Photoallergy to Systemic Quinidine in the Mouse

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Using the mouse, photoallergy to the antiarrhythmic agent quinidine could be induced following systemic administration. After pretreatment with cyclophosphamide 150 mg/kg, groups of 5–10 mice were injected i.p. with quinidine chloride 100 mg/kg on 2 consecutive days, followed by exposure of shaved abdominal skin to UVB 0.1 J/cm^2 and UVA 5.0 J/cm^2. Five days later challenge was performed on the left ear and on the tail, using the same dose of quinidine and UVA 5.0 J/cm^2. The reaction was evaluated 24 h later by measuring the increase in ear thickness as well as the wet weight increase of ear and tail. Significant 24 h reactions could be measured using all three evaluation systems. Control animals treated according to the protocol, but not UV-exposed during induction, were negative, thus excluding a phototoxic reaction. The histology of the left ear at challenge showed a round cell infiltrate preferentially of the exposed outer face of the ear consistent with an immunologic reaction. The time course of the reaction showed a maximum at 24 h. Photosensitization to quinidine could be achieved with UVA alone during the induction phase. Quinidine photoallergy can be induced in the mouse after systemic adminstration, and the reaction measured both at ear and tail. These findings support the assumption that clinical photoreactions to quinidine may have an immunological basis. *Key words: Photosensitivity.* (Received June 25, 1987.)

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Photosensitivity following the systemic use of the cardiac antiarrhythmic agent quinidine, the *d*-isomer of quinine, has been reported by several authors (1–3). Evidence based on the clinical picture, histopathology and photo- or photopatchtesting, suggest an immunologic rather than a phototoxic mechanism. However, the basis for quinidine photosensitivity, whether allergic or toxic, has not yet been established due to lack of suitable experimental techniques for reproducing photoallergy to systemic drugs. Recently a method has been described whereby photoallergy to intraperitoneally administered sulfanilamide and chlorpromazine was induced in the mouse, including the successful adoptive transfer of the sensitivity with cells to naive recipient animals (4).

We have used a modification of this technique to study the capacity of quinidine to induce photoallergy after systemic administration.

MATERIAL AND METHODS

Mice

Female albino mice were purchased from Anticimex, Sollentuna, Sweden. All mice were housed in identical cages and had access to food and fresh water ad libitum. Their weight varied from 23–30 g. In each individual experiment mice delivered at the same time were used. The number of mice in each experimental group was 5–10.

Chemicals

Quinidine chloride was purchased from Sigma, St Louis, Mo., USA, and was used without further purification. Cyclophosphamide was purchased from Lääkefarmos, Turku, Finland.

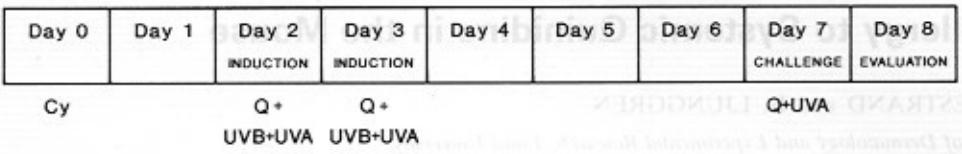


Fig. 1. Schematic illustration of basic experimental protocol.

Ultraviolet radiation

Medium wave radiation (UVB) was obtained from two fluorescent sunlamp tubes (Westinghouse Sunlamp FS 40, 40 W) with an emission peak at 312 nm. The output measured with a photometer (Waldmann AG, Schwenningen, GFR) was 0.7 mW/cm^2. Long-wave ultraviolet radiation (UVA) was obtained from two fluorescent blacklight tubes (Philips TLA 40 W/08) with an emission peak around 360 nm. The output as measured with a PUVA-meter (Waldmann) was 1.6 mW/cm^2. In some experiments a 3 mm window glass pane was inserted to exclude UV light below 320 nm.

Immunoadjuvant

In each experiment 150 mg/kg cyclophosphamide was injected intraperitoneally 2 days prior to photosensitization. Cyclophosphamide was dissolved in sterile normal saline to a total volume of 0.5 ml shortly before injection.

