Free Rad. Res., Vol. 31, pp. 335–340
Reprints available directly from the publisher
Photocopying permitted by license only

Redox Regulation and Oxidant Activation of Heme Oxygenase-1

R. TYRRELL*

Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK

Accepted by Prof. B. Halliwell

(Received 17 March 1999; In revised form 30 March 1999)

The ultraviolet A (UVA, 320–400 nm) component of sunlight has the potential to generate an oxidative stress in cells and tissue so that antioxidants (both endogenous and exogenous) strongly influence the biological effects of UVA. The expression of several genes (including heme oxygenase-1, HO-1; collagenase; the CL100 phosphatase and the nuclear oncogenes, c-fos and c-jun) is induced following physiological doses of UVA to cells and this effect can be strongly enhanced by removing intracellular glutathione or enhancing singlet oxygen lifetime. We have observed that heme is released from microsomal heme-containing proteins by UVA and other oxidants and that activation of HO-1 expression by UVA correlates with levels of heme release. UVA radiation also leads to an increase in labile iron pools (either directly or via HO-1) and eventual increases in ferritin levels. The role of heme oxygenase in protection of skin fibroblasts is probably an emergency inducible defense pathway to remove heme liberated by oxidants. The slower increase in ferritin levels is an adaptive response which serves to keep labile iron pools low and thereby reduce Fenton chemistry and oxidant-induced chain reactions involving lipid peroxidation. In keratinocytes, the primary target of UVA radiation, heme oxygenase levels are constitutively high (because of HO-2 expression). Since there is a corresponding increase in basal levels of ferritin the epidermis appears to be well protected constitutively against the oxidative stress generated by UVA.

Keywords: Oxidative stress, UVA, solar ultraviolet, gene expression, heme oxygenase, antioxidants, glutathione, singlet oxygen, iron, ferritin, protection, skin cells

INTRODUCTION

Solar ultraviolet radiation ranges from approximately 290 to 380 nm. The shorter UVB (290–320 nm) wavelengths are strongly absorbed by DNA and cause direct damage to biomolecules and are considered to be the primary photocarcinogenic wavelengths, at least with regard to non-melanoma skin cancer. UVA (320–380 nm) radiation can also cause many biological effects and these wavelengths are carcinogenic in animal models.[1] An interesting feature of the interaction of UVA radiation with tissue is that it has the potential to generate a strong oxidative stress[2] leading to formation of oxygen radical intermediates and singlet oxygen. As a consequence of this, many of the biological consequences of UVA are the result of oxidative processes and antioxidants

* Tel.: 01225 826793. Fax: 01225 826114. E-mail: R.M.Tyrrell@bath.ac.uk.

(both endogenous and added) are therefore critical determinants of the biological consequences of UVA radiation.

ULTRAVIOLET RADIATION AND MODULATION OF GENE EXPRESSION

Cellular Level

Ultraviolet radiation in the solar range modulates the expression of a wide variety of genes depending on the ultraviolet wavelength range employed, the dosage level and the cell type.[3,4] Where examined, the effects of UVB radiation are similar to those documented for the non-solar and more energetic UVC range (usually involving the mercury line emission at 254 nm). The expression of a large number of genes has been shown to be modified following UVC/UVB irradiation of cells. The explanation almost certainly lies in well-documented observations that 3 major classes of transcription factor (AP-1, NFkβ and p53) are either activated (AP-1 or NFkβ) or stabilised (p53) by short wavelength UV radiation. Several of the stress responsive signalling pathways, notably jun kinases and p38MAP kinases (both stress activated protein kinases, SAPKs), and ERK kinases are activated by short wavelength UV although the early events that trigger the response are still controversial. Since literally hundreds of eucaryotic promotors have *cis*-acting elements that will bind these factors, it is not surprising that the expression of a considerable number of genes can be modulated.

