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The advantages of a novel CoQ10 delivery system in skin photo-protection

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ABSTRACT

Skin photo-ageing induced by ultraviolet (UV) radiation is mainly ascribed to oxidative stress and reactive oxygen species (ROS). Coenzyme Q10 (CoQ10) has been reported as a powerful antioxidant in plasma. However, CoQ10 was barely satisfactory in topical drug delivery because of its lipid solubility. To improve the anti-oxidative efficiency of CoQ10 in skin photo-ageing, the present research prepared a novel CoQ10 nano-structured lipid carrier (CoQ10-NLC) and characterised it by size and freeze-fracture transmission electron microscopy (FF-TEM). In UVA-irradiated fibroblasts, the protection of CoQ10-NLC was more effective than the CoQ10-emulsion as demonstrated by cell viability and morphological changes of the cell body and nucleus. In addition, malondialdehyde (MDA, the product of lipid peroxidation) concentration decreased by 61.5% in the group treated with CoQ10-NLC compared to the group subjected to general CoQ10-emulsion. In the presence of CoQ10-NLC, the activities of the anti-oxidative enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) were reinstated to 81% and 75%, respectively, of the control group. *In vivo*, the CoQ10-NLC displayed a stronger capability to penetrate the stratum corneum and permeate the dermis after a topical skin application. These results reveal that CoQ10-NLC has greater antioxidant properties and topical skin penetration than the CoQ10-emulsion.

1. Introduction

Ultraviolet A (UVA) radiation (320–380 nm), a component that represents 96% of the total solar ultraviolet (UV) spectrum reaching the earth, penetrates through the epidermis, dermis and beyond to subcutaneous tissues, which results in skin photo-ageing, inflammation, skin cancer and other damage (Fuchs et al., 1989; Han et al., 2004). At the cellular level, UVA radiation mainly causes vast oxidative stress due to the generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide anion, hydroxyl radical and hydrogen peroxide (Shindo and Hashimoto, 1998; Basu-Modak et al., 2003). UVA radiation can overwhelm constitutive cellular defences, such as glutathione, ascorbate, superoxide dismutase (SOD) and glutathione peroxidase (GSH-px). As a result of this stress, cells respond to UVA damage with modified gene expression and DNA damage, which results in abnormal cell morphology, cell apoptosis or necrosis and eventually skin ageing (Fuchs et al., 1989; Podda et al., 1998; Ichihashi et al., 2003).

As an essential electron carrier in cellular respiration, Coenzyme Q10 (CoQ10, also called ubiquinone-10) shows potential antioxidant properties by clearing ROS and protecting cells from oxidative stress (Beyer, 1990; Witting et al., 2000; James et al., 2004). Regarding UVA-induced skin ageing, CoQ10 is a potential preventive medication against skin photo-ageing (Tomasetti et al., 1999; Tanino et al., 2005). Considering its lipid solubility and topical drug delivery efficiency, the water-soluble CoQ10 formulation may have greater anti-oxidative ability and topical skin penetration than general CoQ10-emulsion (Fetoni et al., 2009; Zhang and Wang, 2009).

Nano-structured lipid carriers (NLC), most of which are lipid-based particles with a diameter usually less than 100 nm, are novel drug delivery systems with high substance solubility, stability, powerful skin penetration and low skin irritation (Hughes, 2005; Müller et al., 2007; Kotyla et al., 2008; Küchler et al., 2009). Recently, studies have reported that water-soluble lipid carrier formulations could be easily prepared with some poorly water-soluble drugs such as paclitaxel, dexamethasone and amiodarone (Xie et al., 2005; Beck et al., 2007; Lacoeuille et al., 2007). Theoretically, the lipid-soluble vitamin-like substance, CoQ10, can be prepared in water-soluble nano-structured lipid carriers for use in topical drug delivery.

The present work used a UVA-irradiated human skin fibroblasts model to analyse differences in oxidation resistance of a water-soluble CoQ10 nano-structured lipid carrier (CoQ10-NLC) and general CoQ10-emulsion. Nile Red fluorescent dye was used as a model compound to determine the efficiency of CoQ10-NLC penetration in rat skin (Sheihet et al., 2008).

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2. Materials and methods

2.1. Materials

Acetone was used as an analytical reagent. CoQ10 was purchased from Zhejiang Medicine Co., Ltd. (Xinchang Pharmaceutical Factory, Xinchang, Jangsu Province, China). Soybean lecithin was obtained from Cargill Texturizing Emulsions (Deutschland GmbH & Co. KG, Decatur, Illinois, USA). Octyl decyl acid triglyceride (GTCC) was purchased from Croda Co. Ltd. (East Yorkshire, UK). 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), 4′,6-diamidino-2-phenylindole (DAPI) and Nile Red were obtained from Sigma–Aldrich (St. Louis, Missouri, USA). Foetal bovine serum and Dulbecco's modified Eagle's medium (DMEM) were purchased from HyClone (South Logan, Utah, USA).