Photosensitization

For a schematic presentation of the photosensitization protocol, see Fig. 1. Mice were photosensitized to quinidine in the following manner. On day 0 150 mg/kg of cyclophosphamide dissolved in 0.5 ml water was given i.p. On day 2 approximately 4 cm^2 of ventral skin was shaved and quinidine (100 mg/kg) injected i.p. Topical contamination of the quinidine solution was carefully avoided. The mice were kept in the dark for one hour prior to UV exposure. After being anesthetized with pentothal sodium 80 mg/kg, the animals were placed in plastic tubes for fixation used in the mouse tail technique for phototoxicity evaluation (5). The mice were then exposed to UVB 0.1 J/cm^2 followed by UVA 5.0 J/cm^2 on the shaved ventral skin (Fig. 2 A). During the exposure the ears were shielded in the plastic tube. On day 3 the procedure was repeated but the mice were not reshaved. In the group of control mice designed to exclude phototoxicity, an identical procedure was followed, but these animals were not UV-irradiated. On day 4 the baseline ear thickness was measured with a micrometer (NSK Digital, Japan), while the animals were anesthetized with pentothal as above.

Photochallenge

Photosensitized and control mice were photochallenged on day 7 in the following manner: Quinidine (100 mg/kg) was injected i.p. and after one hour in the dark the mice were anesthetized with pentothal. During anesthesia the animals were placed in the plastic tubes with the left ear facing the UV-lamp (Fig. 2 B). The upside ear (left) was exposed to UVA 5.0 J/cm^2. This UV dose did not cause any inflammatory edema in the absence of quinidine. The right ear was carefully shielded from UV light. By using the fixation device according to the standard mouse tail technique the tail was then exposed to UVA for 5 h (29 J/cm^2).

Evaluation

Evaluation was made on day 8 with three different methods. Increased ear thickness was measured using the micrometer. The edematous reaction of the ear tissue was also measured as increased wet weight. After sacrifice, both ears were excised. The relative wet weight of each ear was then established by weighing the ear before and after drying in an oven at 110°C for one hour. The wet weight of the tails was established in a similar way by drying in the oven at 110°C for 3 h. In the time-course experiment the evaluation was made by measuring ear thickness up to 26 days after challenge.

Statistics

Student's *t*-test was used for the statistical analysis.

Histology

Histologic sections through the left, irradiated ear of photosensitized animals sacrificed at 48 h, were routinely processed and stained with hematoxylin-eosin. Sections of control ears were prepared in the same way.

