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Both UVA and UVB induce cytoskeleton-dependent surface blebbing in epidermoid cells

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Abstract

Data on the morphological changes induced by UVA or UVB irradiation of A431 epidermoid cells in culture are presented. After irradiation with different doses of UVB (120–2400 J m^{-2}) or UVA (10^4–10^5 J m^{-2}), the membrane and cytoskeleton of these cells were analysed by immunofluorescence and scanning electron microscopy at different times after exposure (0–48 h). Both UVA and UVB alter microtubules and microfilaments and surface blebs are formed after UV irradiation. In particular, UVB induces multiple small blebs on the cells, while UVA induces one single large bleb on each cell.

Since cytoskeletal damage and surface blebbing of this type are also induced by oxidative stress, these results add to the body of evidence indicating that UV radiation is capable of pro-oxidant behaviour. Specifically, the morphological changes described in this paper are reminiscent of the modifications which accompany epidermal keratinocytes during their transformation to sunburn cells after UV irradiation. The physiological implications of these findings are discussed.

Keywords: UVA; UVB; Cytoskeleton-dependent surface blebbing; Epidermoid cells

1. Introduction

UV-induced damage to cells and cell constituents has been the object of numerous detailed studies. Such damage can be the consequence of direct absorption of UV photons by relevant cell components or an indirect phenomenon mediated by the absorption of UV photons by endogenous photosensitizers [1].

In recent years, a body of evidence has been accumulating indicating that UV radiation induces biological responses which resemble closely the responses elicited by other stresses, such as heat shock and treatment with heavy metals, alkylating agents or hydrogen peroxide. Indeed, it has been observed that exposure to UV radiation elicits the heat shock response in rodent epidermis and cultured cells [2–4], as well as the induction of stress-related genes such as *fos* [5] and haem oxygenase [6].

It has also been reported that UV irradiation promotes membrane damage in cultured fibroblasts [7] in keeping with the observed effect that both UVA and UVB induce the peroxidation of lipids in cultured cells [8,9]. Oxidative mechanisms may also serve to explain the UV-induced damage to adenosine-*N*-1-oxide in cellular DNA [10] or single-strand breaks in purified DNA [11,12]. In keeping with these findings, it has been suggested that nicking of DNA by UV radiation could be the result of an oxidative process triggered by UV-induced electron transfer from DNA to DNA-bound ferric iron. The ferrous iron thus generated could undergo auto-oxidation with the consequent formation of reactive oxygen species which will eventually nick the sugar-phosphodiester backbone of DNA [11].

Since it is known that cells in culture are targets of UV–visible light damage and non-nuclear damage and cell lysis are induced by UV radiation [13,14], this study was undertaken to assess the possible damage induced in A431 epidermoid cells subjected to UVA or UVB radiation. Particular attention was focused on those modifications (i.e. microfilament disruption and surface blebbing) which are known to result after different types of oxidative stress [15].

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2. Materials and methods

2.1. Cell cultures

A431 cells, a human epidermoid carcinoma cell line, were grown in monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (Flow Laboratories, Irvine, UK), 1% non-essential amino acids, 5 mM L-glutamine, penicillin (100 IU ml^{-1}) and streptomycin (100 μg ml^{-1}) at 37 °C in 5% CO_2/95% air. For fluorescence and scanning electron microscopy, control, UVA- and UVB-exposed A431 cells were seeded on 13 mm diameter glass coverslips in separate wells (5×10^4 cells per well).

