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Suction Blister Fluid Histamine in Fixed Drug Eruption

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The histamine concentration was measured from suction blister fluid obtained from normal and lesional skin of 8 patients with fixed drug eruption (FDE) caused by phenazone salicylate and from that of 2 healthy control subjects. In blister fluid samples obtained before peroral challenge with phenazone salicylate, the histamine concentrations were below 5 nmol/l both in uninvolved skin and in sites of previous FDE lesion (sample 0). After challenge, samples were taken from the incipient reaction that was visible after an average of 155 min. Histamine levels were significantly elevated in the blister fluid of 2 out of 8 FDE lesions (200 and 640 nmol/l) but in none of the uninvolved skin (sample 1). Two hours later (sample 2) the histamine levels were elevated in both uninvolved (mean 51.4 nmol/l) and lesional skin (mean 168 nmol/l). After 24 h (sample 3) the corresponding mean value was 25.4 nmol/l for uninvolved skin and 108 nmol/l for lesional skin. The histamine values in the blister fluid from FDE lesions in samples 2 and 3 were significantly higher ($p<0.05$) than those in the control blisters of uninvolved skin. An elevation of histamine levels comparable to that in the uninvolved skin of FDE patients was seen in the 2 healthy control subjects studied. The present study provides direct evidence of early release of histamine from mast cells or basophils in FDE and suggests that histamine is one of the mediators of clinical symptoms of FDE. ***Key words: Histamine; Fixed drug eruption.***

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Fixed drug eruption (FDE) is a cutaneous drug reaction characterized by round erythematous lesions that recur at the same site of the skin or mucous membranes. The pathomechanism of FDE is unknown although there is indirect evidence that immunological mechanisms play a major role in its pathogenesis (1,2).

The aim of this study was to ascertain whether histamine is involved in the pathogenesis of FDE. This was done by producing suction blisters on skin areas of previous FDE, inducing a new FDE by peroral provocation and measuring histamine levels from the blister fluid.

MATERIAL AND METHODS

Patients and controls

The study was approved by the ethics committee of the Department of Dermatology, Helsinki University Central Hospital. Eight patients were selected for the study. All patients had previously had a recurrent FDE caused by phenazone salicylate, which is the most common drug causing FDE in Finland. In all but one patient the reaction had been verified by peroral (3) or topical (4) provocation. The test doses in our study were approximately one third of the original dose during the previous clinical reaction; this is the usual practice in peroral drug provocations in FDE. The patients did not use any other medications while undergoing study. We selected patients with sites of FDE accessible to the suction blister method. We also made suction blisters on the skin of 2 healthy controls, collected blister fluid according to the same schedule and measured the histamine concentration.

Suction blister fluid

The blisters were produced on sites of previous FDE and on normal skin according to the suction blister method of Kiistala (5). When more than one FDE site was available, a set of six blisters was produced on two FDE sites. On normal skin, two sets of blisters were always made on abdominal skin.

The first blister fluid samples from both normal and postlesional skin were taken from newly formed blisters before drug administration (sample 0). The test dose of the drug was administered perorally. The next samples of blister fluid were collected when the FDE appeared clinically (sample 1); the following samples were taken from a prominent reaction 2 h later (sample 2). The last samples were taken 24 h after drug administration (sample 3).

The blister fluid samples were always collected in 0.05 ml glass capillaries in which the samples taken from the first 4 patients were also stored. The samples taken from the later 4 patients and the 2 control subjects were stored in plastic tubes. One blister produced 0.02–0.04 ml of fluid. The blister fluid samples were kept frozen until assayed.

Histamine assay

The histamine concentration was measured from duplicate samples, using the single isotope method described by Dyer et al. (6), in the laboratory of the Department of Allergic Diseases, Helsinki University Central Hospital.

Statistical methods

Logarithmic values of histamine concentrations were used to attain approximate normality of the histamine concentration distributions. Differences in mean logarithmic histamine concentrations between lesions and normal skin were tested by pairwise t-tests, i.e., using each patient as his/her control.

RESULTS

The clinical reactions during the study are shown in Table I. With patient 1, we had to interrupt the test and take the last samples after 9 h because of intense FDE requiring systemic corticosteroid medication. Patients 6 and 7 had no pigment macules left and we had to trace the test areas by photographs taken at the hospital during the previous FDE. The clinical reactions of these patients were of a widespread type without an intense erythema on individual patches. The last, or third, sample could not be collected from 3 patients, because not enough blister fluid was available.

The histamine levels in the blister fluid during FDE are shown in Table II. A statistical summary of the results is presented in Table III. The histamine values in blister fluid

* deceased.

Table I. *The patients and their clinical reactions to oral provocation with phenazone salicylate*

Patient	Sex/Age	Phenazone salicylate dose (mg)	Test reaction spread*	Erythema of individual FDE patches*	Interval to sample 1
1	M 39	50	+++	++	1 h
2	F 17	30	++	++	1 h 30
3	F 25	50	++	++	4 h
4	F 46	50	++	++	1 h 15
5	F 22	40	+	+	4 h 50
6	M 65	70	+++	+	5 h 45
7	M 27	50	+++	++	1 h 15
8	F 25	40	+	++	1 h

* graded from + to +++.

from lesions appearing during the reaction (samples 2 and 3) were significantly higher ($p<0.05$) than those in the control blisters on healthy skin. However, as seen in Table II, only 2 of the patients (P6 and P7) had clearly elevated histamine concentrations of the blister fluid.

