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Research Article

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

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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.

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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-imodazole]-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)-1α, IL-1β, 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 m-RNA 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 ($P2Y_{1-14}$) or ionotropic P2X receptors ($P2X_{1-7}$) among which direct involvement of $P2Y_2$ and $P2X_7$ have been found [25,26]. Recently, $P2Y_6$ 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 prefilled with 2 ml chicken embryo fibroblast medium. After collection, it was transferred to the laboratory within 2 h for further studies.

Ultra-diluted histamine

It was directly procured from Dr. Willmar Schwabe India Pvt. Ltd. A Government recognized company which follows a standard pharmacopoeia for its preparation. We used in the form of histamine 6CH, which contains approximately 1 pg/ml quantity of histamine.

Egg lysozyme and OVA

This was directly purchased from HiMedia, India, in lyophilized powder form at an amount of 1 g each.

Chicken embryo fibroblast media

It was also directly purchased from HiMedia, India.

Experimental procedure

Lung tissues were taken out from the sterile containers and placed in sterile Petri dishes which were previously filled with 20 mL chicken embryo fibroblast medium in each of them. There was no supply of oxygen for the tissue to enable a hypoxic injury followed by damage and inflammation; due to this, it was termed as "restricted organoid culture." OVA and hen egg lysozyme were then reconstituted with 2 ml of chicken embryo fibroblast medium. Then, the lung tissues were divided into two groups, where each group contains four different tissues in four Petri dishes. Petri dish 1–4 belongs to Group A (Table 1) where experiment done with histamine and lysozyme along with control and Petri dish 5–8 belongs to Group B (Table 2) where OVA and histamine were used for the same along with control in similar quantities. Lysozyme is a known anti-inflammatory agent while OVA is a known pro-inflammatory agent which was used as controls along with a blank control as well. The experiment was repeated thrice. Lysozyme and OVA used in 100 µL quantities each, whereas ultra-diluted histamine was used in 1 ml quantities. In combined experiment as given in the tables, either lysozyme or OVA was added first and then after 30 min, histamine was added. The Petri dishes then placed in the incubator and left undisturbed for 24 h. After that, all the tissues were transferred to 10% buffered formol saline and were processed for histopathological examination by standard procedure. A similar experiment with ethyl alcohol was also done separately as the vehicle of ultra-diluted histamine was alcohol.

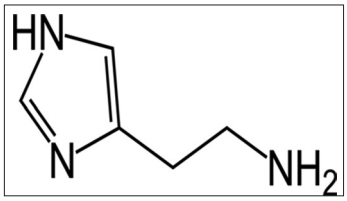

Fig. 1: Chemical structure of histamine

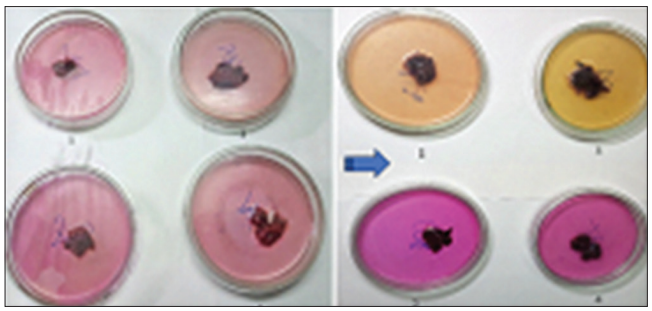

Fig. 2: Macroscopic appearance of the tissue in Petri dishes Group A, on the left at the time of inoculation and on the right after 24 h of inoculation, a significant difference in color was observed due to lowering of pH

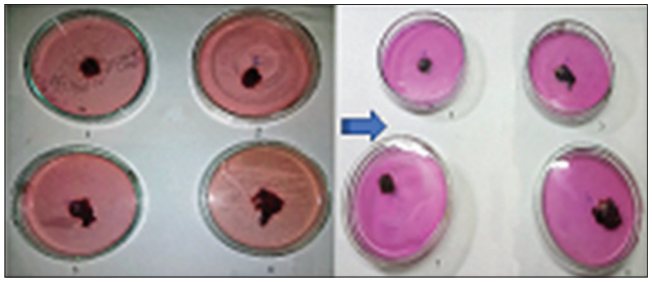

Fig. 3: Macroscopic appearance of tissue in different Petri dishes of Group B, on the left appearance of the tissues at the time of inoculation and appearance after 24 h, significant color changes were seen due to lowering of pH

Observation

Macroscopic observation of the tissue after 24 h

Yellowish-brown tissue after 24 h became dark brown in color; otherwise, no significant color changes were seen. On the other hand, there were color changes of the medium due to alteration of the pH (Fig. 2 and 3).