2.2. Exposure conditions

After a period of 24 h after seeding, the medium was changed and the A431 cell cultures were exposed to UV irradiation in fresh DMEM (supplemented with foetal bovine serum) or in phosphate-buffered saline (PBS), with identical results, using a Philips TL 20 W/12 for UVB irradiation and two Philips TL09 lamps for UVA irradiation localized in a sterile hood. The plastic Petri dishes containing the cells were placed without covers at a vertical distance of 10 cm from the centre of the tube and exposed to either UVB or UVA. In order to eliminate UVC radiation, a Kodak filter (Kodacell TL 401) having an optical density of less than 0.4 for wavelengths above 295 nm and higher than 2.5 for wavelengths below 285 nm was employed and was placed on the Petri dishes during exposure. The emission spectra of the lamps are reported in Fig. 1. In these conditions, the UVB radiant flux density to the cells was 2.2 W m^{-2}, as verified by an Osram Centra UV meter, and the UVA was 30 W m^{-2}. The filter used in our experiments has an optical density of 2.5 at 285 nm, 3 at 280 nm and above 4 for wavelengths below 270 nm. This implies that the intensity of the radiation emitted by the lamps below 285 nm after crossing the filter is reduced by about 300–10 000. Therefore we can estimate that the contamination by UVC to the cells does not exceed 0.003% of the total UVB or 0.0001% of the total UVA.

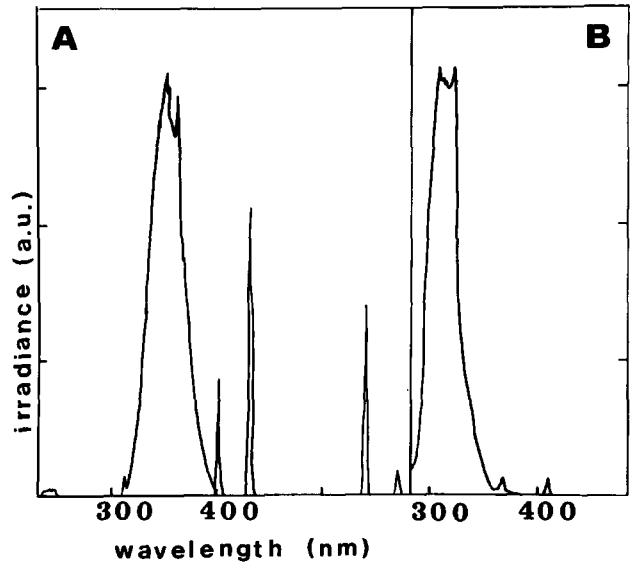

Fig. 1. Emission spectra of the lamps: (A) Philips 20 W/09N (UVA); (B) Philips TL 20 W/12 (UVB) (unfiltered); the irradiances are reported in arbitrary units (a.u.) vs. wavelength.

2.3. Light microscopy

For microscopic evaluation of the cell cultures, cells in Petri dishes were observed at different times after irradiation (3–48 h) and photographed by a Nikon inverted phase contrast microscope at a magnification of 20×. For fluorescence microscopy observations, cells were fixed with 3.7% formaldehyde in PBS (pH 7.4) for 10 min at room temperature. After washing in the same buffer, the cells were permeabilized with 0.5% Triton X-100 (Sigma) in PBS for 5 min at room temperature. For cytoskeletal analyses, A431 cells were stained with fluorescein–phalloidin (Sigma) or with antitubulin antibodies (Sigma) at 37 °C for 30 min. The first is a toxin capable of direct binding to F-actin and is usually linked with a fluorescent marker. The second is a mixture of α and β (1 : 1) antitubulin antibodies capable of reacting with the cell microtubular network. For the detection of tubulin, cells were subsequently incubated with anti-rabbit IgG-fluorescein-linked whole antibody (Amersham International) at 37 °C for 30 min. Finally, after washing, all the samples were mounted with glycerol–PBS (2 : 1) and observed with a Nikon Microphot fluorescence microscope.

2.4. Scanning electron microscopy (SEM)

Control and exposed cells were washed in PBS and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 3% (w/v) sucrose at room temperature for 20 min. Following post-fixation in 1% osmium tetroxide for 30 min, cells were dehydrated through graded ethanols, critical point dried in CO_2 and gold coated by sputtering with a Balzers Union SCD 040 apparatus. The samples were then examined with a Cambridge 360 scanning electron microscope.

