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Preparation and characterization of liposomal coenzyme Q10 for *in vivo* topical application

Wen-Chuan Lee [a], Tung-Hu Tsai [a,b,c,*]

[a] Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan
[b] Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan
[c] Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

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ABSTRACT

Coenzyme Q10 (CoQ10) is an endogenous cellular antioxidant that is used as a nutritional supplement and for medicinal purposes. In recent *in vivo* investigations, cosmetically applied CoQ10 has demonstrated its ability to reduce photoaging, with a corresponding decrease in wrinkle depth. However, the bioavailability of topical CoQ10 is poor; the development of a practical topical formulation is therefore highly desirable. In this study, a liposomal formulation composed of soybean phosphatidylcholine (SPC) and α-tocopherol (Vit E) was utilized to encapsulate CoQ10 for topical application. The liposomes were less than 200 nm in diameter and had a narrow size distribution. Encapsulation of CoQ10 in liposomes composed of SPC and Vit E significantly ($p < 0.05$) enhanced its accumulation (at least twofold) in rat skin, compared with an unencapsulated suspension. Prolonging the treatment time and increasing the content of CoQ10 in the formulation both raised the amount of CoQ10 in rat skin. However, in skin treated with the highest CoQ10 content formulation, insufficient treatment time limited the amount accumulated. This study demonstrates that liposomal CoQ10 is a promising candidate for the topical application of CoQ10. The treatment duration is the key factor limiting penetration following *in vivo* topical application.

1. Introduction

Coenzyme Q10 (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, also called CoQ10 or Q10) is a vitamin-like, oil-soluble molecule, sometimes referred to as vitamin Q. Its chemical structure (Fig. 1), consisting of a p-benzoquinone ring with a polyisoprenoid side-chain, reflects its important role as an electron and proton transporter in the mitochondria. CoQ10 also participates in aerobic cellular respiration by generating energy in the form of ATP (Lenaz et al., 2007). As an ingredient in skin care products, CoQ10 is a popular antioxidant that protects cells against aging induced by free radicals (Fuller et al., 2006). CoQ10 has also been suggested to strongly inhibit oxidative stress in the skin that is induced by ultraviolet B light; possibly by increasing manganese superoxide dismutase and glutathione peroxidase activities (Kim et al., 2007).

In a previous investigation, CoQ10 applied topically to human skin for 6 months was shown to reduce the depth of wrinkles (Hoppe et al., 1999), indicating that direct topical delivery of CoQ10 through the skin is an excellent wrinkle reducing treatment. A short topical CoQ10 application period also improved the stabilization of mitochondrial function, which again correlated with skin aging through influences on age-affected cellular metabolism. Thus, CoQ10 was used for anti-aging at the cellular level by improving mitochondrial function (Prahl et al., 2008). In a recent investigation, CoQ10 was shown to increase production of basement membrane components, to increase fibroblast proliferation, and to protect cells against oxidative stress; all of these reflect the anti-aging actions of CoQ10 on skin (Muta-Takada et al., 2009). Taken together, these findings indicate that CoQ10 is a unique cosmetic substance that protects the skin from early aging, wrinkle formation, and the loss of cell activity.

However, CoQ10 is a highly lipophilic substance, as a consequence of the 10 isoprene units in its chemical structure (Boicelli et al., 1981). This lipophilicity, combined with its large molecular weight (863 Da) and its thermolability (Boreková et al., 2008), limit the topical bioavailability of CoQ10. In some previous investigations, researchers provided different lipid-related formulations for encapsulating CoQ10 for topical application, including nanostructured lipid carriers (Junyaprasert et al., 2009; Teeranachaideekul et al., 2007) and nanoliposomes (Xia et al., 2006, 2007). These studies provided details for the optimization of the composition of the CoQ10 formulations and for its *in vitro* release; however, the *in vivo* topical application of these formulations was not discussed.

* Corresponding author at: National Yang-Ming University, School of Medicine, Institute of Traditional Medicine, 155, Li-Nong Street Section 2, Taipei 112, Taiwan. Tel.: +886 2 2826 7115; fax: +886 2 2822 5044.
E-mail address: thtsai@ym.edu.tw (T.-H. Tsai).

