JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 969–976

Received January 14, 2008
Accepted March 31, 2008
DOI 10.1211/jpp.60.8.0004
ISSN 0022-3573

Cellular effects of long wavelength UV light (UVA) in mammalian cells

T. J. McMillan, E. Leatherman, A. Ridley, J. Shorrocks, S. E. Tobi and J. R. Whiteside

Abstract

UVA should receive significant consideration as a human health risk as it is a large proportion of the solar spectrum that reaches the earth's surface and because of its ability to penetrate human skin. It is only relatively recently that this has been recognized and this previously under-researched part of the UV spectrum is becoming increasingly well characterized at doses that are quite low in relation to those experienced by humans. Absorption of UVA in a cell leads to the production of reactive oxygen and nitrogen species that can damage major biomolecules including DNA and membrane lipids. Various types of damage induced in these molecules lead to significant biological effects including cytotoxicity, mutations and alterations in cell signalling pathways. Longer-term effects such as persistent genomic instability and bystander effects have also been observed following UVA treatment of mammalian cells and, as with ionizing radiation, this changes some of the fundamental thinking around tissue effects of irradiation. Antioxidants have been assessed extensively for their ability to protect against the biological effects of UVA and a number have been shown to be successful at least in-vitro, for example vitamin E and epigallocatechin-3-gallate. Other potential targets for protection are suggested through the increased understanding of some of the signalling mechanisms activated following treatment, for example the inhibition of NADPH oxidase is seen to reduce a bystander effect. The search for appropriate and successful photoprotective agents remains an important area of research.

Introduction

UV light is commonly considered to be the part of the electromagnetic spectrum that runs from 100–400 nm. There has been extensive laboratory work for many years on the cellular effects of the shorter UV wavelengths (UVC 220–280 nm) because these wavelengths are readily absorbed by DNA and are highly genotoxic. More recently, there has been a recognition that the longer UV wavelengths (UVB 280–315 nm; UVA 315–400 nm) are actually more relevant to human exposures due to the elimination of wavelengths below 300 nm by the ozone layer (Frederick et al 1989) and the increase in penetration through the skin that comes with increasing wavelength (Bruls et al 1984). Taking this atmospheric and tissue penetration into account means that UVA is in fact the most potentially damaging portion of the UV spectrum and has even more relevance because of the significant use of sunbeds in the UK and other countries, as the spectrum used in sunbeds is especially high in the UVA region.

Skin cancer is very common, with >70 000 cases reported each year in the UK, of which the most serious form, malignant melanoma, makes up around 10% (Cancer Research UK). In 2005, malignant melanoma caused more than 1800 deaths in the UK. The epidemiological links between patterns of sun exposure and its consequences are well established, although the relative patterns of critical exposure vary between cancer types. Squamous cell carcinomas are associated with high cumulative sun exposure, whereas basal cell carcinoma and malignant melanoma have been linked to intermittent exposures (e.g. Rosso et al 1998; Almahroos & Kurban 2004).

Various animal models have demonstrated the carcinogenicity of UVA. Kelfkens et al (1991) demonstrated the ability of UVA to induce papillomas and squamous cell carcinomas. UVA-induced melanomas have been induced in *Xiphaphorus* fish (Setlow et al 1993) and *Monodelphis domestica* (opossum) (Ley 2001), although a transgenic mouse model

Biomedical Sciences Unit, Department of Biological Sciences, Lancaster University Lancaster, LA1 4YQ, UK

T. J. McMillan, E. Leatherman, A. Ridley, J. Shorrocks, S. E. Tobi, J. R Whiteside

Correspondence:
T. J. McMillan, University House, Lancaster University, Bailrigg, Lancaster, LA1 4YW, UK. Email: t.mcmillan@lancaster.ac.uk

failed to show such induction (De Fabo et al 2004). Such studies are necessarily restricted in the number of conditions (e.g. dose, exposure pattern) that can be examined and so negative results can be difficult to interpret. In humans, some important data have come from the relationship between sunbed use and skin cancer (Young 2004). Gallagher et al (2005) identified a significant link between sunbed use and an increased risk of cutaneous melanoma through a meta-analysis of 13 studies in the literature. For non-melanoma skin cancers, a study in the USA demonstrated a significant increase in risk of basal cell and squamous cell carcinoma associated with any use of tanning devices (Karagas et al 2002). There is now sufficient evidence of the detrimental effects of sunbeds that government regulators in the UK are addressing the issue, with the result that sunbed use by young people in particular will hopefully be reduced in the future.