3. Results

3.1. Cell culture analysis

In order to evaluate possible UV damage, the cells were first observed by light microscopy at different times after UV irradiation (Fig. 2). As can be seen,

control epidermoid cells appear to be perfectly flat, polygonal and with intimate cell-to-cell contacts (Fig. 2(d)). After a period of 3 h after the end of UVB exposure, the first lesions appear (not shown). Cells begin to lose their cell-to-cell contacts, become roundish and some (about 15%) show small, multiple surface blister-like protrusions, or blebs, a well-known marker of cellular damage. With increasing time (6 h after the end of UVB exposure), the cells continue to exhibit altered cell-to-cell contacts (Fig. 2(b)) and the percentage of cells undergoing surface blebbing increases (about 30% blebbed cells). Finally, 24 h after the end of exposure, many of the cells are detached from the substratum and surface blebbing is increased further (80% blebbed cells, Fig. 2(c)). Finally, massive cell death was evaluated by the trypan blue staining test 48 h after UVB exposure (not shown). After UVA irradiation, morphological alterations of the A431 cells were also observed with time (not shown). After a period of 6 h after the end of exposure, the appearance of surface blebbing is observed (Fig. 2(a)). In contrast with the results obtained with UVB, exposure to UVA results in the formation of only a single large bleb on each of the cells. With increasing time (within 24 h), these single blebs detach from the cells and flow free in the culture medium. The cells themselves remain attached to the substratum. The quantitative results are summarized in Table 1 and do not depend on the incubation medium (PBS or DMEM).

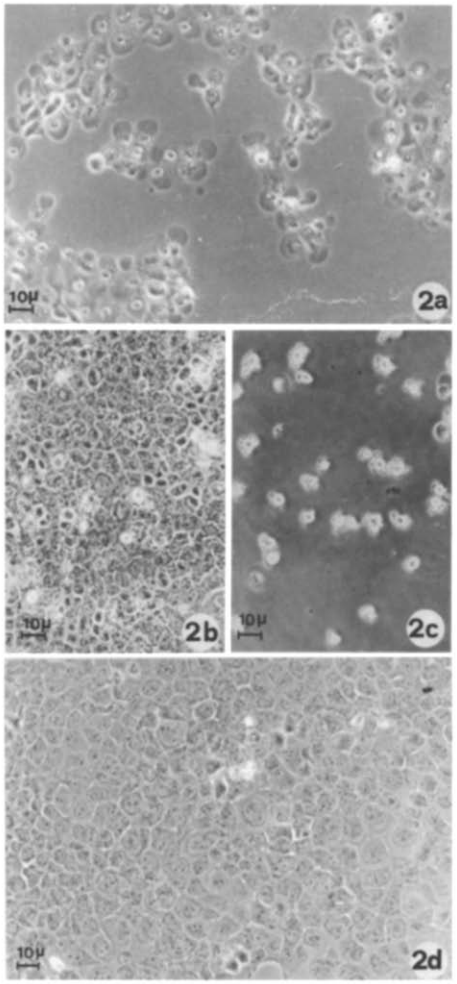

Fig. 2. Light microscopy analysis of A431 cells 6 h after UV exposure. The UVA-irradiated (200 000 J m^{-2}) A431 cells still adhering to the substrate display single, large blebs (a). UVB-irradiated (1200 J m^{-2}) cells tend to detach from the substrate and undergo blebbing (b). Detached cells floating in the medium are shown in (c). Mock irradiated, flat, polygonal cells growing in monolayers are shown in (d). The bar represents 10 μm.

3.2. Cell surface analysis

The results obtained by light microscopy were confirmed by SEM (Fig. 3). Control epidermoid cells appear as flat cells with numerous short microvillous structures and intimate cell-to-cell contacts (Fig. 3(c)). With increasing time after UVB exposure, the cell-to-cell contacts appear markedly altered with respect to the controls and a retraction of the cell body is observed. In addition, numerous small blisters and blebs are visible on the cells which are round in shape and smooth at the surface. After a period of 6 h after the end of exposure, an increase in the number of cells displaying surface blebs is observed (Fig. 3(b)), confirming the light microscopy results reported previously. In contrast, UVA exposure induces different morphological changes. For instance, the formation of a single, large, smooth protrusion on each cell occurs (herniation), while the cell body still remains firmly attached to the substratum (Fig. 3(a)).