2.2. Methods

2.2.1. CoQ10-NLC preparation and characterisation

In the present report, researchers chose CoQ10 and GTCC as an inner lipid core and soybean lecithin as lamellar shell to synthesise CoQ10-NLC by hot high-pressure homogenisation (HPH, NS1001L, Niro Soavi, Italy). Soybean lecithin is an ideal lamellar shell because of its biocompatibility, non-irritating and non-sensitising properties for human skin, balanced between hydrophilic and lipophilic properties (Hoeller et al., 2009). CoQ10 (5%) was dissolved in liquid GTCC (5%) at 60 °C. Soybean lecithin (5%) was added to a glycerol aqueous solution with a glycerol concentration of 45% (w/w). The liquid CoQ10 lipid phase was dispersed in an aqueous lecithin solution at 60 °C and emulsified using a stirrer at 1500 rpm for 1 min. The resulting pre-emulsion was homogenised at 60 °C for 5 cycles at 1800 bar. Lastly, the resulting dispersion was cooled to room temperature under ambient conditions to obtain the CoQ10-NLC. Blank-NLC was prepared in a similar manner by omitting CoQ10. Identical quantities of CoQ10 and GTCC were used to prepare the CoQ10-emulsion; 5% acetone was added to promote CoQ10 dissolution. The mixture was dispersed in glycerol solution 45% (w/w) at 60 °C, emulsified using a stirrer at 1500 rpm for 1 min, and cooled to room temperature. Fluorescent Nile Red was incorporated into the particles and emulsion as a part of the inner lipid core to visualise permeation in rat skin *in vivo* under fluorescent microscopy.

The mean particle size of CoQ10-NLC was determined by photon correlation spectroscopy (PCS) using a Malvern Zetasize 2000 (Malvern Instruments, UK). The particle size was obtained by averaging three measurements at an angle of 90° in 1 cm diameter cells at 25 °C. All samples were diluted 1:50 with distilled water. Morphology of CoQ10-NLC was characterised by freeze-fracture transmission electron microscopy (FF-TEM, BALZERS BAF-400D).

2.2.2. Cell culture

Human embryo skin fibroblasts (ESF-1) were purchased from Cell Resource Centre (IBMS, CAMS/PUMC, China) at generation 10. The cells were cultured routinely at 37 °C in 5% CO_2 in DMEM supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin and 10% FBS as described and split twice per week. For all experiments, cells used were passages 15–20.

2.2.3. UVA-irradiation and addition of CoQ10

ESF cells were cultured in 24- or 6-well plates with 1 or 4 ml complete medium in each well for 24 h and then subjected to UVA. UVA-irradiation was carried out with a UVA light source (wave length: 320–400 nm, wave peak 365 nm, Spectroline, EN-160L/FE, USA) at a dose of 0.72 J/cm^2. Then, these cells were cultured with medium containing CoQ10 or another drug for another 24 h.

2.2.4. MTT test for cell viability

ESF cells were placed in 24-well plates at a density of 4×10^5 cells/well and were allowed to attach and grow for 24 h before treatment with UVA. After UVA radiation, CoQ10 or other drugs were added to wells. Before the MTT test, each well was washed with PBS, 760 μl fresh medium and 0.5% MTT 40 μl were added, and then incubated for 3 h. Finally, DMSO was added to dissolve purple formazan and the absorption was determined by the absorption in an enzyme-linked immunosorbent assay plate reader (Bio-Rad, model 680, US) at $\lambda = 490$ nm and reference wavelength at 630 nm. The results were expressed as percentage of untreated control.

2.2.5. Assay of lipid peroxidation products and intracellular ROS

Lipid peroxidation product MDA was determined by a photometrical method utilising the lipid peroxidation assay kit (Nanjing Institute of Jiancheng Biological Engineering, China), which is based on the method of "TBA test" (Janero, 1990). At low pH and high temperature, MDA readily participates in a nucleophilic addition reaction with 2-thiobarbituric acid (TBA), generating a red fluorescent MDA:TBA (1:2) adduct with absorption maxima at 532 nm.

A kit (Applygen Technologies Inc., Beijing) with fluorescent probe, 2′,7′- dichlorofluorescin diacetate (DCFH-DA), was used to test the cellular ROS level. The principle of this assay is that DCFH-DA is catalysed to non-fluorescent dichlorofluorescin (DCFH); by contrast, DCFH-DA was rapidly oxidised to highly fluorescent dichlorofluorescin (DCF) (Shen et al., 1996). Cells were examined under 100× magnification using a fluorescence microscope (Olympus CK40 microscope, Japan) with a blue excitation filter.

2.2.6. Antioxidant enzyme activities: SOD and GSH-px

After determining the amount of total protein in the cell homogenate, the enzymes SOD (EC 1.15.1.1) and GSH-px (EC 1.11.1.9) were detected using biochemical methods and commercially available assay kits (Nanjing Institute of Jiancheng Biological Engineering, China).

