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PERSPECTIVE

Modulation of gene expression by the oxidative stress generated in human skin cells by UVA radiation and the restoration of redox homeostasis†

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UVA radiation generates a significant oxidative stress in skin cells which is further enhanced by the release of the pro-oxidant catalysts iron and heme, and exacerbated by UVA-mediated destruction of cellular reducing equivalents and the antioxidant enzyme catalase. An important consequence of this altered redox state is the generation of oxidized membrane components in the form of 4-hydroxynonenal, ceramides and oxidized phospholipids, all of which are potent signalling molecules which lead to modulation of the expression of many genes. Transcription factors (such as nuclear factor kappa-light-chain-enhancer of activated B cells) and several genes (*e.g.* interleukins, intercellular adhesion molecule and 1, hemeoxygenase 1) involved in the inflammatory response are dramatically modified by UVA. Levels of both antioxidant and pro-oxidant proteins, including manganese-dependent superoxide dismutase, glutathione peroxidase, hemeoxygenase 1, NADPH oxidase, ferritin, and methionine-S-sulfoxidereductase, are increased by UVA treatment and following moderate dose levels these will contribute to either the restoration or a further perturbation of redox homeostasis. Finally, UVA induces a whole set of matrix metalloproteinases and proteases, primarily in cells of dermal origin, which can contribute to the long-term consequences of UVA exposure of skin.

1 Introduction

A key and essential property of all cells, tissues and organisms is their ability to maintain a state of homeostasis. All cellular and multicellular organisms have evolved stress responses to restore homeostasis as rapidly as possible under adverse physical and chemical conditions. Eucaryotic cells were shown to respond to short wavelength UV (UVC) radiation by inducing a plethora of genes in a phenomenon known as the "UV response", and the genes activated included oncogenes such as c-FOS and c-JUN as well as interstitial collagenase (matrix metalloproteinase 1, MMP-1). Activation of several types of transcription factor were central to the response (*e.g.*, Activator protein 1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and p53) and key upstream events included the activation of several types of mitogen-activated protein kinases (MAPKs). Numerous publications on this topic have appeared over the years but it should be noted that UV doses were often very high and, while similar results were obtained with UVB radiation and the results have been very informative in terms of understanding the cellular signalling response, the doses were frequently out of any reasonable physiological range.

In contrast to the UVB wavelength range, the UVA component of sunlight generates a major oxidative stress in cells at physiologically relevant doses. One of the early events appears to be generation of singlet oxygen. Events that rapidly follow include oxidation of lipids and phospholipids and products include 4-hydroxynonenal (4-HNE) and ceramides which can act as signalling molecules. The latter are believed to be key intermediates in the stimulation of nicotinamide adenine dinucleotide phosphate oxidase 1 (NOX-1) which, at least in keratinocytes, leads to a rapid further increase in reactive oxygen species (ROS), particularly superoxide anion. UVA-mediated oxidative events are further exacerbated by both the immediate and the delayed release of the pro-catalytic factors, free iron and heme, by UVA. As a consequence of these events, a key property of UVA radiation is the perturbation of redox homeostasis in cells and the consequent increase in lipid signalling intermediates leads to activation of various genes. The purpose of this review is to describe these complex consequences of UVA irradiation and to point out, where relevant, how such induced events contribute to restoration of redox homeostasis. The studies described have been undertaken using human skin fibroblasts unless stated otherwise.

2 UVA as an oxidative stress

a Evidence *in vitro* and in cells

It was observed a century ago that glass-filtered radiation (*i.e.* >302 nm) emitted from a mercury source inactivated enzymes such

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as amylase, catalase, trypsin and tyrosinase and that this damage was oxygen-dependent (H. Agulhon, *Ann. Inst. Pasteur Paris*, 1912, **26**, 38–47). Many years later it was shown that the UVA inactivation of *Escherichia coli* was oxygen dependent,[1] and studies followed in several microorganisms and in human cells where almost complete oxygen-dependence of inactivation of colony forming ability was shown.[2,3] Work from several laboratories using components of cells *in vitro* demonstrated that UVA radiation at relevant doses had the potential to generate various ROS in cells and this, together with evidence for the oxygen-dependence of most UVA-mediated biological events, provided the basis for concluding that UVA would generate a strong oxidative stress in cells and tissue (see Fig. 1). Experiments with human fibroblasts demonstrated that reduction in the major source of cellular reducing equivalents (reduced glutathione, GSH) dramatically enhanced UVA-mediated events such as cell death[4] and gene activation,[5] further implicating ROS in these events.

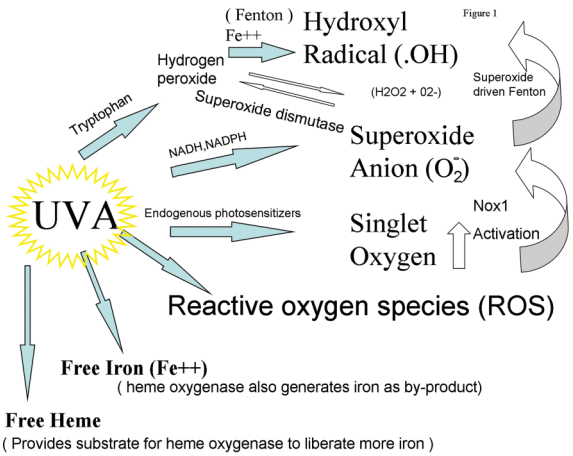

Fig. 1 The generation of reactive oxygen species and the liberation of pro-oxidant catalysts as a consequence of the interaction of UVA radiation with cells.

