

## To Evaluate and Compare the effects of First Generation Anti-Histamine (Chlorpheniramine Maleate) and Second Generation Anti-Histamine (Loratadine) on Isolated Trachea of Rabbit

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### Abstract

**Objective:** The incidence of respiratory allergy has increased gradually over the past several years and current estimates suggest that allergic rhinitis affects approximately 20% of the population. Large scales population surveys indicate that upto 38% of patients with rhinitis have asthma. The allergic response in the airways is an important pathogenesis to cause bronchoconstriction owing to increased responsiveness of tracheo bronchial tree to various stimuli and also causes the release of histamine and other chemical mediators from mast cells. Histamine has been shown to be an important mediator of an allergic reaction in both the upper and lower respiratory airways. Chlorpheniramine maleate is a stable, most potent, sedative first generation anti-histamine and is effective in the treatment of allergic disorders. Loratadine is a highly potent, non-sedating, long acting tricyclic, second generation anti-histamine. It is indicated in allergic rhinitis, chronic idiopathic urticaria and allergic bronchial asthma. The purpose of study was to evaluate the antagonistic effects of chlorpheniramine maleate and loratadine on histamine induced contractions in isolated trachea of rabbit and also to compare the effects of first generation anti-histamine (chlorpheniramine maleate) and second generation anti-histamine (Loratadine).

**Methods:** In this study twenty-four experiments were performed on isolated trachea of rabbit, in the presence of selected standard concentration of histamine dihydrochloride, antagonistic effects of

various concentrations of chlorpheniramine maleate (10-18 to 10-3 gm/ml) and loratadine from concentrations 10-18 to 10-3 gm/ml were recorded by Polygraph Model 7B in terms of rate and amplitude.

**Results:** Chlorpheniramine maleate showed non-significant antagonistic effect from concentrations 10-18 to 10-3 gm/ml in case of rate and 10-18 to 10-8 gm/ml in case of amplitude. Significant response showed from concentrations 10-8 to 10-3 gm/ml in case of rate ( $P < 0.001$ ) and 10-7 to 10-3 gm/ml in case of amplitude ( $P < 0.001$ ) while, loratadine showed non-significant response from concentrations 10-18 to 10-12 gm/ml in case of rate and from concentration 10-18 to 10-14 gm/ml in case of amplitude. Significant response observed from concentrations 10-11 to 10-3 gm/ml in case of rate and 10-13 to 10-3 gm/ml in case of amplitude.

**Conclusion:** It was concluded that chlorpheniramine maleate antagonized the histamine induced contractions 80.65% at concentration 10-3 gm/ml in case of amplitude and 11.35% at concentration 10-3 gm/ml in case of rate and loratadine 76.82% in case of amplitude and 10.59% in case of rate (JPMA 54:556;2004).

### Introduction

Histamine is generally considered as the principal mediator of acute inflammatory process and allergic and anaphylactic reaction<sup>1</sup>, in both the upper and lower respiratory airways.<sup>2</sup> It is the natural candidate as mast cell mediator<sup>3</sup>, also has an important role in gastric acid secretion and function as

neurotransmitter and neuromodulator.<sup>4</sup> It is found in all tissues, but high amount is found in lung, skin and gastrointestinal tract, high concentration in mast cells or basophils.<sup>5</sup> At least three storage sites of histamine are found.

i) In granules of mast cells and basophils, where it is bound with heparin and cannot exert its effects or may be metabolized.<sup>6</sup>

ii) In the mucosal layer of the gastrointestinal tract.

iii) A third fraction is held in the hypothalamus and area postrema of brain.

