DISSOCIATION OF HISTAMINE RELEASE AND CLINICAL IMPROVEMENT FOLLOWING TREATMENT OF ACQUIRED COLD URTICARIA BY PREDNISONE

A. KOBZA BLACK, T.M. KEAHEY*, R.A.J. EADY & M.W. GREAVES

Institute of Dermatology, Homerton Grove, London E9 6BX

- 1 Corticosteroids are known to suppress evoked histamine release from human skin *in vitro*. Therefore the effect of prednisone, on cold evoked histamine release and clinical response was studied in six patients with acquired cold urticaria.
- 2 Following cold challenge all six patients developed an immediate urticarial response of the forearm and elevation of histamine levels, measured by superfusion cascade bioassay of the venous blood draining the forearm.
- 3 After treatment with prednisone, in an oral dose of 20–25 mg for 1 to 5 days, a suppression of evoked histamine release occurred in all but one patient. However, there was no corresponding decrease of erythema or oedema, though pruritus was improved in most patients.
- 4 The lack of clinical improvement in erythema and oedema, despite reduction in histamine levels, suggests that histamine may not be the principal mediator of the vascular response to cold challenge in acquired cold urticaria.

Introduction

Cold urticaria is a disabling skin disorder, in which itching, redness and swelling occur on cold exposed areas of the skin. Systemic symptoms resembling anaphylactic shock may occur (Horton, Brown & Roth, 1936) with headache, wheezing, and syncope. Several mast cell mediators have been implicated in the pathogenesis of acquired cold urticaria. Increased levels of histamine have been demonstrated in patients by ourselves (Bentley-Phillips, Black & Greaves, 1976) and other (Rose, 1954; Kaplan & Beaven, 1976). Increased histamine levels were also shown on cold challenge, in the urine (Granerus *et al.*, 1969) and tissue fluid (Kaplan, Horakova & Katz, 1978). It is this histamine release which is considered to be responsible for the immediate vascular response of the skin, and systemic reactions of cold urticaria. We have previously shown that successful treatment of acquired cold urticaria by induction of cold tolerance, is accompanied by a marked diminution of histamine release (Bentley-Phillips *et al.*, 1976). Other mediators, including eosinophil chemotactic factor (Soter, Wasserman & Austen, 1976) and neutrophil chemotactic factor (Wasserman *et al.*, 1977) have also been demonstrated in venous blood draining cold challenge areas.

Our own work has shown that drugs which raise levels of cyclic AMP (adrenergic agonists and phos-

phodiesterase inhibitors, such as aminophylline) suppress antigen evoked release of histamine, from human skin mast cells *in vitro* (Yamamoto, Greaves & Fairley, 1973). These drugs administered systemically in combination, inhibited evoked venous histamine release *in vivo* in patients with cold urticaria (Keahey & Greaves, 1980).

Glucocorticoids have also been shown to inhibit antigen evoked histamine release from human skin *in vitro*, in a dose-dependent manner (Greaves & Plummer, 1974). We have therefore investigated the action of systemically administered prednisone on cold evoked histamine release, and the ensuing clinical response to cold injury in patients with cold urticaria.

Methods

Subjects

Six patients with acquired cold urticaria were studied. Their ages ranged from 16–74 years and there were three males and three females. They had suffered skin manifestations for 2–16 years, and four patients also suffered from cold-induced syncopal episodes. There was no family history of cold urticaria. There was no evidence of elevation of concentration of cryoglobulins, cold agglutinins or cryofibrinogens in the plasma. No patients had any immunological or

*University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pa. 19104, U.S.A.

haematological evidence of collagen vascular disease. One patient (patient 3) later was found to have developed cutaneous allergic vasculitis following repeated cold challenge, and the second component of complement was also found lacking in this patient on one occasion (Sibbald *et al.*, 1976). No pharmacological investigations were carried out until antihistamines or other drugs had been withdrawn at least 72 h previously.

The patients gave informed consent, and the Institute's Ethical Committee gave approval.

Histamine estimations

In each experiment the antecubital vein of a forearm was cannulated and a control blood sample was collected into a heparinized syringe (25 units to 5 ml blood). The patient's hand and forearm were then immersed in cold water at 10°C for 5 min. Further blood samples were then taken at 2, 5, 7, 10, 12, 15, 20, 25 and 30 min after the end of immersion. Histamine content of the venous blood was immediately measured by means of the cascade superfusion technique of Gaddum (1953) modified by Vane (1964) in which fresh venous blood is cascaded over several isolated smooth muscle preparations mounted in series. Two guinea pig isolated ileum preparations mounted in series were superfused by Krebs's solution. Any smooth muscle contracting activity in the blood samples due to acetylcholine and serotonin was excluded by the addition of specific receptor antagonists. In order to detect and measure histamine, mepyramine was additionally superfused over the lower guinea pig ileum to a tissue concentration of 100 ng ml⁻¹.