UVA leads to an extremely rapid depletion of cellular GSH, the major source of cellular reducing equivalents, in both human fibroblasts[5] as well as human skin.[2] This occurs at routine solar exposure levels and will exacerbate oxidative stress levels in cells. GSH depletion also occurs after UVB but the depletion as a function of other biological events (*e.g.* death) is several times less than UVA. Catalase itself is exquisitely sensitive to UVA radiation because of its heme-containing groups and the solar sensitivity of catalase is well-demonstrated by the seasonal variation of its activity in the stratum corneum of skin where it is much lower in the summer months.[6] Additional studies with broad band UVA and UVB lamps showed that the effect was due to the UVA component. In contrast, superoxide dismutase levels were not affected by seasonal variation.

b Evidence in tissue

ROS have been measured in the skin of hairless rats[7] and in the stratum corneum of human skin after UVA[8,9] with some evidence of ROS generation in deeper layers. These studies of the UVA radiation-induced chemiluminescence of human skin *in vivo* have been complemented[10] by following the decay characteristics of UVA-induced photon emission caused by different radiation doses. This approach provides an extremely sensitive methodology to measure the effectiveness of topically applied antioxidants. There is a considerable literature showing that levels of skin antioxidants and antioxidant enzymes are not only reduced by acute UVA exposures but also decrease with photoaging which again will exacerbate the oxidative stress generated by further exposure to the oxidising components of sunlight[11] and should be measurable by these new methodologies.

c Specific ROS generated

Several lines of evidence indicate that the main ROS species generated in cells and tissue by UVA radiation is singlet oxygen and that this species is the main one involved in UVA effects from cell death to gene activation.[12,13] Although the latter was first demonstrated with the hemeoxygenase 1 (HO-1) gene, studies with other UVA inducible genes reported a similar dependence.[14] Many subsequent studies demonstrated the role of this species in UVA-mediated signalling[15] so that UVA-generated singlet oxygen is now generally accepted as a key early intermediate in cellular signalling pathways.

Recently it has been shown that UVA (presumably *via* singlet oxygen) enhances NADPH oxidase 1 (NOX-1) and that this is a primary source of UVA-induced ROS generation (primarily superoxide anion) in immortalized human skin keratinocytes at sub-lethal doses.[16] In contrast, a study[17] comparing normal primary murine keratinocytes with cells knocked out for phagocyte oxidase, demonstrated that this oxidase played only a limited role in both the induction of ROS and the increased apoptosis that follows UVA irradiation of these cells.

d Disruption of iron and heme homeostasis

In addition to the production of ROS, UVA radiation can influence the levels of pro-oxidant catalysts, particularly iron and heme.[18,19] Iron plays a catalytic role in Fenton chemistry which results in peroxide generation in cells and, although iron is not involved in the initial step of lipid peroxidation, it can play a catalytic role in the lipid peroxidation chain reaction. Reflecting these observations, there have been numerous examples where modulating free cellular iron levels by iron chelation has led to modulation of UVA-mediated effects.[19] Importantly, it has been shown[20] that low dose irradiation of human skin fibroblasts with UVA can increase the levels of labile iron in these cells several-fold. This appears to result from UVA damage to the lysosomal membranes. Subsequent studies have confirmed these findings and shown that free iron is also increased in other skin cell types following UVA[21] and further implicated iron in necrotic cell death.[22] The role of iron in UVA and other oxidant-mediated effects is explored in depth in the article by Aroun *et al.* in this issue.[151]

Free heme can also play a catalytic role in the appropriate cellular environment and is a major UVA absorbing chromophore. UVA can damage heme-containing proteins (*e.g.* catalase, see above) and, more recently, it has been shown that following UVA damage to cells free heme is released.[23] The release of both labile iron and free heme by UVA radiation will further amplify the oxidative stress engendered by direct production of ROS.

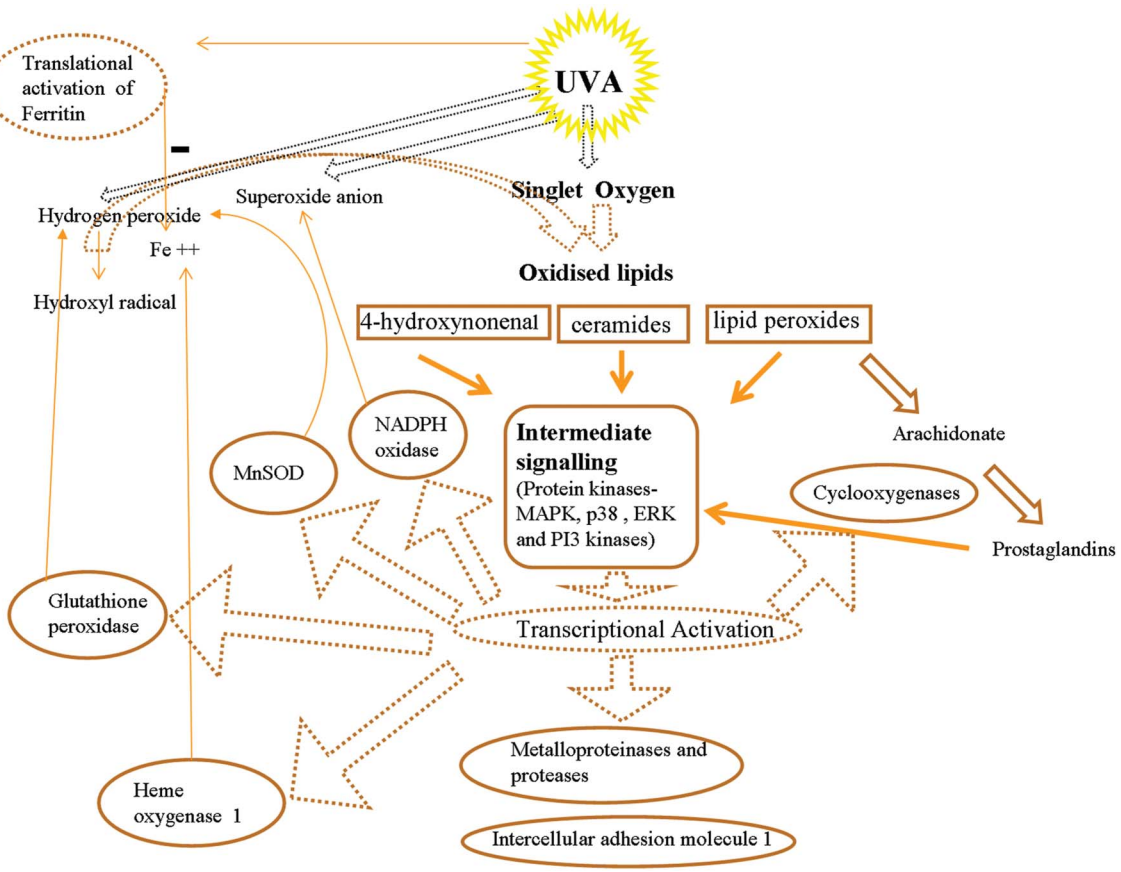

Fig. 2 Generation of oxidised signalling lipids and transcriptional activation as a consequence of UVA irradiation of cells. Reactive oxygen species and free iron may be increased (+) or decreased (–) as a consequence of activation of expression of specific genes with a profound influence on redox homeostasis.

