Accelerated Regeneration of ATP level After Irradiation in Human skin Fibroblasts by Coenzyme Q_{10}

Research that goes under your skin

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Background

Human skin is exposed to a number of harmful agents of which the ultraviolet (UV) component of solar radiation is the most important [1]. UV-induced damages include direct DNA lesions as well as oxidative damage in DNA, proteins and lipids caused by reactive oxygen species (ROS). Being the main site of ROS generation in the cell, mitochondria are particularly affected by photostress [2-3]. The resulting mitochondrial dysfunction may have negative effects on many essential cellular processes [4]. To counteract these effects, coenzyme Q_{10} (CoQ_{10}) is used as a potent therapeutic in a number of diseases, ranging from primary CoQ_{10} deficiencies, to mitochondrial diseases, atherosclerosis, diabetes, cancer, skin aging, cardiovascular or neurodegenerative diseases [5]. Interestingly, in all those disease patterns, low levels of endogenous CoQ_{10} in comparison to healthy patients have been reported. CoQ_{10} deficiency has been associated with impairment of the mitochondrial ETC and ATP synthesis as well as increased ROS production and apoptosis [6-7]. In the present study, we examined the influence of CoQ_{10} on irradiated human skin fibroblasts with respect to mitochondrial function and cellular energy state. To do so, we analyzed the mitochondrial respiration profile, the mitochondrial membrane potential, as well as the cellular ATP-levels in normal human skin fibroblasts after irradiation with physiologically relevant doses of simulated solar light (SSL) at different time points after treatment with or without CoQ_{10}.

Material & Methods

Cell culture

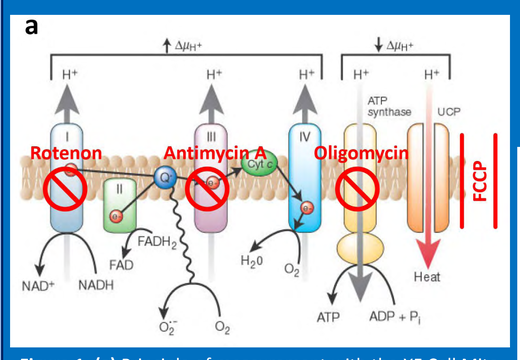
Skin fibroblasts were isolated from skin biopsies, which were received from the Kreiskrankenhaus Sigmaringen, general surgery unit, Germany, or from the Chirurgische Gemeinschaftspraxis Dr. Fuhrer, H. Nonnenmacher, Dr. Astfalk und Dr. Fauser, Reutlingen, Germany. Fibroblasts were isolated as described by Burger *et al.* [8]. CoQ_{10} (QuinoMit® Q_{10} fluid; MSE Pharmazeutika GmbH, Bad Homburg, Germany) was always freshly weighed, diluted with ultrapure water and supplemented to the cell culture media.

Irradiation experiments with simulated solar light (SSL)

Irradiation experiments were carried out with a solar simulator. The cells were either irradiated in 6-well plates or in XF cell culture plates in the presence of PBS. The UVA component of SSL was measured with a handheld UV-meter and a sensor for UVA. To ensure comparability, medium was also changed in control samples. After irradiation, PBS was immediately exchanged with fresh cell culture medium with or without CoQ_{10}.

Mitochondrial respiration measurements

To get a mitochondrial respiration profile, XF24 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA, United States) was used to measure the change of oxygen consumption rate (OCR) and pH in medium surrounding the cells. In order to acquire all key parameters of mitochondrial function, oligomycin (Oligo), FCCP and rotenone + antimycin A (Rot&Anti) were applied at 1 µM concentration (fig. 1 a + b).



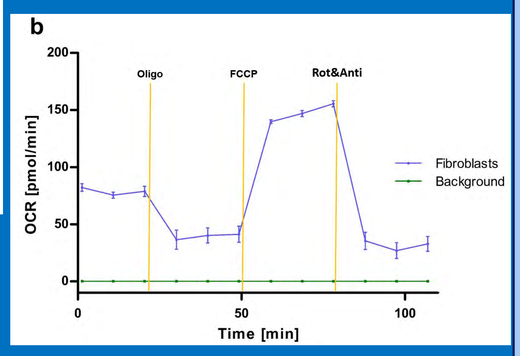


Figure 1. (a) Principle of measurement with the XF Cell Mito Stress Kit. (b) Respiration of human skin fibroblasts with the typical course after addition of stress reagents (Image by Brownlee (2001) modified by D. Schniertshauer).

