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Effects of an Ultraviolet B Radiation Absorber on Photocarcinogenesis in Hairless Albino Mice

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Key Words
Photocarcinogenesis · Ultraviolet radiation · Skin tumor · Uvinul® T 150 · Hairless mice

Abstract
Uvinul® T 150, a UVB absorber, was administered (concentration 5%) in a vehicle to the skin of hairless albino mice before ultraviolet radiation (UVR) exposure for 5 days per week in a photocarcinogenicity study. Uvinul T 150 prolonged the latency period to 50% skin tumor incidence (controls: 21–22 weeks; Uvinul T 150: 36 weeks in males and 31 weeks in females). When Uvinul T 150 was applied in an alternating-exposure procedure (3 days/week before and 2 days/week after UVR), the inhibition of photocarcinogenesis was less marked (latency period 28–30 weeks). The vehicle formulation had no effect (latency period 20–21 weeks). The sensitivity of the test system was demonstrated by a positive control (8-methoxy-psoralene). Although UVB absorption was shown to inhibit photocarcinogenesis, the results also suggest that UVA radiation makes a contribution to skin tumor formation.

Introduction

Non-melanoma skin cancer (NMSC), the most common type of human cancer [1, 2], is mainly caused by solar ultraviolet radiation (UVR) exposure [3, 4]. UVR reaching the surface of the earth contains about 5% UVB (290–320 nm) and 95% UVA (320–400 nm) radiation [5]. Animal studies have implicated UVB as a predominant carcinogenic factor in NMSC [6, 7]. In such animal studies, UVB carcinogenic efficacy has been reported to peak at 293 nm, which is very close to the limit of natural sunlight reaching the earth's surface. At wavelengths ≥340 nm (UVA), the carcinogenic efficacy was reported be a factor of 10^4 lower [8, 9]. Furthermore, UVB has been shown to cause mutations in the p53 tumor suppressor gene, which fosters tumor development by seriously compromising its critical role in the orchestration of the cellular responses to genotoxicity and cytotoxicity; over 90% of human NMSC harbor the p53 mutation [3, 10–14].

Primary prevention of UVR exposure is the most effective means of reducing UVR carcinogenesis [15]. Uvinul® T 150 (ethylhexyl triazone) is a highly effective UVB absorber that is approved in Europe, Japan and Australia for use in sunscreens at concentrations of up to 5%. Its absorption spectrum ranges from 250 to 330 nm, with a

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Table 1. Composition of Uvinul T 150 (5% in vehicle formulation)

Raw Material	Ingredient(s)	Content, %
Phase A		
Eumulgin VL 75	lauryl glucoside, polyglyceryl-2 dipolyhydroxystearate glycerin	4.0
Lanette E	sodium cetearyl sulfate	1.0
Lanette O	cetearyl alcohol	2.0
Cosmacol ETI	di-c12-13 alkyl tartrate	12.0
Finsolv TN	c12-15 alkyl benzoate	8.0
Cetiol B	dibutyl adipate	8.0
Uvinul T 150	ethylhexyl triazone	5.0
Phase B		
Glycerine	glycerin	3.0
Edeta BD	disodium EDTA	0.1
Veegum Ultra	magnesium aluminum silicate	1.5
Keltrol	xanthan gum	0.3
Water, demineralized	water	ad 100
Phase C		
Euxyl K 300	phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben isobutylparaben	1.0

high absorption (A >1) at approximately 295–320 nm and a peak at 314 nm. The US Food and Drug Administration (FDA), however, suggests testing chemicals which absorb UV light for phototoxicity or photocarcinogenicity [16]. Therefore, the ability of Uvinul T 150 to reduce UVR carcinogenesis was investigated in a standard FDA-type photocarcinogenesis test using albino hairless mice [17–19].

Materials and Methods

Test Substance and Vehicle Formulations

Test Substance. Uvinul T 150 (2,4,6-trianilino-*p*-[carbo-2′-ethylhexyl-1′-oxy]-1,3,5-triazine = 2,4,6-tris[*p*-([2′-ethylhexyl]-oxycarbonyl)anilino]-1,3,5-triazine; INCI name: ethylhexyl triazone, $C_{48}H_{66}N_6O_6$, CAS No. 88122-99-0, ELINCS No. 402-070-1) at a concentration of 5% was used in a vehicle formulation. The sun protection factor of the formulation used in the studies reported here was determined to be 14, according to the Colipa II (2003) test method.

Vehicle. The composition of the vehicle formulation is presented in table 1. The vehicle formulation was stored at room temperature prior to use.

The stability of the Uvinul T 150 formulation was checked monthly. Homogeneity and concentration were determined by high-performance liquid chromatography at the beginning and towards the end of the study. No Uvinul T 150 crystals were detected, and the compound was shown to be distributed homogeneously in the product. The Uvinul T 150 concentrations were found to be in the range of 94.5–104.3% of the nominal concentration.

Animals and Maintenance Conditions

Crl:SKH1-hr mice were supplied by Charles River Laboratories, Sulzfeld, Germany. The age of the animals was approximately 6–7 weeks at delivery and 10–11 weeks at the start of treatment. The animals were singly housed in Makrolon® cages, supplied by Becker & Co., Castrop-Rauxel, Germany. The bedding was type 3/4 dust-free, supplied by SSNIFF, Soest, Germany. The animals were maintained in an air-conditioned room at a temperature of 20–24°C, a relative humidity of 30–70% and a 12-hour light/12-hour dark cycle. The animals were maintained on rat/mouse maintenance 'GLP' diet, supplied by Provimi Kliba, Kaiseraugst, Switzerland, and tap water ad libitum. Food was assayed for chemical as well as for microbiological contaminants. Drinking water was regularly assayed for chemical contaminants and the presence of microorganisms. Bedding was regularly assayed for contaminants (chlorinated hydrocarbons and heavy metals).

