ANTI-AGING EFFECTS OF UBIQUINONE-10 (CoQ-10) ON HUMAN SKIN CELLS

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Ubiquinone-10, MMP-1, ROS, UVA, anti-oxidant, anti-aging

Synopsis

Recently several studies have indicated that ubiquinone-10 (CoQ-10) decreases with aging in various organs of the human body. The decrease of ubiquinone-10 in the aging process suggests the reduction of both energy production in mitochondria and anti-oxidation with aging. Based on these findings, Ubiquinone-10 is being focused at as an anti-aging agent in the cosmetic market.

The aim of this study is to clarify the mechanisms of CoQ-10' s activity as anti-aging agent, improvement or prevention of wrinkles, using cell culture system. In general, it is authorized that oxidative stress including UVB, UVA and reactive oxygen stress, is the initiator of aging process. Therefore, in the first step, we evaluated the anti-oxidative profile of CoQ-10 using human fibroblasts and electron spin resonance spectrometry (ESR) spin-trapping method. CoQ-10 showed reducing effects of cell damage against oxidative stress including UVA, UVB and H_2O_2, and successfully quenched singlet oxygens. Also, CoQ-10 decreased DNA damage induced by UVB. From these results, it was indicated that CoQ-10 effectively worked as an ant-oxidant in biological system. In addition, CoQ-10 significantly suppressed the expression of MMP-1 of UVA-irradiated fibroblasts.

These results suggested that ubiquinone-10 can act as an anti-aging agent through the reduction of damage induced by UV or ROS and the suppression of UVA-induced MMP-1 production.

Introduction

Recent advance of medical assistance and equipment has resulted in worldwide increase of elderly population. This tendency caused growing demand for effective skin care agents and supplements to maintain health and beauty. Recently, ubiquinone-10 (CoQ-10) is being focused at as an agent with anti-aging properties (Fig.1). CoQ-10 is known to increase from childhood to maturity and to decrease again significantly in old age (1). It is well known that CoQ-10 plays a critical role in ATP production of mitochondria (2). Therefore, we can suggest that decrease of CoQ-10 with age is one of important factors responsible for lack of ATP production following physiological aging. Furthermore, the reduced form of CoQ-10 exhibits the potential for anti-oxidative activity (3). The skin is located at the surface of the human body, thus, it is strongly affected by environmental

oxidative stress (UV light and reactive oxygen species (ROS) originated by UV light). The decrease of CoQ-10 in the skin following aging causes serious problem in the skin such as the accelerating of photoaging. Hoppe *et al.* has reported that the topical application of CoQ-10 for long term gave excellent results on both improving wrinkles and the turnover rate of the epidermis (4).

These findings are expected to prove the potential of CoQ-10 as an anti-aging agent. The aim of this study was to clarify the potential of CoQ-10 on the prevention of wrinkle formation. We reported in this study that CoQ-10 prevented the wrinkle formation due to its excellent anti-oxidative properties.

Materials and Methods

Cell culture

Human normal dermal fibroblasts (HNDF) were purchased from Kurabo Co. Ltd. (Japan), and cultured with Dulbecco's modified Eagles medium (DMEM) containing 5% fetal bovine serum (FBS).

Reduction of cell damage induced by oxidative stress

To demonstrate the effects of CoQ-10 on anti-oxidation, it was examined whether CoQ-10 can reduce cell damage in cells exposed to oxidative stress, UVB, UVA and hydrogen peroxide (H_2O_2). HNDFs were inoculated in a 96-well plate at a density of 2 x 10^4 cells / well. After 24 hour pretreatment with CoQ-10 at several concentrations, cells were exposed to UVB (30 mJ/cm^2) or UVA (15 J/cm^2) in Hank's buffered solution (HBS). Then, the viability of the cells was evaluated with neutral red assay after 24 hour cultivation. In a case of H_2O_2 exposure, cell viability was estimated by neutral red assay at 2 h after exposing to H_2O_2 (2 mM).

Reduction of DNA damage in cells exposed to UVB

DNA damage was assessed with comet assay. HNDFs were inoculated in a 96-well plate at a density of 2 x 10^4 cells / well. After 24 hour pretreatment with CoQ-10 at several concentrations, cells were exposed to UVB (15 mJ/cm^2) in HBS. At 2 h cultivation after UVB irradiation, the single cell gel electrophoresis assay (comet assay) was carried out (5).

