Stability and Bioequivalence Studies of Two Marketed Formulations of Coenzyme Q₁₀ in Beagle Dogs

Thirumala R. KOMMURI, Mohammad ASHRAF, Mansoor A. KHAN, and Indra K. REDDY

Division of Basic Pharmaceutical Sciences, School of Pharmacy, College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe, LA-71209, U.S.A., Wyeth Ayerst Research, 7 Pearl River, NY-10965, U.S.A., and Texas Tech University Health Sciences Center at Amarillo, School of Pharmacy, Department of Pharmaceutical Sciences; 1300 Coulter, Amarillo, TX-79110, U.S.A. Received January 12, 1999; accepted April 13, 1999

Coenzyme Q₁₀ (CoQ₁₀), a highly lipophilic compound present in the inner mitochondrial membrane, is essential for production of cellular energy in the form of ATP. CoQ₁₀ is used as an antioxidant and also in the treatment of various cardiovascular disorders. The relative bioavailabilities of powder filled capsule (I) and oil-based formulation (II) of CoQ₁₀ were compared in beagle dogs in an open, randomized, multiple dose, cross-over design. Poor and slow absorption characteristics were observed for both the formulations. The AUC/Cₘₙₙₙ and Tₘₙₙₙ for formulation I and II are comparable (p<0.05) where the values for formulation I are 22.84±6.3 μg mL⁻¹ h, 0.51±0.11 μg/mL, and 6.1±2.0 h whereas the values for formulation II are 24.32±5.6 μg mL⁻¹ h, 0.55±0.16 μg/mL, and 6.6±2.3 h, respectively. Stability of CoQ₁₀ at various temperature and humidity conditions and its photostability were studied. Various antioxidants were evaluated to determine the type and amount of antioxidant(s) required to improve the stability of CoQ₁₀. Large extent of degradation was observed at 45°C and 55°C. The effect of humidity conditions on degradation was insignificant. Among the various antioxidants studied, mixture of ascorbic acid (5%) and EDTA (0.1%) offered better protection than phenolic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), or propyl gallate (PG). Further, increasing concentrations of phenolic antioxidants (from 0.1 to 0.3%) accelerated the degradation.

Key words coenzyme Q₁₀, stability; bioavailability; antioxidants

Coenzyme Q₁₀ (CoQ₁₀), also known as ubiquinone (Fig.1), is a highly lipophilic compound present in the inner mitochondrial membrane where it acts as an integral part of oxidative phosphorylation. CoQ₁₀ is essential for production of cellular energy in the form of ATP. CoQ₁₀ is recommended as an antioxidant and also in the treatment of various cardiovascular disorders such as angina pectoris, hypertension, and congestive heart failure. It is poorly and slowly absorbed from gastrointestinal tract due to its high molecular weight and poor aqueous solubility. Currently, CoQ₁₀ is available in the market as nutritional supplement in oil-based and powder filled capsule formulations. The oral bioavailability of these formulations, however, differ widely. Only a few studies have reported the comparisons of bioavailabilities of different CoQ₁₀ formulations. Kishi et al. have reported higher bioavailability for soyabean oil suspension of CoQ₁₀ compared to other formulations such as plain tablets, granules, and sugar-coated tablets. Weis et al. have reported that soyabean oil suspension of CoQ₁₀ without any emulsifiers had higher bioavailability compared to similar formulations containing polysorbate 80 and/or lecithin. However, Chopra et al. have reported higher bioavailability for the new solubilized formulation of CoQ₁₀ containing emulsifiers compared to the oil suspension and powder filled capsule formulations.

CoQ₁₀ is yellow or orange crystalline powder and upon exposure to light, it gradually decomposes and changes its color to dark yellow. Solution preparations of CoQ₁₀ are far more prone to photo degradation than in the solid state. Color darkening of the crystals was attributed to change in the surface condition in addition to the structural changes of CoQ₁₀ molecules. Further, these physicochemical changes affected the UV absorption properties of solid-state samples. Antioxidants are usually added to improve the stability of drug formulations. Antioxidants, when properly used before auto-oxidation sets in, are extremely effective in reducing the initial rate of peroxide build up and hence considerably extend the induction period of the oxidation cycle. The choice of antioxidant(s) will depend on the requirements of the system, i.e., high antioxidant potency at a minimal concentration. The amount of antioxidant needed is usually determined empirically. In many cases, the more antioxidant added, the more protection it offers; for others, acceleration of oxidation can occur at high antioxidant concentration. The objectives of the present study are (1) to compare the bioavailability of two marketed (powder filled capsule and an oil-based) formulations of CoQ₁₀ in beagle dogs; (2) to study the stability of CoQ₁₀ at various temperature, humidity, and light conditions; and (3) to determine the type and amount of antioxidant(s) required to maintain the stability of CoQ₁₀ during its shelf life.