The main aim of studies of the cellular response to UVA is to aid the understanding of the processes by which UVA exerts its biological effects so that potential markers for cutaneous malignancy can be identified and appropriate protective actions can be taken. They also allow the examination of the effects of changing patterns of dose/exposure for example, which are difficult in animal systems, although it needs to be recognized that whole-animal effects such as immunosuppression are likely to be important with UVA and so these need additional consideration (Halliday 2005).

UVA treatment leads to the production of reactive chemical species in the cell

Photons of UVA can be absorbed by molecules including riboflavin, porphyrins and haeme-containing proteins, and these can damage cellular substrates such as DNA, membranes or proteins by either reacting with them directly or by reacting with molecular oxygen to produce reactive oxygen species (ROS) (Foote 1991). These Type I and Type II reactions can lead to the production of superoxide and singlet oxygen. The latter can be dismutated to hydrogen peroxide, which in turn can produce hydroxyl radicals following reaction with metals (e.g. Fenton reaction; Tyrell & Keyse 1990). There is also evidence of the release of nitrogen reactive species (e.g. nitric oxide and peroxynitrite) in UVA-irradiated cells (Villiotou & Deliconstantinos 1995; Podhaisky et al 2002; Paunel et al 2005). In addition to these immediate effects of UVA, there are indications that UVA can stimulate enzymatic production of reactive oxygen and nitrogen species (e.g. Didier et al 1999; Valencia et al 2006) and these have the potential for a longer-term reactive species timescale that may be relevant for delayed effects of the UVA treatment (see below). Thus, there is a range of reactive species produced in response to UVA exposure that have the potential to be involved in all aspects of the biological effects of UVA.

Molecular damage in the cell

Any damage that UVA might cause to DNA is likely to be a significant part of the cellular response, but there is still debate as to which types of DNA lesion are the most important. The lack of direct absorption of UVA by DNA meant that lesions normally associated largely with UVC and UVB (i.e. cylobutane pyrimidine dimers (CPDs) and pyrimidine (6-4)pyrimidone photoproducts) have been traditionally dismissed as being unimportant with UVA. A number of studies failed to detect CPDs in UVA-irradiated DNA or cells (e.g. Rosestein & Mitchell 1987; Cooke et al 2003), but these are now outnumbered by studies in which CPDs have been detected after UVA in isolated DNA (e.g. Matsunaga et al 1991), Chinese Hamster Ovary (CHO) cells (e.g. Rochette et al 2003), human fibroblasts (e.g. Besaratinia et al 2005), human keratinocytes (Courdavault et al 2004) and human skin (Burren et al 1998). Indeed, there is now some evidence that CPDs are produced at levels that are likely to make them at least as significant as oxidatively generated damage (see below), and Mouret et al (2006) have suggested that CPDs produced by UVA are less repairable that those from UVB. CPDs may be formed by direct effects on DNA (Perdiz et al 2000), but it has been suggested that they are more likely produced via a mechanism of photosensitized triplet energy transfer (Douki et al 2003; Rochette et al 2003).

The production of ROS by UVA leads to other DNA lesion types that are potentially detrimental to the cell. Induction of 8-hydroxyguanine has been demonstrated in a variety of cell types (e.g. Kvam & Tyrell 1997; Douki et al 2003). In addition, 5,6-dihydropyrimidines have been observed following UVA irradiation (Kielbassa et al 1997, Pouget et al 2000). UVA can also produce DNA single-strand breaks and alkali labile sites in a range of cell types (e.g. Lehmann et al 1998; Douki et al 2003). In addition, there is indirect evidence that UVA can lead to the production of DNA double-strand breaks (DSBs). We have previously shown that xrs cells, that are defective in non-homologous end rejoining, which is an active repair pathway against DSBs, are sensitive to UVA (Fell et al 2002), suggesting that DSBs are indeed formed in cells after UVA treatment. The production of chromosomal fragments, seen as micronuclei (Fell et al 2002), adds weight to this possibility. Whether these are formed directly or by the breakdown products of other lesion types encountering repair or replication machinery is not known.

