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In vitro–in vivo study of CoQ10-loaded lipid nanoparticles in comparison with nanocrystals

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ABSTRACT

The present work described the effect of CoQ10 dissolution characteristics in nanocrystals and lipid nanoparticles (LNs) on its oral absorption in rats. Nanocrystals and LNs were prepared by melt-high pressure homogenization and sucrose monolaurate was used as a stabilizer in all formulations. Witepsol®W35 and medium-chain triglycerides (MCT) were selected as lipid additives to form $LN_{CoQ10+W35}$ and $LN_{CoQ10+MCT}$, respectively. From the results obtained, the particle size of CoQ10 nanocrystals was 285 nm, while it was reduced to 150 nm by mixture with an equal amount of lipid additives due to their lower melting points. *In vitro* dissolution results indicated that the drug release from two LNs was delayed compared with that from nanocrystals, and $LN_{CoQ10+W35}$ exhibited the highest drug release over 4 h. Finally, *in vivo* evaluation demonstrated that the oral absorption of CoQ10 was markedly increased by using nanocrystals and LNs compared with a coarse suspension. A good relationship was found between the *in vitro* dissolution and *in vivo* evaluation. The enhanced oral absorption of CoQ10 by nanocrystals and LNs was due to improved dissolution. In conclusion, Witepsol®W35 was shown to be a better lipid additive for the preparation of LNs to increase the oral absorption of CoQ10.

1. Introduction

Coenzyme Q10 (CoQ10) is a naturally occurring compound playing a fundamental role in cellular bioenergetics as a cofactor in the mitochondrial electron transport chain (Ernster and Dallner, 1995). It is physiologically important as an antioxidant and has been used to treat cardiovascular disorders (Belardinelli et al., 2006; Langsjoen and Langsjoen, 1999; Pepe et al., 2007), migraine headache (Rozen et al., 2002) and neurodegenerative disease such as Parkinsonism (Beal, 2002; Shults, 2003). In addition, it has been investigated as a treatment for cancer and the relief of some of the side effects of cancer treatment (Sakano et al., 2006). Because of these favorable effects, CoQ10 has been attracted increasing attention from those active in pharmaceutical fields. However, due to its poor solubility in water (4 ng/ml) (Westesen, 2000), CoQ10 is poorly absorbed from gastrointestinal tract (Westesen, 2000). This disadvantage has limited its clinical use (Balakrishnan et al., 2009).

To improve the oral absorption of CoQ10, many studies have focused on self-emulsified drug delivery systems (SEDDS), a kind of lipid-based formulation. Its high lipophilicity makes it easy for CoQ10 to dissolve in lipid materials (e.g. triglycerides or their derivatives) allow formulation of SEDDS (Balakrishnan et al., 2009; Nepal et al., 2010b). These studies indicated that the significant improvement in the oral absorption of CoQ10 could be produced by increasing CoQ10 dissolution by the formation of nanosized emulsions. Other strategies have been proposed to enhance the oral absorption of CoQ10 by the solubilization of CoQ10 in water using a cyclodextrin complex (Bhagavan et al., 2007) or by an improvement in CoQ10 release using solid dispersion techniques (Bhandari et al., 2007; Nazzal et al., 2002; Nepal et al., 2010a). However, these approaches have encountered some problems, such as the low CoQ10 content of these formulations, the toxicity of large amounts of excipients (e.g. surfactants) and the strict conditions required for the preparations with a lower physical stability.