Materials and Methods
1. Materials Standard CoQ₁₀ was purchased from Spectrum Chemicals (Gardena, CA). The internal standard, CoQ₉, was kindly supplied by Eisai Co., (Tokyo, Japan). Sep-Pak silica (100 mg) solid phase extraction cartridges were purchased from Waters (Milford, MA). Antioxidants were purchased from Fluka (Ronkonkoma, NY), and all other chemicals and solvents were purchased from Fisher Scientific (Fair Lawn, NJ).

2. Bioavailability Studies 2.1. Formulations: Two marketed formulations of CoQ₁₀ are selected. Formulation I is a powder filled capsule and Formulation II is an essential oil based solubilized formulation. Both the formu-
lations contained 30 mg of CoQ₁₀ per capsule.

2.2. Study Protocol: The study was open, randomized, multiple-dose, cross-over design. Since CoQ₁₀ is poorly absorbed from gastrointestinal tract, it is necessary to administer multiple doses for a few days to raise the plasma concentrations to quantifiable levels and also to facilitate the comparisons of the formulations. Eight male beagle dogs received each of the two formulations on separate occasions. In the first part of the study, blood samples were collected from all the animals prior to dosing to determine the endogenous levels of CoQ₁₀. Four dogs were randomly assigned to each of the two groups. Each dog in the first group received formulation I and the other group received formulation II twice a day at 8:00 a.m. and 8:00 p.m. for four days. On fifth day, following another dose at 8:00 a.m., blood samples were collected from jugular vein into heparinized tubes at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0, and 72.0 h time intervals. After centrifugation, plasma was separated into amber colored vials and stored at −20°C until further analysis. Following a seven-day wash out period, the two groups were crossed-over to carry out the second part of the study. The dosing and sampling procedure were the same for these two studies.

2.3. Analysis of Plasma Samples: Plasma concentrations of CoQ₁₀ were determined according to our earlier reported method. Since the solutions of CoQ₁₀ are susceptible to photodegradation, studies were carried out under the illumination of yellow light. All the containers used were wrapped with aluminum foil. To a milliliter of plasma, 50 µl of internal standard (CoQ₉, 7.5 mg/ml in hexane) were added and vortex mixed. The plasma was then deproteinized with 1.0 ml of 10% trichloroacetic acid. To this sample, 2 ml of hexane was added, vortex mixed for 5 min, and centrifuged at 2000×g for 5 min. The hexane phase was transferred to 100 mg silica-solid phase extraction cartridge, previously activated with 5 ml of hexane and mounted on vacuum manifold system. The extraction was repeated twice in similar fashion and the hexane layer was collected on silica cartridge. The total volume of hexane (6.0 ml) was passed through the cartridge. The cartridge was dried under vacuum for 2 min and eluted with 0.75 ml of methanol:hexane (85:15 v/v) mixture. A 100 µl volume of the eluted fraction was injected to the RP-HPLC column (C-18) for separation and UV detection.

2.4. Chromatography: The LC system consisted of Isco-2350 pump, isocratic variable absorbance detector. The elute was monitored for absorbance at 275 nm and the detector output was recorded using Isco-Chemsearch (Ver 2.4) Software. A sample of 100 µl volume was injected onto a reversed phase column (Nova-Pak C₁₈, 4 µ, 150×3.9 mm i.d., Waters, Milford, MA) preceded by a guard column (Alpha Bond C₁₈, 7.5×4.6 mm i.d., Alltech, San Jose, CA). The isocratic mobile phase was methanol: n-hexane (98:2 v/v). The flow rate was 1.0 ml·min⁻¹.

2.5. Data Analysis: The plasma concentration–time profile was corrected for endogenous levels of CoQ₁₀ as follows. For each animal, the respective endogenous levels of CoQ₁₀ at time 0 are subtracted from the observed CoQ₁₀ concentrations at each point. The area under the curve, AUC, was calculated by linear trapezoidal rule from zero to the last plasma concentration. The 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 two formulations. Values are reported mean±S.D. and the data were considered statistically significant at p<0.05.

3. Stability Studies: Approximately 750 mg of CoQ₁₀ was placed in each of several glass vials and were kept at various conditions such as 25°C, 37°C, 45°C, 55°C, 37°C–11% RH, 37°C–51% RH, and 37°C–91% RH. These vials were wrapped with aluminum foil to prevent exposure to light. Mixtures of CoQ₁₀ with various antioxidants were taken in transparent glass vials and were exposed to fluorescent light (600 FC) at 25°C in a light stability chamber (Model 9352643, HotPack Inc., Philadelphia, PA). Another set of samples of CoQ₁₀ with various antioxidants were kept at 55°C and these containers were wrapped in aluminum foil to prevent exposure to light. The antioxidants employed in these studies included ascorbic acid (AA), butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG), and ethylenediaminetetraacetic acid (EDTA).