The assay of Cu/Zn–SOD activity is based on the inhibition of the oxidation of oxymine by the xanthine–xanthine oxidase system. Nitrite, the product of this oxidation system, is a red compound with an absorbance maximum at 550 nm. One unit of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%.

The activity of GSH-px was tested by quantifying the amount of H_2O_2-induced oxidation of reduced glutathione to oxidised glutathione. A yellow compound, the product of the reaction of GSH and dithiobisnitrobenzoic acid, has a stable absorbance maxima at 512 nm. One unit of GSH-px activity was defined as the amount that decreased the level of reduced glutathione by 1 μM in 1 min/mg protein (Han et al., 2004).

2.2.7. Cell morphology and apoptosis or necrosis

Cell morphology and apoptosis or necrosis were evaluated by fluorescence microscopy using the DNA fluorescent dye 4′-6-diamidino-2-phenylindole (DAPI). Cells were rinsed with PBS, fixed with 4% paraformaldehyde for 30 min at 4 °C, incubated with 1 μg/ml of DAPI for 10 min, and washed 3 times with PBS. Apoptosis or necrosis (characterised by intensely stained fragmented nuclei, condensed chromatin and loss of membrane integrity) was examined under 400× magnification using a fluorescent microscope (Olympus CK40 microscope, Japan) with a purple excitation filter (Galán et al., 2001; Kuo et al., 2006).

2.2.8. Skin permeation studies

To study the permeation of CoQ10-NLC, Nile Red (2.5 μg/ml) was used as a fluorescent dye incorporated into the oil phase during NLC formulation. 10-week-old female Sprague–Dawley (SD)

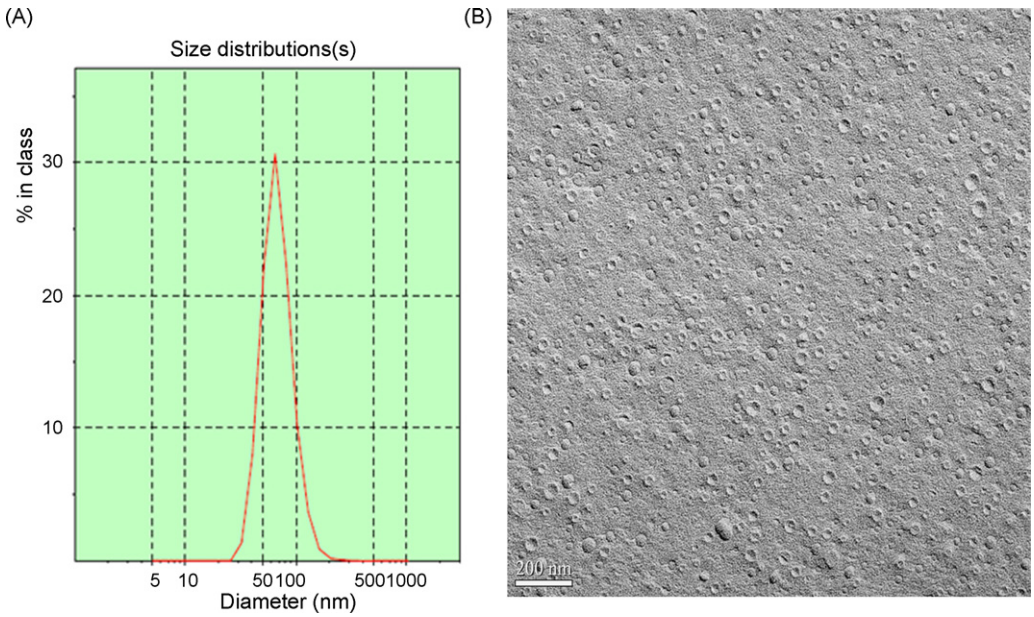

Fig. 1. Characterisation of CoQ10-NLC. (A) The size distribution of CoQ10-NLC as measured by dynamic light scattering photon correlation spectroscopy (PCS) (Zetasize 2000, Malvern Instruments, UK). (B) Freeze-fracture transmission electron microscopy (FF-TEM) images of NLC.

rats (200–250 g; Vitalriver Inc, Beijing, China) were selected as the model animal. Rats were anaesthetised with 2% barbital sodium solution for the duration of the skin permeation experiment. With an electric shaver, the hair of the dorsum was removed carefully to reveal the skin completely. The hairless area was used in transport studies *in vivo*.

For each rat, three hairless areas (approximately 3.14 cm^2 each) were chosen and a 50-mg sample per area was applied to the surface and well distributed. After 0.5, 1, 2, 4, and 8 h, the surplus sample was cleared, the skin surface was washed with physiological saline and immediately dried. In the treated skin area, a 0.5 cm × 0.5 cm piece of skin was cut without muscle and frozen in liquid nitrogen. Each piece of skin was embedded in optimal cutting temperature compound (OCT) and sliced at 25 μm in vertical cross-sections for 10–12 sections using a cryostat microtome (Leica ICACM 1850, Germany). Skin sections were stored at 4 °C until microscopic analysis.

