

Is tyrosine kinase activation involved in basophil histamine release in asthma due to western red cedar?

Frew A, Chan H, Salari H, Chan-Yeung M. Is tyrosine kinase activation involved in basophil histamine release in asthma due to western red cedar? Allergy 1998; 53: 139–143. © Munksgaard 1998.

Occupational asthma due to western red cedar is associated with histamine release from basophils and mast cells on exposure to plicatic acid (PA), but the mechanisms underlying this response remain unclear. Specific kinase inhibitors were used to study the role of tyrosine and serine/threonine kinases in PA-induced histamine release from human basophils. Pretreatment with the tyrosine kinase inhibitor methyl 2,5-dihydroxycinnamate (MDHC) attenuated histamine release from basophils triggered by anti-IgE (29.8% inhibition; $n=15$; $P<0.01$) or grass pollen (48% inhibition; $n=6$; $P<0.01$). Inhibition was concentration-dependent and could be reversed by washing the cells in buffer, while the inactive stereoisomer of MDHC did not affect histamine release. In contrast, the protein kinase C inhibitor staurosporin did not affect histamine release by either anti-IgE or grass pollen. Pretreatment with MDHC partially inhibited PA-induced histamine release from basophils of 6/9 patients with red cedar asthma (25.4% vs 33.8%; $P=NS$). Staurosporin gave a similar level of inhibition of PA-induced histamine release (25.3% vs 33.8%; $P=NS$). Thus, signal transduction of the human basophil $FC_\epsilon RI$ appears to depend upon tyrosine kinase activation, but not on protein kinase C (serine/threonine kinase) activation. The lack of specific effect on plicatic acid-induced histamine release in basophils obtained from patients with occupational asthma due to western red cedar suggests that tyrosine kinases are not as important in this disease as in atopic asthma, and is consistent with the view that histamine release in red cedar asthma is largely IgE-independent.

**A. Frew^{1,2}, H. Chan¹, H. Salari¹,
M. Chan-Yeung^{1,3}**

¹Respiratory Division, Department of Medicine, Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada;

²University Medicine, Southampton General Hospital, Southampton, UK; ³Member of Canadian Respiratory Health Network of Centres of Excellence

Dr A. J. Frew
University Medicine (810)
Southampton General Hospital
Southampton SO16 6YD
UK

Accepted for publication 13 August 1997

Occupational asthma due to western red cedar (*Thuja plicata*) is a common problem in the sawmill industries of the Pacific North-West of America (British Columbia, Oregon, and Washington) (1). Western red cedar asthma (WRCA) is thought to be caused by sensitization to plicatic acid (PA), a low-molecular-weight organic compound which makes up about 40% by weight of cedar dust. In sensitive subjects, inhalation of PA induces bronchoconstriction (1) and local release of histamine and leukotrienes which can be detected in bronchoalveolar lavage (BAL) fluid (2). Plicatic acid releases histamine from basophils and lung tissue of affected subjects *in vitro*, in a dose-dependent manner (3), but serologic and *in vitro* studies have

cast doubt on the role of IgE in this form of allergic asthma (3).

Cross-linking of IgE-Fc receptor molecules ($FC_\epsilon RI$) on the surface of basophils and mast cells leads to degranulation and the release of histamine. Studies of the subcellular mechanisms of IgE-dependent degranulation have shown that histamine release requires the generation of an intracellular calcium flux, which follows phospholipase C-mediated hydrolysis of inositol phospholipids and the liberation of inositol (1, 4, 5)-triphosphate and diacyl glycerol (4). At least five different coupling mechanisms have been described which enable cell-surface receptors to activate phospholipase C (PLC) in various different cell types (5–9).

In rodent models (rat basophilic leukaemia cells and mouse mast-cell lines), it has been shown that cross-linking $Fc_\epsilon RI$ rapidly activates tyrosine kinase with subsequent phosphorylation of tyrosine residues on the $Fc_\epsilon RI$ molecule and subsequently on PLC γ (10–12).

