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RESEARCH PAPER

Bioavailability Assessment of Oral Coenzyme Q10 Formulations in Dogs

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ABSTRACT

*The purpose of this investigation was to compare the bioavailability of three coenzyme Q10 (CoQ10) formulations in dogs using an open, randomized, multiple-dose crossover design. The formulations included a powder-filled capsule (*A*, control) and two soft gelatin formulations (Q-Gel® as the water-miscible form of CoQ10, B and Q-Nol™as the water-miscible form of ubiquinol, the reduced form of CoQ10, C). Formulations were evaluated in pairs, allowing a washout period of 14 days prior to crossing over. Blood samples were collected from each animal prior to dosing to determine the endogenous plasma CoQ10 concentrations. Serial blood samples were collected for 72 hr and plasma CoQ10 concentrations were determined by high-performance liquid chromatography. Plasma concentration–time profiles were corrected for endogenous CoQ10 concentrations. Results showed that the relative bioavailabilities of formulations B and C were approximately 3.6 and 6.2-fold higher than that of control formulation A. The AUC*(μg. hr/mL)±*SD*, C_{max}(μg/mL)±SD, *and* T_{max}(hr)± SD *for formulations A, B, and C were 1.695±0.06, 6.097±0.08, and 10.510±0.10; 0.096±0.035, 0.169±0.038, and 0.402±0.102*; *and 4.2±1.48,*

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4.1±1.57, and 4.5±0.58, respectively. While no significant differences were observed between T_{max} *values of the three formulations, the AUC and* C_{max} *values for formulations B and C were significantly higher than those of the control* ($p < 0.05$). *The present investigation demonstrates that soft gelatin capsules containing water-miscible CoQ10 formulations B (Q-Gel®) and C (Q-Nol™) are superior to powder-filled formulations with regard to their biopharmaceutical characteristics.*

Key Words: *Coenzyme Q10; Bioavailability; Soft gelatin formulation*

INTRODUCTION

Coenzyme Q10 (CoQ10), also known as ubiquinone, is an endogenous compound that exists in two redox forms, namely ubiquinone and ubiquinol. The predominant form in blood and most other tissues is ubiquinol, the reduced form of coenzyme Q10. Coenzyme Q10, in addition to playing a key role in the mitochondrial electron transport chain, is a critical coenzyme in the synthesis of adenosine triphosphate (ATP). It also serves as an important lipid-soluble antioxidant as well as a membrane stabilizer.

Because of its role in energy production, dietary supplements containing CoQ10 have been popular among health-conscious individuals and also among those with ailments directly or indirectly related to mitochondrial function. There are numerous reports documenting the beneficial effect of CoQ10 supplementation in heart disease and several other disorders.[1–3] Commercially available CoQ10 supplements are available as tablets, powder-filled capsules, and oil suspensions in soft gel capsules. Because CoQ10 is water-insoluble and has limited fat solubility, most powder-based formulations exhibit poor bioavailability. In order to improve the bioavailability of CoQ10, Chopra et al.[4–6] developed a hydrosoluble coenzyme Q10 soft gel formulation. This formulation exhibited superior bioavailability characteristics in human subjects when compared with tablets, powder-filled capsules, and soft gel capsules containing an oil suspension. Kommuru et al.[7] using a dog model confirmed this by showing that a simple oil-based suspension of CoQ10 did not improve the poor bioavailability of CoQ10 powder formulations. The same authors later demonstrated that the bioavailability of a self-emulsifying drug delivery system (SEDDS) was superior to that of a powder formulation.[8] In the present study, the bioavailability of two hydrosoluble liquid coenzyme Q10 formulations was compared with that of a powder formulation in dogs using an open, randomized, multiple-dose crossover design.