UVA-induced damage in cell membranes is important in the biological response. At a gross level, changes in membrane fluidity, and hence permeability and function, have been detected (Gaboriau et al 1993) and it is believed that these result from peroxidation of membrane lipids. The UVA-induced ROS lead to lipid hydroperoxide formation, with the additional production of lipid peroxyl radicals that can in turn react with other lipids, thus producing a chain reaction (Girotti 2001). Major by-products of lipid peroxidation such as malondialdehyde and 4-hydroxynonenal are often used as markers of lipid peroxidation, but are also able to diffuse within and between cells and can form adducts with proteins, DNA and phospholipids (Esterbauer et al 1990), although the significance of this within a cell is not known.

Cytotoxicity and mutagenicity

The damaging process of UVA is much less efficient than UVB and UVC because of the ability of UVB and UVC to cause significant direct DNA damage. As a consequence, the high doses of UVA needed to cause cell killing often lead to the conclusion that UVA is irrelevant in the key biological

responses. However, the high levels reaching the lower layers of skin counteracts some of this difference and it is now clear that at doses that are not extreme in environmental terms, UVA can cause significant cell killing.

An important aspect of UVA-induced cytotoxicity that is often overlooked is the impact of the pattern of dose delivery. Most studies concentrate on the effect of a single dose of UVA given over a relatively short time of a few minutes, but there is evidence that fractionated treatment or treatment over prolonged periods can have an altered response. We and others have seen increased cytotoxicity of UVA when the rate at which the treatment was given was reduced (Merwald et al 2005; Shorrocks et al 2008), although Morliere et al (1995) did not find such an effect. In our study, the increased killing at a low dose rate was paralleled by increases in DNA damage and lipid peroxidation, which was consistent with an observed increase in ROS in the low dose rate cells (Shorrocks et al 2008). The wider significance of these results needs to be studied in terms of the influence of dose rate on mutation frequency, but due to the significance of situations in which dose rate can be varied, including during the use of sunscreens, they are potentially important. The correct use of sunscreens protects against UV-induced erythema and DNA damage (Green et al 1999; Bissonauth et al 2000; Liardet et al 2001). The incorrect use of sunscreens, in which the time spent in the sun is increased so that the overall dose may stay the same but the dose rate is decreased, may be detrimental if the above data on cytotoxicity is also reflected in carcinogenic endpoints.

Some UVA-induced cell killing may be associated with DNA damage directly, in that micronuclei (Emri et al 2000; Fell et al 2002) have been detected after treatment and these are associated with cell death in other contexts (e.g. ionizing radiation treatment). There is also evidence, however, that in some cell systems UVA can induce apoptosis (Pourzand et al 1997; Godar 1999; Morley et al 2006). The proposed mechanisms involved in UVA-induced apoptosis include depletion of glutathiones (Larsson et al 2005), generation of oxidative stress (Hirota et al 2005) and induction of Fas expression (Bang et al 2002).

Since apoptosis is an active process in the cell and is mediated through changes in activity of a number of proteins, this raises the general issue of the signalling pathways that are induced by long-wavelength UV and may be important components of the cellular response to treatment. For example, Zhang et al (2002) showed that UVA induces the rapid activation of phosphatidyl 3-kinase-like kinase ataxia telangiectasia mutated (ATM). In addition, they reported that the phosphorylation of p53 at Ser15 and Ser20 and JNK at Ser63 was ATM-dependent and was required for UVA-induced apoptosis. In contrast, UVC-induced apoptosis was shown to occur via an ATR-dependent pathway (Zhang et al 2002). From these data, Zhang et al (2002) suggested that ATM kinase is an oxidative stress sensor, whereas ATR responds to direct DNA damage.

The mitogen-activated protein kinases (MAPKs) have been implicated in orchestrating many aspects of the UV-induced DNA damage response (reviewed by Jinlian et al 2007). More specifically, the generation of singlet oxygen following UVA irradiation is thought to provide a major stimulus for the activation of MAPKs (reviewed by Bachelor & Bowden 2004). MAPKs are members of the proline-directed Ser/Thr kinases that include the c-Jun NH_2-terminal kinases (JNKs), extracellular signal-related kinase (ERK) and p38. The dual phosphorylation of these MAPKs on threonine and tyrosine residues results in the subsequent activation of downstream transcription factors such as c-Jun, JunD and ATF-2 (Cavigelli et al 1995; Rosette & Karin 1996; Wang et al 1999; Li et al 2002). These transcription factors are able to transactivate stress-inducible genes via their interaction with the AP1 or ATF/CRE regulatory sites (Karin et al 1997).

