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Skin Cancer and Solar UV Radiation

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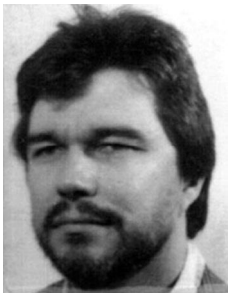

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Ultraviolet (UV) radiation in sunlight is the most prominent and ubiquitous physical carcinogen in our natural environment. It is highly genotoxic but does not penetrate the body any deeper than the skin. Like all organisms regularly exposed to sunlight, the human skin is extremely well adapted to continuous UV stress. Well-pigmented skin is clearly better protected than white Caucasian skin. The sun-seeking habits of white Caucasians in developed countries are likely to have contributed strongly to the increase in skin cancer observed over the last century. Skin cancer is by far the most common type of cancer in the U.S.A. and Australia, which appears to be the result of an 'unnatural displacement' of people with sun-sensitive skin to sub-tropical regions. Although campaigns have been successful in informing people about the risks of sun exposure, general attitudes and behaviour do not yet appear to have changed to the extent that trends in skin cancer morbidity and the corresponding burden on public healthcare will be reversed. The relationship between skin cancer and regular sun exposure was suspected by physicians in the late 19th century, and subsequently substantiated in animal experiments in the early part of the 20th century. UV radiation was found to be highly genotoxic, and DNA repair proved to be crucial in fending off detrimental effects such as mutagenesis and cell death. In fact, around 1940 it was shown that the wavelength dependence of mutagenicity paralleled the UV absorption by DNA. In the 1970s research on UV carcinogenesis received a new impetus from the arising concern about a possible future depletion of the stratospheric ozone layer: the resulting increases in ambient UV loads were expected to raise skin cancer incidences. Epidemiological studies in the last decades of the 20th century have greatly refined our knowledge on the aetiology of skin

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cancers. Analyses of gene mutations in skin carcinomas have identified UV radiation as the cause. The relationship between the most fatal skin cancer, i.e. malignant melanoma and solar UV exposure is, however, still unclear and needs to be clarified to optimise preventive measures and minimise mortality from skin cancers.

Key words: skin cancer, melanoma, incidence trends, UV radiation, DNA damage, gene mutations
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INTRODUCTION

Solar ultraviolet radiation

SUNLIGHT CARRIES the fundamental energy for life on earth by driving photosynthesis, but the ultraviolet (UV) radiation from the sun has clear detrimental effects. The sun emits UV radiation across a broad spectrum from the high-energy UVC band (wavelengths below 280 nm; 1 nm = 10^{-9} m) and the UVB band (280–315 nm) to the UVA band (315–400 nm, bordering the visible band from 400 to 800 nm). Conjugated bonds (alternating single and double bonds) in organic molecules absorb UV radiation of wavelengths around 200 nm and in linear repeats or ring structures the absorption shifts to longer wavelengths (250–300 nm). After absorption of the radiant energy a molecule may become (photo-) chemically reactive, and may be modified or damaged. The broad solar UV spectrum that passed through the earth's primordial anoxic atmosphere was very deleterious to highly evolved organic molecules, such as proteins and DNA. In those conditions life had to evolve in places shielded from UV radiation (i.e. in oceans or under layers of UV-absorbing organic material). By a very fortunate evolutionary event plant-like organisms started to produce oxygen in substantial amounts over 2 billion years ago. In the outer reaches of the atmosphere molecular oxygen (O_2) absorbs short-wave UVC radiation, becomes decomposed (O) in the process and recombines to form trace amounts of ozone (O_3) which absorb wavelengths up to about 310 nm. Thus, all of the detrimental UVC and most of the UVB radiation is absorbed in the 'ozone layer' (a rarified layer spread over altitudes from 12 to 50 km in the stratosphere). Nevertheless, the residual UVB radiation that reaches ground level can still be absorbed by proteins and DNA, and is sufficient to kill unprotected cells. Life on the earth's surface has necessarily adapted itself very well to this residual UV stress (e.g. by UV-absorbing surface layers). Our skin—UV radiation does not penetrate any deeper—shows an impressive variety of passive and active protective features, but it may still develop blistering sunburn hours after being exposed to the sun's UV(B) radiation. This demonstrates that our background level of UV radiation cannot be considered as 'low level'—it can evoke substantial toxic reactions.

