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Physiological concentrations of zinc inhibit the release of histamine from human basophils and lung mast cells

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Abstract

We have previously shown that physiological concentrations of zinc ($\simeq 7 \times 10^{-6}$ *M*) inhibit the release of histamine from human basophil leukocytes (Marone *et al.*, J. Pharmacol. Exp. Ther. 217: 292, 1981). In these experiments we compared the effect of zinc chloride on the release of chemical mediators from human basophils and mast cells isolated from human lung. Preincubation (5 min, 37°C) of human basophils and lung mast cells with zinc chloride (10^{-6}–3×10^{-5} *M*) caused dose-related inhibition of histamine and peptide leukotriene C_4 (LTC_4) release induced by anti-IgE. Increase Ca^{2+} concentrations (0.3 to 6 m*M*) in the extracellular medium completely reversed the inhibitory effect of zinc on anti-IgE-mediated histamine secretion. Zinc chloride was a competitive antagonist of the action of Ca^{2+} in histamine secretion induced by anti-IgE with a dissociation constant (Kd) of about 10^{-5} *M* in both the basophil and mast cell systems. Thus physiological concentrations of zinc inhibit the release of histamine from human basophils and lung mast cells, presumably by blocking Ca^{2+} uptake induced by anti-IgE activation.

Introduction

Human leukocytes [1] and rat mast cells [2] contain high concentrations (2 mcg/10^6 cells) of granule-associated zinc. Hogberg and Uvnäs first showed that zinc prevented histamine release from rat peritoneal mast cells [3]. We have previously shown that physiological concentrations of zinc ($\simeq 7 \times 10^{-6}$ *M*) inhibited the release of histamine from human basophil leukocytes. We also found that zinc was a competitive antagonist of the action of Ca^{2+} in histamine secretion induced by antigen and anti-IgE with a dissociation constant (Kd) of about 10^{-5} *M* [4]. These results suggested that the effect of zinc on histamine release from human basophils might be related to the blockade of Ca^{2+} uptake.

The activation of IgE on human basophils and mast cells leads to a complex sequence of biochemical events causing mediator release. *In vitro* studies have described some of the biochemical mechanisms of the release process, which include, in addition to preformed mediators such as histamine, *de novo* synthesized mediators such as 'Slow Reacting Substance of Anaphylaxis' (SRS-A). SRS-A includes a series of metabolically-related products of arachidonic acid metabolism: peptide leukotrienes C_4 (LTC^4), D^4 (LTD^4), and E^4 (LTE^4) [5]. These sulfido leukotrienes possess several biological properties suggesting that they participate in many aspects of inflammatory reactions [6]. Recent studies indicate that IgE-binding on human basophils and mast cells purified from human lung results in the *de novo* synthesis of immunoreactive and biologically active LTC_4 [7, 8].

Although immunological activation of human basophils and lung mast cells leads to release of both histamine and LTC_4, there are several differences in the mechanisms behind transduction of the cross-linking signal. For example, activation of histamine H_2-receptor on human basophils inhibits histamine release, while mast cells are unresponsive to H_2-agonists [9]. Furthermore, histamine release from mast cells is enhanced by adenosine, whereas basophil histamine release is markedly inhibited [10]. In summary, although human lung mast cells resemble human basophils in several respects, it is becoming clear that there are differences as regards the pharmacological control of medi-

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ator release from these inflammatory cells.

Based on these observations, we studied the effects of zinc chloride on IgE-mediated histamine and LTC_4 release from human basophils and lung mast cells.

Materials and methods

Reagents

Zinc chloride, Pipes were purchased from Sigma Chemical Co. (St. Louis, MO). Rabbit anti-human IgE (anti-IgE) was generously provided by Dr. T. Ishizaka, The Johns Hopkins University (Baltimore, MD). Rabbit anti-LTC_4 and LTC_4 were kindly donated by Dr. Rokach (Merck-Frosst, Canada).

Buffers

The Pipes buffers used in these experiments were made up of 25 m*M* Pipes, pH 7.4, 110 m*M* NaCl, 5 m*M* KCl. This mixture is referred to as P; PC contained, 1.0 m*M* $CaCl_2$ in addition to P [10].