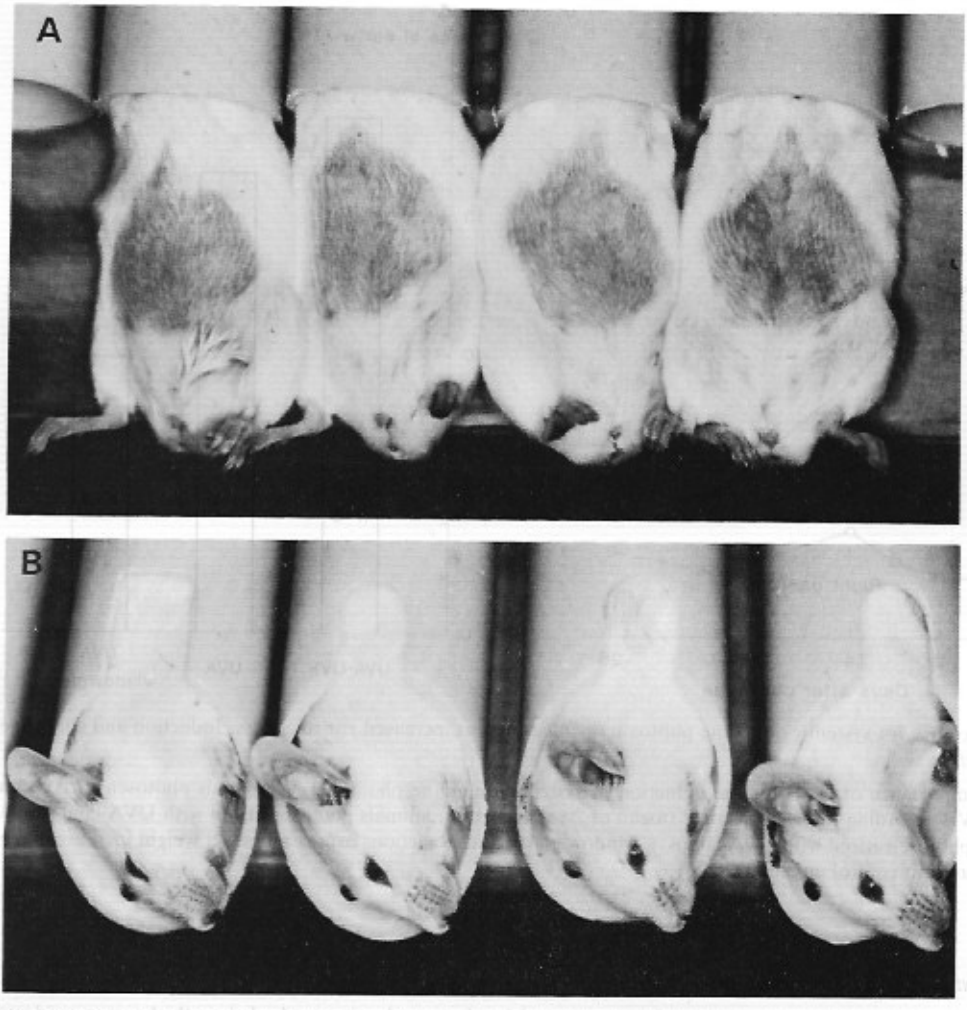


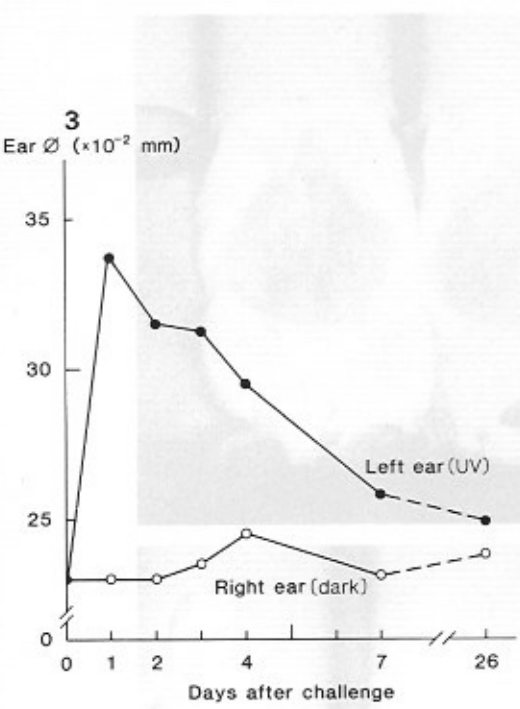
Fig. 2. Mice during induction irradiation (*A*) and during challenge irradiation (*B*).

RESULTS

The results of a typical experiment are shown in Table I. The induction- and challenge-doses of quinidine were 100 mg/kg. Compared with the control group there was a statistically significant ($p<0.001$) increase in left (UV-exposed) ear thickness, in left ear relative wet weight, and in tail relative wet weight in the photosensitized group. A phototoxic reaction could be excluded, since there was no reaction in the control mice, which were unexposed during induction, but were irradiated during challenge.

Time-course

To establish the time-course in quinidine photoallergy, ear thickness was measured at different intervals after challenge (Fig. 3). The strongest reaction (a 47% increase) was obtained 24 h after challenge. With increasing time after challenge a decline in ear thickness was noted, approaching the baseline after 26 days.



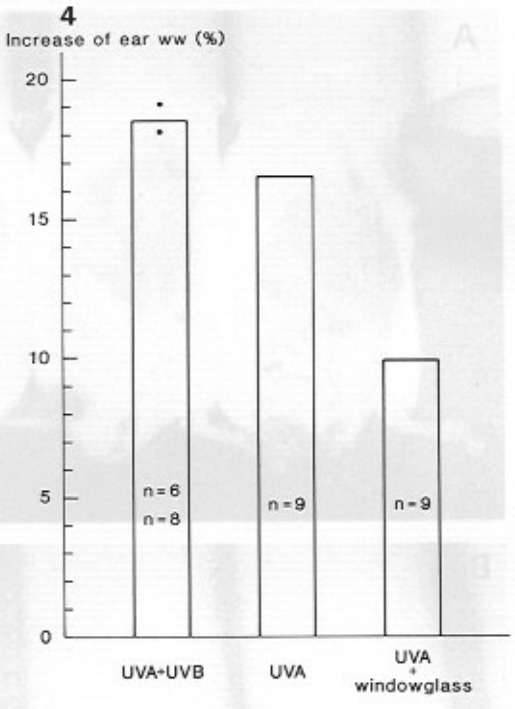


Fig. 3. Time course for systemic quinidine photoallergy measured as increased ear thickness. Induction and challenge doses 100 mg/kg.

