ORIGINAL ARTIC

Comparing the Effects of Diphenhydramine Hydrochloride with Loratadine on Isolated Trachea of Rabbit

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ABSTRACT

Objective: To study and compare the antagonist effects of first generation anti-histamine Diphenhydramine hydrochloride with second generation anti-histamine Loratadine on isolated trachea of rabbit.

Design: Comparative controlled in vitro experimental study.

Place and duration of study: This study was conducted at The Department of Pharmacology and Therapeutics, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC) Karachi for the period of six months.

Material and methods: Isolated tracheal smooth muscles of twenty four rabbits are used. Fresh Kreb's bicarbonate nutritional solution was prepared for each subject. Isolated tracheal smooth muscles were exposed to standard dilution of Histamine, and then they were challenged with serial dilutions (10^{-18} mg/ml to 10^{-3} mg/ml) of Diphenhydramine hydrochloride and Loratadine separately, and effects (rate and amplitude of contractions) were recorded by 7B Grass Polygraph machine.

Results: Diphenhydramine hydrochloride inhibits the rate of histamine induced tracheal contractions ranging from 0.17 to 8.11 % and amplitude from 0.0 to 100 %, while Loratadine inhibit the rate of histamine induced tracheal contractions from 0.85 to 10.59 % and amplitude from 6.5 to 76.82 % .

Conclusion: Diphenhydramine hydrochloride found more potent inhibitor of histamine induced contractions on isolated tracheal smooth muscles of rabbit than Loratadine.

Category: Basic Sciences**.**

Keywords: Histamine, Diphenhydramine hydrochloride, Loratadine and Isolated tracheal smooth muscles.

INTRODUCTION

Allergic rhinitis is a common disease world wide affecting a significant percentage of the global population. Seasonal allergic rhinitis is a source of great discomfort, and can have a major effect on patient's quality of life. Indeed more than 90% of seasonal allergic rhinitis patients believe that their work productivity is negatively affected by allergy symptoms[1].

Histamine is generally considered as the principle mediator of acute inflammatory process and allergic (anaphylactic) reaction[2], in both the upper and lower respiratory airways[3]. It has important role in gastric acid secretion and function as neurotransmitter and neuromodulator[4]. It is found in all tissues, but high amount in lungs, skin, gastrointestinal tract and high concentration in mast cells and basophils[5]. It is also found in animals, in plants, as a component of venoms and secretions from insect stings[6]. The effects of histamine are exerted through three well defined classical G protein coupled histamine receptor subtypes termed H_1R, H_2R and H_3R, and the more recently H_4R. Histamine signaling through H_1R is responsible for the majority of the immediate manifestations of allergic disease[7].

Anti-histamines are the classic H_1 receptors mediated response blockers and competitively block the receptor mediated response of target tissues[8]. They are divided into first and second generation anti-histamines. 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[9].

Diphenhydramine Hydrochloride: It is first generation antihistamine, Ethanolamine derivative, acts by reversible competitive antagonism at H_1 receptors and posses significant antimuscarinic and sedative activity. This drug is well absorbed when

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given orally, reaches maximum concentration in blood in about 2 hours. Diphenhydramine Hydrochloride is widely distributed throughout the body and excreted by kidney. It is effective as antiemetic particularly in motion sickness due to specific depression of conduction in vestibule celebellar path way. It is effective in perennial and seasonal allergic rhinitis, vasomotor rhinitis, conjunctivitis, urticaria and angio-edema. It is used as a sleep aids in a dose of 25-50 mg at bed-time. It is also used to reverse extrapyramidal side effects caused by phenothiazines, in early stages of Parkinson's disease and in allergic reactions by insect bites[10].

Loratadine: It is highly potent, non-sedative and long acting tricyclic[11], second generation anti-histamine[12], with selective competitive peripheral histamine H_1 receptor antagonistic activity[13]. Loratidine. Belonging to the pipridine group and structurally related to azatadine[14]. It is less lipophilic, has no central nervous system activity and is essentially free of sedation[15]. It is proven to be effective in the treatment of seasonal allergic rhinitis, chronic idiopathic urticaria and allergic bronchial asthma[16, 17].

