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Coenzyme Q₁₀, a cutaneous antioxidant and energizer

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Abstract. The processes of aging and photoaging are associated with an increase in cellular oxidation. This may be in part due to a decline in the levels of the endogenous cellular antioxidant coenzyme Q₁₀ (ubiquinone, CoQ₁₀). Therefore, we have investigated whether topical application of CoQ₁₀ has the beneficial effect of preventing photoaging.

We were able to demonstrate that CoQ₁₀ penetrated into the viable layers of the epidermis and reduce the level of oxidation measured by weak photon emission. Furthermore, a reduction in wrinkle depth following CoQ₁₀ application was also shown. CoQ₁₀ was determined to be effective against UVA mediated oxidative stress in human keratinocytes in terms of thiol depletion, activation of specific phosphotyrosine kinases and prevention of oxidative DNA damage. CoQ₁₀ was also able to significantly suppress the expression of collagenase in human dermal fibroblasts following UVA irradiation. These results indicate that CoQ₁₀ has the efficacy to prevent many of the detrimental effects of photoaging.

1. Introduction

The skin is the body's largest organ with an area of approximately 2 m². One of its functions is to protect the body from a hostile environment of toxins, pathogens and UV radiation. UVA is absorbed by a number of molecules in the skin including flavinoids and pheomelanin which initiate the formation of reactive oxygen species within cells. These reactive oxygen species include hydrogen peroxide, singlet oxygen, and hydroxyl radicals, which are the most abundant. The radicals produce oxidative damage to lipids, proteins and DNA [1,2]. To be able to cope with oxidative stress produced by UV light and endogenous metabolism, the skin has both enzymatic and non-enzymatic (antioxidant) mechanisms for protection [27,30]. Examples of enzymes involved in preventing radical damage include superoxide dismutase (SOD), catalase, and glutathione peroxidase. Antioxidants found in the skin include vitamin E, coenzyme Q₁₀ (ubiquinone, CoQ₁₀) and ascorbate [27]. Members of the coenzyme Q family have developed together with biological evolution over millions of years (Fig. 1). CoQ₁₀ is ubiquitous in human tissues, although its level is variable. The level of CoQ₁₀ is highest in organs with high rates of metabolism such as the heart, kidney and liver (114, 66.5 and 54.9 μg/g tissue, respectively), where it functions as an energy transfer molecule [22]. In skin CoQ₁₀ also act as an antioxidant, with 10-fold higher levels in the epidermis than the dermis [27]. From our own data the level of reduced and oxidized CoQ₁₀ from the forearm of a 44 year old male is approximately 300 pmol/cm² or 0.26 μg/cm². Although the epidermis

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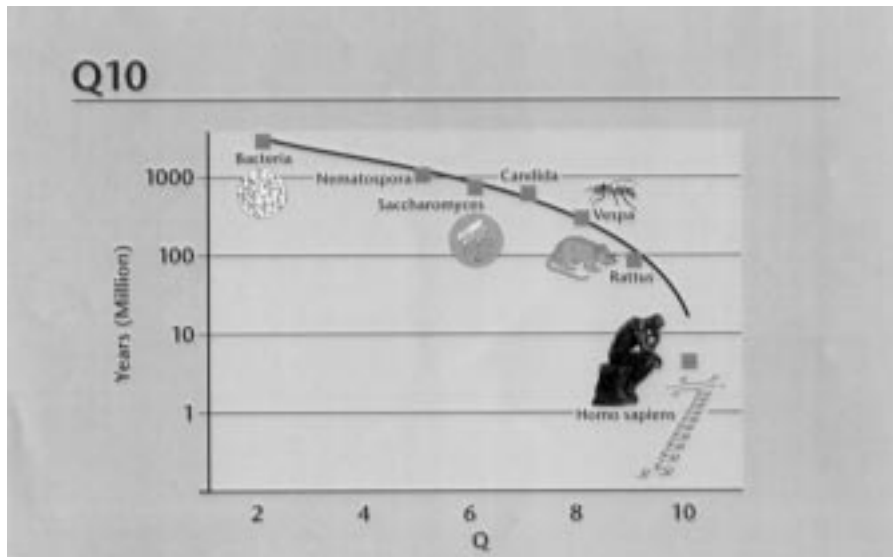


