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# Tracheal Responsiveness to Histamine and Histamine ($H_1$) Receptor Blockade by Chlorpheniramine in an Animal Model of COPD

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**Abstract:** Airway Hyperresponsiveness (AHR) is the main feature of asthma which also exists in Chronic Obstructive Pulmonary Disease (COPD). However the mechanism of AHR is uncertain due to the complexity stimuli action used for measuring AHR. The mechanism of competitive antagonism blockade, which is measured as concentration ratio-1 (CR-1) is far simpler than that of agonists and depends only on drug delivery to the receptor sites and receptor affinity. Therefore, in this study we have examined the histamine ($H_1$) receptor blockade by chlorpheniramine on isolated tracheal chains of a model of COPD compared to control guinea pigs. Experimental models of COPD was induced in guinea pigs by exposing animals to cigarette smoke for three months. The responses of tracheal chains of COPD and control animals (for each group n=7) to cumulative concentrations of histamine (H) in the absence and presence of 10 nM chlorpheniramine were measured and the effective concentrations of H causing 50% of maximum response ($EC_{50}$ H) were obtained. The chlorpheniramine blockade (CR-1) was calculated by: (post chlorpheniramine $EC_{50}$ H/$EC_{50}$ H)-1. In addition, the contractility of tracheal chains due to 50 μM histamine concentration and hematocrit were also measured. The tracheal responses of COPD guinea pigs were significantly higher than those of control animals to histamine ($EC_{50}$ H for COPD and control animals were 19±2.45 and 34.28±2.06 μM, respectively, $p<0.001$). The histamine ($H_1$) receptor blockade by chlorpheniramine (CR-1) was also significantly higher in trachea of COPD compared to that of control animals (2.62±0.51 vs 0.44±0.06, $p<0.001$). There were significant correlations between tracheal response to $EC_{50}$ H and (CR-1) ($r=-0.564$, $p<0.05$).The hematocrit in COPD animal was significantly higher than control animals ($p<0.001$). However there were not significantly difference in contractility response of tracheal chains between COPD and control animals. The enhanced histamine ($H_1$) receptor blockade increased tracheal response to histamine in tracheal chains of COPD animals and significant correlation between these two phenomenan may indicate that increased drug delivery to the receptors could be a determinant factor for bronchial responsiveness to stimuli in COPD.

**Key words:** Tracheal responsiveness, histamine $H_1$ receptors blockade, COPD, guinea pig, chlorpheniramine

## INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a global health problem, reaching almost epidemic proportions in the developing world[1]. The research into understanding of basic mechanisms of the disease and development of new treatment to prevent the progression of this condition presents a major challenge. By leading to a clearer understanding of the key events in the pathophysiology of COPD and enabling short term studies to develop appropriate strategies, animal models can provide a framework for the rational and safe design of expensive and long term clinical studies. In cigarette smokers it is believed that airflow obstruction is caused by parenchyma disease (emphysema) and/or by smoke-induced distortion of the structure of the small airways[2-4]. Recently a model of cigarette smoke-induced lung disease in which guinea pigs develop airflow obstruction and emphysematous lung destruction has been described[5].

There are reports regarding airway hyperresponsiveness to different stimuli in animals exposed to cigarette smoke[6-10], but little is known regarding the mechanism(s) of increased airways responsiveness in animal exposed to cigarette smoke as well as asthmatic patients. This is mainly due to complexity of action of stimuli using in measuring AHR.

The mechanism(s) action of a competitive antagonist measured as the degree of rightward shift or dose ratio or concentration ratio (DR or CR) is far simpler than that of

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an agonist and depends only to concentration of antagonist at the receptor sites [I] and receptor affinity (Ka)[11]. Thus, receptor blockade by a competitive antagonist could be inside the mechanism (s) of airway responsiveness. In previous studies we demonstrated enhanced blockade of different receptors by their antagonists in both asthmatic patients[12-14] and sensitized animals[15,16].

Therefore, in the present study tracheal responsiveness of guinea pigs exposed to cigarette smoke to histamine and histamine ($H_1$) receptor blocked by chlorpheniramine was studied.

