Coenzyme Q<sub>10</sub> Sunscreen Prevents Progression of Ultraviolet-Induced Skin Damage in Mice

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Abbreviations: CoQ10:Coenzyme Q10; DNMT1: DNA (cytosine-5)-methyltransferase 1; ECM: Extracellular matrix; GSH-Px: Glutathione peroxidase; H&E: Hematoxylin and Eosin; IL: Interleukin; MDA: Malondialdehyde; MMP-1: Matrix metalloproteinase-1; MMPs: Matrix metalloproteases; MPO: Myeloperoxidase; PLA2: Phospholipase A2; ROS: Reactive oxygen species; SD: Standard deviation; SOD: Superoxide dismutase; TiO2: Titanium dioxide; UV: Ultraviolet; UVA: Ultraviolet A radiation; UVB: Ultraviolet B radiation; VG: Van Gieson

SYNOPSIS

Background: The level of sunlight reaching the surface of the earth is increasing severely due to the rapid development of the society and environmental destruction. Excessive exposure to ultraviolet radiation causes skin damage and photoaging. Therefore, it has emerged that effective sunscreen to prevent ultraviolet-induced skin damage.

Objective: This study was aimed to investigate the effectiveness of coenzyme Q<sub>10</sub>(CoQ<sub>10</sub>) sunscreen on the prevention of ultraviolet B radiation (UVB)-induced skin damage.

Methods: 3-month-old female mice were used and randomly divided into four groups: control, model, CoQ<sub>10</sub>, and titanium dioxide (TiO<sub>2</sub>; positive control) groups. The control mice were not subjected to any treatment, and the other mice were administered with blank (model) or standardized sunscreen (CoQ<sub>10</sub> or TiO<sub>2</sub>) for 8 weeks and exposed to UVB light for 30 mins everyday. Biochemical assays, histomorphology, and measurement of matrix metalloproteinase-1 (MMP-1) and DNA (cytosine-5)-methyltransferase 1 (DNMT1) levels were performed in skin tissues.

Results: Our results showed that body weight, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, and DNMT1 protein expression were significantly decreased, while malondialdehyde (MDA) activity and MMP-1 level were increased in UVB-treated mice. In contrast, CoQ<sub>10</sub> sunscreen prevented UVB-induced skin damage, as well as reversing SOD, GSH-Px and MDA activities, MMP-1 and DNMT1 levels.

Conclusion: The current study provided further evidences on the prevention of UVB-induced skin damage by CoQ<sub>10</sub> and its underlying mechanisms.

Introduction

Skin is the largest barrier protecting from environmental risk factors that can result in skin aging. Skin aging can be categorized into intrinsic and extrinsic responses. The intrinsic skin aging occurs naturally as time passes [1], while extrinsic factors in skin aging are related with infection, water loss, and ultraviolet ray [2]. Even though only 5% of ultraviolet B radiation (UVB) light reaches the upper dermis of the skin, it is a key risk factor of extrinsic skin aging that affects dermal fibroblasts and skin microenvironment [3]. Collagen, a major component of extracellular matrix (ECM), is associated with extrinsic skin aging. Many studies have reported that collagen is degraded by matrix metalloproteases (MMPs),
including MMP-1, MMP-8, and MMP-13 [4,5]. In particular, MMP-1 is the predominant collagenase in the skin. Since wrinkle formation is evidenced by collagen degradation, the attenuation of MMP-1 activity is an important method for preventing skin aging [6,7]. On the other hand, skin aging is also associated with decreased activity of anti-oxidant enzymes [8].

The system of oxidant and anti-oxidant tends to be balance under normal conditions [9]. However, the levels of reactive oxygen species (ROS) would be produced excessively when the skin was exposed to ultraviolet ray [10,11]. Therefore, the scavenging capacity of the free radicals and the activities of anti-oxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), were reduced, while the amounts of free radicals, malondialdehyde (MDA), were increased. Coenzyme Q_{10} (CoQ_{10}) also known as ubiquinone) was found by Crane et al. in 1957 to be in the mitochondria of the beef heart, and broadly distributed in mammalian tissues [12,13]. CoQ_{10} is a necessary factor for healthy body, plays an important role in cardiovascular disorders and aging, including heart failure, hypertension, and endothelial dysfunction [14]. Growing evidences showed that CoQ_{10} also has a potential role for the prevention and treatment of heart ailments by improving cellular bioenergetics via scavenging free radicals [15]. With regard to ultraviolet A radiation (UVA)-induced skin aging, CoQ_{10} might be a useful preventive medication against skin photo-aging [16].