Cancer

Basic cancer research has evolved to the point where cancer is considered to be a disease stemming from disturbances in signalling pathways that control the cell cycle and differentiation. The most persistent disturbance is introduced by synthesis of dysfunctional signalling proteins or by a complete lack of synthesis of such proteins from miscoding or lost genes. Such defects are passed on to daughter cells, thus propagating the problem of controlling cell growth. Since the ubiquitous solar UV radiation will damage DNA in exposed skin, there is a continuous threat to the integrity of genes in skin cells. The fact that healthy humans do not readily develop skin cancer attests to an impressively adequate adaptation of the human skin.

In the following sections I will present a concise overview of the developments in various fields of research on the relationship between skin cancer and UV radiation (for an extensive earlier review see [1]).

EPIDEMIOLOGY

In the last decade of the 19th century Unna [2] reported on what he called a sailor's skin carcinoma ('Seemanshautcarzinom'), of which he described the precursor stages in chronically sun-exposed skin, starting from hyperkeratosis. Dubreuilh [3] made similar observations in people working in vineyards. Further statistical support for the relationship between sun exposure and these skin carcinomas was provided in the first decade of the 20th century [4]. It was already suspected that solar UV exposure was the cause of skin carcinomas, because other investigators had reported that UV radiation ('chemical rays') evoked skin reactions. This suspicion was substantiated by animal experiments in the 1920s and 1930s (described below).

Three main types of skin cancer can be distinguished: the most common amongst white Caucasians is basal cell carcinoma (approximately 90 cases per 10^5 people per year in NW Europe), followed by squamous cell carcinoma (approximately 15 cases per 10^5 people year^{-1}) and cutaneous malignant melanoma (approximately 10 cases per 10^5 people year^{-1}) [5]. In NW Europe the incidence of skin cancer is approximately equal to that of lung cancer or breast cancer, but in the U.S.A. skin cancer is by far the most common type of cancer (more than 1 million cases per year) [6]. The cutaneous malignant melanoma (CMM, stemming from melanocytes) is the most aggressive type, and can metastasise very rapidly. Although it is less common than basal or squamous cell carcinomas, CMM accounts for the majority of deaths from skin cancer (about 2–3 per 10^5 people year^{-1} in NW Europe, which is comparable to cervical or liver cancer). The other two types of skin cancer (originating from keratinocytes) are less aggressive than CMM. However, they do grow invasively, and when neglected, a squamous cell carcinoma (SCC) can metastasise. In contrast, this occurs very rarely with a basal cell carcinoma (BCC). Most of the BCC (> 99%) and SCC (> 97%) are adequately cured, which makes the dermatologist 'the most successful oncologist'. The big advantage of skin cancers is, of course, that they occur on the body surface where they are easily spotted very early in their development. This greatly enhances the therapeutic success: even for CMM that are removed early (Breslow thickness < 1.5 mm) the 5-year survival is greater than 98% [7]. Although therapeutically successful, the surgical

removal of a skin tumour can leave a patient mutilated, especially on the face where these tumours commonly occur. Moreover, people who have had a skin tumour removed run a substantially increased risk of subsequent occurrences of skin tumours.