## MATERIALS AND METHODS

**Animals and cigarette smoke exposure:** Fourteen adult Dunkin-Hartley guinea pigs (400 to 500 g) of both sexes were divided into two groups of 7 experimental and 7 controls. Experimental animals were exposed to cigarette smoke as previously described[17,18]. The animals were exposed to cigarette smoke in an awake, restrained state and spontaneously breathing in a smoking chamber which was a modification of that described by Simani *et al.*[18]. Animals were placed in a plexiglas box with their heads secured in a compartment (15x12x7 cm). Twenty millilitre puffs of cigarette smoke was drawn out of cigarettes with syringes and then exhausted at a rate of two puffs per minute into the animal's head chamber. Exposure of animals to each cigarette lasted for 8-9 min, with a10 min resting period between cigarettes. The animals were initially exposed to one commercial non-filter cigarette per day and this dose was increased to a maximum of 5 cigarettes per day over a 2-week period. In a pilot study it was observed that animals couldn't tolerate the exposure of cigarette smoke of more than 5 cigarettes per day. The exposure to the smoke of 5 cigarettes per day, 6 days per week, continued for 3 months. The guinea pigs exposed to cigarette smoke were called COPD animals. The control animals were not exposed to cigarette smoke and they were kept in the animal house under normal conditions for the same period of time. The study was approved by the ethical committee of Mashhad University of Medical Sciences.

**Tissue preparations:** Guinea pigs were killed by a blow on the neck and trachea were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle and sutured together to form a tracheal chain[19,20]. Tissue was then suspended in a 10 mL organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing Krebs-Henseleit solution of the following composition (mmol/L): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, $KH_2PO_4$ 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% $O_2$ and 5% $CO_2$. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

**Measurement of tracheal response to histamine and histamine $H_1$ receptor blockade:** In each experiment two cumulative Log Concentration-response Curves (LCRC) of histamine phosphate (MW=308 BDH Chemical Co. Ltd, UK) induced contraction of tracheal chain were obtained, one 10 min after producing 10 nM concentration of chlorpheniramine maleate (MW=391, Sigma Chemical Ltd, UK) in organ bath (adding 0.1 mL of 1 μM chlorpheniramine solution to organ bath= post chlorpheniramine histamine response curve) and the other 10 min after adding the same volume of saline (baseline histamine response curve).

Cumulative log concentration-response curve of tracheal chain to increasing concentrations of histamine (0.1 μM to 10 mM) was obtained with addition of consecutive concentrations every 2 min. To obtain the curve the percentage of contraction of the tracheal smooth muscle due to each concentration of histamine in proportion to the maximum contraction obtained, in baseline histamine response curve, was calculated and plotted against log concentration of histamine.

The effective concentration of histamine causing 50% of maximum response ($EC_{50}$ H) of baseline and post chlorpheniramine histamine response curve in each experiment was measured (expressed as $EC_{50}$ H and post chlorpheniramine $EC_{50}$ H, respectively). The tracheal response to histamine was considered as $EC_{50}$ H. The histamine $H_1$ receptor blockade by chlorpheniramine was assessed as concentration ratio minus one (CR-1) which was calculated by: (post chlorpheniramine $EC_{50}$ H/$EC_{50}$ H)-1.

The experiments for measuring post chlorpheniramine histamine response curve and baseline histamine response curve in each tracheal chain were performed randomly with 1 h resting period between each of the two experiments while washing the tissues every 10 min. Tracheal responses to histamine were tested on incubated tissues with 1.4 μM indomethacin 30 min prior and during obtaining LCRC in the presence of both saline and chlorpheniramine. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and measured after fixation.

**Pathological evaluation:** The lungs of all COPD animals underwent normal pathological evaluation using hematoxin-eosin dye by a pathologist.

**Statistical analysis:** The data of tracheal response to histamine ($EC_{50}$ H), histamine $H_1$ receptor blockade (CR-1) maximum response, muscle contractility and hematocrit were quoted as mean±SEM. In comparing all values between COPD and control guinea pigs unpaired "t" were employed. Tracheal response to histamine ($EC_{50}$ H), was related to (CR-1), using the least square regression. Significance was accepted at $p<0.05$.

## RESULTS

**Histology:** The following pathological changes were observed in the lung of animals exposed to cigarette smoke:

1) Increased intra-alveolar septum in all specimens (infiltration of leukocytes).
2) Increased lymphatic tissue in the lung parenchyma of all specimens.
3) The destruction of alveolar wall and existence of emphysema in the lungs of most animals.
4) Intra-alveolar bleeding in most animal's lungs.

