

Natural skin barriers and immunological mechanisms against sunlight

Werner Voss M.D., Christina Bürger, M.D. *Dermatest*

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Abstract

Consumers have become aware of increasing risk of skin cancer and premature ageing due to unprotected sun exposure; sun protection has been widely advised for many years. Sunscreen is the largest sector of the sun care market and higher SPFs (20+) are increasing in demand. Such products always refer to the “healthy tanned” body ideal. But we should be aware of the variety of effects due to UV-irradiation, which are not always beneficial. UV-irradiation in low doses supports the immune system. In increasing doses it influences the immune system by suppressing the antigenic presenting cells in the skin. The Langerhans cells, which lie in the suprabasal layer of the skin, play a central role in recognising foreign antigens and in activating other effector cells. After UV-exposition a loss of function or even Langerhans cell apoptosis can follow.

As it causes inflammation of the cornea, degeneration of the retina and cataracts, UV-irradiation can also lead to blindness. The skin diseases caused by UV-irradiation are not only skin cancers (malignant melanoma, basalioma and squamous cell carcinoma), but also polymorphic light eruption and phototoxic or photoallergic reactions due to the use of sun cosmetics. One of the most important cosmetic aspects which occurs after years of sun exposure is the presenility of skin, seen as deep wrinkles and lines. Unprotected parts of the skin are often characterised by darker lentigines (lentigines seniles), decreasing elasticity, and reduced water-binding capacity. Two main types of sun-protection strategies – chemical and physical substances – are used in suntan lotions. A lot of the

chemical effects of its components are as yet unknown, especially. the long-term effects on human health. SPF determinations are done *in vivo* according to the COLIPA method: the erythemal dose of UV-B in the skin is measured. The determination of UV-A – according to scientific standards – is still not possible on human skin. There are several methods to determine the SPF *in vitro* but none of these can yet be used as a standard method. Suntan lotions are good for preventing acute sunburns and, in the long run, for reducing chronic sun damage and incidence of skin cancer. But not even the most expensive suntan lotion can prevent early ageing of the skin. Still, the cumulative dose of UV irradiation is the principal reason for chronic skin damages. Use of sun protection lotions invites prolonged sun-exposure and in the end this is followed by increased UV irradiation reaching the skin. Therefore early ageing and other chronic damage caused by the sun can be expected to increase over the next 20 years in spite of the use of sun screens.

1.1 UV Irradiation

It is well-known that life on earth would be impossible without UV irradiation. But what is UV irradiation, and how can the adverse effects induced by sunlight be explained?

Sunlight consists of differing wavelengths of radiation: UV radiation, infra-red radiation and visible radiation. Protection against the harmful effects of solar radiation is important for preserving human health and well-being. Excessive exposure to UV radiation causes sunburn, skin cancer, immune suppression and skin aging.

UV radiation is responsible for skin damage and other affects on human health. Furthermore the wavelength of UV radiation is of great importance. UV radiation consists of UV-A (320-400nm), UV-B (280-320nm) and UV-C (190-280nm).

UV-C, though the most dangerous, can be discounted because it is completely absorbed by stratospheric ozone layers and does not reach the earth's surface.

Three billion years ago, when the ozone layer was not thick enough, there was no terrestrial life on earth. Life was only possible under water. With the formation of the ozone layer, and the consequent elimination of UV-C at the earth's surface, life on the surface became possible.

In recent decades, lifestyle changes of people in industrialized nations have led to an increased exposure to ambient UV irradiation. At the same time, the depletion of stratospheric ozone layer has led to the increase of UV radiation reaching the surface.

1.2 Physiological changes after UV irradiation

High doses of UV irradiation produce vasodilatation and erythema in the human body. The minimal dose of radiation causing erythema is called the minimal erythema dose (MED). The MED has no fixed value, but depends on the individual, her/his skin colour, age and the radiated limb and the season.

Under the influence of UV radiation, melanocytes begin to produce melanin, and the corneous layer thickens. UVB is mainly responsible for this. UV-A radiation is known to produce weaker erythema and pigmentation than UV-B. This suggests that the body's defences respond better to UV-B than to UV-A. Furthermore UVB in particular triggers vitamin D synthesis in the skin. The classic photoprotective mechanisms in human skin after UV exposure are the induction of melanogenesis (tanning) and the enhancement of DNA repair.

1.3 Melaninsynthesis

Pigmentation (the production of melanin) is the body's most important protection mechanism against UV radiation. Furthermore, melanin acts as a scavenger for radicals. The melanocytes are situated at the basal layer. Each melanocyte has direct contact to 36 keratinocytes so that the melanin produced can be dropped into the cytoplasm of these cells. We know two types of melanin, the brown coloured Eumelanin and the yellow to red coloured Phäomelanin. Both are synthesised by tyrosinase and they are responsible for the different skin colours and pigmentation types. After 24-48 hours UV irradiation leads to increased melanin production, called delayed pigmentation, but again the wavelength is critical for the

quality of pigmentation. UV-B induces a pigmentation of the complete epidermis. Melanosomes are deposited in all keratinocytes and even in the corneous layer, in contrast to UV-A radiation where only the basal keratinocytes show melanosomes. The suprabasal cells lie nearly unprotected against irradiation. Besides this delayed melanin synthesis, immediate pigmentation, which appears directly after irradiation, is also seen. The immediate pigment darkening is mainly due to UV-A. It is due to photo-oxidation of melanin precursors but provides no essential protection from the sun.

1.4 Skin types

The susceptibility of individuals to sunlight depends mainly on their skin type. According to Fitzpatrick, we know four pigmentation types, differing in their colour; tanning characteristics after sun exposure, and their capacity to prevent sunburn.

2.1 Acute and chronic damages of the skin

The energy level of UV-A is about 100 times greater than that of UV-B. Because of its wavelength, UV-A reaches the corium and the connective tissue while UV-B irradiation is rarely able to reach deeper layers of the skin. UV-A is not able to initiate skin pigmentation, which, along with erythema, is brought about after brief exposure to UV-B.

