The Effect of Short-term Application of PABA on Photocarcinogenesis

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Photocarcinogenesis was induced in 90 lightly-pigmented hairless mice using a Philips T140 W/12 light source which emits mainly UVB (290–320 nm). During one-third of the induction period (weeks 16–26) a group of 30 mice were protected by topical para-aminobenzoic acid (PABA) and then irradiated again without protection up to week 30 and observed for a further 10 weeks. The application of PABA resulted in a significant delay (p < 0.05) in tumour induction and discontinuation of PABA caused an abrupt decline in the number of tumour-free animals. At the end of the study there was a significant difference in the yield of carcinomas for the PABA group, 20, compared with 78 for non-protected mice (p < 0.05). There was also a statistically significant difference (p < 0.05) between the weight of dorsal skin in non-protected mice compared with the PABA-protected group, the latter showing no difference from a control group of non-irradiated mice. The proportion of benign tumours in the PABA group was significantly (p < 0.05) higher than in the non-protected group, suggesting an inhibition of the photocarcinogenic process. Key words: Sunscreen; Ultraviolet carcinogenesis; Ultraviolet radiation; Mouse skin.

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When applied regularly during the UV exposure period, sunscreens have been shown to protect against carcinogenesis in several studies on hairless mice (1, 2, 3). Most people, however, use sunscreens intermittently and some parts of the body (especially the face, hands and legs) may become heavily exposed to ultraviolet radiation (UVR) prior to the application of a sunscreen.

Photocarcinogenesis in Man and mice is considered to be a dose-dependent process (4, 5, 6). In Man, the dose–effect relationship of solar radiation and the total incidence, I, of non-melanoma skin cancer has been expressed by \( I \sim D^r \), where \( D \) denotes the erythemogenic effective dose per year in a human population in the United States. The constant \( r \) has been calculated to be 2 (4). Likewise, in mice the development of tumours follows the equation \( t_m \sim D^r \), where \( t_m \) is the interval between the first UV exposure and the moment when half of the animals have one or more tumours, \( \sim \) means proportionality, \( D \) stands for the daily dose of UVR and \( r \) is a constant calculated to be \(-0.5\) (3) and \(-0.6\) (6).

In the present study we have tested the inhibiting effect of short-term application of PABA on tumorigenesis in mice, resembling the pattern of usage in humans.

MATERIAL AND METHODS
Female hairless (H/H) mice (Bomholtgaard, Denmark) were 8 to 12 weeks old when entering the experiment. They were fed on standard laboratory feed (Ewos, Sweden) and had free access to water. 180 mice were randomized to six groups of 30 mice each. Five animals were housed in each cage, an 6 cages constituted a group. The treatment schedule of the six groups is described in Table I and one group (C) was treated from week 16 to week 26 in order to observe the effect of short-term application. The sunscreen used was a solution of 5% PABA (Mercador, FRG) in a vehicle consisting of 70% ethanol and 5% glycerol in water.

Immediately after having been painted, the animals were irradiated in their cages with UVR from a Philips T140 W/12 light source. The emission spectrum of this sunlamp and the absorption spectrum of PABA have been determined previously (7).

At a distance of 70 cm the intensity of UVB was 0.86 mW/cm² and of UVA (320–400 nm) 0.1 mW/cm², as measured with an Oertel UV meter (Centra). Due to a 30% lower intensity of radiation at the ends of the lamp compared with the middle part, the cages in each group were rotated before each daily treatment.

The mice were exposed to the light source in a regimen of escalating exposure. According to a prior examination the minimum erythema dose (MED) of our mouse strain has been calculated to be 175 mJ/cm² (3), and we therefore started with a suberythemal dose of 155 mJ/cm² corresponding to 3 min of irradiation. We increased the dose by 25–30% every second week up to a constant dose of 360 mJ/cm², corresponding to 7 min of irradiation. The mice were irradiated 5 days per week for 30 weeks and then observed for 10 weeks. The total UV dose was 49 J/cm² UVB.