Fig. 1. In general as organisms have evolved the structure of the coenzyme molecule has increased in length from 2–10 isoprene units. The correlation coefficient between the evolutionary age of an organism and its coenzyme CoQ form is 0.992.

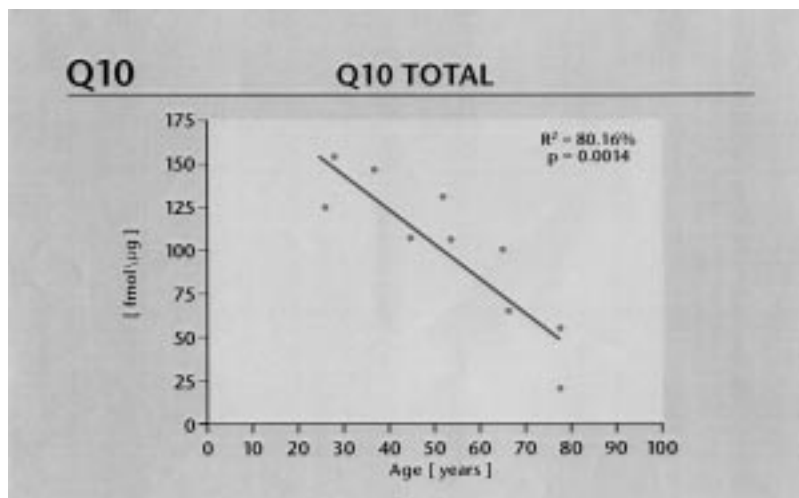


Fig. 2. CoQ₁₀ levels decrease in the epidermis between the ages of 30 and 80 years ($R^2 = 0.802$, $p = 0.0014$). Values are normalized to the cholesterol content of the samples.

is composed mainly of cells, the amount of CoQ₁₀/g of tissue is relatively low. Furthermore, the level of CoQ₁₀/μg cholesterol declines between the ages of 30 and 80 years of age ($R^2 = 0.79$, $p = 0.0012$) (Fig. 2), as it has been reported in organs such as heart and brain [14,29]. The epidermis is therefore a tissue that would potentially benefit from exogenously supplied CoQ₁₀.

Oxidative stress is thought to play a role in the aging process [6]. In the skin there is both chronological and photoaging, although they are two distinct processes, the effects are superimposed on each other [9]. Chronological aging is inherent and genetically determined and possibly with some hormonal influence [23,28]. There are numerous theories as to the cause of chronological aging [33]. One of the

most compelling theories is that of telomere shortening, where aging cells progressively lose part of the DNA at the ends of chromosomes in each cell division [19]. Photoaging, on the other hand, is caused by UVA and UVB. Due to the penetration characteristics of the UV light UVA and UVB have different effects. UVA is able to penetrate further into the dermis whereas UVB penetrates only just through the epidermis. Oxidative stress may result in DNA damage and malignant changes in the epidermis and disorganization in the dermal matrix [16,20]. Photoaged skin is characterized by wrinkles, and lack of tensile strength, which is normally provided by the dermis. In the dermal matrix of aged skin there is an increase in the levels of elastin and a decrease in the levels of glycosaminoglycans and collagen I and III [4]. There is also an increase in collagenase expression which further reduces the levels of collagen. The cumulative result is that the dermis can no longer provide the structural support and elasticity it once had due to the disorganization of the collagen fibers.

