

Systemically Induced Photoallergy to Quinine in the Mouse can be Elicited Topically—and Vice Versa

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Groups of female albino mice were photosensitized and photochallenged to quinine using protocols for either systemic or epicutaneous administration. Compared with control groups, statistically significant inflammatory reactions, measured as wet weight increase in ear tissue, could be obtained both with systemic and epicutaneous administration. Topically induced photoallergy to quinine could be elicited not only by topical, but also by intraperitoneal administration of the drug, and vice versa. The strongest response at challenge was obtained when the induction was performed topically and the challenge by the systemic route. These data suggest that epicutaneous and systemic photoallergy to quinine have mechanisms in common, and that the route of introduction of the sensitizer into the skin is not the crucial factor. This experimental model may be useful in the elucidation of the mechanisms of systemic photoallergy. *Key words: Systemic photoallergy; Photocontact allergy.*

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Quinine, a drug belonging to the group of quinoline methanols, is used in medicine for chloroquine-resistant malaria and for nocturnal cramps. It is reported clinically to cause both systemic (1) and contact (2) photosensitivity. Photocontact allergy to quinine has been successfully induced experimentally in the guinea pig (3). In a report, forming the basis for our own experimental technique, a method was described for the induction of photocontact allergy in the mouse (4). This contact photosensitivity could be passively transferred by lymph node cells to naive recipient animals (4).

Clinical photosensitivity of an eczematous type has been reported following the systemic use of several drugs, such as sulphanilamide (5), hydrochlorothiazide (6), quinidine (7) and quinine (1). The nature of

these photosensitivity reactions, whether allergic or toxic, has not been clearly demonstrated due to lack of suitable experimental techniques for reproducing systemic photosensitivity. However, recently the successful induction of photoallergy to sulphanilamide and chlorpromazine following systemic administration in the mouse was reported (8). The photoallergy could be transferred to naive mice by injecting lymph node cells (8). Using a modification of this technique, we have been able to demonstrate the induction of systemic photoallergy to quinine, and to its *d*-isomer, quinidine (9).

In this study, we induced systemic and photocontact allergy to quinine in different groups of mice, and experimentally combined systemic administration and topical application in the induction and elicitation phases in order to study the relation between these two routes of antigen exposure.

MATERIALS AND METHODS

Mice

Female albino NMRI mice weighing around 25 g were obtained from Anticimex, Sollentuna, Sweden. They had unlimited access to food and water and were housed in identical cages. Mice delivered at the same time were used in each individual experiment. Each experimental group consisted of 7–8 mice.

Chemicals

Quinine hydrochloride was purchased from ACO Läkemedel AB, Solna, Sweden, and was used without further purification. Cyclophosphamide was purchased from Lääkefarmos, Turku, Finland.

Ultraviolet radiation

Two fluorescent sunlamp tubes (Westinghouse Sunlamp FS40, 40 W) with an emission peak at 312 nm were used for medium-wave ultraviolet (UVB) irradiation. The output measured with a photometer (Waldmann AG, Schwenningen, GFR) was 0.7 mW/cm². Two fluorescent blacklight tubes (Philips TLA 40 W/08) with an emission peak around 360 nm were used for long-wave ultraviolet (UVA) irradiation. The output measured with a PUVA-meter (Waldmann AG) was 1.6 mW/cm².

Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
		INDUCTION	INDUCTION						CHALLENGE	EVALUATION
Cy		Q+	Q+						Q+UVA	
		UVB+UVA	UVB+UVA							

Fig. 1. Schematic illustration of basic experimental protocol for epicutaneous and intraperitoneal photosensitization.

Immunoadjuvant

In each experiment 150 mg/kg cyclophosphamide was injected intraperitoneally (i.p.) 2 days prior to photosensitization (day 0). Cyclophosphamide was dissolved in sterile normal saline to a total volume of 0.5 ml shortly before injection.

Systemic photosensitization

Approximately 4 cm² of the ventral skin of the animal was shaved, and quinine (100 mg/kg) injected i.p. on day 2. Topical contamination was carefully avoided. The mice were kept in the dark for one hour and after being anesthetized with pentobarbital 6 mg/ml, 0.35 ml i.p., the mice were placed in plastic tubes for fixation (9). The shaved ventral skin was exposed to UVB 0.1 J/cm² followed by UVA 5.0 J/cm², while the ears were carefully shielded within the plastic tube. On day 3 the same procedure was repeated, but the mice were not reshaved. (For details of the photosensitization protocols, see Fig. 1.)

