Inflamm. res. 48, *Supplement 1* (1999) S17–S18
1023-3830/99/010S17-02 $ 1.50+0.20/0

Inflammation Research

Inhibition of human basophil degranulation by successive histamine dilutions: Results of a European multi-centre trial

P. Belon[1], J. Cumps[2], M. Ennis[3], P.F. Mannaioni[4], J. Sainte-Laudy[5], M. Roberfroid[6] and F.A.C. Wiegant[7]

[1] Boiron, 20 rue de la Libération, F-69110 Sainte-Foy-Les-Lyon, France
[2] UCL 7369, 73 avenue Emmanuel Mounier, B-1220 Brussels, Belgium
[3] Department of Clinical Biochemistry, Institute of Clinical Science, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ, UK, Fax +44 12 32 23 61 43, e-mail: m.ennis@qub.ac.uk
[4] Department of Pharmacology, Viale G. Pieraccini 6, I-50139 Florence, Italy
[5] Cerba, F-95066 Val d'Oise cedex 9, France
[6] Laboratoire de biotoxicologie, UCL 7369, 73 avenue Emmanuel Mounier, B-1220 Brussels, Belgium
[7] University of Utrecht, Department of Molecular Cell Biology, P.O. Box 80.056, NL-3508 TB Utrecht, The Netherlands

Introduction

The biological action of ultra high dilutions is controversial [1, 2]. Inhibition of anti-IgE induced basophil degranulation by successive histamine dilutions is of interest, as it studies a chemically defined compound (histamine) which exerts a negative feed back effect via the histamine H_2 receptor. The biological activity is measured using the human basophil degranulation test, which is relatively simple to perform and does not require specialised equipment. Inhibition of basophil degranulation was observed with histamine dilutions ranging between the 15th and 19th centesimal dilutions. Since most data were originally obtained from only one laboratory, this study aimed to verify these results in a multi-centre trial.

Materials and methods

Laboratories

Four independent laboratories agreed to participate in the trial. Prior to the start of the trial, participants underwent a training period and their results were verified by the French laboratory.

Study protocol

The study was co-ordinated in Brussels and all histamine dilutions were coded randomly by the coordinator, who did not perform any of the tests. The dilutions were prepared in 3 separate laboratories, which did not participate in the trial. The samples were then posted to the trial laboratories. All reagents, including antibodies, histamine, staining solutions, microtitre plates etc., were from the same source. Data were returned to the co-ordinator and analysed independently by a biostatistician, who was not involved in any other part of the trial.

Correspondence to: M. Ennis

Preparation of histamine dilutions

Histamine hydrochloride (50 mg, Sigma) was dissolved in distilled water (5 ml), diluted (1/10, v/v) in distilled water and vortexed for 15 s (full speed). To obtain the dilutions for the trial, this solution was serially diluted (1/100 v/v) up to 19 times, always with vortexing as described. The dilutions 15, 16, 17, 18 and 19 were coded by the co-ordinator. In parallel, dilutions of distilled water alone were prepared in an identical manner and coded (controls). On receipt of the dilutions, each participating laboratory stored them at 4 °C. Prior to use, the solutions were made isotonic by dilution (1/10 v/v) in HEPES buffer (NaCl 127 mM, KCl 5 mM, HEPES 20 mM, pH 7.4).

Human basophil degranulation test

The methods for the selection of volunteers, preincubation of cellular suspensions with the test dilutions and anti-IgE induced basophil degranulation have been described previously [3]. Cell suspensions (250 µl) were mixed with the test dilution (250 µl) and incubated at room temperature for 30 min. After mixing (3 s, medium vortex speed), aliquots (20 µl) were placed in the wells of a microtitre plate and mixed with anti-IgE (polyclonal anti-IgE affinity purified ATAB, USA, 20 µl; 1, 0.2, 0.04 µg/ml). The plates were then covered with a sealer tape (Dynatech Laboratories) and incubated for 30 min at 37 °C. Thereafter, alcian blue (100 µl) was added to each well. Stained basophils (not activated) were counted using a haemocytometer (Fuchs Rosenthal). Approximately 80 cells were counted for each well. Positive and negative controls were included in all cases.