Fig. 4. The importance of UVB for the induction of systemic quinidine photoallergy. Animals photosensitized with UVA and UVB according to basic protocol (mean of 2 experiments), animals photosensitized with UVA lamps only, and animals photosensitized with UVA lamps + window glass pane. Reactions expressed as wet weight increase of ear tissue, compared to control animals.

Table I. *Systemic quinidine photoallergy in the mouse*

Mean reactions, standard deviations and *p*-values vs. control for three evaluation methods (ear thickness, ear edema (ww) and tail edema (ww)) for photosensitized animals and phototoxicity controls

	Photosensitized group ($n=10$)				Control group ($n=10$)			
	Before challenge		After challenge		Before challenge		After challenge	
	Left	Right	Left	Right	Left	Right	Left	Right
Ear thickness								
mm×10^{-2} $\bar{x}$	23.0	22.9	32.5	23.8	22.3	22.2	23.7	23.5
SD	±0.62	±1.03	±2.98	±1.51	±0.75	0.71	±0.94	±0.83
p			<0.001					
ww ears								
% $\bar{x}$			66.3	58.8			57.3	57.2
SD			±1.97	±1.76			±1.90	±2.44
p			<0.001					
ww tails								
% $\bar{x}$			58.6				52.4	
SD			±1.89				±1.05	
p			<0.001					

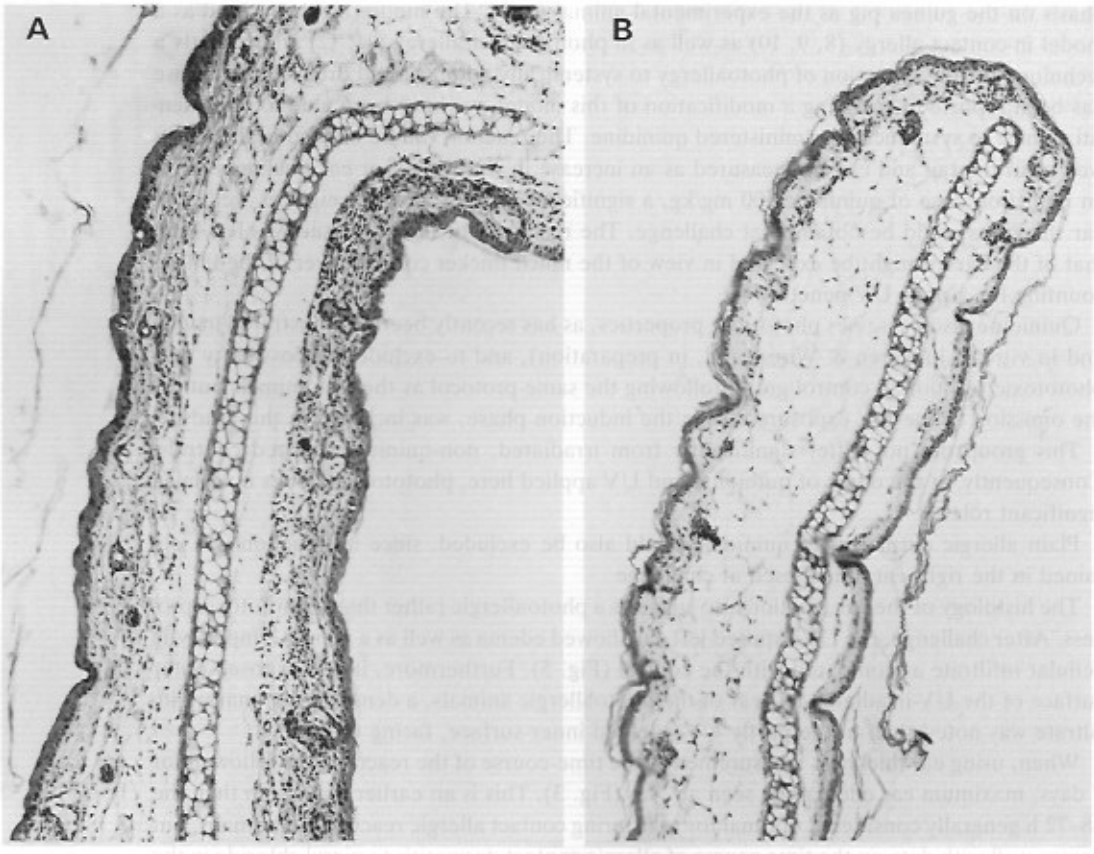


Fig. 5. Histopathologic sections of the left ear of photoallergic animal (*A*) and control animal (*B*) 48 h after challenge (HE ×110).