3 UVA as an oxidant that modulates gene expression

a Introduction

UVA is well-known to activate expression of a variety of genes at doses relevant to natural sunlight exposure (see Fig. 2).[24,25] Of these, the largest and most studied effects are the activation of HO-1[26,27] and a series of metalloproteinases as first observed for (MMP-1).[28] However many other genes have been observed to be activated by UVA including intercellular adhesion molecule 1 (ICAM-1)[29] and cyclooxygenase 2 (COX-2).[30] Much has been learnt recently about the UVA-induced signalling intermediates and the complex cascades of signalling kinases and phosphatases involved in these effects. The pathways are often cell-type dependent.

b UVA-generated signalling intermediates

i Singlet oxygen. Pioneering studies[31] had shown that ROS, particularly peroxide, were involved in modulation of gene expression and there is now a substantial literature on redox regulation of genes *via* a variety of mechanisms. Studies on UVA inactivation of human cells had shown that singlet oxygen was involved in this response, and the demonstration that this intermediate was the primary ROS intermediate in UVA activation of HO-1 gene expression was the first demonstration that singlet oxygen was potentially a key signalling intermediate.[13] Studies soon followed that showed, not surprisingly, that activation of gene expression in photodynamic therapy models (that involved photosensitisers that generated singlet oxygen) also arose *via* this intermediate. Singlet oxygen was also shown to be involved in UVA activation of MMP-1[32] and ICAM-1,[33] as well as most UVA-mediated signalling events subsequently tested. The UVA generation of singlet oxygen is now considered a primary early event in the gene modulation process and is likely to act *via* the generation of oxidised lipids such as ceramides, 4-HNE and oxidised phospholipid, all of which are powerful signalling molecules (Fig. 2).

ii Lipid oxidation products, oxidised phospholipids and 4-HNE. Although ceramide can be generated enzymatically, UVA is believed to act photochemically on sphingomyelin to generate a ceramide intermediate,[34] although there is some controversy in the literature on this issue.[35] Ceramide is a key component in cellular stress responses and has been shown to be implicated in the AP-2 dependent activation of ICAM-1 expression in keratinocytes.[33] The process is likely to involve a ceramide-mediated autocrine loop since the second phase of AP-2/ICAM-1 activation involves activation of serine palmitoyltransferase,[36] which is central to ceramide synthesis. This UVA-mediated signalling response, which appears to involve an early non-enzymatic (photochemical) step, is entirely dependent on the ceramide composition of lipid rafts.[37]

UVA generates oxidized phospholipids from 1-palmitoyl-2-arachidonoyl-sn-*glycero*-3-phosphorylcholine (PAPC) in human

skin fibroblasts.[38] This process depends upon singlet oxygen and these phospholipids are now known to stimulate accumulation of a nuclear factor (NRF-2), a crucial transcription factor which, on migration to the nucleus, leads to up-regulation of a series of antioxidant response genes including aldo-ketoreductases and HO-1.[39] UVA irradiation of arachidonate-containing phospholipids also generated lipid oxidation products which include epoxyisoprostane-phosphatidylcholine and again induce HO-1 expression in skin cells[38] *via* NRF-2.[39]

A crucial UVA-generated lipid oxidation product known to be important in signalling is 4-HNE This compound is a strong inducer of HO-1 and is almost certainly involved in its activation by UVA radiation.[40] Using specific lipid anti-oxidants, this study also indicated that the origin of the lipid signalling intermediates was internal membrane lipids. In subsequent studies, UVA-generated 4-HNE has been shown to underlie sustained activation of a JUN N-terminal kinase (JNK) and apoptosis[41] in a human myelogenous leukaemia cell line (K562). Interestingly, lower 4-HNE levels are observed in cells which over-express glutathione S-transferases and these cells display resistance to apoptosis induced by UVA and other oxidants.[42]

As part of the study on the link between UVA-mediated lipid oxidation products and activation of HO-1 mRNA, the phospholipase metabolites diacylglycerol and arachidonic acid were also shown to be active in modulating gene expression.[40]

c UVA activation of protein kinases and phosphatases

Signalling pathways usually involve a cascade of protein phosphorylation (kinase) reactions. UVA activates a broad spectrum of protein kinases, the specifics of which vary considerably with skin cell type. In studies undertaken in the early days of signalling science, UVA radiation had been shown to activate protein kinase C (PKC, a phospholipid-dependent ser/thr kinase) in murine fibroblasts,[43] normal human epidermal keratinocytes,[44] and rat myeloid leukemia cells.[45] Inhibitor studies later linked PKC signalling to the UVA activation of MMP-1.[46] Activation of p38 MAP kinase and JNK pathways by UVA has been well characterized and will be discussed in depth by Zhang and Bowden elsewhere in this issue.[152] While it is still crucial to define the target genes involved, there are strong arguments to support the notion that activation of kinase-dependent signalling pathways is involved in skin tumor promotion and progression.[47,48]

UVA irradiation is known to compromise gap junctional intercellular communication and this has been associated with hyperphosphorylation and decreased levels of the highly expressed keratinocyte gap junction protein, Connexin 43.[49]

The epidermal growth factor receptor (EGFR), a much-studied oncogene involved in development of many cancers, is a membrane surface receptor which is activated by various ligands (*e.g.* epidermal growth factor) to become an important tyrosine kinase which, in turn, activates several signal transduction pathways (*e.g.* MAPK, AKT kinase and JNK) that lead to DNA synthesis and cell proliferation. Many receptor tyrosine kinases (including EGFR located at the inner side of the plasma membrane) are activated by short wavelength UV[50–53] and UVA leads to a similar activation by inhibiting tyrosine phosphatase activity.[54] A series of studies have examined the downstream consequences of EGFR activation by UVA on signalling kinase activation.[55–59] Unlike EGF, UVA modifies EGFR independently of kinase activity and the receptor down-regulation by UVA may be involved in promoting apoptosis.[59] Activation of EGFR by UVA irradiation of HaCaT cells potentiates anchorage-independent growth of the keratinocytes and this may well be relevant to malignant transformation by UVA radiation.[60] UVA activation of EGFR has been linked with both loss of cell-to-cell contact and the nuclear relocalisation of beta catenin to generate a beta-catenin/T-cell factor 4 (TCF4) complex which mediates activation of MMP-1.[61] Again these events would be crucial in stimulating keratinocyte invasiveness following UVA.