Table 1

Type 1	Always burns, never tans
Type 2	Usually burns, tans with difficulty
Type 3	Sometimes burns, sometimes tans
Type 4	Burns minimally, always tans (Asians, Native Americans, Latin Americans)
Type 5	Rarely burns, tans profusely (African-Americans, east Indians)
Type 6	Never burns, deeply tans (dark-skinned African Americans) may need protection with intense exposure

However, UV-A appears to be the main reason for polymorphic light eruption and in the long-term it is suspected to give rise to denaturation of elastine, a major characteristic of skin ageing. Furthermore, it is known to have a direct influence on the formation of melanoma.

UV-B irradiation is rarely able to reach deeper layers of the skin, therefore it only affects the epidermis. It is responsible for acute skin damage such as sunburn and

polymorphic light eruption. Chronic damage of the skin like photo-ageing is also due to UV-B. Further, there are negative effects on the cornea and retina as described, leading to snowblindness, conjunctivitis, keratitis and cataracts.

UV-B irradiation is also carcinogenic, with SCC and Basalioma both strongly dependent on cumulative exposure to UV-B radiation.

2.2 Sunburn

UV-induced erythema is caused mainly by a wavelength of 300nm, part of the UV-B. It appears 3-5 hours after exposure to the sun, has its maximum effect after 12-24 hours and disappears after 72 hours. The 3-fold MED causes edema of the skin and 6-8 fold MED causes loss of epidermal continuity with blistering. This is the result of sub-epidermal edema, vacuolisation of the basal layer cells and of dyskeratotic keratinocytes, the so-called sunburn cells. This programmed cell death partly prevents mutation and malignant transformation of cells. The small superficial blood vessels show vasodilation. The Langerhans cells diminish, an effect which is seen even after sub-erythematous doses of irradiation. Normal cell counts are not reached until 2-3 weeks later. The exact pathomechanisms underlying UV-B induced erythema are not known but many investigations have elucidated the role of mediators. In the early phase, histamin causes vasodilation; 4-24 hours later prostaglandins lead to erythema and pain, and 12-72 hours after exposure to the sun, Interleukin 6 is responsible for fever and weakness.

Compared to UV-B, UV-A induced erythema requires a 1000-fold dose of radiation. However, it has to be remembered that the intensity of UV-A which reaches the earth's surface is 10-100 fold higher than that of UV-B. UV-A erythema appears immediately after exposure; after 2-4 hours it decreases, then it increases again to a maximum after 6 hours. It depends strongly on skin type. But even after very strong irradiation it never induces blistering. Direct damage to keratinocytes is only due to UV-B irradiation. Vasodilatation and perivascular infiltration with lymphocytes and a few granulocytes occurs, and Langerhans cells decrease. As with UV-B, the underlying pathomechanisms of UV-A induced erythema are not known in detail but in concert with endogenous photosensibilisators, reactive oxidative species are generated and mediate part of the skin damage.

2.3 Sunburn freckles

After acute sunburn, chronic sun exposure or regular use of sunbeds, individuals with pale skin in particular show 'freckles' of persistent medium to dark brown pigmentation, – sunburn freckles. In contrast to sun burn freckles, ephelides are light brown during the winter and change their colour after exposure to the summer sun. Sunburn freckles appear in young people without any signs of skin ageing, whereas senile lentigines are seen in elderly people with photo-aged skin.

2.4 Skin aging

Actinically damaged skin, or photo-aging, is the result of chronic sun exposure. The clinical symptoms include dryness, irregular pigmentation, wrinkling, elastosis and teleangiectasia. These features are predominantly observed in fair-skinned white individuals and of course, exposed areas like the face, neck, or the extensor surface of the upper extremities are more affected than other parts of the body.

Although UV-B photons are much more energetic than UV-A photons and are mostly responsible for sunburn, suntanning and photocarcinogenesis, UV-A is also suspected of playing a substantial role in photo-aging. UV-A induces the formation of reactive oxygen species that readily react with membrane lipids and amino acids. Membrane damage results in the release of arachidonic acid and leads to activation of secondary cytosolic and nuclear messengers that activate UV response genes. Human skin exposed daily for one month to suberythemal doses of only UV-A demonstrated epidermal hyperplasia, stratum corneum thickening, LC depletion, and inflammatory infiltrates with deposition of lysozymes on the elastic fibres. These changes suggest that frequent casual exposure to UV-A radiation results in damage to dermal collagen and elastin in ways expected to produce photo-aging.

2.5 Photocarcinogenesis

Recent investigations have been made to elucidate the role of UV-A vs. UV-B in photocarcinogenesis.

UV-B

UV-B primarily induces DNA damage for DNA with aromatic rings in all of its bases is the main chromophore, the prominent absorber for this wavelength spectrum. UV radiation from 245 to 290nm is absorbed maximally by

DNA. Its four bases (thymine, guanine, cytosine and adenine) have conjugated double-bonds and can absorb UV irradiation and transform to a higher energy level. The radiation induces the formation of cyclobutane pyrimidine dimers and mutagenic photoproducts. CPDs are formed between the C-4 and C-5 carbon atoms of any two adjacent pyrimidines. The double bonds become saturated to produce a four membered ring. Similarly, (6-4) photoproducts are formed between the 5'C-4 position and the 3'C-6 position of two adjacent pyrimidines, most often between TC and CC residues. CPDs are produced three times as often as photoproducts and they are supposed to be the major contributor to mutations in mammals as photoproducts are repaired more quickly in mammalian cells.