There were three aims of this study: (1) To determine if CoQ₁₀ is an antioxidant in cultured skin cells, (2) to demonstrate that CoQ₁₀ can act as an antioxidant when topically applied to human skin, and (3) to investigate whether CoQ₁₀ can prevent or reverse the effects of photoaging.

2. CoQ₁₀ prevents oxidative effects in cultured human skin cells

In all of the following experiments CoQ₁₀ produced by fermentation was used (Kaneka, Japan), which is conformationally identical to human CoQ₁₀. To demonstrate that CoQ₁₀ is an effective antioxidant, either directly or indirectly, we first measured the levels of phosphotyrosine kinase activity and glutathione levels, which are two indicators of oxidative stress in human keratinocytes [8,18,24]. When keratinocytes are exposed to 1 mM hydrogen peroxide for 30 min the activity of the phosphotyrosine kinase increases. This can be significantly suppressed by 150 μ M CoQ₁₀. Conversely glutathione levels decrease by approximately 20% compared to the level of unstressed cells when treated with 1 mM hydrogen peroxide for 30 min. CoQ₁₀ (5–50 μ M) increases glutathione levels in unstressed keratinocytes in a dose responsive manner by approximately 10–20%. Pretreatment of keratinocytes for 24 hours with 50 μ M CoQ₁₀ is able to maintain glutathione levels at about the level found in non-stressed cells. UVA irradiation also reduces the mitochondrial membrane potential [31]. 0.3% CoQ₁₀ has the beneficial effect of suppressing the reduction of the mitochondrial membrane potential following UVA irradiation (20 J/cm²) in fibroblasts from both young and old donors. These experiments demonstrate that CoQ₁₀ can act as an antioxidant in cultured human cells from young or old donors.

To demonstrate that CoQ₁₀ can protect the keratinocytes from UV induced oxidative DNA damage we used the COMET assay [21]. In the comet assay cells are embedded in an agarose gel, irradiated with 5 J/cm² UVA and then treated with alkaline to lyse the cells and unwind the DNA. During electrophoresis the damaged DNA migrates out of the nucleus, and when stained, the DNA looks like a comet. The length of the tail is related to the level of oxidative DNA damage. Using the comet assay we can clearly demonstrate that 24 hours pretreatment with 23 μ M CoQ₁₀ can protect, either immortalized HaCaT keratinocytes, or primary human keratinocytes from UVA induced oxidative DNA damage. The 60–70% decrease in DNA damage is statistically very significant.

3. CoQ₁₀ protects dermal fibroblasts from chronological and photoaging

We next investigated the effects of CoQ₁₀ on dermal matrix synthesis. Chronologically aged skin has lower levels of hyaluronic acid and the collagen fibers are clearly disorganized. Hyaluronic acid is

a glycosaminoglycan comprising alternating D-glycuronic acid and N-acetyl-D-glucosamine residues. Hyaluronic acid has a large hydrated volume which regulates hydration in the dermis and favors a dispersed hydrated organization of collagen fibers. Chronological aging can be mimicked in cell culture. When cells are cultured for a prolonged period they exhibit many of the characteristics of chronologically aged cells [7,15]. Using human fibroblasts that were treated in this way we were able to demonstrate that 50 μ M CoQ₁₀ significantly increased levels of radiolabelled glycosaminoglycan when expressed as the amount of radiolabelled glycosaminoglycan/mg of cell protein. 50 μ M CoQ₁₀ also increased the proliferation of these artificially aged cells by approximately 20%, as measured by the level of radiolabelled thymidine incorporation expressed as per mg of cellular protein.