A number of agents have been developed that selectively inhibit protein kinase activity *in vitro*. These agents have been used successfully to demonstrate the importance of tyrosine phosphorylation in the degranulation of human neutrophils *in vitro* (13). In the present study, we have investigated the role of tyrosine kinase and the serine/threonine kinase, protein kinase C (PKC) in histamine release from human basophils, firstly when induced by stimuli that are known to be IgE-dependent and secondly when induced by plicatic acid.

Material and methods

Chemicals

Unless otherwise stated, all chemicals were purchased from Fisher Scientific Ltd, Vancouver, BC, Canada. Calcium ionophore A23187, human serum albumin, and antibiotics were purchased from Sigma Chemical Co, St Louis, MO, USA. Mixed grass pollen extract (no. 5, containing equal proportions of orchard, redtop, timothy, sweet vernal, and Kentucky bluegrass pollens) was purchased from Hollister-Stier (division of Miles Laboratories, Elkhart, IN, USA). Tyrode's buffer was prepared as a total of sodium chloride 8 g/l, glucose 1 g/l, HEPES 2.4 g/l, disodium hydrogen phosphate 0.06 g/l, and potassium chloride 0.2 g/l, adjusted to pH 7.4, and used for all washing steps. For *in vitro* challenge experiments, Tyrode's buffer was supplemented with magnesium chloride 0.1 g/l, calcium chloride 0.2 g/l, human serum albumin 0.1% w/v, penicillin 100 U/ml, streptomycin 100 μ g/ml, and amphotericin 0.25 μ g/ml. Goat antihuman IgE (Sigma) was reconstituted in the challenge buffer at 30 mg/ml, aliquotted, and stored at -20°C until required.

The tyrosine kinase inhibitor methyl 2,5-dihydroxycinnamate (MDHC) and its inactive stereoisomer were prepared as previously described (14), lyophilized, and stored at -80°C until required. The protein kinase C inhibitor staurosporin was obtained from Biomol, Inc, Philadelphia, PA, USA. At the commencement of the experimental series, these drugs were dissolved at 100 mM/l in dimethylsulphoxide, aliquotted, and stored at -20°C until required. Immediately before use, the drug aliquots were thawed and diluted to 100 μ M/l in challenge buffer.

Subjects

Atopic and nonatopic asthmatic subjects were recruited from patients with asthma attending the University of British Columbia Respiratory Clinic at Vancouver General Hospital. Patients with WRCA were referred for evaluation with a compatible history, and sensitivity to PA was confirmed by formal challenge testing, as previously described (3). Atopic status was determined by skin prick tests to a panel of 14 common airborne allergens, one or more positive skin tests 3 mm or greater than the diluent control being the operational definition of atopy. Nonatopic, nonasthmatic laboratory personnel served as control subjects.

Kinase inhibition experiments

Peripheral blood leukocytes were prepared by dextran sedimentation of heparinized venous blood and washed twice in Tyrode's buffer. Aliquots of 2 million leukocytes were pelleted in polypropylene tubes (Sarstedt, Nurnbrecht, Germany) and resuspended in 1 ml challenge buffer containing MDHC 100 μ M/l, staurosporin 100 μ M/l, or buffer alone. The tubes were capped and then preincubated for 5 min in a water bath at 37°C . After preincubation, the cells were challenged by adding 50- μ l volumes of reagents to give final concentrations of grass pollen of 100 PNU/ml, anti-IgE 10 μ g/ml, or calcium ionophore 1 μ g/ml. Control cells received 50 μ l challenge buffer. All challenges were performed in triplicate. Tubes were then recapped, vortexed, and returned to the water bath. After 30-min incubation at 37°C , the tubes were centrifuged and the supernatants removed and stored at -80°C until assayed for histamine content. The cell pellets were resuspended in 1 ml Tyrode's buffer and then frozen and thawed twice to release residual histamine. Histamine was assayed by a single isotope radioenzymatic assay, as previously described (2, 15). The sensitivity of this assay is 20 pg histamine and the coefficient of variation of repeat measurement less than 2.5%. All histamine measurements were performed in duplicate and results were expressed as the per cent release of the total histamine content. The inhibitory activity of each drug was calculated by comparing the mean net release induced by each reagent in the presence and absence of the drug (after subtracting background histamine release in the appropriate control tubes). In a parallel series of experiments, the specificity and dose-dependence of MDHC-induced inhibition of histamine release were studied by preincubation with a series of concentrations of MDHC and its precursor. The reversibility of MDHC treatment was studied by

