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Mitochondrial tolerance to single and repeat exposure to simulated sunlight in human epidermal and dermal skin cells

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ABSTRACT

Background: Sunlight represents the primary threat to mitochondrial integrity in skin given the unique nature of the mitochondrial genome and its proximity to the electron transport chain. The accumulation of mitochondrial DNA (mtDNA) mutations is a key factor in many human pathologies and this is linked to key roles of mitochondrial function in terms of energy production and cell regulation.
Objective: The main objective of this study was to evaluate solar radiation induced changes in mitochondrial integrity, function and dynamics in human skin cells using a Q-Sun solar simulator to deliver a close match to the intensity of summer sunlight.
Methods: Spontaneously immortalised human skin epidermal keratinocytes (HaCaT) and Human Dermal Fibroblasts (HDFn) were divided into two groups. Group A were irradiated once and Group B twice 7 days apart and evaluated using cell survival, viability and mitochondrial membrane potential (MMP) and mass at 1, 4 and 7 days post one exposure for Group A and 1, 4, 7 and 14 days post second exposure for Group B.
Results: Viability and survival of HaCaT and HDFn cells decreased after repeat exposure to Simulated Sunlight Irradiation (SSI) with no recovery. HDFn cells showed no loss in MMP after one or two exposures to SSI compared to HaCaT cells which showed a periodic loss of MMP after one exposure with a repeat exposure causing a dramatic decrease from which cells did not recover. Mitochondrial Mass in exposed HDFn cells was consistent with control after one or two exposures to SSI; however mitochondrial mass was significantly decreased in HaCaT cells.
Conclusion: Data presented here suggests that mitochondria in epidermal cells are more sensitive to sunlight damage compared to mitochondria in dermal cells, despite their origin, confirming a skin layer specific sensitivity to sunlight, but not as expected.

1. Introduction

Mitochondria are one of the many different types of organelles in the cells of all eukaryotes and are often named as "powerhouses of the cell", and are the most important organelles for ATP supply in nearly all eukaryotic cells [1]. In addition to energy production, mitochondria also play important roles in calcium homeostasis, apoptosis and fatty acid β-oxidation [2,3]. They are of proto-bacterium origin and thus contain their own genome encoding for a subset of mitochondrial oxidative phosphorylation (OXPHOS) components as well as tRNAs and rRNAs necessary for the translation machinery [4–6]. Regulation of mitochondrial DNA (mtDNA), expression is vital for normal OXPHOS function and defective mitochondrial function can cause neurodegenerative diseases, aging, cancer and diabetes [7–15]. Mitochondria consist of two membranes defining four individual compartments within the organelle: the outer membrane, the intermembrane space, the inner membrane, and the matrix, each of them having its specific function. The inner mitochondrial membrane is densely folded to form mitochondrial cristae structures; this provides a big surface to accommodate many copies of the oxidative phosphorylation system [10,16–18]. There are five complexes forming the oxidative phosphorylation system; three of them (Complex I, III and IV) transfer protons from the matrix into the intermembrane space in order to create a proton gradient between the intermembrane space and the matrix [19,20]. Complex I and Complex II transfer electrons from NADH and FADH2, respectively, to an electron carrier, coenzyme Q. Complex III receives electrons from reduced coenzyme Q and in turn forwards single electrons to cytochrome c. Complex IV, the last station for the electrons in the respiratory chain, accepts electrons from cytochrome c and uses them to reduce molecular oxygen to water [19,20]. A translocation of protein from the intermembrane space to the mitochondrial matrix will lead to a dissociation of the ETC and ATP syntheses. As mitochondria utilize oxidizable substrates to produce a proton gradient across the mitochondrial inner membrane, the supply of oxidizable substrates to mitochondria depends on the concentration

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of external growth factors. Withdrawal of growth factors or loss of the extracellular glucose supply will lead to a decline in mitochondrial membrane potential (MMP). If growth factor or glucose deprivation persists, cells ultimately undergo apoptosis that is initiated by cytochrome c release from mitochondria. As the integrity of mitochondria is essential for mitochondrial function, the damage caused by natural sunlight can impair mitochondrial dynamics and recycling, and play a role in tumour initiation [21,22].

Relevant effectors from the sun include ultra violet radiation (UVR), it is the main cause for changes in the skin [23]. UVR is ubiquitous. Almost everyone has some exposure to UVR on a daily basis. It is an exposure we cannot completely evade and to try attempt for zero exposure would produce a huge problem of skeletal disease from vitamin D deficiency. UVR lies between the visible light and X-ray region of the electromagnetic spectrum with wavelength spanning 100–400 nm. UVR is further divided by wavelength into UVA (400–315 nm), UVB (315–280 nm), and UVC (280–100 nm) [24–26]. UVC is totally absorbed by atmospheric ozone, has minimal penetration to the surface of the Earth and thus has little effect on human health [27,28]. Approximately 90% of UVB is absorbed by atmospheric ozone, while UVA passes through the atmosphere with little change. Therefore, the solar ultraviolet radiation of importance to human health consists of UVA and UVB [29,30]. UVA can penetrate deep into the skin reaching the basal layer of the epidermis and dermis, generating reactive oxygen species (ROS), such as the hydroxyl radical which can cause DNA strand breaks and chromosome translocations. Traditionally UVB was thought as being more carcinogenic when compared to UVA because the action spectra for biological responses indicate that it is radiation in the UVB range that is absorbed by DNA – subsequent damage to DNA appears to be a key factor in the initiation of the carcinogenic process in skin. However, a study of UVA and UVB induced mutations in epidermal keratinocytes obtained from squamous cell carcinomas and premalignant solar keratosis showed greater mutations caused by UVA in the basal cell layer highlighting the role of UVA in malignant transformation [31,32].

