



Citation for published version:

Tyrrell, RM 2013, Solar uv damage and skin protection:the boosting of natural defences and healing by cosmeceuticals. in Bulletin Technique Gattefossé N° 106 : fondation Gattefosse. vol. 106, France, pp. 70-79.

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

[Link to publication](#)

University of Bath

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

SOLAR UV DAMAGE AND SKIN PROTECTION: THE BOOSTING OF NATURAL DEFENCES AND HEALING BY COSMECEUTICALS

Rex M. Tyrrell*

Department Pharmacy and Pharmacology, University of Bath, BA2 7AY, United Kingdom

Abstract

The electromagnetic spectrum of solar radiations has been divided into the UVC region (below 290 nm, not incident on the earth's surface) and the UVB (290-320 nm) and UVA (320-400 nm) regions. Wavelengths below 290 nm are blocked by the stratospheric ozone layer. Both UVA and UVB radiations cause damage to skin cells and skin tissue and these events have been linked to acute damage such as sunburn as well as the chronic occurrence of skin cancer and inevitable photoaging. UVA generates distinct types of damage often associated with oxidative stress which is mediated by reactive oxygen species (ROS). UVA also elicits quite distinct stress pathways. The oxidising nature of the interaction of UVA radiation with human skin cells and the implications of this for endogenous and exogenous antioxidant defence will be considered in this overview.

Keywords

Antioxidants, antioxidant enzymes, cancer, cosmeceuticals, formulation, oxidative, photoaging, protection, reactive oxygen species stress, skin, solar, stress proteins, UVA, UVB.

*corresponding author: Email: -
Tel: +44 (0) 1225 386 793.

1. The solar spectrum

The spectrum for absorption of DNA overlaps the region of the solar spectrum which reaches the earth. This region of overlap is indicated in Figure 1 and defines the UVB region of sunlight which has been recognised as dangerous for many years.

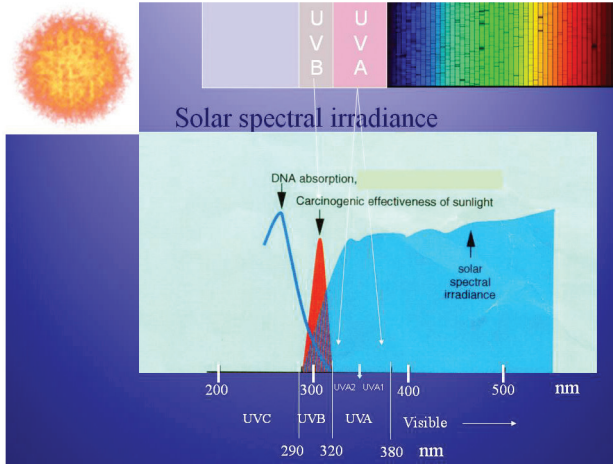


Figure 1. The spectrum of solar UV and visible radiation reaching the surface of the earth and the overlap with DNA absorption.

Because of this absorption overlap, UVB radiations constitute the key cancer-inducing wavelengths in sunlight (Figure 2) and is consequently the region attenuated by conventional optical sunscreens. However, it is now well recognised that the UVA region causes a variety of damage and is also potentially carcinogenic and involved in skin photoaging. Importantly this UVA region interacts with cells and tissue quite differently from UVB and constitutes a much higher percentage (95 %) of the total solar UV radiation reaching the earth's surface.

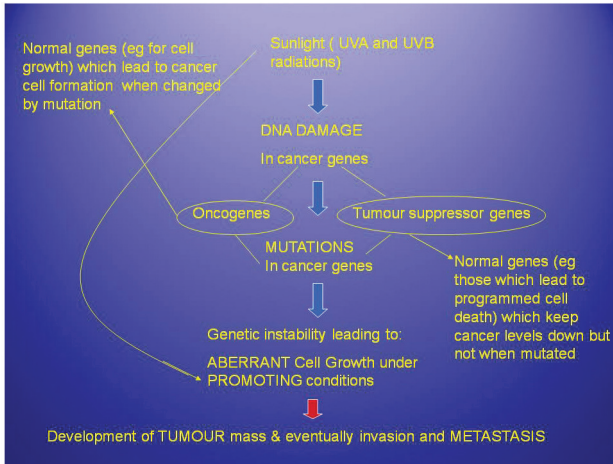


Figure 2. An outline of the carcinogenic pathway following solar UV irradiation of skin.