Another factor contributing to the disorganization of the dermal matrix in photoaging is the degradation of collagen fibers by the enzyme collagenase [26]. Collagenase is produced by the dermal fibroblasts in response to UVA in a dose dependent manner. The expression of collagenase mRNA induced by UVA can be significantly reduced by pretreatment with antioxidants such as vitamin E and CoQ₁₀. 10 μ g/ml CoQ₁₀ is able to reduce collagenase mRNA expression by 50%. CoQ₁₀ (11 μ M) is as effective as 3 mM vitamin E. CoQ₁₀ can also suppress collagenase expression over a longer period of time (6 weeks) with weekly irradiations. These experiments demonstrate that CoQ₁₀ can significantly reduce the detrimental effects of UVA on dermal fibroblasts which maintain the dermal matrix.

4. Topically applied CoQ₁₀ penetrates into the skin

To be able to act as an antioxidant in the skin CoQ₁₀ needs to penetrate into the living layers. The outermost layer of the skin, called the stratum corneum, acts as an effective barrier to many compounds. To determine whether CoQ₁₀ can penetrate into the skin we have used porcine skin, which is very similar to human skin in terms of permeability, and HPLC to quantify levels of CoQ₁₀. Due to the skin not being viable we can measure penetration without the complication of further metabolism of CoQ₁₀. Application of CoQ₁₀, in ethanol as a vehicle, results in penetration into the stratum corneum, with approximately 20% penetrating further into the viable layers of the epidermis, and 2% into the dermis. This data demonstrates that CoQ₁₀ is able to penetrate into the living cell layers in a simple ethanol vehicle.

5. CoQ₁₀ acts as an antioxidant in human skin

Oxidative events in the skin *in vivo* can be detected by means of ultra weak photon emissions [25]. In the basal state, cells emit low levels of photons. When UVA irradiation is applied there is an excited state with a large increase in the level of photons which decays with time. The level of photons emitted is an indication of the antioxidant status of the skin. If there is an increase in antioxidants, the excitation is less and the level of photons emitted will be reduced. The ultra weak photon emission (UPE) was measured after UVA irradiation in two age groups: (1) aged 18–25 years, (2) aged 60–72 years. The level of UPE in the skin was increased in the elderly group by approximately 33% indicating a reduction in the level of antioxidants. Thus demonstrating that the level of antioxidants in the skin decreases with age. To demonstrate that application of CoQ₁₀ can act as an effective antioxidant *in vivo* we measured the UPE of 13 volunteers (mean age 49 ± 6 years) treated on the volar aspect of the forearm, twice daily for 7 days with 0.3% CoQ₁₀ or vehicle alone. Following exposure to 50 mJ/cm² UVA the skin sites treated with 0.3% CoQ₁₀ had significantly lower levels of UPE. Therefore CoQ₁₀ is able to act as an antioxidant *in vivo* against the oxidative effects of UVA.

6. CoQ₁₀ reduces the effects of photoaging

The clearest demonstration of photoaging is the presence of deep wrinkles, as described earlier. To demonstrate the efficacy of CoQ₁₀ against photoaging *in vivo* 0.3% CoQ₁₀ or vehicle control was applied to 20 elderly volunteers, once daily around the eyes for six months. CoQ₁₀ was applied around one eye and vehicle around the other eye. Casts were then prepared for quantitative microtopography [13]. Photographs of the skin before treatment shows deep wrinkles, which are characteristic of photoaging, whereas fine wrinkles are associated with chronological aging. Following treatment with CoQ₁₀ the depth of these deep wrinkles is visibly reduced (Fig. 3). Using microtopography we can measure a reduction in the depth of wrinkles in aged skin. Two important parameters can be calculated from the microtopography. They are: R_t which is the mean peak to valley measurement of a defined unit distance, and R_q which is the intergrated area of the peaks and troughs, this indicates the variation of the microtopography from a flat surface. There was a 27% reduction in the mean peak to valley depth of the skin and a 26% reduction in the R_q value, compared to vehicle treated controls.