The skin is the organ most exposed to environmental sunlight. Many studies on skin cells have established that UVR can damage the skin [25, 29–31,33–40]. This may result in changes of cellular function. DNA is a critical target because its alteration can eventually lead to the development of cancer. There are several types of skin tumours related to sun exposure however, squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanoma develop almost entirely on sun exposed areas of skin with the highest risk for individuals who burn easily [39–41].

Melanoma is the skin cancer most likely to metastasize, typically beginning as a radial proliferation of non-proliferating melanocytes and when the lesion begins growing vertically, metastasis is possible [36, 39,42]. SCC and BCC are tumours of keratinocytes, which are cells that routinely proliferate, differentiate and are then shed from the skin. They usually begin on a background of sun damaged skin, characterized by a loss of elasticity and individual disordered keratinocytes. Continued sun exposure leads to keratinized reddish patches of actinic keratosis, with abnormally differentiating and proliferating cells. These cells usually regress, but can progress to SCC; these tumours are often aneuploidy and can metastasize. BCCs seem to arise without precursors, seemingly from keratinocytes in hair follicles. They are usually diploid and rarely metastasize, although they invade locally [36,39,42]. All three cancers correlate with exposure to sunlight. It has already being demonstrated previously that oxidative mitochondrial mass and mitochondrial membrane potential changes are associated with skin cell response to UVR stress [43].

The main objective of the current study was to evaluate how simulated sunlight, using the irradiance level 0.55 W/m^2 at 340 nm which is a commonly used irradiance levels for Central USA and European summer sun [44,45], can alter cell survival, cell viability, mitochondrial function, mitochondrial mass and mitochondrial membrane potential, in human skin cells.

2. Materials and Methods

2.1. Cell Culture

Spontaneously immortalised human skin epidermal keratinocytes (HaCaT) and Human Dermal Fibroblasts (HDFn) cells were cultured in Dulbecco's Modified Eagle medium (DMEM) (Sigma Aldrich, Dorset, UK) containing 10% Foetal Bovine Serum (FBS) (Sigma Aldrich), 1 μg/ml Penicillin/Streptomycin (Sigma Aldrich) and 2.0 mM L-Glutamine (Sigma Aldrich). Cells were incubated at 37 °C, with 5% CO_2. Petri dishes (Sarstedt, Numbrecht, Germany) were seeded with 6×10^5 cells 24 h prior to exposure to SSI.

2.2. Simulated Sunlight Irradiation

An XE-1 Q-Sun Solar Simulator (Q-Labs, Cleveland, OH, USA) was used to deliver simulated sunlight. This was achieved using the Q-filter and an irradiance output set to 0.55 W/m^2 at 340 nm, equivalent to 1 SED/min. Culture medium was replaced with pre-warmed phosphate buffer saline (PBS) immediately pre exposure. Petri dish lids were removed before samples were exposed once to SSI for 2 min. PBS was removed and was replaced with fresh culture medium post exposure. Controls were handled identically during sham irradiations. For cell cultures to be exposed to SSI for a second time, the progeny of cells exposed to SSI, as just described, were exposed a second time, 7 days later in exactly the same manner.

2.3. Neutral Red Assay

A Neutral Red Assay kit (Sigma Aldrich) was used to measure cell viability and following manufactures instructions. It is based on the ability of viable cells to incorporate and bind the super vital dye neutral red in the lysosomes, non-viable cells do not take up the dye. Briefly, cells were seeded in 96 well plates 24 h prior to analysis. Neutral red uptake into cells, correlating to viability, was evaluated in a FluoStar Optima fluorescent plate reader (BMG Labtech, Ortenberg, Germany) measuring absorbance at 540 nm. Relative viability is expressed as a percentage of control values. Data are presented as the mean ± standard deviation of 3 or more separate experiments.

2.4. MTT Assay

An MTT Assay kit (Sigma Aldrich) was used to measure mitochondrial function and following manufactures instructions. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes, predominantly found within the mitochondrion. Cells were seeded in a 96 well plate 24 h prior to analysis. MTT turnover is related to mitochondrial function and was determined by measuring absorbance in a FluoStar Optima fluorescent plate reader at 540 nm. Relative mitochondrial activity is expressed as a percentage of control values. Data are presented as the mean ± standard deviation of 3 or more separate experiments.

2.5. Cell Survival Assay

Cell count was performed using the Z2 Particle Analyser (Beckman Coulter, FL, USA), 500 μl of cell suspension was suspended in a fixed volume of Isoton in a disposable counting vial and counted using the Z2 Particle analyser. Relative cell survival is expressed as a percentage of control and is presented as the mean ± standard deviation of 3 or more separate experiments with 2 replicates per experiment.