It occurs in plants as well as in animal tissues. Also occurs as a component of venoms and secretions from insect sting. <sup>7</sup> It is formed by decarboxylation of the amino acid L-histadine. In mammalian tissues this reaction is catalyzed by the enzyme histadine decarboxylase. After formation it is either stored or rapidly inactivated. Contractions of smooth muscles by histamine is usually associated with depolarization and increased adenosine phosphate discharge. Histamine also contracts isolated fragments of smooth muscles of cell membrane. In tracheal muscle an increase in cAMP was detected. The anti-histamines are the classic H<sub>1</sub> receptors mediated response blockers and competitively block the receptor mediated response of a target tissue. <sup>8</sup> They are divided into first and second generation anti-histamines. <sup>9</sup> The main distinguishing points between first and second generation anti-histamines are that first generation drugs are widely distributed throughout the body and are more likely to block autonomic receptors and enter the central nervous system readily while the second generation drugs are less lipid soluble and enter the central nervous system with difficulty or not at all, so they show less sedative and anticholinergic effects. <sup>10</sup>

#### Chlorpheniramine Maleate

It is a stable, most potent, first generation, sedative anti-histamine belongs to alkylamine group and acts by reversible competitive antagonism at H<sub>1</sub> receptors. <sup>11</sup> It is widely distributed in the body, including passage into the central nerve system. It is used in the treatment of urticaria, allergic rhinitis, angioedema, conjunctivitis, and in pruritic skin disorders. It is a common ingredient of cough and cold preparations.

#### Loratadine

It is highly potent<sup>2</sup>, non-sedative and long acting tricyclic<sup>11</sup> second generation anti-histamine <sup>12</sup> with selective competitive peripheral histamine H<sub>1</sub> receptor antagonistic activity <sup>13</sup>, belonging to the piperidine group and structurally related to azatadine. <sup>14</sup> It is less lipophilic, has no central nervous system activity and is essentially free of

sedation. <sup>15</sup> It is proven to be effective in the treatment of seasonal allergic rhinitis, chronic idiopathic urticaria and allergic bronchial asthma. <sup>16</sup>

**Study Design**  
The purpose of study was to evaluate the antagonistic effects of chlorpheniramine maleate and loratadine on histamine induced contractions of isolated trachea of rabbit and also to compare the effects of first generation anti-histamine (chlorpheniramine maleate) and second generation anti-histamine (loratadine).

All experimental works were carried out in the Department of Pharmacology and Therapeutics, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.

## Material and Methods

### Preparation of Serial Dilution of Drugs

Serial dilutions were made by taking 1 ml of drug (1 ml/ml) and adding 9 ml of distilled water to make the ratio 1:9. In this way serial dilutions of different drugs were prepared from concentrations 10<sup>-3</sup> to 10<sup>-18</sup> gm/ml.

### Nutrition Solution

In this vitro project Kreb's bicarbonate solution was used for the perfusion of isolated tracheal tissues. <sup>17</sup> For the preparation of 5 liters of Kreb's bicarbonate solution, following quantities of ingredients were used: sodium chloride 34.50 gm, sodium bicarbonate 10.50 gm, D-glucose 10.00 gm, sodium dihydrophosphate 0.60 gm, potassium chloride 1.85 gm, magnesium chloride 0.23 gm, distilled water 5000 ml.

### Preparation and Isolation of Tracheal Smooth Muscle

Twenty-four healthy adult rabbits male and female (non-pregnant), approximately 2 kg weight were selected and used for the present study. <sup>18</sup> The animals were sacrificed, trachea was removed and transferred to petri dish containing aerated (oxygenated) Kreb's bicarbonate solution, where it was cleaned of extraneous tissues. <sup>19</sup> A chain of tracheal section was made by cutting several rings of cartilages and tying them together loosely in such a way that muscles of two rings were at 180° to each other. <sup>20</sup> Chain was suspended vertically in an inner organ bath containing 20 ml Kreb's bicarbonate solution with the help of tissue holder and connected to the Polygraph with the help of force transducer. <sup>21</sup> The nutritional solution was continuously aerated 10-12 bubbles per minute and temperature was maintained at 37°C. The preparations were allowed to equilibrate in Kreb's bicarbonate solution for 90 minutes under an initial lead of 2 gm.