Topical photosensitization

When photosensitization was performed epicutaneously, the ventral skin was shaved on day 2 as described above. The mice were then anesthetized, and 0.02 ml of a solution of quinine 1% (in acetone 30%, ethanol 30% and *N-N*-dimethylacetamide 40%), was applied to the shaved ventral skin and allowed to dry. After being kept in the dark for 30 min the mice were placed in the plastic tubes, and the ventral skin was exposed to the same UV doses as in the systemic photosensitization protocol. On day 3 the same procedure was repeated, but the mice were not reshaved.

Systemic photochallenge

The mice were photochallenged on day 9 by administration of quinine (100 mg/kg) i.p. After one hour in the dark the mice were anesthetized with pentobarbital and placed in the plastic tubes with the left ear facing the UV lamp. The left ear was exposed to UVA 5.0 J/cm² while the right ear was carefully shielded from UV light.

Topical photochallenge

Photochallenge was performed epicutaneously on day 9 when 0.01 ml of the same 1% quinine solution was applied to the left ear. After 30 min in the dark the mice were anesthetized, placed in the plastic tubes, and the left ear exposed to UVA 5.0 J/cm² as described for systemic photochallenge.

Controls

To exclude phototoxicity, control groups were included in all experiments. The control group followed the standard procedure but for the omission of UV irradiation during the induction procedure.

Evaluation

On day 10 the mice were killed and the edematous reaction of the excised ear tissue was measured as increased wet weight. The relative wet weight was established by weighing the excised ears before and after drying in an oven at 110°C for one hour (9). In addition, the reaction was measured as increase in ear diameter using a micrometer (NSK, Digital Japan).

Statistics

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

RESULTS

Individual data from two representative experiments are shown in Table I. Since both photosensitization and photochallenge were performed by the intraperitoneal (IP) and the epicutaneous (EC) protocols, four combinations are possible. The results of experiments representing all these combinations are given in Table II.

Compared to the control group, a statistically significant reaction could be obtained both with the systemic (IP/IP) and the epicutaneous (EC/EC) protocol (Table I), indicating that both routes of administration were effective in inducing and eliciting photosensitivity to quinine hydrochloride.

In the vice versa experiments differences in reactivity were noted. In IP photochallenge, a stronger reaction ($p < 0.001$) was seen with EC than with IP induction. In EC photochallenge, the responses were less strong, but again a tendency towards a stronger reaction with EC induction was noted. Consequently, EC induction resulted in a better response than IP induction for both routes of photochallenge.

In IP photoinduction a stronger reaction ($p < 0.001$) was seen if the photochallenge was also IP as compared with EC. Also with EC photoinduction, a significantly stronger reaction ($p < 0.001$) was seen with IP than with EC photochallenge. Thus IP challenge is superior to EC challenge in both types of induction protocols.

The animals of the control groups did not react with ear edema at challenge, thus ruling out the possi-

Table I. Results from two independent photosensitization experiments with quinine HCl where both induction and challenge were performed by the same route of administration

Left ear UV exposed, right ear shielded. IP = intraperitoneal protocol, EC = epicutaneous protocol, EXP = experimental group, C = control group, identical with EXP but no UV exposure during induction. M WW = mean wet weight, SD = standard deviation

Induction ...	IP				EC			
Challenge ...	IP				EC			
Group	EXP (n=7)		C (n=8)		EXP (n=8)		C (n=7)	
	Left	Right	Left	Right	Left	Right	Left	Right
M WW%	64.9	54.8	51.6	51.0	62.5	55.8	55.2	55.0
SD ±	3.2	1.3	1.1	1.3	3.3	1.7	1.5	1.6
p vs. C	<0.001				<0.001			

bility of a phototoxic mechanism. All data are presented here as relative wet weight increase in ear tissue. The results of the ear diameter measurements closely paralleled the wet weight figures (data not shown).

