Biological effects of exposure to UV radiation relevant to carcinogenesis

A large body of literature documents the effects of UV radiation on different living organisms, including humans, animals and bacteria. Experimental as well as epidemiological data strongly indicate that the spectrum of UV radiation reaching the Earth's surface is involved in the development of melanoma (IARC, 1992).

The biological effects of exposure to UV radiation were described in detail in an IARC Monograph on UV radiation (IARC, 1992), and the molecular effects in recent review articles (Griffiths *et al.,* 1998; Pfeifer *et al.*, 2005). In this section, we summarize the aspects most relevant to the understanding of the biological issues associated with exposure to artificial sources of UV radiation.

Biological lesions induced by UVA and UVB radiation

DNA damage

(a) Experimental systems: UVB is a complete carcinogen that is absorbed by DNA and can directly damage DNA. DNA damage induced by UVB irradiation typically includes the formation of cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4P). If repair mechanisms fail to restore genomic integrity, mutations are likely to occur and persist through subsequent cell divisions. These mutations are $C \rightarrow T$ and $CC \rightarrow TT$ transversions, commonly referred to as "UVB fingerprint" or "UVB signature" mutations. UVB can also induce the formation of singlet oxygen species ($O_2^{.}$), an oxidative compound that is highly reactive and can cause DNA damage indirectly (Griffiths *et al.,* 1998).

UVA is not readily absorbed by DNA and thus has no direct impact on DNA. Instead, UVA induces DNA damage indirectly through the absorption of UVA photons by other cellular structures (chromophores), with formation of reactive oxygen species (such as singlet oxygen and hydrogen peroxide [H_2O_2]) that can transfer the UVA energy to DNA via mutagenic oxidative intermediates such as 8-hydroxydeoxyguanosine (8-OHdG). DNA damage by UVA radiation typically consists of $T \rightarrow G$ transversions, called "UVA fingerprint" or "UVA signature" lesions (Dobretsky *et al.,* 1995).

One study in hamster fibroblasts showed that UVB produces numerous immediate mutations, whereas UVA produces fewer immediate mutations and more delayed mutations than UVB (Dahle & Kvam, 2003).

(b) Effects on humans: The mutagenic properties of UVA in humans have been confirmed in several studies (Robert *et al.*, 1996; see Pfeifer *et al.,* 2005; Halliday, 2005 for reviews). The possibility that indirect DNA damage induced by UVA could play a major role in melanoma occurrence is underlined by reports of multiple cutaneous melanomas developing in patients genetically highly susceptible to oxidative agents (Pavel *et al.*, 2003).

Experiments in human volunteers conducted during the last decade have shown that commercial tanning lamps produce the types of DNA damage associated with photocarcinogenesis in human cells. Volunteers whose skin was exposed to UVA lamps used in tanning appliances show DNA damage, p53 mutations induced by oxidative damage, and alterations of the p53 protein similar to those observed after sun exposure or after UV exposure of experimental animals (Woollons *et al.,* 1997; Whitmore *et al.,* 2001; Persson *et al.,* 2002).

Studies in humans show that a pre-vacation artificially-induced tan offers little or no protection against sun-induced DNA damage (Hemminki *et al.,* 1999; Bykov *et al.,* 2001; Ruegemer *et al.,* 2002).

Cell damage

UVA and UVB radiation can cause cell damage through different mechanisms: both UVA and UVB lead to differential expression of p53 and

bcl-2 proteins, which may play an important role in regulating UV-induced apoptosis (Wang *et al.,* 1998). DNA repair and apoptosis protect the cell's integrity against UV-induced damage. One study conducted in cells from medaka fish suggested that different apoptotic pathways exist depending on the wavelength, i.e. for long- (UVA) and for short- (UVB or UVC) wavelength radiations (Nishigaki *et al.,* 1999). Irradiation of melanocytes with UVA or UVB leads to alterations of different intracellular proteins, suggesting that UVA and UVB may induce initiation of melanoma via separate intracellular pathways (Zhang & Rosdahl, 2003).

UVA, UVB and human skin

In humans UVA penetrates deeper into the skin than does UVB. Because UVA represents the majority of the UV spectrum of tanning appliances and of solar radiation reaching the Earth's surface, far more UVA than UVB reaches the basal layers of the epidermis, where skin keratinocytic stem cells and melanocytes are located. DNA analysis of human squamous cell carcinoma (SCC) and solar keratosis showed that UVA fingerprint mutations are mostly detected in the basal germinative layer of these lesions, whereas UVB fingerprint mutations are found predominantly more superficially in these lesions (Agar *et al.,* 2004).