Experimental Design

The study consisted of 5 groups of 24 male and 24 female hairless mice. The treatment schedule followed largely the protocol described by Fourtanier [20].

Group 0 (no UVR) was not exposed to UVR and served as a negative control.

Group 1 (UVR) was exposed 5 days per week (Monday to Friday) for 31 weeks to UVR from a sunlight simulator for 36 min, corresponding to 60% of the minimal erythema dose (MED) that had been established in a pre-study, and served as the positive control.

Group 2 (vehicle only before UVR) and group 3 (Uvinul T 150 in vehicle, before UVR) received 50 μl vehicle and Uvinul T 150 formulation, respectively, applied to skin on the back and sides with a Multipette and dispersed with a glass rod, 30 min prior to the UVR treatment described above.

Group 4 (Uvinul T 150 in vehicle, alternating before and after UVR) received 50 μl Uvinul T 150 formulation on the back and sides 30 min prior to UVR treatment on Monday, Wednesday and Friday, and 30 min after UVR treatment on Tuesday and Thursday. The purpose of group 4 was to establish a potential dose-response relationship concerning the interaction between UVR and Uvinul T 150. In this group, the total UVR dose and the total amount of Uvinul T 150 applied was the same as in group 3; however, there were 3 days of interaction per week (simultaneous UVR and Uvinul T 150 exposure), rather than 5 times per week (as in group 3).

At the end of the 31-week treatment period, surviving animals were maintained for an additional 6 weeks prior to terminal sacrifice.

Moreover, a second positive control group (group 5) was used to establish the sensitivity of the test system and also to detect enhanced photocarcinogenesis. To this aim, 8-methoxypsoralen, which is a known photocarcinogenic substance, was administered orally at a dose of 10 mg/kg body weight [21, 22] to hairless albino Crl:SKH1-hr mice. The positive control substance was administered 5 times every 2 weeks (according to a repetitive 2-week

dosing sequence for a maximum of 40 weeks, or until 50% of skin tumor incidence). At 1.5 h after the administration of 8-methoxypsoralen, the animals were exposed to UV radiation for 36 min (sunlight simulation).

Irradiation Source and UVR Sensor

Four TQ 1000 Z4 doped water cooled lamps, each one covered by an optical borosilicate filter (QVF Process Systems, UK), were vertically suspended within a circular metal frame at the centre of a special animal room. With the aid of the borosilicate filter, UV emission started at 290 nm with a first peak at 295 nm; thus, providing a UVR source without UVC. There was significant emission of UVA, particularly at wavelengths of 350–380 nm. Consequently, the UVB:UVA ratio was 1:10, which is comparable to natural sunlight. During exposure, the racks holding the animal cages were located approximately 2 m from the UVR source. All racks were irradiated simultaneously, with daily cage rotation in the racks. Representative racks were monitored by customized detector systems, which recorded UVA/UVB intensity as well as UVR biological effectiveness in MED per hour (biologically weighted UVB detector from Solar Light, Glenside, Pa., USA).

Clinical Observations

The general state of health of the animals was checked twice daily on working days and once daily during weekends or public holidays. A detailed inspection of the treated skin was carried out weekly. Skin lesions were recorded using a mapping sheet. Mice with skin tumors ≥10 mm (planar diameter) or (multi-)focal skin ulcers were sacrificed for humane reasons. Body weights were determined at the start of the administration period and at weekly intervals thereafter.

Histopathology

A full necropsy was performed on all animals. The animals were anesthetized under CO_2, weighed, killed by decapitation, exsanguinated and assessed for the presence of gross skin lesions. Treated and untreated skin were fixed in 4% formaldehyde solution and processed. Sections of the skin and gross lesions of the skin were prepared, stained with HE and examined by light microscopy.

Statistics

Body weights were analyzed by comparison of the each group with the control group using the Dunnett's test (two-sided) for the hypothesis of equal means [23, 24]. Tumor incidences and time to 50% tumor incidence were analyzed using the Peto logrank test [24, 25].

Results

Irradiation intensities at the site of the cages were 3.16 ± 0.25 and 0.36 ± 0.02 W/m^2 for UVA and UVB, respectively. On a weekly basis, these levels are equivalent to 341.3 and 38.9 KJ/m^2. There were no treatment-related effects on body weight development (data not shown). Other clinical and microscopic observations are described as follows:

Group 0 (No UVR, Negative Control). Untreated hairless mice developed no macroscopic skin abnormalities during the 37-week observation period, and skin microscopy revealed squamous hyperplasia in 1 female only (table 2).

Group 1 (UVR, Positive Control). Hairless mice exposed to UVR developed a high incidence of (multi-)focal ulcerated skin lesions (table 2) and most group 1 animals were sacrificed for humane reasons before the end of the 31-week treatment period. The median survival time was 26 weeks for both sexes (table 2; fig. 1). The median skin tumor induction times (T_{50}), defined as a 50% prevalence of skin lesions of 5–10 mm in planar diameter, were 21 and 22 weeks for males and females, respectively (table 2; fig. 2). Histopathological examination of exposed skin revealed a high incidence of squamous hyperplasia and squamous cell carcinomas (table 2).