Microscopical examination

i. Control group: In the control experiments, there were marked accumulations of inflammatory exudate in alveoli in wide areas surrounding the bronchioles (Fig. 4a). There were dense accumulations of inflammatory cells mainly polymorphs in the exudates. There were mild edematous changes in the bronchioles
ii. Ultra-diluted histamine group: There was no accumulation of inflammatory exudate in lung alveoli except in few focal areas with mild inflammatory changes (Fig. 4b), bronchioles appeared normal in architecture and mildly dilated
iii. Lysozyme group: The changes were almost similar with the changes seen with ultra-diluted histamine group (Fig. 4c). However, there is no evidence of bronchodilation as it was observed in the histamine group
iv. OVA group: There was moderate to marked accumulation of inflammatory exudate in the lung alveoli (Fig. 4d) and the bronchioles showed markedly edematous changes
v. Alcohol group: Prominent inflammatory changes in the lung tissue with alveoli filled with inflammatory exudate (Fig. 4e) and marked edematous bronchioles were seen
vi. Combined experiments: With lysozyme and histamine the lung tissue showed almost normal features (Fig. 4f) with mild bronchodilation, whereas OVA and histamine experiment showed marked inflammatory changes and edema-like that of alcohol group (Fig. 4g).

DISCUSSION

In this experiment, it was seen that ultra-diluted histamine in ~1 pg/ml concentration showed anti-inflammatory and mild bronchodilatory action in the lung tissue. Immunoregulatory mechanism of histamine is controversial and it appears after the experiment that in ultra-diluted

Table 1: Distribution and contents of Petri dishes of Group A

Petridish	Content
1	Chicken lung tissue only; used as control
2	Chicken lung tissue+1 mL histamine 6 CH
3	Chicken lung tissue+100 µL HEL
4	Chicken lung tissue+100 µL HEL+1 mL histamine 6 CH

Table 2: Distribution and contents of Petri dishes of Group B

Petridish	Content
5	Chicken lung tissue+1 mL Rectified spirit
6	Chicken lung tissue+1 mL histamine 6 CH
7	Chicken lung tissue+100 µL OVA
8	Chicken lung tissue+100 µL OVA+1 mL histamine 6 CH

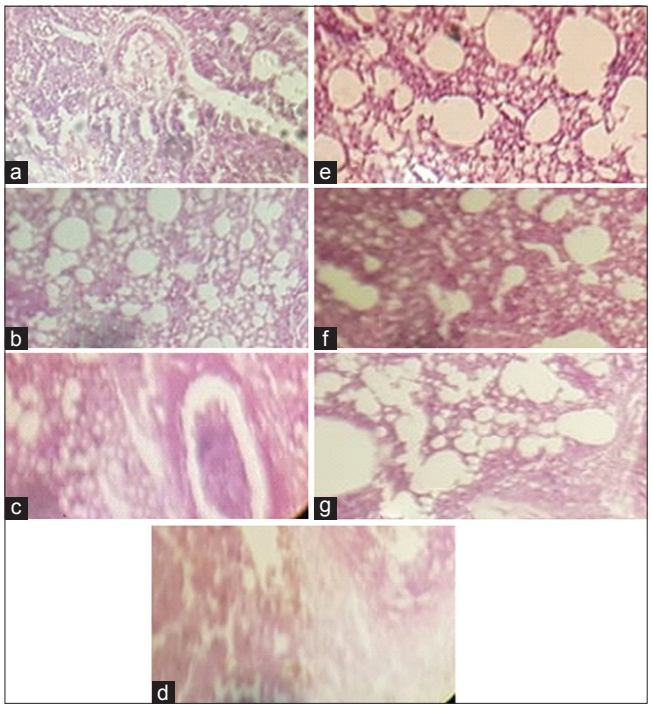

Fig. 4: (a-g) Histopathological changes in chick lung tissue in different experiments, Figs 4b, c, and f showing normal histopathological appearance of the experimental lung tissue

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.

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