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Ultraviolet radiation and free radical damage to skin

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Abstract

Solar UVB (290–320 nm) and particularly UVA (320–380 nm) radiations have a capacity to generate reactive chemical species, including free radicals, in cells. These intermediates have been shown to be involved in various biological effects in cultured human skin cells (e.g. cell death) and skin (e.g. erythema). Endogenous glutathione is a critical molecule in protection against the cytotoxic effects of both wavelength ranges. Although there is evidence from cellular studies for the involvement of an oxidative component of UVC/UVB radiations in activation of several genes, the doses used are generally extremely cytotoxic and could cause aberrant signalling. Genes activated by sublethal doses of UVA radiations (e.g. haem oxygenase 1 and the CL100 phosphatase) are clearly redox regulated. The strong induction of haem oxygenase 1 in human fibroblasts has been implicated in an adaptive response to oxidative membrane damage that involves increased synthesis of the iron storage protein, ferritin.

Introduction

Chronic exposure to sunlight is the major cause of skin cancer in man and a major factor in the ageing of human skin. Epidemiological evidence now clearly links UV exposure to human melanoma and there is provocative evidence from studies using a fish model that the oxidative UVA component could be involved [1]. However, for the most part, the evidence for the involvement of free radicals and oxidative processes in the chronic effects of sunlight on skin is based on animal (usually rodent) experiments involving topical application or dietary supplementation with antioxidants. In contrast, there is evidence from various experimental approaches for the involvement of oxidative processes in the acute effects of UV on cultured mammalian cells and human skin cells in particular and the main purpose of this overview is to draw together some of the evidence in this

area before briefly summarizing our own data related to free radical-mediated gene activation.

Penetration of UV through skin

Solar UV radiation incident at the surface of the earth is composed of UVB (290–320 nm) and UVA (320–380 nm) radiation. UVB radiation is essentially defined by the short wavelength cut-off for solar radiation reaching the surface of the earth and this precise cut-off is largely determined by the thickness of the ozone column. Decreased ozone will lead to increased UVB and this effect is particularly dramatic at the shorter wavelengths. In humans the major targets for UV are the skin and the eyes, and it is important to emphasize that the transmission of UV through these tissues increases with increase in wavelength. In practice this means that, in normal skin, the proportion of UVB penetrating through to the basal layer of the epidermis (at an average of 70 μm from the skin's surface) is of the order of 1–10% (according to wavelength) while approximately 20% of a mid-UVA wavelength (365 nm) can penetrate as far. Indeed, significant levels of UVA radiation penetrate beyond the dermis and into subcutaneous tissue and will undoubtedly be 'seen' by blood components. So, while many of the biological effects of UV diminish dramatically with increase in wavelength, this is compensated for to some extent by tissue penetration. Furthermore, more of the solar UV energy incident on the skin is in the UVA region than in the UVB region. These factors clearly influence the levels, magnitude and effectiveness of UV received by the epidermal (keratinocytes and melanocytes) and dermal (fibroblasts) layers of skin.

UV and the generation of active species including free radicals

Both UVA and UVB radiations interact with several biomolecules to generate free radicals and other active intermediates (reviewed in [2]). Sufficient levels of hydrogen peroxide are almost certainly generated by UV in cells to cause generation of hydroxyl radical in the ferrous ion-catalysed Fenton reaction. The ferric ion produced in this reaction may be continuously reduced by superoxide anion and this is generated not only during normal oxidative metabolism but also as a result of the absorption of UV radiation by molecules such as NADH and NADPH. Another important intermediate is singlet oxygen which is generated as a result of the interaction of UV with endogenous photosensitizer molecules that include flavins and porphyrins. These are classical photodynamic agents which undergo reactions (defined as type II) in which the porphyrin chromophore is excited to a triplet state and transfers energy or an electron to oxygen to generate active oxygen intermediates. Porphyrins are probably the most important endogenous photosensitizers in cells and, since they exhibit a Soret band absorption with a peak around 405 nm, it is assumed that the longer UVA wavelengths are

primarily responsible for generation of singlet oxygen in cells. A good example of a powerful sensitizer is protoporphyrin IX (the immediate precursor to haem) which is found in the mitochondria and whose levels may be elevated under certain conditions. Nevertheless, it should be mentioned that the major UV chromophore in skin is usually melanin which has a broad absorption spectra which ranges through the UVB, UVA and visible ranges. Although melanin is generally considered to be protective by virtue of its UV-absorbing properties, its degradation can lead to the appearance of melanin free radicals whose biological consequences have not yet been fully evaluated.