Group 2 (Vehicle before UVR). The administration of vehicle formulation before UVR treatment (group 2) had no effect on photocarcinogenesis. Median survival and skin tumor induction times were similar to those observed in group 1.

Group 3 (Uvinul T 150 before UVR). The administration of Uvinul T 150 formulation before UVR treatment (group 3) delayed photocarcinogenesis, the median skin tumor induction times being markedly higher than in groups 1 and 2, i.e. 36 and 31 weeks for males and females, respectively (table 2; fig. 2). In contrast to the fate of animals in groups 1 and 2, most group 3 animals survived the 31-week treatment period (fig. 1), and no group 3 animal was sacrificed with ulcerated skin lesions (table 2). Histopathological examination of UVR exposed skin revealed a lower incidence of squamous hyperplasia and squamous cell carcinomas in group 3 than in groups 1 and 2 (table 2).

Group 4 (Uvinul T 150 before or after UVR). When Uvinul T 150 formulation was delivered before irradiation on 3 days and after irradiation on 2 days of the week, the number of animals sacrificed with ulcerated skin lesions was reduced, whereas the number of animals sacrificed with skin tumors (10 mm) was increased in comparison to groups 1 and 2 (table 2). The median survival times in group 4 were 34 and 36 weeks for males and females, respectively (table 2; fig. 1). The median skin tumor induction time in group 4 was higher than that seen in groups 1 and 2, but lower than that seen in group 3, i.e. 28 and 30 weeks for males and females, respectively (table 2; fig. 2). Histopathological examination of exposed skin revealed an incidence of squamous hyperplasia and squamous cell carcinomas in group 4 similar to that seen in groups 1 and 2 (table 2).

Table 2. Clinical and microscopic observations in a photocarcinogenesis study with Uvinul T 150 in hairless mice

	Group 0 (no UVR, negative control)		Group 1 (UVR, positive control[a])		Group 2 (vehicle before UVR[b])		Group 3 (Uvinul T 150 before UVR[c])		Group 4 (Uvinul T 150 before or after UVR[d])	
	males	females	males	females	males	females	males	females	males	females
n	24	24	24	24	24	24	24	24	24	24
Median survival time, weeks	n.d.	n.d.	26	26	26	26	n.d.	n.d.	34	36
Median time to tumor T_{50}, weeks	n.d.	n.d.	21	22	20	21	36	31	28	30
Early deaths, n[e]	0	0	21	22	23	23	4	2	16	15
Due to skin ulcers	0	0	17	20	19	21	0	0	6	7
Due to skin tumors ≥10 mm	0	0	4	2	4	2	4	2	10	8
Animals with skin pathology, n										
Squamous hyperplasia	0	1	24	24	23	23	16	18	23	23
Keratoacanthoma	0	0	0	0	3	0	0	1	1	0
Squamous cell papilloma	0	0	0	0	5	3	6	4	1	3
Squamous cell carcinoma	0	0	24	22	23	23	18	15	23	22

T_{50} = Median skin tumor induction time, defined by a 50% prevalence of skin lesions of 5–10 mm in planar diameter; n.d. = not determinable within experimental observation period (37 weeks).

[a] Exposed daily, 5 days per week (Monday to Friday) for 31 weeks, to UVR from a sunlight simulator for 36 min, corresponding to 60% of the MED.

[b] 50 μl vehicle formulation applied to skin of the back and sides 30 min before UVR treatment.

[c] 50 μl Uvinul T 150 formulation applied to skin of the back and sides 30 min before UVR treatment.

[d] 50 μl Uvinul T 150 formulation on the skin of the back and sides 30 min before (Monday, Wednesday and Friday) or after (Tuesday and Thursday) UVR treatment.

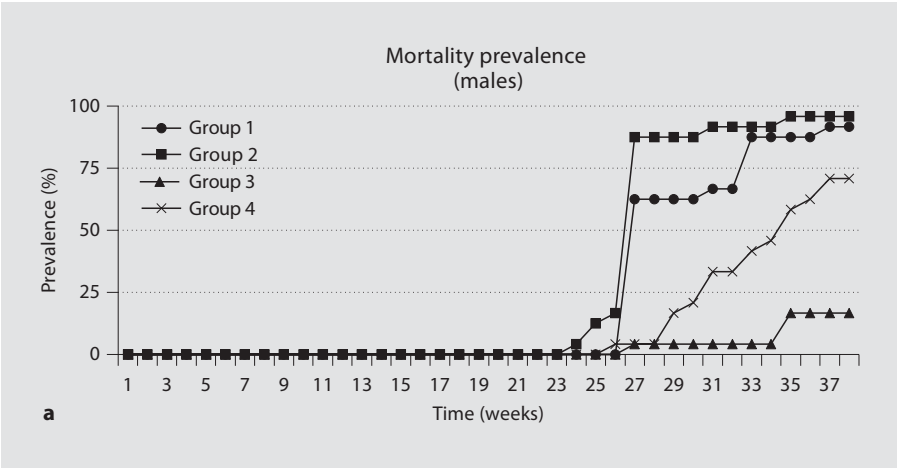
[e] Mice that developed skin tumors ≥10 mm (planar diameter) or (multi-)focal skin ulcers were sacrificed for humane reasons.