Statistical analysis

This study was designed to investigate the inhibitory effect of histamine dilutions on basophil degranulation triggered by anti-IgE. To examine such an effect, the anti-IgE must have caused a degranulation. Thus, all data were validated by calculating the coefficient of variation (cv) of the absolute basophil count in the absence of anti-IgE for each laboratory. The minimum percentage degranulation was set at 3.4 cv for the 0.1% level of risk. Only data exceeding these values were included in the analyses.

Statistical analysis was performed at 0.1% level of risk and two sets of tests were performed based on either parameteric (GLM procedure, multivariate analysis) or non-parametric (Kruskal-Wallis) procedures.

Results and discussion

A total of 3674 datapoints were collected from the 4 laboratories, of which 840 were invalid at the 0.1% level of risk. Combining all data in the presence or absence of histamine dilutions, the overall effect was highly significant ($p < 0.0001$). However, statistical analysis demonstrated that the lowest anti-IgE concentration (0.04 µg/ml) was the best for the observation of inhibition. At this concentration, there were 772 valid data points.

Using the GLM procedure, the overall effect of all histamine dilutions (15^{th}–19^{th} centesimal dilutions) was significant ($p = 0.0001$, $F = 40.07$) (Table 1). The normality of the distribution of the basophil counts was verified using Henri plots for the control but not for the histamine-dilution treated cells (data not shown). Non-parametric analyses (Kruskal-Walis test) confirmed the inhibitory effect of histamine ($p < 0.0001$).

These data confirm previous findings that histamine, at very high dilutions, inhibits anti-IgE induced basophil degranulation. In 3/4 of the independent laboratories a statistically significant inhibition was found and in the fourth laboratory the results approached significance. Overall there was a small but statistically significant percentage inhibition of anti-IgE induced basophil degranulation.

The test solutions were made in independent laboratories, the participants were completely blinded with respect to the content of the test solutions and data analysis was performed by a biostatistician, who was not involved in any other part of the trial. The method to assess basophil degranulation has been a matter of much controversy, however in this study we have attempted to remove areas of potential problems [3]. We are further investigating this phenomenon using flow cytometry [4, 5].

Acknowledgement. Dr. Ennis gratefully acknowledges the financial support from the Royal Society to attend the European Histamine Research Society meeting in Lodz, Poland.

References

[1] Linde K, Clausius N, Ramirez G, Melchart D, Eitel F, Hedges VL, et al. Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. Lancet 1997; 350: 834–43.
[2] Doutremepuich CH, Aguejouf O, Belon P. Effects of ultra-low dose aspirin on embolization in a model of laser-induced thrombus formation. Seminars Thromb Hemostas 1996; 22 Suppl 1: 67–70.
[3] Sainte-Laudy J. Standardization of basophil degranulation for pharmaceutical studies. J Immunol Meth 1987; 98: 279–82.
[4] Sainte-Laudy J, Belon P. Analysis of immunosuppressive activity of serial dilutions of histamine on human basophil activation by flow-cytometry. Inflamm Res 1996: 45 Suppl 1: S33–4.
[5] Sainte-Laudy J, Belon P. Application of flow cytometry to the analysis of the immunosuppressive effect of histamine dilutions on human basophil activation: Effect of cimetidine. Inflamm Res 1997; 46 Suppl 1: S27–8.

Laboratory	Control (% degranulation)	Histamine (% degranulation)	Number	F	p
1	45.8	36.5	123	15.35	0.0002
2	50.2	47.5	312	n.a.	0.065
3	51.6	47.4	183	5.19	0.024
4	47.8	35.7	154	17.01	≤ 0.0001
All	48.8	41.8	772	40.07	≤ 0.0001

Statistical comparisons were made using MANOVA.

Table 1. Comparison of percentage degranulation induced by anti-IgE (0.04 µg/ml) in the absence and presence of histamine dilutions (15^{th}–19^{th} centesimal dilutions).