In an approach to overcome the above limitations, the formation of nanocrystals has been shown to be a promising technique for improving the oral absorption of poorly water soluble drugs (where the rate of dissolution in water is an absorption-limiting step) with less negative effects such as the toxicity of surfactants (Hecq et al., 2005; Keck and Muller, 2006). On the other hand, lipid and lipophilic excipients can have significant and beneficial effects on the absorption and exposure of co-administered lipophilic drugs (Porter et al., 2007). In fact, an effect of lipid additives on the incorporated CoQ10 release from lipid nanoparticles (LNs) has also been reported and CoQ10 release depends strongly on both the type and content of the lipid additives in the lipid carrier (Teeranachaideekul et al., 2007). It is well known that choosing a suitable lipid additive is very important for improving the oral absorption of lipophilic drugs because the characteristics of the co-administrated lipid significantly influence the extent of drug

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absorption (Chakraborty et al., 2009). Although the oral absorption of CoQ10 was improved by the formation of nanosized emulsion using SEDDS, little is known about the potential usefulness of an improvement in the oral absorption of CoQ10 via the formation of nanocrystals and lipid nanoparticles.

In the present study, the main objectives were to investigate the oral absorption of CoQ10 given in the form of LNs in comparison with nanocrystals and to investigate the effect of lipid materials on the *in vitro* dissolution and *in vivo* absorption in rats. Melt-high pressure homogenization was employed to prepare nanocrystals of CoQ10 and LNs with a combination of two types of lipid materials, i.e. medium-chain triglycerides (MCT), which are widely used as a lipid additive for oral administration of CoQ10, and Witepsol®W35 (a mixture of mono-, di- and triglycerides). The results obtained indicate that the oral absorption of CoQ10 is markedly increased by using LNs prepared with Witepsol®W35 compared with nanocrystals and LNs prepared with MCT due to a significant improvement in CoQ10 release.

2. Materials and methods

2.1. Materials

CoQ10 was purchased from Asahi Kasei N&P Co., Ltd. (Tokyo, Japan). Sucrose monolaurate (L1695) was obtained from Mitsubishi Chemical Foods (Tokyo, Japan). Witepsol®W35 (a mixture of mono-, di- and triglycerides) was obtained from Mitsuba Trading Co., Ltd. (Osaka, Japan). Crodamol GTCC (medium-chain triglycerides, MCT) was obtained from Croda Singapore Pte. Ltd. (Singapore). All other chemicals were of analytical reagent grade.

2.2. Preparation of nanocrystals and LNs

The quantitative CoQ10 powder (200 mg) was melted at 60 °C, and then mixed with 20 ml aqueous solutions containing 2.5 mg/ml L1695 at 60 °C followed by high-speed shear agitation (FA-25; FLUKO Equipment Shanghai Co., Shanghai, China) at 10,000 rpm for 1 min. After agitation, the coarse suspensions were processed in a high pressure homogenizer (ATS Engineer Inc., China), applying 20 homogenization cycles at 800 bar. Then the suspensions were cooled in an ice-water bath to obtain nanocrystals of CoQ10. For the preparation of CoQ10-loaded lipid nanoparticles, 200 mg CoQ10 and an appropriate amount of lipid materials (e.g. Witepsol®W35 and MCT) were mixed at 60 °C, and the prepared CoQ10 was loaded into lipid nanoparticles under the same conditions as those used for the preparation of nanocrystals.

2.3. Particle size, zeta potential and morphology

The particle size and size distribution of the nanocrystals and the LNs were determined by laser diffractometry using a Coulter LS 230 instrument (Beckman-Coulter Co. Ltd., USA). The results obtained were evaluated using the volume diameter and span values. In addition, the zeta potential was determined using a Delsa 440SX Zeta potential analyzer (Beckman-Coulter Co. Ltd., USA). The morphology of the nanocrystals and LNs were examined using TEM (H-600, Hitachi, Japan). The samples were stained with 2% (w/v) phosphotungstic acid for 3 min and placed on copper grids with films for viewing.

2.4. X-ray diffraction (XRD) and differential scanning calorimetry (DSC)

The freeze-dried samples and raw crystals were analyzed in an X-ray diffractometer (Rigaku Geigerflex, Japan) with Cu Kα radiation at a wavelength of 1.542 Å, generated at 30 mA and 40 kV. The scanning speed was 4°/min from 5° to 50° of 2θ with a step size of 0.03°. The thermal properties of the powder samples were investigated with a Mettler Toledo DSC-1 differential scanning calorimeter/TAC-1 thermal analysis controller with an intracooler-2 cooling system (Mettler Toledo Instruments, Switzerland). Each sample was heated from 0 to 100 °C at 10 °C/min, using nitrogen as blanket gas.