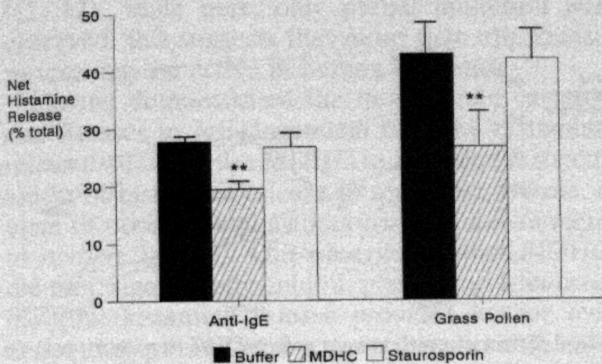


Fig. 1. Net per cent release of histamine from peripheral blood leukocytes pretreated with challenge buffer alone, MDHC 100 μ M/l, or staurosporin 100 μ M/l and then challenged with anti-IgE 10 μ g/ml ($n=15$) and grass pollen 100 PNU/ml ($n=6$). Statistical comparisons of drug-treated and untreated cells by paired t -test: ** $P<0.01$.

preincubating basophils with MDHC for 5 min, and then washing twice in Tyrode's buffer to remove MDHC before challenge.

Ethical approval

This study was approved by the ethics committee of the University of British Columbia.

Results

To be eligible for inclusion in the analysis, untreated basophils had to release at least 15% of total basophil histamine in response to the challenge agent in question. This cutoff was chosen so that there was sufficient signal against which to judge the magnitude of any drug inhibition. Fifteen subjects released $\geq 15\%$ histamine to anti-IgE. When basophils from these subjects were preincubated with MDHC 100 μ M/l, mean histamine release was reduced from 27.8% to 19.2% (mean reduction 29.8%; $P<0.01$). In contrast, staurosporin 100 μ M/l had no effect on anti-IgE-induced histamine release (mean release 26.2% vs 27.8% for control cells; $P=NS$) (Fig. 1). The effect of MDHC on IgE-dependent histamine release was dose-dependent (Fig. 2) and could be completely reversed by washing the cells twice in Tyrode's buffer (Fig. 3). The specificity of this response was confirmed by parallel control studies ($n=3$) using the stereoisomer of MDHC, which did not affect histamine release induced with either anti-IgE or grass pollen (data not shown).

Six atopic subjects were identified from whose cells grass pollen induced release of at least 15% of total leukocyte histamine. Pretreatment with MDHC 100 μ M/l partially inhibited histamine release to

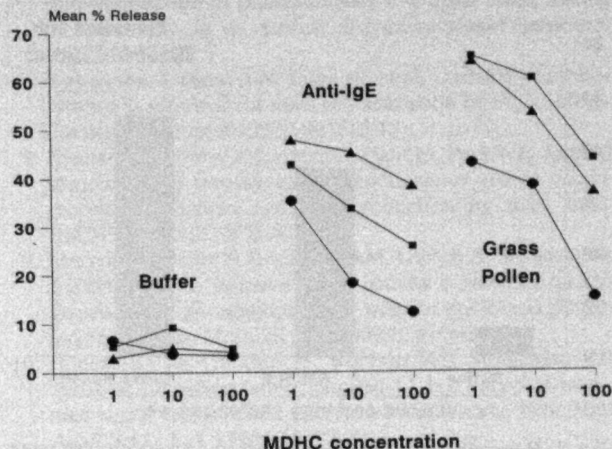


Fig. 2. Dose-dependence of effects of MDHC (1, 10, and 100 μ M/l) on histamine release from basophils of three atopic subjects triggered by buffer, anti-IgE 10 μ g/ml, or grass pollen 100 PNU/ml.

grass pollen in five of these six subjects (mean histamine release 43.1% vs 26.8%; 48% inhibition; $P<0.01$) (Fig. 1). As with anti-IgE, staurosporin 100 μ M/l had no significant effect on grass pollen-induced histamine release (42.2% mean histamine release after staurosporin compared to 43.1% from control cells; $P=NS$) (Fig. 1).

