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Biologic Effects of Light: An Enlighting Prospective

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During the past several decades our knowledge on the effects of light on human health and its underlying mechanisms has been expanded exponentially. These findings have led to an enormous scientific progress including new concepts for prevention and treatment of many diseases such as autoimmune diseases, cardiovascular disease, skin cancer and other malignancies. To summarize our present knowledge on this topic and to stimulate new research initiatives, an international symposium entitled "Biologic Effects of Light", that was organized by J. Reichrath, Th. Vogt and M.F. Holick, and that was supported by Deutsche Forschungsgemeinschaft (DFG), was held June 11/12, 2015 in Homburg/Saar, Germany. This meeting was specially designed to offer scientists and clinicians a platform to discuss the latest developments in this intriguing research area. Plenary and Keynote lectures as well as Round Table Discussions gave an update on carefully selected "hot topics", including vitamin D, skin cancer prevention, UVA radiation and cellular homeostasis, photocarcinogenesis, and photochemical internalization (PCI). Some of the relevant findings and conclusions of this meeting are published in this issue of Anticancer Research (1-13) and can be summarized as follows:

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Biologic Effects of Light for Health (M. Holick)

When human skin is exposed to sunlight or artificial broad-spectrum lighting there exists a wide variety of photochemical processes that occur in the skin that can influence overall health and well-being (1). Exposure to UVB radiation is responsible for the production of vitamin D. The association of living at higher latitudes with increased risk for many chronic illnesses including multiple sclerosis, type 1 diabetes, hypertension and cardiovascular mortality has been linked to vitamin D deficiency and the decreased efficiency of the sun for producing vitamin D (1). People exposed to sunlight have a feeling of well-being. This is, in part, due to the increased expression of the proopiomelanocortin gene that produces beta endorphin, an endogenous opioid that is much more effective than morphine in pain relief (1). Ultraviolet A (UVA) radiation induces the release of nitric oxide in the skin which is a known vasodilator and may help explain why blood pressure is lower in the summer and at lower latitudes (1). Visible radiation and infrared radiation has been used clinically for wound healing and improving skin health. There are a plethora of other photochemical reactions that are occurring in the skin during sun exposure that are only now being appreciated for their potential health benefits (1). This overview (1) provides an insight into many of them.

Photocarcinogenesis: Unravelling the Molecular Mechanisms (S. Emmert)

Cancer is a devastating disease and the second most common cause of death in humans. To this end, skin cancer is by far the most common type of cancer. Basal cell cancer (BCC) followed by squamous cell cancer (SCC) may occur at a

frequency of about 1 per 1,000 Caucasians. In Germany, 15,000 new cases of cutaneous melanoma, are diagnosed per year and approximately every sixth patient may eventually die from melanoma metastases (14). However, the good news is that – depending on the type of cancer, its early detection, and appropriate treatment – mortality from skin cancer is low, even from melanoma.

The reasons for cancer development are quite broad and may include all types of processes that interfere with genomic stability on the cellular level or immune surveillance of de-differentiated cells on the organ level. Concerning skin cancer risk the contribution of chronic life-long sun exposure (non-melanoma skin cancer) or acute sunburns (cutaneous melanoma) is well recognized. This even led to the inclusion of UV-induced SCC in the German ordinance on occupational diseases (15).

On the molecular level the concept of UVB-induced dimer formation, formation of UV fingerprint C-T mutations and subsequently additional mutation acquisition due to knockout of essential cell regulators like p53 as the basis of a multi-step skin cancer development is widely accepted (16).

However, is UVB really the main cause of skin cancer? UVA radiation is much more abundant in the solar spectrum compared to UVB. Indeed, it could be shown that there is a peak in the action spectrum of skin cancer formation at 360 nm in the UVA range (17). It has also been shown that UVA can induce pyrimidine dimers, however, at a much lower rate compared to UVB. In contrast with this, mutations typically found in UVA-induced tumors are comparable to the mutation spectrum of UVB-induced ones (C to T transitions) arising from photodimers. This indicates that a single UVA-induced dimer is more mutagenic than an UVB-induced dimer. To this end, it has been shown that UVA-specific immunosuppressive effects and reduced cell-cycle arrest lead to inhibition of the cellular response to DNA damage (18).

And how about the high cancer risk in organ transplant recipients? About 40% of patients develop skin cancer or precursors in sun-exposed areas within 5 years after transplantation. This is reminiscent of xeroderma pigmentosum patients who cannot properly repair photodimers due to a defect in the nucleotide excision repair pathway. In addition, SCC rather than BCC prevail in organ transplant recipients. And the cancer risk depends on the immunosuppressive medication with calcineurin inhibitors like cyclosporine A (CsA) rendering the highest risk. Indeed, this high risk can be attributed to a reduced immune surveillance but is exaggerated due to CsA-induced reduction of nucleotide excision repair of photodimers. Human papilloma viruses may act as cofactors here (19).

