Inflamm. res. 50, *Supplement 2* (2001) S47–S48
1023-3830/01/02S47-02 $ 1.50+0.20/0

Inflammation Research

Flow-cytometric analysis of basophil activation: inhibition by histamine at conventional and homeopathic concentrations

V. Brown and M. Ennis

Department of Clinical Biochemistry, Institute of Clinical Sciences, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ, UK, Fax: +44 28 90236143, e-mail: m.ennis@qub.ac.uk

Introduction

Human basophils play a pivotal role in the pathogenesis of chronic allergic diseases such as asthma. Activation of basophils by cross-linking of IgE induces fusion of the cytoplasmic granules with the plasma membrane and the subsequent release of potent mediators including histamine. Basophil activation can be quantified by the measurement of secreted histamine [1] and by direct microscopic examination of the percentage of degranulated cells [2]. More recently, flow cytometry has been used to measure basophil activation using the cell surface marker CD63, in conjunction with anti-IgE [3]. CD63 is expressed on intracytoplasmic granules of resting basophils and only weakly expressed on the outside membrane (<5%) [4]. However, basophil activation leads to the upregulation of CD63 on the cell surface, which can be easily quantified using flow cytometry. Both pharmacological concentrations and high dilutions of histamine have been reported to modulate anti-IgE induced basophil degranulation [5]. The aim of this study was to confirm that a 2-colour (anti-IgE FITC and anti-CD63 R-PE) flow cytometric method can quantify basophil activation and to investigate the effects of histamine on basophil activation.

Materials and methods

Leukocyte suspensions were obtained by sedimentation (1–1.5 h) of heparinised blood from healthy control subjects. Cells were washed (2×) with Hepes EDTA buffer and then incubated with equal volumes of histamine solutions (final concentrations: 10^{-2} to 10^{-40} M) for 30 min at room temperature, essentially as previously described [4]. Basophil activation was induced by incubation of 20 µl cells with 20 µl anti-human IgE (0.2 µg/ml, Dako, UK) for 30 min at 37 °C. Cells were washed and labelled with 10 µl anti-IgE FITC (0.5 µg/10^6 cells, Caltag, USA) and 10 µl anti-CD63 PE (1 µg/10^6 cells, Caltag, USA) for 20 min at 4 °C. Basophils were selected by their brightly fluorescent anti-IgE FITC (high mean channel fluorescence MCF). Data from at least 250 basophils (in duplicate) were used to measure the proportion of activated basophils expressing CD63 and each experiment was performed at least 5 times. Negative controls consisted of isotype-matched, directly conjugated non-specific antibodies.

Correspondence to: M. Ennis

Effect of physical treatments of histamine dilutions on their biological activity

Temperature – Histamine dilutions were heated to 70 °C for 30 min. These solutions were tested in parallel with the same solutions that had not undergone heating.

Freezing/thawing – Histamine dilutions were frozen (–70 °C) and then thawed to room temperature (both processes completed twice) and analysed in parallel with histamine dilutions that had not undergone this cycle.

Results and discussion

Activation of basophils with anti-IgE caused a considerable increase in $CD63^+$ cells compared with unstimulated cells (15.2 ± 3.5% and 64.2 ± 4.1% respectively, n = 10, $p < 0.0001$, Wilcoxon signed rank test).

Effect of histamine dilutions on basophil activation

Significant inhibition of CD63 expression was observed after incubation with histamine at 10^{-2}, 10^{-4}, 10^{-6}, 10^{-14}, 10^{-18}, 10^{-20} and 10^{-26} M, with maximal inhibition at 10^{-20} M histamine, 43.8% ± 11.5% p = 0.0005 (Fig. 1).

Effect of physical treatments of histamine dilutions on their biological activity

Heating histamine dilutions (10^{-2} M, 10^{-30} M, and 10^{-36} M) caused a significant decrease in the inhibitory effects of these histamine dilutions on basophil activation, with p = 0.039, p = 0.018, p = 0.0064 respectively.

Little if any effect was observed on the percentage of inhibition of activation following 2 cycles of freezing and thawing of histamine dilutions 10^{-2} M–10^{-40} M (data not shown).

These results firstly confirm that CD63 expression is upregulated on the surface of anti-IgE activated basophils compared with unstimulated cells and also that low doses of histamine have an inhibitory effect on human basophil

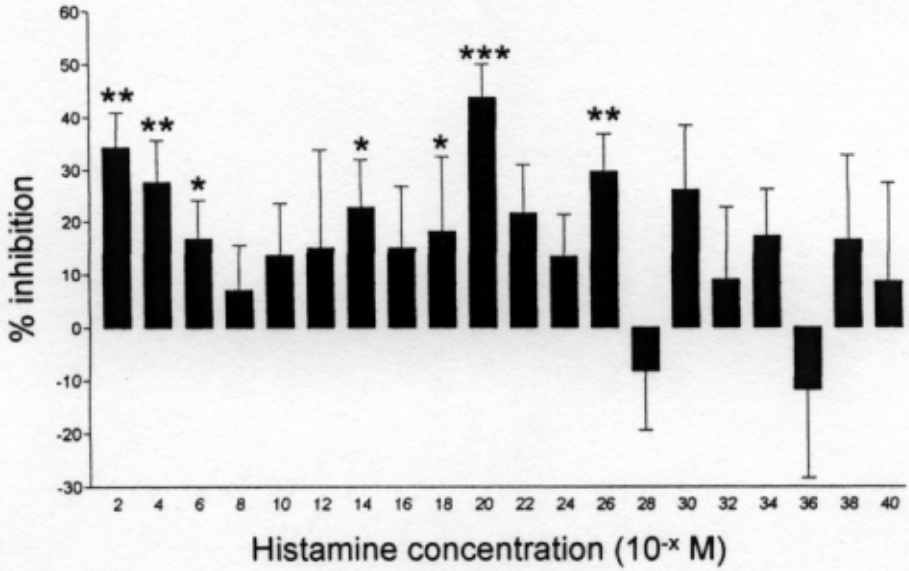

Fig. 1. Effect of histamine on anti-IgE induced expression of CD63 on human basophil membranes. Data are represented as mean ± SEM for n = 10. Statistical analysis was performed using the Wilcoxon signed rank test with * = $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

activation with significant effects observed at 10^{-2}, 10^{-4}, 10^{-6}, 10^{-14}, 10^{-18}, 10^{-20} and 10^{-26} M. We are unable to provide any explanation for the mechanism of action of these high histamine dilutions. However, the incubation of the histamine dilutions at 70 °C reduced the inhibition of basophil activation at most histamine dilutions tested. Thus basophil degranulation appears to be regulated by histamine through a negative feedback process even at homeopathic concentrations.

Acknowledgements: This work was supported by a grant from the French Institute of Homeopathic Research.

References

[1] Pruzansky JJ, Patterson R. Limiting concentrations of human basophil bound antibody required for histamine release. Immunology 1988; 64: 307–310.

[2] Gilgert HS, Ornstein I. Basophil counting with a new staining method using alcian blue. Blood 1975; 42: 279–286.

[3] Sainte-Laudy J, Vallon C, Guerin JC. Analyse de l'expression membranaire du marquer CD63 par activation du basophile humain. Aplication au diagnostic allergologique. Allergie et Immunologie 1994; 26: 211–214

[4] Knol EF, Mul FOJ, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. J Allergy Clin Immunol 1991; 88: 328–348.

[5] Sainte-Laudy J, Belon P. TI: Inhibition of human basophil activation by high dilutions of histamine. Agents Actions 1993; 38: C245–247.