Although the stratum corneum, the outermost layer of the epidermis, is being continuously sloughed off and replaced, it can display evidence of aging in the underlying living cells [11]. The area of corneocytes, which make up the stratum corneum, is proportional to the time taken for the keratinocyte to differentiate and move from the basal layer to the stratum corneum. In aged skin, the time taken to move through the epidermis increases, and corneocytes become larger. The surface can develop fine lines and may become dry and scaly (senile xerosis) [12]. For example, at age 18 the average surface area of a corneocyte is 1020 μm^2 this increases to 1080 μm^2 at age 38. Therefore, the size of the corneocytes

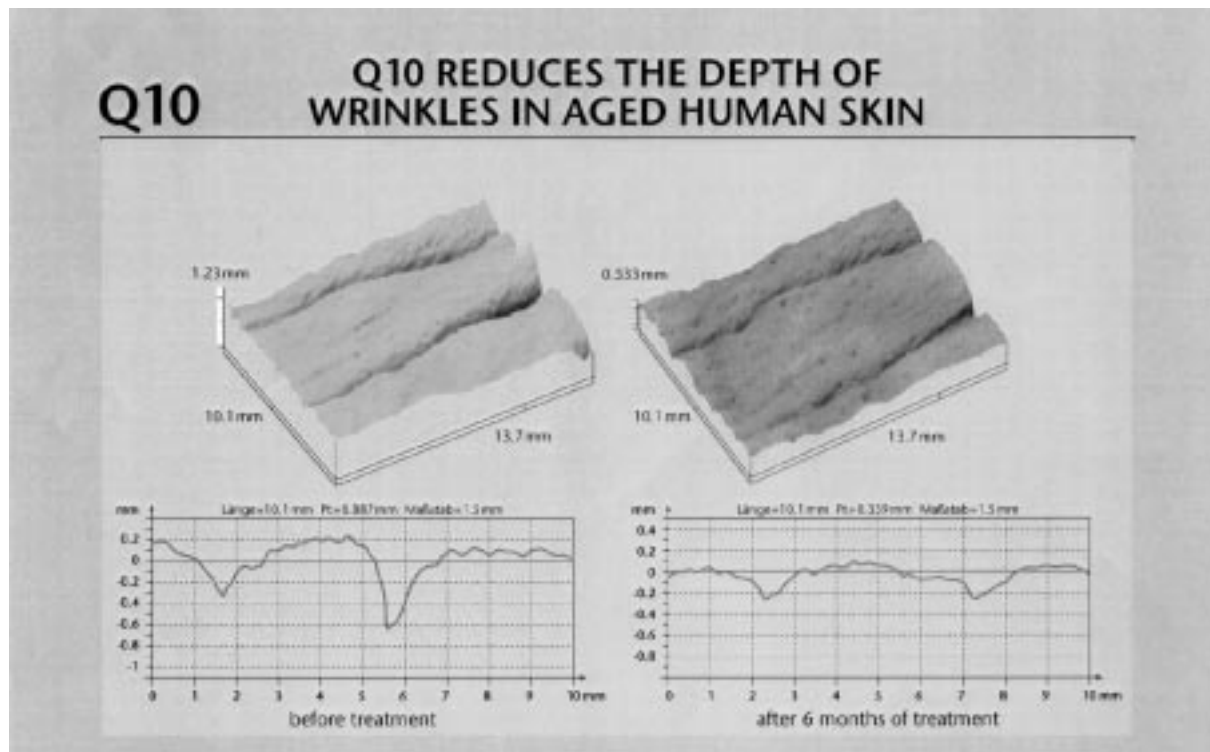


Fig. 3. CoQ₁₀ reduces the depth of wrinkles in the area around the eyes over a 6 month period of use.