Scavenging of ROS by electron spin resonance spectrometry (ESR) spin-trapping method (6)

To evaluate the scavenging profiles of CoQ-10 against ROS (superoxide anion radical, hydroxyl radical, lipid peroxyl radical and singlet oxygen), ESR spin-trapping test was performed. As spin-trapping reagents we used 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO) for superoxide anion radical, hydroxyl radical and lipid peroxyl radical and 2,2,6,6-*tetra*-methyl piperidone hydrocloride (TMPD).

Suppression of Matrix metalloprotanase-1 (MMP-1) production by UVA

HNDFs were inoculated in a 96-well plate at a density of 2 x 10^4 cells / well. After 24 hour pretreatment with CoQ-10 at several concentrations, cells were exposed to UVA (15 J/cm^2) in HBS. After 24 hour cultivation, MMP-1 secreted into the cultured medium was detected by western blotting method.

Results and Discussion

Anti-oxidative effects of CoQ-10

To address the anti-oxidative effects of CoQ-10 in biological system, the effects were examined with HNDF culture system. CoQ-10, which was pre-treated to HNDFs, showed the significant protection against cell damage induced by UVB (Fig.2), UVA (Fig.3) and H_2O_2 (Fig.4). In chemical aspect, the anti-oxidative effects of CoQ-10 (the scavenging abilities against ROS) were measured with ESR spin-trapping method. CoQ-10 did not show any scavenging features against superoxide anion radical, hydroxyl radical and lipid peroxyl radical. However, it was found that CoQ-10 quenched singlet oxygens (Fig. 5).

In general, the anti-oxidative effects of CoQ-10 are originated to its reduced form, ubiquinol-10. It has been authorized that UVB mainly produced superoxide anion radicals by stimulation of mitochondorial respiratory chain reaction (7). Superoxide anion radical spontaneously converts to H_2O_2, and then hydroxyl radical in the presence of metal ions, Fe^{2+} and Cu^{+} (8). We already indicated that the substance of cell damage during the H_2O_2 exposing was hydroxyl radical. From both biological and chemical test results for UVB, H_2O_2 or hydroxyl radical, it was suggested that exogenous CoQ-10 well converted to the reduced form after uptake into cells.

In addition, CoQ-10 showed the suppression of DNA damage in cells exposed to UVB (Fig.6). There are two types of DNA damage caused by UV (UVA and UVB). By absorbing of UVB to DNA molecule, DNA causes photochemical reactions, and yields dimmers such as cyclo-butane type and 6,4-adduct type. On the other hand, DNA is damaged by ROS (hydroxyl radical and singlet oxygen) generated in cells by UVB or UVA. The results of comet assay suggested that CoQ-10 suppressed the DNA damage caused by ROS due to the anti-oxidative property of its reduced form.

Suppression of MMP-1 production

In the process of wrinkle formation, it was acknowledged that the alterations of collagen's amount and character were critical factors. The UV (UVB and UVA) irradiation of the skin caused the alteration in metabolism of collagen matrix. UVA irradiated-fibroblasts showed the decrease of collagen production and the increase of MMP-1 production (9). The repeated reactions caused significant decrease of collagen in the dermis, and led to the formation of very fragile dermis and wrinkles. It was found that CoQ-10 suppressed the excess production of MMP-1 after UVA irradiation (Fig.7). The up-regulating mechanism of MMP-1 by UVA has been studied very well to conclude that singlet oxygen produced into cells by UVA irradiation up-regulated MMP-1 gene transcription through the cytokine network of IL-1 and IL-6 (10). Our results already indicated that CoQ-10 itself quenched singlet oxygen by ESR spin-trapping method. Also, CoQ-10 reduced the damage of NHDFs which were exposed to UVA. The results suggested that exogenous CoQ-10 suppressed the MMP-1 production due to the quenching of singlet oxygen.