ATP bioluminescence assay

Fibroblasts were seeded into 6-well plates and incubated in cell culture medium with or without 100 µM CoQ_{10} until the irradiation took place. Following the irradiation, there was a further incubation of the cells in the incubator for 6, 16, 24, 32 h, respectively. Then the cells were detached and 35,000 cells were collected by centrifugation, washed with PBS and resuspend in ultrapure water. The lysis of the cells was carried out for 5 min at 95 ° C in a thermocycler. The supernatants were analyzed in a bioluminescence measurement, which were carried out as a luciferin-luciferase reaction.

Mitochondrial membrane potential (Δψm) measurements

Fibroblasts were seeded in 6-well plates and the UVA irradiation with a dose of 12 J/cm² took place 7 h or 24 h after seeding. Following the irradiations, the cells were incubated in cell culture medium with or without CoQ_{10}. Prior to measurement, the cells were washed with PBS and stained with JC-1. Immediately after cell detachment, the mitochondrial membrane potential was analyzed using a blue laser (488 nm) and two band pass filters (585 nm; JC-1-aggregates (red) and 530 nm; JC-1-monomers (green)). To calculate a measure for the Δψm, the median of the red fluorescence intensity (medianFIred) was divided by the median of the green fluorescence intensity (medianFIgreen).

$$ratio = \frac{medianFI_{red}}{medianFI_{green}} Normalized\ ratio = \frac{ratio_{sample}}{ratio_{CTRL}}$$

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Results

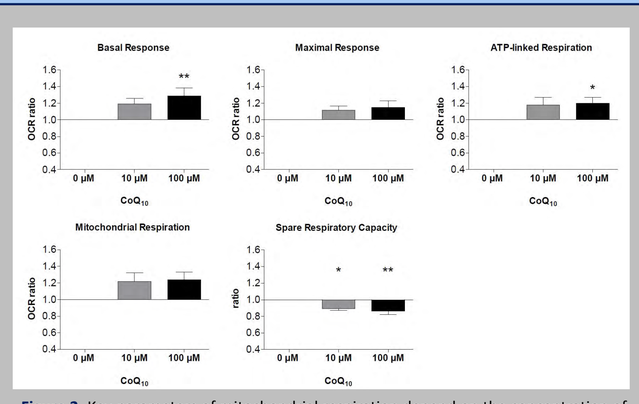

Figure 2. Key parameters of mitochondrial respiration depend on the concentration of CoQ_{10} in fibroblasts. Fibroblasts were incubated overnight with or without 10 or 100 µM CoQ_{10}. After 16 h, the mitochondrial respiration was analyzed with a XF Analyzer.

Key parameters of mitochondrial respiration depending on the concentration of CoQ_{10} in fibroblasts

- CoQ_{10} supplementation induced a concentration-dependent increase in mitochondrial respiration.
- 100 µM CoQ_{10} increased almost all key respiratory parameters.
- No effect of 10 µM CoQ_{10}.
- Significant increase of basal response and ATP-linked respiration with 100 µM CoQ_{10}.

Effect of CoQ_{10} on the mitochondrial respiration of fibroblasts after SSL-UVA irradiation

- Irradiation with 12 J/cm² SSL-UVA induced a rapid decrease in all respiratory key parameters without any effect of CoQ_{10} treatment.
- 16 h after irradiation with 6 J/cm² SSL-UVA, respiration in fibroblasts differed dependent on CoQ_{10} treatment.
- Non-irradiated CoQ_{10} treated cells showed a significantly higher basal response and mitochondrial respiration.
- Irradiated cells showed reduced respiration to an extent as it could not be observed in irradiated and CoQ_{10} treated cells.
- Irradiated cells showed an increased spare respiratory capacity.
- With CoQ_{10} application the cells show improved mitochondrial respiration and ATP production over a period of 16 h after irradiation compared to respiration measurement directly after irradiation.