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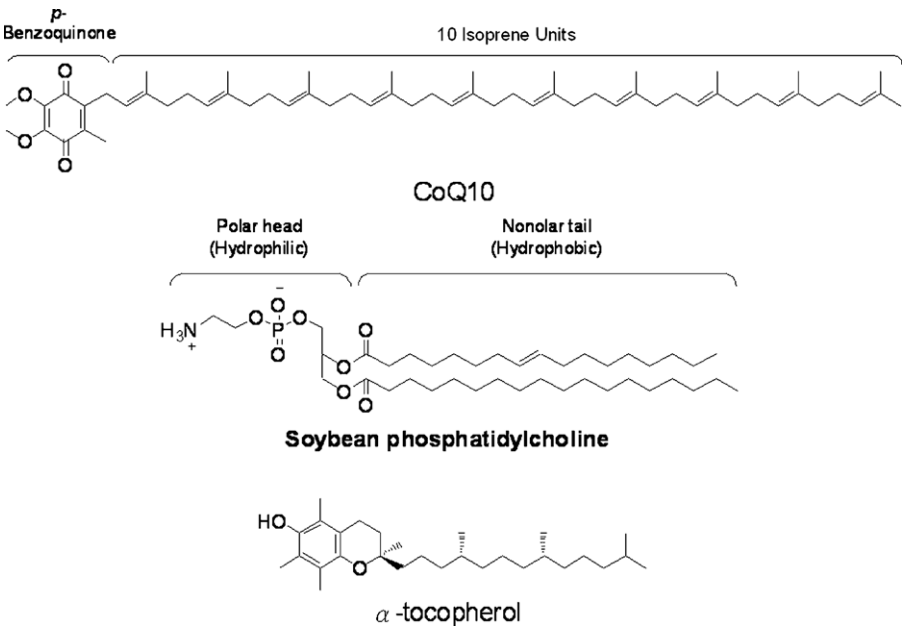

Fig. 1. Chemical structures of CoQ10, soybean phosphatidylcholine and α-tocopherol.

Vit E (α-tocopherol, Fig. 1) is a fat-soluble antioxidant that is widely used as an inexpensive constituent of cosmetics and foods. It is also used as a component in the preparation of liposomes, given its ability to protect lipids from oxidation (Zhang and Wang, 2009). The incorporation of Vit E into soybean phosphatidylcholine (SPC) liposomes has been shown to reduce fibroblast sensitivity toward liposomal phospholipids, compared to pure SPC liposomes (Berrocal et al., 2000). In the present study, Vit E was also selected as a component of liposomal formulation.

Our hypothesis was that liposomal formulations improved the penetration of CoQ10 in the skin. The aim of the present study was to develop a surfactant-free liposomal formulation, to encapsulate CoQ10 within this liposomal formulation, and then to evaluate the effectiveness of liposome-delivered CoQ10 as a form of *in vivo* topical application. The experiment first examined CoQ10 free liposomes, prepared through a solvent injection method with SPC as the basic liposome component and with Vit E as a modifier. Selected formulations were then used to encapsulate CoQ10 and the vesicle size, size distribution, and encapsulation efficiencies were evaluated. The optimal liposomal CoQ10 was then selected for *in vivo* topical application to rats, to study whether liposomal delivery enhances the accumulation of CoQ10 in the skin.

2. Experimental

2.1. Materials

Methanol, ethanol, 2-propanol (all HPLC grade), and sodium chloride (NaCl, ≥99.5%) were obtained from Merck (Darmstadt, Germany). Sodium dodecylsulfate (SDS) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Deionized water (Millipore, Bedford, MA, USA) was used throughout the entire experiment. CoQ10 (≥98%), (±)-α-tocopherol (Vit E, ≥96%), α-chloralose (≤20% β-anomer), urethane (≥99%), and Sephadex G-50 were obtained from Sigma–Aldrich (St. Louis, MO, USA). Soybean phosphatidylcholine (SPC, >95.5%) was purchased from NOF (Tokyo, Japan).

