

## EFFECT OF ULTRA-DILUTED HISTAMINE ON HYPOXIC CHICK LUNG TISSUE INFLAMMATORY CHANGES

PRITAM GOSWAMI<sup>1</sup>, SWAIF ALI SK<sup>1</sup>, SAYAK GHOSH<sup>1</sup>, ANAMIKA BASU<sup>1</sup>, JOYDEEP KHANRA<sup>1</sup>, SATADAL DAS<sup>2\*</sup>

<sup>1</sup>Department of Pathology, Mahesh Bhattacharyya Homoeopathic Medical College and Hospital, Howrah, West Bengal, India. <sup>2</sup>Department of Microbiology, Peerless Hospital and B K Roy Research Centre, Kolkata, West Bengal, India. Email: drsatdas@hotmail.com

Received: 17 April 2019, Revised and Accepted: 24 May 2019

### ABSTRACT

**Objectives:** Since its discovery, the role of histamine in inflammation is controversial; thus, according to some authority, it is mainly pro-inflammatory, and according to others, it is anti-inflammatory in nature. In this scenario, we thought that the contradictory results are dose dependent, thus in this study, our aim was to find the specific role of ultra-diluted histamine in pulmonary inflammation.

**Materials and Methods:** Ultra-diluted histamine (~1 pg/ml) was administered in chick lung hypoxic inflammation in an restricted organoid culture along with lysozyme, ovalbumin, and blank controls.

**Results:** The ultra-diluted histamine showed a significant role as an anti-inflammatory and bronchodilator agent and the anti-inflammatory action was found similar to lysozyme.

**Conclusion:** Ultra-diluted histamine may be used as an anti-inflammatory agent.

**Keywords:** Ultra-diluted histamine, Anti-inflammatory agent, Ovalbumin, Lysozyme, Hypoxia.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i7.33651>

### INTRODUCTION

Human basophils play a key role in allergic diseases by secreting histamine which causes bronchoconstriction with the help of various histamine receptors. The effectivity of histamine in high dilution over basophil degranulation secondary to anti-immunoglobulin E (IgE) induction is itself a matter of controversy [1]. Histamine itself can inhibit activation of basophil through H2 receptor [2,3]. There are series of experiments which show that ultra-diluted histamine is potent enough to prevent degranulation of basophils [4,5]. Ultra-diluted histamine can be used in the treatment of atopic asthmatic patients to prevent bronchoconstriction and cut down the economic burden on asthmatics where it is mostly of allergic origin.

#### Histamine and histamine receptors

Histamine (2-[4-imidazole]-ethylamine) (Fig. 1) is mainly secreted by mast cells and basophils and is one of the most studied biochemical agents in medicine since its discovery in 1911. Histamine is synthesized from histidine of Golgi body, from where it is further stored in the granules for ionic association with glycosaminoglycans of the heparin side chain. The pleiotropic effects of histamine are mainly due to histamine receptors HR1, HR2, HR3, and HR4 which belong to G-protein coupled receptor family. Histamine plays an active role in acute allergic inflammatory condition by increasing secretion of several proinflammatory cytokines, for example, interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6, and chemokines like IL-8 [6-9]. Among the histamine receptors, HR4 is mainly present in bone marrow, peripheral blood cells, neutrophil, eosinophil, and T-cell and moderately expressed in lung, spleen, thymus, and heart [10]. HR4 mRNA is expressed by both basophil and mast cell [11]. Activated HR4 recruits eosinophil and mast cell at the time of inflammation. Histamine itself controls its expression on endothelial cell and controls and influences the pathway of inflammation [12]. Histamine behaves as a classical chemoattractant and it recruits eosinophil through HR4 during the time of inflammation [13]. There are several experiments shown in the past that high dilution of histamine is able to deactivate basophil through CD-63, so it may be used to prevent allergic reactions [4,5,14,15].

