ORIGINAL ARTICLE

Photocarcinogenesis is retarded by a partly photodegraded solution of para-aminobenzoic acid

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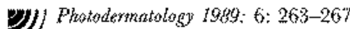
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A solution of para-aminobenzoic acid (PABA) was exposed to ultraviolet (UV) radiation emitted from a Philips TL 40 W/12 sunlamp and the degree of photodegradation following an exposure of 27 J/cm^2 was estimated to be approximately 40%. The formation of the photoproducts was confirmed by mass spectroscopy and UV spectroscopy. The solution was painted on the backs of hairless light-pigmented mice prior to daily UV irradiation by the above sunlamp, and this procedure was continued for 30 weeks. The preirradiated solution of PABA significantly retarded the tumor induction time and reduced significantly the number of squamous cell carcinomas compared with nonprotected controls. This tumor-retarding ability did not differ significantly from the effect achieved when using nonirradiated PABA.

Key words: PABA – sunscreen agent – photocarcinogenesis – photodegradation.

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The electromagnetic energy absorbed by chemicals may be dissipated in the formation of photoproducts by the interaction of the excited molecule with another molecule. Each quantum of energy activates one molecule in the excited state (1). Sunscreens absorb ultraviolet (UV) energy in specific wavelength ranges (2). The structure of the photoproduct of para-aminobenzoic acid (PABA) has recently been identified as *cis*- and *trans*-diazobenzoic acid (DABA) (3), and the absorption spectrum of DABA has been isolated and its rate of formation has been calculated in an artificial UV model (2).

We previously tested the ability of PABA to retard photocarcinogenesis induced by a Philips TL 40/12 sunlamp in hairless mice (4). However, the daily dose of UV radiation (UVR) that has been applied in this and in similar animal studies has not exceeded about one half of the maximum available erythemal exposure during a full sunny day (Canary Islands at 28°N) (4–7). A higher degree of sunscreen photodegradation may therefore occur under outdoor conditions than in laboratory studies on mice, and humans may be exposed to a higher lifetime cumulative dose of photoproducts than are laboratory mice.

Table 1. Treatment schedule for 8 groups of mice

Group	Local treatment	UV irradiation
A	–	–
B	–	+
C	PABA	+
D	PABA	–
E	Vehicle	+
F	Vehicle	–
G	Preirradated PABA	–
H	Preirradated PABA	+

In this study we examined the ability of a partly photodegraded solution of PABA to retard photocarcinogenesis in hairless mice exposed to UVR from a sunlamp that mainly emits UVB (290–320 nm).

Material and methods

Photochemical methods

PABA was obtained from Merck (FRG). A 5% PABA solution in a vehicle composed of 70% ethanol and 5% glycerol in water was exposed to UVR from a Philips TL 40W/ 12 light source (emission spectrum in (4)). The solution was kept in a petri dish, continously stirred by a magnet, and covered by a tight-fitting polyethylene film absorbing approximately 8% of the UV dose. The UV intensity was measured with an Osram UV meter (Sentra) and the solution was exposed to a dose of 27 J/cm^2 of UVB (290–320 nm).

The percentage of photolysis products in the irradiated solution was estimated assuming that: (i) all photons from the light source were absorbed by the solution; (ii) PABA and the photoproducts have nearly identical UV spectra; and (iii) the photodegradation of PABA is according to 2 PABA $\rightarrow$ photoproduct. This leads to the following differential equation for degradation of PABA:

$$d(S)/\,dt = -\,k\,I\,(S)^2/((S) + (P))$$

where k is the quantum efficiency of the photodegradation of the sunscreen, S, and I the irradiation intensity, t the time and P the photoproduct. The above equation can be solved to show that approximately 40% PABA is photodegraded under the experimental conditions given. For comparison, the application of an equal dose leads to a 50% photodegradation of a 10^{-5} M solution of PABA (2).

The first assumption leading to the above equation is clearly valid, as the peak absorbance of a 10^{-5} M PABA solution is about 1.5 (2); the second is obviously not fulfilled. However, the UV spectra of PABA and the photoproduct (azodibenzoic acid (3)) are very similar in terms of shape and peak absorbance (2). The third assumption is in accordance with the findings of Gasparro (3) as well as with mass spectroscopic data obtained in our laboratory.

Animal study

Female hairless (hr/hr) mice (Bomholdsgaard, Denmark) were 8–12 weeks old when they entered the experiments. They were fed standard laboratory food (Ewos®, Sweden) and had free access to water.