**Tracheal response to histamine:** The mean value of $EC_{50}$ H in tracheal chains of COPD animals (19±2.49 μM, range 8-25 μM) was significantly lower than in control animals (34.28 ±2.06 μM, range 28-45 μM, $p<0.001$), (Fig. 1a and Table 1).The most responsive trachea of COPD animals was 5.62 times more sensitive to histamine than the least responsive trachea from control animals.

**Chlorpheniramine blockade (CR-1):** The rightward shift of the post chlorpheniramine histamine response curve compared to the baseline histaminre response curve in tracheal chains of COPD animals was greater than that of control animals (Fig. 2). Mean CR-1 in tracheal chains of COPD animals (2.62±0.51, range 0.3-4.8) was 6 times greater than in control animals (0.44±0.06, range 0.2-0.7, $p<0.001$), (Fig. 1b and Table 1). The value of CR-1 of the most sensitive trachea of COPD animals was 24 times greater than that for the least sensitive trachea from control animals.

**Relationship between bronchial response to histamine and chlorpheniramine blockade:** There was a significant negative correlation between tracheal response to histamine ($EC_{50}$ H) and histamine $H_1$ receptor blockade by chlorpheniramine ($r=-0.564$, $p<0.05$; Fig. 3).

Table 1: Values of tracheal response to histamine ($EC_{50}$ H), histamine ($H_1$) receptor blockade by chlorpheniramine (CR-1), hematocrit and contractility response of tracheal chains (contraction response to 50 μM concentration of histamine) and in tracheal chains of control and COPD, (for each group, n=7) animals and statistical differences between the two groups

| Tracheal response | Control | COPD | Sta. Dif. |
|---|---|---|---|
| $EC_{50}$ (μmol) | 34.28±2.06 | 19.00±2.45 | $p<0.001$ |
| (CR-1) | 0.44±0.06 | 2.62±0.51 | $p<0.001$ |
| Contractility | 0.18±0.03 | 0.20±0.03 | NS |
| Hematocrit | 21.80±0.91 | 34.57±2.12 | $p<0.001$ |

Values are quoted as mean±SEM. Sta. Dif.; Statistical difference

**Contractility:** The mean value of the post chlorpheniramine maximum response in tracheal chains of control and COPD animals (99.71±2.85 and 86.28±11.67, respectively) were not significantly lower than maximum response in the presence of saline (100±0.00% for both cases). The mean value of contractility in tracheal chains of COPD animals (0.20±0.04 g, range 0.07-0.36 g) also was not significantly higher than control animals (0.18±0.03 g, range 0.07-0.33).

**Hematocrit:** The mean value of hematocrit in COPD animals (34.57±2.125%, range 25-44) was significantly higher than control animals (21.8±0.91%, range 20-25, $p<0.001$).

## DISCUSSION

This study showed increased tracheal response to histamine in guinea pig exposed to cigarette smoke (COPD) compared to control animals. The histological findings in animal exposed to cigarette smoke showed increased intra-alveolar septum (infiltration of leukocytes), increased lymphatic tissue, destruction alveolar walls and intra-alveolar bleeding. Therefore, the pathological changes in the lungs of these animals are similar to those of COPD patients[21-23]. The pathological changes in the lungs of animals exposed to cigarette smoke were also similar to those of previous studies[5,24,25]. The hematocrit in COPD animals were also significantly higher than control group. The pathological changes in the lung of the guinea pigs exposed to cigarette smoke and increased hematocrit in these animals confirmed the induction of COPD in the experimental group of animals. Tracheal responsiveness to histamine seen in COPD guinea pigs was similar to the results of our previous study in asthmatic patients[13].

In addition several other studies showed AHR in guinea pigs exposed to cigarette smoke to different stimuli[6-10]. However, the results of the present study also demonstrated an increased histamine $H_1$ receptor blockade by chlorpheniramine (CR-1) in COPD animals. The increased receptor blockade by a competitive