The mice were examined for skin tumours once a week and a skin tumour was defined as a papule \( \geq 1 \times 1 \times 1 \text{ mm} \). At the end of the study the dorsal skin was carefully removed and weighed. All skin tumours were biopsied and all mice autopsied. The right femoral lymph node was biopsied from all mice bearing skin tumours. Light microscopy of routine haematoxylin and eosin stained sections was performed and the
changes were classified into three classes, defined as follows, Class I: Hyperplasia without atypia of the cell nuclei; Class II: Atypical hyperplasia that may include flat but usually were verrucous, papillomatous, or keratoacanthoma-like lesions and Class III: Squamous cell carcinomas with an indisputable stromal invasion.

Statistical methods

The results are expressed either as medians with 95% confidence intervals or means with 95% intervals. The confidence interval for medians was constructed by using the Berrioulli-Wilcoxon procedure (8) and for means by Student's t test. Differences were considered statistically significant when the p-values were less than or equal to a level of 5%. The Kruskal-Wallis test (8) and categorized data analysis were used for comparison of the groups. Time until event was analyzed by using the Kaplan & Meier method (9). Gehan's test (10) was applied for comparison of the groups.

RESULTS

The survival of the groups ranged from 93% to 100% without statistical deviations.

The institution of PABA treatment in week 16 resulted in an increase in tumour induction time which was significant (p < 0.05) following 10 weeks of irradiation (Fig. 1).

While a few tumours less than 2 mm disappeared during the PABA treatment period, tumour induction continued steadily (Fig. 1). The skin pigmentation faded somewhat but the mice did not develop any sunburn reaction when PABA protection was stopped (week 26). Ten animals were tumour-free at the end of the study and this was significantly (p < 0.05) more than in the UV exposed non-protected groups where no animals were found to be tumour-free.

Eleven short-term PABA protected mice (C) had between 1 and 3 class III tumours (squamous cell carcinomas) each, or a total of 20. Twelve animals had between one and four class II tumours, or a total of 18; and 7 animals had one class I tumour each. In contrast to this, 25 animals in the non-PABA group treated with UVR (B) had between 1 and 5 class III tumours each or a total of 78 tumours and 28 animals treated with UVR and the vehicle (E) developed between 1 and 6 class III tumours each, or a total of 75. The yield of class III tumours was significantly (p < 0.05) greater in the non-protected groups (B + E) than in the short-term PABA-treated group (C). No tumours developed in any of the non-irradiated control groups.

The ratios between tumours registered as class I and class III were 7/45 and 7/130 for part-time protected mice (C) and UVR-irradiated non-protected mice (B), respectively. This difference was significant (p < 0.05) (Table II). The use of a vehicle in group E did not influence tumourgenesis of class I, II or III tumours (Table II) or the tumour induction time (Fig. 1) when compared with group B.

![Fig. 1. The probability of tumour-free animals for six groups of hairless mice receiving no treatment (Group A), UVR (Group B), part-time PABA and UVR (Group C), PABA (Group D), vehicle and UVR (Group E) and vehicle (Group F).](image)

![Fig. 2. The mean weight and confidence limits of the dorsal skin of six groups of hairless mice. The shaded area shows the group that received part-time PABA and UVR (Group C). Control (Group A), UVR (Group B), PABA (Group D), vehicle and UVR (Group E) and vehicle (Group F).](image)
Table II. The number of skin tumours in six groups (A–F) of mice according to histological classification

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<td>Class I</td>
<td>0</td>
<td>7</td>
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<td>0</td>
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<tr>
<td>Class II</td>
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<td>45</td>
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<td>Class III</td>
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<td>78</td>
<td>20</td>
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<td>75</td>
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<td>Total</td>
<td>0</td>
<td>130</td>
<td>45</td>
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* The skin tumours were classified as class I, II and III tumours. Class I tumours: Hyperplasia without atypia; class II: atypical hyperplasia; class III: invasive squamous cell carcinoma.

The weighing of the dorsal skin of the animals showed a significantly (p < 0.05) heavier mean weight for UVR-exposed non-protected mice (B + E) than for short-term protected mice (C) and the latter did not differ significantly from non-irradiated controls (A + D + F) which failed to develop any tumours (Fig. 2).

All skin tumours were squamous cell carcinomas. No metastases were found.

DISCUSSION

The institution of PABA protection in week 16 led to a sudden decline in the daily ultraviolet dose penetrating the skin of the mice and we conclude that the delay in tumour induction time is linked to this (5, 6).