Treated skin samples were examined by fluorescent microscopy (Olympus CK40 microscope, Japan). Image-Pro Plus program (ipp, Media Cybernetics, USA) was used for image analysis. From the fluorescence spectrum of Nile Red and CoQ10 formulation, the excitation and emission wavelengths were determined at 543 and 604 nm, respectively. Therefore, green light was used as the excitation light and red light was used as the emission light (Diaz et al., 2008).

2.3. Data analysis

The statistical data were analysed using Student's *t*-test with Origin 7.0. Data are expressed as the mean value ± S.E. (standard error) and $p < 0.01$ was considered to be statistically significant.

3. Results

3.1. CoQ10-NLC characterisation: size and morphology

Electron microscopy revealed the CoQ10-NLC sphere was homogenous with a droplet size of approximately 65 nm (Fig. 1) as demonstrated by dynamic light scattering photon correlation spectroscopy. During the 6-month stability test, this CoQ10-NLC showed a good dispensability and no agglutination was found (data not shown). In terms of the CoQ10-emulsion, the droplet size was over 1000 nm and was easy to agglutinate.

3.2. Protective effect of CoQ10 on UVA-irradiated fibroblasts

The anti-oxidative ability of CoQ10-NLC was studied by performing the MTT and viability tests of fibroblasts. The cells were treated with incremental concentrations of samples (2.32–58 μM) after UVA-irradiation. As a result, 11.6 μM was found to be the most appropriate concentration to protect cells. Cell viability was dramatically reduced by UVA (50% of control) as assessed by the MTT test. Compared with the UVA-irradiation group, fibroblasts treated with CoQ10-NLC had greater viability (increased by 41.7%) than the CoQ10-emulsion treated sample, which only increased by 25% (Fig. 2).

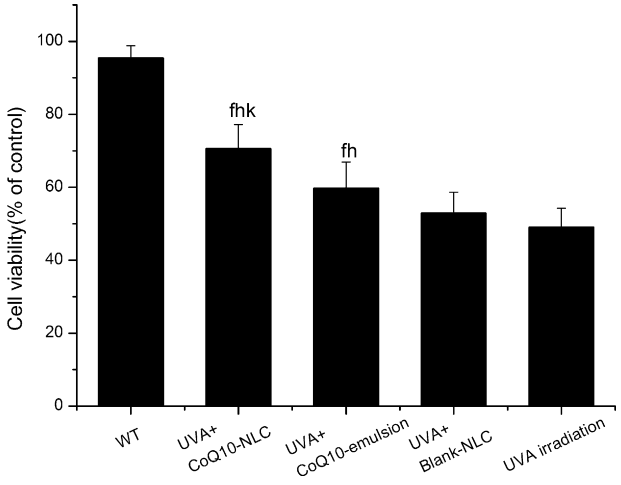

Fig. 2. Protective effect of CoQ10-NLC on UVA-irradiated ESF fibroblasts. Cell viability was assayed with MTT and expressed as percentage of control ($n = 6$). CoQ10 concentration = 11.6 μM. $^{k}p < 0.01$ vs. UVA + CoQ10-emulsion. $^{f}p < 0.01$ vs. WT. $^{h}p < 0.01$ vs. UVA-irradiation. WT group is the control group. The Blank-NLC also showed weak protection to the UV damage, and the following results confirmed this protection.