2. Skin penetration

The longer the wavelength of radiation, the further the penetration in to tissue, as described in Figure 3. Evidently UVA radiation penetrates much further than UVB in to skin and a significant percentage of UVA radiations can penetrate right through the dermal layer and even in to the subcutaneous layer.

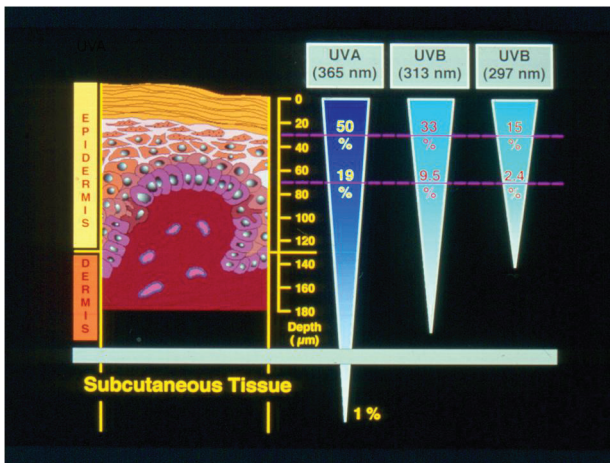


Figure 3. The relative penetration of UVA and UVB radiations through human skin.

3. UVA-induced oxidative stress

The most important characteristic of the interaction of UVA with biological material is that it generates a very significant oxidative stress in cells (Figure 4 and reference 1). The picture is quite complex because there are many cellular molecules which absorb UVA and generate reactive oxygen species (eg tryptophan, nicotinamide adenine dinucleotide phosphate (NADPH), porphyrins etc). Singlet oxygen is undoubtedly the major species generated directly by UVA but other species such as superoxide anion and hydrogen peroxide are also generated and there are many ways in which these species can interconvert to end up with the highly diffusible and reactive oxidant, hydrogen peroxide. UVA also leads to the release of free iron and free heme in cells and these are pro-oxidant catalysts which further exacerbate the oxidative stress [2]. For example, free iron is the catalyst in Fenton chemistry which accelerates the production of the highly reactive hydroxyl radical from hydrogen peroxide. Superoxide anion can also drive this reaction forward by continuously regenerating the ferrous form of iron. Several other reactions are worth noting: Importantly, UVA radiation is able to activate nicotinamide adenine dinucleotide phosphate (NADPH) Oxidase (Nox1) which generates superoxide anion [3]. Furthermore superoxide dismutase can then convert superoxide anion to hydrogen peroxide.

Another point to note is that an important class of cellular molecules which absorb UVA are the porphyrins and these absorb in the longer wavelength UVA region of the spectrum. Since it is this particular molecular interaction with cells that leads to singlet oxygen, the UVA region of sunlight will be of crucial importance to creating ROS and therefore cell damage.