Characteristic pictures of normal skin, skin papillomas and squamous cell carcinomas are shown in figures 3–5.

Group 5 (8-Methoxypsoralen). The substance was administered orally at a dose of 10 mg/kg body weight 5 times every 2 weeks (according to a repetitive 2-week dosing sequence for a maximum of 40 weeks, or until 50% skin tumor incidence) 36 min prior to UV radiation, and resulted in a clear reduction in the median skin tumor induction time. The results of this study are shown in figure 6. For reasons of comparison with the control group, the figures show the incidence of skin-tumor-bearing animals related to the cumulative UV dose.

Discussion

The effect of Uvinul T 150, a highly effective UVB absorber, on photocarcinogenesis was investigated. When administered at a concentration of 5% in a vehicle formulation to the skin of hairless albino mice before UVR exposure at 60% of the MED on 5 days per week (group 3), Uvinul T 150 markedly prolonged the latency period to skin tumor development relative to unprotected or vehicle-only-treated UVR-exposed mice. When Uvinul T 150 was applied before or after UVR exposure on alternate days of the week (group 4), the inhibition of photocarcinogenesis was less marked. This finding was not entirely unexpected. The purpose of group 4 was to establish a potential dose-response relationship concerning the interaction between UVR and Uvinul T 150. In this group, the total UVR dose and the total amount of Uvinul T 150 applied was the same as in group 3 (5 times treatment before UVR), however, there were 3 periods of interaction (simultaneous UVR and Uvinul T 150 exposure) per week, rather than 5 (as in group 3). Thus, if there is an interaction, this should be less pronounced compared to a 5-day (full) protection. The observation of a dose-response relationship consequently strengthens the conclusion of an interaction – in the present case, a protective effect of the test substance. In principle, such a dose-response assessment could also have been achieved by omitting the Uvinul T 150 treatment after the UVR exposure; however, with the present treatment scheme,



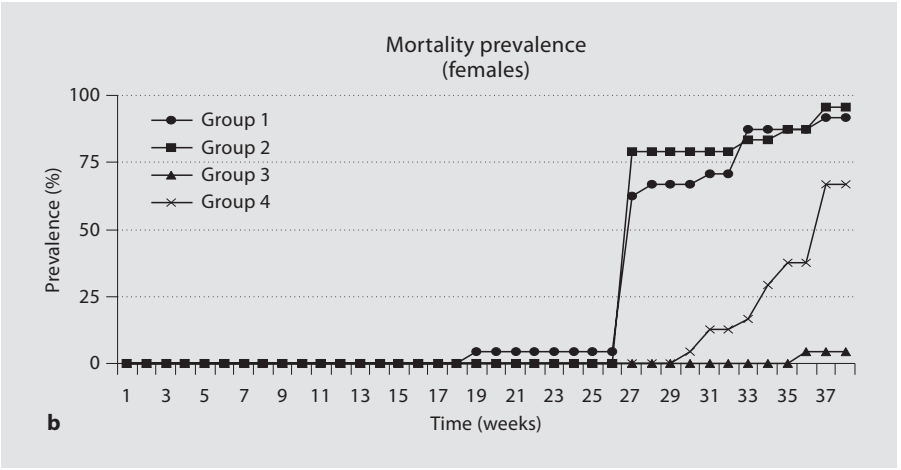


Fig. 1. Mortality rates for male (**a**) and female (**b**) mice. Mice with skin tumors ≥10 mm (planar diameter) or (multi-)focal skin ulcers were sacrificed for humane reasons. Group 1: exposed daily, 5 days per week (Monday to Friday) for 31 weeks, to UVR from a sunlight simulator for 36 min, 60% of the MED. Group 2: 50 μl vehicle formulation applied to skin of the back and sides 30 min prior to UVR treatment. Group 3: 50 μl Uvinul T 150 formulation applied to skin of the back and sides 30 min prior to UVR treatment. Group 4: 50 μl Uvinul T 150 formulation on the skin of the back and sides 30 min prior to (Monday, Wednesday and Friday) or after (Tuesday and Thursday) UVR treatment.

the total dose of the chemical applied to the animal remained constant, which enhances comparability with group 3.

As the total UVR dose and the total amount of Uvinul T 150 was equal in both studies, the difference in latency period is only related to the time of administration (5 administrations/week prior to UVR vs. 2 days/week prior + 3 days/week after UVR treatment). These results indicate that the protective effect is only achieved if Uvinul T 150 is administered prior to UVR exposure. This is not surprising given the fact that Uvinul T 150 absorbs the harmful UVB radiation and protects against sunburn (as a cell proliferation stimulus) and consequently against UVB-induced mutations.