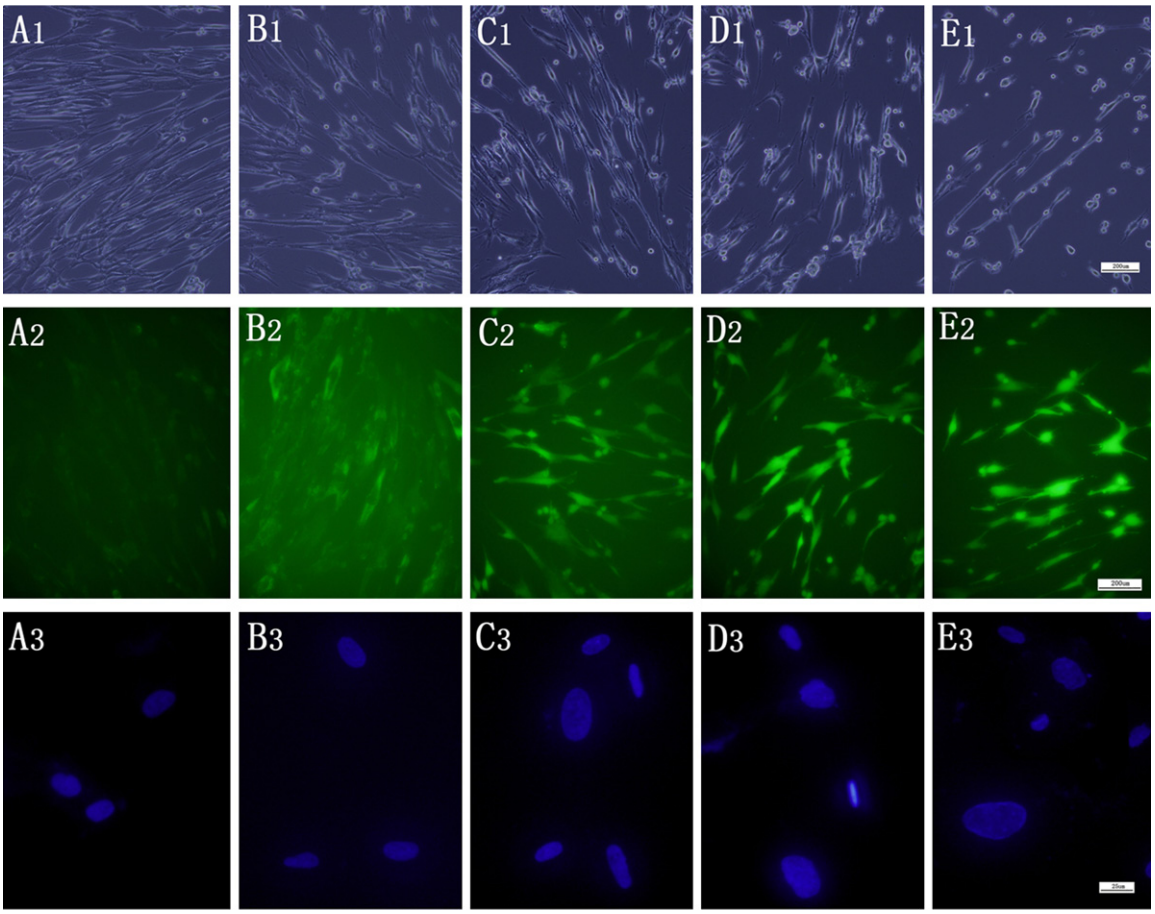

Fig. 3. Cell morphology, ROS level and apoptosis or necrosis after UVA-irradiation and addition of CoQ10. After UVA radiation, some of the fibroblasts were loosely attached, rounded and randomly oriented. Representative images of ROS production of ESF cells were visualised under a fluorescent microscope using green DCF fluorescence. The brighter fluorescence indicates greater ROS production. Compared to the CoQ10-emulsion group, the level of ROS was lower in the cells treated with CoQ10-NLC. The cell chromatin was more condensed and membrane integrity was lost in the set of CoQ10-emulsion. (A) Typical images showing wild type ESF cells. (B) Typical images showing ESF cells with added CoQ10-NLC after UVA-irradiation. (C) Typical images showing ESF cells with added CoQ10-emulsion after UVA-irradiation. (D) Typical images showing ESF cells with added Blank-NLC after UVA-irradiation. (E) Typical images showing ESF cells with nothing added after UVA-irradiation. (X-1) Fibroblasts were observed with a light microscope, X100. (X-2) ROS accumulation in ESF cells, X100. (X-3) Cell nucleus stained with DAPI, X400.

Being rounder and loosely attached, the morphology of UVA-irradiated fibroblasts was different from the control group under the light microscope. Furthermore, abnormal cell nuclei were observed after DAPI staining. Following UVA radiation, cell chromatin was condensed, membrane integrity was lost and cell apoptosis or necrosis was stimulated. However, after treatment with CoQ10-NLC, the number of fibroblasts that had undergone apoptosis or necrosis was less than the control and CoQ10-emulsion groups (Fig. 3).

3.3. Reduction of lipid peroxidation and cellular ROS level

The MDA in supernatant revealed the level of lipid peroxidation in cells. As shown in Fig. 4, MDA in the CoQ10-emulsion group was 1.7-fold higher than in the CoQ10-NLC group. This means that the level of lipid peroxidation induced by UVA in CoQ10-emulsion group was higher and the protective effect of CoQ10-emulsion was weaker than the CoQ10-NLC group. In addition, the Blank-NLC, without CoQ10, showed a feeble protection to this oxidative stress.This same result was obtained in the study of ROS. The green fluorescence in the fibroblasts treated by CoQ10-emulsion was remarkably stronger compared to CoQ10-NLC (Fig. 3, X-2). A high level of intracellular ROS directly resulted in lipid peroxidation aggravation and decreased cell viability.