2.5. Dissolution test and saturation solubility

Dissolution studies were carried out in a drug dissolution test apparatus (Rcz-6B; Huanghai Pharmacy Apparatus Factory, Shanghai, China) using the paddle method. A 200 ml aqueous solution containing 1% Tween-80 was used as the dissolution medium. The temperature was maintained at 37 °C, and the paddle speed was 100 rpm. Accurately weighed samples containing the equivalent of 3 mg CoQ10 were dispersed in the dissolution medium. Then, samples were withdrawn at different times and passed through a 0.1 μm syringe membrane filter (Membrane-solutions, Shanghai, China) and analyzed by HPLC (Hitachi L7110 pump equipped with a L7420 UV-VIS detector, Japan). Analysis was carried out on a Diamonsil™C18 column (5 μm, 250 × 4.6 mm). The mobile phase was composed of methanol and 1-propanol (50:50, v/v) at a flow rate of 1.0 ml/min, and detection was performed at a wavelength of 275 nm.

The solubility of CoQ10 was measured at 37 °C. Excess CoQ10 powder or suspension was added to each vehicle and stirred at 150 rpm for 48 h. The mixture then underwent ultrafiltration using a Microcon Centrifugal Filter with a molecular weight cut-off of 10,000 Da (Millipore Corporation, USA) at 8000 rpm for 10 min. The drug concentration was determined by HPLC.

2.6. Bioavailability studies in Wistar rats

Male Wistar rats (body weight 280–320 g) were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Wistar rats were fasted for 12 h prior to the experiments. CoQ10 was given orally at a dose of 60 mg/kg and a CoQ10 powder suspended in 0.25% (w/v) L1695 aqueous solution was used as a control. Blood (0.4 ml) was sampled by retroorbital puncture at the following time points: 0, 0.5, 1, 2, 4, 6, 8, 12, 24 h. Plasma was obtained from the whole blood, placed in heparinized tubes and frozen at −20 °C until analysis. The plasma samples (each 200 μl) were collected by centrifuging blood samples at 10,000 rpm for 5 min while protected from light. Vitamin k_1, at a concentration of 10 μg/ml, was employed as the internal standard solution. Then, 20 μl vitamin k_1 solution was added to 200 μl plasma and vortex-mixed. Following this, 400 μl ethanol was added to precipitate the protein, then the samples were extracted with 1 ml n-hexane and vortexed for 3 min. After centrifugation at 5000 rpm for 10 min, the organic layer was transferred to another clean tube and evaporated in a vacuum evaporator. The residue was redissolved using 100 μl 1-propanol, and 20 μl of the resulting solution was analyzed by HPLC as described in Section 2.5.

3. Results and discussion

3.1. Preparation of nanocrystals and LNs

Both nanocrystals and LNs were prepared using melt-high pressure homogenization to control the size of nanosized formulations by modifying the production parameters such as temperature, number of cycles and homogenization pressure (Keck and Muller, 2006). Because a higher temperature was required for the preparation of nanocrystals and LNs using the melt-HPH method, sucrose monolaurate (L1695, HLB = 16), a thermostable hydrophilic

Table 1
Composition (w/w), particle size, zeta potential and saturated solubility at 37 °C for the oral formulations ($n = 3$).

