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UV-A Irradiation Induces a Decrease in the Mitochondrial Respiratory Activity of Human NCTC 2544 Keratinocytes

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UV-A irradiation caused a dose-dependent decrease in cellular oxygen consumption (56%) and ATP content (65%) in human NCTC 2544 keratinocytes, one hour after treatment. This effect was partially reversed by maintaining the irradiated cells in normal culture conditions for 24 h. Using malate/glutamate or succinate as substrates for mitochondrial electron transport, the oxygen uptake of digitonin-permeabilised cells was greatly inhibited following UV-A exposure. These results strongly suggest that UV-A irradiation affects the state 3 respiration of the mitochondria. However, under identical conditions, UV-A exposure did not reduce the mitochondrial transmembrane potential. The antioxidant, vitamin E inhibited UV-A-induced lipid peroxidation, but did not significantly prevent the UV-A-mediated changes in cellular respiration nor the decrease in ATP content, suggesting that these effects were not the result of UV-A dependent lipid peroxidation. UV-A irradiation also led to an increase in MnSOD gene expression 24 hours after treatment, indicating that the mitochondrial protection system was enhanced in response to UV-A treatment. These findings provide evidence that impairment of mitochondrial respiratory activity is one of the early results of UV-A irradiation for light doses much lower than the minimal erythemal dose.

Keywords: UV-A, mitochondria, intracellular ATP, oxygen consumption, MnSOD, mitochondrial membrane potential ($\Delta\Psi$)

Abbreviations: ATP, adenosine triphosphate; $DiOC_6(3)$, 3,3-diethyloxacarbocyanin; PI, propidium iodide; UV, ultraviolet; TBARS, thiobarbituric acid reactive substances (lipid peroxidation products); TMPD, *N,N,N′,N′* tetramethyl-

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p-phenylenediamine; $\Delta\psi_m$, mitochondrial transmembrane potential; MnSOD, manganese superoxide dismutase

INTRODUCTION

The role of ultraviolet (UV) radiation (UVB: 290–320 nm, UVA: 320–400 nm) in the photodegenerative processes of the skin is well known, although its mechanism of action is not completely understood.[1,2] Ultraviolet B radiation was long considered to be the only deleterious agent in the solar spectrum because it directly damages nuclear targets.[3,4] Much less attention has been paid to the potential contribution of solar UV-A radiation which is believed to affect different cell components *via* an oxidative reaction.[5] For example, the cellular responses to UV-A exposure in cultured cells include a decrease in the cellular processing of epidermal growth factor,[6] lipid peroxidation,[7,8] specific change in phospholipid metabolism,[9,11] in transcription factor activities [12,13] and gene transcription levels.[14–15] The deleterious effects of UV-A are most probably active oxygen species formed by photosensitisation reactions involving endogenous UV-A chromophores such as flavins or NADH/NADPH.[16] Mitochondria, which contain high concentrations of these co-enzymes, may be therefore important targets of UV-A radiation. Our previous results are consistent with this notion: we have demonstrated that UV-A irradiation mediates a selective decrease in mitochondrial phospholipid (cardiolipin) synthesis.[11] In addition, it has recently been demonstrated that UV-A often induces common mitochondrial deletions.[17] These findings led us to investigate whether UV-A irradiation impaired mitochondrial function. In this work, we investigated the effect of UV-A exposure on respiratory activity, ATP concentration and mitochondrial membrane potential in cultured human NCTC 2544 keratinocytes.

MATERIALS AND METHODS

Chemicals

Dulbecco's modified Minimum Essential Medium (DMEM), Hanks' Salts Solution (HBSS) and fetal calf serum (FCS) were obtained from Gibco (Grand Island, NY, USA). Reagents for ATP determination were obtained from Boehringer Mannhein (Meylan, France). 3,3-Diethyloxacarbocyanine ($DiOC_6(3)$) and propidium iodide (PI) were obtained from Molecular Probes. All other unlabelled chemicals were purchased from Sigma (St Louis, USA) and were of the highest purity available.

Cell Culture

The NCTC 2544 human cell line was established from a single clone of an epidermal cell taken from a healthy human caucasian. Although these cells have been immortalised, they do not have a transformed phenotype. They grow in a monolayer, are inhibited by contact and do not synthesise keratin in excess. Thus, NCTC 2544 keratinocytes can be considered to be a model for undifferentiated keratinocytes of the basal epidermal layer.[18] This cell line was purchased from ICN, France. MRC5 human fibroblast cells were provided by BioMérieux, France. Cells were seeded at a density of $1.5 \times 10^4/cm^2$, and cultured in 100 mm Nunc Petri dishes in DMEM medium supplemented with 10% fetal calf serum and 10 mM HEPES buffer. Cultures were maintained at 37 °C in a humidified 5% CO_2/95% air atmosphere. All experiments were carried out with subconfluent cells.