DNA repair systems

Mammalian cells are equipped with several DNA repair systems that are able to protect the cell from the effects of DNA-damaging compounds by removing DNA lesions. Depending on the primary DNA lesion, one or more repair pathways become active: these pathways include photoreactivation, base excision repair, mismatch repair, double stranded break repair and nucleotide excision repair. In normal cells, most of these photolesions are removed by nucleotide excision repair (NER), a complex repair mechanism. This mechanism is called the "Dark Repair System" because it works in the absence of sunlight. Recent studies have demonstrated that this NER is partly induced by DNA damage itself. NER removes bulky DNA damage in two distinct subpathways: damaged areas of actively transcribed genes are removed by a rapid mechanism called transcription-coupled repair (TCR), and damage to other parts of the genome is removed by a slower pathway called global genome repair (GGR).

Despite the ability of mammalian cells to repair UV-induced DNA damage, some damage will remain. The cells of the skin contain mechanisms to prevent such DNA damage from leading to carcinogenesis. One of these mechanisms is growth arrest followed by DNA repair, the other is cell death by apoptosis.

The central role of the p53 tumour suppressor gene in induction of DNA repair mechanisms, induction of melanogenesis, induction of cell cycle arrest or cell death (apoptosis) could be elucidated, which demonstrates its importance for all photoprotective mechanisms. DNA

damage leads to a dramatic increase of the p53 protein. P53 is induced after acute UV irradiation and stops the cell cycle at the G1 phase (cell cycle arrest), which allows the repair of DNA damage before its replication in the S phase. When DNA damage is too severe, p53 induces apoptosis of the cell in order to inhibit carcinogenesis. But exogenous DNA oligonucleotides have been shown to have the same effect. Even though this is still under investigation, it is a promising option for the future prevention of skin photoaging and carcinogenesis.

Ouhtit *et al.* investigated temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. Mice were irradiated with a single dose of UV and the skin tissues were analysed at various times after irradiation. They found that high doses of radiation induced cells to undergo apoptosis, whereas low doses appeared to stimulate repair of the DNA damage.

In contrast to humans, other biological systems like marine life-forms use a so called "Light Repair System". The enzyme photolyase together with activated light is able to remove the UV-B-induced photo-lesions.

UV-induced mutations

Carcinogenesis caused by UV radiation often involves the activation of one or more tumour suppressor genes or the activation of growth-stimulatory proto-oncogenes. Tumour suppressor genes are negative growth regulators. If they are inactivated, loss of control over cell growth occurs. Proto-oncogenes act to control proliferation and differentiation. Carcinogenesis can result either from the expression of a mutant version or from an altered gene product.

Several genes have been extensively studied and shown to have important roles in skin carcinogenesis, including p53, p16/p19, patched and *ras*.

P53 mutations have been found in a large proportion of human SCCs and BCCs and actinic keratoses and also in skin cancers from patients with xeroderma pigmentosum (XP). Patients suffering from XP, which is based on a deficiency of the nucleotide excision repair complex, are hypersensitive towards induction of mutations in their DNA by UV and show a dramatically enhanced rate of skin cancer. The p53 tumour suppressor gene in skin carcinomas (SCC and BCC) was found to carry point mutations at the di-pyrimidine sites, which are considered to be characteristic of UV-B irradiation. Mutations in the p53 gene appear to be an early genetic change in the

development of UV-induced skin cancers. Recent studies have investigated microscopic clones of keratinocytes mutated in the p53 gene using epidermal sheets. They found that clonal expansion was continually driven by UVB without any additional proliferative mutation, implying that the initial p53 gene mutation in combination with UV irradiation are the essential factors in UV-induced skin carcinogenesis.

Among the various kinds of oncogenes that have been analysed in rodent and human tumours, *ras* oncogenes are most likely to be involved in carcinogenesis. These proteins participate in signal transduction from the cell surface to the nucleus and in growth control through intrinsic GTPase activities. Most *ras* mutations were found in human cancers and lead to activation of *ras* mediated signal transduction. Activated *ras* genes appear to initiate papillomas (benign tumours of the epidermis) and, in cooperation with at least one other genetic alteration, can induce malignant conversion.

UV-A

The carcinogenic potential of UV-A was previously overlooked. In humans, exposure to artificial UVA has been shown to lead to an approximately two-fold increase in melanoma.

UV-A irradiation seems to damage skin more indirectly. This damage arises through UV absorption by other molecules (endogenous photosensitizers) which then form radicals. These reactive oxygen species (ROS) cause oxidative stress to the cell. ROS-mediated DNA damage is found to be relatively more important with long wave UV-A than with UV-B radiation. The precise nature of carcinogenicity of long wave UV-A irradiation is still obscure: the premutual DNA lesions are not as well known as the targeted genes are.

UV can also affect molecular targets located in the cytoplasm or at the cell membrane. It is able directly to activate cell death receptors expressed on the cell membrane independent of the natural ligands. This includes the induction of programmed cell death. UV-induced apoptosis seems to represent a controlled scavenging mechanism which protects cells from malignant transformation in human skin.

Direct activation by UV has also been reported for other receptors, as the cell surface receptor for epidermal growth factor, TNF and interleukin-1.

2.6. UV-induced Immunosuppression

Immuno-compromised individuals are at increased risk of both infectious diseases and malignancies. In renal transplant patients undergoing immuno-suppressive therapy, the increased risk of skin cancer is approximately sevenfold. Squamous cell carcinomas are directly related to sun exposure in these patients.

UV-B has been shown to inhibit cell-mediated immunity (delayed-type hypersensitivity) and immuno-surveillance of transformed epidermal cells.

Exposure to erythemogenic doses of UV radiation decreases the portion of peripheral T cells and reduces the blastogenic response of peripheral blood mononuclear cells to T-cell mitogens. Exposure to 1 hour of summer sunlight on 12 occasions resulted in a significant increase in CD8+ (suppressor/cytotoxic) T cells and a decrease in CD4+ (helper/inducer) T cells in peripheral blood.