However, it is crucial to note that a majority of the studies have been done at very high (supralethal) UV doses and while they have been extremely informative about the signalling cascades involving protein kinases, many of the effects observed are not relevant to normal, physiological exposures. However, there are important exceptions and both metalloproteinases and the jun component of the AP-1 complex are activated both in cultured human skin cells and in human skin irradiated *in vivo*[5–8] at physiologically relevant dose levels. Furthermore relatively low levels of UVB radiation can activate cytokine production (e.g. IL1, IL6, TNFα) in both primary and transfected human keratinocyte cell lines (for review see Ref. [9]). UVB can mimic growth factor responses (e.g. by causing dimerisation of growth factor receptors[10]) and the proteins that are increased after UVB treatment are often involved in growth stimulation.

Studies with UVA radiation have usually been designed to involve physiologically relevant doses. UVA radiation significantly up-regulates expression of several genes in cultured human skin fibroblasts including collagenase, intercellular adhesion molecule 1, CL100 phosphatase and heme oxygenase 1 (HO-1), the latter being the most dramatic oxidant-mediated up-regulation of any higher eucaryotic gene so far observed. In recent studies we have observed strong up-regulation of both c-fos[8] and c-jun (Soriani and Tyrrell, unpublished results) expression by UVA irradiation of cultured fibroblasts. These proteins are nuclear oncogenes as well as transcription factor components of the AP-1 complex. Induction of various cytokines is also up-regulated by UVA radiation.[9]

The Skin

For the most part, similar patterns of UV modulation of gene expression are also observed in skin irradiated *in vivo*.[3] Much of this work has been done with solar simulated radiation which is predominantly UVB and information is available in both rodents and humans.

Increases in p53 protein, collagenase and ornithine decarboxylase have all been observed in skin after broad band UVB radiation and increases in HO-1 protein and ferritin have been observed in human skin following broad spectrum UVA radiation. Although there are several studies showing that the AP-1 components that include fos and jun, are increased to some extent in skin following UV treatment, recent studies

using shave biopsies of human skin treated with radiation *in vivo* have shown that only the jun protein shows significant increases and that fos is expressed constitutively at significant levels.[7] The induction is inhibited by retinoic acid, but this is not an effect on signal transduction and occurs at the post-transcriptional level. Work from the same group[6] has convincingly shown that metalloproteinases are strongly induced in human epidermis at UVB doses as low as 0.2 of a minimal erythemal dose. The transcriptional regulation of the metalloproteinase enzyme collagenase, involves the AP-1 *cis*-acting response element in the promotor of the gene which corresponds to the DNA sequence originally observed as a phorbol ester responsive element.[11]

There is also evidence that HO-1 protein is increased in human skin following UVA radiation (Applegate, Frenck and Tyrrell unpublished results) and that this is accompanied by increased ferritin levels (as predicted from experiments with cultured cells[12]). It has been known for some time that iron accumulates in UV exposed skin.[13] Furthermore these observations appear to be related to data from studies with genetically modified mice lacking HO-1[14] which strongly indicate that this enzyme is intimately linked with iron utilisation and storage.

REDUCING EQUIVALENTS AND OXIDANT-INDUCIBLE GENES

Since UVA provides such a controllable agent for generating cellular oxidative stress (see above) and the activation of expression of the HO-1 gene by UVA radiation provides a dramatic example of oxidant-inducible gene expression in higher eucaryotes, this phenomenon may be used to demonstrate some general hallmarks of oxidant-inducible genes such as the dependence of their expression on cellular reducing equivalents.

Both UVA and peroxide inducibility of HO-1 gene expression (as monitored by mRNA accumulation) can be enhanced several-fold in human fibroblasts by depleting cellular glutathione.[15] This is done in practice by long (overnight) exposure to low concentrations (1–5 μM) of the gamma-glutamylcysteine synthetase inhibitor, buthionine-S, R-sulfoximine, which reduces levels of reduced glutathione to a minimum. Similar observations have been made with other UVA-inducible genes that have been tested including the two AP-1 transcription factor components c-fos and c-jun[8] and the Cl100 phosphatase (Keyse, personal communication). Interestingly, the basal level of expression of HO-1 is also reproducibly enhanced five-fold under conditions of glutathione depletion. This suggests that intermediates generated during cellular metabolism and normally quenched by cellular reducing equivalents may actually be triggering the expression of constitutive levels of the mRNA. Clearly, it would be of interest to know if this effect on basal levels of gene expression also occurred for other oxidant-inducible genes.