All responses were recorded, using force displacement transducers, on a Devices Recorder. Histamine was deemed to be present if the response of the mepyramine-free ileum, was significantly greater than the response of the mepyramine-treated ileum to the same sample. The responses to histamine in the blood samples were measured in terms of responses to authentic reference standards, by the bracketing method of bioassay (Schild, 1942). Histamine concentrations of 1 ng ml⁻¹ were reliably detected by the bioassay method, the assay being reproducible within ± 2.5 ng/ml. Nearly all the contractile response was blocked by mepyramine, therefore histamine was the major pharmacological substance detected.

Treatment

Three patients were treated for periods of three to five days with prednisone 20 mg each morning. One of these patients underwent three separate treatment trials over a 6 week period. Three other patients were given a single dose of 20 or 25 mg prednisone in the morning, depending on body weight.

Cold challenge was carried out 4–6 h after the last dose of prednisone. The previously unchallenged forearm was used for the post-treatment experiment to avoid the possibility of spuriously low mediator levels, due to induction of partial tolerance from the previous immersion. If this was not possible, a period of at least seven days was allowed before rechallenge of the same arm. The contractile response of the guinea pig ileum to histamine was not affected by prednisone in a concentration of 100 µg/ml.

Clinical response

The clinical response following cold challenge was measured subjectively and was not done 'blind'. Erythema and oedema were visually assessed by T.K. or A.B. Pruritus or pain was graded by the patient. The grading system is as follows:

- oedema: 0 = absent
1 = mild, without sharp margination
2 = moderate, without sharp margination
3 = intense, with sharp margination
- erythema: 0 = absent
1 = blotchy pink
2 = confluent pink-red
3 = dusky pink
- pruritus or pain: 0 = absent
1 = mild discomfort initially lasting less than 1 min
2 = mild discomfort lasting greater than one but less than 5 min
3 = mild to moderate discomfort lasting greater than 5 min

In two patients oedema was assessed objectively before and after therapy using a cryoprobe (Curnow, 1978). The probe applied to the skin consisted of a raised metal plate the temperature of which could be set and maintained over a range of 2.5° to 20°C. The probe was applied at 10°C on symmetrical areas of the thighs for different periods of time, to determine minimum contact time necessary to produce wealing.

Histamine content of forearm skin

One patient underwent punch biopsies (4 mm diameter) for assay of whole skin histamine content, prior to and following therapy. Following four quadrant peripheral injections of 1% xylocaine, punch biopsies from midvolar forearm skin were taken and deep frozen. These were later freeze dried, weighed and boiled individually in 4 ml Tyrode solution for 5 min. Protein was precipitated with 0.4 N perchloric acid and the suspension centrifuged at 5000 g for 15 min. The supernatant was assayed for histamine using a Technicon AA II autoanalyser and the modified fluorometric technique of Evans, Lewis & Thomson

Table 1 The levels (ng ml^{-1}) and timing (min) of maximum venous histamine concentrations post immersion, and areas under the plots of venous histamine concentrations against time in arbitrary units, before and after prednisone therapy.

Patients	Pretreatment			Post-treatment		
	Histamine	Min	Units	Histamine	Min	Units
1*	14.0 ± 2.5 (s.e. mean)	5	21.0 ± 2.2 (s.e. mean)	2.5	2-15	3
2	19.0	7	24.2	1	—	—
3	11.0	7	6.2	20	7	27.7
4	18.5	2	14	5.7	7	7
5	51.0	5	41	17.6	5	12
6	4.4	5	5.0	1	—	—

*mean of three tests.

(1973). Histamine content was expressed in ng mg^{-1} dry weight.

Histamine release

All patients showed increased levels of histamine release on challenge prior to therapy. Prednisone caused significant suppression of cold-evoked histamine release in five out of six patients. Individual patient results are illustrated in Table 1. Patient 1 underwent three treatment trials, incorporating nine separate tests over a 6 week period, with total suppression of histamine release to baseline concentrations following two treatment levels and to 2.5 ng ml^{-1} per unit time histamine release following the third and final trial. Elevated histamine concentrations following cold challenge could not be detected for 7 to 11 days following prednisone treatment.

