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Experimental Photoallergic Contact Dermatitis to Bisphenol A

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A single clinical report describes photoallergic contact dermatitis to bisphenol A, with subsequent persistent light reactivity, in a group of eight outdoor workers. In a mouse model we have induced photoallergic contact dermatitis to bisphenol A; sites of photochallenge reactions flared when later tested with UVA alone. Attempts to induce photoallergy to bisphenol A in guinea pigs failed. (Received October 24, 1987.)

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Bisphenol A, a common chemical in the plastics industry, is a component of most epoxy resins (1). Despite its wide use, bisphenol A is a rare sensitizer, although bisphenol A may serve as a cross-reacting allergen, the primary sensitivity being induced by another substance (2–5). In the Magnusson-Kligman Guinea Pig Maximization Test, bisphenol A has been reported to be a non-allergen (6). Our interest in bisphenol A derived from a clinical report by Allen & Kaidbey of 8 cases of severe, extensive photoallergic contact dermatitis that occurred in workers doing outdoor pipe repair with an epoxy resin (7). The photoallergen appeared to be bisphenol A. Photo-patch tests were positive for bisphenol A, classical patch tests for conventional contact allergy to bisphenol A were negative. In these workers, the photoreactivity did not disappear when the exposure to bisphenol A ceased; in all 8 men, ultraviolet light continued to induce a disabling dermatitis where there had been a previous eruption. These workers were persistent light reactors.

Several years ago, Maguire & Kaidbey and, independently, Takigawa & Miyachi described a mouse model for photoallergic contact dermatitis (8, 9). We have used this model to explore the capability of bisphenol A to induce photoallergic contact dermatitis and persistent light reactivity. Bisphenol A readily photosensitizes mice of a variety of different strains (all strains examined); however, to date, we have been unable to induce photoallergic contact dermatitis to bisphenol A in the guinea pig.

MATERIALS AND METHODS

Chemicals

Separate lots of bisphenol A (4,4-isopropylidenediphenol) were purchased from Eastman Kodak (Rochester, NY) and from Sigma (St. Louis, Mo.). Absorption spectroscopy of bisphenol A in 95% ethanol gave identical readings for the two samples. Peak absorption was at about 285 nm; there was major absorption in the UVB, with only a small absorption in the UVA and negligible absorption above 400 nm. For sensitization and challenge, we used a vehicle consisting of 4 : 1 acetone : corn oil. Cyclophosphamide was purchased as a bulk powder from Sigma. Heat-killed *C. parvum* (*P. acnes*) organisms in saline at a stock concentration of 7 mg/ml was purchased from the Burroughs Wellcome Company (Triangle Park, NC).

Ultraviolet sources

Fluorescent tube UVA and UVB sources were, respectively, banks of fluorescent blacklight tubes (F-20 BL) and banks of FS-20 sunlamp fluorescent tubes, as described in reference 8.

Animals

At different times mice were purchased from the Institute for Cancer Research (Fox-Chase, Philadelphia, Pa.), the Jackson Laboratories (Bar Harbor, Maine), and Sprague Dawley Co. (Indianapolis, Indiana). The mice were fed Purina Mouse Chow and had access to acidified water (pH 3.0) 24 h a day. They were housed in a temperature-controlled, light-cycled room.

Photosensitization and photochallenge

The methods were similar to those previously described (8). Briefly, age-matched female mice were photosensitized to bisphenol A by the application to a clipped site on the rear flank of 0.02 ml of bisphenol A solution, followed by radiation of the site with UVB (0.1 J/cm^2) followed by UVA (11 J/cm^2). For the UV radiation, mice were anesthetized with intraperitoneal Nembutal® and then positioned with adhesive tape on a wooden board. Their ears were protected from UVB damage by the interposition of paper towelling between the UVB source and the ears. In some instances, immunological adjuvants were used, viz. cyclophosphamide, 50 mg/k IP, given 2 days prior to photosensitization, or *C. parvum* (*P. acnes*) 30 µg injected into or adjacent to the sensitization site on Day 1 (10). Five or more days after their first exposure the mice were photochallenged to bisphenol A in the following manner: baseline ear thickness was determined using a metal calipers bearing a tension-sensitive rachet. Then 0.01 ml of bisphenol A solution was applied to the left ear followed by UVA (11 J/cm^2) to both ears; after this the right ear was challenged with 0.01 ml of the same bisphenol A solution without further UVA. Measurements of ear thickness of both ears were taken at 24 and 48 h post-photochallenge.

