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# Aberrant Cutaneous Weal and Flare Responses in Chronic Urticaria

L. SHALL and E. M. SAIHAN

*Department of Dermatology, University Hospital, Queen's Medical Centre, Nottingham, UK*

**This study examined cutaneous mast cell behaviour in 14 patients with chronic urticaria but no dermographism and 11 healthy controls, by measuring cutaneous weal and flare reactions evoked in response to intradermal challenge injections of 0.1 ml isotonic saline, histamine (20 μg), codeine phosphate (10 μg) and compound 48/80 (10 μg). Five minutes after each injection, the area of the resulting weal and flare was calculated by computer-aided planimetry. The process was repeated at 15, 30 and 45 min following the injection. In patients, saline flares were significantly larger than those of the volunteers at 15, 30 and 45 min ($p < 0.05$). However, histamine and codeine flare areas were significantly smaller in the patients when compared to the controls at 15, 30 and 45 min ($p < 0.05$). Compound 48/80 produced smaller reactions in the patients without reaching statistical significance.** ***Key words: Histamine; Codeine; Compound 48/80.***

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L. Shall, Department of Dermatology, St. Bartholomew's Hospital, New Road, Rochester, Kent ME1 1BS, UK.

Urticaria encompasses a wide variety of afflictions that share transient cutaneous wealing and erythema as a hallmark of the disease. Urticaria should therefore be thought of as a cutaneous reaction that can be precipitated by a wide variety of triggers and which involves the release of histamine and other mediators from dermal mast cells.

Despite the fact that some of the precipitating factors have been identified, most urticaria remains classified as idiopathic, and chronic urticaria is defined as typical episodic skin lesions occurring for more than 6 weeks in the absence of obvious immunological, biochemical or physical causes. It is plausible that the mast cells of individuals with chronic urticaria are inherently unstable and degranulate more readily than those in the skin of healthy individuals.

The aim of this study was to determine whether patients with chronic urticaria reacted differently from healthy volunteers after intradermal challenge injections of saline, histamine and the experimental mast cell degranulators, codeine and compound 48/80.

## MATERIALS AND METHODS

### *Patients*

For the purposes of this study, the following inclusion criteria were applied to the patients. Urticaria was present for 6 months or longer and symptomatic dermographism, as demonstrated by firm stroking of the skin of the back with a blunt instrument to produce itchy weals, was not present. The interval between attacks was no longer than 24 h. Antihistaminics, systemic steroids and non-steroidal antiinflammatory therapy were stopped 1 week before the start of the study. No patients had received long-acting antihistaminics such as astemizole.

Fourteen patients (7 females, 7 males; age range 27–57, mean 40.7 years) with urticaria (but no dermographism) for 8 months to 18 years were recruited. Eleven healthy non-atopic individuals (10 females, 1 male; age range 18–62, mean 39.5 years) on no medication were recruited as controls. Both patients and volunteers gave witnessed informed consent for the study, which was approved by the ethics committee of the University Hospital, Nottingham.

### *Methods*

Each individual was challenged with intradermal injections of 4 substances, namely 0.9% saline (Phoenix Pharmaceuticals Ltd.), histamine acid phosphate (Macarthy Medical Ltd.), codeine phosphate (Macarthy Medical Ltd.) and compound 48/80 (Sigma (London) Chemical Co.). Twenty micrograms of histamine and 10 μg of both 48/80 and codeine were diluted in 0.1 ml saline and injected intradermally into the volar surface of the forearms (histamine and saline into the right, codeine and 48/80 into the left) as far apart as possible, using 0.5 ml single use insulin syringes (B-D Lodose) fitted with a micro-fine III needle (0.36 mm × 12.7 mm). Five minutes after each injection, the perimeter of the resulting weal and flare was traced onto a transparent acetate sheet and the areas subsequently calculated by computer-aided planimetry (Seescan Imaging, Seescan Limited, Cambridge, UK). The process of tracing and measurement was repeated at 15, 30 and 45 min following the injection.

### *Statistics*

In order to correct for the skewness of the distribution, a square root transformation was used for the areas of both the weals and flares.