Bath solutions were changed after every 15 minutes. The drugs were added in small quantities (1 ml) at each interval to inner organ bath from lower concentration 10<sup>-18</sup> gm/ml to higher concentration 10<sup>-3</sup> gm/ml according to experimental protocol and responses from each dilution were recorded on Grass Polygraph under resting tension of 1 gm. 22

#### Methodology

First of all spontaneous contractions of tracheal smooth muscles were recorded as a baseline. In group I, tissues were challenged with serial dilutions 10<sup>-18</sup> to 10<sup>-3</sup> gm/ml of histamine dihydrochloride and responses were recorded. From those responses, standard concentration of histamine dihydrochloride was selected, which had produced maximum response.

In group II, tissues were challenged with serial dilutions 10<sup>-18</sup> to 10<sup>-3</sup> gm/ml of chlorpheniramine maleate in the presence of selected standard concentration of histamine dihydrochloride and responses were recorded for each dilution, respectively.

In group III, tissues were challenged with serial dilutions 10<sup>-18</sup> to 10<sup>-3</sup> gm/ml of loratadine in the presence of selected standard concentration of histamine dihydrochloride and responses were recorded for each dilution, respectively. After taking response of each concentration the tissues were washed and giving rest for 3 minutes before applying the next concentration.

## Observations and Results

### Effects of Histamine on Isolated Trachea of Rabbit (Group I)

In this group, eight experiments were performed and eight rabbits were used in each group. First of all spontaneous contractions of tracheal smooth muscles were recorded as a baseline. Then responses of different concentrations of histamine from 10<sup>-18</sup> to 10<sup>-3</sup> gm/ml were recorded. The results obtained in these experiments were expressed in terms of rate and amplitude (mm).

The difference between the mean values of these two data showed that histamine dose dependently increased the amplitude 11.38 mm at maximum concentration that was 10<sup>-3</sup> gm/ml and increased the rate 26.99% as shown in Table 1(a) and 1(b). Statistically: The increase in rate at the concentrations 10<sup>-18</sup> to 10<sup>-8</sup> gm/ml was non-significant, while at the concentrations 10<sup>-7</sup> to 10<sup>-3</sup> gm/ml, showed the significant response (P<0.001).

On the other hand, the increase in amplitude at the concentrations 10<sup>-18</sup> to 10<sup>-9</sup> gm/ml was non-

significant, while at the concentrations 10<sup>-8</sup> to 10<sup>-3</sup> gm/ml showed the significant response (P<0.001).

### Effects of Chlorpheniramine Maleate on Histamine Induced Contractions in Isolated Trachea of Rabbit (Group II)

In this group, eight experiments were performed and eight rabbits were used in each group. After recording the baseline, selected standard concentration of histamine dihydrochloride was used to record the agonistic effects. Then different concentrations from 10<sup>-18</sup> to 10<sup>-3</sup> gm/ml of chlorpheniramine maleate were used to record the antagonistic effects. The difference between mean values of these two data showed that chlorpheniramine dose dependently antagonized the rate and amplitude 11.35% and 80.65%, respectively a maximum concentration that was 10<sup>-3</sup> gm/ml as shown in Figure 1(a) and 1(b).

Statistically the antagonism in rate by chlorpheniramine maleate from concentrations 10<sup>-18</sup> to 10<sup>-9</sup> gm/ml were non-significant, while from concentrations 10<sup>-8</sup> to 10<sup>-3</sup> gm/ml, it was significant (P<0.001). The antagonism in amplitude by chlorpheniramine maleate from concentrations 10<sup>-18</sup> to 10<sup>-8</sup> gm/ml were non-significant, while from 10<sup>-7</sup> to 10<sup>-3</sup> gm/ml it was significant (P<0.001).

### Effects of Loratadine on Histamine Induced Contractions in Isolated Trachea of Rabbit (Group III)

In this group, eight experiments were performed and eight rabbits were used in each group. First of all spontaneous contractions of tracheal smooth muscles were recorded as a baseline. Then responses of different concentrations of histamine from 10<sup>-18</sup> to 10<sup>-3</sup> gm/ml were recorded. The results obtained in these experiments were expressed in terms of rate and amplitude (mm). The Table 1(a). Effects of histamine on isolated trachea of rabbit in group 1 (amplitude in mm).