MATERIALS AND METHODS

Materials

Pure CoQ10 (standard) was kindly supplied by Tishcon (Westbury, NY). The internal standard CoQ9 was purchased from Sigma-Aldrich (St. Louis, MO). Sep-Pak silica (100 mg) solid-phase extraction cartridges were purchased from Waters (Milford, MA). All other chemicals and solvents were purchased from Fisher Scientific (Fair Lawn, NJ). The three CoQ10 formulations tested were labeled as A, B, and C, where A was a powder-filled capsules of CoQ10 available commercially (control), B was Q-Gel® Forte (hydrosoluble CoQ10) soft gelatin capsules, and C was Q-Nol™ (hydrosoluble ubiquinol, the reduced form of CoQ10) soft gelatin capsules (the latter two products were obtained from Tishcon Corp.), each containing 30 mg of CoQ10 per capsule. The hydrosoluble Q-Gel® and Q-Nol™ are produced by the patented Biosolv process which uses a solubilizer and an edible polyhydric alcohol to enhance dissolution. Dogs (coonhounds) were obtained from a local vendor of purpose-bred animals.

Bioavailability Study

The bioavailability of the three formulations of CoQ10 (A, B, and C) was compared in dogs. All the three formulations contained 30 mg of CoQ10 per capsule. The study was an open, randomized, multiple-dose crossover design. Since CoQ10 is poorly absorbed from gastrointestinal tract, it was necessary to administer multiple doses for a period of five days to raise baseline plasma concentrations to quantifiable levels and thus facilitate comparison of

the formulations. Formulations were evaluated in pairs (formulations A & B and A & C), where formulation A served as the control. Ten coonhounds, each weighing approximately 23 kg, were used for the study. All the formulations were administered as whole capsules and were not mixed with the food. Blood samples were collected from each animal prior to dosing to determine endogenous levels of CoQ10. Five dogs were assigned to each of the two groups, with those in the first group receiving formulation A and the other receiving formulation B. The CoQ10 formulations were administered twice daily (a.m. and p.m.) for four days. On day 5, following the administration of the first dose (a.m.), blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hr from the cephalic vein into heparinized tubes. Samples were stored on ice and protected from light until plasma was separated by centrifugation. Plasma samples were transferred into amber-colored vials and stored at -20°C until further analysis. Following a two-week washout period, the two groups were crossed over and the second pair of formulations (A & C), evaluated in a similar fashion. Plasma concentration–time profiles were corrected for endogenous levels of CoQ10, and areas under the curves (AUC) were calculated using the linear trapezoidal rule from zero to the last plasma concentration. Maximum plasma concentration, C_{max}, and the time of its occurrence, T_{max}, were compiled from the concentration–time data. Analysis of variance (ANOVA) and *t*-tests were performed to evaluate significant differences between the three formulations. Values are reported as mean±SD and the data were considered statistically significant at $p < 0.05$.

Plasma Sample Analysis

Plasma concentrations of CoQ10 were determined according to the method reported by Kommuru et al.[9] Frozen plasma samples were thawed in the dark just prior to analysis. Extractions were performed using $115 \times 10\,mm^2$ screw top borosilicate glass tubes. One milliliter of plasma was placed in a test tube along with 25 µL of internal standard (CoQ9, 4 mg/50 mL in hexane) and the contents vortex-mixed. Plasma was deproteinized with 1 mL of 5% trichloroacetic acid and vortex-mixed for 30 sec. Two milliliters of hexane were then added and the contents were vortex-mixed for an additional 5 min. Samples were then centrifuged at 3000 rpm for 5 min to separate the hexane layer. A 100-mg silica solid-phase extraction cartridge (wide mouth) was mounted on a vacuum manifold system, and activated with 5 mL of hexane. The hexane layer was allowed to pass, except for a small volume kept on the top of the silica. The hexane extract was then carefully transferred from the tube onto the silica cartridge. The extraction process was repeated twice (2×2 mL *n*-hexane), and all the hexane extractions (a total of 6.0 mL) collected on the silica extraction cartridge. Vacuum was applied for approximately 2 min until the cartridge was dry, and then eluted with 0.75 mL of methanol:*n*-hexane (85:15) mixture. The eluant was collected into a snap vial and a 100-µL aliquot injected onto the HPLC column. The samples were analyzed in duplicate.

Chromatography

A component high-performance liquid chromatography (HPLC) system (Waters Corp., Milford, MA) consisted of a model 510 solvent delivery system, model 484 tunable ultraviolet absorbance detector, and a model 715 Ultra Wisp sample processor. A Nova-Pak C18 (4 µm), 150×3.9 mm column (Waters Corp., Milford, MA) was used for the chromatographic analysis. A 7.5×4.6 mm guard column, Alpha Bond C18 (Alletch, CA) was used. A mobile phase of methanol:*n*-hexane 90:10 v/v was delivered at a flow rate of 1.5 mL/min, and monitored at 275 nm. Under these conditions, the retention times for CoQ10 and CoQ9 were approximately 7 and 5 min, respectively.