Several health consequences of this immuno-suppression have been recognized. HSV-I and II are re-activated by ultraviolet exposure, in a dose-response fashion. UV-B can affect the course of infectious diseases of the skin such as onchocerciasis and dermatophytosis, and diseases where the skin is a portal of entry such as Leishmaniasis. In sandfly-borne Leishmaniasis, parasite replication occurs in the dermis or epidermis, and is followed by hematologic dissemination and diffuse incurable disease. In a murine model of cutaneous Leishmaniasis, Giannini found that low doses of UV-B radiation increase the likelihood of systemic spread or re-activation of the protozoa. Additionally, UV-B does not diminish the viability of parasites in the skin. These findings are consistent with diminished epidermal defence mechanisms and suggestive of reduced systemic immunity.

The immuno-suppressive properties of UV irradiation are of major biological relevance since suppression of the immune system by UV irradiation is not only responsible for the exacerbation of infectious diseases following UV exposure, but also contributes to the induction of skin cancer.

Suppression of tumour cell rejection has been specifically linked to UV-B, while UV-A does not show such suppression. Moreover, in animal transplant studies, it was found that UV-B-induced tumours fail to grow in normal recipient mice, but continue to progress when transplanted into irradiated mice.

It has been revealed that contact hypersensitivity (CHS) to epicutaneous application to low molecular weight

allergens, called haptens, can be suppressed by preceding UV irradiation. In human epidermis the Langerhans cells are located in the suprabasal layer of the skin and their density is between 460 and 1000/mm² depending on anatomical location. Although LCs account for about 2% of the epidermal cell population, they are able to survey most events within the whole epidermis by virtue of their long dendrites. They play a central role in recognising foreign antigens and activating other effector cells. Inhibition of Contact hypersensitivity correlates with a reduction of Langerhans cells at the site of exposure and with changes in their morphology. Ultraviolet radiation has effects on their characteristic cell-surface molecules, such as ATPase and MHC II antigens. *In vitro* studies showed that UV-mediated alterations of Langerhans cells, which are the crucial cells in the epidermis for sensation, are associated with a loss of antigen presenting function of these cells. However, some of these studies have been conducted with non physiological and unrealistic UV doses and relatively few studies have addressed the effect of UVA irradiation in humans. Recently, a study was undertaken to clarify the effects of physiological UV doses in human epidermal LC populations. UV-A doses administered were between 15 and 60 J/cm² (20 J/cm² is equivalent to about 1 hour received from sunlight on the French Riviera at noon in summer on a plain sun exposed horizontal surface). The number of LC decreased linearly with the UV-A dose and the cells displayed fewer, shorter and partially fragmented dendrites and a rounding up of the cell body with less intense staining for MHC II surface antigen compared to unexposed control cells. This observation indicates a decrease in the length of LCs after UV-A exposure and not simply a reduction of LC surface markers. The cell contact between LCs and keratinocytes appeared to be slackened. These observations seemed to be independent from the individual UV sensitivity of volunteers. All these data clearly demonstrate that LC surface markers and cells themselves are particularly susceptible to UV irradiation but with differing UV waveband effects. It can be emphasised that different mechanisms are involved. The fact that UV-SSR and UV-A radiation induce LC alteration within the epidermis suggests that satisfactory photoprotection requires the use of broad-spectrum, highly protective sunscreens.

Immunosuppression is seen not only at the site of irradiated skin, but also at non-UV exposed sites. This type

is called a systemic immuno-suppression. For a long time it remained unclear how UV light can affect immune responses in skin areas not directly affected by UV radiation. A major breakthrough was the observation that keratinocytes after UV exposure release mediators with immuno-suppressive properties. These cytokines can shift cellular immune responses of many kinds and are not limited to only local effects.

The effect of UV radiation on molecular targets such as causing DNA damage, triggering surface receptors, affecting target cells at the cell membrane, and phosphorylation of important signal transducing proteins leads to UV-induced immuno-suppression.

2.7. Skin cancer

As mentioned above, skin cancer can be caused by UV-radiation. There is no doubt that the level of UV-irradiation is closely connected to the appearance and frequency of skin cancer.

Non-melanomatous skin cancer and superficial spreading melanoma are closely connected with cumulative sunlight exposure. Although there is no direct relationship between cumulative ultraviolet exposure and melanoma, excessive UV exposure during childhood and sunburns before the age of eighteen are associated with greater incidence of this type of skin cancer. In the U.S., malignant melanoma incidences among the white male population show the steepest rise of all neoplasms, and increased by 86.3% between 1973 and 1988. More than one million Americans suffered from skin tumours in 1998.

According to the cancer registry of the Saarland, the incidence of malignant melanoma increased between 1970 and 1995 from approximately 3/100.000 to almost 7/100.000. For every 1% decline in the stratospheric ozone layer, a 3-5% rise in squamous cell carcinoma, 2-3% rise in basal cell carcinoma, and 1-2% increase in melanoma are expected. Scientists in the USA claim that the daily use of sunscreen with at least LSP 12 during the first 18 years of life will decrease the incidence of skin cancer by about 80%.

The correlation between sun exposure and skin cancer is demonstrated by the following observations:

- People with dark skin are rarely troubled by skin cancer, even in areas frequently exposed to sunlight;
- Conversely, fair-skinned people are more liable to suffer from skin cancers;

- The white-skinned citizens of Australia show skin cancer much more often than white-skinned people in western Europe. Australia shows 50% of all skin cancers in the world.
- 95% of all skin cancers are found at light-exposed skin areas (face, neck);
- White-skinned people who spend a lot of time outside develop skin cancer much more often than office workers;
- In Asia, light skin is considered to be beautiful; sunbathing is therefore uncommon, and skin cancer is rare.

Skin cancers differ in their clinical aspects, their growth characteristics and their malignancy.