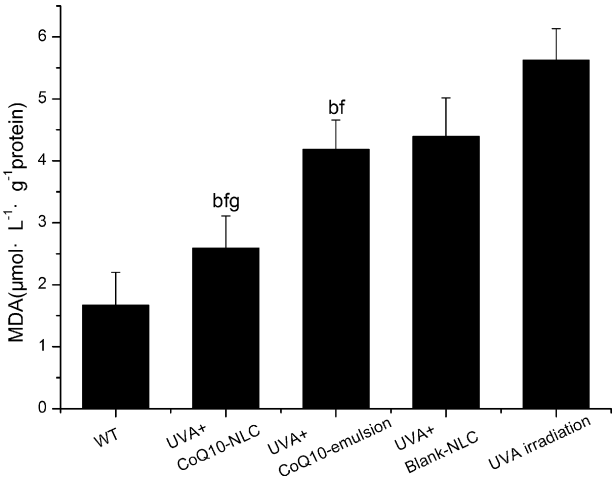

Fig. 4. UVA-induced lipid peroxidation in fibroblasts and CoQ10 anti-oxidative ability. The lipid peroxidation of cells was determined by measuring MDA formation ($n = 6$). CoQ10 concentration = 11.6 μM. $^{g}p < 0.01$ vs. UVA + CoQ10-emulsion. $^{b}p < 0.01$ vs. WT. $^{f}p < 0.01$ vs. UVA-irradiation.

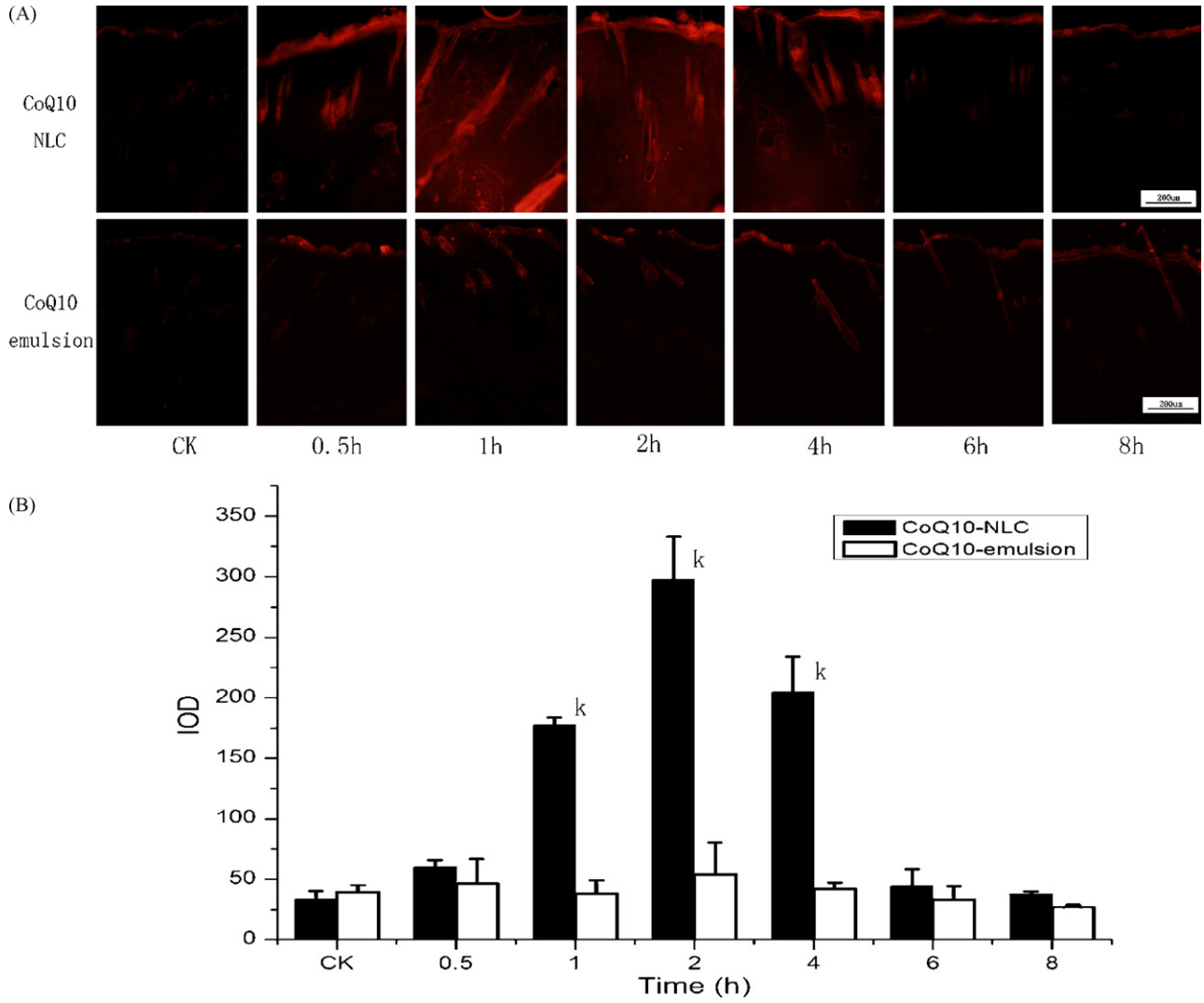

Fig. 5. The skin penetration capability of CoQ10-NLC and CoQ10-emulsion. (A) The fluorescence of Nile Red increased during the first 2 h and then weakened with time (X100). (B) The optical density of the red fluorescence was measured and analysed with Image-Pro Plus 6.0. $^{k}p < 0.01$ ($n = 6$).