	CoQ10:lipids:L1695	Mean size (±SD; nm)	Span value (±SD)	Zeta potential (±SD; mV)	Saturated solubility (±SD; μg/ml)
Nanocrystals	1:0:0.25	282 ± 15	0.55 ± 0. 09	−34.7 ± 1.3	0.19 ± 0.02
$LN_{CoQ10+W35}$	1:1:0.25	150 ± 10	0.37 ± 0. 07	−24.2 ± 1.2	1.43 ± 0.07
$LN_{CoQ10+MCT}$	1:1:0.25	143 ± 10	0.36 ± 0.06	−31.1 ± 1.2	0.46 ± 0.03

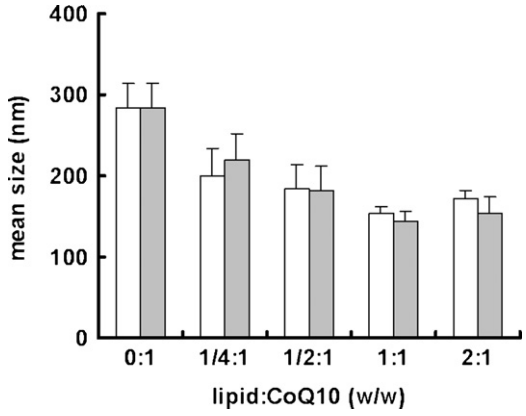

Fig. 1. Effects of the lipid additive concentration [(□) Witepsol®W35; (■) MCT] on the particle size of LNs (all the formulations were prepared using HPH at 800 bar and 20 cycles).

surfactant, was selected as a stabilizer, and the L1695 content of the final formulations was selected as 2.5 mg/ml because addition of a lower or higher amount would lead to an increase in the size distribution of CoQ10 nanocrystals (data not shown). As shown in Fig. 1, the particle size of CoQ10 nanocrystals prepared without addition of lipid additives was 285 nm at 800 bar for 20 homogenization cycles. To investigate the effect of the amount of lipid additives on the size of nanoparticles, different weight ratios of lipid additives (i.e. Witepsol®W35 or MCT) to CoQ10, e.g. 1/4:1, 1/2:1, 1:1 and 2:1, were used. The particle size of LNs prepared with the lipid additives was reduced by increasing the weight ratio to 1:1, but a higher concentration of lipid additives did not help reduce the particle size. The melting point of the mixture of CoQ10 and lipid additives was reduced by increasing the proportion of lipid additives. As a result, less energy was required to crush the particles by high pressure homogenization (Nepal et al., 2010b). Hence, the most suitable formulation of LNs consisted of CoQ10, lipid additives (i.e. Witepsol®W35 or MCT) and L1695 in the weight ratio of 1:1:1/4, designated as $LN_{CoQ10+W35}$ and $LN_{CoQ10+MCT}$, respectively (shown in Table 1). TEM analysis was also performed and the nanocrystals and LNs had a spherical shape (Fig. 2).

The zeta potential is important for the storage stability of colloidal dispersions. As shown in Table 1, the zeta potential of nanocrystals, $LN_{CoQ10+W35}$ and $LN_{CoQ10+MCT}$ were in the range −25 mV to −35 mV. Regarding physical stability, no change in the size of any of the formulations occurred over 2 months at 4 °C (data not shown).

3.2. XRD and DSC investigations

In order to confirm the physical state, XRD was used to analyze potential changes in the inner structure of CoQ10 samples. As shown in Fig. 3a, it was confirmed that a typical drug peak was found in nanocrystals and LNs because their powder X-ray diffraction patterns were consistent with the pattern of the raw crystals. However, the differences in the relative intensities of the peaks could be due to differences in the crystallinity of the samples. One possible reason was that the drug recrystallized in the lipid phase after cooling of the melted lipid particles.