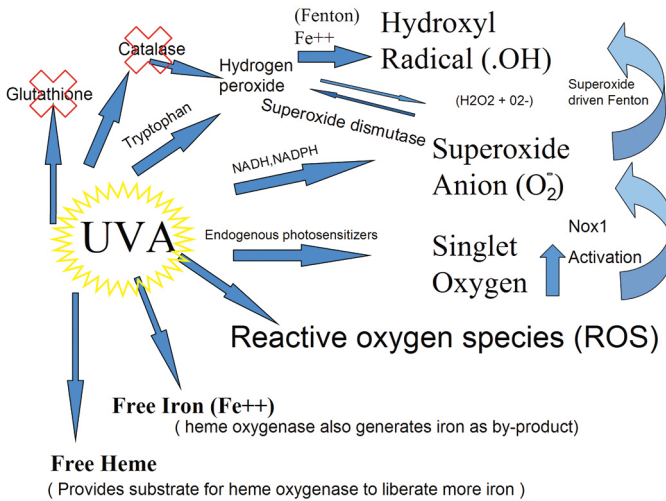


Figure 4. A schematic representation of the reactive oxygen species generated and pro-oxidant moieties released following UVA irradiation of cells and tissue.

As mentioned in the introduction, a major effect of UVB at biologically relevant doses is damage to DNA. However, an important consequence of the dramatic UVA-mediated oxidative stress is that, unlike UVB, a major effect of UVA radiation at the doses which cause observable damage to skin is to oxidise lipids and proteins throughout the epidermis and the dermis.

Damage to proteins will clearly involve those of the extracellular matrix, such as fibrillin, and since these will not always be repaired or replaced, long periods of sustained low level UVA exposure are likely to lead to a slow oxidation of these crucial structures. Lipids are also oxidised by low levels of UVA and this can set off chain reactions which can be sustained. Here it is important to note that this process can generate many lipid messenger molecules (such as ceramides and 4-hydroxynonenal). These signalling intermediates activate other proteins and enzymes and this includes not only enzymes such as oxidases which generate additional ROS but crucially, metalloproteinases. The sustained activation of these metalloproteinases, in addition to the accumulation of protein damage, is clearly going to be central to the photoaging process [4].

4. UVA activation of gene expression

Several genes/proteins are activated, some very strongly, as a result of irradiation of skin cells with UVB and UVA (Figure 5 and reference 5) and although most observations were originally made in cells, similar data was later obtained in human skin. By virtue of the oxidative stress generated by UVA, wavelengths in this range activate a unique set of enzymes. The first and strongest activation of gene expression to be observed was the activation of heme oxygenase 1 in human skin fibroblasts (reviewed in reference 6). This activation is due to the strong transcriptional up-regulation of the gene which is at least 15-20 fold over basal levels even at sub-lethal doses and can reach 100 times the basal level transcription rate of the gene. This is clearly an oxidative stress response since the activation is strongly dependent on the levels of endogenous glutathione. Furthermore the activation was the first demonstration of gene activation by singlet oxygen (already known to be generated by UVA). Activation of this gene was soon shown to protect against oxidative membrane damage and there is now a considerable interest in the protein since this oxidative stress response is known to be anti-inflammatory as well as antioxidant and has been implicated in many human pathologies. One of the by-products of heme oxygenase catabolism of heme is the release of iron which together with the iron released directly by UVA radiation leads to the post-transcriptional activation of ferritin, the major iron scavenging protein in cells. Clearly a major effect of UVA radiation is to disturb heme and iron homeostasis and the resultant gene activation would appear to be a strong cellular response to restore homeostasis [2]). Activation of heme oxygenase 1 only occurs in fibroblasts and not in human skin keratinocytes and it has been shown recently by gene silencing experiments that this difference is entirely due to the activity of the transcriptional suppressor protein, Bach1 [7].

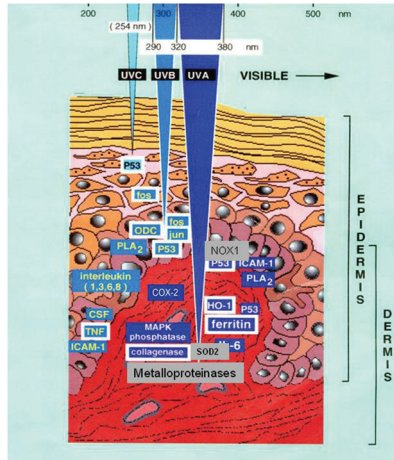


Figure 5. Differential activation of gene expression as a function of UV wavelength range (adapted from reference 5). There is major induction of the stress protein heme oxygenase 1 (HO-1) in the dermal layer of human skin.

