Polyherbal formulation Bresol® protects the mast cells against compound 48/80-induced disruption and histamine release: a non-immunological mechanism of mast cell stabilization

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OBJECTIVE: Present study was aimed to evaluate the protective effect of Bresol®, a polyherbal formulation, on mast cell degranulation and histamine release from mast cells.

METHODS: Mast cell-stabilizing activity of Bresol® was evaluated against compound 48/80-induced mast cell degranulation and histamine release from rat peritoneal mast cells in ex vivo conditions.

RESULTS: Microscopy of the control group smears showed more of intact mast cells, with very minimum number of degranulated mast cells and negligible amount of histamine release. In contrast, incubation of mast cells with compound 48/80 caused significant degranulation of the mast cells associated with release of high concentration of histamine in the positive control group. Furthermore, Bresol® at 100 mg/L showed a significant inhibition of compound 48/80-induced mast cell degranulation. In addition, Bresol® significantly and dose-dependently inhibited compound 48/80-induced histamine release.

CONCLUSION: Bresol® inhibits compound 48/80-induced mast cell degranulation and histamine release in ex vivo conditions. The present findings could be one of the non-immunological mechanisms responsible for usefulness of Bresol® in various allergic conditions.

KEYWORDS: medicine, Ayurvedic; plants, medicinal; compound 48/80; mast cells; cell degranulation; histamine release; in vitro
Allergy is one of the common diseases that affect mankind. The prevalence of allergic disease has dramatically increased over the recent years. Depending on the amount of allergen entering into the body, it will induce different kinds of changes including running nose, sneezing, cutaneous wheal and flare reaction and wheezing [3]. Allergic rhinitis, allergic conjunctivitis, asthma, atopic eczema and food allergy are some of the allergic diseases [1, 5, 6].

Recently it has been reported that in immediate type of allergic reaction, the allergens trigger B cells to produce immune globulin (IgE) and IgG antibodies which react with the allergens and bind to high-affinity receptors for IgE (FcεRI) along with circulating basophils and tissue mast cells [3]. In late-phase reaction the allergens express multiple epitopes recognized by specific IgEs and IgGs; the membrane-bound IgEs induce receptor aggregation, triggering a signalling cascade leading to the production and release of allergic and inflammatory mediators such as histamine, leukotrienes, chemokines and cytokines responsible for the symptoms of allergic diseases [5]. Mast cells are well known as critically important components in various biological processes of allergic diseases. These are found relatively large numbers in the mucosa of respiratory, gastrointestinal and urinary tract, skin and near blood or lymphatic vessels. These cells are supposed to express surface membrane receptors with high affinity and specificity for IgE [3].

Ayurveda, an ancient system of Indian medicine, has described several drugs from indigenous plant sources that can be used for the treatment of immediate hypersensitive diseases such as bronchial asthma, rhinitis and other allergic conditions. Bresol® is one such polyherbal formulation approved by the Government of India’s Drug Regulatory Authority (Department of AYUSH, Ministry of Health and Family Welfare). Bresol® contains extracts of Curcuma longa, Ocimum sanctum, Adhatoda vasica, Trikatu, Triphala, Embelia ribes, Ceyperus rotundus, Cinnamomum zeylanicum, Elettaria cardamomum, Cinnamomum tamala, and Mesua ferrea. It has been tested for its quality and consistency in each and every step of manufacturing as per the accepted principles of good manufacturing practice (GMP) and good laboratory practice (GLP). Bresol® has been standardized with respect to various parameters which comply with the stringent quality check specification of the finished product. The product is standardized by physical and chemical parameters. Every batch of Bresol® is compared with the reference control in terms of various quality parameters and also by unique thin layer chromatography finger-printing. Bresol® is well proved for its clinical effectiveness in the treatment of allergic conditions. It has been reported to act on different pathways leading to hypersensitivity and inflammation [4, 7].

With this background, the present study was undertaken to evaluate the protective effect of Bresol® against compound 48/80-induced mast cell degranulation and histamine release from rat peritoneal mast cells in ex vivo conditions.

1 Materials and methods
1.1 Materials Histamine (Sigma Aldrich, Bangalore), toluidine blue (Loba Chemie, Mumbai, India); other chemicals and reagents were procured from the Himedia Laboratories Limited, Mumbai, India.