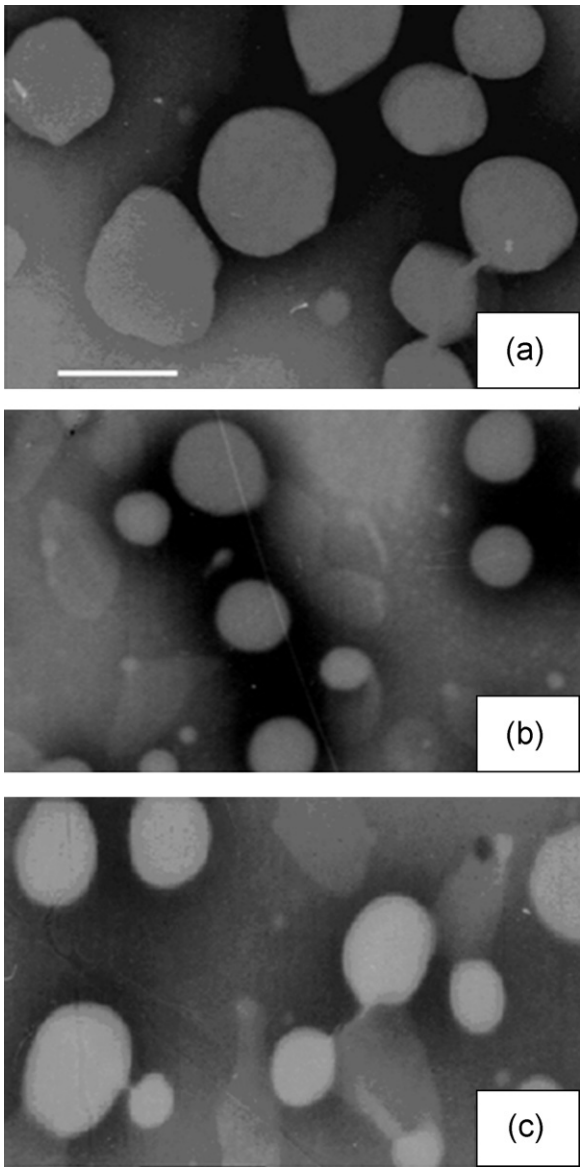

Fig. 2. The morphology of nanocrystals (a), $LN_{CoQ10+MCT}$ (b) and $LN_{CoQ10+W35}$ (c) observed using TEM (bar = 200 nm).

In order to further confirm the physical state, DSC was also performed to investigate the different samples. In Fig. 3b, it can be seen that DSC scans of pure CoQ10 and nanocrystals showed a single sharp endothermic peak, which indicated that there was no substantial crystalline change. However, the melting point of nanocrystals was lower than that of the raw crystals and this might be due to the size reduction in the crystals. When Witepsol®W35 or MCT was incorporated in LNs, the endothermic peak of CoQ10 shifted to a lower temperature compared with that of

Table 2
Pharmacokinetic parameters following an oral administration of CoQ10 (60 mg/kg) in four different formulations to rats (mean ± S.D., $n = 5$).

	Coarse suspensions	Nanocrystals	$LN_{CoQ10+W35}$	$LN_{CoQ10+MCT}$
T_{max} (h)	1.5 ± 0.71	1.33 ± 0.58	1.67 ± 0.58	1.67 ± 0.58
C_{max} (ng/ml)	274.34 ± 71.64	437.8 ± 66.6	402.8 ± 77	316.85 ± 69.8
AUC_{0-24h} (ng h/ml)	1480.5 ± 625.8	3672.7 ± 1913.6	4376.6 ± 1263.1	2722.7 ± 2215.9