UVB and UVA radiation were both shown to activate interstitial collagenase (metalloproteinase 1, MMP1) in human skin fibroblasts. It is now known that UVA irradiation activates a series of metalloproteinases in human skin fibroblasts and that these will play a key role in elimination of damaged/oxidised proteins and in remodelling of damaged skin [4]. Chronic activation of these multiple proteases in the dermis together with sustained oxidative protein damage will undoubtedly lead to irreversible damage to the extracellular matrix and contribute to the photoaging process.

An interesting recent observation is the UVA activation of NADPH oxidase (Nox1) in human keratinocytes [3]. Importantly this activation leads to the generation of reactive oxygen species (presumably superoxide anion) and this UVA generation of ROS is entirely prevented by using SiRNA targeted at the Nox1 gene. Our own recent experiments have shown that, although originally observed in keratinocytes, this activation occurs to an even greater extent in cultured fibroblasts (Zhang and Tyrrell, unpublished data).

In addition to these proteins, UVA also activates superoxide dismutase 2 (SOD2 which generates hydrogen peroxide from superoxide anion), intercellular adhesion molecule 1 and a protein that repairs oxidised protein, methionine-S-sulfoxide reductase [8]. It should be noted that the activation of these stress proteins is an emergency response to restore cellular homeostasis and this can have damaging side effects which further exacerbate the oxidative stress generated by UVA. For example, Nox1 generates superoxide which, in turn can be converted to hydrogen peroxide by the induced SOD2 and other SODs. The release of free iron by heme oxygenase can then contribute to the catalytic breakdown of hydrogen peroxide to generate hydroxyl radical.

5. Strategies for added protection

While constitutive and inducible pathways are able to strongly protect skin against oxidative insult, there are arguments that the supplementation of standard UV absorbing optical sunscreens with antioxidants could provide added protection. Given the large choice of antioxidants available, a key factor in selecting an antioxidant mix is to ensure that it is possible to formulate these such that the active compounds reach the target sites. This crucial aspect is often overlooked but there are also several non-formulation issues to be considered when deciding on the appropriate antioxidants to add to the mix as outlined below.

5.1 Do the selected compounds give added protection against ROS and how do you measure this?

A useful measure of protection against ROS in skin is provided by Electron Spin Resonance (ESR) technology. A study by Wang *et al* [9] compares ESR signals between UVA treated porcine skin (*ex vivo*) which had been pre-treated or not with a series of protective topical preparations already on the market. These were mostly optical sunscreens plus tocopheryl acetate although some preparations had added flavonoid-containing plant extracts. Almost no reduction in ROS as measured by ESR was observed over and above that provided by the optical absorption (SPF) provided by the preparation. Since at present no product could be marketed as a sunscreen without the optical absorbing component, these observations provide an interesting challenge.

Another useful marker of oxidative stress is activation of gene expression. An EU project from a decade ago (Prevention Biomarkers QLK4-1999-01590) included a closing study which exploited several marker genes (heme oxygenase, intracellular adhesion molecule 1 and metalloproteinase 1) in a cross-over study in humans fed either a low or a high flavonoid-rich diet (green tea polyphenols plus oranges). After a suitable time on the diet the skin of subjects was irradiated with a standard dose of UVA radiation. Biopsies were taken 24 hours later and gene activation measured. A significant number of subjects showed suppressed gene activation when on a flavonoid rich diet. Although this study was an indication that such a flavonoid diet may provide health benefits, the study was too small to reach definitive conclusions. The key point to stress is that it did demonstrate the feasibility of using such markers to measure protection in human skin.

