Anti-inflammatory drugs modulate histamine release from mast cells induced by fibrinogen degradation products

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SUMMARY The products resulting from proteolytic degradation of human fibrinogen (FDP) were found to induce the release of histamine from rat peritoneal mast cells. Low molecular weight, dialysable peptides (FDP) showed the highest dose dependent, histamine releasing activity. Histamine release induced by FDP was effectively inhibited by the gold compound auranofin at a concentration of 10^{-5} – 10^{-7} mol/l and also by the non-steroidal anti-inflammatory drugs BW 755c, timegadine, medosan, naproxen, and aspirin at the higher concentration range of 10^{-4} – 10^{-6} mol/l. It is concluded that the release of histamine from mast cells may be modulated to some extent by anti-inflammatory drugs, especially auranofin, BW 755c and timegadine, a functional property which may be beneficial in the management of joint disease.

Low molecular weight fibrinogen degradation products (LMW-FDP) derived from plasmin digestion exhibit a variety of biological activities. These peptides inhibit platelet aggregation,¹ are chemotactic to polymorph neutrophils,² suppress immunoreactivity,³ damage kidney⁴ and endothelial cells in culture,⁵ increase the progression of some experimental tumours,⁶ and enhance the permeability of capillaries and the blood-brain barrier.⁷ The recently reported release of histamine from mast cells by peptides cleaved from fibrinogen may explain their effects on the microvasculature.⁸

Interest in the role of mast cells in joint disease has increased since reports of their increased numbers in the rheumatoid synovium,^{9,10} and their demonstration at sites of cartilage erosion.^{11,12} Indeed, the measurement of histamine in synovial fluids from both rheumatoid and osteoarthritic patients,^{13,14} and the report that synovial fibroblasts and articular chondrocytes possess histamine receptors,^{15–17} support the concept that pharmacological control of histamine release in joint disease may be beneficial.

The purpose of the present work was to compare the activity of various peptides cleaved by plasmin

from human fibrinogen as inducers of histamine release from mast cells and to examine the influence of anti-inflammatory drugs on this process.

Materials and methods

Peritoneal fluids were collected from male Wistar rats (200–350 g), and the cell suspension containing about 5% mast cells was purified by centrifugation in Percoll.¹⁸ The purity of the final mast cell preparation was greater than 95% and viability averaged 98% as assessed by trypan blue exclusion. The mast cell suspension contained 2×10^5 cells/0.2 ml of 145 mM NaCl, 0.9 mM CaCl₂, 2.4 mM KCl, 0.1% glucose, and 0.1% human serum albumin adjusted to pH 7.4 with Sørensen phosphate buffer. The suspension was exposed to various concentrations of FDP and the samples were incubated for 30 min at 37°C. Cells were separated from the medium by centrifugation (400 g for five minutes) at 4°C, and histamine was determined fluorimetrically in both the supernatant and cells using the *o*-phthalaldehyde method of Short *et al.*¹⁹ Histamine released into the supernatant was expressed as a percentage of the total cellular histamine content. All values were corrected for the spontaneous histamine release (5–8%) which occurred in the absence of any inducer.

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For the experiments with anti-inflammatory drugs the mast cell suspensions were preincubated for 15 min at 37°C with various concentrations of the substances tested before the addition of FDP. The results were expressed as the percentage inhibition of histamine released by control incubations.

FDP were obtained by digestion of human fibrinogen by plasmin (human plasminogen activated by streptokinase). Fibrinogen was digested with plasmin (100:1) at 37°C for various time intervals and proteolysis was stopped by addition of soybean trypsin inhibitor (0.1 mg/ml). FDP samples were fractionated by gel filtration on a Sephadex G-25 column (2×75 cm), eluted with 0.14 M NaCl at a flow rate of 30 ml/h, and monitored at 280 nm. The column was calibrated under the same conditions using bradykinin (mol. wt 1240), trasyolol (mol. wt 6500) and cytochrome c (mol. wt 12 600) as standards. The FDP eluted in individual peaks of absorbancy 280 nm was pooled.

The chemicals and reagents were obtained as follows: fibrinogen (Kabi, Uppsala, Sweden), auranofin (Smith Kline & French Laboratories Philadelphia, USA), BW 755c (Wellcome, Temple Hill, UK), naproxen (Syntex, Basle, Switzerland), timegadine (Leo Pharmaceutical Products, Ballerup, Denmark), medosan (Sigma Tau, Rome, Italy), and aspirin (Polfa, Warsaw, Poland). Streptokinase (Biomed, Warsaw, Poland) contains streptodornase (4:1). The other reagents were commercial products of analytical grade.

Results

The effect of unfractionated mixtures of fibrinogen derived peptides on histamine release from mast cells is shown in Fig. 1. The histamine releasing potency increased with the length of fibrinogen digestion, a 24 hour digestion producing more than a fourfold increase over that of the initial preparation.