These two examples highlight the principles of primary skin cancer prevention strategies: (i) photolesion reduction, and (ii) enhancement of cellular responses to DNA damage. Lesion reduction can be achieved by simple UV avoidance strategies – avoid midday sun, textile sun protection, sun blockers in the UVB and UVA range. Strategies to enhance cellular responses to DNA damage are manifold and still developing. DNA repair creams have been successfully applied in high-risk xeroderma pigmentosum patients. More recently, certain antibiotics may overcome stop codon mutations in repair genes and re-constitute cellular repair capacity at least to some extent (20). Other signaling pathways including vitamin D may emerge as contributors with this respect in the future.

Finally, the beauty of skin is its easy accessibility. This renders secondary prevention strategies like skin cancer screening a quick, and non-invasive task – given one knows what he sees and draws the right conclusions. Skin examination with the naked eye is simple, suspicious lesions may further be evaluated with dermoscopy or – in high-risk cancer patients with digital dermoscopy and regular follow-up intervals. Confocal laser scan microscopy offers the option of "non-invasive histology" *in vivo*.

These and other aspects surely alleviate the fear of sun exposure and pave the way for a safe and healthy utilization of the beneficial aspects of solar radiation.

UV - or Light protection: New Concepts for Skin Cancer Prevention (L. Zastrow, J. Lademann)

Solar radiation is an evolutionary basis of human life, inducing not only the synthesis of vitamin D but also generating physical and mental well-being. Excessive exposure to and high doses of solar radiation lead to complex damages, such as immunosuppression, sunburn, photoaging and skin cancer. The ultraviolet light (UV) is perceived by the general public to be responsible for the almost epidemical rise in cancer incidence. In 2006, the European Commission recommended to approve sunscreens only if their already existing strong protective efficacy against UVB (280-300 nm) radiation is complemented by an adequate protection against carcinogenic-free radicals induced in the UVA (320–400 nm) spectral range (21).

Therefore, the efficacy of current sunscreens is excellent in this partial range of the complete solar spectrum incident on the earth's surface (280-3,000 nm).

In 2009 Zastrow *et al*. (22) published a spectrum of action, proving for the first time that excessive free radical formation is the general biophysical response to solar radiation in the range between UVB (280 nm) and near infrared (NIR; 1,600 nm). The induced mixtures (23) of short-lived reactive oxygen radicals (ROS) and long-lived lipid peroxide radicals (LOS) behave exactly like the UVA-induced free radicals, which are classified as carcinogenetic. Surprisingly it was found that the amount of free radicals induced by the complete solar spectrum (22) more than doubled compared to the amount induced by the UV alone. For the first time (24) also a free radical threshold value (FRTV) could be detected, which functions as a "universal

body constant". Thereby, the numeric value of this FRTV corresponds to the amount of ROS/LOS induced by the light dose required for a healthy supply with vitamin D.

Below the FRTV, *i.e.*, in the "physiologically active" region of the excessive radical formation, the "primary" ROS are dominant in the ROS/LOS mixture (23). Above the FRTV, *i.e.*, at light doses reaching and exceeding the minimal erythema dose (MED), the ratio is reversed. The amount of "secondary" LOS exceeds that of the "primary" ROS, with the lipid peroxide-based cascade of damages extending from biomolecular cell injury to clinically evaluable organ damage-like sunburn. Consequently, the free radical concentration above the FRTV is "destructive" and, therefore, may be called "pathophysiologic".

It is well-known that people applying sunscreens stay considerably longer in the sun. Although reduced in the UV solar range, the radical formation in the unprotected VIS and NIR ranges overcomes the FRTV within such short time that the "pathophysiological" effect takes place in parallel with the UVB-induced cascade of damage. As sun protection is usually not provided in the VIS and NIR spectral ranges, the radical-induced damage to the skin is much bigger than previously supposed. In light of the globally rising skin cancer incidence, the existing UV protective systems seem to be almost ineffective.

In consideration of these facts, there is an urgent need for the development of innovative concepts starting from an intelligent behavior in the sun, *via* the utilization of our natural protective mechanisms, *i.e.*, hyperkeratosis, tanning and anti-oxidative potential, up to novel radiation-reducing materials for the VIS and NIR spectral ranges. Due to the immense impact of radiation-induced ROS/LOS on skin carcinogenesis, anti-oxidants are of key importance in new sunscreen formulations to ward-off these excess free radicals.

Lademann *et al*. consider specific modifications of the optical properties, *i.e.*, reflectance and scattering, to be very promising. Supposedly based on such modifications, although not purposefully developed, a protective efficacy in the VIS/IR spectral ranges could be detected in some commercial sunscreens (24).

