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Reflections in Mutation Research

The rise and fall of photomutagenesis☆

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ABSTRACT

UV is the most abundant human carcinogen, and protection from extensive exposure to it is a widespread human health issue. The use of chemicals (sunscreens) for protection is intuitive and efficacious. However, these chemicals may become activated to reactive intermediates when absorbing energy from UV, thus producing damage themselves, which may manifest itself in phototoxic, photoallergenic or photocarcinogenic reactions in humans. The development of safe sunscreens for humans is of high interest. Similar issues have been observed for some therapeutically used principles such as PUVA therapy for psoriasis or porphyrins for phototherapy of human cancers. Photoactivation has also been reported as a side effect of various pharmaceuticals such as the antibacterial fluoroquinolones. In this context, the authors have been involved over more than 20 years in the development and refinement of assays to test for photomutagenicity as an unwanted side effect of UV-mediated activation of such chemicals for cosmetic or pharmaceutical use. The initial years of great hopes for simple mammalian cell-based assays for photomutagenicity to screen out substances of concern for human use were followed by many years of collaborative trials to achieve standardization. However, it is now realized that this topic, albeit of human safety relevance, is highly complex and subject to many artificial modifiers, especially in vitro in mammalian cell culture. Thus, it is not really suitable for being engineered into a general testing framework within cosmetic or pharmaceutical testing guidelines. Much knowledge has been generated over the years to arrive at the conclusion that yes, photomutagenicity does exist with the use of chemicals, but how to best test for it will require a sophisticated case-by-case approach. Moreover, in comparison to the properties and risks of exposure to UV itself, it remains a comparatively minor human safety risk to address. In considering risks and benefits, we should also acknowledge beneficial effects of UV on human health, including an essential role in the production of Vitamin D. Thus, the interrelationships between UV, chemicals and human health remain a fascinating topic of research.

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1. Introduction

Photochemical mutagenesis is fascinating science. Selecting the optimal experimental setup is a challenge to the experimenter, as effects mediated by a chemical coexist with the effects of light itself, in particular UVB light. Critical variables include appropriate concentrations of the chemical and appropriate spectra and doses of the irradiation. Interpretation of the data in relation to the effects of the UV light alone, and possibly even 'dark' mutagenicity of the test chemical, has many intriguing facets.

When choosing our title for this article in the Reflections series in Mutation Research, we did not want to make a judgment on the scientific value of the field. Rather, we refer to the sudden increase in attention that this field received for pharmaceuticals in the wake of the studies of photochemical mutagenicity and carcinogenicity of fluoroquinolone antibiotics, followed by a decline when difficulties of interpretation of photoclastogenicity assays became increasingly apparent. Lately, it is disputed whether data from in vitro photogenotoxicity testing provide any 'added value' beyond that of data on in vitro phototoxicity testing. Before telling the story from our personal experience as regulatory and industrial genotoxicologists, it is worthwhile to clarify a few principles in the field of photomutagenesis.

Photomutagenicity (or photogenotoxicity) in a strict sense refers to the ability of UV light to induce mutations or chromosomal aberrations after direct absorption by the DNA molecule. This property has long been known. The mechanisms have been investigated in fine detail, and the relevance to human health is well established. This aspect of irradiation will not be

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discussed here. Beneficial effects of UV on mental, socioeconomic or immunological health [1] and its essential role in Vitamin D production [2] also fall outside our scope. Instead, we focus on "photomutagenesis" or "photogenotoxicity" as commonly used to describe the 'indirect' induction of mutations or chromosomal aberrations after transfer of energy or charge from a light-absorbing molecule other than DNA. This includes the genotoxic effects elicited by degradation products and/or radicals generated by light of visible and ultraviolet wavelengths. Here, a more specific term is photochemical mutagenesis, but we will use "photomutagenicity" to describe the indirect mode of action, as this is consistent with common usage [3]. In many respects, the activation of a small molecule by light to reach a higher, reactive status resembles what happens to many promutagens by enzymatic metabolic activation.

Prior to regulatory actions on photomutagenesis, several academic groups had recognized the photogenotoxicity of a few photomutagens, notably the furocoumarins (e.g. [4–7]) and the chlorinated phenothiazine tranquilizers [8]. The impact of these studies on regulatory action, if any, was slow to emerge.

