

Studies on Mast Cells and Histamine Release in Psoriasis: The Effect of Ranitidine

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The purpose of this study was to investigate histamine and skin mast cells in psoriasis before and during 6 months of treatment with high-dose ranitidine. Sixteen psoriasis patients, presenting a mean PASI score of 15.4, were compared with 13 age- and sex-matched healthy controls. Resting extracellular skin levels of histamine and histamine release to mast cell secretagogues, as measured by the microdialysis technique, were increased in involved psoriasis skin compared to normal skin in the controls. Plasma histamine, but not basophil histamine release, was significantly increased in the patients. Mast cells and lymphocytes were significantly increased in numbers in involved versus non-involved skin in the patients, the lymphocytes being predominantly T-lymphocytes expressing HLA-DR activation. During 6 months of ranitidine treatment, mean PASI score of 15.4 decreased to 5.8. The lymphocyte infiltration, but not mast cell numbers, was significantly reduced during treatment, and histamine release to mast cell secretagogues was normalized. These observations suggest that skin mast cells in active psoriasis are functionally hyperreactive. The biochemical findings together with the clinical effect of ranitidine indicate that histamine may be involved in the pathophysiology of psoriasis.

(Accepted December 10, 1997.)

Acta Derm Venereol (Stockh) 1998; 78: 190–193.

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Studies have indicated that psoriasis is a T-cell dependent autoimmune disease which develops primarily in genetic susceptible subjects. A variety of effector cells and inflammatory and proinflammatory mediators may be involved in the pathophysiology (1, 2).

Based on circumstantial evidence, mast cells and histamine may be involved in the pathophysiology of psoriasis. First, dermal infiltration of mast cells is one of the earliest findings in developing psoriasis lesions, and mast cells are consistently demonstrated in increased numbers in active psoriasis plaques (3, 4). Second, sensory nerve cell–mast cell interactions are altered in psoriasis. The number of neuropeptide containing sensory nerves is increasingly expressed in psoriatic skin, and the numbers of contact sites between nerve fibres and mast cells are increased (5). Neuropeptides may interact with mast cells bi-directionally. Upon intradermal injection in human skin, neuropeptides, including substance P and vasoactive intestinal peptide, cause vasodilation and plasma extravasation, entirely or partially, by their ability to release histamine from human skin mast cells as shown *in vitro* and *in vivo* (6). In addition to

being activated by neuropeptides, mast cells may regulate neuropeptide activity. Mast cell proteases, such as tryptase and chymase, may play a crucial role in neurogenic inflammation in psoriasis because of their neuropeptide-degrading activities, which seemed to be impaired in psoriasis (7). Third, the involvement of histamine in the pathogenesis of psoriasis is substantiated by clinical improvement during long-term treatment with H₂-receptor antagonists (8, 9). The possible roles of histamine in psoriasis have been discussed recently (10).

The purpose of this study was to investigate histamine, mast cells and basophils in psoriasis and to follow these parameters during therapy with high-dose ranitidine.

MATERIALS AND METHODS

Subjects

Sixteen patients suffering from psoriasis vulgaris participated in the study. Median PASI score at entry was 15.4 (range 9.0–24.7). The patients are described in detail elsewhere (9). The control group consisted of 13 healthy, non-medicated age- and sex-matched subjects. All participants gave informed consent, and the study was approved by the ethics committee of Copenhagen hospitals and the Danish National Board of Health.

Microdialysis and instrumentation

A microdialysis technique was used to measure histamine levels in intact skin. Single dialysis fibres of 216 m diameter, 2 kDa molecular weight cut off, and 20 mm length for diffusion were inserted in EMLA-treated skin (11, 12). Three dialysis fibres were inserted at a distance of at least 3 cm between adjacent fibres. In most patients, dialysis fibres were inserted in separate psoriasis plaques in the same anatomical region. A reconstitution phase of 2 h was interposed before any measurements (12). Local anaesthetics do not effect histamine release (13).