Experimental Conditions for Cell Irradiation

UV-A irradiation was carried out using a Vilber Lourmat table (Torcy, France) equipped with TF-20L tubes emitting maximally at 365 nm, at a dose rate of $3.5 \pm 0.2\,mW/cm^2$. Our irradiation equipment was designed to avoid UV-B contam-

ination: a piece of glass (4 mm thick) was placed 20 mm above the table to absorb UV-B light (transmission < 0.01% at 310 nm). Before irradiation, cells were washed 3 times in PBS and covered with HBSS without additive. The cells were irradiated, left in the dark for 1 h and the irradiation medium was then replaced by DMEM supplemented with 10% FCS. Sham-irradiated cells were treated in the same way but were not exposed to UV-A. To study the reversibility of the UV-A effect on cell respiration and ATP content, cells were covered with fresh whole medium after the incubation for one hour in the dark after irradiation. They were then left for 24 h at 37 °C in the CO_2 incubator before measurements. The cytotoxic effect of UV-A irradiation was evaluated 1 h or 24 hours after irradiation using two viability tests: the Trypan Blue exclusion test, and the Neutral Red assay described by Quiec *et al.*[10]

Oxygen Consumption Measurements

Oxygen consumption was carried out with an A Clarke Type electrode in a 1.6 ml-chamber of a Gilson 5/6 oxygraph (Gilson Medical Electronics) at 37 °C. Cells were collected and transferred into the oxygraph chamber. The decrease in oxygen concentration was monitored using a linear recorder. Cellular respiration rate was expressed as nanograms of oxygen atoms consumed/min/mg of cell protein (ng O atoms/min/mg cell prot.). For the determination of mitochondrial respiration, we used the method described by Hofhaus *et al.*,[19] which allows the measurement of mitochondrial respiratory activity in cells without isolation of the mitochondria. Treatment of cells with a low concentration of digitonin selectively permeabilises the plasma membrane leaving mitochondria and other cell organelles intact. Therefore, the permeabilised cells can carry out state 3 respiration (i.e. under phosphorylating condition) if suspended in buffer containing ADP, phosphate, and a respiratory substrate. The polarographic measurements reflect the activity of the respiratory enzymes. It is therefore possible to identify the defective steps in oxidative phosphorylation. Briefly, 2×10^7 cells were collected in 2 ml of buffer A (20 mM HEPES, 250 mM sucrose, 10 mM $MgCl_2$, pH 7.1) and rapidly diluted in 8 ml buffer A supplemented with digitonin (50 µg/ml). After 1 min, digitonin was diluted by adding 5 volumes of buffer A. This cell suspension was then centrifuged and the pellet washed twice with buffer A. The final cell pellet was resuspended in 200 µl of respiratory medium (buffer A + 1 mM ADP + 2 mM potassium phosphate). An aliquot of 80 µl of the above cell suspension was introduced into the oxygraph chamber. After achieving a constant baseline reading, oxygen consumption was assayed by the sequential addition of various respiratory substrates and inhibitors of different sites in the respiratory chain system: 5 mM glutamate + 5 mM malate, then 0.1 µM rotenone + 5 mM succinate, and finally 20 nM antimycin A + 200 µM TMPD + 10 mM ascorbate. Protein determination was carried out by the method of Lowry as modified by Peterson.[20] The integrity of the outer mitochondrial membrane of digitonin-permeabilised cells was evaluated by measuring the activity of citrate synthase activity, a mitochondrial matrix marker. We found that the permeabilisation of cells with 50 µg/ml digitonin preserved the outer mitochondrial membrane in both UV-A-irradiated and controls cells (data not shown).

Determination of Mitochondrial Membrane Potential ($\Delta\psi_m$)

NCTC 2544 cells were incubated at 37 °C for 1 to 24 hours after UV-A exposure. Where indicated cells were treated, with 1 µM staurosporine for 24 hours.

$\Delta\psi_m$ was analysed as previously described.[21,22] $DiOC_6(3)$ is a lipophilic cation that selectively enters mitochondria. A stock solution of 0.5 mM $DiOC_6(3)$ in ethanol ($\lambda_{ex\,max}$: 484; $\lambda_{Em\,max}$: 501 nm) was prepared and kept in the dark at 20 °C.

For mitochondrial membrane potential measurements, cells (10^6/ml) were incubated in culture medium with 0.1 mM of $DiOC_6(3)$ for 30 min at 37 °C. In parallel, plasma membrane integrity was assessed by propidium iodide staining (PI, 10 μg/ml). The samples were analysed by flow cytometry (Becton Dickinson) using the Cell Quest software. PI-positive cells were excluded from the analysis of $DiOC_6(3)$ fluorescence for each sample.

ATP Determination

After irradiation and incubation in the dark for 1 h, cells were harvested with a rubber policeman. ATP concentration was determined in an aliquot of the cell suspension by the luciferase method, using the ATP Bioluminescence Kit from Boerhinger Mannheim and a Berthold 9501 luminometer. Results are expressed as a percentage of controls (sham-irradiated cells).

TBARS Measurement

Lipid peroxidation products (TBARS) were determined 1 h after cell irradiation by the fluorometric method described by Yagi.[23] The results were expressed as nmol MDA equivalents/mg of cell protein. The effect of the antioxidant, vitamin E, was tested in some experiments. Vitamin E was introduced at a final concentration of 50 μM in the culture 24 h before irradiation.