The mean histamine concentrations in the six patients, after the end of immersion, before and after prednisone therapy are shown in Figure 1. With a paired *t*-test, there was significant reduction of histamine concentrations at 5 and 10 min after cold immersion ($P < 0.05$ and $P < 0.025$ respectively). Oedema and erythema were visually assessed and graded by the observers. Pruritus or pain was subjectively graded by the patient (Table 2). There was no significant post-treatment improvement in either clinically evident erythema or oedema following cold challenge. Complete or partial suppression of post challenge pruritus or pain was noted by all six patients. Patient 3 was noted to have significant residual oedema up to eight hours following cold challenge. This patient was later shown to have the histological appearance of vasculitis in a skin biopsy after local ice challenges. Objectively, there was no change in the minimum time for wealing in patient 5

(90 s at 10°C) and patient 6 (6 min at 10°C) using the cryoprobe.

Skin histamine content

There was no significant change in histamine content of the skin before prednisone therapy, and after prednisone therapy, both before and after cold challenge (Table 3).

Figure 1 Venous histamine concentrations $\pm$ s.e. mean plotted against time following cold challenge before and after treatment with prednisone. Each point represents mean of eight experiments in six patients. Solid line indicates pre-treatment values; dotted line post-treatment values.

Table 2 Clinical response following prednisone

Patient	Oedema		Erythema		Pruritus	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
1 (a)	3	2 ¹	3	2	3	0
		3 ²		3		1
(b)	3 ³	2 ⁴	3	2	2	1
		2 ⁵		2		1
		3 ⁶		3		1
(c)	3 ⁷	3 ⁸	3	3	2	1
2	2	2 ⁹	2	2	1	0
3	3	3 ¹⁰	3	3	1	0
4	3	3 ¹¹	3	3	2	1
5	2	2 ¹¹	2	2	2	1
6	1	1 ¹²	1	1	2	2

Key:

1. Prednisone 20 mg for 3 days. 2. No prednisone for 4 days. 3. No prednisone for 7 days. 4. Prednisone for 3 days. 5. Prednisone for 5 days. 6. No prednisone for 4 days. 7. No prednisone for 11 days. 8. Prednisone for 3 days. 9. Prednisone for 4 days. 10. Prednisone for 3 days. 11. Prednisone 20 mg for 1 day. 12. Prednisone 25 mg for 1 day.

Discussion

We have demonstrated that prednisone, which has previously been shown to inhibit histamine release *in vitro*, also inhibits histamine release evoked by contact of the skin with cold in patients with cold urticaria. The results of skin histamine determination carried out before and after prednisone therapy suggested that it acts by inhibiting histamine release from mast cells, and not by depleting endogenous histamine stores.

The mode of action of corticosteroids in inhibiting histamine release is not fully understood. Glucocorticoids may act by raising cyclic adenosine monophosphate (cyclic AMP) levels in mast cells, and we have previously shown that drugs which increase cyclic AMP suppress evoked histamine release from human skin (Yamamoto *et al.*, 1973). Alternatively, corticosteroids may owe their inhibitory action on histamine release to their well-recognised ability to 'stabilise' intracellular organelle membranes including lysosomal membranes (Weissman & Thomas, 1963).

Inhibition of lysosomal phospholipase A2 secretion, leading in turn to reduced formation of arachidonic acid metabolites, and to reduced concentrations of cellular cyclic AMP, could cause enhanced histamine release in this situation. However, none of these proposed mechanisms explain the observation, in our patient one, that suppression of histamine release occurred for 7–11 days after ceasing prednisone treatment.

We were surprised that there was generally no correlation between suppression of histamine release and clinical improvement of erythema and oedema, though pruritus was improved. That clinical improvement did not occur in association with diminished histamine release is especially obvious in patients 1 and 6 where suppression of histamine release to baseline concentration was achieved without any effect on erythema or wealing in response to cold challenge. To implicate histamine as the main mediator of the vascular response in cold urticaria, inhibition of release of histamine should reduce erythema and oedema following cold challenge, but this was not the case either in the present or previous studies

Table 3 Whole skin histamine content in ng mg⁻¹ of 4 mm punch biopsies taken from forearm skin before cold challenge prior prednisone treatment, and before and after cold challenge following prednisone treatment, in one patient.

Histamine (ng mg ⁻¹)		
Pre-prednisone therapy	Post-prednisone therapy	
Before cold challenge	Before cold challenge	After cold challenge
28.4	25.6	27.0

(Bentley-Phillips *et al.*, 1978; Keahey & Greaves, 1980).