Table 1
Compositions and characteristics of liposomes.

Formulations	SPC (mg/mL)	Vit E (mg/mL)	CoQ10 (mg/mL)	Vesicle size (nm)	PDI[a]	EE (%)[b]
L	5	–	–	176 ± 14.6	0.235 ± 0.038	–
L-A	5	1	–	167 ± 11.0	0.184 ± 0.013*	–
L-B	5	2.5	–	202 ± 26.8	0.166 ± 0.010*	–
L-C	5	5	–	233 ± 8.6*	0.211 ± 0.018	–
L-1	5	–	0.1	162 ± 15.3	0.231 ± 0.039	91.0 ± 3.15
L-2	5	–	0.25	167 ± 10.1	0.223 ± 0.024	90.3 ± 10.1
L-3	5	–	0.5	150 ± 17.4	0.247 ± 0.016	96.2 ± 5.80
L-A1	5	1	0.1	169 ± 8.7	0.168 ± 0.012	79.4 ± 3.80**
L-A2	5	1	0.25	177 ± 16.9	0.181 ± 0.018	74.7 ± 6.11**
L-A3	5	1	0.5	173 ± 7.2	0.183 ± 0.009	87.4 ± 10.5
L-B1	5	2.5	0.1	187 ± 14.3**	0.179 ± 0.015	54.4 ± 18.6**
L-B2	5	2.5	0.25	194 ± 16.4**	0.189 ± 0.041	51.0 ± 10.9**
L-B3	5	2.5	0.5	200 ± 18.9**	0.166 ± 0.017	59.7 ± 10.2**
CoQ10 suspension	–	–	0.1	–	–	

Data expressed in mean ± standard deviation ($n = 4$).
[a] PDI indicated the polydispersity indexes of liposomes.
[b] EE indicated the encapsulation efficiency of CoQ10.
* $p < 0.05$, significantly different from L.
** $p < 0.05$, significantly different between corresponding groups.

2.2. Preparation and characterization of liposomal CoQ10

A solvent injection method was used to prepare the liposomes (Lasic, 1993). Selected amounts of SPC, Vit E, and CoQ10 were dissolved in ethanol (1 mL) and injected through a 23-gauge needle into deionized water (10 mL). During injection into the aqueous solution, an ultrasonic processor (XL2210, Misonix Inc., CT, USA) was used for dispersion. The sonication was carried out for 5 min with cycle 0.5 (i.e., 0.5 s pulse, 0.5 s pause). The mixture was then stirred at 1000 rpm overnight to vaporize the ethanol, and the volume of the mixture was replenished with deionized water to 10 mL. The final compositions are shown in Table 1 and they consisted of SPC liposomes (L), and SPC liposomes containing 1 mg/mL (L-A), 2.5 mg/mL (L-B), and 5 mg/mL (L-C) of Vit E. The numerals indicate the different CoQ10 amounts in each composition. Prepared liposomes were then stored at 4 °C for further use. The stability of CoQ10 amount and the particle size of the CoQ-loaded liposome were examined by freeze thaw test. A 0.1 mg/mL CoQ10 suspension formulation, prepared by adding CoQ10 to a 1% (w/w) of SDS aqueous solution, served as the control treatment.

The vesicles sizes and their distribution (polydispersity index, PDI) were determined at 25 °C using a laser particle analyzer (Photon LPA3100, Otsuka Electronics, Osaka, Japan) with a photon correlator (Photon LPA3000, Otsuka Electronics, Osaka, Japan). The encapsulation efficiency (EE%) of CoQ10 in the liposomes was determined by the ratio of total CoQ10 minus unencapsulated CoQ10 relative to total CoQ10, expressed as a percentage. The prepared liposomal CoQ10 was mixed with 2-propanol to disrupt the liposomal structure and the amount of CoQ10 was estimated as being the total CoQ10 amount. Prepared liposomes were passed through a Sephadex G-50 packed column, with deionized water as an eluant, to separate liposomal CoQ10 and unencapsulated CoQ10. The total CoQ10 amount in the liposomes and the amount of unencapsulated CoQ10 were quantified by high performance liquid chromatography (HPLC).