RNA Extraction and Northern Blot Analysis

Total RNA was isolated using a Trizol kit (Gibco BRL). RNA (10 μg) was subjected to electrophoresis in a 1.2% (w/v) agarose gel and transferred to a Hybond-C nitrocellulose membrane (Amersham). The membranes were hybridised overnight at 42 °C with the probe labelled with [α-^{32}P] dCTP using the Megaprime DNA labelling system (Amersham). The hybridised membranes were washed and exposed to Hyperfilm-Mp (Amersham). The RNA blot was stripped by boiling in 0.1% (w/v) SDS and probed again with a β-actin probe to check the amounts of RNA loaded in each lane. Results are expressed as times induction over control cells.

RESULTS

UV-A Mediated Decrease in Cellular Respiration Rate

The effect of UV-A irradiation on the cellular respiration rate was assessed one hour after treatment in two different cell types, NCTC

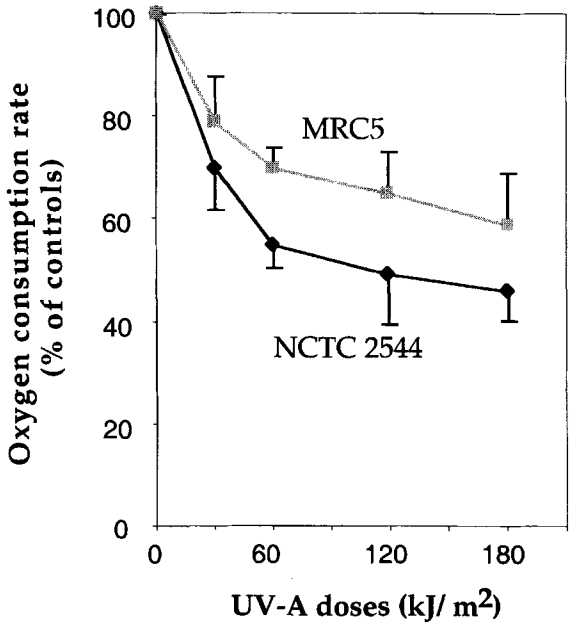

FIGURE 1 *Dose dependence of UV-A-mediated inhibition of cellular respiration rate.* NCTC 2544 keratinocytes and MRC5 fibroblasts were subjected to various UV-A doses. Oxygen consumption was measured one hour after treatment, in intact cells, as described in Material and Methods. Results are expressed as a percentage of those for sham-irradiated controls, corresponding to the mean of 6 experimental values ± s.d. (3 experiments in duplicate). Oxygen consumption (100%) = 52 ± 7 ng O atoms/min/mg of cell protein for NCTC 2544 and 29 ± 5 ng O atoms/min/mg of cell protein for MRC5 fibroblasts.

TABLE I Effect of UV-A irradiation on cellular respiration rate and cell viability

	Time after UV-A treatment (h)	
	1	24
Oxygen consumption (% of control)	57 ± 4.7	85 ± 7
Cell number	96 ± 3.4	93 ± 5.7
Cell viability (% of control)		
Trypan Blue	91 ± 8.1	83 ± 7.3
Neutral Red	88 ± 5.3	79 ± 6.6

NCTC 2544 keratinocytes were irradiated with 180 kJ/m^2 UV-A. Cellular oxygen consumption rate, cell number and cell viability were determined 1 h or 24 h after treatment (see Material and Methods).

2544 keratinocytes and MRC5 fibroblasts. UV-A irradiation caused a dose-dependent inhibition of oxygen consumption in both cell lines (Figure 1). Significant effects were already observed with UV-A doses as low as 30 kJ/m^2. At the maximum UV-A dose tested, oxygen consumption was 56% ± 6 lower than that for sham-irradiated cells for NCTC 2544 keratinocytes and 40% ± 7 lower for MRC5 fibroblasts. The reversibility of the phenomenon was also investigated (Table I) by placing NCTC 2544 cells in fresh medium after the 1 h dark period following UV-A exposure (180 kJ/m^2). The cellular oxygen consumption rate was then measured after a further overnight incubation in normal culture conditions. Cell respiration was partially restored in NCTC 2544 keratinocytes: the oxygen consumption rate increased to 83% that of sham-irradiated controls (Table I). The number of cells measured 1 h or 24 hours after UV-A exposure was equivalent to the sham-irradiated control (Table I). In addition, UV-A irradiated cells showed a slight but not significant increase in thymidine incorporation (+14% after UV-A exposure). It is noteworthy that the cell viability