Preparation of peripheral blood leukocytes (containing basophils)

Venous blood was obtained from normal volunteers who had given informed consent. Leukocytes (containing basophils) were isolated by dextran sedimentation as previously described [10].

Preparation of dispersed lung cells (containing mast cells)

Mast cells were purified from human lung tissue obtained from thoracotomy specimens from patients undergoing surgery for lung cancer. Mast cells were isolated as described elsewhere [11]. The preparation used in the present experiments contained approximately 3–35% mast cells.

Histamine release assay and radioimmunoassay of LTC_4

In all experiments 0.4 ml of the cell suspensions were placed in Falcon 12 × 75 mm polyethylene tubes and warmed to 37°C; 0.2 ml of the prewarmed (37°C) releasing stimulus was added and incubation was continued at 37°C for 45 min. After centrifugation (1000 × g, 22°C, 5 min), the cell free supernatants were assayed for histamine by an automated fluorometric technique [4, 10]. Immunoreactive LTC_4 was measured as described previously [12], using dextran-coated charcoal for separation.

Results

We have previously shown that zinc caused dose-related inhibition of anti-IgE-induced histamine release from human basophils [4]. This inhibition was observed at a zinc concentration of about 3×10^{-6} *M* and was maximal at 10^{-5} *M*. Similar concentrations of zinc chloride also inhibited *de novo* synthesis of LTC_4 from human basophils challenged with anti-IgE (data not shown). In this range of concentrations zinc had no effect on cell viability and caused no spontaneous histamine release.

We therefore investigated the effect of zinc on IgE-mediated histamine and LTC_4 release from human lung mast cells. Table I shows that zinc induced dose-related inhibition of histamine release and *de novo* synthesis of LTC_4 by human lung mast cells.

We have previously shown that zinc inhibited IgE-mediated release of histamine competing with Ca^{2+} for the same binding site (*i.e.*, Ca^{2+} channel) activated by these stimuli [4]. We therefore investigated the interaction between Ca^{2+} and zinc on histamine release from human lung mast cells. Figure 1 shows the inhibition of anti-IgE-induced histamine release at various Ca^{2+} concentrations (from 0.1 to 6 m*M*); increasing the concentrations of zinc shifted the dose-response curves to the right.

The parallel shift of the dose-response curves suggested that zinc might act as a competitive inhibitor of Ca^{+2}. We therefore carried out experiments to measure the affinity constant of the Ca^{2+}-receptor interaction.

Assuming that calcium (C) and zinc (Z) compete for a receptor site and that the ion-receptor (R) interaction is freely reversible, one can write the following equations:

Table 1
Effects of zinc chloride on anti-IgE-induced histamine and LTC_4 release from human lung mast cells.[a]

	Exp. 1		Exp. 2		Exp. 3	
	LTC_4	Histamine	LTC_4	Histamine	LTC_4	Histamine
Zinc chloride, *M*						
0	68	42	121	56	48	38
3×10^{-6}	41	38	76	41	24	18
10^{-5}	12	8	6	16	9	2
3×10^{-5}	0	1	0	0	0	0

[a] Anti-IgE final concentration (3 mcg/ml). Cells were preincubated with zinc chloride for 10 min before anti-IgE addition. Values are expressed as LTC_4 ng-equivalents/10^6 mast cells and percent histamine release. Each value is the mean of duplicate determinations.

$$Z + R \rightleftharpoons Z - R \text{ complex}$$
$$C + R \rightleftharpoons C - R \text{ complex}$$

where the Z-receptor (Z–R) complex inhibits histamine release and the calcium receptor (C–R) complex promotes histamine release. At equilibrium, it can be shown [13] that $(X - 1) = K_d$ where K_d is the dissociation constant of the zinc-receptor complex and X is the ratio of the calcium concentrations in the presence and absence of zinc required to produce the same effect. Plotting the logarithm of $(X - 1)$ against the logarithm concentrations (a 'Schild plot') gives, in the case of a competitive inhibitor, a straight line with a slope of 1 and an intercept on the abscissa corresponding to $-\log K_d$.