Among CoQ_{10}-loaded conventional carriers, ultra-small lipid nanoparticles-CoQ_{10} exhibited reduced capacity in free radical formation compared with non-nano carrier-treated cells. Therefore ultra-small lipid nanoparticles containing CoQ_{10} were shown to be suitable to increase the anti-oxidant capacity of the skin [17]. Moreover, CoQ_{10} significantly reduced the levels of myeloperoxidase (MPO), phospholipase A2 (PLA2) and MDA, while it increased SOD levels in vivo and in vitro [18]. A study found that CoQ_{10} might rejuvenate wrinkled skin through inhibiting the degradation of dermal fiber components and stimulating the paracrine of dermal fiber via up-regulation of interleukin (IL)-6 and MMP secretion [19]. As systemic delivery of anti-oxidants to the skin is poor, it may be beneficial to penetrate the skin with sufficient amount as topical application [20,21]. The present study explored the preventive effects of CoQ_{10} sunscreen against skin damage induced by UVB at topical application on mouse skin.

Materials and Methods

Materials

CoQ_{10} was provided by Runhe Biology Co. (Guangzhou, China), while titanium dioxide (TiO_{2}) was purchased from Kemao Chemical Co. (Dongguan, China). Ointment base was made by our laboratory, which contained purified water, petrolatum, Tween 80, cetostearyl alcohol, and did not contain any drug.

Animals and Treatments

This study was carried out according to the Guide for the Care and Use of Laboratory Animals of Guangdong Laboratory Animal Monitoring Institute, the National Laboratory Animal Monitoring Institute of China. All the procedures performed were in accordance with the ethical standards of the Academic Committee on the Ethics of Animal Experiments of Guangdong Medical University. 36 specific pathogen-free female Kunming mice were acclimated to local vivarium conditions (temperature 24-26°C, humidity 67%) and allowed to free access of water and diets containing 1.11% calcium, 0.74% phosphorus. The average weight of mice was about 27.66 g ± 0.56. CoQ_{10} sunscreen was composed of CoQ_{10} and ointment base at the concentration of 10 mg/g. TiO_{2} sunscreen was used as a positive control, and composed of TiO_{2} and ointment base at the concentration of 50 mg/g [18,19]. Mice were randomly divided into four groups: control group (n=9), aging model group (ointment base without additives; n=9), CoQ_{10} group (CoQ_{10} sunscreen; n=9), and TiO_{2} group (TiO_{2} sunscreen; n=9). The hair on the back of each mouse was shaved, and 0.5 g of ointment was topically applied to 3 × 3 cm^2 of the skin once daily for 8 weeks. Except the control group, the other groups were exposed to ultraviolet B radiation (UVB; 303 nm and 1522.7 μW/cm^2) under diffused UV light (Sentry Optronics CORP, Taiwan) for 30 mins everyday.

Sample Collection

All the mice were weighed weekly. At the end of the treatment, the mice were sacrificed and blood was collected from the eyeballs. Firstly, the serum was collected from the blood by centrifugation, and it was used for biochemical assays. And then, the dorsal skin, heart, liver, kidney, and brain were isolated, weighed, and it was used for biochemical assays. And then, the dorsal skin tissues were immediately collected for histological, biochemical, and quantitative real-time PCR analyses.

Biochemical Analysis

The degree of skin damage exposed to UVB could be determined by evaluating the activities of MDA, SOD, and GSH-Px [22]. They are the most frequently used biomarkers of oxidative stress (imbalance between oxidant and anti-oxidant systems) in the skin tissues. MDA levels in the dorsal skin were measured using a MDA detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. The activities of SOD and GSH-Px in the skin tissues were detected using a commercial kit (Nanjing Jiancheng Bioengineering Institute).