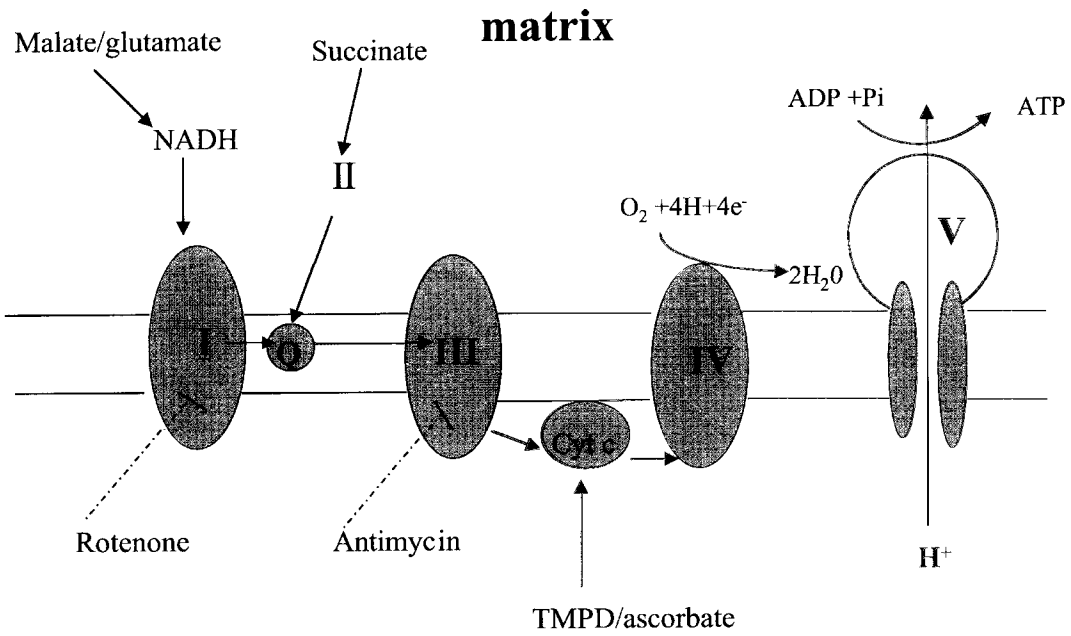

FIGURE 2 *Mitochondrial respiratory chain.* This diagram shows complexes I–V, ubiquinone (coenzyme Q), cytochrome *c*, sites of substrate entry and the sites of action of the inhibitors used in these experiments.

TABLE II Effect of UV-A exposure on oxygen consumption in cells permeabilised with digitonin

	oxygen consumption rate		
	Malate/glutamate	Succinate	TMPD/ascorbate
S	20.6 ± 2.2	31 ± 3.1	63.5 ± 12.2
UV-A	9 ± 1.1	10 ± 0.6	68 ± 9.9

UV-A-treated (180 kJ/m^2) or sham-irradiated cells were permeabilised with digitonin as described in Materials and Methods. The state 3 respiration of the mitochondria was determined by polarographic assay using various specific substrates and inhibitors of different segments of the mitochondrial electron transport system, as described in Material and Methods. Results are expressed as ng O atom/min/mg of cell protein and correspond to the means of five independent experiments. S: Sham-irradiated control cells; UV-A: UV-A irradiated cells.

was not significantly affected after UV-A treatment (180 kJ/m^2) (Table I).

The decrease in cellular respiration suggests that UV-A may affect the mitochondrial electron transport, which is involved mainly in cellular oxygen consumption.[24,25] Mitochondrial electron transport is mediated by four multisubunit complexes (I–IV) in the inner mitochondrial membrane (Figure 2). We assayed the function of these complexes in mitochondria by sequentially adding substrates and inhibitors which are specific for various steps in electron transport after permeabilisation of the cells with digitonin. We then measured the respiratory activity in state 3, i.e. in phosphorylating conditions (presence of ADP in the medium). We used malate/glutamate to assess electron transport from complex I to complex IV (cytochrome *c* oxidase): UV-A light strongly decreased respiratory activity (57%) as shown in Table II. Similarly, succinate, which passes electrons to oxygen *via* II→III→IV induced a marked decrease (67%) in respiratory activity. In contrast, if TMPD/ascorbate was used to assess electron transport from cytochrome *c* oxidase (complex IV) the oxygen consumption by UV-A irradiation was not affected.[26,27] These results with permeabilised cells are consistent with those obtained with intact cells and provide evidence that state 3 respiration of mitochondria was strongly inhibited by UV-A exposure.

UV-A Induced a Decrease in Intracellular ATP Content

Electron transport in mitochondria is tightly coupled to the biosynthesis of ATP by the ATP-synthase (complex V). To determine whether UV-A irradiation affected ATP synthesis, we measured cellular ATP content after UV-A exposure. UV-A irradiation caused a dose-dependent decrease in intracellular ATP concentration in NCTC 2544 keratinocytes (Figure 3A). Exposure to 180 kJ/m^2 resulted in intracellular ATP levels 65% lower than those of sham-irradiated cells. As for cellular respiration, studies were performed with this maximum dose (180 kJ/m^2) to investigate the reversibility of the phenomenon. We found that 24 h after irradiation, ATP content was almost restored to normal levels (20 to 10% inhibition compared to sham-irradiated controls) whereas it was only 57 ± 6.6% lower than those of sham-irradiated measured 1 h after irradiation (Figure 3B).