Evidence that certain biological effects of UV involve free radical species and active oxygen intermediates

Most of the evidence that UV generates active intermediates in skin cells is indirect and is based on either observed oxygen effects or experiments designed to study the modifying effects of endogenous or exogenous antioxidants (free radical scavengers).

Oxygen effects

There are only a few reports (e.g. [3]) of dose-modifying effects of aerobic versus anaerobic irradiation with UVB in cultured cells. However, there are numerous examples of oxygen effects on the biological action of UVA radiation. These include induction of damage to macromolecules such as DNA (e.g. strand breaks and DNA–protein cross-links) and proteins (including many enzymes), as well as a strong oxygen-dependence for inactivation of populations of both cultured bacterial cells [4,5] and mammalian cells [6]. However, even whole organ effects such as UVA-induced erythema in human skin appear to depend on the presence of oxygen. Such observations provide firm evidence that active species are generated by UVA in the presence of molecular oxygen.

Endogenous antioxidants

In addition to antioxidant vitamins, cells cultured from human skin and other organs contain high (3–5 mM) levels of reduced glutathione, a ubiquitous sulphydryl tripeptide with strong reducing properties. Depletion of cellular glutathione levels using the highly specific γ-glutamyl synthetase inhibitor, buthionine *S,R*-sulphoximine, strongly sensitizes cells to the lethal action of both UVB and UVA radiations [7,8]. These observations clearly implicate active intermediates in cytotoxicity caused by both wavelength regions. It is also important to note that glutathione levels in both dermis and epidermis are rapidly depleted as a result of UVA irradiation [9], a clear indicator of oxidative stress. UVA also depletes glutathione in cultured dermal fibroblasts [10], but epidermal keratinocytes appear to be relatively resistant to UVA depletion (L. Laurent-Applegate and R.M.Tyrrell, unpublished work). It is worthy of note that glutathione depletion of newborn rodents leads to a strong disposition to cataract [11].

Inhibition of antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase, does not appear to have significant effects on the

UVA sensitivity of cultured human fibroblasts [12] but simultaneous inhibition of a combination of enzymes has not been tried. Similarly, addition of antioxidant vitamins does not normally increase protection of cell cultures against UVB or UVA irradiation, although there is a single report that UVA-sensitive cells cultured from actinic reticuloid patients can be protected by Trolox-C, a water-soluble derivative of vitamin E [13]. In one report, α-tocopherol has been shown to protect against UV-induced cytotoxicity but not DNA damage [14]. A brief summary of the possible evidence of a photoprotective role for vitamin E in animal cells and tissues [15] includes evidence that vitamin E protects lens epithelial tissue from the oxidizing component of UV radiation. Furthermore, there is evidence that UVB-induced erythema and sunburn in the skin of mice can be prevented by topical application of α-tocopherol acetate [16].

Modulation of UV effects by exogenously added agents

In addition to antioxidant vitamins, which have occasionally shown protective effects (see above), other antioxidants or free-radical scavengers may be used to test the involvement of radical intermediates in UV effects. Indeed, the antioxidant drug *N*-acetylcysteine has been increasingly used after it was shown that it can prevent the UVC-activated signal transduction pathway involving membrane-bound tyrosine kinases [17]. This is almost the only evidence for an oxidative component of UVC radiation in a biological system and it is possible that the high doses of UVC employed rapidly disrupt cellular function and increase oxidative processes which cause aberrant signalling. As discussed above, there is evidence for an oxidative component of UVB radiation but most experiments using chemical modulating agents have involved longer UVA wavelengths. Classical hydroxyl-radical scavengers such as dimethyl sulphoxide (DMSO) and mannitol have little effect on the cytotoxic action of UVA [12] but, in the same study, significant sensitization to the lethal effects of UVA radiation on human fibroblasts was obtained by irradiating them in deuterium oxide, an agent which enhances singlet oxygen lifetime. Interestingly, singlet oxygen has also been implicated as the effector species in the UVA activation of the haem oxygenase (HO)-1 gene (see next section).