New concepts for the prevention of skin carcinogenesis indispensably require a transfer from mere UV to holistic light protection in all spectral ranges (25). It gives hope that some producers are already selling sunscreens claiming "light protection".

Light for Treatment of Cancer and Other Diseases: Photochemical Internalization (PCI), and Beyond (K. Berg)

A large number of photosensitizers are currently in use or under clinical development for treatment of various types of cancers based on the principle of photodynamic therapy (PDT). PDT is under continuous development and has also recently been further developed for enhancing the activity of other therapeutic agents, such as macromolecular therapeutics, a technology named photochemical internalization (PCI) (26). These macromolecular therapeutics have been and are currently under development to improve treatment specificity in cancer and other diseases. The utilization of macromolecules in the therapy of cancer and other diseases is becoming increasingly important. Recent advances in molecular biology and biotechnology have made it possible to improve targeting and design of cytotoxic agents, DNA complexes and other macromolecules for clinical applications. To achieve the expected biological effect of these macromolecules in many cases internalization to the cell cytosol is crucial. At an intracellular level, the most fundamental obstruction for cytosolic delivery of therapeutic macromolecules is the membrane-barrier of the endocytic vesicles (27). PCI is a novel technology for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in membranes of endocytic vesicles that upon activation by light induce rupture of the endocytic vesicles and thereby release of the macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), gene-encoding plasmids, adenovirus, oligonucleotides and the chemotherapeutic agent bleomycin. For clinical utilization a novel photosensitizer has been developed and evaluated for PCI of bleomycin. Early-phase clinical trials have shown promising results on several advanced cancers despite the low specificity of bleomycin. A phase II clinical trial for the treatment of cholangiocarcinoma has recently been initiated (NCT01900158).

Future development of PCI for delivery of targeted macromolecules to cancers should be expected to enhance treatment specificity, but thus far this has only been evaluated pre-clinically. The PCI technology may be utilized in principle on all solid cancers by delivering light by external-beam radiation, through optical fibers to all hollow organs, by interstitial delivery of light and during surgical procedures. PCI is designed to primarily be a once-off treatment with approximately one week hospitalization. PCI could also be repeated as has been shown in pre-clinical settings, *e.g.* for gene therapeutic transfection of *p53*. One may envision that PCI in the future could also be utilized for treatment of non-cancerous diseases such as rheumatoid arthritis, but also to treat monogene loss-of-function diseases. Current PCI treatment requires a low cytotoxic effect induced by the photochemical treatment to cause an efficient intracellular translocation of macromolecular therapeutics. By inhibiting the photochemical cytotoxicity without perturbing the rupture of endocytic vesicles PCI could be

used repeatedly for many diseases other than cancer. Also very encouraging is the possible contributions to improve development of cancer vaccines by PCI-enhanced cross-presentations in antigen-presenting cells. Efficient activation of $CD8^+$ T-cells requires a strong presentation of tumor-associated antigens by antigen-presenting cells such as dendritic cells and macrophages. In order to induce a sufficient antigen presentation the antigens need to enter the cytosol in order to be processed and transferred to the endoplasmic reticulum where the antigen peptides can bind to MHC class I proteins and transferred to the plasma membrane for recognition by the immature $CD8^+$ T-cells. The translocation of the antigens into the cytosol from the endocytic vesicles appears to be a bottleneck that may be substantially reduced by PCI. Furthermore, the penetration of therapeutics through the plasma membrane and into the cytosol is a major limitation for the design of new therapeutics with intracellular targets and is the main physico-chemical restriction for the design of new chemical entities for clinical treatments. PCI may contribute in this search for new and specific medicines.

Solar Simulators for Human Health: Challenge and Promise (G. Seckmeyer)

Humans are exposed to solar radiation which can be described by the physical quantity radiance that is a complex function of many parameters: Location (latitude, longitude, height above sea level), time (diurnal, yearly variation), wavelength, incident angle and azimuth angle. This function varies with numerous factors. The solar exposure of humans is, therefore, influenced by solar elevation, cloud cover, cloud type, cloud distribution on the sky, aerosols, trace gases in the atmosphere (*e.g.* ozone), obstructions by buildings, reflections from the ground and human behavior including variable clothing. Since the exposure is very complex, it is a great challenge to artificially simulate natural solar radiation. However, by doing so, it can offer unique possibilities to study the influence of various factors in controlled experiments on the exposure that may or may not have a great impact on human health.

Outlook (M.F. Holick, Th. Vogt, J. Reichrath)

Analyzing the effects of light on human health and the underlying mechanisms has developed in recent years into a fascinating research area. It is likely that in upcoming years our knowledge on this topic will continue to grow and will lead to a continuous scientific progress including new promising concepts for the prevention and treatment of many challenging diseases such as skin cancer and other malignancies.

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