For determination of histamine levels in unchallenged skin sites, each fibre was perfused with Krebs Ringer bicarbonate at a rate of 1.0 l/min for 60 min. Control experiments were performed to compare diffusion characteristics of extracellular compounds in psoriatic and normal skin. The dialysate/plasma glucose concentration ratio was selected as an indicator for diffusion characteristics since glucose rapidly equilibrates within the extracellular water space and skin concentrations are identical with plasma concentrations during steady state (11).

After measurement of histamine levels in unchallenged skin, histamine release by codeine and substance P was studied. The perfusion rate was 3.0 l/min and sampling fractions were 6 µl, which allowed a detailed characterization of histamine release (12). Fifty µl aliquots of codeine (250 mM), substance P (2 µM), or vehicle (saline with 0.02% human serum albumin) were injected intradermally above individual fibres. Dialysates were collected in 6 µl fractions before (–4 to 0 min) and from 0 to 14 min after the injection at 2-min intervals.

Histological examinations and immunohistochemistry

Histological examinations were performed in the patients, not in the corresponding controls. Four 3-mm punch biopsies were taken from

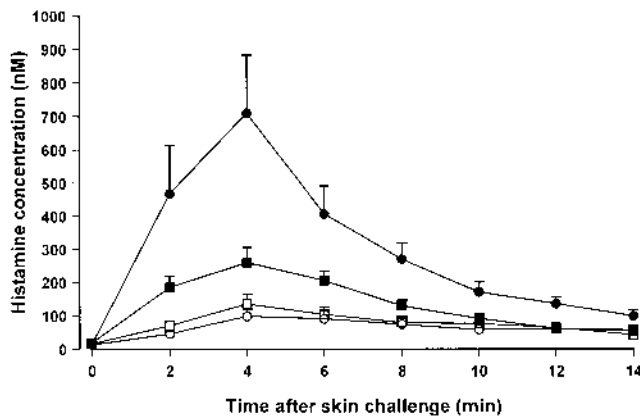


Fig. 1. The time course of histamine release in involved skin of untreated psoriasis patients (filled markers) and healthy controls (open markers) following the intradermal injection of 2 µM of substance P (squares) and 250 mM of codeine (circles). Values are mean $\pm$ SEM (16 patients and 13 controls).

involved and non-involved skin before and after 3 and 6 months of treatment. The biopsy locations were symmetrically chosen and, for each individual patient, they were performed in the same anatomical region every time.

One set of biopsies was prepared for immunohistochemical analysis. The other set was formalin-fixed and stained with haematoxylin-eosin, chloroacetate esterase, or the antibodies UCHL-1 (anti-CD45R0, 1:200), L-26 (anti-CD20, 1:100) and anti-HLA-DR 1:25 (DAKO, Glostrup, Denmark). Chloroacetate esterase stained mast cells as well as granulocytes, but granulocytes were infrequently found in psoriatic skin and they could be easily recognized by their lobulated nuclei. Cryostat skin sections were stained with anti-CD4 and anti-CD8 antibodies (DAKO).

An Olympus BHS microscope and a computerized optical dissector video system (GRID[®] Medicosoft, Silkeborg, Denmark) was used. In each biopsy, 30 test grids (0.17 mm²) were counted. Frames were counted from the basement membrane, 3 rows of 10 adjacent frames perpendicular to the epidermis were visualized. The count were expressed as cells/mm². A 4-point semiquantitative score from zero to 3+ was used to describe HLA-DR staining, representing no staining, weak staining, moderate staining and strong staining. The CD4⁺/CD8⁺ ratio was expressed as 1:1, 2:1 or 3:1 respectively.