RESULTS AND DISCUSSION

The three CoQ10 formulations investigated in this study with dogs were powder-filled capsules as the reference product (A), the hydrosoluble liquid in soft gel capsules (B), and the hydrosoluble liquid containing the reduced form of CoQ10, i.e., ubiquinol, in soft gel capsules (C). The mean endogenous plasma CoQ10 in dogs prior to the oral administration of CoQ10 formulations was 0.21±0.07 µg/mL. The reported CoQ10 values have been corrected for the endogenous levels by subtracting them from the actual values at each time course measurement. Corrected plasma CoQ10 concentration vs. time profiles are shown in Fig. 1. The C_{max} (µg/mL) and T_{max} (hr) values (mean+SD) for the three formulations A, B, and C (Table 1) were respectively 0.096+0.345

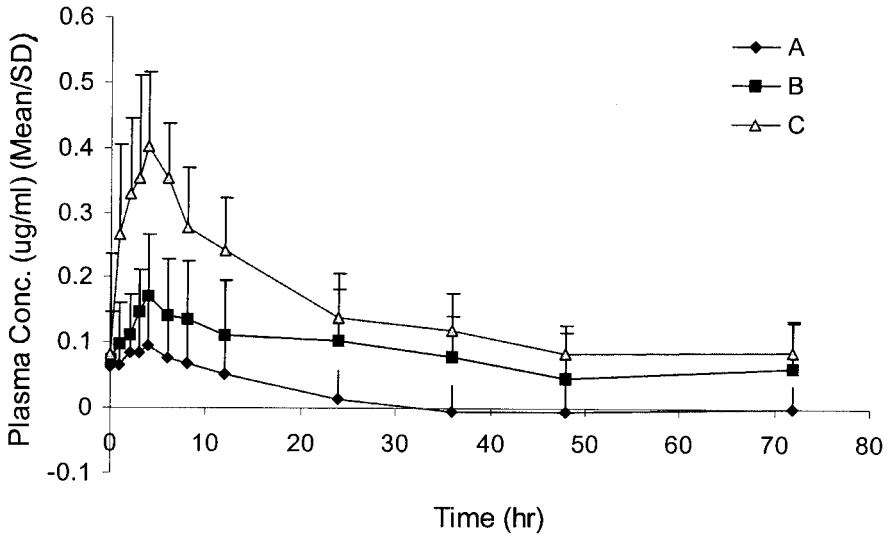

Figure 1. Mean plasma concentration and time profile of CoQ10 formulations: $n = 10$, except for the "zero" time point where $n = 8$.

Table 1

Pharmacokinetic Parameters of CoQ10 Formulations

Formulation	AUC Mean±SD (μg. hr/mL)	C_{max} Mean±SD (μg/mL)	T_{max} Mean±SD (hr)	Relative Bioavailability (%)
A	1.695±0.06	0.096±0.345	4.2±1.48	—
B	6.097±0.08	0.169±0.038	4.1±1.57	359.71
C	10.510±0.10	0.402±0.102	4.5±0.58	620.06

and 4.2+1.48, 0.169+0.038 and 4.1+1.57, and 0.402+0.102 and 4.5+0.58, respectively. While there was no difference in the T_{max} between the three formulations, the C_{max} values of formulations B and C were significantly higher than that of the reference product formulation A ($p<0.05$). The AUC values (mean+SD) were 1.695+0.06, 6.097+0.08, and 10.510+0.10 (Table 1), and the differences between the reference product A and the two hydrosoluble products B and C were highly significant ($p<0.05$) (Fig. 2). Furthermore, the relative bioavailability of formulation C containing the hydrosoluble ubiquinol was significantly higher than that of fomulation B containing the hydrosoluble CoQ10 ($p<0.05$).