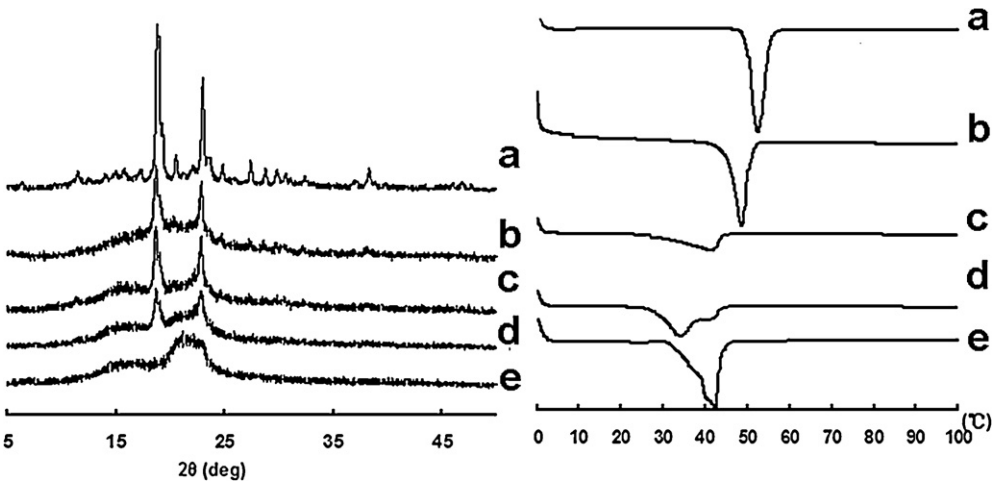

Fig. 3. XRD (left) and DSC (right) curves: (a) CoQ10 powder; (b) nanocrystals; (c) $LN_{CoQ10+MCT}$; (d) $LN_{CoQ10+W35}$; (e) pure Witepsol®W35.

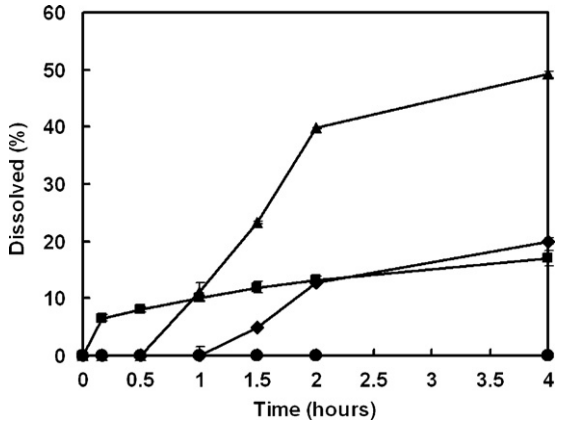

Fig. 4. Dissolution profile of CoQ10 formulations [(●) coarse suspensions; (■) nanocrystals; (▲) $LN_{CoQ10+W35}$; (♦) $LN_{CoQ10+MCT}$] (mean ± SD, $n = 3$).

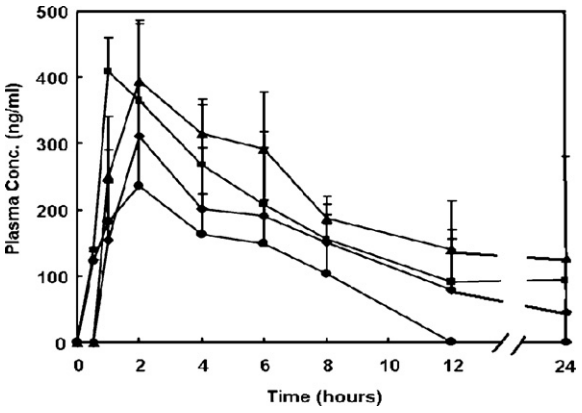

Fig. 5. Mean plasma concentration *vs* time curves [(●) coarse suspensions; (■) nanocrystals; (▲) $LN_{CoQ10+W35}$; (♦) $LN_{CoQ10+MCT}$] (mean ± SD, $n = 5$).

nanocrystals. Possible explanations were that there was a size reduction and formation of a crystal lattice that was not as orderly as the pure components (Stott et al., 2001).