- Basalioma develop from the basal layer of the epidermis and grow very slowly. Basalioma primarily grow on light exposed skin and do not develop on precancerous skin. Metastases never occur but nevertheless they can cause severe damage by local growth. They can destroy deeper layers of the skin, the underlying bones and lead to a loss of function depending on their localisation. For example basaliomas situated near the eye can lead to blindness. Therefore early surgery is the most important treatment and recovery can be expected in 97% of cases. Because of their correlation with cumulative exposure to the sun, basaliomas often occur with elderly patients, especially those with pale skin.
- Squamous cell carcinoma develop from the spinous layer of the epidermis. They grow faster than basalioma, generalisation is possible, but with timely excision, complete recovery rates of 95% are reached. They often develop on precancerous skin areas, such as actinic keratoses and Bowens disease. Likewise, the squamous cell carcinoma are seen with elderly patients.
- Melanoma is the most malicious cancer of the skin. In the beginning melanoma spread horizontally, while no generalisation occurs. But after a time melanoma begin to grow vertically. This rapid and deep growth leads to metastases in the whole body. There are different types of melanoma with different prognoses. The ones that grow horizontally for a long time like the superficially spreading melanoma have better cure rates. Its correlation with sun exposure is not as close as seen with other skin cancers, but nevertheless excessive UV exposure at an the age up to 18 years, which goes hand in hand with one or more severe sunburns, is often the cause for SSM (superficially spreading melanoma) and NM (nodular melanoma)

between the 20th and the 40th year of life.

Another important factor is the number of pigmented lesions and naevi. People with a great number of naevi have a greater risk of developing melanomas.

Further, congenital naevi, bigger than 1.5 centimeters, have a 6% likelihood of becoming malignant after the 18th year of life.

Recent epidemiological studies indicate that the regular use of sunbeds is associated with a greater risk of melanoma. In 1993 Richard Setlow *et al.* used a Xiphophorus fish-model to prove that UV-B is more likely to induce melanoma than UV-A. Extrapolating the critical spectrum on humans the wavelength which is responsible for about 90% of the solar-induced melanoma is 365nm.

Nucleotide excision repair mechanisms play a central role in preventing carcinogenesis. Xeroderma pigmentosum (XP) is a rare genetic condition, involving a defect in this nucleotide excisional DNA repair system. A reduced capacity to excise UVB-induced pyrimidine dimers leaves these patients at a 2000-fold increased risk of developing melanoma, and a 4800-fold increased risk of forming squamous and basal cell carcinomas by the age of 20. The mean age at which the cutaneous symptoms of XP appear, such as parchment and freckles, is two years. Basal cell carcinomas, squamous cell carcinomas and, less frequently, melanomas appear almost exclusively in sun-exposed areas.

A study by Ley *et al.* supports the evidence for an inverse correlation between UV-B repair mechanisms and development of cutaneous melanoma. The authors conclude that UV-B can function as a complete carcinogen for the initiation and promotion of a malignant melanoma, specifically via DNA pyrimidine dimer formation. (Inference from this photo-reactive repair model might help explain why higher melanoma risk is related more to episodic than prolonged exposures).

3.1. Therapeutic aspects of UV irradiation

Notwithstanding the negative effects which follow non-critical UV-exposure, there are several therapeutic indications for UV-irradiation in dermatological diseases. The slight immuno-suppression due to UV-irradiation, for example, is very useful in the treatment of atopic dermatitis and psoriasis. Furthermore in the case of sun allergy "light hardening" is a well known and common therapeutic option. Vitiligo sometimes can be treated

successfully with PUV-A, the combination of previous photosensibilisation with methoxypsoralen and subsequent UV-A irradiation.

3.2. The psychology of sun protection

A suntan has been a status symbol among Caucasians since the industrial revolution. Before that time, pale skin was fashionable because it indicated wealth and no need to work out-of-doors. With industrialisation, however, the status of tanned skin reversed. It was now considered a sign of abundance of leisure time to spend outdoors. Suntanning as a fashion statement began in the 1940s, promoted by Coco Chanel, the French fashion designer. In the early 1900s the suntan's association with health began a treatment known as "heliotherapy". Though mostly discredited in the 1940s and 1950s, the belief that sun exposure as a cure-all have persisted. The popularity of suntanning as a symbol of health, wealth and fashion has risen almost unabated since the end of World War II. Only recently, spurred by a rapid rise in skin cancers and the decline in the ozone layer, has there been an attempt to reverse this popularity. The campaign has been difficult – not so much in spreading information, but in affecting change in beliefs and behaviour. While the level of knowledge concerning skin protection and the dangers of skin cancer is considered high, many continue to believe that the risks are outweighed by the benefits of a suntan. Even for those who have a good knowledge of the dangers, changes in behaviour are reluctant. A factor is the "optimistic bias" whereby an individual believes that something negative is less likely to happen to them than to their peers. There is a marked difference in health beliefs, behaviour and choice of sun protection among groups segregated by occupation, age and/or gender. For example, younger people are more likely to rely on a sunscreen for sun protection while older people prefer to cover-up with clothing. Such considerations are important when designing education campaigns. Women are more likely to be concerned with health and preventative behaviour than men, and women generally have better knowledge of the effects of sun on the skin. However, this does not seem to have a marked change in their desire for a suntan nor their belief that a tan is healthy. In summary, at present people have good knowledge of the dangers of over-exposure to the sun, but many people still want a suntan, and some go to great lengths to get a suntan. Some of the

major barriers in this area are that having a suntan is seen as being both healthy and attractive, and it is not "cool" to cover-up.

It has also been observed that UV radiation has a positive influence on psychological variables such as mood and emotional state and it is suggested that opioid peptides may be responsible for this effect. Plasma levels of beta-endorphin and met-enkephalin in UV-exposed and non-exposed healthy volunteers have been determined but no significant differences were seen. It can be concluded that UV-A irradiation does not elevate levels of circulating opioid peptides.

4.1. Sunscreens

The regular use of sunscreens is growing amongst sunbathers. According to epidemiological studies in the USA, the regular use of a sunscreen with at least LSF12 during the first 18 years of life reduces the incidence of skin tumours by about 80%. There are two basic types of sunscreen options available: chemical sunscreens that act by absorbing ultraviolet light, and physical sunscreens that reflect or scatter light in both the visible and the UV. The effectiveness of sunscreens depends on their ability to absorb or reflect sunlight, their concentration, their formulation, and their ability to withstand contact with water (through swimming) or perspiration.