For assessment of the role of tyrosine kinase activation in histamine release induced by plicatic acid, blood was obtained from a total of 20 patients with occupational asthma due to western red cedar. Nine of these subjects released 15% or more of total basophil histamine when challenged *in vitro* with plicatic acid 100 μ g/ml. Pretreatment with MDHC 100 μ M/ml partially inhibited histamine release to plicatic acid in six of the nine subjects (mean histamine release 25.4% vs 33.8% for buffer-treated cells; 24.7% inhibition; $P=NS$) (Fig. 4). In contrast to the pattern of drug effects seen with pollen and anti-IgE, staurosporin 100 μ M/ml also partially attenuated plicatic acid-induced histamine release in six of the nine subjects (mean histamine release 25.3% vs 33.8%; mean inhibition 25.1%; $P=NS$) (Fig. 4). Five of these subjects were partially inhibited by both MDHC and staurosporin, two were unaffected by either drug, and one each was inhibited by MDHC or staurosporin alone.

Discussion

Pretreatment with the specific tyrosine kinase inhibitor MDHC attenuated allergen- and IgE-mediated histamine release from basophils in a reversible and dose-dependent manner. The serine/threonine kinase (PKC) inhibitor staurosporin did not affect histamine release induced by these stimuli. In contrast, both MDHC and staurosporin

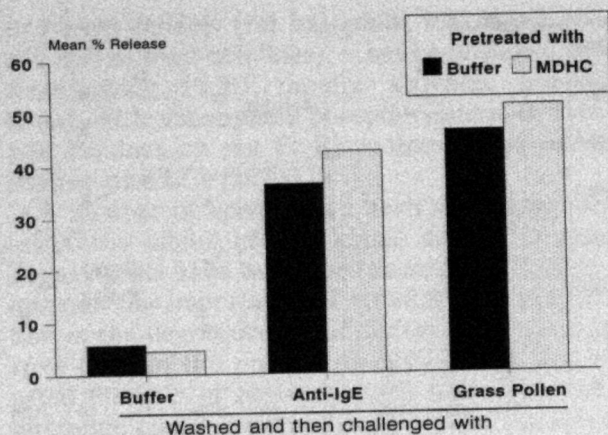


Fig. 3. Reversibility by washing of effect of MDHC 100 μ M/l on histamine release from blood leukocytes triggered by anti-IgE 10 μ g/ml, calcium ionophore 1 μ g/ml, or grass pollen 100 PNU/ml ($n=2$).

partially attenuated basophil histamine release induced by plicatic acid, the causative agent of occupational asthma due to western red cedar. Taken together, these results indicate that tyrosine kinase activation is an important coupling mechanism in IgE-dependent degranulation of human basophils, and that plicatic acid-induced basophil degranulation probably operates through a different pathway or pathways.

Previous experiments have demonstrated that MDHC is a highly specific inhibitor of tyrosine kinases (14); therefore, it is reasonable to conclude that the effects observed here were due to tyrosine kinase inhibition. One limitation of this study was the use of unfractionated leukocytes: however, the triggering system and the readout used are basophil-specific. Both anti-IgE and allergen operate through the IgE molecule and its high-affinity Fc receptor, and in human atopic blood, these are found only on basophils. Similarly, the principal readout was histamine release, and histamine is released only from basophils, and not from other formed blood elements.

Degranulation of both basophils and mast cells is thought to require activation of phospholipase C- γ , hydrolysis of inositol-containing phospholipids, and liberation of inositol triphosphate and 1-2-diacylglycerol (16). Inositol triphosphate is a second messenger molecule which has been shown in several cell types to elicit a rise in intracellular calcium concentrations, which then combine with diacylglycerol to trigger degranulation. However, this process appears to have some biologic redundancy in that activation of PLC- γ and protein kinase C, together with an increase in intracellular calcium, is necessary but not sufficient to account for the full secretory signal of the mast cell (17).

