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Effect of Loratadine on Guinea Pig Mast Cells: A Pharmacological and Histological Study

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Abstract

Loratadine (Claritine) is a new antihistaminic drug that blocks the peripheral H_1-receptors. This study was conducted to speculate whether it may possess other properties than merely being an antagonist at the H_1-receptors. Its effect on mast cells stabilization and mediator release was investigated both pharmacologically and histologically. The pharmacological part comprised the study of the histamine induced contraction of the isolated guinea pig trachea obtained from three groups of animals. These groups were: the control group, the sensitized challenged group and the sensitized challenged loratadine-treated group receiving the drug orally daily for 3 weeks before anaphylaxis. The histological part comprised demonstrating mast cells in the three groups in the connective tissue, trachea and lung. The percentage of degranulated cells in the connective tissue was calculated in each group. The results obtained from both studies showed a mast cell stabilizing effect and inhibition of mediator release which may contribute to its anti-allergic effect especially in patients with bronchial asthma.

Introduction

LORATADINE is a potent new antihistaminic with low toxicity, no measurable C. N. S. activity and a low potency for binding to serotonin and cholinergic receptors [1, 2].

Reports concerning the effect of loratadine on mast cells and mediator release were rather conflicting. Mann et al. [3] reported that the drug competitively blocks histamine receptor sites rather than inhibiting histamine release. Also, Hoshiko et al.

[4] concluded that inhibition of the development of airway hyperactivity is not a characteristic of loratadine.

On the other hand, Hagermark et al. [5] reported that loratadine did not only inhibit histamine effects at H_1-receptor level, but have additional suppressive effects, probably due to inhibition of mast cell degranulation.

In view of these reports, the aim of this work was to study the effect of loratadine on the mast cells and histamine release. The study comprised a pharmacological part on the isolated guinea pig trachea and a histological part on the mast cells in connective tissue, trachea and lung.

Materials and Methods

Fifteen adult male guinea pigs (350-500 gm) were used. They were divided into three groups, each containing five animals.

Group 1 (The Control Group):

Five animals were maintained on ordinary diet and did not receive any drug or antigenic material.

Group II (The Sensitized Challenged Group):

The animals were injected with 100 mg of egg albumin intraperitoneally and 100 mg subcutaneously. After three weeks, they were challenged by injecting 500 mg egg albumin dissolved in saline intraperitoneally. They were sacrificed 3 hours after challenge to give chance for the antigen to be absorbed and to reach antibodies fixed to mast cells.

Group III (The Loratadine Treated Group):

The animals of this group were first treated as those of group II. Then they were given loratadine 0.3 mg daily for 3 weeks [this dose was extrapolated from the proposed human therapeutic dose 10 mg/day according to the method of Paget and Barnes [6]. Loratadine tablets were dissolved in distilled water and given by gastric intubation. The last dose was given one hour before they were challenged. They were sacrificed 3 hours later. By the end of the 3rd week all animals were sacrificed. The trachea, lung and pieces of connective tissue from abdominal side of the thigh were excised. The following was done:

(A) Pharmacological Study:

Isolated spiral strip of guinea pig trachea were prepared according to Dungan and Lish [7]. Histamine in doses of 1, 2, 4 ug/kg was tested on the isolated trachea obtained from animals of the three different groups. For each group, the experiment was repeated 5 times. The results were expressed as means ± SE. Statistical evaluation of the data was done by Student's "t" test ($p > 0.05$).

"n" = number of experiments which was "5" for all.

B) Histological Study:

1. Spreads of subcutaneous connective tissue were prepared immediately for the

abdominal side of the thigh, fixed in Carnoy's solution and freshly stained with toluidine blue.

2. The trachea and the lung were fixed in Carnoy's solution and paraffin blocks were prepared. Thin paraffin sections of 7 u were cut and stained with toluidine blue stain.

In all preparations, the mast cells were examined. The mean percentage of degranulation of these cells was calculated by counting 100 cells and calculating the percentage of cells presenting granules extrusion in these 100 cells in the connective tissue spreads. This was done using a Meopta microscope with magnification X 1000 (oil immersion lens).

The results obtained from the control and experimental groups were statistically evaluated by the Student's "t" test, a probability "P" of 0.05 or less was accepted as significant [8].