Histopathology of challenge

After sacrifice, ear specimens were obtained and preserved in neutral buffered formalin. The specimens were processed to slides by routine procedures and stained with hematoxylin and eosin.

RESULTS

In a typical experiment, outlined in Table I, a group of 6 female ICR mice were photosensitized to 1% bisphenol A (bis-A) on days 0 and 1 and photochallenged with 1% bisphenol A on day 6. Measurements of ear thickness at 24 and 48 h showed positive reactions only in the mice (group I) that had been photosensitized to bisphenol A and at sites challenged with chemical followed by UVA irradiation. Lack of ear swelling in the toxicity group demonstrates the absence of phototoxicity of bisphenol A under the conditions of photochallenge. The lack of reactivity of the chemical applied to the right ear after UVA, in the group I photosensitized mice, demonstrates the absence of classical allergic contact dermatitis to bisphenol A in these mice. It has been our consistent finding that UVA has to be given after, not before, application of bisphenol A to the challenge site in order to obtain a positive result in bisphenol A photosensitized animals.

Table I. *Photosensitization with bisphenol-A*

				Day 7	
Group	Days 0, 1	Day 1	Day 6	L ear	R ear
I	bis-A UVR	*C. parvum*	*L ear:* bis-A, UVA	4.0[a]	0.2
II	–	–	*R ear:* UVA, bis-A	0.3	0.0

[a] Mean ↑ in ear thickness (mm×10^{-2}).
Left ear: I>II, $p<0.02$.

The left ears were excised from several mice of each group after the 24 h measurements. In specimens from the control group, histopathology showed no inflammation. In contrast, the 24 h reactions in mice of the experimental group showed edema and a mononuclear cellular infiltrate; the histopathology was similar to that of reactions of photoallergic contact dermatitis to other compounds in mice that we have examined as well as to classical allergic contact dermatitis in mice (8, 11).

Photosensitization to bisphenol A requires UVB

In several experiments we examined the requirement for UVB at the time of photosensitization. In a typical experiment, 6 female Swiss-Webster mice were photosensitized to bisphenol A utilizing cyclophosphamide (50 mg/k) and *C. parvum* immunopotentiation (Table II). A comparison was made between groups that presumptively were photosensitized using UVB plus UVA, UVA alone, and no ultraviolet light. All three groups, as well as a toxicity group, were photochallenged simultaneously on Day 6. Only those mice photosensitized with UVB plus UVA showed positive photochallenge reactions. This has been a reproducible finding and accords with the observation that the dominant absorption of bisphenol A is in the UVB spectrum.

Adoptive transfer of photoallergy to bisphenol A

Successful cellular transfer was obtained in female Balb/C×A/J mice. In one experiment, donor mice were injected with cyclophosphamide (50 mg/k) on day −2 and then photosensitized by the application of 5% bisphenol A to clipped sites on each dorsal quadrant on days 0 and 1, followed by UVB-UVA. In addition, on day 1, each of the four photosensitization sites was injected with 10 μg of *C. parvum* suspension. After sacrifice on day 4, regional lymph nodes were taken and a cell suspension made by cutting and teasing lymph node fragments in Hanks' balanced salt solution containing 5% fetal calf serum. Red blood cells were lysed by exposure for 90 s to distilled water followed by acute restoration of isotonicity by the addition of hypertonic saline. The white cells were washed in media three times, and a cell count made at that time. By trypan blue dye exclusion, the viability of the cells was over 90%. Recipient animals were injected by tail-vein, under diethyl ether anesthesia, with 50 million donor lymphoid cells. The recipients had been treated with 150 rad whole-body X-ray one day previously. Photochallenge (bis-A, UVA) was made about 1 h after cellular transfer. The mean increase in ear thickness ($mm \times 10^{-2}$) of the cell recipient mice was 5.3, while that of a toxicity control group of female Balb/C×A/J mice that was tested in parallel was 1.2; the difference was statistically significant ($p<0.05$).