Differential effects of UVA and UVB on skin cancers

Experimental systems

Several studies showed that UVA could induce squamous cell cancers in nude mice, but the ability of UVA alone (without exogenous photosensitizers such as those used in PUVA therapy — see Page 41) to induce squamous cell skin cancers was about 5000 to 10000 times lower than that of UVB alone (IARC, 1992; de Laat *et al.,* 1997; Griffiths *et al.,* 1998). Both in-vitro experiments and epidemiological studies have demonstrated that long-lasting, chronic exposure to UVB is the main cause of SCC of the skin (see IARC, 1992; Brash *et al.,* 1996 for reviews).

Accordingly, before 1990, only UVB, and not UVA, was considered to be carcinogenic.

In the 1990s, studies in newborn rodents and on human foreskin grafted on immunosuppressed nude mice have provided compelling evidence that high UVB doses were required in the genesis of melanoma or of melanocytic tumours considered to be precursor lesions of melanoma (Mintz & Silvers, 1993; Atillasoy *et al.,* 1998; Robinson *et al.,* 1998; Sauter *et al.,* 1998; Robinson *et al.,* 2000a; Noonan *et al.,* 2001; van Schanke *et al.,* 2005). At the same time, several in-vivo studies showed that UVA can induce melanoma in backcross hybrids of freshwater fishes of the genus *Xiphophorus* (platyfish and swordtail; Setlow *et al.,* 1993) and melanocytic tumours in the South American opossum *Monodelphis domestica* (Ley, 1997, 2001). However, UVA was less efficient than UVB for the induction of melanocytic tumours in *Monodelphis domestica* (Ley 2001), and experiments with UVA on newborn rodents and on human foreskin could not reproduce the results obtained with UVB (Robinson *et al.,* 2000b; Berking *et al.,* 2002; de Fabo *et al.,* 2004; van Schanke *et al.,* 2005).

Other studies showed that radiation emitted by lamps used in tanning appliances (mainly UVA) could significantly increase the carcinogenic effect of broad-spectrum UV radiation (Bech-Thomsen *et al.,* 1991, 1992), indicating the possibility of a complex interplay between UVA and UVB radiation in human skin.

Relevance of experimental data to human skin cancers

To date, evidence obtained from experimental studies on the involvement of high UVB doses in the causation of SCC is consistent with observations in humans. In contrast, experimental studies provide conflicting results on an implication of UVB and UVA in the induction of melanoma in humans. The same uncertainties hold true for basal cell carcinoma (BCC), a type of tumour that shares many of the epidemiological characteristics of melanoma.

The relevance of animal models for elucidating the biological mechanisms involved in the development of melanoma and BCC remains

questionable, as even engineered mice with multiple deficiencies in key genes involved in cell cycle regulation and growth factor synthesis do not represent a model equivalent to the human skin. In addition, experiments on animals cannot reproduce the complex relationship existing in individuals between highly variable natural susceptibilities to UV radiation, different sun exposure behaviours, and exposure to various sources of UV radiation. In the case of indoor tanning, such relationships may be critical, as users are more inclined than the average population to engage in outdoor tanning activities (Autier et al., 1991), and indoor tanning sessions often precede or follow active sun exposure or outdoor tanning.

Changes in immune response

Several reports (IARC, 1992, 2001; Ullrich, 2005) have extensively reviewed the studies on the effects of UV on the immune system and of the underlying mechanisms. This section only refers to studies relevant to UVA and use of indoor tanning facilities.

Experimental systems

Both UVA and UVB radiation can affect the immune response that may be involved in the promotion of melanoma (Kripke, 1974; Singh et al., 1995), but the two types of radiation seem to act differently. UVB can induce immune suppression at both local and systemic levels whereas UVA does not induce systemic immune suppression. However, studies have shown that a number of local responses induced by UVB radiation on the skin could be suppressed by a UVB filter, but the melanoma growth stimulation effect could not be suppressed (Donawho et al., 1994; Wolf et al., 1994). This result suggests that UVA may influence local immune responses different from those influenced by UVB.