All the data was assessed using a repeated measures analysis of variance with injected compounds and time as fixed effects and subject as random effect. If the F-test between the injected compounds in the analysis of variance gave a significant result, then unpaired *t*-tests were carried out between volunteers and patients for each compound.

## RESULTS

### *Normal saline*

The areas of both the weal and the flare at 5 min in both groups were similar (Table I). Flare areas following intradermal saline challenges were minimal and similar in all the volunteers. However, in the patients the flare areas increased in size between 15 and 30 min and at 45 min were still considerably larger than when initially measured. *T*-tests comparing the effects of intradermal saline on volunteers and patients demonstrated a statistically significant difference at 15, 30 and 45 min ($p < 0.05$).

Over the 45 min test period the weals in the volunteers gradually diminished and by the end of the experiment had virtually disappeared. In the patient group the weals persisted for much longer, initially increasing slightly in size but remaining approximately constant during the experiment. Although there was a definite difference between the 2 groups it was not significant.

### *Histamine acid phosphate*

Flare areas were smaller in the patient group than in the controls (Table I). Comparing patients to controls, the flare areas ran a parallel course maximum at 15 min and then

Table I. *Summary statistics of patients and controls showing mean and standard error of the mean (sem) following intradermal challenges of normal saline, histamine acid phosphate, codeine phosphate and compound 48/80*

| Time | 5 min | | 15 min | | 30 min | | 45 min | |
|---|---|---|---|---|---|---|---|---|
| | Patients | Controls | Patients | Controls | Patients | Controls | Patients | Controls |
| *Saline* | | | | | | | | |
| Weal area mm² (sem) | 85.1 (13.2) | 63.1 (13.6) | 78.4 (13.3) | 37.9 (10.8) | 60.1 (13.8) | 32.2 (20) | 64.8 (15.4) | 14.7 (13.1) |
| Flare area mm² (sem) | 82.5(48) | 0.2 (0.1) | 91.5 (65.1) | 0.03 (0.03) | 106.2 (70.2) | 0.1 (0.1) | 78.5 (51.2) | 0.1 (0.1) |
| *Histamine* | | | | | | | | |
| Weal area mm² (sem) | 241.1 (23.7) | 257 (18.5) | 296.2 (22.8) | 352 (20.6) | 359 (37.2) | 415.8 (28.6) | 362 (30.9) | 430.3 (43.7) |
| Flare area mm² (sem) | 2082.6 (245.5) | 1672.6 (160.1) | 2123.2 (223) | 2094.2 (170.8) | 1671.4 (232.5) | 1839.3 (172.1) | 1416.6 (212.4) | 1526.7 (142.1) |
| *Codeine phosphate* | | | | | | | | |
| Weal area mm² (sem) | 121.5 (8.5) | 159 (17.5) | 139.6 (11.7) | 181.9 (31.5) | 130.1 (9.1) | 168.4 (25.8) | 144.8 (14.8) | 148.1 (25.5) |
| Flare area mm² (sem) | 1249.2 (203.9) | 890.2 (177.5) | 1035.1 (179.9) | 1186.3 (173.8) | 731.8 (151.8) | 989.7 (107.2) | 591.8 (132) | 925.9 (108) |
| *Compound 48/80* | | | | | | | | |
| Weal area mm² (sem) | 66 (8.7) | 99.5 (16.5) | 91.3 (11) | 111.5 (20.2) | 102.8 (14.1) | 100 (14.7) | 100.6 (13.8) | 103.5 (20) |
| Flare area mm² (sem) | 565.7 (142.4) | 751.6 (160.2) | 708.7 (169.9) | 653 (156) | 380 (149.6) | 552.2 (114) | 341.7 (145.6) | 375 (93.1) |

declining towards 45 min. Using *t*-tests to estimate the effects of histamine induced flares in urticarial patients and healthy volunteers revealed a significant difference at 15, 30 and 45 min ($p < 0.05$).

The difference in weal area was not significant, maximal weal size being reached in the patients at 30 min and in the controls at any time between 15 and 45 min.

*Codeine phosphate*

Again, flare areas were smaller in the patient group (Table I). In the volunteer group, flare area reached a maximum at 15 min and then slowly decreased in size whereas in the urticarial group it reached maximum size at 5 min. Resolution of flare area was more rapid in the volunteers. A statistically significant difference existed between the flare areas of the 2 groups at 15, 30 and 45 min ($p < 0.05$). Weal area was smaller in the patients than in the volunteers at all times besides 45 min, but this was not statistically significant.