In the past, the body has only been protected from UV-B radiation, which is responsible for sunburns and acute sun damage. The sun protection factors were determined by the protection against UV-B irradiation: UV-A protection was not considered.

4.2. Methods for determining the protection factor of sunscreens

UVB

At present, the determination of SPF, is done by radiating the backs of probands and estimating the minimal erythema dose by producing a sunburn. In 1956 R. Schulze defined the Sun Protection Factor as the ratio of the Minimum Erythema Dose (MED) of protected versus unprotected skin visually assessed about 24 hours after irradiation. This definition of the SPF has gained a world-wide acceptance over a period of 25 years.

Based on today's knowledge, instead of the term "Sun Protection Factor" scientists prefer the term "Erythema Protection Factor" or "Sunburn Protection Factor" because

only the 24 hour response is considered in the SPF determination and calculation. Sub-erythemally, sunlight induced effects and chronic effects are probably not adequately considered (e.g. immunosuppression, skin ageing, skin cancer).

There are different definitions in different countries for estimating the erythral threshold irradiation time. Countries define SPF according to the regulations to which they are subject. In Europe, the Colipa recommendation determines the SPF as follows: 10-20 probands with skin types I-III have to be tested over an area greater than 35 cm². Two milligrammes /cm² (-/+0,04 mg) of the product under test has to be applied on the skin 15 minutes before irradiation. The reading of the MED has to be done after 20 (+/-4) hours visually and with a colorimeter. The MED is usually reached after the exposure to 0.038-0.053 J/cm² UV-B.

There are a few methods available to determine the SPF *in vitro*, but these are difficult to use and the results are not widely accepted.

UVA

The definition of UV-A protection is far from universal. There is no standardization of either testing or labelling.

There are still unanswered questions concerning UV-A protection, e.g.:

- Which is the most relevant and measurable skin response to indicate UV-A induced skin damage?
- Which absorption profile should an ideal sun-care product have?
- Which UV-B/UV-A ratio should be considered to meet different irradiation situations?
- What level of UV-A protection is adequate?

In 1994 the German health authorities invited experts to discuss UV-A protection issues. It was concluded that all sun-protection products should have “adequate” UVA protection.

In vitro test methods used until now are:

- Australian standard AS;
- The Boots star rating system;
- The Broad Spectrum Rating;
- The APP – Method/UV-A Protection Percentage.

These methods are all based on transmission/absorption

measurements. They differ in detail and the method of calculation. The results are usually used as indicator of UV-A protection or breadth of the absorption characteristics.

The *in vivo* methods are based on the determination of UV-A induced skin responses like pigmentation or erythema. The UV-A protection factor is calculated analogue to the UV-B protection factor SPF according to Colipa. But the determination of the minimal erythral dose of UV-A radiation is not really suitable because UV-A irradiation hardly ever leads to erythema in physiological conditions. Skin has to be radiated with the 1000-fold dose of radiation to produce an erythema from UV-A. Therefore pigmentation seems to be a more appropriate endpoint for determining the UV-A protection factor. The results obtained diverge significantly between methods, and may also lead to different efficiency rankings.

In vivo methods are:

- IPD – method (Immediate Pigment Darkening)
- PPD – method (Persistent Pigment Darkening)
- APF – method (Erythral UVA- Protection Factor)
- PPF – method (Phototoxic Protection Factor)

As the *in vivo* method can not measure the target skin responses (ageing, immuno-suppression, carcinogenicity) directly, the results can only be seen as indicative.

Therefore, the value of and necessity of *in vivo* UV-A testing has been questioned by several scientists, especially the additional benefit of the *in vitro* measurements.

For the customer, having just become familiar with the SPF system, the existing UVA labelling (“Contains UVA Protection”, “Broad-spectrum protection”, “PA+-PA+++” and “B20A6” or “SPF60-IPD 55-PPD 12”) is more confusing than informative.

A simple and easy-to-understand system is required, like the *in vitro* determination of “Broad-spectrum” criterion.

UV-A index–relative UV-A protection of sun care products Despite the intensive debate on appropriate measurement and labelling of UV-A protection, up to now no proposal has been officially accepted in Europe and the US. A determination was published recently of *in vitro* PPD protection factor which was identified as a candidate for future, harmonized UV-A protection measurement. (Wendel V *et al*, IFSCC Magazine vol.5 no3/2002) The method combines the merits of *in vitro* as

well as of *in-vivo* determinations. Seven laboratories participated in the Round Robin study and five marketed sunscreens were tested. The UV-A Index is defined as the relation between the *in vitro* PPD factor and the labeled *in vivo* SPF. Based on the simple transmission measurement and a calculation that takes into account the *in vivo* effectiveness as references, it provides a rapid and valid procedure to evaluate and to differentiate between the UV-A protection of sunscreens. By inclusion of the *in vivo* SPF in the calculation, the dependency on the sample layer thickness is now negligible. Compared to other *in vitro* UV-A protection measurements the UV-A Index leads to better product differentiation. Reproducibility and relevance are also very positive aspects of the UV-A Index. According to these findings the UV-A Index may be a potential candidate for UV-A determinations in the future.

4.3 Chemical versus physical filters

Sunscreens have been available for 60 years. Safety and effectiveness are dependent on quantity, and the formulation. Principally, there are two ways to protect the skin from UV irradiation. Chemical filters absorb radiation whereas physical filters work with microparticles which prevent UV radiation from penetrating the skin by reflecting them.

Physical sunscreens are made from nonorganic pigments like zinc oxide or titanium dioxide, which reflect or diffuse the radiation. In micronised form (size: 10-100nm) the particles even absorb UV radiation. In Europe the concentration of physical filters is not limited like the chemical filters are. Even the smallest particles do not penetrate the skin and are chemically inert. Therefore they do not produce allergies and work immediately after application.