#### Biological action of ovalbumin (OVA) and hen egg lysozyme

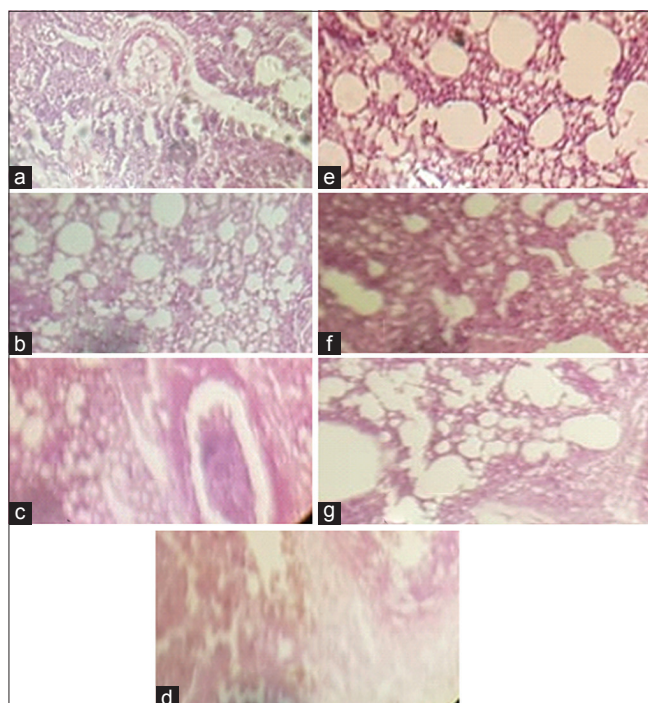
Adverse immunological reaction in the body caused by egg proteins, particularly OVA, may be IgE mediated or non-IgE mediated, which are mainly Type I hypersensitivity [16]. There are different types of allergens present in egg-like OVA (54%) the most abundant one and others are being ovotransferrin (12%), ovomucoid (11%), and lysozyme (3.4%) [17-20]. The capability of any substance to produce allergy depends on their ability to excite the immune response and their stability [21,22]. The sustained allergic reactions are mainly due to their binding with the epitopes of T-cell and B-cell. In this process of airway hyperresponsiveness along with different cytokines and chemokines involvement of some extracellular nucleotides such as uridine diphosphate (UDP), uridine triphosphate (UTP), and adenosine triphosphate has been observed in recent times [23,24]. This extracellular nucleotides mainly act with the help of transmembrane purinergic receptors of P2Y family (P2Y<sub>1-14</sub>) or ionotropic P2X receptors (P2X<sub>1-7</sub>) among which direct involvement of P2Y<sub>2</sub> and P2X<sub>7</sub> have been found [25,26]. Recently, P2Y<sub>6</sub> a group of G-protein coupled receptor has been found to play influential role through UDP or UTP and resulting into activation of neutrophils, mast cells, macrophages, and T-cells [27-31]. Secreted histamine attached with histamine receptors expressed by dendritic cells, which are specialized antigen presenting cells which, in turn, interferes with the involvement of Th1 and Th2 [32-34]. The net effect of these various changes by OVA finally leads to inflammation with development of bronchoconstriction and congestion in the lung endothelium along with mild-to-moderate tissue degeneration. Lysozyme present in the white part of the egg although in much less quantity is primarily an anti-inflammatory agent.

### MATERIALS AND METHODS

#### Collection of lung tissue

From a local shop, *Gallus gallus domesticus* lung tissue was taken from a freshly dissected chicken with the help of sterile scissor and transferred within a sterile container which was pre-filled with 2 ml chicken embryo fibroblast medium. After collection, it was transferred to the laboratory within 2 h for further studies.





**Fig. 4: (a-g) Histopathological changes in chick lung tissue in different experiments, Figs 4b, c, and f showing normal histopathological appearance of the experimental lung tissue**

concentration, it is anti-inflammatory and a potent bronchodilator. Thus, probably at usual concentration, it is pro-inflammatory in nature. The action depends on activities of histamine receptors. It is important to note that *in vitro* study with histamine is extremely difficult, due to this in most studies, histamine receptor blockers were used assuming a normal histamine concentration in the tissue. In histidine decarboxylase gene, knockout mice controlled granulomatous type of inflammation occurs in the lungs along with activation of CD4 type of T-cells and dendritic cells. In this condition, there is downregulation of IL-17, IL-6, and TNF cytokines. Due to all these difficulties in this experiment, we have used organoid cultures of lung which appears to be a good experimental model to study the action of histamine [35]. This simple experiment also indicates a positive alternative and/or adjuvant therapy in relation to antibiotics as they are now becoming mostly inactive. Lysozyme present in different tissue is a naturally occurring antimicrobial peptide [36]. It usually increases in microbial infections [37] and it acts on peptidoglycans layer of microbial cell wall [38,39]. In this study, they may destroy the invading microorganisms in an hypoxic damaged tissue along with other activities. However, receptor study and chemometric studies [40,41] may help to find out the exact mechanism. Thus, considering all these points, we may conclude that ultra-diluted histamine is an anti-inflammatory and bronchodilator agent which may be effective in pulmonary inflammatory diseases.