Basophil histamine release

Histamine release from washed whole blood was performed as previously described (14). In short, blood was drawn into heparinized glass, mixed with Pipes buffer, gently centrifuged, the supernatant was discharged, and the blood cells were resuspended in Pipes buffer. The blood cells were challenged with anti-IgE (0.4–400 U/ml) and buffer. Basophil sensitivity (the concentration of anti-IgE causing the release of >20% of total basophil histamine content), maximum histamine release to anti-IgE, and total basophil histamine content were analysed.

Mediator assays

Histamine concentrations, except for those in plasma, were analysed using a glass fibre-based spectrofluorometric assay (15). The detection limit of this assay is 25 nM histamine (12). Plasma concentrations of histamine and stem cell factor were analysed using commercially available enzyme immunoassays without modifications (histamine: Immunotech, Marseille, France, sensitivity 0.2 nM; stem cell factor: Amersham, Buckinghamshire, U.K., sensitivity 3 pg/ml). Glucose concentrations were analysed on a Beckman II Analyzer (Beckman Instruments, Fullerton, CA).

Treatment

The patients, not the controls, were treated with the H₂-receptor antagonist ranitidine (Zantac[®], Glaxo) 300 mg orally twice daily for 6 months as previously described (9). Systemic drugs and ultraviolet irradiation were stopped 4 weeks before entry, so were local drugs 2 weeks before entry.

Statistics

Descriptive statistics included calculation of mean $\pm$ SEM if not otherwise stated. The non-parametric Friedman and Kruskal-Wallis analysis of variance tests were used for paired and unpaired measurements of more than two sets of data, whereas the Wilcoxon and Mann-Whitney tests were used in the case of paired or unpaired two-sample data. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Clinical outcome

Mean PASI score declined from 15.4 to 12.7, 9.1 and 5.8 after 1, 3 and 6 months of treatment, respectively (*p* < 0.001). Further details of the clinical results of these patients (patients 1–16) plus four additional patients have been presented in detail elsewhere (9).

Histamine measurements

In this section, data from involved skin in the patients were compared with data from normal skin of healthy volunteers. Histamine levels in unchallenged skin in the patients before treatment were 50.3 $\pm$ 8.1 nM compared with 31.3 $\pm$ 2.7 nM in the controls (*p* < 0.05). The dialysis efficacy, estimated as the dialysate/plasma glucose ratio, was identical in the patients and in the controls, the ratios being 0.45 $\pm$ 0.03 and 0.44 $\pm$ 0.02, respectively. Plasma histamine concentrations of 3.71 $\pm$ 0.33 nM in the patients were significantly increased compared to control values of 2.55 $\pm$ 0.31 nM (*p* < 0.01). In patients and controls, skin histamine levels were 12–14 fold the values in venous plasma (*p* < 0.001). During treatment skin histamine concentrations rose from 50.3 $\pm$ 8.1 nM to 76.7 $\pm$ 10.8, 74.5 $\pm$ 7.6, and 68.1 $\pm$ 11.9 nM after 1, 3 and 6 months of treatment [not statistically significant (NS)]. Plasma histamine decreased from 3.71 $\pm$ 0.33 nM to 2.99 $\pm$ 0.26, 2.70 $\pm$ 0.20, and 2.76 $\pm$ 0.36 nM at 1, 3 and 6 months (NS). Skin/plasma glucose ratio was constant throughout the 6-month study period (data not shown).

Total histamine content was 188.9 $\pm$ 34.2 and 132.5 $\pm$ 22.1 ng histamine in 100 mg wet weight skin in the patients before and after 6 months of treatment (NS).

There were no statistically significant differences between patient and controls regarding basophil sensitivity or maximum histamine release to anti-IgE, or total basophil histamine content (data not shown). Further, plasma concentrations of stem cell factor in the patients (1183 $\pm$ 51 ng/ml) were similar to control values (1162 $\pm$ 45 ng/ml).