Studies in humans

Observations in human volunteers have demonstrated that UV exposure suppresses the induction of immunity (Cooper et al., 1992; Tie et

al., 1995; Kelly et al., 1998). Few studies have specifically investigated the effects of exposure to tanning appliances on the systemic and local immune systems. UV lamps similar to those used in tanning appliances are used without concomitant use of photosensitizer for treating skin conditions such as dermatitis and sun allergies, illustrating the effect of that radiation spectrum on the skin immune system.

Studies in volunteers have shown that exposure to tanning appliances induces reductions in blood lymphocyte counts, changes in proportion of lymphocyte subpopulations, immune response to known carcinogens applied to the skin, and changes in the skin immune system (Hersey et al., 1983, 1988; Rivers et al., 1989; Clingen et al., 2001). These studies also indicated that UVA and UVB would affect the immune system via interacting and overlapping mechanisms, depending on the amount of UVA and UVB emitted (Clingen et al., 2001), which would then lead to the suppression of known immune reactions (Nghiem et al., 2001, 2002). Hence, these studies indicate that UVA can suppress established immune reactions at the skin level, but it remains to be established how these effects relate to the induction of neoplastic processes.

Effects of natural and artificial UV radiation on human skin
Variety of skin types

There is a considerable range of susceptibility of the human skin to the carcinogenic effects of UV radiation, and in humans, there is an estimated 1000-fold variability in DNA repair capacity after UV exposure (Hemminki et al., 2001). Susceptibility to sun-induced skin damage is closely related to pigmentary traits, and subjects having the following characteristics are at increased risk for developing a skin cancer (melanoma, SCC and BCC):

- Red hair, followed by blond hair, followed by light brown hair.

- Skin phototype (Fitzpatrick, 1988): subjects who always burn and never tan when going

unprotected in the sun (skin phototype I) have a much higher risk for skin cancer than subjects who never burn and always develop a deep tan (skin phototype IV). Intermediate risk categories are subjects who always burn then develop a light tan (skin phototype II), and subjects who sometimes burn and always develop a tan (skin phototype III). Subjects of skin phototypes V and VI belong to populations with natural brown or black skin, and are resistant to sunlight.

- Freckles (ephelides) on the face, arms or shoulders. The skin cancer risk increases with increasing sensitivity to freckling.

- Skin colour: pale colour, followed by increasing depth of pigmentation.

- Eye colour: blue, followed by grey/green eyes, then by brown eyes.

Subjects with red hair, many freckles and who never tan are at particularly high risk for skin cancer.

Sunburn

Sunburn is the occurrence of painful erythemal reaction after exposure to UV radiation. Sunburn during childhood or during adulthood is a risk factor for melanoma, and the risk increases with increasing number of sunburns (IARC, 1992). Skin erythema or sunburns are reported by 18–55% of users of indoor tanning facilities in Europe and North America (reviewed in Autier, 2004). Although UVB is more potent than UVA for triggering sunburn, high fluxes of UVA are capable of inducing skin erythemal reactions after 10 to 20 minutes in subjects susceptible to sunlight and having moderate tanning ability (Fitzpatrick skin phototype II).

Tan acquisition

The production of melanin (tanning) accounts for part of the protection against UV radiation, but there is mounting scientific evidence that facultative tan is triggered by UV-induced DNA damage in the skin (Pedeux et al., 1998; Gilchrest & Eller 1999 for a review). Facultative tanning is now considered a better indicator of inducible DNA repair capacity than of efficient photoprotective skin reaction. Inducible DNA repair capacity rather than pigmentation itself could result in the lower incidence of skin cancer observed in darker-skinned individuals (Young et al., 1998; Agar & Young, 2005; Bohm et al., 2005).

In subjects who tan easily, exposure to tanning appliances will first lead to the oxidation of melanin already present in superficial keratinocytic layers of the skin (i.e. immediate pigment darkening [IPD]). IPD is essentially triggered by UVA (Young, 2004). It develops rapidly after exposure during an indoor tanning session, and fades away after a few hours. A more permanent tan is acquired with accumulation of exposure, depending on tanning ability and on the amount of UVB present in the UV spectrum of the lamps. The permanent tan conferred by "UVA-tanning" has a uniform and less deep brown appearance than the tan acquired in the sun.

IPD has no photoprotective effect against UV-induced erythema (Black et al., 1985). A UVA-induced permanent tan provides practically no photoprotection either (Gange et al., 1985; Rivers et al., 1989), and UVA-induced moderate skin thickening would afford even less photoprotection than tanning (Seehan et al., 1998).