*Compound 48/80*

Although flare areas were smaller in the patient group, this did not reach significance at any time (Table I). Weal area ran a similar course to that produced by histamine in both patients and controls with no significant difference shown.

## DISCUSSION

Weal response is dependent upon the direct action of histamine on capillary permeability. Flare response depends on multiple co-ordinated activities: the intactness of the cutaneous nerve fibre, sufficient neurotransmitter, responsive mast cells near the nerve fibres and cutaneous blood vessels that can dilate.

A model for the mechanisms of the weal and flare proposes that mast cell degranulation occurs by one of several possible mechanisms and results in release of histamine, which directly increases capillary permeability and the resultant weal formation. It also binds to histamine-sensitive receptors on the C-fibres, which in turn promotes neuropeptide release, such as substance P, from terminal nerve endings. The flare pattern follows the cutaneous innervation because substance P causes vasodilation of vessels near the fibre endings (1).

Human cutaneous mast cells appear to have opiate receptors that, when stimulated, cause mast cells to degranulate. Five types of opioid receptors have been described in mammalian tissue (2). Morphine sulphate, codeine phosphate and endogenous opioid peptides are specific agonists for these receptors and their intradermal injection results in weal and flare reactions. Naloxone, a non-specific opiod receptor antagonist, when mixed with morphine causes a dose dependent inhibition of morphine induced weal reaction but fails to inhibit the endogenous opioid, dynorphin, induced weal response (3), suggesting that both opioid and non-opioid receptors may be involved in the immediate skin response.

The non-immunological secretagogues of histamine, such as compound 48/80, appear to involve a receptor-independent mechanism in mast cells by a direct interaction with G-proteins to release histamine (4).

In the present study, intradermal saline resulted in larger and more persistent weals and flares in the urticarial group compared to the volunteers. This finding is consistent with either a vascular instability in chronic urticaria or the postulate that mast cells of patients with urticaria are more sensitive and

degranulate more readily following a large injected volume (100 µl) with its resulting local pressure, as is seen in symptomatic dermographism, than those of healthy individuals. The resulting cascade of responses liberates histamine, neuropeptides and other proinflammatory mediators, which expresses itself clinically as large and long lasting weals and flares.

The results following intradermal codeine phosphate and compound 48/80 were unexpected, as it was thought that these results would follow a similar pattern to that of intradermal saline. However, weals and flares were smaller in the patient group but still significantly larger than that caused by saline in the same group of people. Possible explanations include the depletion of vasoactive substances from mast cells or nerve endings following repeated insults resulting in a diminished response to the agonist. Tachyphylaxis of the end organ to histamine may occur alone or in combination with mast cell depletion of mediators, or higher concentrations of histamine may be required for depolarization due to reduced responsiveness of nerve endings. A further possibility is that the usual mast cell receptors for attachment of codeine and 48/80 may become blocked by binding to a serum factor (5) released during the active phase of urticaria, resulting in a smaller weal and flare reaction than would be expected.

Intradermal histamine produced similar results to that of codeine and 48/80. This is contrary to the experience of Krause & Shuster (6). The concentration of histamine required to depolarize the nerve endings may gradually increase during the active phase of urticaria, which will result in reduced flare areas in the patients with urticaria following an intradermal challenge. The raised histamine levels demonstrated in urticarial skin (7–9) and in perilesional blister fluid following intradermal challenges of compound 48/80 in chronic idiopathic urticaria (10) add some weight to this supposition.

In conclusion we have demonstrated a sustained and greater response to intradermal saline in patients with chronic idiopathic urticaria as well as a diminished weal and flare response to intradermal codeine and histamine compared to healthy, nonatopic individuals. In order to validate further our conclusions, dose response curves using the drugs tested in both population groups are necessary to confirm that the dose response curve in either group has not been shifted to the right or left and so show that urticaria patients are not more or less responsive to the tested urticants. It is also possible that the maximal flare reactions in the patients with urticaria occurred earlier than at our first measurement time. These 2 areas will be addressed in future studies.

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