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 Tl 40 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 amimals. 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 photocarcinogenesis 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.

Photocarcinogensis 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 (5) 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 (Hr/Hr) mice (Bomholdsgaard, 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 (Merck®, 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 Tl 40W/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/cm^2 and of UVA (320–400 nm) 0.1 mW/cm^2, as measured with an Osram UV meter (Centra®). Due to a 30% lower intensity of radiation at the ends of the lamps 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/cm^2 (3), and we therefore started with a suberythemal dose of 155 mJ/cm^2 corresponding to 3 min of irradiation. We increased the dose by 25–30% every second week up to a constant dose of 360 mJ/cm^2, 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/cm^2 UVB.

The mice were examined for skin tumours once a week and a skin tumour was defined as a papule $\geq 1 \times 1 \times 1$ 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

Table I. *Treatment schedule for six groups of 30 mice*

Group	Local treatment	UV irradiation
A	–	–
B	–	+
C	PABA week 16–26	+
D	PABA week 1–30	–
E	Vehicle 1–30	+
F	Vehicle 1–30	–

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 keratoaceanthoma-like lesions and Class III: Squamous cell carcinomas with an indisputable stromal invation.

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 Bernoulli-Wilcoxon procedure (8) and for means by Student's 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 analysed by using the Kaplun & 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 UVR 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.

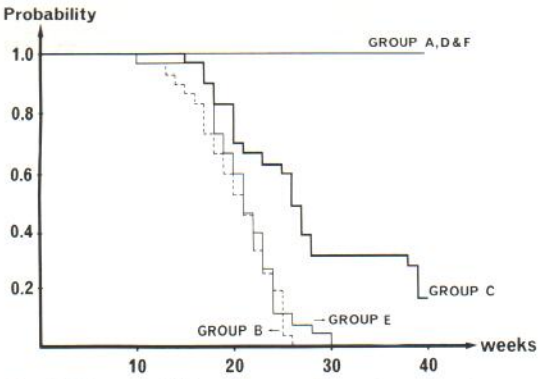

Fig. 1. The probability of tumour-free animals for six groups of hairless mice receiving no treatment (Group A), UVR (Group B), part-time PABA and UVR (Group C), PABA (Group D), vehicle and UVR (Group E) and vehicle (Group F).

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 tumourigenesis of class I, II or III tumours (Table II) or the tumour induction time (Fig. 1) when compared with group B.

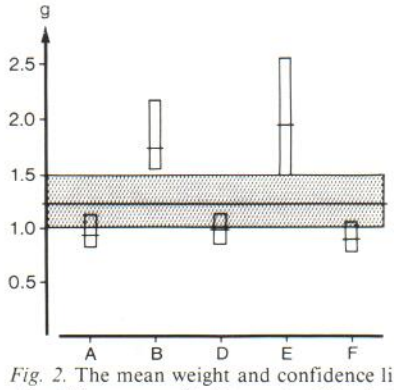

Fig. 2. The mean weight and confidence limits of the dorsal skin of six groups of hairless mice. The shaded area shows the group that received part-time PABA and UVB (Group C). Control (Group A), UVR (Group B), PABA (Group D), vehicle and UVR (Group E) and vehicle (Group F).

Table II. *The number of skin tumours in six groups (A–F) of mice according to histological classification*

Histological classification	Group of mice					
	A Control	B UVR	C UVR/PABA	D PABA	E UVR/Veh.	F Veh.
Class I	0	7	7	0	2	0
Class II	0	45	18	0	35	0
Class III	0	78	20	0	75	0
Total	0	130	45	0	112	0

[a] 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 signficantly ($p<0.05$) 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 proportion 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 phototumourigenic effect of UVR (12), thus reducing the tumour-retarding effect of various sunscreens. However, we were unable to show any tumourigenic effect due to our vehicle. We did observe that the vehicle-treated group pigmentated 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 intermittantly.

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