Another interesting kinase that is stimulated by UVA is the DNA damage-inducible Ataxia Telangiectasia mutated (ATM) kinase which regulates cell survival and cell cycle checkpoints and again ATM-dependent p53 and JNK pathways have been linked to UVA apoptosis.[62] Abelson-related gene (Arg) is also strongly up-regulated by UVA in normal human keratinocytes.[63] Finally, PKR (double-stranded RNA-dependent protein kinase R) has also been shown to be activated by extracellular stress including UVA[64] with potential anti-viral and apoptotic consequences.

UVA radiation is known to increase the expression of DUSP1/MKP-a specific protein-tyrosine phosphatase,[65,66] which can down-regulate mitogen-activated protein kinases by dephosphorylation. However UVA (and UVB) radiations can also degrade and irreversibly inactivate protein tyrosine phosphatases by the combined effect of substrate oxidation and activation of calpain, a calcium-dependent non-lysosomal cysteine protease.[67]

d UVA modulation of transcription factors

i UVA modulation of the transcription factors NRF-2 and BTB and CNC homology 1, basic leucine zipper transcription factor 1 (BACH-1). Hemeoxygenase 1 is controlled at the transcriptional level by the dynamic interaction of the activating NRF-2/MAFK heterodynamic complex and the repressing BACH-1/MAFK type complex.[68] Of all the factors involved in cellular stress responses, NRF-2 is the most studied. This factor is involved in the activation of phase 2 proteins which have been strongly implicated in chemoprevention of carcinogenesis. Under normal (non-stress) conditions Kelch-like-ECH-associated protein 1 KEAP-1 binds to NRF-2 in the cytoplasm and it is continuously degraded through the ubiquitination pathway. Stress (including UVA irradiation) stabilizes the protein which migrates to the nucleus where it binds to small MAF proteins (typically MAFK). This powerful heterodimeric transcription factor complex can then bind to the MARE (MAF associated regulatory element) which is present in the promoter of the HO-1 gene and other stress-activated genes. Apparently, the activation of the NRF-2 stabilisation response by UVA correlates with the UVA generation of oxidized phospholipids[39] The biological relevance of NRF-2 activation in light/UVA-induced stress has been shown by using rodent retinal pigment epithelial cells knocked out for NRF-2 or with the repressor KEAP1 disrupted and demonstrating the photosensitivity (300–400 nm) of these cells compared to wild type.[69] Activation of NRF-2 by various agents (*e.g.* sulforafane and flavonoid antioxidants such as quercetin) has been implicated in photoprotection.[70]

UVA radiation leads to stabilization and nuclear accumulation of NRF-2 both in rodent[71] and human[72] in skin fibroblasts.

In the latter study, specific inhibitory small interfering RNAs(SiRNAs)were used to show that NRF-2 is involved in protection against UVA-induced membrane damage. The second study[72] also showed that the UVA/NRF-2 activation response is dependent on heme levels. The DNA binding activity and therefore functionality of the transcriptional repressor protein BACH-1 is also dependent on heme.[73] Heme also promotes degradation of BACH-1[74] and its nuclear transport.[75] Like NRF-2, BACH-1 forms a heterodimeric transcription factor by binding to MAFK but the BACH-1/MAFK complex acts as a negative regulatory protein and binds to the MARE site of HO-1 to prevent transcription.[68] BACH-1 bound to the HO-1 promoter prevents UVA activation of HO-1 under low heme conditions and knock-down of BACH-1 increases resistance of keratinocytes to UVA-induced membrane damage.[76]

ii NFκB. NFκB has provided a classical example of redox sensitivity of transcription factors.[31] This factor, which has a central role in many inflammatory events, would be expected to be responsive to UVA and indeed it was shown that relatively low doses of UVA activate NFκB binding to DNA in human skin fibroblasts,[77] whereas activation of NFκB by UVC and UVB radiations was only observed at very high doses or where there was evidence of significant membrane damage. It was later shown[78] that the kinetics of appearance of NFκB accumulation in the nucleus was slow and that this was related to labile iron-dependent nuclear membrane damage. Indeed, this work supported the concept that oxidised lipids are a crucial intermediate in UVA-mediated NFκB activation and may underlie the role of UVA in skin inflammation. There is also evidence that UVA can enhance NFκB binding to DNA in HaCaT cells[79] and a human epithelial cell line,[80] but studies in normal human melanocytes report both negative[79] and positive[81] results.

iii AP-1. AP-1 which is crucially involved in metalloproteinase activation has long been known to be redox sensitive and the REF-1 protein has been strongly implicated in redox regulation of transcription factors including the AP-1 component proteins, c-FOS and c-JUN.[82,83] Apparently UVA can alter the redox state of and activate AP-1 components.[84] UVA radiation strongly activates c-FOS gene expression in human dermal fibroblasts at physiologically relevant doses,[85] thus providing a mechanism by which genes (*e.g.* those coding for stromelysin and MMP-1)[86] are UVA-activated *via* the AP-1 binding elements in their respective promoters. However, as detailed in the section on metalloproteinases below, the pathways leading to metalloproteinase activation by UVA are varied and complex.