The HPLC system consisted of a chromatographic pump (LC-20AT, Shimadzu, Kyoto, Japan), an autosampler (SIL-20AC, Shimadzu, Kyoto, Japan), a diode array detector (DAD, SPD-M20A, Shimadzu, Kyoto, Japan), a degasser (DGU-20A5, Shimadzu, Kyoto, Japan) and a reversed-phase HC-C18 column (150 mm × 4.6 mm i.d.; particle size 5 μm, Agilent, USA). The mobile phase consisted of 2-propanol and methanol (60:40, v/v), filtered through a 0.22 μm filter (DURAPORE® membrane filters, Millipore, Ireland BV, Cork., Ireland) and degassed for 10 min by sonication (2510, Branson, CT, USA) before use. The flow-rate was set at 1 mL/min, the UV wavelength was set at 278 nm for peak integration, and the sample injection volume was 20 μL.

2.3. In vivo topical application

Rats were chosen as the animal model for topical application since, among rodents, rat skin reportedly shows the closest anatomical similarity to human skin (El Maghraby et al., 2008). Adult male Sprague–Dawley rats (350–400 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). The animals were specifically pathogen-free and were allowed to acclimate in their environmentally controlled quarters (24 ± 1 °C and 12–12 h light–dark cycle). Food (Laboratory Rodent Diet no. 5001, PMI Nutrition International LLC, MO, USA) and water were available *ad libitum*. All experimental animal surgery procedures were reviewed and approved by Institutional Animal Experimentation Committee of National Yang-Ming University. The rats were initially anesthetized with urethane 1.0 g/mL and α-chloralose 0.1 g/mL (1 mL/kg, i.p.) and remained anesthetized throughout the experimental period. Body temperature was maintained by a heating pad set at 37 °C. The fur on each rat's back was shaved away and the dorsal skin was cleaned with tape to ensure that all of the cut hairs were removed. The dorsal skin was divided into isolated areas and treated with liposomes in each area. After topical treatment with CoQ10 for 1, 2, and 4 h, the skin was cleaned to remove excess CoQ10. The rats were then sacrificed with an overdose of anesthetic and the dorsal skin was cut into areas corresponding to the CoQ10 treatment areas. After careful removal of subcutaneous fat, the skin samples were weighed and preserved at −20 °C for further sample preparation.

2.4. Skin sample preparation

CoQ10 in rat skin was extracted using liquid–liquid extraction. The skin samples were first cut into small pieces and homogenized at a ratio of 1:10 (w/v) in a solution of 0.1 M SDS and 0.15 M NaCl at a ratio of 1:1 (v/v). The homogenized solution was then centrifuged at 2000 rpm for 5 min. The supernatant was collected and 1 mL of supernatant was mixed with 1 mL of 2-propanol, centrifuged at 6000 rpm for 5 min, and 1 mL supernatant was collected. The residue was extracted by repeating the same procedure two more times.

The collected supernatant was then dried by a centrifugal vaporizer system composed of a pump (GVD-050A, ULVAC, Japan), a trap (UNI TRAP UT-1000, EYELA, Tokyo, Japan), and a centrifugal vaporizer (CVE-200D, EYELA, Tokyo, Japan). The dried supernatant was re-dissolved in 1 mL 2-propanol before HPLC analysis. After thorough mixing and centrifugation, the supernatant was collected and analyzed by HPCL-DAD, as previously described.

2.5. Method validation

The calibration curves were established by spiking CoQ10 into samples of homogenized rat skin and assaying six replicates on 6 separate days for inter-day linearity. Intra-day linearity was evaluated for six samples assayed on the same day. Calibration curves were constructed using least-squares regression analysis; correlating the concentration of each diluted standard solution to the area of a corresponding peak on the chromatogram. The relative error (RE), or accuracy, was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentrations (C_{obs}) using the equation: RE (%) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The precision was calculated from the equation: % relative standard deviation (RSD, %) = [standard deviation (STD)/C_{obs}] × 100.

