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Broncho-Vaxom® inhibits histamine release from rat mast cells induced by compound 48/80 and ionophore A23187

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Abstract

Broncho-Vaxom (BV) inhibited in dose-dependent manner the release of histamine from and degranulation of isolated rat peritoneal mast cells stimulated with compound 48/80 and the ionophore A23187. Inhibition persisted after removal of BV from the incubation medium before stimulation, but did not occur when bovine serum albumin (BSA) was used instead of BV. Binding of BV to mast cells was observed by electron microscopy on cells that had been incubated with colloidal-gold labelled BV. There was no significant difference between the binding of BV gold and BSA gold to the mast cells. Washing before fixation removed most of the BV gold from the cells. This study establishes BV as an *in vitro* histamine release inhibitor.

Introduction

The mechanism of action of Broncho-Vaxom (BV) is not yet fully understood. We know, for example, that it enhances immune responses, both cellular [1] and humoral [2, 3], restores defective membrane properties in the T-lymphocytes of IgA-deficient subjects [1], stimulates interferon production and T-lymphocyte blastogenic transformation [4], and raises concentrations of secretory IgA in saliva and of IgG and IgM in serum [5]. But these are only a few of the pieces in a complex mosaic.

In a study of BV in the treatment of respiratory infections in patients with or without bronchial asthma [6], the response to BV was better in the non-asthmatic than in the asthmatic subjects. This could be because in some asthmatics there are factors other than infection that act detrimentally on cough and dyspnoea, and it suggests that when BV has a beneficial effect in patients with asthma it does so simply by curing associated infection. It cannot, however, be excluded that BV may also act on some factor that is responsible for the allergic phenomena of asthma, the most likely such factor being the mediator histamine. Certain substances, disodium cromoglycate [7] and ketotifen [8], for example, are known to act beneficially in allergic asthma by inhibiting mediator release from mast cells. Might BV have an analogous action? This question prompted us to investigate BV in an *in vitro* test model in which histamine release from isolated rat peritoneal mast cells is induced by Compound 48/80 or by ionophore A23187.

Materials and methods

Rat peritoneal mast cells were incubated with BV before addition of the mast cell histamine-releasing agents compound 48/80 and ionophore A23187. The amount of histamine released from the mast cells was determined fluorimetrically [9]

and expressed as a percentage of the total histamine content of the cells. For comparison, histamine release (a) after removal of BV from the incubation medium before stimulation, and (b) with bovine serum albumin (BSA) in the medium instead of BV, was also determined. Degranulation was estimated under the light microscope on cells stained with ruthenium red. Binding of BV to mast cells was followed morphologically by labelling BV (and BSA as control) with colloidal gold, the BV and BSA being adsorbed to 10 nm colloidal gold particles [10]. After incubation of the concentrated BV-gold and BSA-gold with the mast cell pellets, electron microscope investigation was performed. The quantity and distribution of the bound BV-gold and BSA-gold, and the number of gold particles remaining on the mast cells when BV-gold was washed out before fixation, was noted.

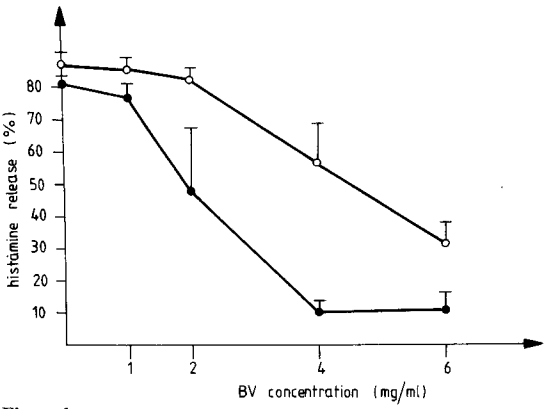

Figure 1
Inhibition of histamine release from rat peritoneal mast cells stimulated by compound 48/80 (1 μg per ml for 1 min): ●—● after preincubation of the cells with increasing concentrations of BV; the concentration of BV that inhibited 48/80-induced histamine release by 50% of its maximum value (i.e., the IC_{50} value) was 2.3 mg per ml; o—o after removal of BV from the incubation medium by one washing with phosphate buffered saline; the IC_{50} value was then 5.0 mg BV per ml.

Results and discussion

These *in vitro* experiments showed that BV exerts a dose-dependent inhibition of histamine release from rat peritoneal mast cells stimulated by compound 48/80 (Fig. 1) and by ionophore A23187 (Fig. 2). Complete or nearly complete inhibition required a BV concentration of 6 mg per ml. Even a dosage of 1 adult (7-mg) capsule of BV per day would not produce such a concentration as this *in vivo* in the neighbourhood of the mast cells. The inhibitory *in vitro* concentrations of disodium cromoglycate [7] and of ketotifen [8], both compounds which also reduce histamine release from mast cells, are likewise far above the concentrations that are accessible therapeutically. The time factor must also be considered. In our experiments, the mast cells were incubated in BV for 10 min, but disodium cromoglycate and ketotifen exert their beneficial effects only after 2 to 3 weeks of administration.

The fact that removal of BV from the incubation medium before stimulation by 48/80 (Fig. 1) did not abolish (although it did weaken) the inhibition of 48/80-induced histamine release suggests a stable binding of BV to the surface of the mast cells.

The weakness of the histamine inhibition exerted by BSA compared with that exerted by BV in equal concentration (6 mg per ml, Fig. 2) excludes the

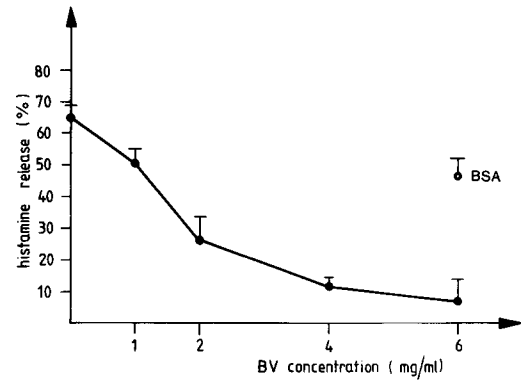

Figure 2
Inhibition of histamine release from rat peritoneal mast cells stimulated by ionophore A23187 (5×10^{-5} *M* for 15 min): ●—● after preincubation with BV; IC_{50} value was 1.7 mg BV per ml; o after preincubation with bovine serum albumin (6 mg per ml for 10 min).

possibility that the action of BV in such high concentrations might be a non-specific one consisting simply in forming a protective coat round the cells.

BV preincubation decreased the number of 48/80-degranulated mast cells, the decrease being proportional to increasing concentrations of BV.

No significant difference between the binding of BV-gold and BSA-gold to the surface of mast cells

was detected. If BV-gold was washed out before fixation, most of the BV-gold particles were found to have disappeared from the mast cell surface, a fact which does not support the theory of stable binding of BV to the mast cells.

In conclusion, we know that airway infection may exacerbate asthma and that the long-term seasonal administration of BV prevents airway infection, and thereby benefits asthma. By demonstrating the dose-dependent inhibition of histamine release by BV, this study has shown that there is no risk of BV having an adverse effect in asthma by causing bronchospasm. However, whether BV can relieve allergic asthma unassociated with airway infection is a question that calls for long-term clinical studies.

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