In a recent study it has been confirmed that low-dose UVA increased nuclear levels of c-JUN but not c-FOS in JB6 C141 epidermal cells and increased reporter activity of AP-1 promoter elements.[87] Importantly, these authors showed that PIN-1, a peptidylprolylisomerase that is overexpressed in most types of cancer tissues, is activated by UVA irradiation of both the skin of hairless mice and epidermal cells. PIN-1 inhibition blocked proliferation and cyclin D1 expression of epidermal cells following UVA treatment.

iv STATs. Signal transducers and activators of transcription (STATs) which regulate genes involved in cell proliferation, survival, differentiation, angiogenesis and invasion are among the most studied classes of transcription factors because they are often inappropriately activated in cancer and have been implicated in the carcinogenesis process. STAT-1 binding to its target promoter was enhanced by low UVA doses in a keratinocyte cell line (NCTC 2544) but inhibited by higher doses.[88] Inhibitor studies indicated that phosphorylation by both serine/threonine kinases and MEK was involved. Nuclear transport of STAT-1 has been observed after UVA.[89] Phosphorylation of STAT-3 (at Ser 727) has been observed in a mouse epidermal cell line (JB6) following UVA radiation and this occurs *via* mitogen and stress-activated protein kinase (MSK-1) and JNKs but not extracellular signal-regulated kinases (ERKs) or p38 kinase.[90] The biological relevance of UVA modulation of STAT activity remains to be elucidated.

v Activating transcription factor 3 (ATF-3). Transcription of ATF-3, a member of the cyclic AMP response element binding (CREB) family of transcription factors is substantially induced by relatively low (non-lethal) doses of UVA radiation.[91] Again the biological relevance of this UVA-dependent response is not yet clear but ATF-3 dependent genes are involved in immunity and implicated in cancer development.

e UVA activation of genes influencing cellular redox homeostasis and inflammation

i Hemeoxygenase 1. HO-1 was the first gene to be shown to be activated by an imbalance in cellular redox state in mammalian cells and the initial reports in human skin fibroblasts[26,27] derived from studies which used UVA radiation as a natural oxidising carcinogen at biologically relevant doses. It is by far the most dramatic gene activation response to oxidative stress observed in mammalian cells and, like other UVA-mediated gene activation responses, the first event appears to be the UVA generation of singlet oxygen. HO-1 levels (basal and induced) are exquisitely dependent on cellular redox status and can be manipulated by altering GSH levels in cells.[5] As mentioned above, UVA is a powerful lipid oxidant and HO-1 is involved in protection against oxidative membrane damage.[92] However it has also been recognized as a major anti-apoptotic and anti-inflammatory protein and has been implicated in numerous pathologies.[93,94]

The central role of hemeoxygenases is in heme catabolism. This activity is essential to degrade heme in a controlled fashion during erythrocyte turnover but it is also an essential cellular factor to keep free heme levels low. Oxidants including UVA can release free heme in cells[23] and this must be removed to maintain heme and redox homeostasis (Fig. 3).

As described above, the promoters of both the rodent and human HO-1 genes contain crucial response elements (MAREs) and a dynamic equilibrium between activation and repression of transcription of the gene is maintained by the relative activities of the NRF-2 and BACH-1 heterodimeric transcription factors respectively.[68] The demonstration of the crucial nature of these factors in HO-1 expression allowed us to further investigate our earlier hypothesis[95] that heme was a crucial factor in modulating the activity of this gene. This study showed that NRF-2 is stabilised by UVA radiation and that this stabilisation is mediated by the UVA release of heme.[72] The heme molecule is central to the repression and UVA released heme will bind to BACH-1 and release its binding to the HO-1 promoter.[76,96] Together, these studies evidence the central role of free heme in HO-1 gene expression.

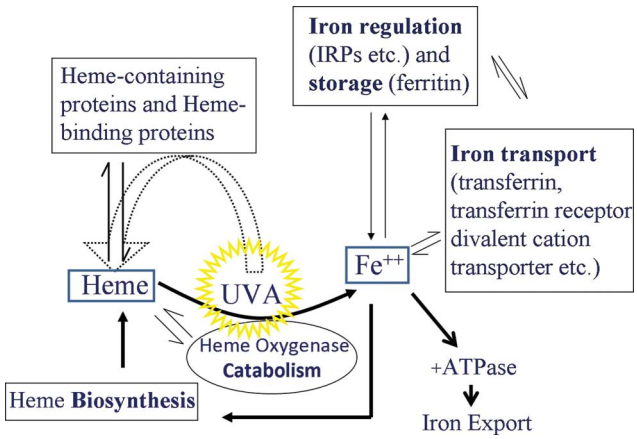

Fig. 3 Disturbance and restoration of heme and iron homeostasis following UVA irradiation.

In contrast to skin fibroblasts and melanocytes,[27,97] epidermal keratinocytes appear not to require the inducible HO-1 pathway since they show constitutive expression of HO-2.[98] Again, HO-2 was believed to prevent UVA activation of HO-1 by mopping up free heme but the role of HO-2 in suppressing HO-1 activation has only recently been verified.[76] Specific silencing of HO-2 by SiRNA knockdown leads to strong basal and UVA-induced expression of HO-1 even in epidermal keratinocytes. Importantly, it has been shown that it is BACH-1 that is central to the suppression of HO-1 in fibroblasts since SiRNA knockdown of expression of this protein leads to a massive constitutive expression of HO-1. The expression level cannot be further increased by inducers such as UVA radiation or by knocking down HO-2. Finally, a central role for heme in mediating the expression level of HO-1 could also explain the strong refractoriness to UVA induction of HO-1 by a second delayed dose of UVA[95] since a few hours after UVA irradiation, induced HO-1 levels will be high and free heme levels very low.