Drug concentration	Baseline Mean	Baseline SEM	Histamine Mean	Histamine SEM	Baseline to histamine (%)	P value
10 <sup>-18</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-17</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-16</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-15</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-14</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-13</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-12</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-11</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-10</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-9</sup>	1.00	0.00	1.00	0.00	0.00	NS
10 <sup>-8</sup>	1.13	0.13	2.25	0.16	99.01	0.001
10 <sup>-7</sup>	1.38	0.18	3.25	0.16	135.50	0.001
10 <sup>-6</sup>	2.00	0.00	4.50	0.19	125.00	0.001
10 <sup>-5</sup>	2.00	0.00	6.75	0.25	237.50	0.001
10 <sup>-4</sup>	2.00	0.00	10.25	0.37	412.50	0.001
10 <sup>-3</sup>	2.13	0.13	11.38	0.38	434.22	0.001

SEM = Standard error of mean

NS = Non-significant.

gm/ml = Gram per milliliter

Table 1 (b). Effects of histamine on isolated trachea of rabbit in group 1 (rate).

Drug concentration	Baseline		Histamine		Baseline to histamine (%)	P value
	Mean	SEM	Mean	SEM		
10-18	28.50	0.42	29.38	0.46	0.03	NS
10-17	28.50	0.42	29.50	0.42	3.50	NS
10-16	28.75	0.45	29.75	0.53	3.47	NS
10-15	29.25	0.53	30.13	0.52	0.88	NS
10-14	29.50	0.42	30.50	0.42	3.38	NS
10-13	30.25	0.45	31.00	0.42	2.47	NS
10-12	30.50	0.42	31.50	0.42	3.27	NS
10-11	31.00	0.46	32.13	0.40	3.64	NS
10-10	31.38	0.46	32.38	0.46	3.18	NS
10-9	31.88	0.52	32.88	0.52	3.13	NS
10-8	32.25	0.53	33.75	0.59	4.65	NS
10-7	32.88	0.55	36.63	0.46	11.40	0.001
10-6	33.63	0.53	39.13	0.67	16.35	0.001
10-5	34.50	0.60	41.25	0.90	19.56	0.001
10-4	34.75	0.49	42.75	0.77	23.02	0.001
10-3	35.63	0.56	45.25	0.84	26.99	0.001

SEM = Standard error of mean

NS = Non-significant.

gm/ml = Gram per milliliter

[(0)]

difference between the mean values of these two data showed that loratadine dose dependently antagonized the

[(1)]

rate and amplitude 10.59% and 76.82%, respectively as shown in Figure 2(a) and 2(b).

Statistically: The antagonism in rate at the concentrations of 10-18 to 10-12 gm/ml was non-significant, while the concentrations 10-11 to 10-3 gm/ml showed the significant response ( $P < 0.001$ ).

The antagonism in amplitude at the concentrations 10-18 to 10-14 gm/ml was non-significant, while at the concentrations 10-13 to 10-3 gm/ml showed significant response ( $P < 0.001$ ).

## Discussion

In the present in vitro study, we have observed the effects of first generation anti-histamine (chlorpheniramine maleate) and second generation anti-histamine (loratadine), on histamine induced contractions of tracheal smooth muscles. In this study histamine produced a prominent action on the smooth muscles of the tracheal tissues of the rabbit characterized by contractions and both the anti-histamines showed significant antagonistic effects, but the effects of chlorpheniramine maleate were more than the loratadine. This study is according to in vivo study done by Druce et al 12 in allergic rhinitis who has compared the symptomatic effects of brompheniramine (belongs to same group as chlorpheniramine maleate) and loratadine. He found that brompheniramine was superior than loratadine in clinical efficacy. Still there is no in vitro study available on loratadine to observe its effects on the respiratory tract. More work on this drug is

necessary.

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