Histological Analysis

Each part of the skin samples (1×1 cm^2) was fixed with 4% paraformaldehyde for Van Gieson (VG), and hematoxylin and eosin
(H&E) staining. H&E staining was used to assess the skin structure alteration, while VG staining was applied to detect the presence of collagen fibers. All the stained skin specimens were observed and photographed using an optical microscope (Nikon Eclipse Boi, Nokon Corporation, Japan).

**Quantitative Real-Time PCR**

Total RNA was extracted from the dorsal skin tissues using Trizol reagent (TaKaRa Bio, Otsu, Japan) as recommended by the manufacturer. Total RNA was reverse transcribed to cDNA using a commercial kit (Takara Bio, Otsu, Japan) according to the protocol of the manufacturer. Target genes were amplified with SYBR® Premix Ex Taq™ (Takara Bio, Otsu, Japan) using a PikoRel 96 Real-Time PCR System (Thermo Fisher Scientific, Vantaa, Finland). The sequences of the forward and reverse primer sets (Shenggong Bio. Co., Shanghai, China) were shown in Table 1. To confirm the specificity of the amplification, PCR products were evaluated by melting curve analysis. mRNA expression was determined based on the cycle threshold values, which were normalized to that of β-actin, and calculated using the 2−ΔΔCT method [23].

Table 1: Primer sequences for quantitative real-time-polymerase chain reaction.

| Gene       | Forward primer sequences (5' to 3') | Reverse primer sequences (5' to 3') |
|------------|-------------------------------------|------------------------------------|
| β-actin    | GCCAACCGTGAAGATGAC                  | ACGAGGACTACAGGGACAG                |
| MMP-1      | CCCAAATCCCATCCAGGCAA                | ATAAATGAGCTCAGGTCG                |
| GC         |                                     |                                    |

**Western Blotting**

The total protein was extracted from the dorsal skin tissues with ice-cold lysis buffer. The protein concentrations of the lysates were measured by the bicinchoninic acid kit (Pierce, France). Equal amounts of proteins were used and separated by SDS-PAGE gels, and were then transferred onto the nitrocellulose membranes. Next, the membranes were incubated with the primary DNMT1 antibody (Cell Signaling, USA), and anti-rabbit-HRP antibody (Cell Signaling, USA). The blots were developed by enhanced chemiluminescence (GE Healthcare Life Sciences, USA) with aChemiDoc™ MP System (Bio-Rad Laboratories, USA). α-tubulin antibody (Cell Signaling, USA) was used as a housekeeping control.

**Statistical Analysis**

The results were expressed as mean ± standard deviation (SD). The data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences between groups was evaluated by one-way or two-way ANOVA, and p<0.05 was considered statistically significant.

**Results**

**CoQ10 Sunscreen Altered Anti-Oxidant Enzyme Activities in UVB-Treated Skin**

We investigated the activities of anti-oxidant enzymes in UVB-treated skin in response to CoQ10 sunscreen treatment. Compared with control group, the MDA activity was increased in the dorsal skin of the model group (Figure 2). Interestingly, the MDA activity was significantly decreased in CoQ10 and TiO2 groups compared with the model group. Moreover, the activities of T-SOD and GSH-Px were decreased in the dorsal skin of the model group compared with control group (Figure 2). The reductions of these activities were significantly attenuated in CoQ10 group compared to the model group. Similarly, TiO2 group, as a positive control, also slightly attenuated these reductions compared to the model group (Figure 2).