The recovery of CoQ10 in rat skin was also evaluated by triplicate analysis at each of the three concentrations within the range of the standard curve (0.1, 1 and 10 μg/mL). The recovery rate was determined by comparing the mean peak area of the standard samples to the corresponding stock solution (100 μL) spike in normal saline (900 μL), followed by the liquid–liquid extraction, as previously described.

2.6. Statistical method

The data are presented as mean values ± standard deviation. Differences between the series were determined by Student's *t*-test, with $p < 0.05$ values considered to be statistically significant.

3. Results and discussion

3.1. Preparation of liposomal formulations

The composition, vesicle sizes, and polydispersity indexes of the CoQ10 free liposomes are shown in Table 1. The mean diameters

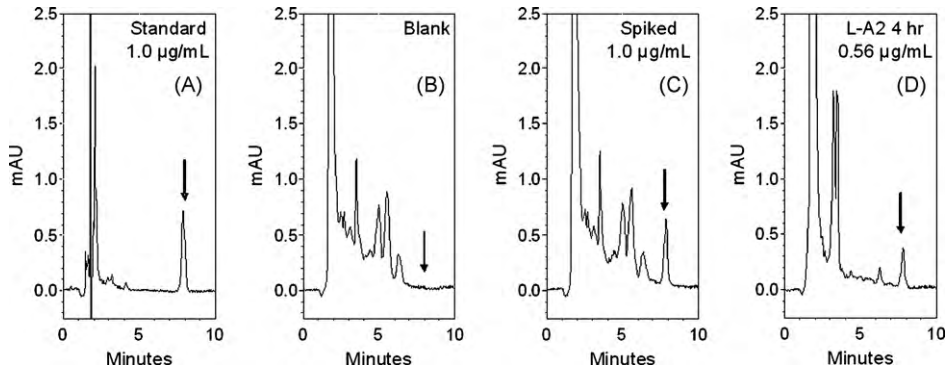

Fig. 2. Typical chromatograms of CoQ10. (A) Standard CoQ10 solution (1 μg/mL), (B) homogenized solution of blank skin, (C) homogenized solution of blank skin spiked with CoQ10 (1 μg/mL), and (D) homogenized solution of skin containing CoQ10 (0.56 μg/mL) after L-A 2 h treatment. The arrow indicates CoQ10 peak.

of the four CoQ10 free liposomes (L, L-A, L-B, and L-C) ranged from 167 ± 11.0 to 233 ± 8.6 nm. The PDI values were all lower than 0.3, indicating that these liposomal formulations had a relatively narrow size distribution.

In Vit E-containing liposomes, the L-C liposomes were the only group with a vesicle size significantly larger ($p < 0.05$) than that of the L liposomes prepared with SPC only. The PDI of both the L-A and L-B liposomes was significantly smaller ($p < 0.05$) than that of the L liposomes. These results implied that Vit E participated in the formation of the liposomes, altered the vesicle size, and was responsible for the uniform size distribution. Vit E molecules in liposomes have been reported to locate near the membrane surface (Urano et al., 1992) and this indicated that Vit E occupied the hydrophobic shell of the liposomes and led to the larger vesicle size. The vesicle size of L-C liposomes was significantly larger than 200 nm, and the PDI of the L and L-C liposomes were higher than 0.2. Subsequent liposomal CoQ10 was therefore prepared by the L-A and L-B compositions, in order to maintain a smaller size and narrower size distribution.

The vesicle sizes of the liposomal CoQ10 composed of SPC ranged from 150 to 167 nm. Those of the L-A series, including L-A1, L-A2, and L-A3, were in the range of 169–177 nm, and the L-B series, including L-B1, L-B2, and L-B3, were in the range of 187–200 nm.

Previous investigations have reported liposomal CoQ10 with sizes of less than 200 nm (Verma et al., 2007; Xia et al., 2006, 2007). However, preparation with surfactants or membranes with a 200 nm pore size have also been utilized in these investigations. In the present investigation, SPC, with or without Vit E, and CoQ10 were the only components in the formulation. Regardless of the amount of CoQ10 in the liposomes, the vesicle sizes and size distribution did not change significantly ($p > 0.05$).