Results

Pharmacological Results:

Dose-response curves were elicited using histamine in doses of 1, 2, 4, & 8 ug/kg on the isolated guinea pig tracheas obtained from the different groups of animals. It was found that the histamine induced contractions of the isolated trachea from the animal which were sensitized and undergone anaphylaxis without receiving the drug (group II) were significantly higher than the controls. The means of the heights of contractions are represented in table (1). The dose response curves of such results showed a parallel shift to the left compared to the control curve, indicating an enhancement of contractile reactivity (Fig. 1).

Table (I): Comparative Estimates of Histamine Induced Cintractile Responses of Perfused Isolated g.p. Tracheal Strips of controls, Undergone Anaphylaxis and Animals Treated with Loratidine Before Anaphylaxis.

Graded histamine test conctration µg / ml.	Mean values ± S.E.M. of histamine induced contractile responses (cm).	
	Contarol	Loratidine ± anaphylaxis
1	1.20 ± 0.08	0.60 ± 0.03*
2	2.44 ± 0.04	0.88 ± 0.04*
4	3.52 ± 0.037	1.64 ± 0.04*
8	3.56 ± 0.05	1.64 ± 0.02*

P > 0.05 N=S *Significant

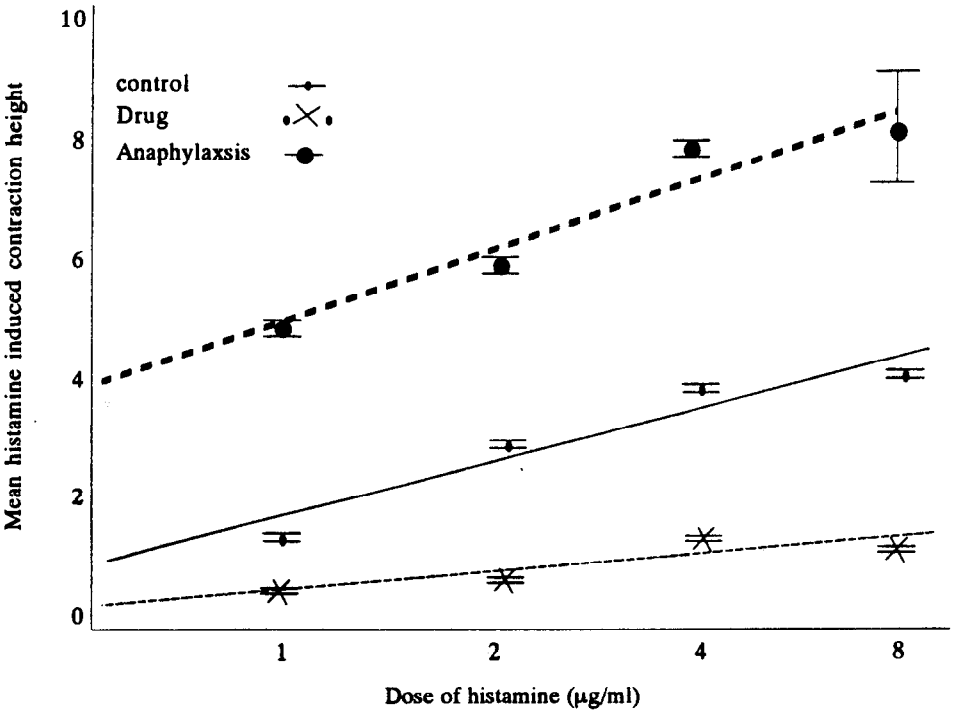

Fig.(1): Log contraction response curve of isolated g.p. spirals of controls, animals undergone anaphylaxis and animals receiving loratadine before anaphylaxis to different histamine concentrations.

On the other hand, it was found that the histamine induced contractions of the isolated tracheas obtained from animals receiving loratadine for 3 weeks before anaphylaxis (group "3") were significantly lower than the controls. The means of the heights of contractions are represented in table 1. Meanwhile, the dose response curve of such results showed a parallel shift to the right compared to the control indicating an inhibition of contractile reactivity.

Histological Results:

(I) *Mast Cells in the Control Group* :

1. *Subcutaneous Connective Tissue:*

Mast cells were clearly demonstrated in large numbers in the connective tissue spreads especially around blood vessels. They stained metachromatically with toluidine blue. Most of the cells were intact and appeared rounded or oval with large pale central nuclei (Fig. 1a). Other cells were stuffed with large numbers of

Table (II): The Percentage of Degranulated Mast in the Three Groups in the Connective Tissue.