Experiments such as that shown in Figure 1 were used to construct Schild plots from

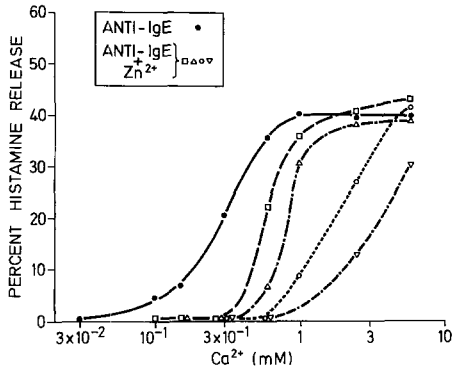

Figure 1
Effects of varying concentrations of Ca^{2+} alone (●), and in combination with zinc (□ 2×10^{-6} *M*; △ 4×10^{-6} *M*; ○ 1×10^{-5} *M*; ▽ 2×10^{-5} *M*) on anti-IgE-induced histamine release from human lung mast cells. The cells were preincubated 10 min with zinc and Ca^{2+}. Anti-IgE (3 mcg/ml) was then added and the cells incubated for 30 min.

which it was found that the K_d of the zinc-receptor complex was (mean ± S.E.M.) $1.2 \pm 0.3 \times 10^{-5}$ *M* (N = 3). The slopes were not significantly different from 1 (data not shown).

Discussion

Zinc is present in plasma at a concentration of 7×10^{-6} *M* [14], enough to cause approximately 40% inhibition of anti-IgE-induced histamine and LTC_4 release from human basophils and lung mast cells.

The precise mechanism by which zinc inhibits the immunological release of chemical mediators from human basophils and mast cells is not clear. Previous reports from our laboratory indicate that zinc is a competitive antagonist of the Ca^{2+}-dependent IgE-mediated histamine release from human basophils [4]. The present results indicate that zinc is also a competitive antagonist of anti-IgE-induced histamine release from human mast cells with a dissociation constant of about 10^{-5} *M*, similar to that in the basophil system. This suggests that zinc acts on the IgE-activated phase of the release process, present in both basophils and lung mast cells.

The plasma level of zinc in man is approximately 1 mcg/ml ($\simeq 7 \times 10^{-6}$ *M*) [14] and this concentration would be expected to inhibit mediator release from human basophils and mast cells. Thus, zinc may play a homeostatic role in the mechanism which *in vivo* controls the release of inflammatory mediators. Additional evidence suggests that zinc modulates several aspects of inflammation including serotonin release from platelets [15], macrophage and neutrophil phagocytosis [16], lymphocyte proliferation [17], and immune hemolysis [18].

Zinc has been used in humans for the treatment of a variety of diseases with little or no side effects [19]. Our results indicate that zinc inhibits IgE-mediated release of histamine and LTC_4 from human basophils and lung mast cells. Whether this can be manipulated therapeutically is not known and merits further investigations.

References

[1] R. JAROM, T.A. HALL and A. POLLIACK, *Electron microscopic X-ray microanalysis of normal and leukemic human lymphocytes*, Proc. Natl. Acad. Sci. USA *73*, 3690–3694 (1976).

[2] A.M. ANGYAL and G.T. ARCHER, *The zinc content of rat mast cells*, Aust. J. Exp. Biol. Med. Sci. *46*, 119–121 (1968).

[3] B. HÖGBERG and B. UVNÄS, *Further observations on the disruption of rat mesentery mast cells caused by compound 48/80, antigen-antibody reaction, lecithinase A and decylamine*, Acta Physiol. Scand. *48*, 133–145 (1960).

[4] G. MARONE, S.R. FINDLAY and L.M. LICHTENSTEIN, *Modulation of histamine release from human basophils in vitro by physiological concentrations of zinc*, J. Pharmacol. Exp. Ther. *217*, 292–298 (1981).

[5] S. HAMMARSTRÖM, Leukotrienes. In *Advances in Clinical Immunology. The Role of Chemical Mediators in Pulmonary and Cardiac Diseases*, pp. 77–90 (Eds M. CONDORELLI, G. MARONE and L.M. LICHTENSTEIN). O.I.C. Medical Press, Florence 1984.