2. SCC guideline (1990)

Protection of the naked skin by applying a UV-absorbing preparation is an intuitive way to reduce sunlight-inflicted DNA damage if extensive exposure to sunlight is unavoidable. Human societies have practiced this for hundreds if not thousands of years. At one time, fair skin was considered an ideal of beauty, as suntanned skin was associated with hard, manual work outdoors and exposure to the harsh conditions of nature. In modern times, this has completely reversed at least in the so-called western societies, and the conflict between obtaining a 'healthy' suntanned look and the avoidance of such long-term consequences as skin cancer is a matter of constant debate. Contrary to the immediate benefit of sunscreen application, the inherent difficulty exists that UV absorption by the sunscreen molecules produces aggressive chemicals, including radicals that may damage the genetic material of the skin cells, as does UV itself. The original intention of protecting against UV damage would thus be lost. The evaluation of the photomutagenic potential of UV-absorbing sunscreens and topically applied cosmetics was therefore a sensible step in the characterization of their genotoxic potential. In 1990 the European Scientific Committtee for Cosmetology (SCC) published a guideline requesting such studies [9]. Since the genetic toxicologists working in the labs of cosmetics companies did not have much experience with the adaptation of standard assays to UV-activation, a working group was established by Colipa, the European trade association for the cosmetic, toiletry and perfumery industry. At the same time, SCC contracted validation studies to Covance (then Hazelton Microtest), UK [10,11]. Experts in the pharmaceutical industry became involved primarily because of partnerships between cosmetics and pharmaceutical companies, not because photoactivation was a major concern for pharmaceuticals.

The genetic toxicology literature soon reflected the growing interest in photomutagenesis [10–16]. The known photogenotoxicants 8-methoxypsoralen (8-MOP) and chlorpromazine were used as positive controls and were active in bacterial strains TA102 and TA1537, respectively, as well as in a mammalian cell chromosomal aberration test. These two test systems, bacterial reverse mutation and chromosomal aberrations, were preferred because they belonged to the test battery recommended for 'standard' genotoxicity testing. A problematic question for these tests was whether to include UVB light. The exquisite sensitivity of the excision-repair-deficient tester strains only allowed UV doses corresponding to minutes of natural sunlight, and the common practice of testing to very high concentrations in in vitro genotoxicity assays could therefore not be extended to the irradiation doses. We were surprised to see that holding the agar plate of strain TA100 for 15 s in the sunlight outside our Basel laboratory induced about a doubling of the number of colonies in the plate. The excision-proficient strain TA102 was about 30-fold less sensitive than TA100, and the sensitivity of mammalian cells and yeast was about 100-fold less than TA100. In these systems higher UVB doses could be applied, but they still corresponded to only minutes of intense sunlight.

Initial studies on the photomutagenicity of psoralens [5,6,17] had largely been conducted with baker's yeast, and *Saccharomyces cerevisiae* strain D7 detected 8-MOP and chlorpromazine with high sensitivity in the validation exercises [12]. However, yeast had lost favor as an object of study in genetic toxicology testing laboratories, and its utility in photomutagenesis investigations was not extensively pursued.

3. Irradiation spectra and interlaboratory comparability of light sources and doses

It was much discussed whether inclusion of the UVB part of the solar-simulator light spectrum would be needed to detect photomutagenicity. Obviously, a UVB sunscreen has an absorbance maximum in the UVB part of the spectrum. UVB might therefore be expected to be especially effective for activating photogenotoxicants. On the other hand, increased absorption of UVB in the irradiated solution, which would protect against the direct UVB-induced genotoxicity, would most likely be the dominant effect [18]. Indeed, it was observed that addition of a sunscreen agent reduced the genotoxic effects observed in the irradiated sample to the level of the 'dark' control [12,16]. Similar findings had been made in phototoxicity testing with such tests as the 3T3-NRU assay [19]. In this case, attenuation of the UVB wavelengths was recommended in the guidelines as a means of reducing the direct lethal effects of the irradiation. A UVA/UVB ratio of 20:1 was suggested for photogenotoxicity testing, as this comes close to the ratio in the solar radiation that reaches the earth's surface [18].

Further discussions centered on follow-up testing. There was little agreement on how the relevance of in vitro findings should be assessed, given that no in vivo system for photomutagenicity had been established. The lack of 'gold-standard' photocarcinogens also made validation studies disputable. We knew about 8-MOP plus UVA as a human photocarcinogen [20] but there were insufficient studies in animal models. Disparate results were reported for chlorpromazine, and no studies were available for any other phototoxicants with conclusive photocarcinogenicity data in animals.

4. The case of the fluoroquinolones

Reports on the phototoxicity of the new class of fluoroquinolone antibiotics, including clinical observations, appeared in the literature starting in 1988 [21,22]. Cutaneous phototoxicity in mice had been attributed to reactive oxygen species (ROS) as causative agents [23,24]. A comparative photocarcinogenity study of three fluoroquinolones was initiated at Roche in the only model available at the time – measuring the induction of papillomas in mice as a basis for risk assessment for a fluoroquinolone in development [25]. German authorities, knowing about the ongoing photomutagenesis validation efforts in the company, requested further photomutagenicity investigations of the fluoroquinolones to obtain evidence about the mechanism of photocarcinogenicity [3]. We employed the Ames test and chromosomal aberration tests. ROS-related mutagenicity is most sensitively detected in strain TA102. However, strain TA102 is also exquisitely sensitive to

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