Fig. 1 The effect of unfractionated fibrinogen degradation products (FDP) on histamine release from mast cells. Results are the mean of six experiments.

Dialysable peptides were more active and showed the highest dose dependent, histamine releasing activity (data not shown).

Analysis of 24 hour plasmin digested fibrinogen samples on Sephadex G-25 indicated that peptides of various sizes were generated (Fig. 2). The major peaks observed were peak I eluting at the void volume and peaks II and III eluting at approximately 4300 and 1400 daltons respectively. The effect of these fractions on the release of histamine from mast cells is shown in Fig. 3. Fraction III had a strong histamine releasing potency, whereas fraction I was inactive. Fraction II showed only weak activity and therefore was not studied further. The release of histamine by fraction III was dependent on

Fig. 2 Molecular sieve chromatography of 24 hour FDP on Sephadex G-25. The flow rate was 30 ml/h and 4 ml fractions were collected.

concentration and approximately 85% of the total mast cell histamine was released at a concentration of 10^{-3} mol/l. The time course of the fraction III mediated histamine release was examined at a concentration of 10^{-4} mol/l and was apparently completed within 20 minutes (Fig. 4). The effects of the anti-inflammatory drugs auranofin, timegadine, medosan, naproxen, BW 755c, and aspirin on the FDP (fraction III) mediated histamine release from mast cells were subsequently examined and are shown in Fig. 5. Exposure of mast cells to different

Fig. 3 Concentration dependent effect of FDP (fractions I-III) on histamine release from mast cells. Results are the mean of three experiments.

Fig. 4 Time course of histamine release from mast cells by FDP (fraction III). Results are the mean of three experiments.

Fig. 5 Effect of anti-inflammatory drugs on FDP (fraction III) induced histamine release from mast cells. Results are the mean of five experiments.

concentrations of auranofin from 10^{-4} to 10^{-8} mol/l caused dose dependent inhibition of histamine release. The effect was maximal, with 100% inhibition at an auranofin concentration of 10^{-5} mol/l. Lower concentrations of 10^{-6} and 10^{-7} mol/l produced 80% and 40% inhibition of histamine release respectively. The highest concentration of each drug used had no adverse effects on cell viability, at least over the time course of these experiments. All the compounds examined, including the dual inhibitors of cyclo-oxygenase and lipoxygenase activity (BW 755c, timegadine, and medosan), inhibited histamine release, with almost complete inhibition being achieved at drug concentrations of 10^{-4} to 10^{-5} mol/l. After auranofin BW 755c was the most effective blocker of histamine release. Naproxen and aspirin, compounds inhibiting cyclo-oxygenase activity, were less effective. These drugs only partially blocked histamine release at concentrations many times greater than those at which they are known to be effective as cyclo-oxygenase inhibitors.

Discussion

The results of this study support the hypothesis that LMW-FDP should be added to the list of agents capable of inducing the release of histamine from mast cells. Although it is uncertain whether similar concentrations of these fibrinogen degradation products occur in vivo, their potential physiological action should not be excluded. Recent reports have shown that FDP can liberate some constituents or factors from other cell types, for example, small peptides derived from fibrinogen release PG I₂ from vascular segments,^{20 21} and fragment D of human fibrinogen stimulates macrophages to induce a factor related to the hepatic synthesis of antithrombin III and fibrinogen.²² The effect of LMW-FDP

on microvascular permeability may be mediated in part by the release of histamine from mast cells, especially as the former may be inhibited by histamine H₁ receptor antagonists.⁸ Other LMW-FDP activities, such as PG I₂ release²¹ and the potentiation of the effects of various natural vasoactive compounds,²³ including that of the bradykinin action,²⁴ may also contribute to the mechanism underlying increased permeability of the natural biological barrier.

Our study shows that the histamine release from mast cells induced by LMW-FDP was suppressed by the dual cyclo-oxygenase and lipoxygenase blockers BW 755c, timegadine, and medosan, but not by cyclo-oxygenase inhibitors.²⁵ The most effective inhibitor was auranofin. It is known that different gold compounds inhibit the cyclo-oxygenase pathway of arachidonic acid metabolism,²⁶ but auranofin has no inhibitory action on the synthesis of lipoxygenase products.²⁷ This compound is also reported to inhibit the release of collagenolytic enzymes from neutrophils,^{28,29} histamine release from basophils and mast cells,³⁰ and is effective in reducing the carrageenin induced rat paw oedema.³¹ It was suggested, therefore, that auranofin modulates the release of the inflammatory mediators from basophils and mast cells by inhibiting a common metabolic step,³⁰ but the mechanism of action of auranofin on these cells has yet to be resolved.

We conclude that FDP effectively induce the release of histamine from rat peritoneal mast cells, and that this response is abrogated by auranofin and other non-steroidal anti-inflammatory drugs. This observation may be relevant to the mode of action of these compounds in the management of inflammatory joint diseases.

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