DISCUSSION

We first studied a set of 4 patients and later, after preliminary results, another set of 4 patients. Because of technical problems we could not arrange identical storage for the samples of the two different sets. Histamine is adsorbed by glass surfaces (7), as is apparent in the lower histamine concentrations in the samples stored in glass capillary tubes. For our study the absolute numeric values were not relevant because we studied the difference between histamine values in normal skin and lesional skin in individual patients. This difference was also seen in the first set of samples studied by the less sensitive "glass capillary method".

The histamine values were highest for cases 6 and 7, who also showed clinically widespread reactions. In mild FDE, the histamine values were often low. We did not detect any connection between the intensity of erythema in the individual test reactions and the histamine levels. Some patients showed clear increases in histamine concentrations not only in FDE but also on normal skin. However, the increase was not seen regularly. Therefore it cannot indicate that histamine liberating cells diffundate over time in all skin blisters, an error we had sought to eliminate by raising control blisters on clinically healthy skin. The implication is either that histamine can be liberated in tissue injury such as suction blister, or that a systemic (humoral) histamine release takes place in these cases.

Our study provides direct evidence that histamine is released in both normal and lesional skin in patients with FDE and suggests that histamine release is one of the factors causing the clinical symptoms of FDE, i.e., erythema and pruritus. Histamine concentrations have earlier been determined from suction blister fluid in UVA-treated subjects. The study provided evidence that UVA is able to induce histamine release from mast cells/basophils of the skin (8). Increased histamine concentrations have also been detected in delayed type skin reactions; in this study histamine determination was done from biopsies of positive patch test reactions (9).

Histamine release cannot explain the prolonged presence of the FDE lesions. The prolonged clinical symptoms of eryth-

Table II. *Histamine concentrations (nmol/l) in blister fluid samples during peroral provocation*

Patient (P) /Control (C)	Sample							
	0 (normal/lesion)		1 (normal/lesion)		2 (normal/lesion)		3 (normal/lesion)	
P1	0	0	0	0	0	7	2	38
P2	0	0	0	0	0	3	4	47
P3	0	0	1	7	4	69	–	–
P4	2	1	0	0	25	18	55	73
P5	0	0	0	0	23	31	4	5
P6	0	5	7	640	345	480	62	375
P7	0	0	8	200	13	730	–	–
P8	0	0	0	0	1	4	–	–
C1	2		0		5		31	
C2	1		0		39		67	

Sample 0: before drug administration.
Sample 1: incipient reaction.
Sample 2: prominent reaction (2 h later).
Sample 3: 24 h reaction (except patient 1 taken after 9 h).

Table III. *Summary of the results in Table II and results from pairwise t- tests*

	Sample 0 (normal/lesion)		Sample 1 (normal/lesion)		Sample 2 (normal/lesion)		Sample 3 (normal/lesion)	
N	8	8	8	8	8	8	5	5
Mean	0.25	0.75	2.00	106	51.4	168	25.4	108
Range	0–2	0–5	0–8	0–640	0–345	3–730	2–62	5–375
Mean of difference of log. values	0.07		0.48		0.6		0.61	
S.E.M.	0.10		0.26		0.22		0.22	
t-value	0.73		1.81		2.81		2.83	
p	0.49		0.11		0.026*		0.047*	

* significant at $p<0.05$ level.

ema and itch might be attributed to release of other proinflammatory mediators from basophils or mast cells.

There is preferential expression of intercellular adhesion molecule 1 (ICAM-1) by keratinocytes in FDE but not in neighbouring skin (2). This has been taken as evidence that FDE could be a T-lymphocyte mediated disease, as ICAM-1 expression by keratinocytes is induced by adhesion molecule LFA-1 (10,11). The early onset of the FDE-reaction, from 15 min to a few hours in our patients, suggests, however, that immediate type mechanisms are involved. It remains to be established whether the factor release from basophils/mast cells is IgE-mediated, i.e., whether the reaction is an anaphylactic type of reaction or whether nonimmunological mechanisms through direct factor release from the histamine containing cells are involved. Histamine release from basophils/mast cells can be evoked by a variety of immunological and physical stimuli (12).

We have found complement depositions at the basement zone in skin biopsies of patients with FDE (unpublished data), suggesting that complement may be involved in the pathogenesis of FDE. Previous studies suggest that complement activation by antigen-antibody complexes at the basement membrane zone could be responsible for mast cell degranulation in diseases such as bullous pemphigoid (13). Accumulation of basophils is seen not only in acute-phase reactions but also in delayed-type hypersensitivity reactions (14), where the extent of basophil accumulation in the skin correlates well with the histamine content of the skin (15). Only a few studies have been conducted on basophils and mast cells in FDE. Stubb (16) has shown that at the height of FDE reaction there is a drop in the number of peripheral blood basophils with an ensuing increase in their number after the reaction has subsided. This is indirect evidence that these cells could be recruited to sites of FDE lesion. No direct studies on the number of basophils have been performed on tissue sections of FDE lesions. The number of dermal mast cells is not increased in FDE (17).

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