3.3. *In vitro dissolution and saturated solubility test*

The dissolution profiles of coarse suspensions, nanocrystals and LNs ($LN_{CoQ10+W35}$ & $LN_{CoQ10+MCT}$) are shown in Fig. 4. Almost no drug was dissolved in the case of the coarse suspensions over the 240 min testing period, while approximately 17% drug was dissolved in the case of the nanocrystals. In the case of $LN_{CoQ10+MCT}$ and $LN_{CoQ10+W35}$, 19% and 49% drug was dissolved, respectively. The enhanced dissolution can be attributed to the small particle size with a higher surface area available for dissolution and the reduced diffusion layer thickness (Hecq et al., 2005). However, a lag-time was observed during the initial stage of dissolution. Witepsol®W35 and MCT produced a delay in the diffusion of CoQ10 into the aqueous phase during the initial stage. These dissolution profiles can be explained by the drug-enriched core model (Wissing et al., 2004). In such a model, the drug-enriched core is surrounded by a practically drug-free lipid shell. Due to the increased diffusion distance and effects being reduced by the surrounding lipid shell, the drug was released only after the lipid had dispersed in the dissolution medium. The lag-time of $LN_{CoQ10+MCT}$ was longer than that of $LN_{CoQ10+W35}$. The phenomenon could be explained by the different interaction between drug-lipid molecules. The solubility of CoQ10 in MCT and Witepsol®W35 was determined, and it was found that the solubility of CoQ10 in MCT was higher than Witepsol®W35 (322 mg/g in MCT, 133 mg/g in Witepsol®W35), showing that CoQ10 has a higher affinity for MCT than Witepsol®W35. Thus, it was more difficult for the drug to be released from $LN_{CoQ10+MCT}$ than from $LN_{CoQ10+W35}$.

The solubility of CoQ10 in the nanocrystals, $LN_{CoQ10+W35}$ and $LN_{CoQ10+MCT}$ was determined, and is shown in Table 1. The solubility in $LN_{CoQ10+W35}$ was significantly higher than in the other two systems. Witepsol®W35 appeared to affect the solubility of CoQ10.

3.4. Bioavailability study

To confirm the usefulness of nanocrystals and LNs in improving the bioavailability of CoQ10, an *in vivo* test was carried out using rats. The average plasma concentration–time curves of CoQ10 after oral administration are shown in Fig. 5. CoQ10 levels in the blood were found to be very low when the coarse suspension was administered orally, and the C_{max} and AUC values were 274.3 ng/ml and 1480.5 ng h/ml, respectively. According to the pharmacokinetic parameters of four dosage forms (Table 2), LNs were slowly absorbed with a T_{max} of about 2 h, while nanocrystals rapidly produced a plasma peak within about 1 h. These results were in agreement with the initial *in vitro* dissolution data. An improvement in C_{max} and AUC was observed in nanocrystals and LNs compared with coarse suspensions. The C_{max} and AUC of nanocrystals were 1.6-fold and 2.5-fold higher than that of coarse suspensions, respectively, indicating that oral absorption of CoQ10 was enhanced by the particle size reduction and the dissolution was increased. In the case of $LN_{CoQ10+W35}$, the C_{max} and AUC values were found to be 1.5-fold and 3.0-fold greater than the values obtained with coarse suspensions, while those of $LN_{CoQ10+MCT}$ were increased 1.2-fold and 1.9-fold. Although both LNs had a similar particle size, different lipids produced different improvements in bioavailability. Unlike MCT, which contains caprylic/capric triacylglycerols, Witepsol®W35 contains short fatty acid chains and considerable amounts of mono- and diglycerides which possess surface active properties (Mehnert, 2001), resulting in both significant drug dissolution and absorption. Thus, the enhanced bioavailability exhibited by $LN_{CoQ10+W35}$ might be attributed to the higher saturated solubility and the greater drug dissolution enhancement compared with nanocrystals and $LN_{CoQ10+MCT}$ due to surface active properties and possible direct uptake of nanoparticles from the gastrointestinal tract and lymphatic transport.

4. Conclusions

CoQ10 dissolution was enhanced by the preparation of nanocrystals, $LN_{CoQ10+MCT}$ and $LN_{CoQ10+W35}$. The *in vivo* study in rats demonstrated that the oral bioavailability of nanocrystals and $LN_{CoQ10+MCT}$ was approximately 2.5-fold, 2.0-fold higher than that of coarse suspensions, respectively, while the corresponding value for $LN_{CoQ10+W35}$ was 3.0-fold higher. $LN_{CoQ10+W35}$ was proved to be a better formulation than the others, and offered an excellent method to improve the oral bioavailability of CoQ10.

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