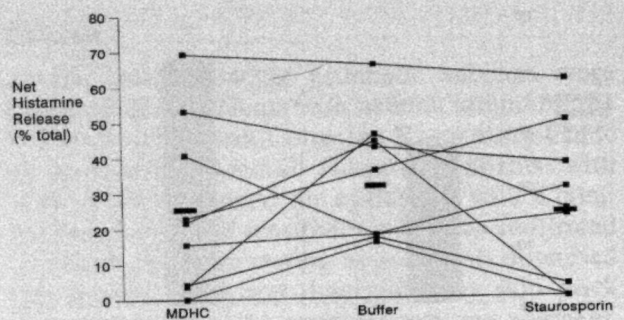


Fig. 4. Effect of pretreatment with MDHC or staurosporin on plicatic-acid-induced histamine release from leukocytes obtained from patients with Western red cedar asthma. Linked points represent data from same subject. Bars show mean values. No significant difference between groups.

Evidence from rat basophilic leukaemia cells suggests that tyrosine phosphorylation may affect not only Fc ϵ R function but also the activity of PLC and the availability of substrate for PLC (8, 18). A similar sequence of events occurs in PLC activation via the T-cell antigen receptor and the epidermal growth factor receptor (19). However, while the T-cell antigen receptor and the epidermal growth factor receptor have intrinsic tyrosine kinase activity, current evidence suggests that the Fc ϵ RI molecule lacks any tyrosine kinase activity, although tyrosine kinases have been coprecipitated with the Fc ϵ RI molecule (20, 21).

In rodents, ligation of Fc ϵ RI by anti-IgE leads to phosphorylation of tyrosine, serine, and threonine residues on the Fc ϵ RI molecule (11, 16). The present data suggest that the serine and threonine phosphorylation is not critical to Fc ϵ RI function in that IgE-dependent degranulation was not inhibited when basophils were pretreated with staurosporin. In a separate study, we found that phorbol esters, which activate serine/threonine kinases (PKC), did not cause any increase in histamine release (Salari et al., unpublished observations), while, in the present study, the PKC inhibitor staurosporin was unable to block anti-IgE-induced histamine release. These results suggest that although PKC is activated by Fc ϵ RI ligation, it does not play an important role in histamine release from human basophils. In addition, the lack of effect of the precursor of MDHC confirms the involvement of a specific mechanism in IgE-induced activation of tyrosine kinases. It remains unclear whether the tyrosine kinase activity is associated with the Fc ϵ RI molecule or is associated with some postreceptor signalling group such as PLC. In rodents, pretreatment with tyrosine kinase inhibitors completely abolished histamine release from mast cells and leukaemic basophils

(11, 12), while here, only partial inhibition was observed: this suggests that more than one kinase system may be active in human basophils.

Having demonstrated the involvement of tyrosine kinases in IgE-dependent basophil histamine release, we could use MDHC to explore the mechanism of histamine release in red cedar asthma, a form of occupational asthma which is not thought to involve IgE (3). Our observation that MDHC did not significantly inhibit plicatic acid-induced basophil histamine release provides further evidence that plicatic acid triggers histamine release through IgE-independent mechanism(s). It would be interesting to apply this same approach to other forms of IgE-independent occupational asthma such as asthma due to isocyanates.

These findings offer a potential avenue for the drug treatment of atopic disease, if it proves possible to identify and inhibit tyrosine kinases that are specific for $Fc_\epsilon RI$ of mast cells or basophils. Tyrosine kinase inhibitors may also be a useful tool for dissecting mechanisms of basophil degranulation by flagging proteins relevant to the degranulation process.

Acknowledgments

The expert technical assistance of Ann Chadderton for the histamine assay is gratefully acknowledged. This work was supported by grants from the Medical Research Council of Canada.