**CoQ10 Sunscreen Protected Epidermis in UVB-Treated Skin**

To investigate the effect of CoQ10 sunscreen on the epidermis in UVB-treated skin, H&E staining was used, and the thickness of the dermis was also assessed. Our results showed that the epidermis of control mice was unbroken and its corneum was not shed, but the epidermis of model mice was injured and its corneum was shed obviously (Figure 3). Compared with model group, there were no differences in CoQ10 and TiO2 groups. Interestingly, the skin of CoQ10 mice was not injured and its corneum was not shed, but the skin of TiO2 mice appeared to be a little bit broken. Furthermore, the corneum of TiO2 mice was shed slightly, but it looked healthier than that of model mice. In addition, the thickness of the dermis in the model mice was decreased compared with control mice, and this reduction in thickness was significantly attenuated in CoQ10 mice (Figure 3).
Figure 1: Body and organ weight changes with Coenzyme Q10 (CoQ10) sunscreen treatment in response to ultraviolet B radiation (UVB). The weights of
a) Body,
b) Liver, and
c) Heart
in response to UVB. CON, control group without exposing to UVB; MOD, model group with ointment base exposed to UVB; CoQ10, treatment with CoQ10 sunscreen exposed to UVB; TiO2, positive control with titanium dioxide (TiO2) sunscreen exposed to UVB. n≥3 for each group. Results were shown as mean± SD. *p<0.05 vs. CON, ***p<0.001 CON, CoQ10, TiO2 vs. MOD.

Figure 2: The anti-oxidant enzyme activities were altered by Coenzyme Q10 (CoQ10) sunscreen treatment in ultraviolet B radiation (UVB)-treated skin. The activities of
a) Malondialdehyde (MDA),
b) Superoxide dismutase (SOD), and
c) Glutathione peroxidase (GSH-Px)
in the UVB-treated skin tissue in response to CoQ10 sunscreen treatment. CON, control group without exposing to UVB; MOD, model group with ointment base exposed to UVB; CoQ10, treatment with CoQ10 sunscreen exposed to UVB; TiO2, positive control with titanium dioxide (TiO2) sunscreen exposed to UVB. n≥3 for each group. Results were shown as mean ± SD. *p<0.05 vs. MOD.

CoQ10 Sunscreen Prevented Degradation of Collagen in UVB-Treated Skin

In order to investigate the effect of CoQ10 sunscreen on collagen degradation in UVB-treated skin, Van Gieson staining was performed. As shown in Figure 4, we found that the collagen fibers of control mice was deposited neatly, while there were
less collagen fibers in model mice they rowed irregularly. Moreover, its corneum was shed obviously in model group. Compared with model group, the skin of CoQ$_{10}$ mice was not injured and its collagen fibers rowed regularly. However, the skin of TiO$_2$ mice appeared to be a little bit broken, its corneum was shed slightly, and its collagen fibers rowed regularly.

![Figure 3](image-url)

**Figure 3:** Coenzyme Q10 (CoQ10) restored ultraviolet B radiation (UVB)-induced damage in the epidermis of the skin.

- **a)** Hematoxylin and Eosin (H&E) staining of the epidermis and dermis on the skin. The picture was captured at 10× magnification using an electron microscope.
- **b)** The thickness of the dermis was measured in response to CoQ10 treatment.

CON, control group without exposing to UVB; MOD, model group with ointment base exposed to UVB; CoQ10, treatment with CoQ10 sunscreen exposed to UVB; TiO$_2$, positive control with titanium dioxide (TiO$_2$) sunscreen exposed to UVB. n≥3. Results were shown as mean ± SD. *p<0.05 vs. MOD.

**CoQ$_{10}$ Sunscreen Modulated MMP-1 Expression in UVB-Teated Skin**

Skin collagen degradation is mainly regulated by MMP-1 [24]. We found that the mRNA level of MMP-1 of model group was increased compared with control group (Figure 5). CoQ$_{10}$ treatment significantly attenuated this up-regulation of MMP-1 level induced by UVB. Conversely, the MMP-1 level was not decreased in TiO$_2$ group compared with the model group.

**CoQ$_{10}$ Sunscreen Prevented DNMT1 Down-Regulation in UVB-Treated Skin**

DNMT1 was shown to be associated with UV-induced photo-aging [25]. Next, we investigated the effect of CoQ$_{10}$ sunscreen on DNMT1 protein expression in UVB-treated skin. Our results showed that DNMT1 expression was decreased in the model group compared to control group (Figure 6). Besides, CoQ$_{10}$ and TiO$_2$ sunscreen treatment significantly suppressed this down-regulation induced by UVB.