The vehicle formulation had no effect on photocarcinogenesis. The absence of a vehicle effect on photocarcinogenesis is not simply a matter of course [18]. Sambuco et al. [18] reported vehicle-enhanced skin tumor development in 3 out of 8 photocarcinogenicity studies, both in male and female albino hairless mice. The latency periods of 21 and 22 weeks in male and female UVR controls and 20 and 21 weeks for the UVR vehicle groups are similar to those reported by others. Sambuco et al. [18] reported, for a group exposed to 1,200 RBU (Robertson-Berger units), T_{50} values of approximately 25 and 24 weeks for males and females, respectively. Ananthaswamy [14] reported a T_{50} value of 25 weeks for female C3H mice [20] and a T_{50} value of 20 weeks when exposed to 2.7 MED/week. A shorter latency period (of approximately 16–17 weeks) was reported by van Kranen et al. [26] in hairless mice. These differences in T_{50} values can be partly explained by the strain of mouse used, the nature of UVR treatment, as well as by the criteria used to diagnose skin tumors (i.e. the size of non-melanoma skin tumors). The effect of skin tumor size on positive diagnosis was elegantly demonstrated by Sambuco et al. [18] showed skin tumor prevalence curves over time for skin tumor sizes of >1, >2 and >4 mm run virtually parallel, but were

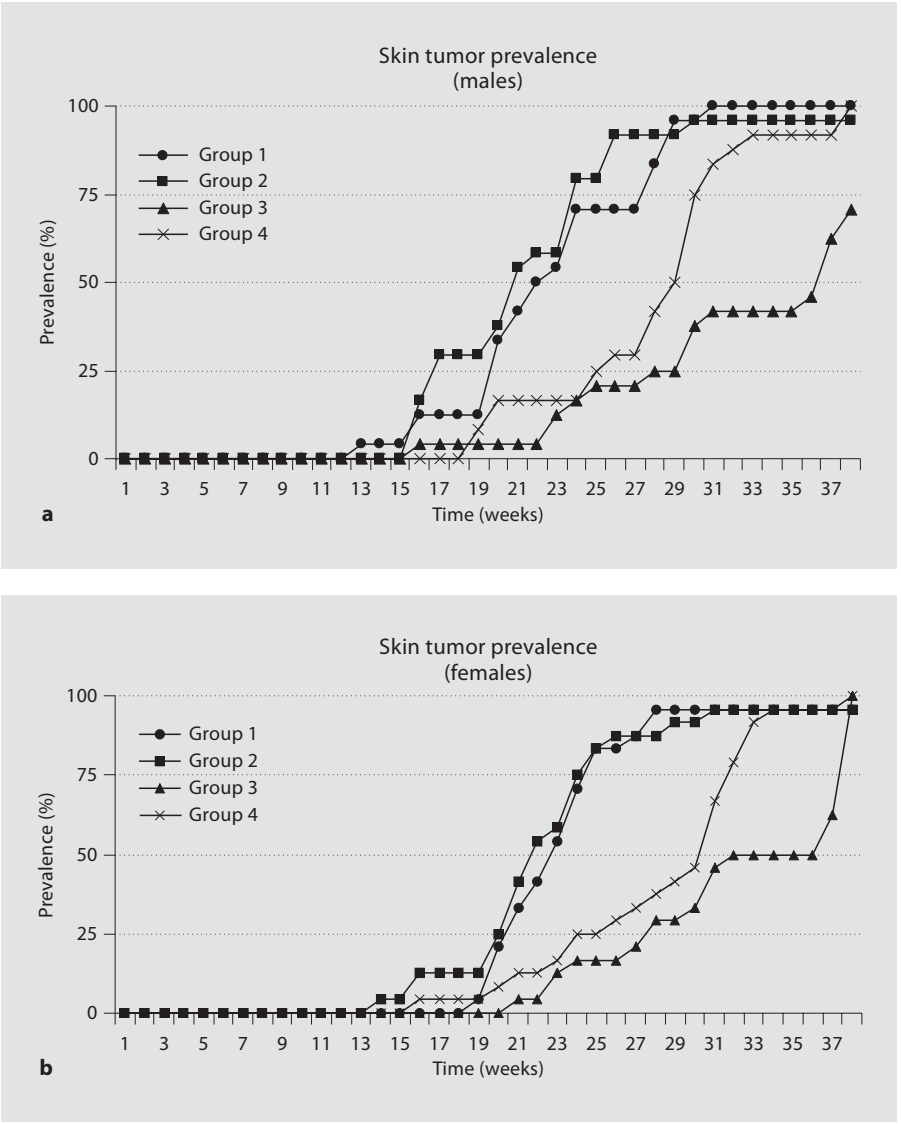


Fig. 2. Tumor rates for male (**a**) and female (**b**) mice (tumors ≥5 to ≤10 mm in planar diameter). Group 1: exposed daily, 5 days per week (Monday to Friday) for 31 weeks, to UVR from a sunlight simulator for 36 min, 60% of the MED. Group 2: 50 μl vehicle formulation applied to skin of back and sides 30 min prior to UVR treatment. Group 3: 50 μl Uvinul T 150 formulation applied to back and sides 30 min prior to UVR treatment. Group 4: 50 μl Uvinul T 150 formulation on back and sides 30 min prior to (Monday, Wednesday and Friday) or after (Tuesday and Thursday) UVR treatment.

delayed by about 4 weeks (i.e. longer latency period) with each larger size of tumors used to determine the T_{50} value. Consequently, tumor size would not appear to be critical for diagnosis of treatment-related effects within a study. However, standardization of animal strain, UVR dose and tumor size would greatly facilitate inter-study comparisons.