SINGLET OXYGEN AND OXIDANT-INDUCIBLE GENES

Another factor common to oxidant (UVA)-inducible gene expression is that the generation of singlet oxygen appears to be a crucial early event in the activation response. Again, this was first proposed for the HO-1 gene[16] when it was observed that irradiation in buffer made with deuterum oxide rather strongly enhanced the UVA-inducible mRNA accumulation. Singlet oxygen scavengers (histidine, azide) suppressed the response whereas agents that would modify other intermediates (e.g. hydroxyl radicals) had no effect. Later we showed[17] that other agents such as protoporphyrin IX, which is a well-known photodynamic agent that will generate singlet oxygen in the presence of red light, is a very strong inducer of the heme oxygenase response. Studies in other laboratories have now shown that singlet oxygen is involved in the UVA activation of other genes such as collagenase, and intercellular adhesion molecule 1 (ICAM1) by UVA radiation[18,19]

and that the induction can be mimicked by exposure of cells to singlet oxygen generated by the thermal decomposition of a compound which is the 1,4-endoperoxide of disodium 3,3′-(1,4-naphthylidene) diproprionate.[20]

MEMBRANE DAMAGE AND SIGNALLING BY UVA

The generation of singlet oxygen appears to be an important early event in the signalling pathway that eventually leads to altered gene expression following UVA irradiation.[16–21] Membrane lipids are an important target of singlet oxygen and UVA radiation leads to extensive membrane lipid peroxidation that is dependent on iron. Oxidised lipids such as 4-hydroxynonenal are potent inducers of HO-1 mRNA accumulation.[22] However, it is also known that phospholipase A2 is active after UVA irradiation so that there is a rapid build-up of arachidonate. We questioned whether metabolites of arachidonate, such as prostaglandins and leucotrienes, are involved in the signalling pathway. Indomethacin (a cycloxygenase inhibitor) strongly inhibits UVA-induced accumulation of HO-1 mRNA in a concentration dependent fashion whereas a lipoxygenase inhibitor enhances the response.[22] These results point to prostaglandins as important intermediates in the signalling response and indeed an independent study had already shown that prostaglandin A1 could induce HO-1 in myoblasts.[23] There is little known concerning the involvement of the cycloxygenase pathway in activation of other genes by oxidative stress.

THE UP-REGULATION OF HO-1 – FUNCTIONAL SIGNIFICANCE

UVA radiation and other oxidants up-regulate HO-1 expression in a wide variety of human cell types. The up-regulation will lead to heme breakdown and iron release which in turn will convert the cytoplasmic iron regulatory protein from its active to inactive form and lead to increased ferritin synthesis.[12,24] All these events have been shown to occur after UVA irradiation, and using anti-sense oligonucleotides, we have linked HO-1 not only to the increase in ferritin but also to an adaptive response that leads to protection against oxidative membrane damage.[25] Maintenance of low labile iron pools by increased ferritin levels appears to play a key role in this type of cellular antioxidant defense. There has also been a suggestion that the products of heme breakdown, biliverdin and bilirubin, may contribute to protection since they are powerful antioxidants.[26] However, although the compounds are likely to play an important role in the plasma antioxidant defense system, it would appear unlikely that this low level generation of such compounds would play a role in cellular defense.