#### CONTRIBUTION OF AUTHORS

PG (experiment and manuscript preparation), SSA (experiment), JK (experiment), SG (experiment), AB (experiment), and SD (experiment design + analysis).

#### CONFLICTS OF INTEREST

There are no conflicts of interest of any author.

#### REFERENCES

1. Linde K, Clausius N, Ramirez G, Melchart D, Eitel F, Hedges LV, *et al.* Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. *Lancet* 1997;350:834-43.

2. Lichtenstein LM, Gillespie E. The effects of the H1 and H2 antihistamines on "allergic" histamine release and its inhibition by histamine. *J Pharmacol Exp Ther* 1975;192:441-50.
3. Masini E, Blandina P, Brunelleschi S, Mannaioni PF. Evidence for H2-receptor-mediated inhibition of histamine release from isolated rat mast cells. *Agents Actions* 1982;12:85-8.
4. Sainte-Laudy J, Belon P. Inhibition of human basophil activation by high dilutions of histamine. *Agents Actions* 1993;38:C245-7.
5. 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.
6. Vannier E, Dinarello CA. Histamine enhances interleukin (IL)-1-induced IL-1 gene expression and protein synthesis via H2 receptors in peripheral blood mononuclear cells. Comparison with IL-1 receptor antagonist. *J Clin Invest* 1993;92:281-7.
7. Meréty K, Falus A, Taga T, Kishimoto T. Histamine influences the expression of the interleukin-6 receptor on human lymphoid, monocytoid and hepatoma cell lines. *Agents Actions* 1991;33:189-91.
8. Jeannin P, Delneste Y, Gosset P, Molet S, Lassalle P, Hamid Q, *et al.* Histamine induces interleukin-8 secretion by endothelial cells. *Blood* 1994;84:2229-33.
9. Bayram H, Devalia JL, Khair OA, Abdelaziz MM, Sapsford RJ, Czarlewski W, *et al.* Effect of loratadine on nitrogen dioxide-induced changes in electrical resistance and release of inflammatory mediators from cultured human bronchial epithelial cells. *J Allergy Clin Immunol* 1999;104:93-9.
10. Nakamura T, Itadani H, Hidaka Y, Ohta M, Tanaka K. Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem Biophys Res Commun* 2000;279:615-20.
11. Zhu Y, Michalovich D, Wu H, Tan KB, Dytko GM, Mannan IJ, *et al.* Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol* 2001;59:434-41.
12. Schaefer U, Schmitz V, Schneider A, Neugebauer E. Histamine induced homologous and heterologous regulation of histamine receptor subtype mRNA expression in cultured endothelial cells. *Shock* 1999;12:309-15.
13. O'Reilly M, Alpert R, Jenkinson S, Gladue RP, Foo S, Trim S, *et al.* Identification of a histamine H4 receptor on human eosinophils role in eosinophil chemotaxis. *J Recept Signal Transduct Res* 2002;22:431-48.
14. Brown V, Ennis M. Flow-cytometric analysis of basophil activation: Inhibition by histamine at conventional and homeopathic concentrations. *Inflamm Res* 2001;50 Suppl 2:S47-8.
15. 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.
16. Hill DJ, Hosking CS, de Benedictis FM, Oranje AP, Diepgen TL, Bauchau V, *et al.* Confirmation of the association between high levels of immunoglobulin E food sensitization and eczema in infancy: An international study. *Clin Exp Allergy* 2008;38:161-8.
17. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994;93:1047-59.
18. Miller H, Campbell DH. Skin test reactions to various chemical fractions of egg white and their possible clinical significance. *J Allergy* 1950;21:522-4.
19. Bleumink E, Young E. Studies on the atopic allergen in hen's egg. II. Further characterization of the skin-reactive fraction in egg-white; immuno-electrophoretic studies. *Int Arch Allergy Appl Immunol* 1971;40:72-88.
20. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997;159:2026-32.
21. Heine RG, Laske N, Hill DJ. The diagnosis and management of egg allergy. *Curr Allergy Asthma Rep* 2006;6:145-52.
22. Astwood JD, Leach JN, Fuchs RL. Stability of food allergens to digestion *in vitro*. *Nat Biotechnol* 1996;14:1269-73.
23. Vitiello L, Gorini S, Rosano G, la Sala A. Immunoregulation through extracellular nucleotides. *Blood* 2012;120:511-8.
24. Eltzschig HK, Sitkovsky MV, Robson SC. Purinergic signaling during inflammation. *N Engl J Med* 2012;367:2322-33.
25. Müller T, Vieira RP, Grimm M, Dürk T, Cicko S, Zeiser R, *et al.* A potential role for P2X7R in allergic airway inflammation in mice and humans. *Am J Respir Cell Mol Biol* 2011;44:456-64.
26. Müller T, Robaye B, Vieira RP, Ferrari D, Grimm M, Jakob T, *et al.* The purinergic receptor P2Y2 receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation. *Allergy* 2010;65:1545-53.
27. Stachon P, Peikert A, Michel NA, Hergeth S, Marchini T, Wolf D, *et al.*