UV-A Exposure did not Affect Mitochondrial Membrane Potential

We studied the effect of UV-A radiation on mitochondrial membrane potential in individual cells by carrying out flow cytometry analysis with $DioC_6(3)$, a lipophilic cation taken up by mitochondria as a function of $\Delta\psi_m$. Plasma membrane integrity was assessed in parallel by PI staining and was not significantly affected by UV-A treatment. Figure (4A) illustrates the cytometric distribution of $DiOC_6(3)$ in UV-A-irradiated and sham-irradiated cells. UV-A radiation did not affect the mitochondrial membrane potential, $\Delta\psi_m$, of NCTC 2544 cells, measured 1 h after exposure. Indeed, $\Delta\psi_m$ remained unchanged 3, 5 and 24 hours after UV-A treatment (Table III). In contrast, staurosporine used as a positive control efficiently induced a marked decrease in $\Delta\psi_m$

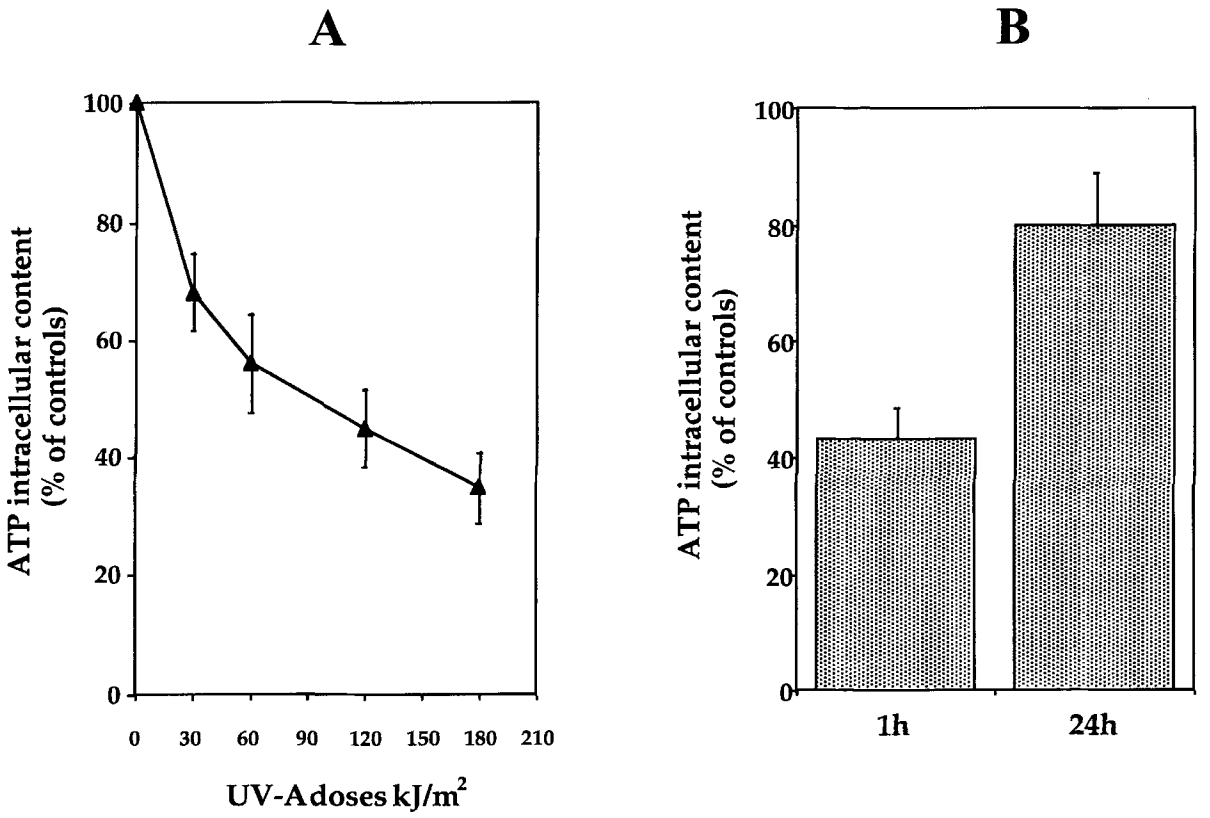

FIGURE 3 *UV-A induced decrease in ATP content in NCTC Cells.* (A) NCTC cells were irradiated with UV-A doses of up to 180 kJ/m^2 then left for one hour in the dark at 37 °C. Intracellular ATP was determined as described in Materials and Methods. (B) Cells were irradiated with 180 kJ/m^{2}UV-A. The intracellular ATP content was determined 1 h or 24 h after treatment. Results (A, B) are expressed as percentages of those for sham-irradiated controls, corresponding to the mean of 3 independent experiments. ATP content (100%) = 2.9 ± 0.4 mM.