Oxidants can lead to heme protein breakdown and release of heme which, when present in membranes, can exert a strong pro-oxidant effect and cause cellular damage.[27] Recently we have observed that UVA radiation immediately leads to a rapid release of heme from microsomal cytochrome proteins.[28] Furthermore we have observed a direct correlation between the degree of heme release under different treatment conditions with the level of activation of HO-1 mRNA accumulation. Such observations are consistent with the hypothesis that a major role of the dramatic increase in HO-1 is to purge the cell membranes of the potentially damaging heme molecules and that this is essentially an emergency response. In fibroblasts, the increased activity of HO-1 apparently increases cellular labile iron pools which in turn leads to an eventual increase in ferritin levels which will further lower free iron levels. Epidermal keratinocytes have low levels of HO-1 and the enzyme is not induced following UVA treatment. However, they do have high levels of the constitutive HO-2 enzyme and correspondingly higher levels of ferritin.[29] The keratinocytes are also dramatically more resistant to oxidative membrane damage than the corresponding fibroblasts. Thus it appears

that keratinocytes, which are the first cellular target of UVA radiations in the skin, are constitutively better equipped to breakdown the heme liberated by oxidising treatments.

Cellular studies point to a protective role of HO-1[25] and these now include a series of experiments using cells derived from genetically modified mice lacking the gene.[30] Several animal studies also point to a protective role. For example, heme-induced damage to rat kidneys exposed to haemoglobin or myoglobin was prevented by induction of an adaptive response by haemoglobin itself[31] which apparently involved HO-1. In a quite different experiment, chemically induced inflammation in the pleural cavity of mice was exacerbated when heme oxygenase inhibitors were used,[32] pointing to a role for heme oxygenase induction in preventing inflammation. Such studies cannot be definitive because of doubts as to the specificity of the metalloporphyrins which are used as chemical inhibitors of heme oxygenase. However, in recent studies it has been found that HO-1 is a determinant of cardiac xenograft survival. Prolonged transplant survival and accommodation are prevented in HO-1 knock-out mice[33] and in an independent study it was shown that induction of high levels of HO-1 expression protected grafts from arteriosclerosis[34] a major cause of eventual transplant failure.

Heme oxygenase is expressed constitutively only in skin keratinocytes which are in the front line of attack by oxidising UVA (see above). We have also observed that, even in fibroblasts, where the inducible HO-1 response is extremely strong, a total refractoriness to further UVA induction developed within 48 h.[35] Such a strong down-regulation response (which also occurs when heme is the inducing agent) suggests that continuous overexpression of the enzyme could be harmful to the cells and is probably linked to the observation that no HO-1 was found in heart transplants undergoing chronic rejection (see above). Among the possible negative effects of HO-1 up-regulation, the continual release of labile iron as a result of heme breakdown is almost certainly significant. Indeed in recent studies (Ryter *et al.*, this laboratory, submitted), we have shown that cell lines that overexpress heme oxygenase several-fold become considerably more sensitive to oxidative (including UVA-induced) membrane damage when heme is supplied as a substrate for the enzyme and that the enhanced toxicity is directly related to iron release. A detailed mechanistic description of the relationship of heme and iron release to the down-side of heme oxygenase up-regulation is described in detail elsewhere (Ryter and Tyrrell, in preparation).

Acknowledgements

The work of the author is supported by the UK Department of Health (Contract 121/6378), the Association for International Cancer Research (UK) and the European Union 4th framework environment programme (env4-CT-95–0184) administered by the Swiss Office of Education and Science (OFES 95.0509).