3.1. Analysis of Stability Samples: Approximately 50 mg of CoQ₁₀ were accurately weighed into 100 ml volumetric flask, dissolved in n-hexane, and made up to volume with n-hexane. Three milliliters of the hexane solution was further diluted with methanol to 50 µl of the methanolic solution was injected into the HPLC. The chromatographic conditions are the same as described in section 2.4, except with a flow rate of 1.5 ml per minutes.

Results and Discussion

Bioavailability Studies: The endogenous plasma levels of CoQ₁₀ were determined in the animals of the two study groups and were found to be 0.20±0.06 and 0.22±0.05 µg/ml, respectively. The plasma concentration–time profiles of formulation I and II, calculated by subtracting the endogenous levels of CoQ₁₀ at each time point, are shown in Fig. 2. The pharmacokinetic parameters are summarized in Table 1. The AUC, C_max, and T_max values of formulation I and II are comparable (p<0.05) and therefore, the powder filled capsule and oil based formulation are bioequivalent. These results are in agreement with the study reported by Kaikonen et al. In which no significant differences were found between the bioavailabilities of granular and oil-based formulation in humans. Also, Bogentoff et al. have reported no significant differences in the bioavailabilities of solid dosage form (tablet) and soybean oil (soft gelatin capsule) formulation of CoQ₁₀. In a study using deuterium labeled CoQ₁₀ in humans, Tomono et al. have reported that CoQ₁₀ undergoes enteroheliepatic recycling and thereby increasing the plasma CoQ₁₀ levels 24 h after oral administration. In the present investigations, however, no such rise in plasma concentra-
tions was observed. These variations may be attributed to differences in analytical techniques and/or species differences.

Following an oral dose of 60 mg per day for four days, the rise in plasma concentrations from the endogenous levels for formulation I and II were 0.51±0.11 and 0.55±0.16 μg/ml, respectively. Such a small increase (2.5 fold from the base line) in plasma concentration reflects the poor absorption characteristics of CoQ₁₀ with a reported half-life of 34 h.  

Sannam et al. have reported similar results in a study with beagle dogs, where an increase in plasma concentrations of CoQ₁₀ from 0.4 to 1.7 μg/ml following oral administration of 10 mg/kg/d for four days was observed. Further, poor absorption characteristics were also reported in humans. Langsjoen et al. reported mean plasma levels of 1.99 μg/ml, increased from 0.85 μg/ml, in patients with congestive heart failure following oral administration of 100 mg/d for 3 months. In present studies, the T_max value was about 6 h and is in agreement with the reported value.  

Poor and slow absorption characteristic of CoQ₁₀ are attributed to its high molecular weight and high lipophilicity.  

Highly lipophilic compounds including CoQ₁₀ are shown to be absorbed mainly by the route of intestinal lymphatics. The absorption of these compounds depend on lipoprotein synthesis by the enterocyte. It is, therefore, advantageous to administer these drugs formulated in oils/lipids. In the present investigations, the oil based formulation (II) showed slightly higher, but statistically not significant, bioavailability than the powder filled capsule. Since surfactants/emulsifiers are absent in the oil-based formulation (II), it did not form fine emulsion upon dilution with water, which otherwise could have provided greater surface area for drug absorption. Incorporation of emulsifiers in the oil based formulations have been reported to improve the oral absorption of CoQ₁₀. However, in contrast, Weis et al. reported higher bioavailability with soyabean oil supension of CoQ₁₀ without any emulsifiers in comparison with similar formulations containing polysorbate 80 and/or lecithin. These observations, may be explained on the basis of formation of micelles or mixed micelles in the intestine with the formulations containing emulsifiers which subsequently decreased the solubilization with bile salts.  

Stability Studies Effect of Temperature and Humidity: Glass vials containing CoQ₁₀ were wrapped with aluminum foil and kept at various temperature and humidity conditions. The degradation profiles and the first-order rate constants are shown in Fig. 3. Degradation to a large extent was observed at 45 °C and 55 °C while it is relatively stable at 37 °C. Degradation rate constant at room temperature, calculated from the Arrhenius plot of log K vs. 1/T (not shown) was found to be 0.0014 month⁻¹. The predicted shelf life at room temperature (time to 90% potency) was 6.3 years. However, the effects of light, excipients, and formulation processing conditions were not considered in these observations which otherwise might have affected the degradation. Our preliminary studies indicated that some of the commercially available products are not stable during their shelf life (unpublished results). Humidity conditions (11% RH, 51% RH, 91% RH) at 37 °C did not cause significant degradation (data not shown) over a period of 12 months.