The light source was a Philips TL 40 W/12 lamp (emission spectrum (4)). The intensity of UVR was measured with a Osram UV meter (Sentra) and was found to be 0.86 mW/cm^2 (UVB) (290-320 nm) and 0.01 mW/cm^2 (UVA) (320-400 nm) at a distance of 70 cm.

The MED of the laboratory mice has been shown previously to be 175 mJ/cm^2 (150-200) (4), using the same sunlamp.

A total of 240 mice were randomized into 8 groups of 30 mice each. The groups consisted of 6 cages with 5 mice housed in each. They were treated as shown in Table 1. The sunscreen was painted at the backs of the animals and they were immediately irradiated. The cages were rotated before each daily treatment in order to compensate for a 30% lower intensity at the ends of the lamps compared with the middle part and for differences in penetration time of the topical agent before irradiation.

The mice were exposed to the light source in a regimen of escalating exposure. The starting dose was suberythemal (155 mJ/cm^2) and increased by 25–30% every second week during the first 8 weeks and then remained constant at 360 mJ/cm^2 corresponding to an increase in time

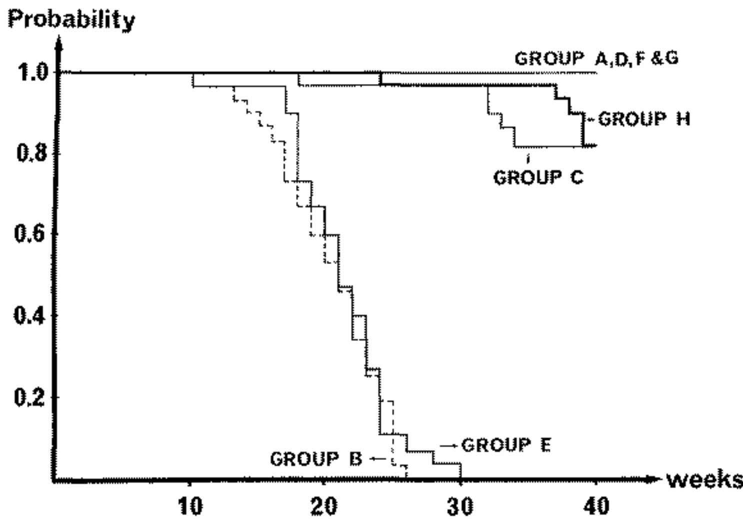

Fig. 1. The probability of tumor-free animals in 8 groups of hairless mice receiving no treatment (A), UVR (B), PABA and UVR (C), PABA (D), vehicle and UVR (E), vehicle (F), preirradiated PABA (G) and UVR and preirradiated PABA (H).

of irradiation from 3 min to 7 min. The total dose was about 49 J/cm^2.

The animals were irradiated once daily for 30 weeks 5 days per week and then observed for 10 weeks. Each animal was examined for tumor development once a week and a tumor was defined as a papule $\geq 1\times1\times1$ mm. At the end of the study the dorsal skin was removed and weighed to evaluate the tumor burden and the tumors were studied histologically by light microscopy and classified as described elsewhere (4).

Statistical methods

The results are expressed either as medians with 95% confidence interval or means with 95% intervals. The confidence intervals for medians were constructed by using the Bernoulli-Wilcoxon procedure (8) and for means by Student's t-test. Based on a priori knowledge all tests were carried out one-tailed and differences were considered statistically significant when $P \leq 0.05$. The Kruskal-Wallis test (8) and categorized data analysis were used to compare the groups. Time until event was analyzed by using the Kaplun & Meier method (9). Gehan's test was applied to compare the groups (10).

Results

A few animals died during the study. The percentage of survivors according to their groups were: A: 100%, B: 93%, C: 83%, D: 97%, E: 93%, F: 93%, G: 93% and H: 93%. The death rate did not differ significantly between the groups.

No animals in nonirradiated groups (A, D, F, G) developed tumors (Fig. 1). All animals in the UVR-exposed group (B) and the UVR-exposed vehicle-treated group (E) developed tumors and these 2 groups did not differ significantly in terms of tumor induction time. The application of a preirradiated solution of PABA in group H resulted in a delay in tumor induction time compared with nonprotected groups (B+E) ($P<0.05$), but did not differ from the group treated with nonirradiated PABA (C).