The sensitivity and ability of this photocarcinogenicity study to detect an enhancement in skin tumor formation was demonstrated by the positive control 8-methoxypsoralen. In both males and females, there was a clear and statistically significant reduction in the tumor latency period. The regulatory background of our study is the evaluation of the interaction between UVR and the sunscreen. This interaction can be protective, which may be expected of a sunscreen; however, the modification of the sunscreen by the absorption of UVR could, in principle, also result in an activation of the UV-absorbing chemical, resulting in an enhancement of skin tumor formation. Some compounds, such as the psoralenes, are known for such a reaction. Thus, to demonstrate that the test system (the mouse strain and treatment conditions) used in these investigations was sensitive enough to detect a reduction in the latency time, 8-methoxypsoralen was used as a positive control. As the UVR system employed in this study has an emission spectrum that also

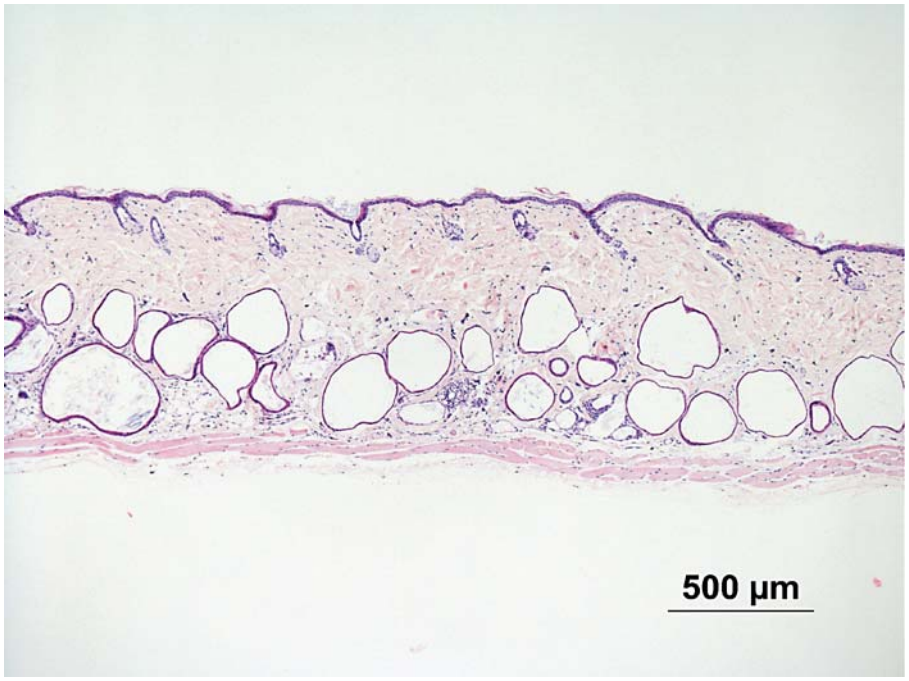


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Fig. 3. Skin of male control hairless albino mouse, without UV radiation. HE. ×40.

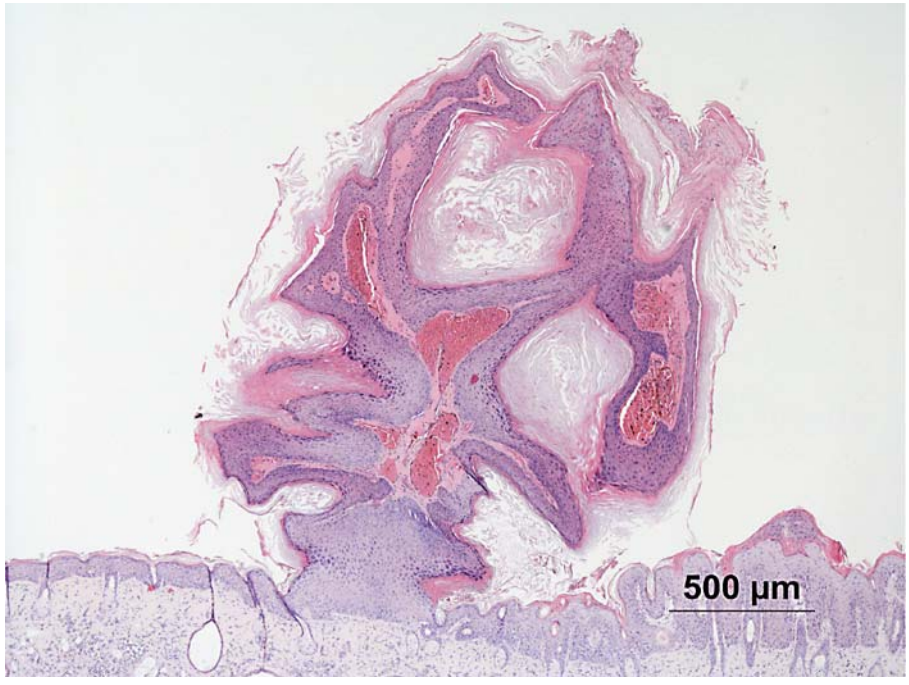

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Fig. 4. Skin of female hairless albino mouse after UV radiation; squamous cell papilloma. HE. ×40.

covers UVA, 8-methoxypsoralen (which enhanced skin tumor formation upon exposure to UVA) was a suitable compound for this purpose.