Substance P and codeine released significant amounts of histamine in the patients as well as in the controls compared to buffer sites (*p* < 0.001) (Fig. 1). Peak histamine release by substance P of 270.8 $\pm$ 60.5 nM in the patients before treatment were almost twice that seen in the controls (150.2 $\pm$ 34.6 nM), but this difference did not reach statistical significance. Total histamine release was 4.06 $\pm$ 1.04 and 2.81 $\pm$ 0.67 pmol in 14 min (NS). Codeine-induced peak histamine release of

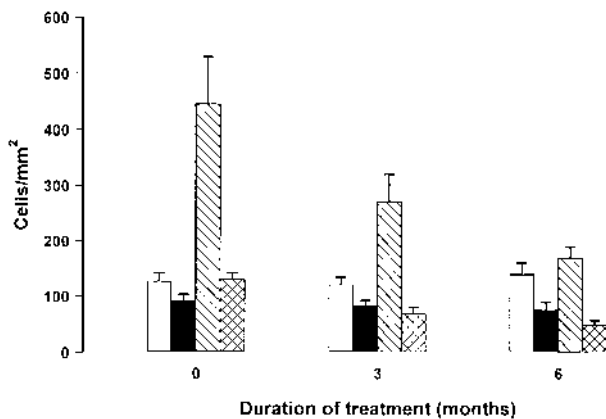


Fig. 2. Numbers of cells/mm² in psoriasis skin before and after 3 and 6 months of treatment. Values are mean ± SEM of 16 patients. Open bars: mast cells in involved skin; closed bars: mast cells in non-involved skin; single hatched bars: lymphocytes in involved skin; double-hatched bars: lymphocytes in non-involved skin.

725.9 ± 195.1 nM in untreated patients was significantly larger than control values of 99.5 ± 22.7 nM ($p < 0.01$). Total histamine releases were 9.77 ± 2.33 and 1.67 ± 0.40 pmol ($p < 0.01$). In most cases, peak histamine concentrations were found 4 min after skin challenge in patients and controls. After 6 months of treatment, substance P-induced and codeine-induced peak histamine release was reduced from 270.8 ± 60.5 to 104.2 ± 11.9 nM ($p < 0.05$) and 725.9 ± 195.1 to 122.1 ± 25.9 nM ($p < 0.01$).

Histological examinations

In this section, data from involved and non-involved skin in the patients were compared. Fig. 2 shows mast cell and lymphocyte numbers before and during treatment. Before treatment, the numbers of mast cells in involved and non-involved skin were 127 ± 15 and 91 ± 13 cells/mm² ($p < 0.05$). There were no significant changes in mast cell numbers in either involved or non-involved skin during treatment. There was a pronounced infiltration of lymphocytes in involved skin (446 ± 84 cells/mm²) compared to non-involved skin (131 ± 12 cells/mm²) before treatment ($p < 0.001$). During treatment the numbers of lymphocytes decreased in both involved ($p < 0.001$) and non-involved skin ($p < 0.01$).

Immunohistochemical staining in involved skin showed that the lymphocytes represented predominantly T-lymphocytes. The CD4+/CD8+ ratio was 23% (1 : 1), 46% (2 : 1), and 31% (3 : 1); this did not change significantly during treatment. Values in non-involved skin were not analysed. HLA-DR immunostaining was significantly increased in involved skin (0: 7%, +: 36%, ++: 36%, and +++: 21%) compared to non-involved skin (0: 15%, +: 85%) ($p < 0.05$). No significant changes were observed during treatment.

DISCUSSION

Several findings in this study suggested alterations in mast cell characteristics and functions in psoriatic skin. Compared to control skin in healthy volunteers, mast cells were higher in

numbers, skin and plasma histamine concentrations were increased, and histamine release to mast cells secretagogues were raised.