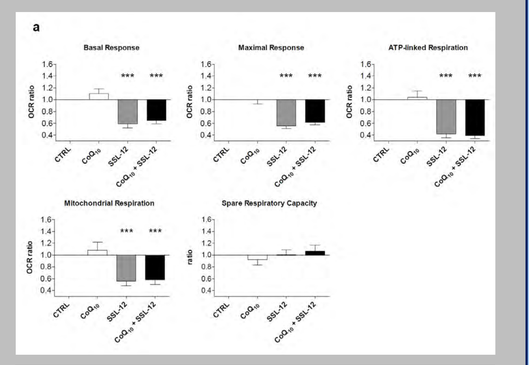

Figure 3. Effect of CoQ_{10} on the mitochondrial respiration of fibroblasts after SSL. (a) Fibroblasts were incubated overnight in cell culture medium with or without 100 µM CoQ_{10}. After 16 h the cells were irradiated with 12 J/cm² SSL-UVA (SSL-12) and then mitochondrial respiration was analyzed with a XF Analyzer. (b) Fibroblasts were incubated for 1 h in cell culture medium with or without 100 µM CoQ_{10} and then irradiated with 6 J/cm² SSL-UVA (SSL-6) 16 h prior to mitochondrial respiration measurement and afterwards kept in CoQ_{10} supplemented medium.

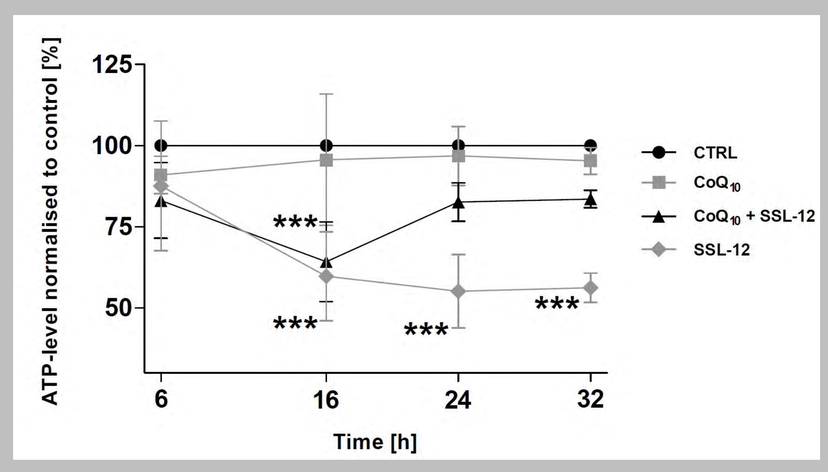

Figure 4. Effect of CoQ10 on the cellular ATP-level of fibroblasts after SSL (simulated solar light)-UVA irradiation. Fibroblasts were incubated in cell culture medium with or without 100 µM CoQ10, irradiated with 12 J/cm2 SSL-UVA (SSL-12) 16 h after seeding. Following the irradiation, there was a further incubation of the cells in the incubator for 6, 16, 24, 32 h, respectively. After reaching the intended incubation time points, cells were harvested and viable cells were quantified. 35,000 cells then were used for the determination of the ATP-level over different time points in cell lysates by a luminescent reaction.

Effect of CoQ_{10} on the cellular ATP level of fibroblasts after SSL-UVA irradiation

- ATP levels of the cells treated only with CoQ_{10} were almost identical to the controls.
- After irradiation ATP-levels decreased down to 60 % of control cells.
- In CoQ_{10} treated samples, ATP levels regenerated much faster and were near control levels after 24 h.
- 8 h later there was no significant difference between the control cells and the cells treated with CoQ_{10} and additionally irradiated.
- After 32 hours the ATP level of the untreated and irradiated cells was dramatically reduced.

Effects of CoQ_{10} on the mitochondrial membrane potential (Δψm) of fibroblasts after SSL-UVA irradiation

- Immediately after treatment Figure 4a) as well as 17 hours post irradiation (Figure 4b) the normalized ratio decreased due to depolarization of mitochondria.
- When CoQ_{10} containing solution was added to the cells prior to irradiation and directly after irradiation no significant change in the normalized ratio was detectable.