3.3. Cytoskeleton analysis

Because of the importance of the cytoskeleton in the maintenance of the cell shape, experiments were conducted to determine whether UVB or UVA radiation is capable of inducing changes in actin microfilaments (Fig. 4) or in the microtubular network (Fig. 5). These

Table 1
Experiments conducted at different doses. Δt is the time interval after irradiation where the maximum number of cells with blebs was observed

UV	Dose (J m^{-2})	Δt (h)	Blebbing cells (%)
A	<60000	–	0
A	100000	2	5–10
A	200000	4	40–50
A	300000	0.1	100
B	<240	–	0
B	500	5	10–20
B	1200	5	80
B	2400	5	100

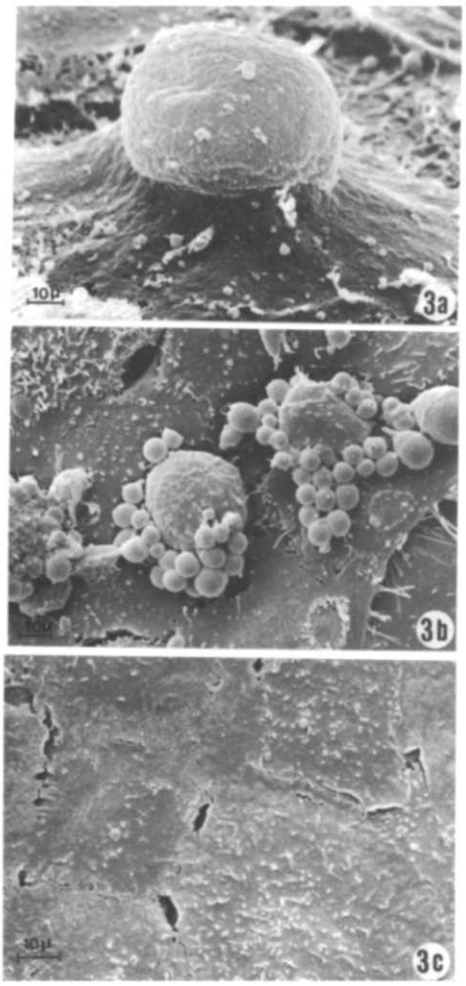

Fig. 3. SEM analysis of A431 cells 6 h after UV exposure. A large single bleb appearing after UVA irradiation (200 000 J m^{-2}) (a). Small multiple blebs appearing after 1200 J m^{-2} UVB; the figure shows the retraction of the cell body (b). Adhering epidermoid control cells showing numerous, short microvilli (c). The bar represents 10 μm.

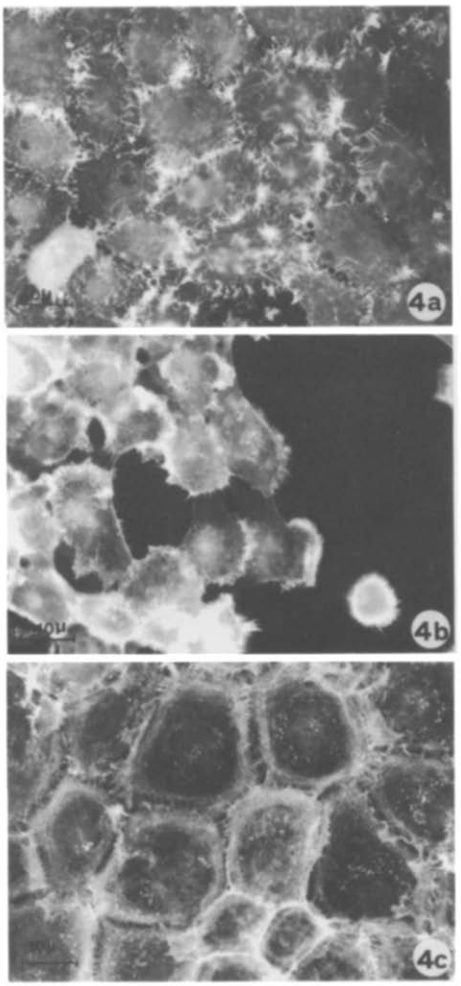

Fig. 4. Cytoskeletal actin decorated with fluorescein isothiocyanate (FITC)–phalloidin. Cortical microfilament rearrangement 3 h after UVA (200 000 J m^{-2}) irradiation (a), after 1200 J m^{-2} UVB (b) and after mock irradiation (c). The bar represents 10 μm.