Table II. *Photosensitization of mice to bisphenol A requires UVB*

					Day 7	
Group	Day −2	Days 0, 1	Day 1	Day 6	L ear	R ear
I	Cy	bis-A, UVB-UVA	*C. parvum*	*Left ear:* bis-A, UVA	5.3[a]	1.0
II	Cy	bis-A, *No* UVR	*C. parvum*	*Right ear:* bis-A only	1.5	0.5
III	Cy	bis-A, UVA only	*C. parvum*		1.1	0.3
IV	Cy	–	–		1.4	−0.3

[a] Mean ↑ in ear thickness ($mm \times 10^{-2}$).
Left ear: I>II, III or IV, $p<0.02$.

Rechallenge of mice with UVA alone

In order to provide a model for the photoreactivity shown by the patients of Allen & Kaidbey, we photosensitized and photochallenged mice to bisphenol A in the usual way, rested them for one week to allow the photochallenged ears to return to normal size and then rechallenged the mice with UVA alone (no further bisphenol A). The results of a typical experiment are outlined in Table III. UVA irradiation of previously positive challenge sites caused these old photoallergy test sites to flare, evidenced by increased ear thickness. Interestingly, the increase in ear thickness began within a few hours of UV exposure. For instance, in one experiment, ear swelling was clearly evident when the first measurement was taken 5 1/2 h after UVA challenge (data not shown). This is in contrast to classical photochallenge reactions (photoallergen, then UVA) in which inflammation develops much more slowly (8). Ear swelling was limited to previously positive photochallenge reactions, in particular, ears previously challenged by UVA *followed* by bisphenol A did not become thickened following UVA alone challenge. In control experiments (not shown) DNFB-sensitized mice, whose resolved challenged sites were similarly exposed to UVA, had no ear thickening.

DISCUSSION

We have found that photoallergic contact dermatitis—but not classical allergic contact dermatitis—can readily be induced in mice with bisphenol A. The histology of the photochallenge sites, and the cellular transfer of the bisphenol A hypersensitivity are entirely congruent with our previous studies on photoallergic contact dermatitis to other photoallergens, such as TCSA and chlorpromazine, in mice and imply that we are dealing with delayed-type hypersensitivity. Positive reactions to conventional photochallenge were seen at 24 and 48 h but not at 6 h (data not shown). These results confirm and extend the clinical finding implying that bisphenol A is a photosensitizer (7).

The guinea pig is the classical experimental animal for the study of photoallergy; several prospective tests for photoallergens in man have been developed that utilize the guinea pig (12, 13). Curiously, our best efforts (four experiments), using maximization methods successful with other photosensitizers failed to induce any photosensitization of the guinea pig to bisphenol A. What accounts for this disparity between the mouse and guinea pig, with reference to bisphenol A, is not apparent.

We challenged with UVA alone, mice that were previously sensitized and photochallenged with bisphenol A. Positive ear reactions were limited to challenge sites that previously had received bisphenol A, then UVA; control sites that had been challenged with UVA then bis A failed to respond. We hypothesize that the binding of the bisphenol A photoproduct in the photochallenge sites causes a concomitant binding of native bisphenol A in the site and that this residual bisphenol A is converted to allergic reactive photoproduct with subsequent exposure to UVA. Control studies with DNFB and other clinical contact allergens in the mouse exclude the likelihood that the persistent light reactivity represents a non-specific

Table III. *Rechallenge with UVA only of mice photosensitized and photochallenged with bis-A*

Group	Day −2	Days 0, 1	Day 1	Day 6	Day 13	Day 14
I	Cy (50 mg/k)	1% bis-A, UVB-UVA	*C. parvum*	*Left ear:* 1% bis-A, UVA	UVA *Left ear:*	+7.1[a]
II	Cy (50 mg/k)	–	–			+0.5

[a] Mean ↑ in ear thickness ($mm \times 10^{-2}$); I>II, $p<0.02$.

flare of a previously inflamed test site. In their patients with epoxy photoallergy and persistent light reactivity, Allen & Kaidbey observed that an old positive photopatch test to bisphenol A could be reactivated by exposure to UVA alone, even 6 months later. Interestingly, this flare in their patients occurred within hours of the exposure to UVA, just as in the UVA photoreactivation in our bisphenol A photosensitized mice. It is likely that in general, the binding of a photoallergen in areas of photoallergic contact dermatitis accounts for a significant subset of persistent light reactions.

In a recent study Peltonen et al. (14) describe the formation of free radicals when bisphenol A was UV irradiated. These radicals may react covalently with nearby macromolecules to form the ultimate antigen that is responsible for photoallergy to bisphenol A.

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