However, there are some negative aspects concerning physical filters. In pigmented form they whiten the skin and are not very effective against UV radiation. As micro-particles they tend to agglomerate and aggregate due to electrostatic effects, which means an enormous loss in efficiency. Therefore the micropigments have to be coated and kept in dispersion, which is still a great challenge for the cosmetic industry.

In chemical filters, UV radiation activates electrons in the filters from the passive to a short-time active state. With the return to the stable state, energy is emitted as heat or fluorescent radiation.

If the sun protecting factors are not photostable, secondary photo-reactions like photolysis, photo-isomerisation and photo-dimerisation take place.

Typical chemical UV filters are: octyl triazone, urocanic

acid, octyl methoxycinnamate, methylbenzylidene camphor, 3-benzylidene sulfonic acid and PABA. They all have deficiencies. Some work well in UV-B wavelengths are less effective in the UV-A. Others are not photostable and are likely to induce allergies. Recently developed filters are benzophenone-3, benzophenone-4, butylmethoxydibenzoylmethane (Parsol 1789), terephthalylidene sulfonic acid, Mexoryl SX and Mexoryl XL. The major advantage of Mexoryl is its photostability and its capacity to absorb the whole UV-B spectrum (290-320nm) and wavelengths between 320-360nm with maximum efficiency at 344nm.

To provide the best possible protection against sunlight it is reasonable to combine different chemical and physical sunscreens in one product.

4.4 Antioxidants

Solar radiation in the ultraviolet consists of 5% UV-B and 95% UV-A. UV-B radiation is directly harmful to DNA. UV-A radiation operates indirectly by producing free radicals and reactive oxygen species. These cause the peroxydation of fatty acids generating inflammatory reactions. They also polymerise proteins in the dermis leading to complex brittle structures, which lead to slack wrinkled skin.

To diminish the destructive effects of UV induced oxygen radicals, antioxidants are added to sunscreens. These effects can be observed *in vitro* using vitamins (A, E, C), minerals (Selenium, Zinc) or reduced glutathione. But there are problems with the solubility and photostability of the antioxidants mentioned above. Often these substances only protect the sunscreens from oxidation and have no significant effect on the skin. Further investigations have been made by using nothing but antioxidants as sunscreens. In this case the SPF is limited to the factor 5.

Natural antioxidants are getting more and more *en vogue*. Various anti-free radical materials like extracts from sunflowers (Helioxine); green apple skins (Bioprotectyl); Lutein, a mixture of carotinoids found in the chlorophyll complex of many green leafed plants and also in human skin; and Pronalen Sunlife, an antioxidant derived from malt, ginkgo biloba extract, vitis vinefera (grape) seed extract and soy isoflavone extract, are available for inclusion in sun care products.

Another approach to optimize UV-protection is the oral administration of carotinoids. Carotinoids are effective antioxidants. They can be found in numerous vegetables, such as carrots, tomatoes, paprika, maize, broccoli, and fruits like peaches, oranges, grapefruit, melons and apricots. The

most important carotinoids in humans are Lutein, Zeaxanthin, Lycopin and the Provitamin- A-carotinoids β -Carotin, α -Carotin, and Cryptoxanthin. Recently it has been shown that the daily oral administration of 24mg β -Carotin leads to a reduction of UV-induced erythema compared to the control group. Daily administration of 40g of tomato paste correlates with significantly higher levels of Lycopin in the serum of test subjects and likewise higher doses of UV-radiation are necessary to induce erythema compared to the control group.

5.1 A new approach in photo-protection

A new approach in photo-protection is to repair DNA damage after sunexposure. The T4 endonuclease V is a DNA repair enzyme with specificity for UV-induced cyclobutane pyrimidine dimers. Recent studies have shown that liposomes can be used for topical intracellular delivery of small proteins to human skin and suggest that liposomes which contain DNA repair enzymes like the T4 endonuclease V may provide a new avenue for photoprotection against some forms of ultraviolet skin damage. The ability of this enzyme in a liposomal delivery vehicle applied topically to lower the rate of skin cancers in patients with xeroderma pigmentosum was investigated in a recent study. The topical application of T4N5-lotion (T4 endonuclease V) lowered the rate of actinic keratoses and basal cell carcinomas during a year of treatment.

Further approaches in the development of sunscreens have been made with liposomes containing photolyase. In the presence of sunlight this enzyme is very efficient in removing UV-B induced cyclobutane pyrimidine dimers.

A recently published study in Nature Cell Biology demonstrates that interleukin 12 is capable of inducing the nucleotide excision repair complex. (Schwarz A, Ständer S, Berneburg M *et al.*) The possibility of inducing the endogenous nucleotide excision repair system by exogenous applied cytokines could be a promising pathway in the development of new sunscreens in future.

Further studies involve the exogenous application of DNA oligonucleotides. Like endogenous DNA damage, this induces the p53 tumour suppressor gene and thereby the nucleotide excision repair complex. Though still under investigation, this is a promising option for the future.

6.1 Deficiencies and pitfalls in using sunscreens

Erythema is a measurable biological endpoint of UV exposure and is used to determine sunscreen effectiveness.

"Sun Protection Factor"(SPF) is rated by the length of time required to elicit a "minimal erythematous dose" (MED). It has also been shown that sunscreens partially prevent immuno-suppression due to sunlight, but with no correlation with their SPF designations. While histologic signs of erythema are blocked by the application of sunscreen, the protective agents may fail to prevent UV-B induced tumour susceptibility. One study, suggests that the dose of UV-B necessary to increase animal susceptibility to cancer by immuno-suppression is a tenth of the amount needed for overt carcinogenesis by molecular damage.