The encapsulation efficiencies of the L, L-A, and L-B series are summarized in Table 1. Higher Vit E content resulted in lower amounts of CoQ10 encapsulation in the liposomes. A previous investigation (Qin et al., 2009) showed that Vit E with hydrophobic characteristics is located in the lipid tails layer, the hydrophobic part of the liposome structure. The basic characteristic of CoQ10 is also hydrophobic, so it may locate in the same space. As the result, encapsulation efficiencies decreased as the amount of Vit E in the liposome increased.

The L series provided smaller vesicle sizes and higher encapsulation efficiencies, but larger size distributions. The L-B series provided larger vesicle size and lower encapsulation efficiencies, but smaller size distributions. The L-A series provided appropriate vesicle size, PDI, and encapsulation efficiencies. Therefore, these liposomes (L-A1, L-A2, and L-A3) were chosen to test the *in vivo* topical application of CoQ10.

3.2. Chromatography

To investigate the amount of CoQ10 in the skin, HPLC coupled with a UV system was used to separate endogenous interfering substances and to quantify CoQ10. The chromatograms of CoQ10 in rat skin are shown in Fig. 2. The CoQ10 standard (1.0 μg/mL) (Fig. 2A), a blank skin sample (Fig. 2B), the standard CoQ10 (1.0 μg/mL) spiked into a blank skin sample (Fig. 2C), and the skin sample containing CoQ10 (0.56 μg/mL) collected 4 h after L-A2 administration (Fig. 2D) were analyzed. With an isocratic mobile phase containing 2-propanol–methanol (60:40, v/v), no endogenous interference was detected close to the retention time of CoQ10 (RT 7.85 min). Thus, the HPLC conditions used here were acceptable for the analysis and quantification of CoQ10 and demonstrated the successful separation of endogenous interfering substances and CoQ10.

3.3. Method validation—linearity, accuracy, precision, and recovery

Method validation was established using a blank homogenized skin solution spiked with various concentrations of CoQ10. The calibration curve of CoQ10 was constructed over a concentration range of 0.1–20 μg/mL prior to the analysis of real samples using HPLC-DAD. The limit of detection (LOD) was 0.02 μg/mL, with a signal-to-noise ratio of 3. All calibration curves were considered linear, with a coefficient of determination (r^2) greater than 0.995. The limit of quantitation (LOQ) was defined as the lowest concentration of CoQ10 in the calibration curve. The LOQ of CoQ10 for skin samples was 0.1 μg/mL, which was met acceptable criterion for accuracy and precision.

Intra- and inter-day precisions (RSD%) ranged from 0.8 to 10.9% and 0.4 to 16.4%, respectively, and accuracies (RE%) for intra- and inter-day assay ranged from −1.0 to 12.7% and from −4.3 to 18.0%, respectively. The recovery of the liquid–liquid extraction was tested by spiking CoQ10 with three blank homogenized skin samples at three different concentration levels (0.1, 1 and 10 μg/mL) and by analyzing the samples in triplicate. The resulting recoveries were 77.6 ± 4.7%, 79.8 ± 9.3%, and 70.9 ± 0.9% for 0.1, 1 and 10 μg/mL, respectively, and the average recovery was 76.1 ± 5.0%. The intra- and inter-day precision and accuracy, and the recovery demonstrated repeatability and reliability. These results are summarized in Table 2.

The drug stability in the CoQ-loaded liposome at room temperature and in skin homogenate have examined by freeze thaw testing for days. The CoQ10 amount and the particle size of the CoQ-loaded liposome had been test for stable at least for a month at 4 °C preservation. Meanwhile, CoQ10 amount in the skin homogenate was detected stable for at least a month at −20 °C.

Table 2
Intra-day and inter-day assay for accuracy and precision for the determination of CoQ10 in the skin.