References

1. Chan-Yeung M, Lam S, Koener S. Clinical features and natural history of occupational asthma due to western red cedar (*Thuja plicata*). *Am J Med* 1982;72:411-15.
2. Chan-Yeung M, Chan H, Tse KS, Salari H, Lam S. Histamine and leukotriene release in bronchoalveolar fluid during plicatic acid-induced bronchoconstriction. *J Allergy Clin Immunol* 1989;84:762-8.
3. Frew AJ, Chan H, Dryden P, Salari H, Lam S, Chan-Yeung M. Immunologic studies of the mechanisms of occupational asthma due to western red cedar. *J Allergy Clin Immunol* 1993;92:466-78.
4. Beaven MA, Moore JP, Smith GA, Hesketh RT, Metcalfe JC. The calcium signal and phosphatidylinositol breakdown in 2H3 cells. *J Biol Chem* 1984;259:7137-42.
5. Benhamou M, Siraganian RP. Protein-tyrosine phosphorylation: an essential component of $Fc_\epsilon RI$ signalling. *Immunol Today* 1992;13:195-7.
6. Hempel WM, Schatzman RC, DeFranco AL. Tyrosine

- phosphorylation of phospholipase C- γ upon cross linking of membrane Ig on murine B-lymphocytes. *J Immunol* 1992;148:3021-7.
7. Kawakami T, Inagaki N, Takei M, et al. Tyrosine phosphorylation is required for mast cell activation by $Fc_\epsilon RI$ cross-linking. *J Immunol* 1992;148:3513-19.
8. Keegan AD, Pierce JH, Artrip J, Plaut M, Paul WE. Ligand stimulation of transfected and endogenous growth factor receptors enhances cytokine production by mast cells. *EMBO J* 1991;10:3675-82.
9. Margolis B, Bellot F, Honegger AM, Ullrich A, Schlessinger J, Zilberstein A. Tyrosine kinase activity is essential for the association of phospholipase C- γ with the epidermal growth factor receptor. *Mol Cell Biol* 1990;10:435-41.
10. Benhamou M, Gutkind JS, Robbins KC, Siraganian RP. Tyrosine phosphorylation coupled to IgE receptor-mediated signal transduction and histamine release. *Proc Natl Acad Sci U S A* 1990;87:5327-30.
11. Paolini R, Jouvin M-H, Kinet J-P. Phosphorylation and dephosphorylation of the high affinity receptor for IgE immediately after receptor engagement and disengagement. *Nature* 1991;353:855-8.
12. Park DJ, Min HK, Rhee SG. IgE-induced tyrosine phosphorylation of phospholipase C- γ in rat basophilic leukemia cells. *J Biol Chem* 1991;266:24237-40.
13. Burt HM, Jackson JK, Dryden P, Salari H. Crystal-induced protein tyrosine phosphorylation in neutrophils and the effect of a tyrosine kinase inhibitor on neutrophil responses. *J Mol Pharmacol* 1993;43:30-6.
14. Umezawa K, Hori T, Tajima H, Imoto M, Isshiki K, Takeuchi T. Inhibition of epidermal growth factor-induced DNA synthesis by tyrosine kinase inhibitors. *FEBS Lett* 1990;260:198-200.
15. Verburg KM, Bowsher RR, Henry DP. A new radioenzymatic assay for histamine using purified histamine N-methyltransferase. *Life Sci* 1983;32:2855-67.
16. Scharenberg AM, Kinet J-P. Initial events in $Fc_\epsilon RI$ signal transduction. *J Allergy Clin Immunol* 1994;94:1142-6.
17. Beaven MA, Guthrie DF, Moore JP, Smith GA, Hesketh TR, Metcalfe JC. Synergistic signals in the mechanisms of antigen-induced exocytosis in 2H3 cells: evidence for an unidentified signal required for histamine release. *J Cell Biol* 1987;105:1129-36.
18. Schneider H, Cohen-Dayag A, Pecht I. Tyrosine phosphorylation of phospholipase C- γ couples the Fc_ϵ receptor mediated signal to mast cell secretion. *Int Immunol* 1992;4:447-53.
19. Mustelin T, Coggeshall KM, Isakov N, Altman A. T cell antigen receptor-mediated activation of phospholipase C requires tyrosine phosphorylation. *Science* 1990;247:1584-5.
20. Eisemann E, Bolen JB. Engagement of the high affinity IgE receptor activates *src* protein-related tyrosine kinases. *Nature* 1992;355:78-80.
21. Quarto R, Metzger H. The receptor for immunoglobulin E: examination for kinase activity and as a substrate for kinases. *Mol Immunol* 1986;23:1215-23.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.