(Figure 4B), consistent with previous studies in other cell lines.[28]

Effect of Antioxidants on Cellular Respiration Rate and ATP Concentration

Finally, we investigated whether there was a relationship between the lipid peroxidation induced by UV-A and changes in cellular respiration rate. In our conditions, vitamin E efficiently reduced (by about 85%) the UV-A-induced formation of TBARS (Figure 5A). This result is consistent with previous studies.[7,8] In contrast, this antioxidant did not significantly prevent changes in levels of oxygen consumption or cellular ATP content (Figure 5B), demonstrating that these effects were not the result of

TABLE III Effect of UV-A irradiation and staurosporine on mitochondrial membrane potential

	$\Delta\psi_m$ (% of control)
UV-A (1 h)	102 ± 3
UV-A (3 h)	98 ± 2.6
UV-A (5 h)	98 ± 3.2
UV-A (24 h)	101 ± 2
Staurosporine (24 h)	27 ± 4

Cells were treated with UV-A (180 kJ/m^2) or staurosporine (1 μM) and incubated for 1 to 24 hours at 37 °C as described in Figure 4. The level of $DiOC_6$ incorporation (see Figure 4) is expressed as a percentage of control cells and corresponds to two independent experiments.

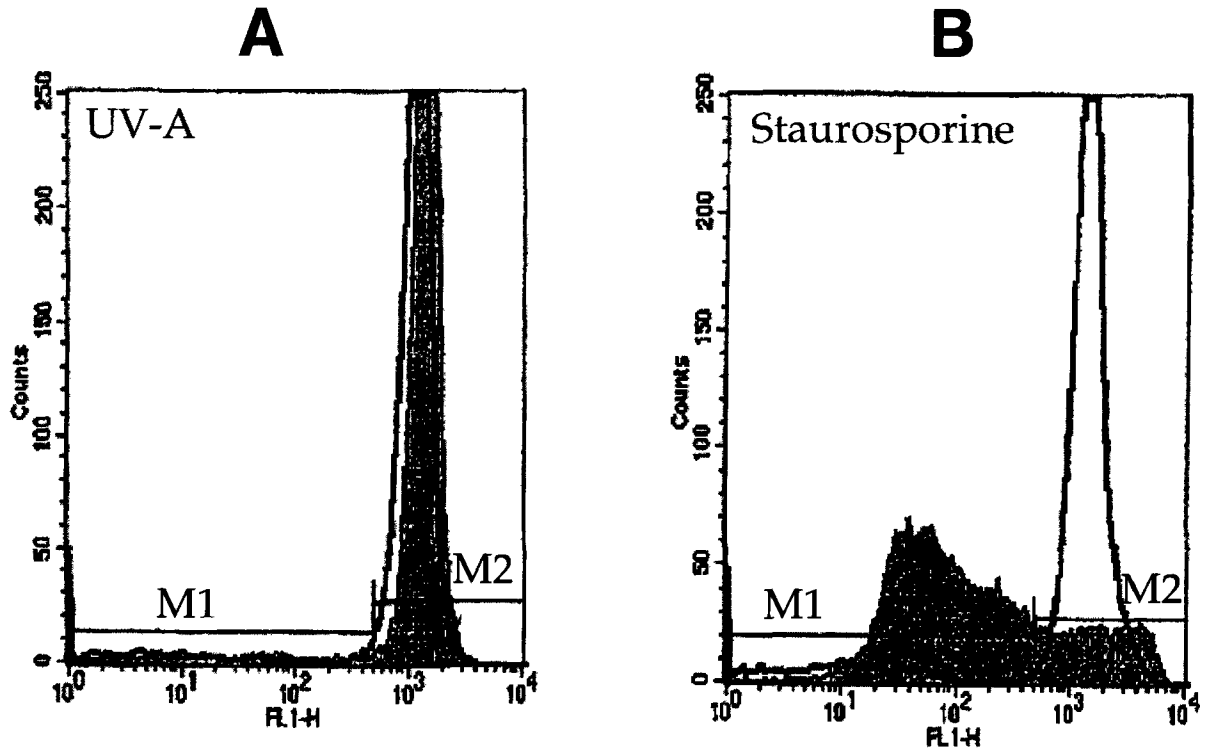

FIGURE 4 *$DiOC_6(3)$ fluorescence distribution of UV-A irradiated cells.* NCTC 2544 cells were sham-irradiated or irradiated with UV-A (180 kJ/m^2) and incubated for 1 to 24 hours at 37 °C. In a parallel experiment, cells were treated with 1 μM staurosporine for 24 hours. Cells were treated after incubation with $DiOC_6(3)$, but before the addition of PI and analyzed with a flow cytometer. Histograms corresponding to $DiOC_6(3)$ fluorescence distribution are shown (x axis: number of cells; y axis: $DiOC_6(3)$ fluorescence, M1: PI-negative cells with low levels of $DiOC_6(3)$ incorporation, M2: PI-negative cells with high levels of $DiOC_6(3)$ incorporation.) The fluorescence intensities of control and sham-irradiated cells are indicated by white curves; UV-A-irradiated cells and strausporine-treated cells are illustrated by dark curves. Identical results were obtained in three independent experiments.

UV-A dependent lipid peroxidation but rather mediated by other oxidative reactions.