The propensity for the development of UV induced skin cancer in PABA-protected mice did not alter. When PABA treatment was stopped, they developed tumours in a similar manner to previously non-protected animals and the time-lapse remained unchanged during the UV exposure period.

Epidermal proliferation which gradually becomes papillomatous and is accompanied by cellular atypia leading to invasive carcinomas is the sequence of skin changes seen in mouse skin exposed to UV irradiation (11). We have observed a significantly greater promotion of benign tumours in the short-term PABA-protected group than in the non-protected group, a finding which suggests that PABA can inhibit the progression of skin tumours towards invasive carcinomas.

Some vehicles can exacerbate the phototumorigenic effect of UVR (12), thus reducing the tumour retardant effect of various sunscreens. However, we were unable to show any tumour repellent effect due to our vehicle. We did observe that the vehicle-treated group pigmented more intensively during the first months of irradiation compared with non-treated groups and at the end of the study the mean weight of the dorsal skin samples from this group was greater than in the non-treated group, but the difference was not statistically significant.

Actinic keratoses may appear on heavily UV-exposed skin of Caucasians and may proceed to invasive squamous cell carcinoma. We conclude that sunscreens delay the development of skin malignancies both in normal human skin and in skin with premalignant changes, even if only used intermittently.

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REFERENCES


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Hydrogen Peroxide Cream for the Prevention of White Pressure Areas in UVA Sunbeds

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A single application of 1% hydrogen peroxide in a stabilized lipid cream during 2 min before UVA exposure prevents white spots in anoxic pressure areas in sunbed use, causing an almost normal pigmentation. During maintenance exposure with UVA once a week this pigmentation will remain unchanged if the pressure areas are pretreated with hydrogen peroxide before each irradiation. White spots will appear 3–4 weeks after finishing hydrogen peroxide pretreatment in the pressure areas thus exposed to UVA alone.

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Pressure areas on the back (scapular region and medial sacral region) remain unpigmented on the reclining subject upon exposure to UVA from sunbeds, probably due to a lack of oxygen (1, 2). It has earlier been demonstrated that the application of a stabilized hydrogen peroxide cream prior to UVA exposure could induce delayed tanning on the pressure areas, while the placebo-treated contralateral sites remained unpigmented (3). This finding provides evidence for the role of oxygen in delayed pigmentation by UVA (4). The hydrogen peroxide cream was used ad libitum with repeated applications during a half hour period before exposure to UVA (3).

The aim of the present study was to evaluate the pigment promoting effect of a single application of the cream a short time before exposure to UV light and to study the pigmentation during maintenance treatment with UVA with and without hydrogen peroxide.

MATERIAL AND METHODS

Twenty-one healthy individuals, 18 men and 3 women, in the age range 23–35 years, who tan easily in the summer sun (skin types III and IV), were selected for the study.

Light exposure

A Philips sunbed was used (Phillips TL 85 W/09 T) with an emission spectrum of 310–420 nm and a peak emission at 355 nm. The skin was exposed for 30 min per day, 4–5 times per week (6–12 exposures in all during the initial treatment phase) and then during the maintenance treatment phase once a week.

Cremes

The two active creams contained 2% and 1% hydrogen peroxide in a stabilized lipid cream base of 21% mononystan, 7% monolaunin and 70% water. The two creams used in series II were not identifiable by appearance or smell and were supplied in identical tubes with a code sign and marked “left” and “right”.

“Treatment”

Series I: Comparison 2 and 5 min application of 2% H₂O₂ cream. The subjects were irradiated with UVA from below while reclining fairly still on the hard acrylic surface of the sunbed. Before each sunbed session the 2% H₂O₂ cream was applied to symmetrical areas known to remain hypopigmented after exposure to UVA on sunbeds (scapular areas and sacro-gluteal areas) during periods of 5 min and 2 min before irradiation, randomly on the left and right sides. The cream was washed off with lukewarm water just before light exposure.

Series II: Comparison 2% and 1% H₂O₂ cream. In this study 2% and 1% hydrogen peroxide creams were applied with a double-blind technique on the selected symmetrical areas (scapular and sacro-gluteal areas) 2 min before irradiation. The cream was washed off just before light exposure.

Series III: Maintenance treatment. After the provocation of delayed tanning with H₂O₂ and UVA on the pressure sites, maintenance treatment followed once a week. Before each

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