People who sunburn easily and never tan run the highest risk of all three types of skin cancer. The incidences of all three types of tumours go up with increasing ambient UV exposure over the U.S.A. [8]; SCC shows the steepest increase and CMM the least. SCC appears to be straightforwardly related to sun exposure: these tumours occur on the most regularly exposed skin (face, neck and back of hands) [8] and the risk goes up with life-long accumulated exposure [9, 10]. In older epidemiological data BCC and SCC were lumped together as 'non-melanoma skin cancers' and their aetiology was believed to be largely similar, but this proved to be a fallacy. Although BCC do occur on the most regularly exposed skin (80–90% on the head) [8] and the risk has been reported to go up with accumulated exposure [9], they hardly ever occur on the backs of the hands (relatively more on the trunk) and the accumulated sun exposure over the decades prior to removal of the tumour does not appear to contribute to the risk [10]. BCC appear to show a predilection for certain sebaceous skin areas and sun exposure would appear to play a role in the early stages of tumour development. More recent Australian data show that recollected numbers of sunburn episodes are positively related to BCC risk, especially sunburn in childhood [11]. In many respects the aetiology of BCC resembles that of CMM. Except for the Hutchinson melanotic freckle melanoma (a minority of CMM of approximately 10% with an aetiology more like SCC), CMM is not related to accumulated UV exposure, but more to intermittent over-exposure and high levels of ambient UV during childhood [12]. The latter was also proven to increase the number of moles that children develop [13] which is known to be an important risk factor for CMM. The risk from childhood exposure is based on analyses of records on migration from temperate to sub-tropical regions, whereas the data on sunburn episodes are solely based on recall and, therefore, very susceptible to patient-bias. Moreover, sunburn is heavily confounded by an individual's inborn sensitivity, for which statistical corrections need to be made. An assessment of personal exposures is an apparent weakness in the epidemiological studies.

Many cancer registries have shown increases in skin cancer, most strongly in CMM. The upward trend in CMM mortality has been traced to an increase in successive birth cohorts [14], and this trend in the birth cohorts appears to have slowed down. More recent data from the U.S.A. appear to show a discontinuation of this birth cohort trend [15] which is predicted to result in a levelling off of the mortality and possibly a reversal of the trend in overall mortality by the second decade of the next century. The reason for these trends in successive birth cohorts remains obscure.

DNA DAMAGE, REPAIR AND MUTATION

Based on an analysis of the wavelength dependency, Gates [16] concluded in 1928 that the bactericidal effect of UV radiation corresponded to the absorption by DNA, and approximately 12 years later a similar analysis led Hollaender and Emmons [17] to the conclusion that UV-induced mutations in fungi were also related to absorption by DNA. These experiments are clearly amongst the earliest to indicate the vital role of DNA in cell survival and transformation (see historical reviews [18, 19]). Beukers and colleagues [20] in 1960 identified a UV-induced modification of DNA bases: they established that thymine bases in a frozen aqueous solution formed cyclobutane dimers upon UVC irradiation. It was subsequently found that cyclobutane pyrimidine dimers, CPD, were induced by UVC and UVB radiation between neighbouring pyrimidine bases in DNA strands [21]. Later studies identified another UVC/B-induced dimer at di-pyrimidine sites, the 6–4 photoproduct (6–4 PP) [22]. In the early 1960s it was found that pyrimidine dimers were removed by what is now called 'nucleotide excision repair' (NER) [23] which entailed 'unscheduled DNA synthesis' (UDS) [24] to fill in the gaps left after excision of the oligonucleotides containing the dimers.

Patients with the rare hereditary disorder *Xeroderma pigmentosum* were known to be very sun-sensitive and to run a dramatically increased risk of all the types of skin cancer previously described: if these patients do not strictly avoid sunlight they succumb to their skin cancers before reaching adulthood. In 1968 Cleaver [25] found that fibroblasts from these patients failed to show UDS after UV exposure. Consequently, these patients appear to be deficient in NER. Cleaver's finding underlines that UV radiation is a prominent carcinogenic agent in our environment and that NER is an immensely important line of defence.

From this early work on DNA repair, a very fruitful area of research has sprouted which has identified various repair pathways with cascades of multiple interacting enzymes in dynamic repair complexes [26]. NER appears to operate differentially on various DNA lesions: e.g. 6–4 PP are removed faster than CPD and lesions in transcribed DNA strands are repaired faster than in non-transcribed strands (i.e. transcription-coupled repair, TCR) [27]. In contrast to human epidermis, murine epidermis appears to remove CPD only by TCR, leaving the CPD in the non-transcribed strands [28]. This lack of CPD removal by NER in mice probably contributes importantly to their susceptibility to UV carcinogenesis.