Nominal conc. (μg/mL)	Observed conc. (μg/mL)	RE (%)[a]	RSD (%)[b]
Intra-day assay			
0.1	0.11 ± 0.01	13	9.1
0.2	0.20 ± 0.02	−0.5	10
1	1.01 ± 0.10	1.4	9.9
2	1.98 ± 0.09	−1.0	4.5
10	9.99 ± 0.11	−0.1	1.1
20	20.1 ± 0.16	0.4	0.8
Inter-day assay			
0.1	0.12 ± 0.02	18	17
0.2	0.21 ± 0.01	3.4	4.8
1	0.96 ± 0.04	−4.3	4.2
2	1.95 ± 0.06	−2.5	3.1
10	10.1 ± 0.16	1.0	1.6
20	20.0 ± 0.07	−0.2	0.4

Data are expressed as mean ± standard deviation ($n = 6$).
[a] Accuracy was expressed in relative error (RE%), RE(%) = [($C_{obs} - C_{nom}$)/C_{nom}] × 100.
[b] Precision was expressed in % relative standard deviation (RSD (%)), RSD(%) = [standard deviation (STD)/C_{obs}] × 100.

3.4. *In vivo* topical application of CoQ10

To investigate the topical application of CoQ10, both a CoQ10 suspension and liposomal CoQ10 formulations were used for *in vivo* topical application. The results are shown in Fig. 3. When these two formulations were applied to the skin, the amount of CoQ10 in the skin of L-A1 was significantly higher than that of the CoQ10 suspension formulation. At each time point, there was at least a twofold enhancement in liposomal CoQ10 compared to the CoQ10 suspension application. This result indicates that the liposomal formulation enhanced CoQ10 accumulation in the skin.

A previous report established five possible mechanisms for the penetration of liposome encapsulated drugs into the skin: (1) a free drug mechanism, (2) a penetration enhancing mechanism, (3) vesicle adsorption to and/or fusion with the stratum corneum, (4) an intact vesicular skin penetration mechanism, and (5) transappendageal penetration (El Maghraby et al., 2008).

In this investigation, the CoQ10 suspension formulation provides evidence for (1), the free drug mechanism for skin penetration, although it has been reported that SDS can enhance transdermal penetration by perturbing the skin barrier, the stratum corneum (Ghosh and Blankschtein, 2007). Liposomes have been previously shown to interact with the stratum corneum and then perturb its membrane properties (Fang et al., 2001) SPC also acts to directly lower the permeability barrier of the stratum corneum (Kato et al., 1987; Yokomizo and Sagitani, 1996). These results are examples of (2) a penetration enhancing mechanism. Liposomal CoQ10 contained both SPC and Vit E, which are natural compounds and compatible with the composition of the skin, making the direct fusion of the liposomes with the upper layer of the skin possible and thereby enhancing the skin permeation of drugs (Choi and Maibach, 2005), in agreement with (3) vesicle adsorption to and/or fusion with the stratum corneum. This is based on the fact that the structure of liposome is flexible and liposomal CoQ10 follows (4) an intact vesicular skin penetration mechanism to promote CoQ10 penetration. The sizes of these liposomal CoQ10 formulations were smaller than 200 nm, and the small size vesicle also improved drug deposition into and penetration through the skin (Valenta and Janisch, 2003; Verma et al., 2003).

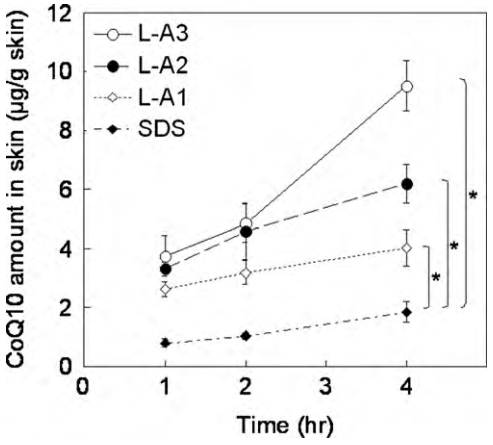

Fig. 3. Time (h) versus CoQ10 amount (μg/g of skin) in the skin after treatments with different CoQ10 formulations ($n = 6$). *$p < 0.05$ compared with CoQ10 suspension formulation.