Maintaining low levels of free heme is crucial to preventing cellular damage and it would appear that the primary role for the activation of HO-1 following oxidative stress, including that induced by UVA radiation, is the restoration of heme homeostasis (Fig. 3). However, it is important to note that the catabolism of free heme results in the release of iron and that this will add to the level of labile iron released immediately after UVA treatment as a result of lysosomal destruction.[20] UVA-mediated oxidative stress therefore leads not only to a disruption of heme homeostasis but also to a disruption of iron homeostasis. Enhanced free iron levels will exacerbate oxidative stress by acting as a catalyst in Fenton chemistry and the lipid peroxidation chain reaction. The release of iron following UVA treatment will activate another regulatory protein, the iron responsive protein, which results in the strong induction of the iron storage protein ferritin.[99] The link between UVA and iron homeostasis is further described by Aroun *et al.* in this issue.[151] It is evident that the immediate induction of HO-1 and the delayed induction of ferritin following UVA are intimately linked and are crucial to restoring heme and iron homeostasis in cells and thereby maintaining cellular redox homeostasis.

ii Antioxidant enzymes. The classical cellular antioxidant enzymes, superoxide dismutases (SODs, which convert superoxide anion to hydrogen peroxide), glutathione peroxidases (GPx, which remove cellular peroxides including hydrogen peroxide), peroxiredoxins (which reduce a broad range of peroxides) and catalase (which converts hydrogen peroxide to water) are available in most cells to provide essential antioxidant defense against UVA irradiation and back up the constitutive defense provided by reduced GSH (which is present in skin tissue at millimolor levels) and other anti-oxidant components including anti-oxidant vitamins. Glutathione is progressively destroyed with increasing doses of UVA. The activity of catalase, which contains a UVA-absorbing heme-containing chromophore, is also reduced by UVA radiation (see above) and so the activity of the other antioxidant enzymes following UVA becomes particularly relevant.

A series of studies have mapped changes in antioxidant enzyme activity in different skin tissue[100] and showed gradients of activity.[101] Work in human skin fibroblasts[102] had indicated that acute exposure to UVA and incubation for several days led to little overall change in SOD and GPx activity in living human fibroblasts but did result in loss of catalase activity. Chronic irradiation of rodent skin[103] had shown a similar outcome. However, studies using cultured cells demonstrated that repeated acute doses of UVA (at an appropriate interval, 12 h but not 24 h) led to an approximately 5-fold increase in manganese-dependent superoxide dismutase (MnSOD) in human skin fibroblasts and that this was protective against high dose UVA exposure.[104] In complementary work shortly afterwards,[105] it was shown that similar repeat dose UVA irradiation conditions led to a substantial upregulation of selenium-dependent GPx and that under selenium-supplemented conditions this activity complemented the protection provided by the enhanced MnSOD levels. Although similar studies have not been carried out in human skin, the prediction is that both induced MnSOD and GPx will add to the antioxidant defense required against solar UVA and compensate, at least in part, for the loss of catalase and GSH activity.

iii NADPH oxidase 1. UVA is now known to activate NOX-1 and this is a primary source of UVA-induced ROS generation (primarily superoxide anion) in cells at sublethal doses.[16] A direct consequence of this increase in ROS (as shown by SiRNA knockdown experiments) is rapid initiation of prostaglandin E2 synthesis.

iv Thioredoxin interacting protein. Thioredoxin is an important cellular reducing agent which is inhibited by the thioredoxin interacting protein (TXNIP). UVA exposure up-regulates TXNIP gene expression in cutaneous melanoma cells thereby lowering thioreoxin activity and increasing cellular ROS which, in turn, leads to intravasation of melanoma cells.[106]

v Methionine-S-sulfoxidereductase. The enzyme methionine-S-sulfoxidereductase which repairs oxidized proteins is expressed in human epidermis and upregulated by low doses of UVA radiation (and hydrogen peroxide), but not by UVB.[107] Its expression was also shown to be increased by repetitive exposure of human skin to simulated sunlight. The enzyme reduces methionine sulfoxide to methionine, thus reversing the inactivation of proteins caused by oxidation of critical methionine residues. It is the only enzyme so far identified in human skin that is able to repair oxidative protein damage.

vi Intercellular adhesion molecule 1. ICAM-1 plays an important role in mediating leucocyte/keratinocyte adhesion and is therefore crucial to the inflammatory response. UVA induces

ICAM-1 in human skin keratinocytes[29] and the activation of the AP-2 transcription factor on the ICAM-1 promoter of the human keratinocyte ICAM-1 gene was shown to be the mechanism underlying this response.[33] This model system was also used to show the crucial role of ceramide in UVA signalling (see above). Interestingly, ICAM-1 (and E-Selectin but not VCAM-1) was induced by UVA but not UVB in human dermal microvascular epithelial cells,[108] indicating an important role for these cells in skin inflammation. Further studies from the same group[109] demonstrated that repeated exposure to UVA actually abrogated leucocyte adhesion to endothelial cells. In contrast, UVA has been shown to lead to a decrease in ICAM-1 expression in both epidermis and epidermal keratinocytes and although some increase in ICAM-1 was observed in dermal fibroblasts, ICAM-staining in the dermis was specific for vascular structures,[110] a finding consistent with previous data.[108]

vii Cyclooxygenase-2 (COX-2). Cyclooxygenases generate the inflammatory mediators known as prostaglandins from arachidonate. Although such intermediates have been implicated in UVB induction of erythema, studies using indomethacin (a COX inhibitor) indicated that prostaglandins were not involved in UVA erythema.[111] Nevertheless, COX activity was measured in human and murine skin fibroblasts[30] following UVA irradiation and activation of COX-2 has been clearly demonstrated in HaCaT cells[23] and in artificial human epidermis.[112] Activation in keratinocytes appears to be mediated by p38 stabilisation of the COX-2 mRNA.[113] These authors conclude that MAPKs, JNK and p38 are all involved in COX-2 stimulation following UVA activation and that AP-1 is a major factor.

f UVA activation of metalloproteinases and other proteases

i Metalloproteinases. UVA irradiation activates a series of metalloproteinases in human skin fibroblasts (Table 1) and it is believed that these play a key role in elimination of damaged/oxidised proteins and in remodelling of damaged skin. Chronic activation of these multiple proteases in the dermis together with sustained oxidative protein damage will very likely lead to irreversible damage to the extracellular matrix and contribute to the photoaging process.