[6] R.P. SCHLEIMER, D.W. MACGLASHAN, JR., S.P. PETERS, K.A. KNAUER, E.S. SCHULMAN, G.K. ADAMS, III, A. KAGEY-SOBOTKA and L.M. LICHTENSTEIN, *In vivo*

and *in vitro* studies of human anaphylaxis. In *Advances in Clinical Immunology. The Role of Chemical Mediators in Pulmonary and Cardiac Diseases*, pp. 65–76 (Eds M. CONDORELLI, G. MARONE and L.M. LICHTENSTEIN). O.I.C. Medical Press, Florence 1984.

[7] D.W. MACGLASHAN, JR., R.P. SCHLEIMER, S.P. PETERS, E.S. SCHULMAN, G.K. ADAMS, III, H.H. NEWBALL and L.M. LICHTENSTEIN, *Generation of leukotrienes by purified human lung mast cells*, J. Clin. Invest. *70*, 747–751 (1982).

[8] D.W. MACGLASHAN, JR. and L.M. LICHTENSTEIN, *Dimeric IgE may not represent the unit signal for the release of all mediators in human basophils* (abstr.), Fed. Proc. *43*, 1663 (1984).

[9] G. MARONE, *The role of basophils and mast cells in the pathogenesis of pulmonary diseases*, Int. Arch. Allergy Appl. Immunol. *76* (suppl. 1), 70–82 (1985).

[10] G. MARONE, S. VIGORITA, C. ANTONELLI, G. TORELLA, A. GENOVESE and M. CONDORELLI, *Evidence for an adenosine A_2/R_a receptor on human basophils*, Life Sci. *36*, 339–345 (1985).

[11] E.S. SCHULMAN, D.W. MACGLASHAN, JR., S.P. PETERS, R.P. SCHLEIMER, H.H. NEWBALL and L.M. LICHTENSTEIN, *Human lung mast cells: purification and characterization*, J. Immun. *129*, 2662–2667 (1982).

[12] E.C. HAYES, D.L. LOMBARDO, Y. GIRARD, A.L. MAYCOCK, J. ROKACH, A.S. ROSENTHAL, R.N. YOUNG, R.W. EGAN and H.J. ZWEERINK, *Measuring leukotrienes of Slow Reacting Substance of Anaphylaxis: development of a specific radioimmunoassay*, J. Immun. *131*, 429–433 (1983).

[13] R.J. TALLARIDA, A. COWAN and M.W. ADLER, *pA_2 and receptor differentiation: a statistical analysis of competitive antagonism*, Life Sci. *25*, 637–644 (1979).

[14] M.D. STEVENS, W.F. MAC KENZIE and V.D. ANAND, *A simplified method for determination of zinc in whole blood, plasma, and erithrocytes by atomic absorption spectrophotometry*, Biochem. Med. *18*, 158–163 (1977).

[15] M. CHVAPIL, P.L. WELDY, L. STANKOVA, D.S. CLARK and C.F. ZUKOSKI, *Inhibitory effect of zinc ions on platelet aggregation and serotonin release reaction*, Life Sci. *16*, 561–572 (1975).

[16] M. CHVAPIL, L. STANKOVA, C. ZUKOSKI, IV and C. ZUKOSKI III, *Inhibition of some functions of polymorphonuclear leukocytes by in vitro zinc*, J. Lab. Clin. Med. *89*, 135–146 (1977).

[17] K.M.K. RAO, S.A. SCHWARTZ and R.A. GOOD, *Age-dependent effects of zinc on the transformation response of human lymphocytes to mitogens*, Cell. Immunol. *42*, 270–278 (1979).

[18] M.D.P. BOYLE, J.J. LANGONE and T. BORSOS, *Studies on the terminal stages of immune hemolysis. IV. Effect of metal salts*, J. Immun. *122*, 1209–1213 (1979).

[19] E.F. GORDON, R.C. GORDON and D.B. PASSAL, *Zinc metabolism: basic, clinical, and behavioral aspects*, J. Pediatr. *99*, 341–349 (1981).