- P2Y6 deficiency limits vascular inflammation and atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 2014;34:2237-45.
28. Vieira RP, Müller T, Grimm M, von Gernler V, Vetter B, Dürk T, *et al.* Purinergic receptor type 6 contributes to airway inflammation and remodeling in experimental allergic airway inflammation. *Am J Respir Crit Care Med* 2011;184:215-23.
  29. Liu GD, Ding JQ, Xiao Q, Chen SD. P2Y6 receptor and immunoinflammation. *Neurosci Bull* 2009;25:161-4.
  30. Ben Yebdri F, Kukulski F, Tremblay A, Sévigny J. Concomitant activation of P2Y(2) and P2Y(6) receptors on monocytes is required for TLR1/2-induced neutrophil migration by regulating IL-8 secretion. *Eur J Immunol* 2009;39:2885-94.
  31. Zhang Z, Wang Z, Ren H, Yue M, Huang K, Gu H, *et al.* P2Y(6) agonist uridine 5'-diphosphate promotes host defense against bacterial infection via monocyte chemoattractant protein-1-mediated monocytes/ macrophages recruitment. *J Immunol* 2011;186:5376-87.
  32. Idzko M, la Sala A, Ferrari D, Panther E, Herouy Y, Dichmann S, *et al.* Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 2002;109:839-46.
  33. Gutzmer R, Langer K, Lisewski M, Mommert S, Rieckborn D, Kapp A, *et al.* Expression and function of histamine receptors 1 and 2 on human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 2002;109:524-31.
  34. Caron G, Delneste Y, Roelandts E, Duez C, Bonnefoy JY, Pestel J, *et al.* Histamine polarizes human dendritic cells into th2 cell-promoting effector dendritic cells. *J Immunol* 2001;167:3682-6.
  35. Hori Y, Nihei Y, Kurokawa Y, Kuramasu A, Makabe-Kobayashi Y, Terui T, *et al.* Accelerated clearance of *Escherichia coli* in experimental peritonitis of histamine-deficient mice. *J Immunol* 2002;169:1978-83.
  36. Taubes G. The bacteria fight back. *Science* 2008;321:356-61.
  37. Callewaert L, Michiels CW. Lysozymes in the animal kingdom. *J Biosci* 2010;35:127-60.
  38. Sagel SD, Sontag MK, Accurso FJ. Relationship between antimicrobial proteins and airway inflammation and infection in cystic fibrosis. *Pediatr Pulmonol* 2009;44:402-9.
  39. Koch AL. Bacterial wall as target for attack: Past, present, and future research. *Clin Microbiol Rev* 2003;16:673-87.
  40. Kanthiah S, Kannappan V. Chemometric screening and optimization of liquid chromatographic method for simultaneous determination of seven antihistamines. *Int J Pharm Pharm Sci* 2016;8:288-95.
  41. Aboelyazeed H, El-haggar S, Okasha K. Comparative study between the effect of histamine receptor antagonists of type II (Famotidine) and proton pump inhibitors (omeprazole) on the efficacy of calcium carbonate as phosphate binder in haemodialysis patient. *Int J Pharm Pharm Sci* 2017;9:10-4.