Effect of Antioxidants at 55 °C: Samples of CoQ₁₀ with
Fig. 5. Effect of Phenolic Antioxidants on the Stability of CoQ₁₀ at 55 °C

Fig. 6. Effect of Antioxidants on the Photo Stability of CoQ₁₀ (at 600 ft, 25 °C)

various antioxidants were taken in clear glass vials and kept at 55 °C. The degradation profiles are shown in Figs. 4 and 5. The antioxidant mixtures of AA and EDTA at 1—5% range and 0.1% were investigated. It was found that higher concentration of AA (5%) offered better protection than at lower concentration (1%) (Fig. 4). Chelating agents are usually employed in the concentration range of 0.005—0.1% to enhance the stability and to inhibit possible pro-oxidation effects, especially in presence of trace metal ions, by AA. The inhibition of autoxidation by AA can be explained by several mechanisms. In general, in the presence of metal ions, ascorbate monoanion reacts rapidly with oxygen and forms ascorbate radical which further oxidizes to dehydroascorbic acid. This is usually considered the primary mechanism for AA, especially in closed systems. In addition, AA can also act as a chain breaking antioxidant since it can form a stable free radicals.

PG at 0.1% level increased the degradation of CoQ₁₀ compared to control and an increase in PG concentration from 0.1 to 0.3% further accelerated the degradation process (Fig. 4). This pro-oxidation effect of PG may be explained as follows. During the oxidation process, antioxidants convert the activated drug molecules (free radicals) to thermodynamically less active or inactive forms. The radical antioxidants formed in this process react with one another and terminate the reaction, but under normal circumstances they do not react with the drug molecules. However, if the antioxidant radicals are reactive towards substrate, then autoxidation of substrate accelerates. This reaction is unlikely for stable phenolic radicals especially those with bulky groups around the radical center. In PG, the radical center is not surrounded by bulky groups, as in cases of BHT and BHA and, therefore, may be more reactive towards CoQ₁₀ molecules leading to pro-oxidation effects. In addition, BHT in the concentration range of 0.1—0.3% and BHA at 0.1% offered better protection (Fig. 5). However, increasing concentrations of BHA from 0.1% to 0.4% accelerated the degradation of CoQ₁₀. When an antioxidant is present in excess, it may promote acceleration rather than inhibition by promoting the decomposition of peroxides which in turn promote the oxidation process.

Photostability studies: The fluorescent light (typically 400—800 lx) provides reasonable simulation of natural light and widely used in photostability testing. Samples of CoQ₁₀ with various antioxidants were kept in clear glass vials and exposed to fluorescent light (600 lx at 25 °C) in a light stability chamber. The degradation profiles are shown in Fig. 6. The antioxidant mixture of AA (5%) and EDTA (0.1%) offered better protection than other antioxidants such as BHA (0.1%), BHT (0.3%), or PG (0.1%). AA in the concentrations of 1—5% and in combination with EDTA (0.1%) offered protection to the same extent (data not shown). Further, increasing concentrations of BHA and PG from 0.1 to 0.3% did not improve the photostability (data not shown). In Fig. 6, increase in degradation rate can be observed in all the profiles after a 7-day period. This period is the lag phase or induction period during which sufficient concentration of the free radicals build-up to propagate the reaction. The length of the induction period varies depending on the an-
tioxidant(s) used and the stability of the compound.\textsuperscript{71}

In conclusion, bioavailability of powder filled capsule and oil-based formulations of CoQ\textsubscript{10} are comparable ($p<0.95$). Poor and slow absorption of CoQ\textsubscript{10} were observed for both the formulations. Following oral administration of 60 mg/d for four days, the mean plasma concentrations were raised by 0.51 and 0.55 $\mu$g/ml for formulation I and II, respectively. In stability studies of CoQ\textsubscript{10}, large extent of degradation was observed at 45 °C and 55 °C, and humidity conditions did not affect the stability of CoQ\textsubscript{10}. Among the various antioxidants evaluated, mixture of AA (5%) and EDTA (0.1%) offered better protection of CoQ\textsubscript{10} when exposed to light (6001x, 25°C) or to a higher temperature (55 °C). Increasing concentrations of phenolic antioxidants (from 0.1% to 0.3%) such as BHA and PG accelerated the degradation of CoQ\textsubscript{10}. Stable formulations of CoQ\textsubscript{10} may be developed incorporating ascorbic acid (5%) and EDTA (0.1%) as an antioxidant system.

References