Group of animals	No. of intact cells	No. of degranulated cells	% ofmast cell degranulated	Mean % of mast cell day + S.D.
I. Control group	90	10	10	10.6 + 2.7
	91	9	9	
	89	11	11	
	85	15	15	
	92	8	8	
II. Challenged group	20	80	80	82.4 + 5.59*
	15	85	85	
	18	82	82	
	19	81	81	
	16	84	84	
III. Loratadine treated group	60	40	40	32.6 + 2.07
	75	25	25	
	70	30	30	
	65	35	35	
	67	33	33	

*Significant $p < 0.05$ n=s

granules that sometimes masked the nucleus (Fig. 1b). The mean percentage of degranulated cells showing extrusion of granules were found to be 10.6 + 2.7 (Table II).

2. In Trachea:

Mast cells are found in the lamina propria in between the tracheal glands, most cells are rounded and intact. In the trachialis muscle, they appeared oval in shape (Fig. 1c).

3. In Lung and Bronchioles:

Mast cells were seldom found in connective tissue septa between the alveoli (Fig. 1d). In the bronchiole, they were found in the lamina propria and smooth muscle layer.

(II) Group II: The Challenged Group

Guinea pigs challenged with the second dose of the antigen showed widespread

degranulation in mast cells in all the examined specimens. The stages of degranulation varied from vacuolation of the cells up to their complete disruption with dispersion of their granules (Figs. II a, b, and c). The mean percentage of disrupted mast cells in the connective tissue was 82.4±5.59 (Table II).

(III) Group III: Loratadine-Treated Group:

The number of degranulated mast cells in this group decreased in comparison to those in challenged group. Many cells in the connective tissue were intact (Fig. III a, b, and c). The mean percentage of degranulation in this group was 32.6 ± 2.07 (Table II). Statistical analysis of the results showed significant difference between the drugtreated group and the challenged one. Similarly, in the trachea some cells were degranulated while many cells were intact (Fig. IV a and b).

In the lung, some cells were degranulated while others were intact. However, the intact cells were not densely packed with granules as those of the control group (Fig. IV c).

Discussion

The present results, both pharmacological and histological, showed that loratadine had a significant mast cell stabilizing effect and produced inhibition of histamine release. The pharmacological results showed a significant difference between the histamine-induced contraction of the isolated trachea of the animals that underwent sensitization and anaphylaxis and those taking the drug for 3 weeks before anaphylaxis. This may indicate that the drug loratadine has stabilized the mast cell membrane and prevented to a significant extent the release of histamine. This was shown by the parallel shift to the right of the curve of animals receiving the drug compared to the controls and compared to the animals undergone anaphylaxis and not receiving the drug. To ensure this finding, histological examination of mast cells in the connective tissue, trachea and lungs of the different experimental groups was done.

The present study showed that a second dose of the antigen elicited mast cell degranulation in all tissues examined in animals of group II. Some cells showed vacuolization of their cytoplasm while other were completely disrupted with dispersion of their bluish-violet metachromatic granules. These findings were in accordance with those of Mota [9]. To study the effect of loratadine as a mast cell stabilizer, the animals of group III were pretreated with the drug before being challenged with the second dose of the antigen. Most of the mast cells in the trachea and lungs of group III were intact while other cells were degranulated. The mean percentage of degranulation of mast cells was calculated in the connective tissue spreads as it was the best site for counting the cells and assessment of the percentage of degranulation.

This study showed that loratadine caused a decrease in the number of degranulated mast cells in the connective tissue of the