Dialysate histamine levels in psoriatic skin were higher than in the skin of healthy controls. Since the diffusion capacity was similar in patients and controls, this observation seems to reflect increased extracellular histamine levels in psoriatic skin. Skin histamine levels of histamine were higher than plasma levels in patients and controls, suggesting a low incessant histamine release from skin mast cells in both groups. Since mast cell numbers and total skin histamine content in the healthy controls, as well as histamine metabolism in general, were not measured, we can only speculate whether the increased plasma histamine levels reflected increased incessant skin mast cell degranulation in the patients. During treatment with ranitidine, skin histamine concentrations remained unchanged or increased slightly, and histamine release to substance P and codeine was normalized. Mast cell numbers did not change significantly, nor did total skin histamine content. The finding of a constant increased number of mast cells during successful anti-psoriatic treatment is in accordance with previous findings (16, 17). The findings of unaltered mast cell numbers and total skin histamine content along with decreased histamine release to mast cell secretagogues during treatment suggest a decrease in histamine releasability during remission.

Previously, increased histamine releasability has been demonstrated in bronchoalveolar mast cells in atopic asthma patients, and in basophil and eosinophil granulocytes in atopics (18, 19). Increased mast cell releasability has been shown in the skin of chronic urticaria patients and this increased releasability normalized during spontaneous remissions (20, 21). A consistent histological finding in chronic urticaria skin sites as well as in psoriasis plaques is a cellular infiltrate dominated by T-lymphocytes. This has led some authors to hypothesize that increased mast cell releasability in urticaria is closely related to lymphocyte-derived histamine-releasing factors which locally modify the degranulation processes. In this study the number of T-lymphocytes decreased significantly along with decreased histamine releasability, but the data do not provide a causative explanation for these alterations. The histamine-releasing factors remain to be determined. However, neither peripheral blood mononuclear cell-derived histamine-releasing factors nor the most potent of these factors, the CC-chemokines in human cloned forms, release histamine from human skin mast cells (22, 23). Stem cell factor, which is a potent histamine-releasing cytokine of skin mast cells (22), was not found in increased concentrations in the systemic circulation in the patients compared to healthy controls. Skin stem cell factor concentrations could not be assessed because of methodological aspects. Increased skin mast cell releasability seemed not to be related to atopic skin diseases exclusively, but may be triggered by local factors. The possible effects of extracellular matrix and cellular components on mast cells functions *in vivo* warrant further studies.

The mast cell secretagogues codeine and substance P induced histamine release in the patients compared to the controls 10-fold and 2-fold, respectively. Immunohistochemical reports have suggested impaired degradation of substance P in the skin of psoriatics (7). Histamine release to substance P in patients and controls was not significantly different, which suggests a normal neuropeptide-induced mast cell activation in psoriasis. The exact mechanism by which these compounds

degranulate mast cells remains unsolved, but it appears to occur via the same, non-receptor mediated, G-protein activation (6). Our data allow no valid explanation for the difference in magnitude of histamine release in the patients compared to the controls for these two substances.

We are aware of the fact that the design of this study makes it difficult to interpret changes in clinical and biochemical data during treatment. It remains to be elucidated whether the observed biochemical changes occurred exclusively as a consequence of treatment effect. It is generally believed, however, that H₂-receptor antagonists do not modulate histamine release from human skin mast cells (24). Also, non-involved skin in psoriasis may show signs of altered biochemical functions and cellular composition. The design of the present study did not allow detailed studies of such alterations. Additional studies may further reveal the pathophysiological roles of histamine in evolving psoriasis, and explore the therapeutical use of H₂-antihistamines in controlled clinical trials.

ACKNOWLEDGEMENTS

Dr. Petersen holds a research fellowship from the University of Copenhagen. The work was supported by the Novo Foundation Committee, the Aage Bang Foundation, the Allergy Foundation of 1981, Glaxo Wellcome A/S, the Health Foundation "Danmark", and the Danish Psoriasis Research Foundation. The laboratory assistants I. Aakilde, K. Vangsgaard and B. S. Nielsen, as well as the staff at the Reference Laboratory, are thanked for their skilful technical assistance.

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