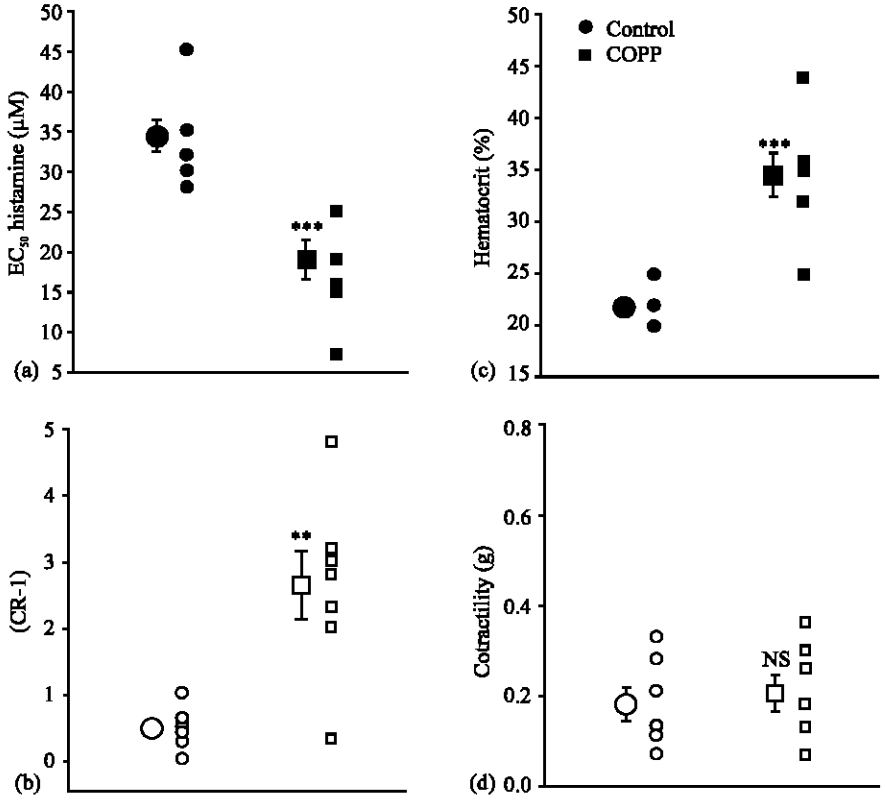


Fig. 1: Individual values and mean±SEM (big symbols with bars) of tracheal response to histamine ($EC_{50}$ H) (a), histamine ($H_1$) receptor blockade by chlorpheniramine (CR-1) (b), hematocrit (c) and contractility response of tracheal chains (contraction response to 50 μM concentration of histamine) (d), in tracheal chains of control (circle symbols) and COPD (square symbols) (for each group, n=7). Statistical differences between values in COPD with those of control animals: NS; Non-significant, *; $p<0.05$, **; $p<0.01$, ***; $p<0.001$

antagonist was similar to the results of our previous *in vitro* results indicating increased muscarinic and histamine ($H_1$) receptors by atropine[16] and chlorpheniramine[15] in sensitized guinea pigs, respectively. The consistent increase in antagonist blockade in the present study and our previous studies in sensitized animals[15,16] indicated that the cause of enhanced receptor blocked is increase of either receptor affinity (Ka) or drug delivery to the receptors ([I]) or both of these factors[1]. The fact that two receptor systems showed enhanced competitive antagonist blockade in sensitized animals[15,16] and one receptor system in cigarette exposed animals in *in vitro* studies suggests that the abnormality lies with [I] rather than Ka. This conclusion is also supported by *in vitro* experiments, which predict that receptor affinity for a given antagonist shows little variation between species and tissues[26]. We therefore suggest that this enhanced antagonist blockade may be caused by epithelial damage leading to increased epithelial permeability and accessibility of ligands to the receptor sites.

In fact, the increased airway epithelial permeability to different agents has been demonstrated in animals exposed to cigarette smoke[18,27] and in smokers[28]. The existence of airway inflammation in animal exposed to cigarette smoke[17,29,30], smokers[28,31] and COPD[32,33] is well documented. Thus airway inflammation can cause epithelial damage; and this, in turn, can result in better access of ligands to the active sites in the airways, causing increased receptor blockade by competitive antagonists.

However, our previous studies[12-14] also showed enhanced blockade when pharmacological antagonists were administered by i.v. injection in asthmatic patients and this cannot be due to increased epithelial permeability. The strongest possibility is increased tissue permeability due to airway inflammation. If the permeability of a physical barrier of some kind close to the