Conclusion

It has been authorized that endogenous or exogenous oxidative stress is a critical factor in

the aging process. In the study, it was elucidated that exogenous CoQ-10 showed excellent anti-oxidative effects and suppressed the excess MMP-1 production after UVA irradiation. In the process of wrinkle formation, MMP-1 plays an important role in the decrease of collagen. Our results demonstrated that exogenous CoQ-10 was the effective agent for anti-aging on the following points. Exogenous CoQ-10, which converted well to its reduced form, effectively works as an anti-oxidant, and suppresses the UVA-induced MMP-1 expression. It has been well studied that endogenous CoQ-10 decreased with aging in several organs of human being including the skin and the decrease of endogenous CoQ-10 is responsible for the reduction of energy production following aging.

Basing on these findings, CoQ-10 can be expected to act as a new anti-aging agent through the recovering of the energy production and the potent anti-oxidative properties.

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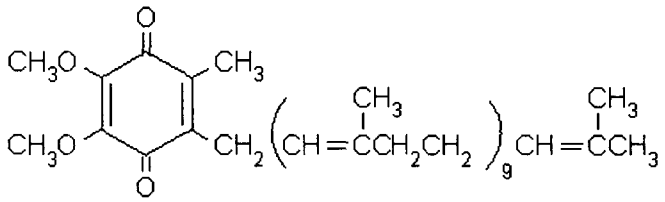

Fig. 1 Chemical structure of ubiquinone-10 (CoQ-10)

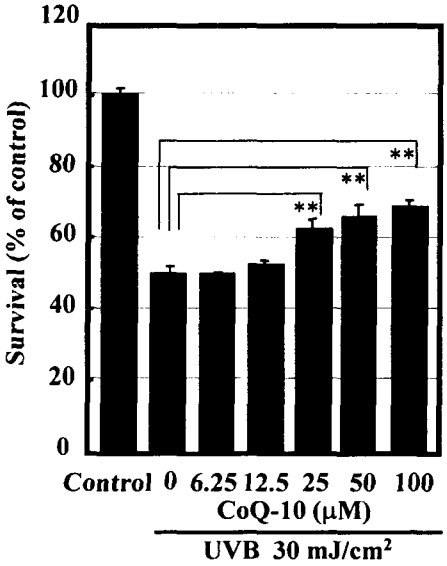

Fig. 2 Proteictive effects of CoQ-10 on cell damage induced by UVB After 24 h pretreatment with CoQ-10 at several concentrations, HNDFs were exposed to UVB (30 mJ/cm^2) in Hank's buffered solution (HBS). Then, the viability of the cells was evaluated with neutral red assay after 24 hour cultivation. Siginificance * $p<0.05$, ** $p<0.01$

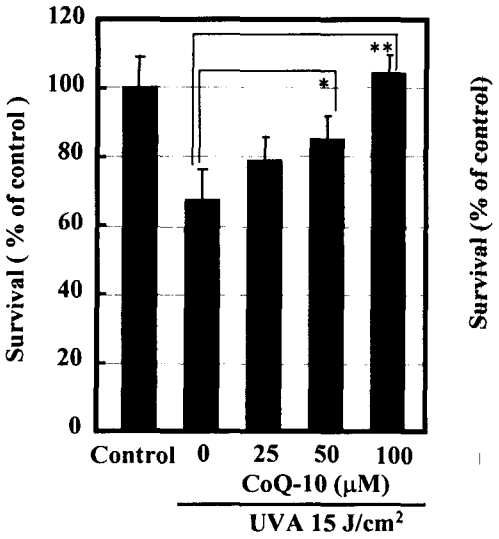

Fig. 3 Proteictive effects of CoQ-10 on cell damage induced by UVA After 24 h pretreatment with CoQ-10 at several concentrations, HNDFs were exposed to UVA (15 J/cm^2) in Hank's buffered solution (HBS). Then, the viability of the cells was evaluated with neutral red assay after 24 hour cultivation. Siginificance * $p<0.05$, ** $p<0.01$