PURPOSE OF STUDY

The purpose of study was to evaluate the antagonistic effects of Diphenhydramine hydrochloride and Loratadine on histamine induced contractions in isolated trachea of rabbit. Also to compare the antagonistic effects of first generation anti-histamine Diphenhydramine hydrochloride and second generation anti-histamine Loratadine on isolated trachea of the rabbit.

MATERIAL AND METHODS

All the experimental works were carried out for the period of three months, in the Department of Pharmacology and Therapeutics, Basic Medical Science Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi.

Preparation of serial dilutions of drugs: Serial dilutions were made by taking 1 ml of drug and adding 9ml of distilled water to make the ratio 1:9. In this way serial dilutions of drugs were prepared from concentration 10^{-3} to 10^{-18} gm/ml.

Nutrition solution: In this invitro project, Kreb's bicarbonate solution was used for the perfusion of isolated tracheal tissue. 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 1.85gm, Magnesium Chloride 0.23 gm and Distilled Water 5000 ml.

Preparation and isolation of tracheal smooth muscle: Twenty-four healthy adult rabbits, male and female (non-pregnant), approximately 2kg weight were selected and used for the present study. The animals were sacrificed; trachea was removed and transferred to Petri dish containing aerated (oxygenated) Kreb's bicarbonate solutions, where it was cleaned of extraneous tissues. 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^{0} to each other. 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 Grass Polygraph machine with the help of force transducer. The nutritional solution was continuously aerated by 10-12 bubbles of oxygen per minutes and temperature was maintained at 37^{0}C. The preparations were allowed to equilibrate in Kreb's bicarbonate solution for 90 minutes. Bath solution was changed after every 15 minutes. The drugs were added in small quantities (1ml) at each interval to inner organ bath from lower concentration 10^{-18} gm/ml to higher concentration 10^{-3} gm/ml according to experimental protocol and response from each dilution were recorded on Grass Polygraph machine under resting tension of 1 gm.

Methodology: Experimental subjects were divided into three groups. Eight animals were used for eight experiments in each group. In *group-I*, first of all spontaneous contraction of isolated tracheal smooth muscles were recorded than tissue were challenged with a serial dilution of histamine dihydrochloride (from 10^{-18} to 10^{-3} gm/ml) and responses were recorded. From these responses, standard concentration of histamine dihydrochloride (10^{-3} gm/ml) was selected, which had produced maximum response. In *group-II*, tissues were challenged with serial dilution of Diphenhydramine hydrochloride (from10^{-18} to 10^{-3} gm/ml) in the presence of selected standard concentration of histamine dihydrochloride and responses were recorded for each dilution. After taking response of each concentration the tissue were washed and given rest for 3 minutes before applying the next concentration. In *group III*, tissue were challenged with serial dilution of Loratadine (from 10^{-18} to 10^{-3} gm/ml) in the presence of selected standard concentration of histamine dihydrochloride and responses were recorded for each dilution. After taking response of each concentration the tissue were washed and given rest for 3 minutes before applying the next concentration.

RESULTS

The responses were recorded as Rate and Amplitude of tracheal smooth muscle contractions.

Table 1: Effects of Diphenhydramine Hydrochloride on Histamine induced Contractions in Isolated Trachea of Rabbit (Group-II): (Rate of contraction)