Although numerous studies have characterized several functions of MAPK activation in UVA signalling, the precise role of MAPKs remains controversial. For example, a rapid and transient activation of p38 MAP kinase and JNK, but not ERK, was observed in human skin fibroblasts (Klotz et al 1999; Silvers et al 2003). In conflict with these data, the activation of JNK, p38 MAP kinase and ERK was reported in the NCTC 2544 human keratinocyte cell line and the mouse epidermal JB6 promotion-sensitive CI 41 cell line (Maziere et al 2001; Zhang et al 2001a, 2001b). However, it has been shown that the activation of p38 MAPK following UVA irradiation has a role in the cutaneous inflammatory response in HaCaT keratinocytes via the stabilization of cycloxygenase 2 (COX-2) mRNA, resulting in an increase in COX-2 protein levels (Bachelor et al 2002). In addition, p38 MAPK has been implicated in the survival of HaCaT human keratinocytes via the modulation of Bcl-XL, an anti-apoptotic member of the Bcl-2 family (Bachelor & Bowden 2004). Thus, the induction of cell death by UVA can be a complex process that remains poorly understood.

UVA has long been known to be mutagenic. Standard mammalian cell mutation assays such as the HPRT assay have identified the increased mutation frequency after non-extreme doses of UVA (e.g. Tobi et al 2002). Detailed analysis of the nature of the mutations induced by UVA have identified G-to-T transversions and small tandem base deletions as being dominant after wavelengths >340 nm (Besaratinia et al 2004; Pfiefer et al 2005). This is a spectrum similar to that of oxidative base lesions such as 8-oxo-7,8-dihydro-2′-deoxyguanosine, although it may be important to note that these data were from a system in which no CPDs were detectable after UVA treatment. On the other hand, the site-specific CPD damage spectrum observed within the adenine phosphoribosyl transferase (aprt) locus in CHO cells following UVA exposure has recently been demonstrated to correlate with the UVA-induced mutation spectrum observed within the same sequence (Rochette et al 2003). Importantly, Agar et al (2004) showed that mutations more commonly associated with UVA rather than UVB were found to be dominant in human squamous cell carcinomas and also showed a tendency to be in the lower skin layers.

Indirect effects of UVA: persistent genomic instability and bystander effects

Led by the study of ionizing radiation, there have been some important changes in the way that radiation effects are considered in recent years (Lorrimore & Wright 2003). It is increasingly recognized that not all biological effects are a

direct effect of the radiation, but that irradiated cells maintain a level of instability that results in new chromosome damage and mutations being produced several cell generations after the original treatment. It has also been shown in some systems that irradiated cells can pass on a signal to unirradiated cells in the near vicinity that results in significant damage to the latter. These phenomena of persistent genomic instability and bystander effects have recently been identified in the cellular response to UVA.

As indicators of persistent genomic instability, O'Reilly & Mothersill (1997) reported that human cells had a prolonged reduction in plating efficiency after UVA irradiation. This was also seen by Phillipson et al (2002) who observed a continued reduction in plating efficiency in the survivors of UVA treatment up to 3 weeks after treatment and that hprt mutations and micronuclei were still being produced at least 7 days after treatment of HaCaT cells. Subsequently, Dahle & Kvam (2003) also found a long-term induction of mutations in V79 Chinese Hamster fibroblast cells after both UVA and UVB. Thus, although not well characterized at this stage, there is evidence that persistent genomic instability is a consequence of UVA treatment and this has potential implications for approaches to chemically reduce the effect of UVA (see below).

In studies with ionizing radiation, the bystander effect has been demonstrated using a number of different experimental protocols. These include conventional low-dose high-LET irradiation, where only a small number of cells are traversed by an α particle (Nagasawa & Little 1992), the precise specific irradiation of individual cells in a population using a microbeam with the subsequent examination of adjacent cells (Prise et al 1998), and the transfer of conditioned medium from irradiated cells onto unirradiated cells (Lyng et al 2002). We have used a co-incubation system in which irradiated cells plated on the bottom of a well in a 6-well tissue culture plate are incubated in the same medium as unirradiated cells that are plated on a permeable insert so that the medium and any molecules released into it is shared between the two cell populations but they do not come into direct physical contact. Figure 1 shows the results from such an experiment in which the number of colonies from HaCaT human skin keratinocytes is significantly reduced when co-incubated with irradiated cells compared with when they are co-incubated with unirradiated cells. The effect is relatively small but highly consistent and remains to be examined with respect to mutation, although there is evidence that DNA damage can result in the unirradiated cells.