References

[1] F.R. de Gruujil and P.D. Forbes (1995) UV induced skin cancer in a hairless mouse model. *Bioessays* **17**, 651–660.
[2] R.M. Tyrrell (1991) UVA (320–380 nm) radiation as an oxidative stress in *Oxidative Stress: Oxidants and antioxidants* (Ed. Sies, H.), pp. 57–83, Academic Press, London.
[3] R.M. Tyrrell (1996) Activation of mammalian genes by the UV component of sunlight – from models to reality. *Bioessays* **18**, 139–148.
[4] R.M. Tyrrell (1996) UV activation of mammalian stress proteins in *Stress-inducible Cellular Responses* (Feige, U., Morimoto, R.I., Yahara, I. and Polla, B.S. Eds.), pp. 255–272, Birkhäuser Verlag, Basel.
[5] K. Scharfetter, Wlaschek, M. Hogg, K. Bolsen, A. Schothorst, G. Goerz, T. Krieg and C. Plewig (1991) UVA irradiation induced collagenase in human dermal fibroblasts *in vitro* and *in vivo*. *Archives of Dermatological Research* **283**, 506–511.
[6] G.J. Fisher, S.C. Datta, H.S. Talwar, Z.Q. Wang, J. Varani, S. Kang and J.J. Voorhees (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* **379**, 335–339.
[7] G.J. Fisher, H.S. Talwar, J. Lin, P. Lin, F. McPhillips, Z.Q. Wang, X. Li, Y. Wan, S. Kang and J.J. Voorhees (1998) Retinoic acid inhibits induction of c-jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin *in vivo*. *Journal of Clinical Investigation* **101**, 1432–1440.