UVB and UVA radiation both activate MMP-1 in human skin fibroblasts. Studies with UVB radiation led to the proposal several years ago that tissue-degrading metalloproteinases are involved in photoaging[114,115] and strong activation by UVA radiation will clearly contribute to this process. The enzymatic capacity for extracellular matrix synthesis and degradation mostly resides in the dermis. MMPs (28 zinc metallo-endopeptidases are currently recognised) break down collagen, gelatine and other matrix proteins and include collagenases (collagen), gelatinases (gelatine), stromelysins and matrilysins (non-secreted membrane type family). It is important to note that MMPs are zymogens, and the signal sequence polypeptides are removed during activation.

MMP-1 was shown to be activated in primary fibroblasts but not in keratinocytes where UVA actually slightly lowered MMP-1 levels.[116,117] However UVA activation of MMP-1 was later shown in transformed cells that included a squamous cell carcinoma cell line and HaCaT cells.[118–120]

Crucially very low doses of UVA radiation lead to activation of MMP-1 in human skin fibroblasts,[116] an activation that has again been shown to depend on singlet oxygen.[32] At least in cultured fibroblasts, the response is biphasic[121] with a first peak of induction at approximately 3 h and a second peak a day later. The first peak is believed to be due to the rapid post-translational activation of interleukin 1 (IL-1) which stimulates IL-6 and the second peak also involves IL-1 and IL-6 but is due to transcriptional activation of these cytokines. There are several studies related to protein kinase involvement in the process. For example, it has been shown that serine/threonine kinase is crucial to MMP-1 activation (at least for UVB) but it is at the level of translational control.[122] However, a key finding[123] is that UVA activation of MMP-1 is abrogated by phospholipid-hydroperoxide glutathione peroxidase clearly demonstrating the involvement of oxidised lipid in this response, *e.g. via* lipid peroxide-mediated activation of NFκB which itself is essential to IL-6 activation. Interestingly iron is involved in both NFκB activation by UVA[78] and the second peak (24 h) of UVA activation of MMP-1.[124]

Several intermediates have been implicated in MMP-1 activation by UVA in dermal fibroblasts including macrophage migration inhibitory factor (MIF),[125] which is controlled by various upstream protein kinase pathways (PKC, PKA, SRC family tyrosine kinases, MAPK and c-JUN but not p38). A more recent study using knock-out mice has confirmed that the IL-1 beta/MIF is involved in UVA activation of MMP-1.[126]

New findings on redox regulation of MMP-1 have emerged recently. For example, the antioxidant oxidoreductase thioredoxin-1 has been shown to inhibit UVA-induced MMP-1 activation.[127] Although these authors concluded that this was an effect on transcription, redox regulation of MMP-1 could occur at the post-translational level. Reduced thiols such as cysteine or glutathione substantially reduced MMP-1 activity[128] and this effect was reversed by oxidising radicals (*e.g.* the trolox radical). The authors concluded that these observations reflected redox control *via* derivatization or oxidation of a GSH/MMP-1 complex. As for HO-1, lipid signalling (involving the UVA generation of

Table 1 UVA activation of a variety of metalloproteinases in human skin cells

	Fibroblasts	Keratinocytes
Metalloproteinase 1 (MMP-1) collagenase	***	Lowered (normal cells); enhanced in transformed cells, *e.g.* HaCaT
Metalloproteinase 2 (MMP-2) type iv collagenase, gelatinase A	**	Lowered
Metalloproteinase 3 (MMP-3) stromelysin 1	**	*
Metalloproteinase 9 (MMP-9) gelatinase B		Lowered (acute) in normal human keratinocytes. Increased secretion (chronic, HaCaT)
Metalloproteinase 10 (MMP-10) stromelysin 2		Enhanced in transformed cells, *e.g.* HaCaT

*, **, *** refer to the relative levels of activation (low, medium or high respectively.

lipid peroxides) has been implicated in MMP-1 activation in skin fibroblasts.[124] Repeated (chronic) exposure of human skin fibroblasts to UVA radiation leads to a sustained overexpression of MMP-1.[129] Recently UVA has been shown to down-regulate the forkhead box gene, group O (FOXO) transcription factor (crucial in regulating insulin/insulin-like growth factor-1 signalling)[130] and since knock-down of FOXO-1A expression led to increased expression of MMP-1 and -2 this pathway is also implicated in regulating metalloproteinase expression.

Among agents that have been shown to protect against MMP-1 activation by UVA are flavonoids.[21] Beta carotene can also prevent UVA-mediated MMP-1 activation in skin cells[120] and dietary beta carotene can suppress MMP-9 activation in mice.[131] The authors conclude that this systemic effect is due to the prevention of cholesterol peroxidation which is known to be mediated by UVA and to be a trigger for gene activation.

The proteasome has also been implicated in the UVA stress response involving metalloproteinases[132] since overexpression of either the proteasome or methionine sulfoxide reductase (which repairs oxidized proteins) prevents UVA activation of MMP-1. Under conditions where the proteasome is inhibited, MMP-1 signalling pathways are activated (*via* c-JUN phosphorylation and AP-1 activation).

As well as MMP-1, metalloproteinases 2 (type IV collagenase, gelatinase A) and 3 (stromelysin 1) have also been reported to be induced in human fibroblasts after UVA treatment.[28] Activation of MMP-2 (and reduction in tissue inhibitor of metalloproteinase 2, TIMP-2, activity) has been linked to increased secretion of thioredoxin following UVA treatment by UVA. Although other studies have independently reported UVA induction of MMP-2 in fibroblasts,[133,134] a study in normal human keratinocytes[135] showed that as for MMP-1, UVA quite significantly lowered expression of both MMP-2 and MMP-9 (gelatinase B). No change in secretion of these proteins was seen[136] but chronic (18 week) re-exposure of HaCaT cells to UVA treatment[137] actually led to increased secretion of matrix metalloproteinase 9 (MMP-9). In studies with human microvascular endothelial cells, UVA was shown to up-regulate MMP-1 but down-regulate MMP-2[138] and this has been linked to the impaired angiogenic phenotype of the dermal endothelial cells. The induction of MMP-3 in fibroblasts by UVA has been reported and, as for MMP-1, the crucial *cis*-acting promoter element is an AP-1 site.[86] MMP-3 is also induced in HaCaT cells.[120] Induction of MMP-10 (stromelysin-2) by UVA has been observed in a human squamous cell carcinoma line[118] and in HaCaT cells.[119]