Several possibilities may explain the apparent dissociation of histamine release and the clinical response to cold injury. Overall histamine liberation, as detected in the venous drainage, may be less relevant than local release. Alternatively, other mediators of inflammation, including polymorphonuclear chemotactic factor and eosinophil chemotactic factor, bradykinin, or prostaglandins may be involved. With more sophisticated techniques increased concentra-

tions of these or other known or as yet unrecognised mediators may be detected.

Cold urticaria, like many other types of urticaria, has traditionally been classified as a histamine mediated skin disorder. Our findings suggest that the role of histamine in the pathogenesis of urticarias needs to be reconsidered.

We thank Mrs June Isaacs and Mrs Deborah Russell for their technical assistance, and the Medical Research Council and the Sir Herbert Dunhill Trust for their continued support.

References

- BENTLEY-PHILLIPS, C.B., BLACK, A. & GREAVES, M.W. (1976). Induced tolerance in cold urticaria caused by cold-evoked histamine release. *Lancet*, **ii**, 63–66.
- BENTLEY-PHILLIPS, C.B., EADY, R.A.J. & GREAVES, M.W. (1978). Cold urticaria; inhibition of cold-induced histamine release by doxantrazole. *J. invest. Derm.*, **71**, 266–268.
- CURNOW, J.S.H. (1978). Stable low temperature source to test for cold urticaria. *Med. Biol. Comp.*, **16**, 215–216.
- EVANS, D.P., LEWIS, J.A. & THOMSON, D.S. (1973). An automated fluorometric assay for rapid determination of histamine in biological fluids. *Life Sci.*, **12**, 327–336.
- GADDUM, J.H. (1953). The technique of superfusion. *Br. J. Pharmacol.*, **8**, 321–326.
- GRANERUS, G., SVENSSON, S.E., WETTERQVIST, H. & WHITE, T. (1969). The metabolism of histamine in a case of cold urticaria. *Acta Allerg.*, **24**, 258–260.
- GREAVES, M.W. & PLUMMER, V. (1974). Glucocorticoid inhibition of antigen-evoked histamine release from human skin. *Immunology*, **27**, 359–364.
- HORTON, B.T., BROWN, G.E. & ROTH, G.M. (1936). Hypersensitiveness to cold; with local and systemic manifestations of a histamine-like character: its amenability to treatment. *J. Am. med. Ass.*, **107**, 1263–1269.
- KAPLAN, A.P., HORAKOWA, Z. & KATZ, S.I. (1978). Assessment of tissue fluid histamine in patients with urticaria. *J. Allergy clin. Immunol.*, **61**, 350–354.
- KAPLAN, A.P. & BEAVEN, M.A. (1976). *In vivo* studies of cold urticaria, cholinergic urticaria and vibration-induced swelling. *J. invest. Derm.*, **67**, 322–327.
- KEAHEY, T.M. & GREAVES, M.W. (1980). Cold urticaria: dissociation of cold-evoked histamine release and urticaria following cold challenge. *Arch. Derm.*, **116**, 174–177.
- ROSE, B. (1954). Histamine, hormones and hypersensitivity. *J. Allergy*, **25**, 168–189.
- SCHILD, H.O. (1942). A method of conducting a biological assay on a preparation giving repeated graded response illustrated by the estimation of histamine. *J. Physiol.*, **101**, 115–130.
- SIBBALD, R.G., EADY, R.A.J., KOBZA-BLACK, A. & GREAVES, M.W. (1979). Cold urticaria and complement deficiency. *Br. J. Derm.*, **101**, Suppl, 17, 45.
- SOTER, N.A., WASSERMAN, S.I. & AUSTEN, K.F. (1976). Cold urticaria: release into the circulation of histamine and eosinophil chemotactic factor of anaphylaxis during cold challenge. *New Engl. J. Med.*, **294**, 687–690.
- VANE, J.R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmacol.*, **23**, 360–373.
- WASSERMAN, S.I., SOTER, N.A., CENTER, D.M. & AUSTEN, K.F. (1977). Cold urticaria: Recognition and characterisation of neutrophil chemotactic factor which appears in serum during experimental cold challenge. *J. clin. Invest.*, **60**, 189–196.
- WEISSMAN, G. & THOMAS, L. (1963). Studies on lysosomes II. The effect of cortisone on the release of acid hydrolases from a large granule fraction of rabbit liver induced by an excess of vitamin A. *J. clin. Invest.*, **42**, 661–669.
- YAMAMOTO, S., GREAVES, M.W. & FAIRLEY, V.M. (1973). Cyclic AMP-induced inhibition of IgE-mediated hypersensitivity in human skin. *Immunology*, **24**, 77–83.

(Received September 29, 1980)