UV activation of gene expression and cellular defence

Numerous genes are activated by non-solar UVC radiation and to the extent that these studies have been extrapolated to the UVB region a similar set of genes is involved. Clearly, UVB activates the AP-1/*ras* signalling pathway and this is linked to activation of many genes including collagenase [18] and c-*fos* [19], a critical component of the AP-1 transcription factor complex. UVB also activates the c-*jun* gene [20] and leads to enhanced production of the corresponding protein that constitutes another component of the AP-1 transcription factor. Activation of c-*fos* and c-*jun* also occurs in rodent skin after treatment with solar-simulated UV [21]. Both UVC and UVB radiations enhance levels of the p53 tumour-suppressor protein in skin [22,23], a phenomenon which is believed to reflect

increased p53 protein lifetime [24] rather than modulation of messenger RNA levels.

Activation of gene expression by UVC and UVB radiation often requires very high doses and there is little evidence that these phenomena are actually related to enhanced cellular defence, either in terms of altered DNA repair or elevated antioxidant activity. Based on studies with ionizing radiation, the p53 protein has been postulated to act in a cell-cycle checkpoint pathway which would allow time for enhanced DNA repair [25]. Interestingly, UVA radiation also enhances p53 protein levels in skin, but, for reasons yet to be clarified, this phenomenon is restricted to the basal layer of the epidermis [22].

In my laboratory, we have been particularly concerned with elucidating the functional role of the activation of the HO-1 gene by UVA and other oxidants. The activation which results in a strong enhancement of the corresponding enzymic activity appears to be a general response to oxidative stress in mammalian cells and occurs to high levels in cultured human skin fibroblasts.

Several lines of evidence, in addition to activation of the gene by oxidants such as hydrogen peroxide, are consistent with the conclusion that this gene is redox-regulated. Perhaps most significant is the observation that both basal levels of expression and the extent of transcriptional activation are strongly influenced by cellular glutathione levels [10]. More recently we have observed that antioxidants such as *N*-acetylcysteine suppress activation of the gene [26]. Furthermore, there is evidence that both iron and singlet oxygen are critical to the activation response [27,28].

The normal role of HO in tissue is the breakdown of haem proteins (and particularly haemoglobin) to generate biliverdin which is then normally converted into bilirubin. Although these breakdown products are considered important antioxidants in plasma [29], they are unlikely to play a crucial role in cells where the actual levels generated are low. Rather we have proposed a model in which the final result of UVA activation of the HO-1 gene is increased levels of ferritin. This protein appears to play a critical role in cellular antioxidant defence by keeping the levels of free intracellular iron, a catalyst in many oxidative reactions (see above), to a minimum. We now have strong experimental support for this model [30,31] which is summarized below.

Low fluences of UVA radiation lead to a dramatic increase in HO-1 transcription rate and specific HO-1 mRNA accumulation [32] which is followed several hours later by the predicted several-fold increase in HO enzymic activity. Corresponding to this increase in activity, there is a sharp increase in haem catabolism which will lead to release of chelatable iron. As expected from the known role of iron in stimulating ferritin translation via its interaction with iron responsive factor, this increase in HO activity is followed by a two-fold increase in ferritin levels [30]. We have now observed a UVA-inducible protective response against oxidative damage to membranes, consistent with a lowering of the pro-oxidant state of the cell as a result of increased scavenging of free intracellular iron [31]. Both the increase in ferritin levels and the newly observed adaptive response appear to be mediated via the transient stimulation of HO activity that results from oxidative stress, since these processes are prevented by antisense oligonucleotides targeted to the start site of the HO-1 gene. We have also isolated

cDNA for the constitutively expressed HO-2 gene (A. Noël, unpublished work) and compared HO-1 and HO-2 mRNA accumulation in human fibroblasts and keratinocytes [33]. While HO-2 levels are expressed at very low levels in human fibroblasts, this gene is expressed at high levels in keratinocytes. In contrast, keratinocytes show little constitutive or inducible HO-1 activity. The total basal level of HO enzymic activity is three times higher in keratinocytes than in fibroblasts and this correlates with relative levels of ferritin. We propose that HO plays a crucial role in cellular defence against oxidative stress in both types of skin cells, and that the pattern of differential expression of the two HO genes between the different cell types is related to the higher levels of UVB and UVA radiation that penetrate to the basal layer of the epidermis as compared with penetration to the dermal fibroblasts.

In summary, we have shown that glutathione plays a crucial role in protecting cultured skin cells against the oxidative stress generated by both UVA and UVB radiations in cultured skin cells. UVA radiation also leads to an enhancement in ferritin levels which clearly provides increased protection against oxidative damage to membranes. The relevance of this observation to other cellular targets is clearly an important question for the future and one which has direct relevance to understanding degenerative oxidative processes in the skin.

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