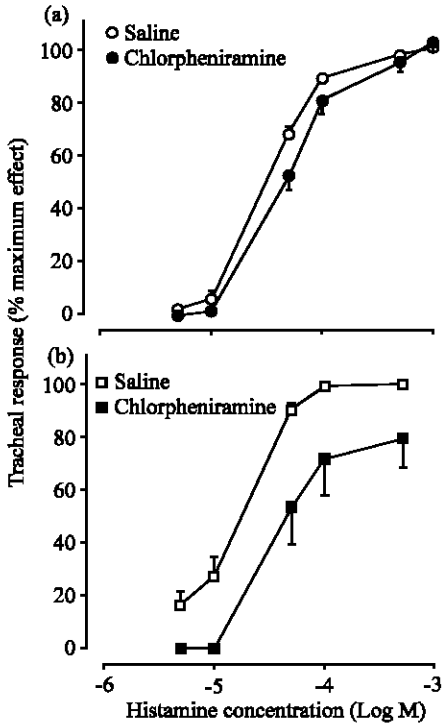


Fig. 2: Cumulative log concentration-response curves of histamine induced contraction of isolated trachea in the presence of saline and chlorpheniramine of control (a) and COPD animals (b), (for each group, n=7)

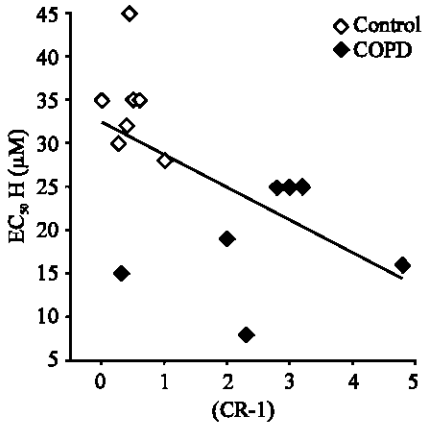


Fig. 3: Correlation between tracheal response to histamine ($EC_{50}$ H): and muscarinic receptor blockade by chlorpheniramine (CR-1) in all control and COPD guinea pigs (n=14): r=-0.542, p<0.01

receptor was increased in COPD and asthma, it could increase diffusion of antagonist ligands administered by either way and would explain the variation in (CR-1) produced by both routes of administration. In addition, the results of the present *in vitro* study cannot be fully explained by increased epithelial permeability because the barrier role of epithelium against ligands diffusion[34] may be appreciated only when perfused tracheal or bronchial tubes are exposed to ligands from the mucosal sides but not in the model used in this study, using tracheal rings. A stripe of tracheal smooth muscle is covered by epithelium only from one side and easily exposed to chemicals from the remaining three sides; this mostly excludes the barrier function of the epithelium. The increased chlorpheniramine blockade in tracheal chains of COPD guinea pigs exposed to cigarette smoke shown in this study, as well as increased chlorpheniramine, atropine and propranolol blockade in our previous *in vivo* studies in asthmatic patients[12-14] and enhanced atropine and chlorpheniramine blockade in tracheal chains of sensitized animal studies[15,16], is perhaps due to higher concentration of antagonists at the receptor sites achieved by an increased epithelial and tissue permeability leading to an increase in [I]. In addition, destruction of lung parenchyma is also documented in exposed animals to cigarette smoke[17]. This can support an increased tissue permeability and better accessibility of ligands to the receptor sites.

The results of this study also showed significant correlations between histamine $H_1$ receptor blockade by chlorpheniramine (CR-1) and tracheal response to histamine. The significant correlation between (CR-1) and tracheal response to histamine in the present study, as well as blockade by other antagonists and agonist responsiveness in previous studies[12-16], indicates that bronchial hyperresponsiveness to different stimuli in COPD and asthma, at least in part, is due to increased bronchial epithelial and tissue permeability which is perhaps due to airway inflammation in these diseases. If this is true, the treatment of these diseases should be focused on preventing this increased permeability.

The present *in vitro* study indicated higher chlorpheniramine blockade at histamine $H_1$ receptors than tracheal response to histamine in tracheal chains of COPD guinea pigs compared to control animals (5.9 times for chlorpheniramine blockade and 1.7 times for histamine response).

In addition the contractility response of tracheal chains to histamine in COPD group was similar to the control animals.These findings suggested that mainly the prejunctional mechanisms were responsible for increased tracheal responsiveness in COPD animals[35]. These mechanisms include: epithelial damage, neural control, inflammatory process and metabolism or absorption which affect antagonism blockade more than response to an agonist.

In conclusion, this study demonstrated enhanced histamine $H_1$ receptor blockade by chlorpheniramine in tracheal chains of COPD guinea pigs exposed to cigarette smoke. The cause of this enhanced antagonist blockade at the tracheal chains is perhaps increased epithelial and tissue permeability due to airway inflammation. The increased epithelial and tissue permeability of the airways appears , at least in part, is responsible for airway hyper responsiveness.

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