Five animals protected with preirradiated PABA before exposure to UVR (group H) devel-

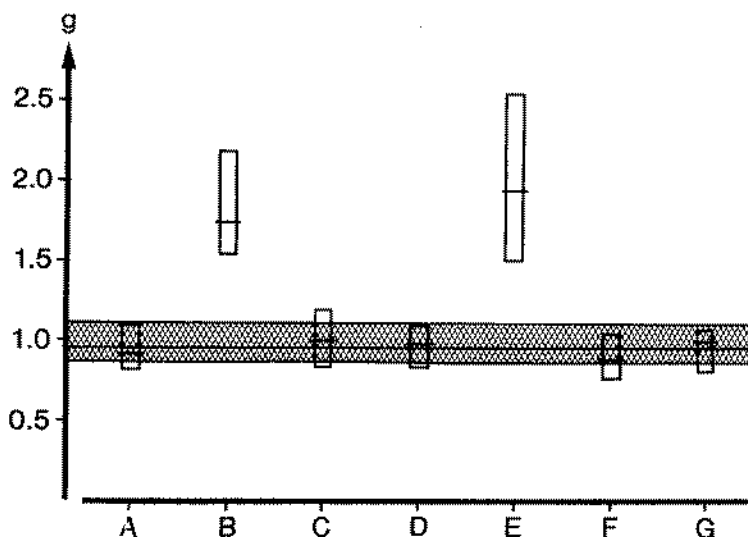

Fig. 2. The mean weight of the dorsal skin of 8 groups of mice receiving no treatment (A), UVR (B) , PABA and UVR (C), PABA (D), vehicle and UVR (E), vehicle (F) and preirradiated PABA (G). The shaded area shows the confidence interval of the mean weight of group H treated with preirradiated PABA and UVR.

oped 1 papilloma each and no animals developed squamous cell carcinomas. In the group treated with nonirradiated PABA before exposure to UVR (C), 3 mice developed 1 papilloma each and a single squamous cell carcinoma. These 2 groups did not differ significantly in terms of carcinoma yield and both groups had a lower carcinoma yield compared with UVR-exposed nonprotected controls (B+E), which developed 78 and 75 squamous cell carcinomas, respectively ($P < 0.05$).

The weighing of the dorsal skin of the animals (Fig. 2) showed a lighter mean weight for groups protected by PABA (C) and preirradiated PABA (H) than for UVR-exposed nonprotected mice (B+E) ($P < 0.05$). The group protected by preirradiated PABA (H) did not differ from the group protected with nonpreirradiated PABA (C) , or nonirradiated controls (A, D, F, G).

All cancers were registered as definite squamous cell carcinomas. No basocellular carcinomas or malignant melanomas were found. No metastases were identified.

Discussion

We have previously depicted the absorption spectra of PABA and DABA (2). Both compounds absorb strongly in the UVB range (290–320 nm). In addition, DABA was shown to absorb weakly in the UVA range (320–400 nm). However, only trace amounts of DABA are formed under outdoor conditions compared with the maximum available UV doses measured at near equatorial zones (7).

The preirradiation procedure used in this study differs from normal conditions on the skin in terms of the chemical milieu, and the photoexcited PABA molecule may therefore form photoproducts on the skin different from those formed under the experimental conditions. Likewise, various vehicles (at least in theory) may form other photoproducts that can interfere with the photoactivated active ingredient. Further studies might therefore be required if PABA had been dissolved in another vehicle. However, our initial UV spectroscopic studies of the photodegradation of PABA revealed no differences between the spectra obtained from a PABA and

water solution and an ethanolic solution with or without glycerol (unpublished data).

The irradiance level used to photodegrade the solution of PABA was extremely high (7) and was chosen to illustrate the tumor-retarding effect of an exceedingly photodegraded solution.

Certain chemicals may become co-carcinogenic together with UVR, either as sensitizers (11), additively (12) or as promoters (13). Any topical chemical preparation used in connection with prolonged and intensive UV exposure, including its photoproducts, should therefore be carefully tested in order to exclude a carcinogenic or co-carcinogenic effect. Sunscreens are examples of such topical preparations, and although they have inhibited photocarcinogenesis in various animal studies (4–6), the effects on carcinogenesis by a cumulative exposure to their photoproducts might not always be inhibitory. The UVB (290–320 nm) model used here failed to show any carcinogenic side effect of DABA at a 40% concentration. From the absorption spectra of DABA, with a peak in the UVB (2), one would expect the maximum photodynamic action to be found in this range. However, the sunlamp used in this study emits very little UVA and a photodynamic effect here cannot be excluded from our experiments.

We conclude that an exceedingly photodegraded solution of PABA significantly retards the tumorigenesis induced by an artificial sunlamp in hairless mice and recommend that other sunscreen agents be tested in a similar way.

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