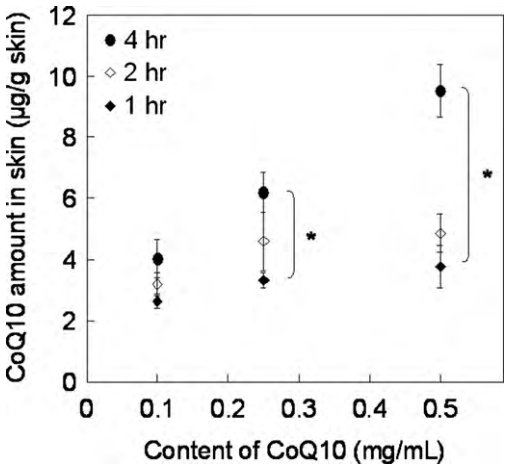

Fig. 4. Content of CoQ10 (mg/mL) in liposomal CoQ10 versus amount of CoQ10 accumulated in the skin (μg/g of skin) after liposomal CoQ10 treatment. *$p < 0.05$ compared with 1 h group.

Raising the content of CoQ10 in the liposomes also caused more CoQ10 to accumulate in the skin, compared with the CoQ10 suspension formulation (Fig. 3). Comparing the three liposomal formulations, the L-A3 liposomal formulations resulted in the highest amount of CoQ10 in the skin. The amounts of CoQ10 in the skin followed the same trend as the initial loading amount: L-A3 > L-A2 > L-A1. However, the vesicle sizes, PDIs, and encapsulation efficiencies of these three liposomal CoQ10 formulations were similar ($p > 0.05$) (Table 1). The major difference between these three liposomal CoQ10 was the content of CoQ10. The content of CoQ10 therefore must have been the key factor affecting the *in vivo* amount of CoQ10 accumulation in the skin; indicating that penetration of CoQ10 was concentration dependent. This result was similar to a previous report (Giovannini et al., 1988).

Fig. 4 demonstrates a clear correlation between the content of CoQ10 in liposomes and the amount of CoQ10 accumulated in the skin at different treatment durations. It shows that with a 1 h treatment time, even when the content of CoQ10 in liposomal formulation was as high as 0.5 mg/mL, the accumulated amount of CoQ10 in the skin was limited. After 4 h of treatment, the amount of CoQ10 increased significantly ($p < 0.05$) compared with only 1 h of treatment. Even with a high content of CoQ10 in the liposomal CoQ10, CoQ10 accumulation in the skin was limited after only 1 h or 2 h of treatment. Therefore, although SPC, Vit E, and even the SDS used for suspending CoQ10 enhance the penetration ability of CoQ10, these molecules need time to interact with the stratum corneum, regardless of which mechanism is ultimately employed to facilitate drug penetration into the skin.

4. Conclusions

In this study, a solvent injection method for liposomal preparation was used to prepare liposomal CoQ10, and it yielded vesicle sizes smaller than 200 nm, with a narrow size distribution. The encapsulation efficiencies of CoQ10 in these liposomes were modulated by the Vit E content in the formulations, which was a result of hydrophobic Vit E occupying the hydrophobic shell of the liposomes. High Vit E content reduced the capacity of the liposomes and led to a lower encapsulation efficiency of CoQ10. Following *in vivo* topical application, liposomal CoQ10 formulations enhanced the penetration of CoQ10, indicating that a liposomal formulation improved the topical penetration ability of CoQ10. The penetration ability of CoQ10 was altered by the concentration in the formulation and the duration of treatment. A long duration and high CoQ10 content in the formulation enhanced CoQ10 distribution in the skin. However, insufficient treatment time limited the amount of CoQ10 in the skin, regardless of the concentration. In conclusion: (1) liposomal formulations promoted CoQ10 penetration through the stratum corneum, compared with CoQ10 suspension formulations; (2) higher CoQ10 loading amounts increased accumulation in the skin; (3) longer treatment times enhanced the accumulation of CoQ10 in the skin; and (4) adequate treatment time was necessary in order to allow a high amount of CoQ10 to penetrate the skin.

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