There has been much debate about the importance of persistent genomic instability and bystander effects in cancer initiation by ionizing radiation in humans. The potential to amplify the biological effect of treatment either in terms of the number of cells affected or in the response in irradiated cells is not clear. The significance of these probably does not lie initially in increasing the perceived carcinogenic effects of exposure as these mechanisms will have already contributed to epidemiological risk assessments. The shape of dose–response relationships and the timing of biological effect relative to the time of exposure are, however, potentially important when cancer incidence data are being extrapolated or when protective or therapeutic interventions are being considered. Both of these areas require more extensive investigation.

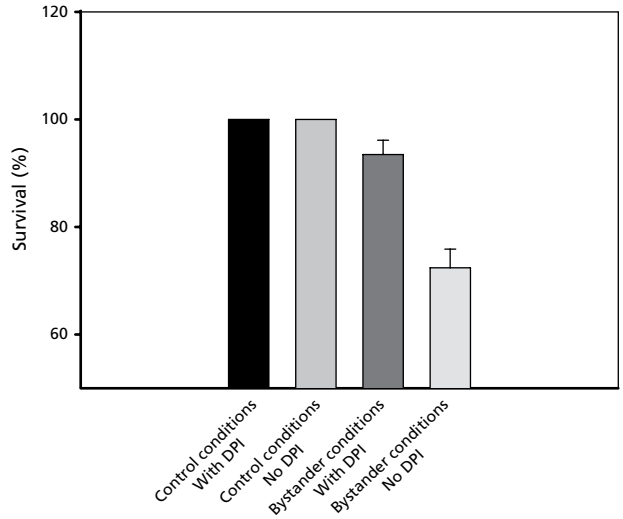

Figure 1 Changes in clonogenic survival of unirradiated HaCaT bystander cells with and without incubation (18 h) with 2 μM diphenyleneiodoium (DPI) followed by 7 days incubation in the presence of UVA-treated (1×10^5 J m^{-2}) cells. Data are means ± s.d. of three experiments. Control conditions were unirradiated cells on both plate and insert; bystander conditions were irradiated cells on the plate and unirradiated on the insert. The survival data for cells on the insert are presented.

Chemical modification of the effects of UVA

The ability to protect against the deleterious effects of UVA is increasingly recognized as an important aspect of the design of sunscreens, which now commonly include an indication of the performance against UVA. Set against the role of ROS in the biological effects of UVA, there have been a significant number of studies on the ability of various antioxidants to protect against UVA effects. Vitamin E has been widely examined and this does provide protection against lipid peroxidation and small reactive species, and consequently against biological endpoints including cell survival (e.g. Shorrocks et al 2008).

It has been shown that modification of the cellular antioxidant status by using buthionine sulphoximine to reduce the level of glutathione can increase UVA-induced oxidative species (e.g. Tobi et al 2000) and there have been numerous studies, especially of vitamins and plant-derived compounds, investigating their ability to protect against the effects of UV exposure. For example, Besaratinia et al (2007) have reported a reduction in UVA-induced mutation frequency through the addition of vitamin C in a marker gene in mouse embryonic fibroblasts. Also, the primary antioxidant in green tea, epigallocatechin-3-gallate, has been shown to modify the levels of oxidative species in cells and consequently to reduce mutation induction, cell killing and DNA damage induced by UVA (Tobi et al 2002). Figure 2 reproduces data from Tobi et al (2002) in which the alkaline single-cell gel electrophoresis (comet) assay was used to quantify DNA single-strand breaks

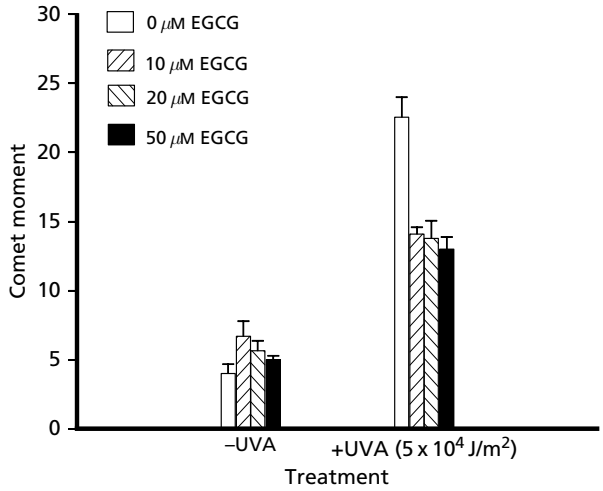

Figure 2 Protection by epigallocatechin-3-gallate (EGCG) against UVA-induced DNA strand breakage (reproduced with permission from Tobi et al 2002).