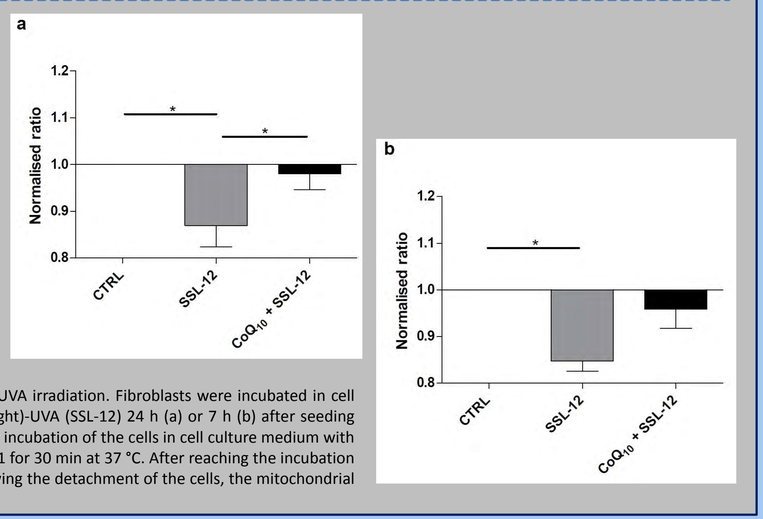

Figure 5. Effects of CoQ_{10} on the mitochondrial membrane potential (Δψm) of fibroblasts after UVA irradiation. Fibroblasts were incubated in cell culture medium with or without 100 µM CoQ_{10}, irradiated with 12 J/cm2 SSL (simulated solar light)-UVA (SSL-12) 24 h (a) or 7 h (b) after seeding respectively. If the cells were irradiated after 7 hours, following the irradiation there was a further incubation of the cells in cell culture medium with or without 100 µM CoQ_{10}. 24 h after seeding the cells were washed with PBS and stained with JC-1 for 30 min at 37 °C. After reaching the incubation time, the cells were detached from the culture dishes with 10× trypsine-EDTA. Immediately following the detachment of the cells, the mitochondrial membrane potential were analyzed with a Fluorescence Activated Cell Sorter.

Conclusion

We analyzed the mitochondrial respiration profile, the mitochondrial membrane potential and cellular ATP level in skin fibroblasts after irradiation. We observed an accelerated regeneration of cellular ATP level, a decrease in mitochondrial dysfunction as well as a preservation of the mitochondrial membrane potential after irradiation in human skin fibroblasts by treatment with CoQ_{10}. We conclude that the faster regeneration of the ATP level was achieved by a preservation of mitochondrial function by the addition of CoQ_{10} and that the protective effect of CoQ_{10} is primarily mediated via its antioxidative function. We suggest also that it might be further dependent on a stimulation of DNA repair enzymes by CoQ_{10} (unpublished data).

References

[1] Gebhard, D., Matt, K., Burger K., Bergemann, J. (2014) Shortwave UV-Induced Damage as Part of the Solar Damage Spectrum Is Not a Major Contributor to Mitochondrial Dysfunction. *J Biochem Molecular Toxicology* **28(6)**, 256-262.

[2] Furda, A.M., Marrangoni, A.M., Lokshin, A., Van Houten, B. (2012) Oxidants and not alkylating agents induce rapid mtDNA loss and mitochondrial dysfunction. *DNA Repair (Amst.)* **11**, 684-692.

[3] Yoshida, T., Goto, S., Kawakatsu, M., Urata, Y., Li, T.S. (2012) Mitochondrial dysfunction, a probable cause of persistent oxidative stress after exposure to ionizing radiation. *Free Radic.* **46**, 147-153.

[4] Harman, D. (1972) The biologic clock: the mitochondria? *Journal of the American Geriatrics Society* **20(4)**, 145-147.

[5] Garrido-Maraver, J., Cordero, M.D., Oropesa-Ávila, M., Fernández Vega, A., de la Mata, M., Delgado Pavón, A., de Miguel, M., Pérez Calero, C., Villanueva Paz, M., Cotán, D., Sánchez-Alcázar, J.A. (2014) Coenzyme Q_{10} Therapy. *Mol Syndromol* **5**, 187-197.

[6] Rötig, A., Appelkvist, E.L., Geromel, V., Chretien, D., Kadhom, N., Edery, P., Lebideau, M., Dallner, G., Munnich, A., Ernster, L., and Rustin, P. (2000) Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. *Lancet* **356(9227)**, 391-395.

[7] Quinzii, C.M., Tadesse, S., Naini, A., and Hirano, M. (2012) Effects of Inhibiting CoQ_{10} Biosynthesis with 4-nitrobenzoate in Human Fibroblasts. *PloS one*, **7(2)**.

[8] Burger, K., Matt, K., Kieser, N., Gebhard, D., Bergemann, J. (2010) A modified fluorimetric host cell reactivation assay to determine the repair capacity of primary keratinocytes, melanocytes and fibroblasts. *BMC Biotechnol* **10**, 46.