UV-induced mutations were found to be associated with di-pyrimidine sites [29], the typical UV target site for DNA damage. However, adjacent thymines did not appear to yield any mutations, possibly by default insertion of an adenine opposite a non-informative base, the 'A' rule [30]. Strikingly, these UV types of mutations were later found in abundance in the *TP53* tumour suppressor gene from human SCC [31] and BCC [32]. Thus, solar UVB radiation appeared to have left its 'signature' in the genome of these tumours. Similar *TP53* mutations were found in experimentally UV-induced murine skin cancers [33] and cell lines thereof [34]. Although a low frequency was first reported [33], high frequencies of these mutations (50–70%) were later found with improved techniques in UV-induced squamous cell carcinomas and precursors from hairless mice [35]. In contrast to human carcinomas, the murine carcinomas showed a particular association with di-pyrimidine sites on the non-transcribed strand of the *TP53* gene, which is most likely attributable to the lack of removal CPD from this strand in mice. Microscopic clusters of cells with high levels of p53 protein in mutant conformation could be detected in UV-irradiated skin of hairless mice long before the occurrence of tumours [36], and similar foci of cells with *TP53* mutations were found in normal human skin [37, 38]. These data indicate that a UV-induced *TP53* mutation can be an early step in the

development of skin carcinomas (not a step that will necessarily progress to carcinoma, nor a step that is necessary in the development of a carcinoma, as can be inferred from the very low percentage, 15%, of *TP53* mutations in UVA-induced carcinomas [39]).

Other tumour suppressor genes and oncogenes must be involved in the development of (UV-induced) skin cancers. With knowledge of the many potentially involved pathways and with the continuous deciphering of genes coding for proteins in these pathways, 'the hunt for genes is on' with ever more refined and more elaborate techniques. A fruitful approach in identifying tumour suppressor genes has been based on genetic analyses of familial skin cancers: thus, CMM was found to be related to the *INK4a/CDKN2A* locus and BCC to the *PTCH* gene. These genes were also found to be mutated in sporadic tumours, but only in a fraction of BCC [40] and mostly in cell lines from CMM [41]. These mutations could have been caused partly by solar UVB radiation, but the causal role of UV does not appear to be as clear cut as for the *TP53* mutations.

EXPERIMENTAL UV CARCINOGENESIS

The first experimental proof that UV radiation is carcinogenic stems from experiments with mice carried out by Findlay in the 1920s [42]. In the 1930s Roffo showed that sunlight could induce skin cancer in rats, and he showed that this carcinogenic action was blocked by coloured and colourless glass, which commonly filters out UVB radiation [43]. In the 1940s Blum, Kirby-Smith and Grady carried out an elaborate research programme on experimental skin carcinogenesis by chronic UV exposure in which they carefully determined the quantitative relationships between tumour induction and UV exposure schedules [44, 45]. The quantitative relationships they found were very similar to those found by Druckrey [46] with chronic application of chemical carcinogens to the skin and to those found by Raabe and colleagues [47] for bone cancer induction by radium: a common result was that $D^r.t_m = \text{constant}$, where t_m is the median tumour latency period, r is a power constant which depends on the carcinogen ($0 < r < 1$) and D is the average daily dose (or a monthly or yearly average dose). This formula implies that a 2-fold higher daily dose will not shorten the tumour induction time by a factor of two, but by a factor less than two. This is entirely in agreement with the contention that tumour development is a process of multiple rate-limiting steps, e.g. successive mutations, of which only some are directly dependent on the carcinogen and/or a process in which protective mechanisms become more and more activated as the daily dosages are increased.