The emission spectrum of the UVR system used in these studies has a first peak at 295 nm, with minor amounts of UVR emitted at a wavelength of 290 nm. It could thus be argued that this extreme part of the UVB spectrum may not have been adequately tested. However, as the absorption of UVR by Uvinul T 150 already starts at 250 nm, and is quite pronounced at 290 nm ($A > 0.75$) it is unlikely that the minor UVR exposure at 290 nm would have affected the study. Increased UVR exposure

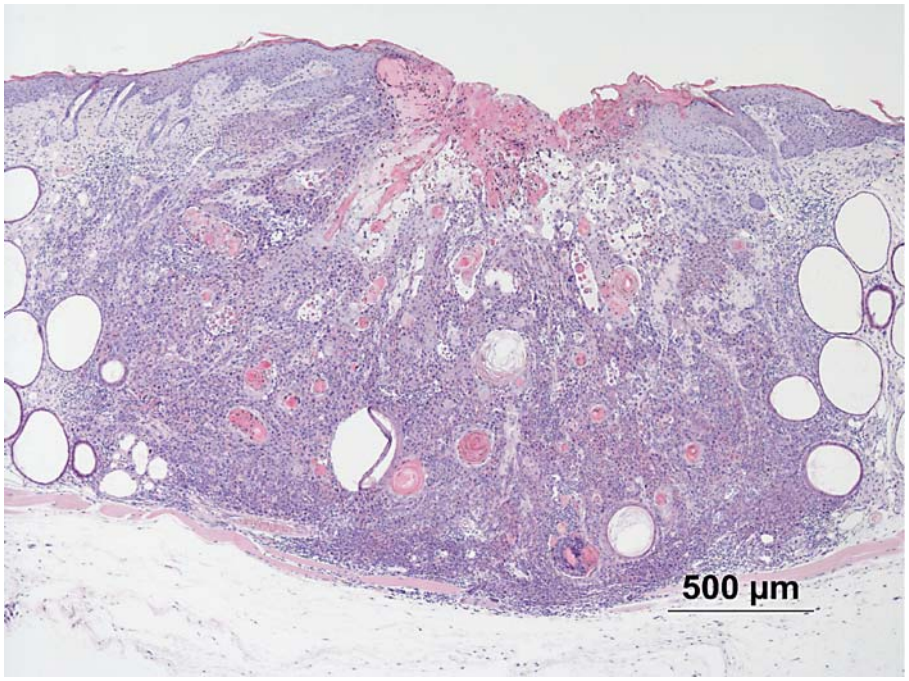

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Fig. 5. Skin of female hairless albino mouse after UV radiation; squamous cell carcinoma. HE. ×40.

at 290 nm, however, would certainly have resulted in a reduced latency period for unprotected control animals.

Our results indicate that Uvinul T 150 delayed photocarcinogenesis by UVB absorption. However, despite the significant increase in time T_{50}, Uvinul T 150 did not fully prevent UVR-induced skin tumor development. Consequently, the results of the present study suggest that UVA also could contribute to skin cancer induction. Although most studies conducted so far have focused on UVB as inducer of NMSC, there is experimental evidence that UVA1 may also cause DNA damage (possibly via reactive oxygen species) as well as squamous cell carcinomas in albino hairless mice [8, 9, 27–30]. The quantitative aspects of UVA1- and UVB-induced skin carcinogenesis are of interest in this context. The relationship between the daily dose (D) and the median skin tumor induction time (T) for both UVA1 [9] and UVB [27] has been demonstrated to be:

$$D^r \times T = \text{constant} \quad (1)$$

or

$$D \times T^{1/r} = \text{constant} \quad (2)$$

where r = 0.62 for UVB and 0.35 for UVA1 and 1/r = 1.6 for UVB and 2.9 for UVA1. Equation 2 is identical to that established for chemical carcinogens in a single dose and chronic experiments by Druckrey et al. [32–34]:

$$D \times T^n = \text{constant} \quad (3)$$

where the exponent n reflects time-associated acceleration of the carcinogenic process, and is always greater than 1.

UVR carcinogenic efficacy has been reported to peak at 293 nm (UVB) and to be a factor of 10^4 lower with UVA1 at wavelengths ≥340 nm ([8, 9], but the Druckrey equation (2) indicates much stronger time-associated acceleration of the carcinogenic process with UVA1 (where n = 2.9) than with UVB (where n = 1.6). Some reports indicate that the contribution of solar UVA to human NMSC may have been underestimated [34]. Based on sequencing of the p53 gene in keratinocytes from solar keratoses and squamous cell carcinomas, it was shown that UVA and UVB caused similar numbers of p53 gene mutations in both benign and malignant human skin tumors, with UVB-induced mutations being restricted to the upper areas of the tumors and UVA-induced mutations predominating at the basal germinal layer [35, 36]. Penetration of UVA to the dividing basal/stem cell layer may be important to fix acute DNA damage as heritable genomic mutations. Hence, the UVA waveband of sunlight is likely to contribute to skin tumor development in humans. Several lines of research also point in this direction; UVA radiation is associated with the intracellular formation of oxygen radicals resulting in both cellular as well as genetic damage, e.g. DNA strand breaks and T–G