The importance of UVB exposure

To elucidate whether UVB in addition to UVA is necessary to induce photoallergy to quinidine this experiment was performed. The strongest reaction was obtained when mice were exposed to both UVB and UVA during induction. When mice were exposed to the UVA source only, the reaction was slightly weaker. When, in a third experiment the UVA radiation was filtered through a window glass pane, a weaker but still statistically significant ($p<0.001$) reaction was obtained (Fig. 4). This shows that UVB is not essential for the induction of photoallergy to quinidine.

Histology

The UV-exposed left ear of photosensitized animal showed at 48 h a dense cellular infiltrate consisting mainly of round cells with a preferential location to the outer aspect of the ear facing the UV source. The control ear showed no inflammatory reaction (Fig. 5 A, B).

DISCUSSION

Clinical photoreactions to quinidine generally have been of the delayed type, morphologically and also histopathologically, suggesting a photoallergic rather than a phototoxic mechanism. However, experimental data in support of this assumption have been lacking.

For the study of photocontact allergy, several in vivo models have been used with an em-

phasis on the guinea pig as the experimental animal (6, 7). The mouse has been used as a model in contact allergy (8, 9, 10) as well as in photocontact allergy (11, 12) and recently a technique for the induction of photoallergy to systemically administered drugs in the mouse has been reported (4). Using a modification of this model, we have been able to photosensitize mice to systemically administered quinidine. The reaction can be elicited in the ear as well as in the tail and can be measured as an increase in wet weight or ear thickness. With an induction dose of quinidine 100 mg/kg, a significant increase in wet weight as well as in ear thickness could be obtained at challenge. The reactivity of the tail tissue was less than that of the ear as might be expected in view of the much thicker corneal layer of the tail accounting for a poor UV penetration.

Quinidine also possesses phototoxic properties, as has recently been demonstrated in vivo and in vitro (Ljunggren & Wirestrand, in preparation), and to exclude the possibility of a phototoxic reaction, a control group following the same protocol as the test animals but for the omission of the UV exposure during the induction phase, was included in this study.

This group did not differ significantly from irradiated, non-quinidine-treated controls. Consequently, at the doses of quinidine and UV applied here, phototoxicity does not play a significant role.

Plain allergic dermatitis to quinidine could also be excluded, since no reaction was obtained in the right ear, unexposed at challenge.

The histology of the ear reaction also suggests a photoallergic rather than a phototoxic process. After challenge, the UV-exposed left ear showed edema as well as a mainly lymphocytic cellular infiltrate as compared with the control (Fig. 5). Furthermore, in the exposed outer surface of the UV-irradiated left ear of the photoallergic animals, a denser inflammatory infiltrate was noted than in the partly UV-shielded inner surface, facing the skull.

When, using ear thickness measurements, the time-course of the reaction was followed for 7 days, maximum ear edema was seen at 24 h (Fig. 3). This is an earlier maximum than the 48–72 h generally considered optimal for registering contact allergic reactions in humans, but agrees well with data on the time course of allergic contact dermatitis to picryl chloride in the mouse (10).

For the induction of photocontact sensitivity, UVB is generally considered necessary in addition to UVA, although for some substances, such as TCSA, the attempt to induce photoallergy with UVA only has been successful (12, 13). We studied the effect of decreasing and eventually eliminating the UVB dose in the induction phase by using, in a first step, the UVA lamp only, which emits a small amount of UVB as well, and, in a second step, by inserting a window glass pane, eliminating all radiation below 320 nm. As can be seen in Fig. 4, the relative wet weight of the ear tissue decreased with these measures, but left a still significant inflammatory edema when UVA alone was applied during induction. Systemic quinidine photoallergy can thus be elicited by exposure to wavelengths beyond 320 nm. This is in contrast to sulfanilamide and chlorpromazine, where UVA without UVB failed to induce systemic photoallergy (4).

This study thus demonstrates the capacity of quinidine to induce photosensitivity of an allergic type after systemic administration, supporting the assumption that clinical photoreactions to quinidine may have an immunologic basis. For the assessment of the inflammatory reaction in the mouse, both the ear and the tail can be used. Further studies are in progress to investigate the dynamics of the reaction.

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