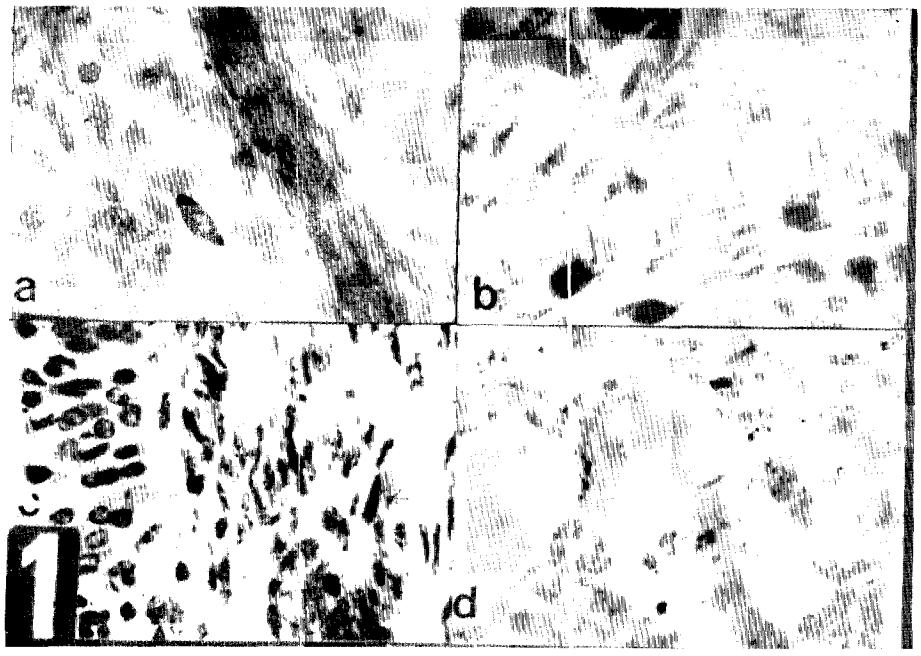

Fig. (I): (a) A connective tissue spread of a control guinea pig showing an intact mast cell. The cytoplasm is metachromatically stained bluish-violet. (Toluidine blue, original mag. X 1000).

(b) A connective tissue spread of a control guinea pig showing an intact mast cell. Stuffed with granules that masked the nucleus. (Toluidine blue, original mag. X 1000).

(c) Trachea of a control guinea pig showing an intact mast cell in the corium (arrow). (Toluidine blue, original mag. X 1000).

(d) The lung of a control animal showing an intact mast cell. (Toluidine blue, original mag. X 1000).

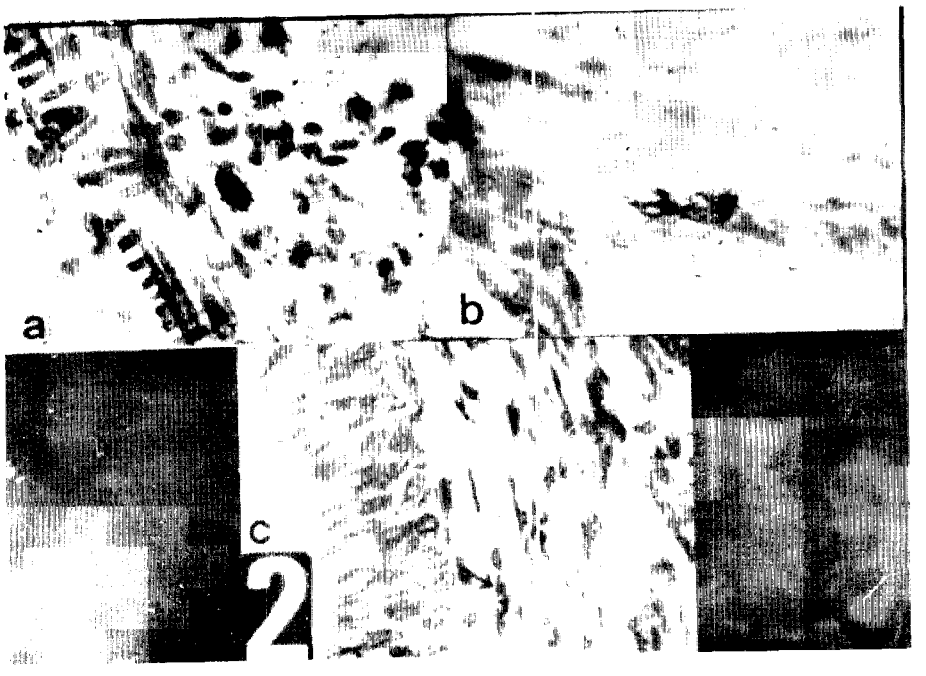

Fig. (II): (a) A connective tissue spread of a guinea pig challenged with the antigen showing a slightly degranulated mast cell. (Toluidine blue, original mag. X 1000).

(b) A connective tissue spread of challenged animal showing two disrupted mast cells. (Toluidine blue, original mag. X 1000).

(c) The trachea of a challenged animal showing a slightly vacuolated degranulated mast cell in the lamina propria (arrow). (Toluidine blue, original mag. X 1000).