changes are important since, if present, they can contribute to cell blebbing. After a period of 3 h after the end of exposure to both UVA (Fig. 4(a)) and UVB (Fig. 4(b)), a reduction in cell-to-cell contacts due to cell retraction and reorganization of the microfilament network is observed. In particular, although in control cells a cortical boundary network of well-organized actin filaments is detected (Fig. 4(c)), the exposure to UVB radiation induces, as an early lesion, a remarkable alteration of actin filament organization. It is possible that this type of damage may be responsible for the cell retraction and detachment from the substratum observed previously by inverted microscopy in A431 cells (Figs. 2(b), 2(c)). Parallel experiments conducted with UVA radiation show that this type of treatment is also capable of modifying slightly the actin assembly (Fig. 4(a)). As an early indicator of cell damage, peripheral cortical patching of actin filaments is observed in cells which are still strongly adherent to the substrate. Experiments were also carried out to evaluate the effects of UV radiation on the microtubular network. In control cells, this network is well-developed and organized (Fig. 5(c)). In contrast, the collapse of these structures towards the cell nucleus is detected in UVB-exposed cells (Fig. 5(b)), while a minor yet important rearrangement is also observed in UVA-treated cells. In addition, these cells also display a characteristic modification of the nuclei. This alteration, which is observed 6 h after the end of exposure to UVA in numerous cells, may be considered as a nuclear blebbing phenomenon (Fig. 5(a)).

4. Discussion

This paper reports data on the morphological changes induced in cultured A431 human epidermoid cells after UV irradiation. The experiments were undertaken to

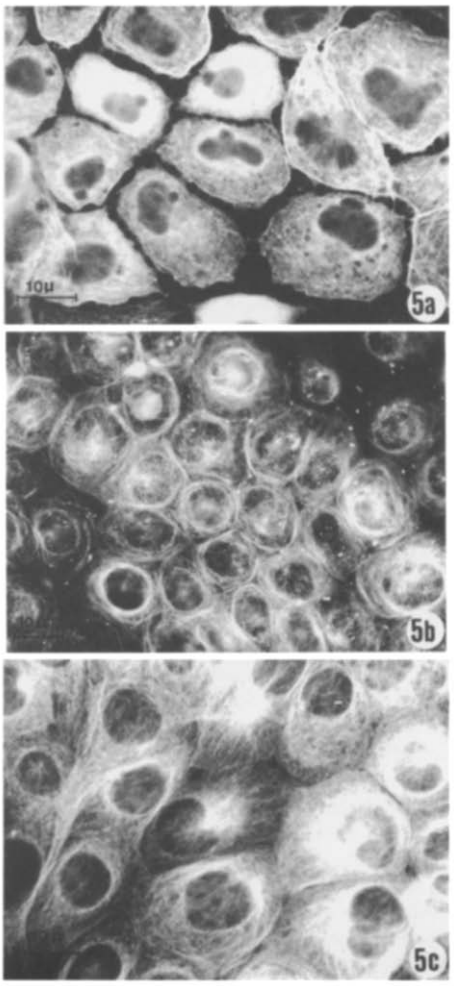

Fig. 5. Microtubule immunostaining. Cytoskeletal and nuclear morphological alterations 6 h after UVA (200 000 J m^{-2}) irradiation (note the microtubule network rearrangement as well as the nuclear membrane protrusions) (a). Collapsed cytoskeletal network 6 h after 1200 J m^{-2} UVB (b). Microtubular network in normal cells after mock irradiation (c). The bar represents 10 μm.

determine the pleiotropic effects of UV on living matter. Lamps emitting in the UVB or UVA range were chosen to investigate the possible differences of the effects of photons belonging to these different wavebands. For instance, as pointed out by Zamansky and Chou [16], cytoskeletal elements are disrupted in human keratinocytes after exposure to UVB, but not after exposure to UVA.