DISCUSSION

Evidence for an immunologic basis of contact photoallergy is accumulating (10). Following the absorption of photons, the photoactive chemical is transformed into a hapten which, after protein binding, will evoke an immune response. Sometimes the only role of the

UV energy is to produce a photoproduct, which will subsequently induce ordinary contact allergy, but it is unlikely that this mechanism accounts for all instances of photoallergy. Transfer of contact photosensitivity has been achieved experimentally by the injection of lymph node cells obtained from sensitized animals into naive recipients (4, 11, 12).

Photosensitivity following the oral, or parenteral, introduction of the photoactive agent has been less well studied and characterized. Most systemic photo-reactions are caused by phototoxicity, which can be defined and reproduced experimentally. However, a smaller group of clinically non-phototoxic reactions remains, where the pattern is eczematous, the reaction is delayed, and the photosensitivity may be reproduced by photopatch testing. These observations suggest that photoallergy can also be produced systemically. Experimental evidence now exists to support this theory. Photoallergy has been induced in the mouse by several systemically administered drugs, such as sulphanilamide, chlorpromazine, quinidine and quinine (8, 9, 13), and the sensitivity has been transferred to naive mice by lymphoid cells (8). Whether contact and systemic photoallergy have a common or a dissimilar pathogenetic mechanism is not clear. In one report, however, photoallergy to intradermal sulphanilamide in the mouse could be elicited using intraperitoneal administration (14).

With these mouse models, using cyclophosphamide prior to induction in order to inhibit suppression of the response, we could elicit topically induced photoallergy to quinine by introducing this agent systemically, and vice versa. In fact, parenteral administration caused a stronger response ($p < 0.001$) than was

Table II. Photoinduction and photochallenge with quinine HCl in the mouse with IP (intraperitoneal) or EC (epicutaneous) protocol

Each bar represents a comparison between two combinations of protocols, with the experiments performed at the same time. The asterisks are given in the experimental column yielding the strongest reaction

Induction ...	IP	IP	EC	EC	Controls
Challenge ...	IP	EC	EC	IP	
	***	-----	-----	-----	
	***	-----	-----	-----	
	**	-----	-----	-----	
	-----	-----	-----	***	
	NS	-----	-----	-----	
	-----	-----	-----	***	
	*	-----	-----	-----	
	-----	-----	***	-----	
	-----	-----	-----	***	
	-----	-----	-----	***	

NS, $p > 0.05$, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

seen when quinine was applied topically at challenge. The lowest reactivity was obtained with epicutaneous challenge, while epicutaneous sensitization combined with systemic challenge yielded the strongest response of all combinations.

The possibility of a phototoxic reaction could be ruled out, since control animals, tested according to the two protocols, but not irradiated during the induction phase, did not react with edema at challenge. In addition, studies on the phototoxic properties of quinine *in vivo* in the mouse were negative (15).

However, to compare the degree of reactivity at the target organ, the ear, may be difficult, since the quinine doses administered epicutaneously and intraperitoneally cannot be expected to give identical tissue concentrations in abdominal and ear skin. In topical induction, a total quinine dose of 0.1 mg/cm² is delivered to abdominal skin divided on 2 consecutive days. In the systemic protocol each animal receives a quinine dose of about 2.5 mg i.p. on 2 consecutive days. At i.p. photochallenge, the same amount is given as a single dose, whereas in the topical protocol the quinine dose to the ear is approximately 0.1 mg/cm². The i.p. dose would be roughly equivalent to the epicutaneous dose if it could be assumed that around 4% of the i.p. dose were delivered to each cm² of mouse skin. If, however, the stronger reactivity after epicutaneous application at induction were merely an effect of a higher quinine concentration due to this route of administration, the same would be expected to occur at challenge. Here, however, epicutaneous application proved inferior to i.p. administration. And, consequently, if skin concentrations were higher after i.p. injection, this would explain the stronger reactivity with this mode of application at challenge, but not the poor performance of the systemic route at induction.

From this it appears likely that the way quinine is introduced into the skin has important consequences for the process of both induction and elicitation of photoallergy. These animal experiments seem to indicate a close relationship between topical and systemic photosensitivity. The critical event—the photochemical alteration of quinine, or its products, in the skin following UV exposure and the subsequent exposure of these products to the immune competent cells of the skin—can be achieved when quinine reaches the skin from the exterior as well as from the interior.

This experimental model may be helpful in further

studies to elucidate the immunological mechanisms responsible for systemic photoallergy.

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