1.2 Animals Inbred Wistar rats (200 to 220 g) of either sex housed in standard conditions of temperature (22 ± 3°C), relative humidity (55% ± 5%) and a 12-h light/dark cycle. They were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the animal experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

1.3 Preparation of rat peritoneal mast cells A total of 10 mL of Tyrode’s buffer (pH 7.4) containing 0.1% bovine serum albumin (BSA) and 5 IU/mL of heparin was injected intraperitoneally to Wistar rats. After gently massaging the

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abdomen for 90 s, peritoneal fluid containing peritoneal mast cells (RPMCs) was collected; it was centrifuged at 600 x g for 10 min at 4 °C. The supernatant was discarded and the pellet containing mast cells was washed three times and resuspended with Tyrode's buffer. The cells count was determined by mixing 50 μL of 0.5% toluidine blue in saline with equal volume of the cell suspension. The stained mast cells were counted microscopically using a counting chamber. Mast cell count in the suspension was adjusted to 4 x 10⁶ cells/mL for histamine-release experiment[1].

1.4 Mast cell stabilization activity of Bresol®

Purified RPMCs were preincubated for 10 min at 37 °C for stabilization before the addition of compound 48/80. The cells were incubated with the test drug for 10 min at 37 °C, followed by next 10-min additional incubation at 37 °C with compound 48/80 (10 μg/mL). Smears of mast cells were prepared, fixed in methanol and stained with 0.1% toluidine blue for 10 min. The morphology of the cells was observed under a light microscope and graded based on the extent of degranulation[1]. The assays were carried out for six times during each run; the absolute mast cell count of each type of mast cells (as described above) was recorded and the percentage of different types of mast cells was further arrived.

1.5 Effect of Bresol® on histamine release from mast cells

Separate set of ex vivo studies was carried out to study the effect of Bresol® on compound 48/80-induced histamine release from mast cells. Purified RPMCs were preincubated for 10 min at 37 °C for stabilization before the addition of compound 48/80. The cells were incubated with various concentrations of Bresol® (at concentrations 19.5, 9.8, 4.9 and 1.2 μg/mL) for 10 min at 37 °C, followed by next 10-min additional incubation at 37 °C with compound 48/80 (10 μg/mL). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifuging at 400 x g for 5 min at 4 °C. The released histamine was estimated by orthophthalaldehyde (OPA) spectrofluorimetric method[16,17]. The fluorescent intensity was measured at excitation and emission wavelengths of 380 nm and 460 nm respectively using Synergy Biotek enzyme-linked immunoassorbent assay reader. A standard histamine curve was obtained using pure histamine, which used to calculate the histamine release from the mast cells. The histamine release assays were carried out for six times during each run; the concentration of histamine released from mast cells were calculated from standard histamine curve, and the percentage inhibition was calculated with respect to control histamine release (without drug).

1.6 Statistical analysis

The results of study were expressed as mean ± standard error of mean and analyzed statistically using one-way analysis of variance followed by Tukey's multiple comparison test to find out the level of significance. The minimum level of significance was fixed at 95% confidence limit. The analysis was performed using Graphpad Prism software package (Version 4.03).

2 Results

2.1 Effect of Bresol® on compound 48/80-induced mast cell degranulation

The morphology of the cells was observed under a light microscope and graded as Type-I (intact mast cells) (Figure 1A), Type-II (partially degranulated mast cells) (Figure 1B) and Type-III (completely degranulated mast cells) (Figure 1C). Microscopic evaluation of mast cell smear stained with toluidine blue showed presence of 70% to 80% of Type-I cells, 10% to 20% of Type-II cells and 8% to 10% of Type-III cells in the control group. Compound 48/80-induced a significant reduction in Type-I cells and an increase in the degranulated Type-II and Type-III cells compared to the control group (P < 0.05). Bresol® at 100 μg/mL concentration significantly inhibited the compound 48/80-induced mast cell degranulation in ex vivo conditions (P < 0.05) (Figure 2).

2.2 Effect of Bresol® on compound 48/80-induced histamine release from mast cells

A standard histamine curve was obtained using pure histamine. It showed the assay chosen was linear and can be used to calculate histamine even at low concentrations (Figure 3). Incubation of mast cells with compound 48/80 in the positive control group showed a significant increase in the release of histamine compared to the normal control. In contrast, preincubation of mast cells with Bresol® showed a significant and dose-dependent inhibition of mast cell degranulation and prevented the histamine release induced by compound 48/80, indicating its mast cell-stabilizing activity (Figure 4).