The effects of different delivered doses on A431 cells and the kinetic response involved were considered. These two parameters play a major role in the morphological modification of cells after UV irradiation, as noted by Lubart et al. [13], who found that the number of mitoses in mouse fibroblasts 24 h after visible or UVA irradiation first increases with increasing delivered dose and then decreases.

In this paper, we examined A431 cells exposed to UVA or UVB radiation focusing particularly on those morphological modifications already known to be induced by several types of oxidative stress [15]. Both UVA and UVB induce actin microfilament and microtubule rearrangement and, subsequently, remarkable surface blebbing within hours after irradiation in both PBS and DMEM. The blebbing appears to be the consequence of microfilament network disassembly. In particular, we followed the modification of the cellular contacts between the irradiated cells. Separation of each individual cell from the surrounding cells, disruption of cell–cell communication and eventual rounding of the plasma membrane occur. These modifications are also likely to take place in vivo on UV irradiation, as for instance in human epidermis. Indeed, it is tempting to speculate that these morphological modifications may precede the eventual formation of sunburn cells. It is evident that there is no way to compare the object of our analysis (living, blebbing cells still adhering to the substratum) with sunburn cells (presumably dead cells in a physiological environment). Yet, the modifications observed here after UV irradiation are reminiscent of the description provided by Wilgram et al. [17] for the sequence of ultrastructural events leading to the formation of sunburn cells, and quoted by Young [18] as: "perinuclear clumping of tonofilaments with gradual dissociation from desmosomes, perinuclear halo formation, intracellular vacuolization, nuclear disintegration and the reduction of the number of desmosomes finally resulting in a mixture of disintegrated nuclear material and tonofilaments". The sunburn cell, as quoted by Young [18], has been described by Daniels et al. [19] as having "a shrunken, homogenized, densely-staining glassy cytoplasm and a hyperchromatic condensed pyknotic nucleus" and is formed as early as 30 min after irradiation but peaks in human skin 24 h after irradiation with broad-spectrum Hanovia hot quartz lamps [20]. It could be argued that the comparison between the observations made here and sunburn cells is not completely correct since UVA, in the absence of photosensitizers, is not known to induce sunburn cells. However, it is interesting to note that blebs form after UVA irradiation even though these blebs may not be directly related to sunburn cell formation. In addition, it is also important to observe that the single, large bleb provoked by UVA detaches from the cell which remains adhered to the plastic dish, while the small blebs which form after UVB exposure accompany the cell to its death when the cell itself detaches from the dish into the medium. Finally, it should also be considered that an alteration of the relationships between different cytoskeletal components can represent

the basic mechanism leading to surface blebbing. In particular, it has been suggested that UV can alter the interconnections between keratin and actin filaments [21] in keratinocytes. We can hypothesize that this may be a more general effect of UV radiation which alters the microfilament–intermediate filament interactions as well as modifying certain specific binding proteins (e.g. actin binding proteins).

The similarity between the ultrastructural modifications observed in A431 cells and those described in the literature for the formation of sunburn cells seems to be relevant to the in vivo situation. Indeed, the number of sunburn cells found in epidermis on UV irradiation is an indicator of the extent of the sunburn and can be diminished by treatment with antioxidants such as superoxide dismutase [22,23]. Therefore our observation that UV induces cytoskeletal and membrane changes which are similar to those induced by oxidative stress provides further evidence of the pro-oxidant behaviour of UV photons. These observations might be helpful in suggesting strategies for avoiding deleterious and undesirable effects in organs and organisms exposed to excessive UV radiation, such as human epidermis exposed to sunlight. Protection against sunlight should ideally be made against each target of UV photons, but since the different targets of UV radiation in cells remain unknown and they are possibly large in number, mixing antioxidants with photostable sunscreens may be a winning strategy in fighting erythema and actinic aging of the skin.

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