plasma CoQ\(_{10}\) levels because CoQ\(_{10}\) may be absorbed from the food. For humans, the major dietary sources of CoQ\(_{9}\) are meats, fish, and some vegetables. The motion, time of day, human race, age, gender, drug dosage forms may also influence the levels of CoQ\(_{10}\). The endogenous levels of CoQ\(_{10}\) in different human race may be different, due to the different diet, size, etc. Furthermore, the plasma CoQ\(_{10}\) levels are higher\(^6\) in men than in women.

Endogenous CoQ\(_{10}\) fluctuates at different time-points in the day and night. When a small amount of CoQ\(_{10}\) was administered to subject, the elimination phase may not so clear in the concentration–time curve, thus leading to difficulty for evaluation of exact elimination rate constant. Very high dosing of CoQ\(_{10}\) to humans is not suitable due to medical, ethical and legislation reasons. However, concentrations following multiple dosing can provide a clear profile of CoQ\(_{10}\) in plasma, which will provide a useful information for clinical practice. The concentration–time profiles for both formulations showed a slow rise in concentrations. This may be due to the rapid distribution of CoQ\(_{10}\) into cells and tissues and does not necessarily mean that CoQ\(_{10}\) was sparsely absorbed from gastric intestine tract. In humans, CoQ\(_{10}\) is found in relatively high concentrations in the heart, liver, kidney and pancreas. Total body content of CoQ\(_{10}\) has been estimated to be 0.5—1.5 g.\(^4\) The intracellular distribution of CoQ\(_{10}\) is described as follows: nucleus 25—30%; mitochondria 40—50% (inner mitochondria membrane); microsomal 15—20%; cytosolic 5—10%.

Following ingestion of 100 mg of CoQ\(_{10}\), peak plasma levels occur between 5 and 10 h, indicating a slow absorption from the gastrointestinal tract due to its high molecular weight and low water solubility. After the initial dose for both formulations, the $C_{\text{max}}$ (1.20 mg/l vs. 1.15 mg/l) and $T_{\text{max}}$ (6 h vs. 6 h) were very similar. This suggests that the sustained release did not significantly delay the absorption rate, and indicated that dissolution is probably the more important factor rather than release and the absorption rate like that reported\(^{4}\) seemed slow. Mean concentrations of CoQ\(_{10}\), following multiple oral administration sustained release tablets are higher but not significantly, than that after regular tablets. Similar results were obtained from comparison of AUCs, though higher mean values for sustained release tablets are found in $AUC_{\text{p}}$, $AUC_{\text{pr}}$, $AUC_{\text{cor}}$, compared to regular tablets, no significant differences were observed between the two pharmaceutical preparations. The paired $t$-test results demonstrated that both pharmaceutical preparations improved the systemic exposure of CoQ\(_{10}\) by comparing $AUC_{\text{p}}$ with $AUC_{\text{pr}}$.

In conclusion, the oral administration of both CoQ\(_{10}\) tablets improved the CoQ\(_{10}\) plasma levels and systemic exposure in Asian men. The morning baseline CoQ\(_{10}\) level of 0.88±0.48 mg/l in Asian men was similar to that in Europeans. Following 1 week 50 mg/d dosing of CoQ\(_{10}\), plasma CoQ\(_{10}\) concentrations increased to 1.85±1.03 mg/l for sustained release tablets and up to 1.37±0.74 mg/l for regular tablets. The net increment proportion in $AUC$ for sustained release and regular tablets are 148.26±176.56%, 102.57±130.00%, respectively. Although the sustained release preparation provided longer systemic exposure of CoQ\(_{10}\), the effect is not statistically significant compared to the regular preparation.

REFERENCES