Figure 1 The morphologies of the mast cells (Light microscopy, ×400)

A: Type-I cells (intact mast cells); B: Type-II cells (partially degranulated mast cells); C: Type-III cells (completely degranulated mast cells).
Mast cells are capable of regulating inflammation, host defense and innate immunity. After their development from the bone marrow-derived progenitor cells that are primed with stem cell factor, mast cells continue their maturation and differentiation in peripheral tissue, developing into two subsets of cells, MC\textsubscript{T} and MC\textsubscript{C} cells\textsuperscript{[11]}. Mast cells can be stimulated to degranulate by various mechanisms, namely, physical or chemical methods by using opioids, alcohols and compound 48/80, cross-linking of IgE receptors or by activated complement proteins\textsuperscript{[12]}.

Compound 48/80, a condensation product of \(n\)-methoxy-phenylamine with formaldehyde, is a well-known histamine releaser\textsuperscript{[13]}. Compound 48/80 activates the mast cells and degranulates by aggregation of FceRI cross linking of IgE by polyvalent antigens, leading to the activation of serine-like cytosolic protein tyrosine kinases (PTKs)\textsuperscript{[14]} and producing rapid tyrosine phosphorylation of the FceRI \(\beta\) and \(\gamma\) subunits. This will enable the recruitment and activation of additional cytosolic PTKs and tyrosine phosphorylation of a number of protein substrates, including phospholipase-C gamma (PLC\(\gamma\)). Even PLC\(\gamma\) is activated in mast cells. Phosphotidyl-4, 5-diphosphate (PIP\(_2\)) is hydrolyzed to inositol 1, 4, 5-triphosphate (IP\(_3\)) and diacylglycerol (DAG)\textsuperscript{[16,17]}. IP\(_3\) binds to its receptors and releases the intracellular calcium from smooth endoplasmic reticulum. The released Ca\(^{2+}\) activates DAG and protein kinases C (PKC), which in turn increases intracellular Ca\(^{2+}\) level and activation of PKC along with PI-3-Kinase that contributes to the release of histamine. Therefore compound 48/80 is employed as a classic mast cell activator and induces about 90\% release of histamine from mast cells\textsuperscript{[18,19]}. In present study, Bresol\textsuperscript{5}, an ayurvedic proprietary medicine, at 100 \(\mu\)g/mL concentration inhibited the compound 48/80-induced mast cell degranulation. Furthermore, Bresol\textsuperscript{5} also inhibited the histamine release significantly and dose-dependently.

4 Conclusion

From the findings of the present study it can be concluded that Bresol\textsuperscript{5}, a polyherbal formulation, is an effective and nonspecific mast cell stabilizer, as well as a potent inhibitor of release of histamine and other mediators of hypersensitivity from mast cells.

5 Acknowledgements

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6 Conflicts of interests

Formulation of Bresol\textsuperscript{5} was received from the Formulation and Development Division of Research and Development Center, The Himalaya Drug Company, Bangalore, India and all the experiments
were conducted in the Department of Pharmacology, Research and Development Center, The Himalaya Drug Company, Bangalore, India. The authors express no conflict of interests in any form.

REFERENCES


草药复方 Bresol® 抵抗化合物 48/80 诱导的肥大细胞脱颗粒及组胺释放; 促使肥大细胞稳定的一种非免疫机制

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目的: 研究一种草药复方制剂 Bresol® 对于肥大细胞脱颗粒以及组胺释放的保护作用。

方法: 使用大鼠腹膜内肥大细胞，在体外经化合物 48/80 诱导肥大细胞脱颗粒及组胺释放，评估 Bresol® 稳定肥大细胞的作用。

结果: 显微镜下正常对照组涂片显示较多完整的肥大细胞，有极少量的脱颗粒肥大细胞和微量的组胺释放。阳性对照组中化合物 48/80 培养的肥大细胞出现了显著的肥大细胞脱颗粒现象以及高浓度的组胺释放。而 100 mg/L 浓度的 Bresol® 明显抑制了化合物 48/80 诱导的肥大细胞脱颗粒。此外，Bresol® 可有效抑制化合物 48/80 诱导的组胺释放，且抑制效果与剂量有关。

结论: Bresol® 能够在体外抑制化合物 48/80 诱导的肥大细胞脱颗粒及组胺释放。本研究的发现可解释 Bresol® 对多种过敏疾病有效可能是通过一种非免疫机制。

关键词: 试验，印度传统；植物，药用；化合物 48/80；肥大细胞；脱颗粒；组胺释放；体重研究