In most of the early experiments UV exposure induced fibrosarcomas and carcinomas on the ears of the haired animals. In the 1960s hairless mice became available which showed a consistent UV induction of SCC with actinic keratoses as precursor lesions, very similar to the lesions observed in chronically exposed human skin. This model was extensively studied at the former Skin and Cancer Hospital in Philadelphia (from which the hairless strain 'SKH' originates) and at the department of Dermatology of the University Hospital (AZU) in Utrecht; for a review see [48]. The wavelength dependency (i.e. the 'action spectrum') of the induction of SCC could be mathematically derived from the accumulated data obtained with UV sources of various spectral compositions [49]. The result was dubbed the 'SCUP-m' action spectrum (SCUP stands for Skin Cancer Utrecht-Philadelphia, and the '-m' for murine). Based on SCUP-m a SCUP-h action spectrum ('-h' for human) could be estimated by correcting for differences in UV transmissions between human and murine epidermis [50] (the differences are largest below 300 nm). The result is depicted in Figure 1 together with the directly measured action spectrum of UV-induced DNA damage (CPD) in human skin [51]. The resemblance between these two action spectra clearly indicates the importance of UVB-induced pyrimidine dimers in the formation of SCC, which is fully in line with the nature of the mutations found in the *TP53* gene (see previous section). Based on the *TP53* gene mutations found in human BCC, it may be assumed that the same action spectrum applies to BCC. Surprisingly, the action spectrum for the induction of melanoma in certain hybrid fish [52] appears to be quite different from the SCUP-m action spectrum, i.e. less difference between UVA and UVB. However, the data on melanoma induction in opossums do not appear to confirm the data in fish. UVB radiation induces melanoma in opossums, and although UVA radiation induces precursor lesions, it does not appear to cause a conversion to malignancy [53].

As in other areas of basic cancer research, great advances are presently being made in basic skin cancer research through the use of transgenic mice. In relation to UV carcinogenesis, for example, my group in close collaboration with other groups has shown that mice with a complete deficiency in NER develop their UV-induced tumours approximately four times faster than their repair proficient littermates [54]. Interestingly, the complete NER-defect appears to cause a relative shift from *TP53* to *ras* mutations (more UVB-induced mutations originating from the transcribed strand), and a corresponding shift from carcinomas to benign papillomas [55]. Immune-deficient RAG-1 mice have been used to host human skin grafts in which skin tumours were subsequently induced by chronic UV exposure, in combination with DMBA applications a melanoma arose in one of 48 grafts [56].

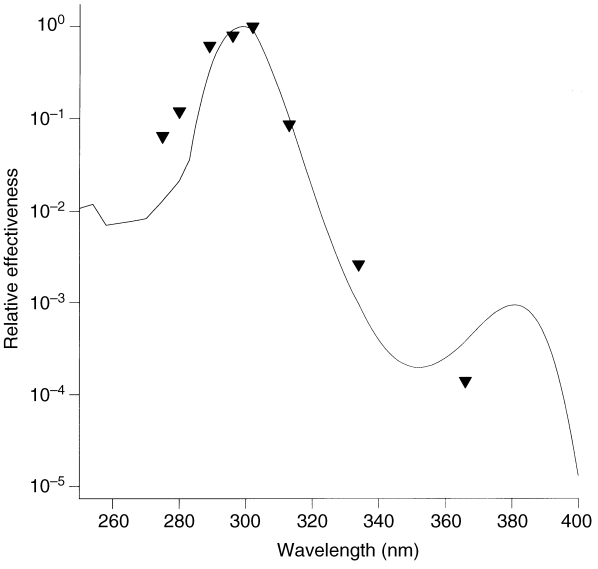

Figure 1. Comparison of the SCUP-h action spectrum (line) [50] with the wavelength dependence of the induction of cyclobutane pyrimidine dimers in human skin (▼) [51].

PHOTO-IMMUNOLOGY

UV-induced skin tumours proved to be very antigenic upon transplantation in syngeic mice, but a prior course of sub-carcinogenic UV exposure rendered the host incapable of rejecting such a tumour implant [57]. This UV-induced immunosuppression appeared to be specific for UV-induced tumours, i.e. a UV tumour-specific tolerance was induced. Although the concept of 'T-suppressor cells' appears to have been banned by main-stream immunologists, the splenic cells with which this tolerance could be transferred seem to fit the bill perfectly [58, 59]. The immune reactivity against autologous primary UV-induced tumours would appear to imply an effective immune surveillance against these tumours [59]. Thus, besides causing the tumours, UV exposure unfortunately also appears to compromise the immune reactivity against these tumours: UV radiation is thus a double-edged sword.