**Discussion**

Skin is regarded as the first line of defense against infection and environmental factors such as ultraviolet (UV) radiation. Sunlight is the main source of UV radiation, which can induce skin senescence, inflammation, aging and cancer [26]. Therefore protecting the skin with sunscreen is very important to avoid skin damage. CoQ$_{10}$ was shown to be an anti-oxidant molecule which could prevent UV-induced DNA damage [19]. In this study, we further investigated the preventive effects of CoQ$_{10}$ sunscreen on UVB-induced skin damage in mice, and its underlying mechanisms. In this study, we found that mouse skin was damaged by UVB. This was shown by the decrease in growth rate of body weight and liver weight in UVB-treated mice. Besides, UVB decreased the activities of SOD and GSH-Px, and increased MDA activity in the mouse skin. This suggested that the balance between oxidant and anti-oxidant systems was impaired when mouse skin was exposed to UVB only. During the aging process, the skin dermis becomes thin and damaged [27], due to the degradation of the collagen matrix [28,29].
Figure 4: Coenzyme Q10 (CoQ10) sunscreen restored collagen degradation in ultraviolet B radiation (UVB)-treated skin. Van Gieson staining was used to detect collagen on the skin. The picture was captured at 10× magnification using an electron microscope. CON, control group without exposing to UVB; MOD, model group with ointment base exposed to UVB; CoQ10, treatment with CoQ10 sunscreen exposed to UVB; TiO2, positive control with titanium dioxide (TiO2) sunscreen exposed to UVB.

Figure 5: Coenzyme Q10 (CoQ10) sunscreen altered MMP-1 mRNA level in ultraviolet B radiation (UVB)-treated skin. The MMP-1 level of mouse skin was measured by real-time PCR. CON, control group without exposing to UVB; MOD, model group with ointment base exposed to UVB; CoQ10, treatment with CoQ10 sunscreen exposed to UVB; TiO2, positive control with titanium dioxide (TiO2) sunscreen exposed to UVB. n≥3. Results were shown as mean ± SD. **p<0.01 vs. MOD.
We also showed that the corneum of mouse skin that was exposed to UVB was shed through regulating collagen via up-regulation of MMP-1 expression. Taken together, our mouse UVB model could be a suitable model for skin aging. In the current study, we demonstrated that topical application of CoQ10 sunscreen could alleviate the alterations of collagen in the mouse skin induced by UVB, and the corneum of mouse skin was not shed. MMP-1 expression has been shown to be increased with age, which is a major factor that causes collagen breakdown and wrinkling problems [30,31]. Indeed, aging is the primary consequence of aerobic metabolism, which produces excess ROS and exceeds the capacity of cellular anti-oxidant defense [32]. Therefore, oxidants are important mediators of aging [33]. In fact, ROS production is also related to age-associated up-regulation of MMP-1 [27]. Interestingly, we showed that CoQ10 sunscreen treatment could inhibit MMP-1 up-regulation and collagen degradation induced by UVB in the mouse skin. Similarly, CoQ10 sunscreen was also shown to reduce MMP-1 levels in dermal fibroblasts [19].

Moreover, anti-oxidant enzymes in the skin, including SOD and GSH-Px, can counteract ROS [34]. Our results showed that the activities of SOD and GSH-Px were significantly increased by CoQ10 sunscreen in the mouse skin. Furthermore, MDA is a biomarker of cell membrane damage caused by free radicals [35]. We found that CoQ10 sunscreen could attenuate MDA activity induced by UVB. Taken together, this suggested that CoQ10 sunscreen might have anti-oxidant activities against UVB damage in mouse skin, and prevented collagen degradation by suppressing MDA activity and MMP-1 levels, and enhancing SOD and GSH-Px activities. In summary, our findings indicated that topical application of CoQ10 sunscreen prevented UVB-induced skin damage by enhancing the anti-oxidative capacity against photo-aging skin and delay the breakdown of collagen through suppressing MMP-1 level and MDA activity in the mouse skin. Therefore, we suggested that CoQ10 sunscreen might have beneficial effects in anti-aging, and topical application of CoQ10 sunscreen could be potential protection against UVB-induced photo-aging.

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Conflict of interest

The authors declare that there is no competing conflict of interests.
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