3.4. The change of cellular anti-oxidative enzyme system

The change of biochemical parameters and antioxidant enzyme activity in fibroblasts was also tested. The activities of SOD and GSH-px in the UVA-irradiation group were only one-third to one-half of the control group (Table 1). Treatment of CoQ10 and other reagents increased SOD and GSH-px activities to different extents. Overall, the CoQ10-NLC group, which reinstated SOD and GSH-px activity to 81% and 75% of the control group, respectively, had the best performance among all samples.

Table 1
Effect of CoQ10 formulation on cellular SOD and GSH-px ($n = 6$).

Groups	SOD (kN U g^{-1} protein)	GSH-px (kU g^{-1} protein)
WT	16.05 ± 1.39	103.16 ± 14.95
UVA + CoQ10-NLC	13.11 ± 1.92^{b,c,k}	75.20 ± 23.48^{b,c,k}
UVA + CoQ10-emulsion	8.25 ± 2.23^{c,k}	39.53 ± 11.51^{c,k}
UVA + Blank-NLC	9.66 ± 1.53	41.26 ± 8.83
UVA-irradiation	6.68 ± 2.00	31.62 ± 11.09

All values are means ± S.D. CoQ10 concentration = 11.6 μM.
[b] $p < 0.01$ vs. UVA + CoQ10-emulsion.
[c] $p < 0.01$ vs. WT.
[k] $p < 0.01$ vs. UVA-irradiation.

3.5. In vivo skin penetration

To analyse the transport of CoQ10-NLC, Nile Red was used as a probe to trace CoQ10-NLC penetration in rat skin. Fig. 5A depicts representative examples of fluorescence microscopy images of vertically cross-sectioned skin following topical application of CoQ10-NLC and CoQ10-emulsion from 0.5 to 8 h. The dermis skin fluorescence measurement of Nile Red is expressed in arbitrary units (ABU) and is shown in Fig. 5B. In the captured fluorescence images, red fluorescence (Nile Red and oil) was enhanced during the first 4 h then the fluorescence started to fade. The intensity of the red fluorescence in the CoQ10-NLC group was stronger at 1, 2 and 4 h than the CoQ10-emulsion group (4.6-, 5.5-, and 4.9-fold higher, respectively). This means the CoQ10-NLC passed more easily through the stratum corneum and permeated dermis skin more than the CoQ10-emulsion group.

4. Discussion

In the present study, the enhanced effect of CoQ10-NLC on UVA-induced oxidative damage in both human skin fibroblasts *in vitro* and skin penetration *in vivo* was characterised. It was clearly demonstrated that CoQ10-NLC possesses advanced antioxidant effects and stronger permeation capability than general CoQ10-

emulsion. Thus, CoQ10-NLC could be an ideal formulation for CoQ10 to inhibit the damage of UVA radiation on human skin.

Comparing the compositions and characteristics of CoQ10-NLC, Blank-NLC and CoQ10-emulsion, it was the participation of soybean lecithin that resulted in the different delivery efficiencies and the increased anti-oxidative capability of CoQ10-NLC. Because of its strong ability to absorb on biomembranes and to enhance biomembrane permeability, soybean lecithin was a formulation stabilising factor and permeation enhancer, which could interact with the skin lipid and promote the penetration of nano-particles (Müller et al., 2002, Spernath et al., 2006). Therefore, lecithin was the main factor to the high efficiency of drug delivery in this work.

The results of the MTT test showed that CoQ10-NLC exhibited a potent protection against UVA-induced fibroblast damage and apoptosis. UV, especially UVA, induces oxidative stress in cells, speeding up the ageing process of human skin (Han et al., 2004). At the cellular level, UVA radiation causes significant oxidative stress due to the generation of ROS, such as superoxide, singlet oxygen, hydroxyl radicals, nitric oxide and hydrogen peroxide. However, most of the harmful cellular effects of UVA seem to be mediated by singlet oxygen (Herrling et al., 2006). Recent research demonstrated that ROS, particularly the highly reactive hydroxyl radicals, will lead to DNA strand breaks and replication mistakes, affecting protein conformation, initiating lipid peroxidation and triggering apoptotic cellular death and necrosis (Latonen and Laiho, 2005). Antioxidants such as glutathione, α-tocopherol, ascorbate, superoxide dismutase and heme oxygenase are able to decrease the extent of damage and protect cells from death by suppressing or scavenging ROS (Basu-Modak et al., 2003). These results show that cells treated with the formulation of antioxidant CoQ10-NLC displayed stronger proliferation than the cells treated with CoQ10-emulsion.