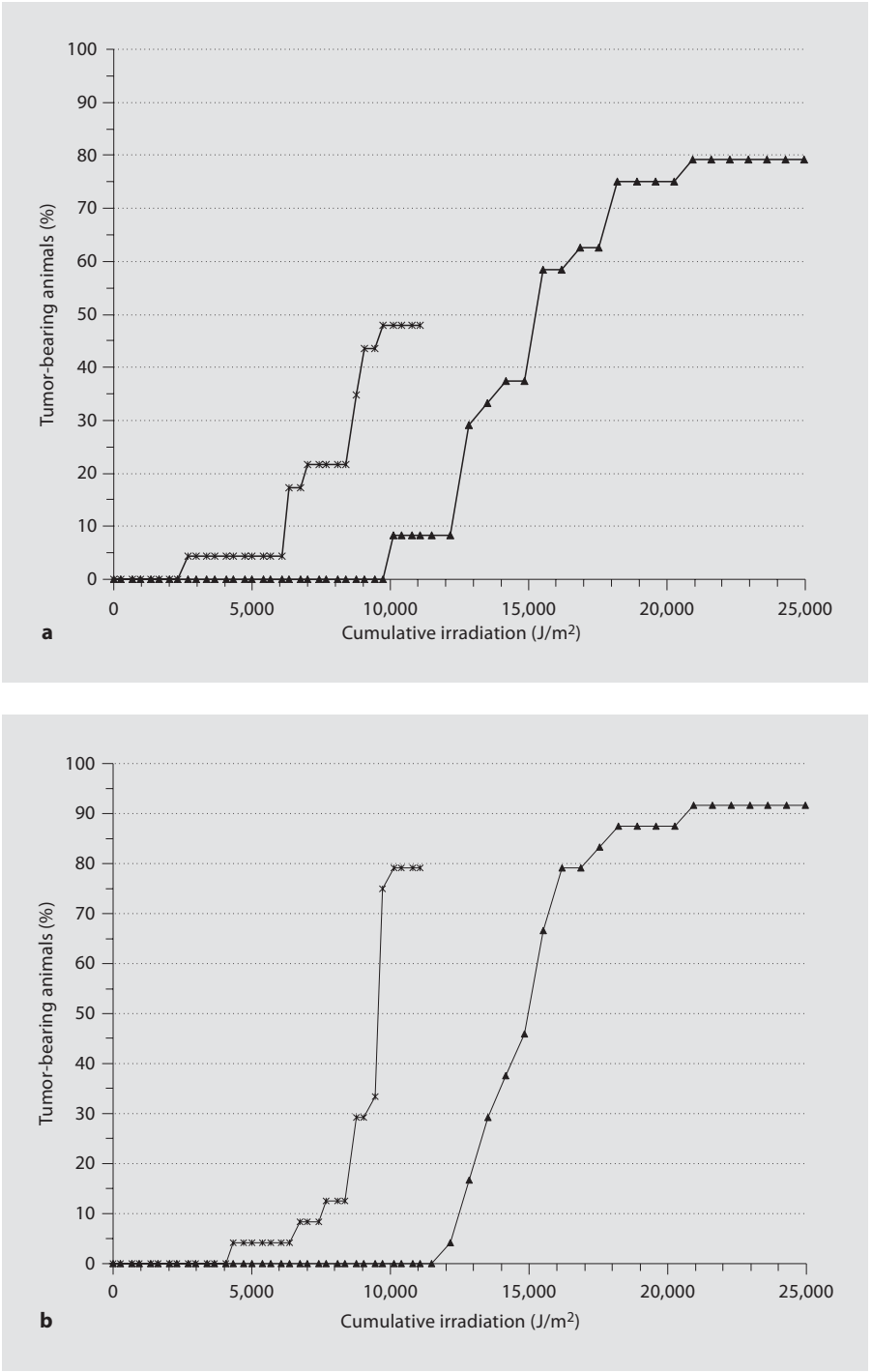

Fig. 6. Impact of 8-methoxypsoralen on skin tumors in hairless male (**a**) and female (**b**) mice. ▲ = Control + UV; * = 8-methoxypsoralen + UV.

transitions in the p53 gene [5, 37]. An additional aspect, which is likely to be involved in UVR enhancement of skin carcinogenesis, and particularly melanoma development, is its induction of immunosuppression [38]. Consequently, it is likely that the combination of several properties of UVA radiation (tumor promotion – induced by cellular damage and protein kinase C induction [39], immunosuppression and a potentially weak genotoxic effect, most likely related to oxidative DNA damage) is the cause of skin tumor development. The particular impor-

tance of oxidative stress in UVR-induced skin carcinogenesis has been demonstrated [40]. In this study, UVR-treated hairless mice were given either control or lutein/zeaxanthin-enriched diets. The carotenoids lutein and zeaxanthin are structurally related to β-carotene and have superior antioxidant properties. β-Carotene and vitamin C have demonstrated their antioxidative properties in chemoprevention trials. The results of the UVR studies showed a increased tumor-free survival time, an increased T_{50} value (approximately 17 vs. 20 weeks) and reduced tumor multiplicity and tumor volume in animals receiving the lutein/zeaxanthin-enriched diets [41]. Moreover, Fourtanier [20] reported inhibition of photocarcinogenesis in hairless mice by topical application of the broad UVA absorber Mexoryl SX, whereas the inhibitory effects of the UVB absorber 2-EHMC (2-ethylhexyl-p-methoxycinnamate) were found to be less marked. Ananthaswamy and colleagues [42] reported inhibition of solar simulation-induced p53 mutations and protection against skin cancer development in C3H mice by sunscreens with human sun protection factors 15–22 using formulations with UVB absorbers only, as well as those containing UVA and UVB absorbers. These authors inferred that blocking out UVA did not provide increased photoprotection against p53 mutations or skin cancer development. However, their data showed that it may have been difficult to estimate the added protective effect of UVA sunscreens because of the small number of affected animals.

In conclusion, the results of the present study indicate that the UVB filter Uvinul T 150 provides protection against UVR-induced skin cancer, but also that a combination of UVA and UVB absorbers merits further investigation.

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