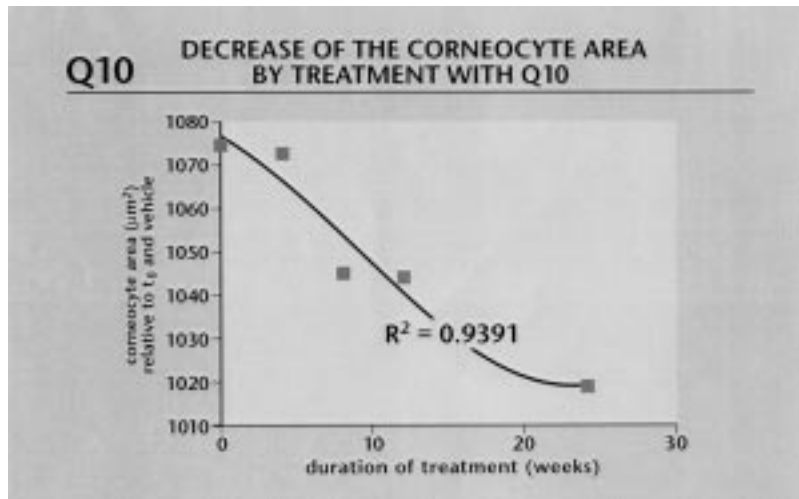


Fig. 4. CoQ₁₀ reduces the corneocyte area over a 24 week period of use.

indicates the effectiveness of a treatment to decrease the transit time. Following treatment of the volar aspect of the forearm daily with 0.3% CoQ₁₀ we can demonstrate a decrease in the corneocyte area over time (Fig. 4). The decrease in corneocyte area is equivalent to a difference of 20 years, from 38 years old to 18 years.

7. CoQ₁₀ is suitable for cosmetic use

CoQ₁₀ demonstrates no cytotoxicity in cultured keratinocytes even at 200 µg/ml CoQ₁₀ which is the limit of solubility. To determine the irritancy potential *in vivo* occlusive patch tests were conducted on volunteers in a double blind randomized trial. The vehicle and 0.3% CoQ₁₀ gave similar results and had no irritancy potential. In another study we were also able to demonstrate that CoQ₁₀ can be tolerated by people who have sensitive skin, and can suffer from stinging around the nose when certain cosmetics are applied [5].

CoQ₁₀ is essentially photostable. Using a Sol 500 source (Dr Hönle, Germany) and HPLC measurement. CoQ₁₀ demonstrated only 10% degradation at 5 times the minimal erythema dose (MED), that is approximately 150 mJ/cm² UVB, and 60% degradation at 10 times the minimal erythema dose. Both of these UVB doses are well above the range normally encountered. CoQ₁₀ is stable in the presence of oxygen (under pressure) for up to 30 min at 75°C.

8. Conclusion

Our experiments have demonstrated that CoQ₁₀ is able to act as an antioxidant against the effects of both hydrogen peroxide and UVA in cultured epidermal keratinocytes and UVA in dermal fibroblasts. The use of ultra weak photon emission has allowed us to demonstrate a significant reduction in oxidation *in vivo*. CoQ₁₀ is highly effective at protecting keratinocytes from DNA damage induced by UVA.

CoQ₁₀ was also clearly effective at reducing photoaging *in vivo* with a reduction in wrinkle depth and a decrease in the turnover time of the epithelium. CoQ₁₀ is also stable in a formulation suitable for

topical use and is well tolerated with no irritation or stinging. CoQ₁₀ is clearly able to penetrate into the living layers of the epidermis, where it must be reduced from ubiquinone to ubiquinol, to be acting as an antioxidant. We know that the epidermis has relatively high levels of NADPH quinone reductase (EC 1.6.99.2), which has been postulated to produce the reduced form of CoQ₁₀ [3,32]. CoQ₁₀ is clearly able to protect cells from the adverse effects of UVA, both in cell culture, where we have demonstrated the protection from DNA damage and effects on dermal matrix turnover, and also *in vivo* where we have demonstrated a reduction in wrinkles and increased epidermal turnover. CoQ₁₀ has efficacy in the skin following topical application without the adverse effects, such as irritation, which are associated with the current treatments for photoaging, such as all-trans retinoic acid [10,17].

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