Recent studies in mice[139] demonstrated that the UVA activation of MMP-9 correlated with increased peroxidised cholesterol formation and increased sagging and wrinkling of the skin. Direct injection of cholesterol hydroperoxides also led to MMP-9 activation supporting the concept that lipid peroxides are key to the UVA induced activation of metalloproteinases.[131]

ii Other proteases. Recently, there has been considerable interest in the cathepsin family of proteases. The lysosomal protease, cathepsin K (CATK), is a potent mammalian elastase and may play a role (among other elastases) in formation of solar elastosis. UVA activation of this potent elastase has been observed in human fibroblasts from young donors both *in vitro* and *in vivo*.[140] Importantly the authors observed much reduced inducible elastase activity in fibroblasts derived from the skin of aging donors. They suggested that this age-related decline in CATK activity, which was also evident after UVA exposure, may "promote the formation of actinic elastosis through a decline of orderly intracellular elastin degradation and subsequent accumulation of elastin in the extracellular space."

An interesting study in human skin fibroblasts from Wondrak's group[141] has shown that the enzymatic activity of cathepsin B (CATB), a lysosomal cysteine protease is substantially reduced by repeated UVA irradiation which also compromised CATB maturation. The consequent impairment of lysosomal capacity to remove lipofuscin would be a clear source of dermal damage. A more recent study reported in this issue (Lamore and Wondrak) has indicated that autophagic-lysosomal alterations observed after UVA are the result of impaired autophagy downstream of CATB inactivation.[153]

Another lysosomal protease, CATL, which degrades matrix proteins was not modified by acute exposure of fibroblasts to UVA but chronic irradiation using repeated exposure led to significant extracellular release of the protein.[142] Furthermore, this was associated with a reduction in intracellular processed CATL and an accumulation of unprocessed cathepsin.

UVA may stimulate proteases *via* activation of p38 kinase (see above) in human skin keratinocytes. For example, UVA activation of p38 MAPK in keratinocytes leads to granzyme B (a serine protease) and this will facilitate degradation of extracellular matrix components.[143] Redox-dependent MIF release appears to be involved.

The serious perturbation of metalloproteinase and other types of protease activity/maturation by UVA radiation will undoubtedly prove to have dramatic long-term consequences in skin. The imbalance in synthesis of MMPs, TIMPs and other proteases following acute and chronic UVA is likely to be a major factor in the dissolution of dermal and basement structures and eventual photoaging.

g Modulation of expression of other genes by UVA

An interesting preliminary report[144] indicated a UVA-mediated up-regulation of N-Cadherin and a down-regulation of E-cadherin in a murine melanoma cell line. This was associated with a decrease in self-adhesion of melanoma cells and an increase in melanoma adhesion to epithelial cells with evident implications for metastatic potential. These authors also demonstrated the UVA-induced metastatic potential of these melanoma cells in mice.[145] However, in a study of melanocyte proliferation in hairless SKH-2 mice, it was found that while a single (but not fractionated) dose of UVB led to a strong enhancement in proliferation, no change in proliferation rate was observed following any type of UVA dose regimen.[146] In a study comparing UVA and UVB induced gene expression in primary human melanocytes,[147] proteins involved in cell growth (p73 and Nup88) were notably enhanced in UVA-irradiated cells. Induction of expression of these genes has not been noted in other cell types.

UVA irradiation of mouse epidermis or a murine keratinocyte cell line has been shown to result in induction of an oxidant-susceptible calcium-binding protein (S100A8) of significance in human psoriasis.[148]

Finally, UVA has been shown[149] to induce a reduction in neuron-specific enolase levels in acrosclerosis (a type of autoimmune rheumatic disorder). UVA has been used as an adjuvant treatment in moderately active systemic lupus erythematosus.[150] Such treatment has been associated with softening of sclerotic lesions but other UVA-mediated events such as T-cell apoptosis, metalloproteinase induction and angiogenesis may all also be involved.

Concluding remarks

While normal low level exposures to the UVA component of sunlight will generate a significant oxidative stress throughout the skin and this is further exacerbated by the UVA-mediated release of pro-oxidant catalysts (iron and heme), a consequence of this altered redox state is the activation of several enzymes which act to restore the redox balance (Fig. 2). The activation of MnSOD and GPx will reduce superoxide and peroxide levels respectively thus directly contributing to the restoration of redox homeostasis. Enhanced hemeoxygenase activity results in the mopping up of free heme and a consequence of this appears to be the partial reversal of oxidative membrane damage. However a by-product of heme catabolism is free iron which, together with the labile iron directly released in cells by UVA radiation, will fuel Fenton chemistry, enhance hydroxyl radical generation and catalyse the lipid peroxidation chain reaction. A further contribution to the oxidative stress, at least in epidermal cells, will be the activation of membrane-associated NOX-1 oxidase which will generate enhanced superoxide anion levels. Clearly while cells and tissue have developed ways to counter redox stress, including that generated by UVA radiation, the potential exists for these systems to be overwhelmed and result in various types of oxidative damage. Damage to skin itself may be further exacerbated by the activation of many proteases, including matrix metalloproteinases, which will progressively erode the structure of skin on repeated and chronic exposure to the UVA component of sunlight.

List of abbreviations

4-HNE	4-hydroxynonenal
AP-1	Activator protein 1
ATM	Ataxia Telangiectasia mutated
BACH-1	BTB and CNC homology 1, basic leucine zipper transcription factor 1
CATK	cathepsin K
EGFR	epidermal growth factor receptor
GPx	glutathione peroxidases
GSH	glutathione
HO-1	hemeoxygenase 1
ICAM-1	intercellular adhesion molecule
IL-1	interleukin 1
JNK	JUN N-terminal kinase
MARE	MAF associated regulatory element
MIF	migration inhibitory factor
MMP-1	matrix metalloproteinase 1
MnSOD	manganese-dependent superoxide dismutase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NOX-1	NADPH oxidase 1
NRF-2	nuclear factor (erythroid-derived 2)–like 2
PKC	protein kinase C
ROS	reactive oxygen species
SiRNA	small interfering RNAs
STATs	signal transducers and activators of transcription

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