Streilein and coworkers [60] found that contact hypersensitivity (CHS) in humans could be suppressed by UV exposure, and that people who had skin cancer removed were particularly susceptible to this UV-induced suppression. This indicates that UV-induced immunosuppression is also a risk factor for skin cancer in humans. It was later established that CHS can be suppressed in all healthy volunteers with sufficiently high UV dosages [61]. This latter finding indicates that the UV-induced suppression is actually a sound physiological response to avoid illicit immune reactions against the UV-irradiated skin, i.e. avoid a 'sun allergy' (e.g. Polymorphic Light Eruption) for which indeed some evidence has been found [62].

The importance of the immune system in keeping skin cancers at bay is further demonstrated by the strongly elevated risk of SCC in renal transplant patients who receive immunosuppressive medication [63]. These SCC occur in sun-exposed skin in association with viral warts and often contain certain types of human papilloma viruses [64].

RISK ASSESSMENTS

Extensive sunbathing, the use of sunbeds and the threat of a thinning ozone layer all raise the question of how skin cancer risk will be affected. The lack of precise human data on personal exposure histories and corresponding risks hampers a precise estimate of such risks. However, approximate estimates can be made based on available epidemiological data and (animal) experimental data. The wavelength dependence for tumour induction is needed to estimate carcinogenic dosages from various UV sources. This wavelength dependence cannot be measured in humans and, therefore, has to be inferred indirectly, e.g. as done in estimating the SCUP-h action spectrum. The time or age dependencies of SCC in humans and hairless mice show similarities, albeit that the mice get their tumours approximately 250 times faster under comparable average UVB exposures [65]. An estimate of the UV dose dependence in humans can be based on the relationship between incidences and ambient UV levels, e.g. as available for the U.S.A. [8]. The dose-dependence for SCC over the U.S.A. is less than that found in albino hairless mice ($r = 0.6$) and more like that found in pigmented hairless mice ($r = 0.3$) [65]. Based on these data an estimate can be made of how much higher the incidence in a certain population would have been if the ozone layer was x% thinner over the last, say, 70 years; i.e. an estimate for two stationary situations with different amounts of stratospheric ozone but all other conditions being equal (*ceteris paribus*). Assuming that the SCUP-h action spectrum is applicable to all three types of cancer, one then finds that the incidences would be 3x% higher for SCC, 1.7x% for BCC and 0.5–1.0x% for CMM [66]. An estimate of what will happen over time as the ozone layer becomes thinner is much harder: it requires additional information, most of all on whether the UV radiation acts early or late in the development of the tumour, or perhaps throughout the development. Based on epidemiology it appears reasonable to assume that UV radiation plays a role throughout the development of SCC, and mainly in early stages of BCC and CMM (although sunburn at later stages might speed up the process) [65]. These premises were used in scenario studies on projected changes in the ozone layer. It was thus calculated that skin cancer incidences in NW Europe and the U.S.A. could have doubled by 2100 under the initial international agreement on restricting ozone-depleting substances. The most recent amendments to this agreement are projected to result in a peak increase by 10% over current levels around the year 2060 [67]. This underlines the importance of compliance with the agreements.

CONCLUSIONS

As skin tumours are easily spotted and accessible they are ideally suited for experimental studies in rodents and comparative studies in humans. These tumours, therefore, provide a convenient model for molecular cancer research which appears to progress at an ever accelerating pace. In their own right, skin cancers pose a serious public health problem which has been aggravated by the increases observed in the 20th Century. National campaigns to curb these increases by promoting people to moderate their (solar) UV exposures appear to be worthwhile, especially in sub-tropical regions where many sun-sensitive and sun-seeking people live, e.g. the U.S.A. and Australia. Campaigns in Australia have had some success [68]. However, despite some improvements in attitudes and sun protection, the frequency of recent sunburn amongst the people in Queensland interviewed in telephone surveys remained the same [69]. Clearly, such campaigns need to aim for long-term success by addressing children and parents, and they need to be tailored to the local situation and continuously modernised.

The UV aetiology of melanoma needs to be better understood better in order to improve advice to the public, preventive measures and targeting of these preventive measures to well-identified high risk groups.

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