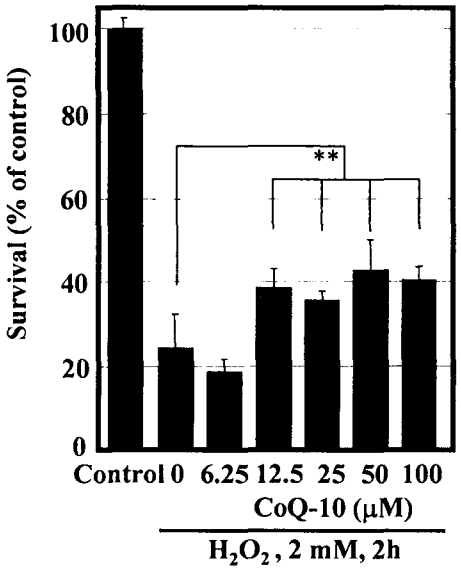

Fig. 4 Proteictive effects of CoQ-10 on cell damage induced by H_2O_2 After 24 h pretreatment with CoQ-10 at several concentrations, HNDFs were exposed to H_2O_2 for 2 h in Hank's buffered solution (HBS). Then, the viability of the cells was evaluated with neutral red assay. Siginificance * $p<0.05$, ** $p<0.01$

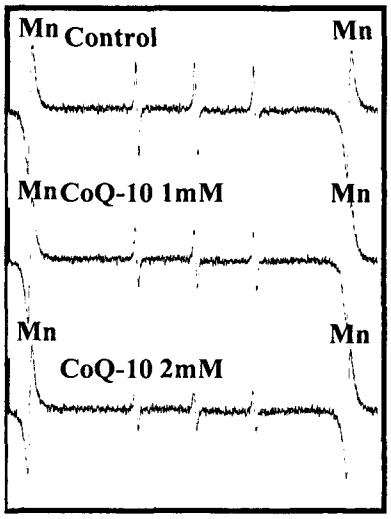

Fig. 5 Quenching ability of CoQ-10 against singlet oxygen by ESR spin-trapping method ESR spin-trapping test was performed. As spin-trapping reagents we used 2,2,6,6-*tetra*-methyl piperidone hydrocloride (TMPD).

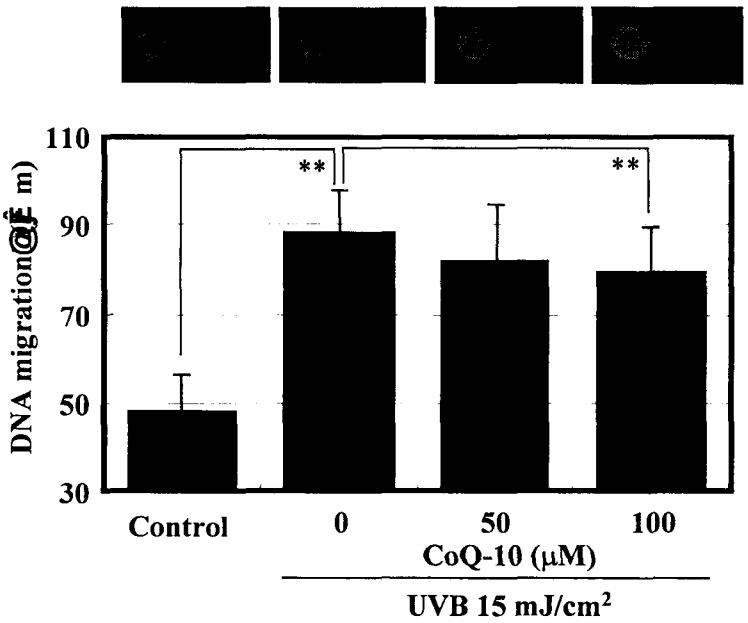

Fig. 6 Reduction of DNA damage in cells exposed to UVB DNA damage was assessed with comet assay. After 24 hour pretreatment with CoQ-10 at several concentrations, HNDFs were exposed to UVB (15 mJ/cm^2) in HBS. At 2 h cultivation after UVB irradiation, the single cell gel electrophoresis assay (comet assay) was carried out. Significance ** $p<0.01$.

Control @0@@ @@25@@@50

CoQ-10@(μM)

UVA@15 J/cm^2

Fig. 7 Suppression of Matrix metalloprotanase-1 (MMP-1) production by UVA

After 24 hour pretreatment with CoQ-10 at several concentrations, HNDFs were exposed to UVA (15 J/cm^2) in HBS. After 24 hour cultivation, MMP-1 secreted into the cultured medium was detected by western blotting method.