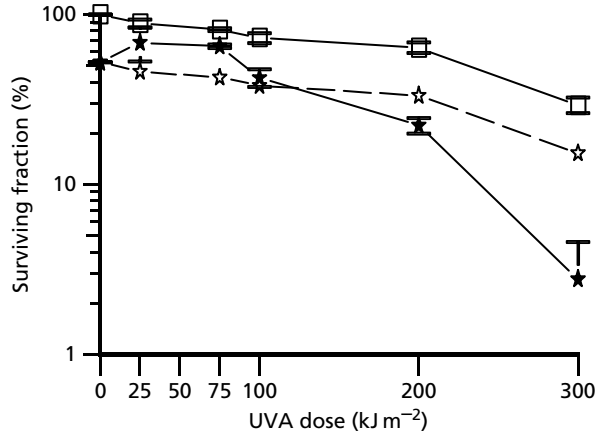

Figure 3 The effect of *trans*-resveratrol pretreatment (24 h with 20 μM) on the cytotoxicity of UVA. □, UV alone; ☆, theoretical curve calculated as the simple addition of the cell killing by UVA and 20 μM of *trans*-resveratrol; ★, UVA + *trans*-resveratrol. Data are means ± s.e.m. of four experiments.

after a dose of 5×10^4 J m^{-2} UVA following 24 h pretreatment with various doses of epigallocatechin-3-gallate. The ability of UVA to induce breaks is clear but these are substantially reduced by the antioxidant treatment. Another agent that has received considerable attention as a chemopreventative agent is resveratrol (*trans*-3,5,4′-trihydroxystilbene). It has a number of proposed modes of action, including direct growth inhibitory effects, extensive changes in the expression of a number of genes and the suppression of angiogenesis (Aggarwal et al 2004). However, it is as a strong antioxidant that it has perhaps received most attention and this led us to consider its efficacy against UVA-induced cytotoxicity in HaCaT cells. Interestingly, at low UVA doses (25 and 75 kJ m^{-2}) the cell survival was greater than expected on the basis of the simple addition of the killing effects of the UVA and resveratrol, indicating a small amount of chemoprotection, but the most striking result was an increase in toxicity (Figure 3), which is likely to be due to the absorption of UVA by resveratrol and the consequent formation of a reactive form of resveratrol. Thus, care needs to be taken when considering resveratrol in the context of UV exposure.

As we learn more about the indirect effects of UVA treatment new avenues of protection are likely to emerge. Studies of persistent genomic instability have seen that ROS scavengers can reduce the persistent genomic instability phenotype. For example, both Phillipson et al (2002) and Dahle et al (2005) found that catalase treatment reduced instability. These modifiers were all applied several days after treatment in these studies, so they add an extra dimension to the photoprotection field. We have recently found that treatment of cells with diphenyleneiodonium (DPI), which inhibits NADPH oxidase, can reduce the induction of the bystander effect when the unirradiated cells are pretreated with the agent prior to co-incubation with irradiated cells (Figure 1). This suggests that the products of NADPH oxidase, namely ROS, play some role in the bystander effect after UVA exposure. Since most ROS processes are very rapid, the effect of protectors on the immediate effects of UV exposure have been the main focus of attention and, while they are likely to remain the dominant factor, the persistent genomic instability and bystander results suggest that there is a role for longer-term protective approaches.

Conclusion

UVA is an under-researched portion of the electromagnetic spectrum. This has been largely due to the fact that the doses of UV required to produce a biological effect are much higher than those for UVB and UVC. However, the recognition that UVA penetrates both the ozone layer and human skin more effectively than shorter wavelengths means that its significance for human health is no longer in doubt. Most of the effects of UVA are mediated through the indirect production of reactive species in the cell, which in turn damage larger biomolecules. DNA damage and lipid peroxidation are at the heart of key biological effects, although the alteration of cell behaviour through changes in cell signalling pathways are also likely to be important. Linked to these, the recognition that UVA can induce a bystander effect and persistent genomic instability adds a new dimension to its effect and opens up potential new approaches to photoprotection, which should not concentrate exclusively on protectors present at the time of UV exposure.

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