5.2 Does the antioxidant mix protect against all species?

There is now a large body of literature which describes scavenging of both radical and non-radical species by a variety of natural antioxidants, usually flavonoids and carotenoids. These can be supplemented with both vitamin C and Vitamin E analogues with the appropriate lipid/aqueous partition coefficients. It is therefore possible to design relatively simple mixtures with the potential to scavenge all solar UV generated ROS and with properties that allow functional formulation.

5.3 Is excess free iron taken care of?

Flavonoids are polyphenolic compounds and since two adjacent hydroxyl groups will confer various levels of iron chelation, the addition of flavonoids in a formulation intended for protection will also confer iron scavenging potential. This can be examined by the measurement of free iron levels with and without the addition of the protective compound(s). The example in Figure 6 (adapted from reference 10) shows the dose-dependent release of labile iron in cultured human skin fibroblasts as a function of increasing fluence. The results with epicatechin, a common flavonoid found in green tea and red wine etc, demonstrates that there is a concentration-dependent reduction in the levels of labile iron detected. Even at 1 and 3 micromolar concentrations of the polyphenolic compound, the protection is very significant.

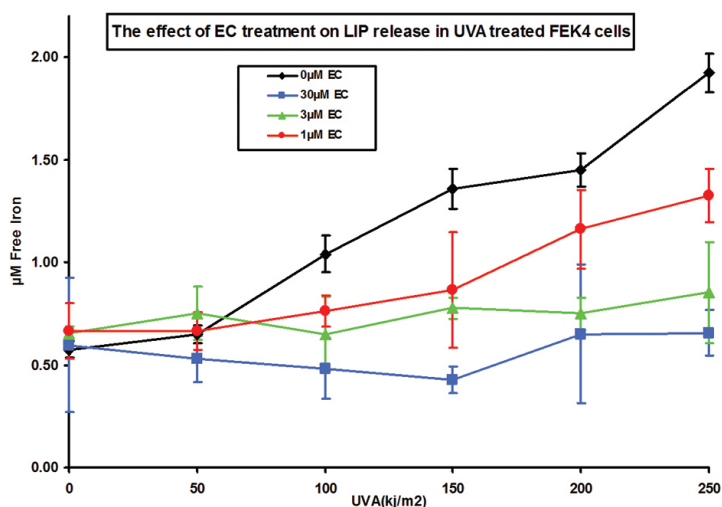


Figure 6. UVA-mediated release of labile iron as a function of UVA fluence and its suppression by a series of concentrations of epicatechin (EC, adapted from reference 10).

5.4 Are the active ingredients doing what is intended and do they have other properties?

The data shown in Figure 6 could lead to the conclusion that epicatechin is acting as an iron chelator. However, additional experiments [10] have shown that the reduction in labile iron levels is not due to iron chelation but results from protection of lysosomal membranes against oxidising damage. The iron reduction and protection occurs regardless of whether one of the hydroxyl groups of epicatechin is substituted by a methyl group, a modification that would be expected to compromise iron chelating efficiency.

Indeed, although polyphenolic flavonoids are used as antioxidants in numerous topical preparations, most of these are powerful cell signalling agents and exert effects at much lower concentrations than the concentrations that would endow them with significant antioxidant properties. When developing products intended for protection, it is essential to be aware of the often complex pharmacology of the components.

5.5 What are the best compounds to use other than vitamins A and C and derivatives?

In practice, many types of preparation have now been shown to provide *in vivo* protection against UV-mediated skin damage in both rodent models and humans and complement endogenous protection [11]. For example, carotenoids have been shown to protect both topically and systemically against UV damage to humans and rodents [12]. Several polyphenols (notably catechins from green tea) have been shown to provide protection often in combination with Vitamins E and C (eg. reference 13) or analogues. This remains an active area of development with many possibilities and formulation is likely to be the limiting factor in generating new and effective photo-protective agents using these natural ingredients [14].