Effect on MnSOD

Manganese superoxide dismutase (MnSOD) is an essential mitochondrial antioxidant enzyme. We therefore investigated the effect of UV-A on MnSOD gene expression. MnSOD gene expression was induced by a factor of about 2.6 (Figure 5C) 24 hours after UV-A treatment (180 kJ/m^2). As a positive control, we treated cells with TNFα, which resulted in a significant increase in the level of MnSOD gene expression, consistent with previous studies.[29]

DISCUSSION

Our results demonstrate that UV-A doses much lower than the minimal erythemal dose are sufficient to cause significant changes in cellular respiration rate, affecting the energetic status of the cell. In addition, we measured oxygen consumption in digitonin permeabilised cells in the presence of ADP in order to evaluate the contribution of the various steps in the mitochondrial respiratory chain after UV-A treatment. Under these conditions, we measured the mitochondrial respiration in state 3, which reflects the *in vivo* situation. We found that UV-A exposure caused a marked reduction in

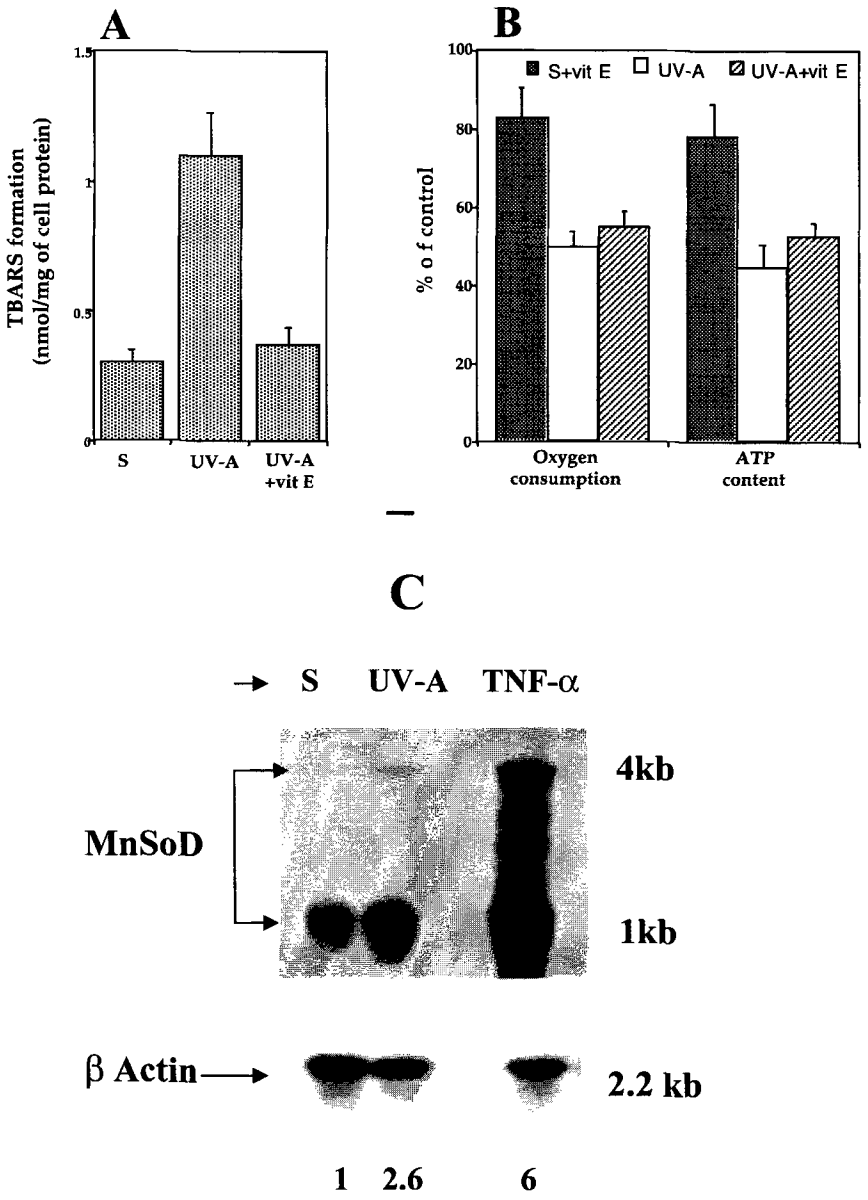

FIGURE 5 *Effect of antioxidants on TBARS formation, oxygen consumption and ATP intracellular content in human NCTC 2544 keratinocytes: potential antioxidant role of MnSOD gene expression.* (A, B) Cells were first cultured for 24 h in the presence or absence of 50 μM vitamin E. They were then washed and irradiated with 180 kJ/m^2 UV-A. TBARS formation (A), oxygen consumption (B), ATP concentration (B) were determined as described in Materials and Methods. The results of TBARS measurements are expressed as nmol MDA equivalents/mg of cell protein. The results of oxygen consumption and ATP concentration are expressed as percentages of those for sham-irradiated cells cultured in normal medium (without antioxidants). (C) NCTC cells were sham-irradiated or irradiated with UV-A (24 h, 180 kJ/m^2) or treated with TNFα (6 h, 40 ng/ml), harvested and RNA was extracted from them. Total RNA (10 μg) was subjected to electrophoresis in a 1.2% (w/v) agarose gel and hybridised to a ^{32}P-labelled MnSOD cDNA probe. A β-actin probe was used to check the amount of RNA loaded in each lane. MnSOD mRNA content was determined by calculating the ratio of the absorbance of the MnSOD mRNA band to that of the actin band. The results are expressed as times induction over control cells (S). S = sham-irradiated control cells; S+VitE = sham-irradiated cells first cultured for 24 h in medium supplemented with 50 μM vitamin E; UV-A = irradiated cells cultured in the absence of vitamin E; UV-A + vit E = irradiated cells first cultured with vitamin E. Resuts are representative of three (A, B) or two (C) independent experiments.