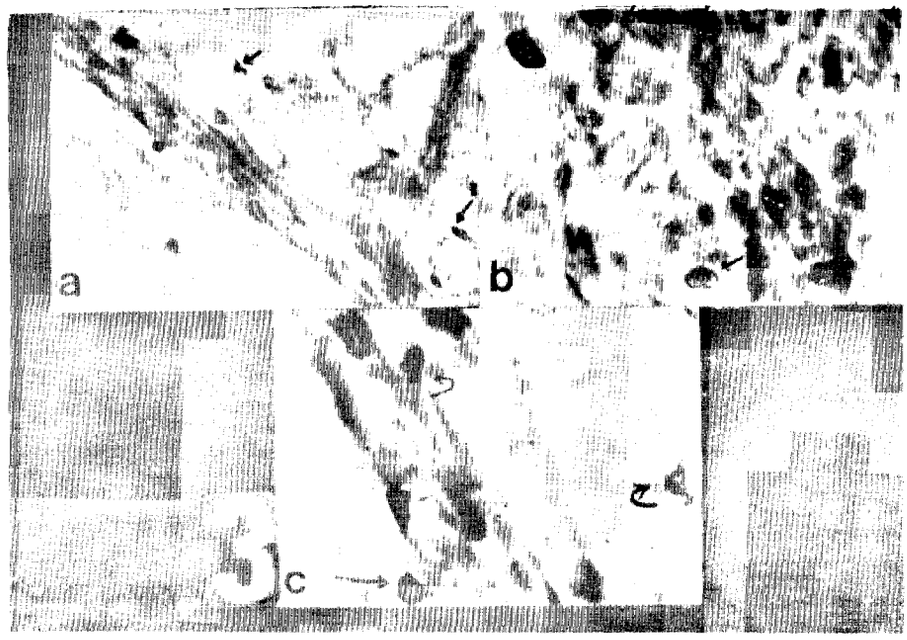

Fig. (III): (a) The connective tissue spread of a guinea pig receiving loratadine for 3 weeks showing many mast cells, some are rounded, others are spindle-shaped. Many of these cells are intact while some of them are degranulated (arrow). (Toluidine blue, original mag. X 1000).

(b) Connective tissue spread of a guinea pig that received loratadine for 3 weeks showing three intact mast cells, and another degranulated one (arrow). (Toluidine blue, original mag. X 1000).

(c) Connective tissue spread of an animal pretreated with loratadine showing an intact mast cell (small arrow), a vacuolated one (large arrow) while others are disrupted with dispersed granules (curved arrow). (Toluidine blue, original mag. X 1000).

Fig. (IV): (a) A section in the trachea of a guinea pig that received loratadine for 3 weeks showing rounded mast cells in the connective tissue corium of the mucosa. Mast cells in the muscle layer are spindle-shaped and some of them are faintly stained. (Toluidine blue, original mag. X 1000).

(b) The trachea of a guinea pig receiving loratadine for 3 weeks showing two oval or spindle-shaped mast cells, the one on the right side is degranulated. (Toluidine blue, original mag. X 1000).

(c) A bronchiole in the section of the lung of a guinea pig treated with the drug showing two mast cells in its wall. The cells appear intact but less densely packed with granules. (Toluidine blue, original mag. X 1000).

pretreated group when compared to that of the challenged one. This decrease was statistically significant. This indicated that the drug has a mast cell stabilizing effect but not to a significant extent.

Most of the literature obtained about loratadine was clinical investigations. The results of this work are in accordance with Naclerio [10] who reported that loratadine significantly reduced sneezing indicating inhibition of mediator release during the early reaction to antigen. Also, Hagermark et al. [5] reported that loratadine does not only inhibit histamine effects at H_1-receptor level but have additional suppressive effect, probably due to inhibition of mast cell degranulation. Ciprandi et al. [11] concluded that loratadine exerts a significant protective effect on the early phase of conjunctival reaction induced by allergen in atopic patients. These reports agreed with those of Anderson et al. [12] who reported that loratadine reduced the allergen-induced release of histamine into the nasal cavity. Moreover Emery et al [1], concluded that loratadine possesses anti-allergic properties as shown by its ability to inhibit mediator release from human mast cells.

From the results of this experimental work, it may be suggested that loratadine may be used in prophylaxis of bronchial asthma and not only in the treatment of the acute attack due to its effect on inhibition of mediator release from the mast cells.

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