6. Concluding remarks

The UVA region of sunlight induces a major oxidative stress in cells that is exacerbated by the UVA-mediated release of the pro-oxidant catalysts, iron and heme. This substantially enhances the potential of sunlight to cause major structural damage in skin so that UVA is implicated in both the photoaging and the photocarcinogenic processes. Skin cells and tissue contain a panoply of complementary antioxidant defence mechanisms, both constitutive and induced, which can neutralise the UVA-mediated oxidative stress. There is a view that it would be beneficial to complement these defences by topical (or even systemic) treatment with natural products with antioxidant properties. There is now a good background for choosing such compounds and a primary issue will be the generation of suitable formulations to ensure their efficacy at damaged sites in the skin.

7. References

- [1] Tyrrell, R.M. UVA (320-380 nm) radiation as an oxidative stress in "Oxidative Stress : Oxidants and antioxidants" (ed. H. Sies), (1991) pp 57-83, Academic Press, London.
- [2] Tyrrell, R. M. Heme oxygenase – a crucial enzyme in iron and heme homeostasis in "Heme oxygenase: The elegant orchestration of its products in medicine (2005) pp 333-349. (eds, L. Otterbein and B. S. Zuckerbraun). Nova Science Publishers, New York.
- [3] Valencia A and Kochevar IE Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. *J Invest Dermatol.* 128, (2008), 214-222.
- [4] Quan T, Qin Z Xia W, Shao Y, J. Voorhees JJ , and G J. Fisher Matrix-degrading Metalloproteinases in Photoaging *J Invest Dermatol Symp Proc.* 14, (2009), 20–24.

- [5] Tyrrell, R.M. Activation of mammalian gene expression by the UV component of sunlight - from models to reality. *Bioessays* 18, (1996), 139-148.
- [6] Tyrrell, R. M. Solar ultraviolet A radiation – an oxidising skin carcinogen that activates heme oxygenase 1. *Antioxidants and Redox Signalling*, 6, (2004), 835-840.
- [7] Zhong, J.L , Raval, C., Edwards, G.P and Tyrrell, R.M. A role for Bach1 and HO-2 in suppression of basal and UVA-induced HO-1 expression in human keratinocytes. *Free Radic. Biol. Med.* 48, (2010), 196–206).
- [8] Ogawa F, Sander CS, Hansel A, Oehrl W, Kasperczyk H, Elsner P, Shimizu K, Heinemann and SH, Thiele JJ. The repair enzyme peptide methionine-s-sulfoxide reductase is expressed in human epidermis and upregulated by UVA radiation. *J. Inv.Dermatol.* 126, (2006), 1128-1134.
- [9] Wang SQ, Osterwalder U and K Jung *Ex vivo* evaluation of radical sun protection factor in popular sunscreens with antioxidants. *J Am Acad Dermatol* 65, (2011), 525-530.
- [10] Basu-Modak, S., Ali, D., Gordon, M., Polte, T., Yiakouvaki, A., Pourzand, C., Rice-Evans, C. and R.M.Tyrrell. Suppression of UVA-mediated release of labile iron by epicatechin – a link to lysosomal protection. *Free Radic. Bio. Med.* 41, (2006), 1197-1204.
- [11] Reeve,V. and R.Tyrrell UVA and inducible protection in “Biophysical and Physiological Effects of Solar Radiation on Human Skin”(2007) pp 293-310 (ed.P.Giacomoni) RSC publications, Cambridge, UK.
- [12] Stahl W and Sies H β -Carotene and other carotenoids in protection from sunlight. *Am J Clin Nutr.* 2012 96 1179S-1184S.
- [13] Murray JC, Burch JA, Streilein RD, Iannacchione MA, Hall RP and SR Pinnell *J Am Acad Dermatol.* A topical antioxidant solution containing vitamins C and E stabilized by ferulic acid provides protection for human skin against damage caused by ultraviolet irradiation. 59, (2008), 418-425.
- [14] Oresajo C, Pillai S, Manco M, Yatskayer M, McDaniel D. Antioxidants and the skin: understanding formulation and efficacy. *Dermatol Ther.* 25, (2012), 252-259.