mitochondrial oxygen consumption in response to malate/glutamate and succinate but not to TMPD/ascorbate. These results provide evidence that UV-A radiation rapidly impairs the state 3 respiratory activity of the mitochondria and suggest that damage to cytochrome *c* oxidase is not the rate-limiting factor in this UV-A effect. These results are consistent with those of Davies and co-workers [30] and Salet *et al.*[31] demonstrating that mitochondria are affected by various oxidative stresses whereas cytochrome *c* oxidase is resistant to the same agents. They are also consistent with our previous findings indicating that UV-A irradiation results in the inhibition of cardiolipin synthesis[11] because this phospholipid has been shown to be absolutely required for the regeneration of the enzymatic activity from a phospholipid-depleted preparation of various mitochondrial electron transport complexes.[32] UV-A irradiation also induced a decrease in cellular respiration in human fibroblasts (MRC5) suggesting that this response is not cell type-specific. However, this effect was less pronounced in MRC5 fibroblast cells than in NCTC 2544, in good agreement with the report of Leccia *et al.*[33] suggesting a difference in cell sensitivity to UV-A irradiation.

We also show that UV-A irradiation caused a marked decrease in intracellular ATP content which was closely related to the reduction in oxygen consumption. This data is consistent with the coupling of electron transport to ATP biosynthesis, but does not exclude a direct effect of UV-A on the mitochondrial ATPase or ADP/ATP translocators. Thus the effects of UV-A irradiation on the activity of the mitochondrial ATPase and ADP/ATP translocators need to be particulary investigated as these complexes are known to be inactivated by a variety of oxidative stresses.[30,34]

On the other hand, we found that UV-A irradiation had no significant effect on mitochondrial membrane potential. It is possible that UV-A irradiation affects respiratory capacity but not enough to prevent the formation and maintenance of an electrochemical proton gradient. Consistent with these results, Salet *et al.*[35] showed that the oxidative stress induced by photosensitisation of isolated mitochondria by photofrin totally inhibited the state 3 mitochondrial respiratory function but had only a slight effect on mitochondrial membrane potential. Our results are also consistent with no cytochrome *c* being released into the cytosol upon UV-A treatment (data not shown): the loss of mitochondrial potential often being associated with a release of cytochrome *c* into the cytosol.[36] In contrast, our results conflict with those of Tada-Oikawa *et al.*,[37] showing a loss of $\Delta\psi_m$ after UV-A exposure in HL-60 cells. These divergent results may be due to difference in the irradiation conditions and cell types used. In their studies, Tada-Oikawa *et al.* irradiated HL-60 cells in phenol-red-free RPMI medium containing 6% FCS. This medium contains many potential UV-A photosensitisers molecules that may increase the effects of UV-A. To avoid this artefact, we carried out UV-A irradiation in Hank's buffer without UV-A chromophores, thereby preventing extracellular photosentisation.

It has been demonstrated that UV-A exposure triggers the formation of lipid peroxidation products.[7,8] We show here that vitamin E effectively prevented UV-A-induced lipid peroxidation, but had no significant effect on the UV-A-mediated inhibition of oxygen consumption and ATP intracellular content. In their study on hepatocytes irradiated with visible light (400–720 nm), Cheng and Packer also found that vitamin E did not significantly prevent the decrease in succinate dehydrogenase activity.[38] Similar findings were obtained by Zhang *et al.*[30] suggesting that lipid peroxidation is unrelated to electron transport chain inactivation due to hydroxyl and superoxide anion radicals. However, the role of reactive oxygen species in the UV-A-induced decrease in oxygen consumption and ATP content requires further investigation.

MnSOD is an intrinsic mitochondrial antioxidant enzyme. We therefore explored the effect of UV-A exposure on MnSOD gene expression. UV-A irradiation increased the level of MnSOD gene expression to 2–3 times compared to sham-irradiated cells suggesting that certain defence mechanisms against UV-A injury are stimulated in mitochondria. This effect is weaker than that of TNFα on MnSOD gene expression, but may account at least partially, for the recovery in oxygen consumption and intracellular ATP content observed 24 hours after UV-A treatment.

Overall, our results indicate that mitochondrial respiratory activity is rapidly affected by UV-A treatment. The maximum UV-A dose used here (180 kJ/m^2) represents only one third to one half of the minimal erythemal dose [MED] of fair-skinned caucasian subjects and did not significantly affect the viability of human NCTC 2544 keratinocytes. It is possible that repeated exposure to UVA results in additive stresses that cannot be controlled by cellular antioxidant systems and which may therefore cause irreversible damage to mitochondrial function. As mitochondria are a key metabolic compartment of mammalian cells, such changes may play a critical role in the degenerative processes of the skin induced by prolonged and repetitive sun exposure.

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