[8] B. Bose, M. Soriani and R. Tyrrell (1999) Activation of expression of the c-fos oncogene by UVA irradiation in cultured human skin fibroblasts. *Photochemistry and Photobiology* **69**, 489–493.
[9] S.E. Ullrich (1995) The role of epidermal cytokines in the generation of cutaneous immune reactions and ultraviolet radiation-induced immune suppression. *Photochemistry and Photobiology* **62**, 389–401.
[10] C.S. Miller, P. Hale and P.P. Pentland (1994) Ultraviolet B radiation injury increase prostaglandin synthesis through a tyrosine-kinase dependent pathway. Evidence for UVB-induced epidermal growth factor receptor activation. *Journal of Biological Chemistry* **269**, 3529–3533.
[11] P. Angel, M. Imagawa, R. Chiu, B. Stein, R.J. Imbra, H.J. Rahmsdorf, C. Jonat, P. Herrlich and M. Karin (1987) Phorbol ester-inducible genes contain a common cis element recognished by a TPA-modulated trans-acting factor. *Cell* **49**, 729–739.
[12] G.E. Vile and R.M. Tyrrell (1993) Oxidative stress resulting from ultraviolet A irradiation of human skin fibroblasts leads to a heme oxygenase-dependent increase in ferritin. *Journal of Biological Chemistry* **268**, 14 678–14 681.
[13] Bissett *et al.* (1991) Chronic ultraviolet radiation-induced increase in skin iron and the photoprotective effect of topically applied iron chelators. *Photochemistry and Photobiology* **54**, 215–223.
[14] K.D. Poss and S. Tonegawa (1997) Heme oxygenase 1 is required for mammalian iron reutilization. *Proceedings of the National Academy of Sciences USA* **94**, 10 919–10 924.
[15] D. Lautier, P. Lüscher and R.M. Tyrrell (1992) Endogenous glutathione levels modulate both constitutive and UVA radiation/oxidant-inducible expression of the human heme oxygenase gene. *Carcinogenesis* **13**, 227–232.
[16] S. Basu-Modak and R.M. Tyrrell (1993) Singlet oxygen. A primary effector in the UVA/near visible light induction of the human heme oxygenase gene. *Cancer Research* **53**, 4505–4510.
[17] S. Ryter, and R.M. Tyrrell (1998) Singlet molecular oxygen ($^1O_2$): A possible effector of eukaryotic gene expression. *Free Radical Biology and Medicine* **24**, 1–12.
[18] M. Wlaschek, J. Wenk, P. Brenneisen, K. Briviba, A. Schwarz, H. Sies, and K. Scharffetter-Kochanek (1997) Singlet oxygen is an early intermediate in cytokine-dependent UV induction of interstitial collagenase in human dermal fibroblasts. *FEBS Letters* **413**, 239–242.
[19] S. Grether-Beck, S. Olaizola-Horn, H. Schmitt, M. Grewe, A. Jahnke, J.P. Johnson, K. Briviba, H. Sies and J. Krutmann (1996) Activation of transcription factor AP-2 mediates UVA radiation- and singlet oxygen-induced expression of the human intercellular adhesion molecule 1 gene. *Proceedings of the National Academy of Sciences USA* **93**, 14 586–14 591.
[20] K. Scharffetter-Kochanek, M. Wlaschek, K. Briviba and H. Sies (1993) Singlet oxygen induces collagenase expression in human skin fibroblasts. *FEBS Letters* **331**, 304–306.
[21] K. Briviba, L.O. Klotz and H. Sies (1997) Toxic and signaling effects of chemically or photochemically generated singlet oxygen in biological systems. *Biological Chemistry* **378**, 1259–1265.
[22] S. Basu-Modak, P. Lüscher and R.M. Tyrrell (1996) Lipid metabolite involvement in the activation of the human heme oxygenase-1 gene. *Free Radical Biology and Medicine* **20**, 887–897.
[23] A. Rossi and M.G. Santoro (1995) Induction by prostaglandin A1 of haem oxygenase in myoblastic cells: An effect independent of expression of the 70 kDa heat shock protein. *Biochemistry Journal* **308**, 455–463.
[24] G. Balla, H.S. Jacob, J. Balla, M. Rosenberg, K. Nath, F. Apple, J.W. Eaton and G.M. Vercollotti (1992) Ferritin: a cytoprotective antioxidant stratagem of endothelium. *Journal of Biological Chemistry* **267**, 18 148–18 153.
[25] G.F. Vile, S. Basu-Modak, C. Waltner and R.M. Tyrrell (1994) Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proceedings of the National Academy of Sciences USA* **91**, 2607–2610.
[26] R. Stocker, A.F. McDonagh, A.N. Glazer and B.N. Ames (1990) Antioxidant activity of bile pigments: biliverdinand bilirubin. *Methods in Enzymology* **186**, 301–309.
[27] B. Halliwell and J.M.C. Gutteridge (1988) *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford.
[28] E. Kvam, A. Noel, S. Basu-Modak and R.M. Tyrrell (1999) Cycloxygenase dependent release of heme from microsomal heme proteins correlates with induction of heme oxygenase 1 transcription in human fibroblasts. *Free Radical Biology and Medicine* **26**, 511–517.
[29] L.A. Applegate, A. Noël, G.F. Vile, E. Frenk and R.M. Tyrrell (1995) Two genes contribute to different extents to the heme oxygenase enzyme activity measured in cultured human skin fibroblasts and keratinocytes: implications for protection against oxidant stress. *Photochemistry and Photobiology* **61**, 285–291.
[30] K.D. Poss and S. Tonegawa (1997) Reduced stress defense in heme oxygenase-1 deficient cells. *Proceedings of the National Academy of Sciences USA* **94**, 10 925–10 930.
[31] K.A. Nath *et al.* (1992) Induction of heme oxygenase is a rapid, protective response in rhabdomyiolysis in the rat. *Journal of Clinical Investigation* **90**, 267–270.
[32] D. Willis, A.R. Moore, R. Frederick and D.A. Willoughby (1996) Heme oxygenase: A novel target for the modulation of the inflammatory response. *Nature Medicine* **2**, 87–90.
[33] M.P. Soares, Y. Lin, J. Anrather, E. Caizmadia, K. Takigami, K. Sato, S.T. Grey, R.B. Colvin, A.M. Choi, K.D. Poss and F.H. Bach (1998) Expression of heme oxygenase 1 can determine cardiac xenograft survival. *Nature Medicine* **4**, 1073–1077.
[34] W.W. Hancock, R. Bnuelow, M.H. Sayegh, and L.A. Turka (1998) Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptolic genes. *Nature Medicine* **4**, 1392–1396.
[35] A. Nöel and R.M. Tyrrell (1997) Development of refractoriness of induced human heme oxygenase-1 gene expression to re-induction by UVA irradiation and hemin. *Photochemistry and Photobiology* **66**, 456–463.