The data from this study clearly indicate that the two hydrosoluble formulations B and C are superior to formulation A in their bioavailability, as shown by their plasma AUC values. Formulation A, the reference product used in this study, is a powder-based formulation in two-piece hard-shell capsules and it represents a typical commercially available product on the market. Formulation B is CoQ10 as a hydrosoluble liquid in soft gel capsules (Q-Gel®), and formulation C is the reduced form of CoQ10, i.e., ubiquinol, as a hydrosoluble liquid in soft gel capsules (Q-Nol™). In the USP dissolution test, formulation A showed zero dissolution whereas both formulations B and C showed 100% dissolution. This was clearly indicative of their superior in vivo bioavailability and is borne out by the data presented here. The data on formulation B (hydrosoluble CoQ10) is consistent with the previous findings with human subjects, where a comparison of powder-filled capsules, tablets, and soft gel capsules

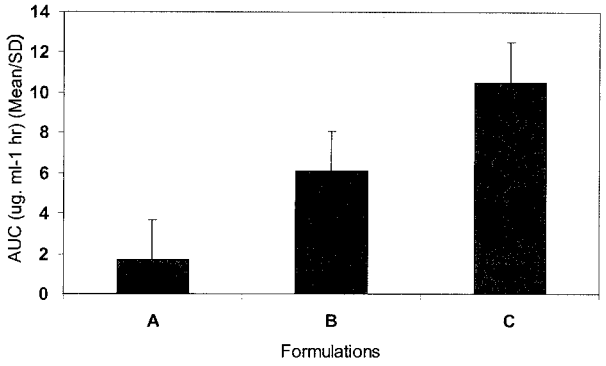

Figure 2. AUC of CoQ10 formulations in dogs (which were dosed 60 mg/day for four days and 30 mg on fifth day).

containing an oil suspension of CoQ10 with the hydrosoluble CoQ10 was made.[4–6] In this study, the hydrosoluble CoQ10 liquid formulation was found to be about threefold higher than any of the other three products. The superiority of the hydrosoluble ubiquinol over the hydrosoluble CoQ10 in the present study may be attributed to the fact that ubiquinol is slightly more polar than CoQ10. This finding is consistent with the findings of a human study by Chopra et al.[6]

There are only a few reports in the literature comparing the bioavailability of CoQ10 formulations. Weis et al.[10] conducted a study with human subjects comparing four products, i.e., a powder-filled capsule, and three soft gelatin capsules containing a suspension in soybean oil, one without and two with emulsifiers. This was a single-dose study with 100 mg, and the data showed that the soybean oil suspension without the emulsifiers was superior to the others, with a peak serum coenzyme Q10 value of about 1.3 μg/mL. Why the emulsifier did not improve bioavailability is hard to explain. In a long-term study, Folkers et al.[11] administered 90 mg of CoQ10 as a suspension in soybean oil and observed that blood concentrations reached a steady-state value of 2.03 μg/mL after three months. No further change was observed when the supplementation was continued to nine months. In a recent report, Kaikkonen et al.[12] compared CoQ10 as an oil suspension with a powder-filled capsule at a dose of 90 mg for two months (along with a placebo group) and found no difference in bioavailability between the two CoQ10 products. They also conducted a single-dose pharmacokinetic study using 30 mg of coenzyme Q10 and found the absorption to be slow and incomplete. In a controlled, single-dose study using dogs,[7] we showed that the absorption characteristics of CoQ10 in powder-filled capsules and oil suspensions were both slow and poor. This is consistent with the finding of Chopra et al.[4–6] All these studies demonstrate, despite vast differences in the experimental design, that the bioavailability of coenzyme Q10 in powder-filled capsules is very poor and suspensions in oil do not confer any significant improvement. One common feature shared by all these products, whether they are powders or suspensions in oil, is the large particle size. The poor bioavailability of these products could be attributed to their poor solubility in water and very limited solubility in fats, coupled with their large particle size. This is why powder-based products failed the USP dissolution test, whereas the hydrosoluble liquids B (CoQ10) and C (ubiquinol) showed 100% dissolution. Moreover, differences in dissolution were reflected in their relative bioavailabilities. The data from this study suggest that the superior bioavailability of hydrosoluble liquid CoQ10 formulations renders them the preferred dosage form for therapeutic usage, and that in vivo performance must be recognized as an important consideration in the development of nutritional products, especially for clinical applications.

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