Exposure to UV-B in tanning salons can cause subsequent failure to respond to antigen challenge, and suppresses enzyme repair of DNA lesions. A major public health concern is that by reducing the erythematous reaction, sunscreens may deceive people into remaining exposed to solar rays and their immuno-suppressive effects for longer periods.

Measuring DNA nucleotide excisional repair may be a method for predicting subpopulations at risk of developing skin cancer. An assay has been developed to measure this mechanism, which is found to be significantly reduced in skin cancer cases. Xeroderma pigmentosum patients have provided a model for testing this assay, as the defect in their repair mechanism varies phenotypically considerably within this subpopulation.

A recent study revealed the protection against ultraviolet-induced immuno-suppression by commercial sunscreens. The ultraviolet radiation wavelengths transmitted by the sunscreen were determined *in vivo* and showed that sunscreens are primarily UV-B absorbers with relatively poor absorption in the UV-A region. The sunscreen protected the subjects against both erythema and immuno-suppression but protection against immuno-suppression was less than half of that for erythema. This was probably due to the immuno-suppression by UV-A, the part of the solar spectrum that does not readily cause sunburn. Because of the dangerous carcinogenic effects of longterm exposure to suberythemal doses of UV radiation, the need for sunscreen products which provide efficient photoprotection throughout the entire UV spectrum is evident.

6.2 Sun protection factor: the higher, the better?

Sun screen products have been available since the 1950s and 1960s. At first a SPF of 4 or 5 was considered to be highly protective. Beginning in the 1980s, SPFs up to 20 were introduced. Now, all major producers offer SPFs up

to 30. In 1998, the Japanese market offered a product with a SPF of 123. No end to this SPF-inflation is in sight.

But is there a logic to ever higher SPFs? First of all the necessary SPF is a factor of the situation in which a sunscreen is used. How much erythral radiation can reach the skin during one day of sunshine? The available data shows erythral doses of up to 32 MED/day (maximum 6 MED/h at high noon) in the case of sensitive skin. This would require a continuous exposure to the sun during the whole day in an unchanged position. In practice, the realistic number of MEDs would clearly be lower. Considering this, an SPF of 15 to 20 would be sufficient protection as long as the product was used under the same conditions as in the Sun Protection Factor test.

But that is exactly the problem. The test conditions are normally not identical with those in practice, and so the labelled SPF is higher than that achieved in practice. The SPF represents the mean of a biological test with typically large deviations. Therefore it can be expected that about 50% of all consumers are overprotected and the other 50% are underprotected. Individual protection might deviate considerably from the mean value. A product with a mean SPF of 30 can, even under standardised test conditions, show individual protection factors between 15 and 45.

Another significant point is the amount of sunscreen applied. In tests, 2mg/cm² are applied. Under realistic conditions it varies from 0,5mg/cm² to 3mg/cm², depending on the user's habits. The SPF varies accordingly. Finally water resistance is an important criteria for effective sunscreens since nobody wants to have to re-apply a sunscreen after bathing.

6.4 Do we need high protection factors?

Considering these points above, it is obvious that the realistic outdoor SPF achieved is often much lower than the theoretical SPF declared on the label. Therefore, high SPF values between 20 and 30 are well justified, but require careful interpretation by the consumer. The real exposure time has to be multiplied by the SPF. This creates errors for the consumer, because higher SPF screens show greater deviation. Furthermore, the self-protection time according to the individual skin type has to be estimated correctly by the consumer. Otherwise, multiplication of the declared SPF with the self-protection time results in a sun exposure time which is often too long.

Another important factor is that the function for

estimating the percentage of UV reduction according to the SPF runs asymptotic, which means only minor increases in reduction with high SPFs. Due to the characteristics of the function, very high protection factors are subject to considerable variations even in only slightly different test conditions. Therefore, a product with an SPF of 30 (97,6 % elimination of erythral active irradiation) can have a mean value of 20 (95%) or even 40 (97,5%).

Because of these facts, a labelling with 4 SPF groups, defining low (2-5), moderate (6-11), high (12-19) and very high (20 and higher) protection makes sense.

7.1 Sun protection - practical guidelines

No matter what the weather is like or what you are doing, protect your skin from ultraviolet irradiation! Make sure your children are protected, too. Would you send a child out in the rain without a raincoat or an umbrella? Would you send a child to play in the snow without gloves or boots? Would you send a child out in the sun without sunscreens and a hat? And consumers should observe the following common-sense guidelines:

1. Don't sunbathe between noon and 4 pm;
2. Get used to the sun slowly; don't overdo it on the first day!;
3. Wear closely-woven covering clothing (long sleeves, long trousers, or a long skirt);
4. Look out for clothing with a label stating that its UPF (protection factor) is greater than 40. Tests have shown sun clothing blocks ultraviolet radiation very effectively, much better than sunscreens;
5. Wear a broad-brimmed hat;
6. Try to keep in the shade;
7. Apply a sunblock lightly to all uncovered skin before going out;
8. Apply sunscreens 30 minutes before sun exposure, and re-apply after swimming or perspiring;
9. Do not use chemical sunscreen products on children less than 6 months of age. Physical protection from the sun is best for this age group;
10. Don't use a sunscreen if a rash appears;
11. Products with PABA (see ingredient classification) may permanently stain clothing yellow; avoid PABA products if sensitive to benzocaine, procaine, sulfonamides, thiazides or PABA
12. Always wear eye protection (sunglasses) as UV light can damage the cornea;

13. Remember that certain maintenance medications can cause photosensitivity;

14. Be aware of ground surfaces. Ground surfaces such as sand, cement and white painted surfaces reflect the sun. In the water, ultraviolet light penetrates to three feet (approx. 1 meter) deep;

15. Are you at risk: fair skin?, light hair? tendency to freckle? tendency to burn easily? a family history of the disease? chronic sun exposure throughout life? blistering sunburns as a child or adolescent?

Avoidance of sunburn is no guarantee of the avoidance of the long-term effects of sun exposure like photo-aging and photocarcinogenesis. There is really no safe way to tan.

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