Previously, CoQ10 has been used as a lipid-soluble antioxidant in heart failure treatment, ischemic heart disease and myocardial protection during heart surgery (Sinatra, 1997; Kumar et al., 2009). Clinically, the antioxidant properties of CoQ10 can decrease the oxidation of low-density lipoprotein (LDL) and/or improve arterial endothelial function (Raitakari et al., 2000). It is well known that the ROS induced by UVA radiation often attack lipids and protein molecules in plasma and mitochondrial membranes, especially of unsaturated fatty acids. Since MDA is one product of this free radical reaction, it was hypothesised that the UVA-irradiated fibroblasts would accumulate more MDA and this trend would be reversed when CoQ10-NLC was added. The data confirm this hypothesis. All the cells supplemented with CoQ10 had stronger defences to oxygen radicals, and the MDA level in the cells with CoQ10-NLC was remarkably lower than the group with CoQ10-emulsion.

As ROS level reflects the UVA-induced damage in cell proliferation, apoptosis and necrotic death, it is possible to directly study the potential of antioxidants by this criterion. In this study, the level of ROS showed the same trend as MDA in cells. The level of ROS increased after UVA exposure in UVA-irradiated fibroblasts, while it declined significantly when the antioxidant (CoQ10) was presented. Furthermore, compared to the CoQ10-emulsion groups, cells with CoQ10-NLC exhibited reduced ROS damage as observed by green fluorescence.

Antioxidant enzymes such as SOD, GSH-px, xanthine oxidase and catalase can be activated by exposing cells to UVA radiation. Among these enzymes, SOD and GSH-px are the most common and important enzymes for scavenging ROS generated by UVA-irradiation (Basu-Modak et al., 2003; Han et al., 2004).

Existing in cytoplasm, mitochondria and extracellularly, SOD catalyses two O_2^- into H_2O_2 and O_2 and protects cells from the UV-induced damage. The substrate H_2O_2, another reactive oxidant, is broken down by GSH-px and CAT. GSH-px is primarily found in the cytoplasm, catalysing the destruction of H_2O_2 with concomitant conversion of reduced glutathione (GSH) to glutathione disulfide (GSSG) (Schrader and Fahimi, 2006). Interestingly, the present results indicate that the activities of SOD and GSH-px are significantly different among fibroblasts experiments. The enzyme activities of UVA-irradiated cells with CoQ10-NLC added were greater than activities in the cells only treated with general CoQ10-emulsion. This suggests that CoQ10 may exert some protective effects on fibroblasts through enzyme systems, particularly SOD, which is in accordance with other clinical reports (Kalpravidh et al., 2005; Belardinelli et al., 2008).

However, cell defence systems would be exhausted if the UVA radiation is overdosed. Then, with the ROS attacking, plasma membrane fluidity decreases, gene expression is altered and fibroblasts undergo a pathway of apoptosis or necrosis. Oxidative damage results in depletion of glutathione and ATP as well as extensive peroxidation of lipids. These changes promote the onset of mitochondrial permeability transition, a common event in both types of cell death. It has been proposed that the damage generated by ROS in UVA-irradiated cells causes them to undergo apoptosis rapidly in the early stage. Soon after, some of the fibroblasts take the fate of necrosis (Basu-Modak et al., 2003). In this research, cell shrinking, nuclear fragmentation, and chromatin condensation have been observed and cell viability demonstrated by MTT assay. When CoQ10 was added, especially CoQ10-NLC, the apoptotic and necrotic rates of fibroblasts were significantly decreased in a dose-dependent manner after 24 h UVA-irradiation.

Because the CoQ10-NLC displayed such powerful anti-oxidative ability compared to the general CoQ10-emulsion despite the lipid-soluble property of CoQ10, the NLC formulation is considered to be the key to the efficient delivery of CoQ10. Recent studies have also reported that a nano drug delivery system is a new and potent drug transport pathway for the slow-release, special-targeting and efficient delivery of drugs (Hughes, 2005; Shah et al., 2007). Therefore, it was important and meaningful to study the skin penetration ability of CoQ10-NLC *in vivo*. As for the skin penetration experiment, the CoQ10-NLC group showed stronger fluorescence than the general CoQ10-emulsion group in rat dermis. All of these results clearly show that with the NLC delivery system, the lipid-soluble CoQ10 can sufficiently pass the epidermis barricade, penetrate to the dermis and exhibit greater anti-oxidative ability.

5. Conclusions

CoQ10-NLC significantly enhanced the anti-oxidative capacity of fibroblasts in which oxidative stress was induced by UVA-irradiation. CoQ10-NLC also improved skin penetration *in vitro* and *in vivo* compared to a non-particulate formulation at the same concentration. Considering the advantage of CoQ10-NLC in protecting fibroblasts from UVA-irradiation and its strong penetration ability, this formulation can be used in topical drug delivery. Due to the possibility that CoQ10 may act with Vitamin E to further enhance its anti-oxidative ability, enfolding these two factors or more is an interesting idea for future research (Lippa et al., 2000; Ognjanovic et al., 2006). This NLC offers an efficacious tool for the topical skin delivery of lipophilic drugs such as resveratrol and ceramide and could be widely used in cosmetic dermal products (Müller et al., 2007).

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