Drug concen-tration	Agonist		Antagonist		Agonist to antagonist	
gm/ml	Mean	SEM	Mean	SEM	%age	P-value
10^{-18}	29.25	0.59	29.20	0.59	0.17	n.s
10^{-17}	31.12	0.66	31.00	0.68	0.38	n.s
10^{-16}	31.87	0.61	31.62	0.65	0.78	n.s
10^{-15}	32.37	0.53	31.87	0.51	1.54	n.s
10^{-14}	33.25	0.64	32.75	0.59	1.50	n.s
10^{-13}	33.75	0.45	32.62	0.56	3.34	n.s
10^{-12}	34.37	0.62	33.37	0.73	2.90	n.s
10^{-11}	35.00	0.53	33.87	0.76	3.22	n.s
10^{-10}	35.25	0.52	33.75	0.61	4.80	n.s
10^{-9}	35.5	0.62	33.87	0.83	4.59	n.s
10^{-8}	36.37	0.82	34.37	0.59	5.87	n.s
10^{-7}	36.25	0.59	34.12	0.44	5.37	<0.001
10^{-6}	35.75	0.81	33	0.46	7.69	<0.001
10^{-5}	34.75	0.86	32	0.65	7.91	<0.001
10^{-4}	33.87	0.71	31.12	0.61	8.11	<0.001
10^{-3}	33.37	0.65	31.12	0.54	6.74	<0.001

Table 2: EEffects of Diphenhydramine Hydrochloride on Histamine induced Contractions in Isolated Trachea of Rabbit (Group-II): (Amplitude in mm)

Drug concen-tration	Agonist		Antagonist		Agonist to antagonist	
gm/ml	Mean	SEM	Mean	SEM	%age	P-value
10^{-18}	1.5	0.18	1.5	0.18	0.00	n.s
10^{-17}	1.87	0.22	1.87	0.22	0.00	n.s
10^{-16}	2.12	0.12	2.12	0.12	0.00	n.s
10^{-15}	2.87	0.12	2.87	0.12	0.00	n.s
10^{-14}	3.12	0.12	3.12	0.12	0.00	n.s
10^{-13}	4.00	0.00	4.00	0.00	0.00	n.s
10^{-12}	5.25	0.26	5.25	0.26	0.00	n.s
10^{-11}	6.00	0.00	5.75	0.16	4.16	n.s
10^{-10}	6.87	0.12	6.00	0.18	12.66	<0.001
10^{-9}	7.12	0.12	6.00	0.18	12.46	<0.001
10^{-8}	7.75	0.16	5.37	0.18	30.70	<0.001
10^{-7}	8.00	0.00	4.25	0.16	46.87	<0.001
10^{-6}	8.00	0.00	2.25	0.16	71.87	<0.001
10^{-5}	9.00	0.00	0.25	0.16	91.66	<0.001
10^{-4}	9.25	0.16	0.10	0.00	100.00	<0.001
10^{-3}	9.25	0.16	0.75	0.16	91.89	<0.001

Effects of Diphenhydramine Hydrochloride on Histamine induced Contractions in Isolated Trachea of Rabbit (Group-II):

Rate: Diphenhydramine hydrochloride antagonized the rate of histamine induced contractions of isolated tracheal smooth muscles from 0.07 to 5.49 % (non-significantly) at the concentrations 10^{-18} to 10^{-8} gm/ml while from 5.87 to 8.11 % (significantly $p<0.001$) at concentrations 10^{-7} to 10^{-3} gm/ml.

Amplitude: Diphenhydramine hydrochloride antagonized the amplitude of histamine induced contractions of tracheal smooth muscles from 0.0 to 4.16 % (non-significantly) at the concentrations 10^{-18} to 10^{-11} gm/ml and from 12.66 to 100 % (significantly $p<0.001$) at concentrations 10^{-10} to 10^{-3} gm/ml.

Table 3: Effects of Loratadine on Histamine induced Contractions in Isolated Trachea of Rabbit (Group-III): (Rate of contraction)

Drug concen-tration	Agonist		Antagonist		Agonist to antagonist	
gm/ml	Mean	SEM	Mean	SEM	%age	P-value
10^{-18}	29.37	0.49	29.62	0.37	0.85	0.01
10^{-17}	30.25	0.55	30.37	0.46	0.39	n.s
10^{-16}	31.5	0.62	31.37	0.53	0.41	n.s
10^{-15}	31.87	0.54	31.87	0.54	0.00	n.s
10^{-14}	33.75	0.86	33.25	0.61	0.74	n.s
10^{-13}	35.37	0.77	34.25	0.64	3.16	n.s
10^{-12}	36.37	0.70	35	0.5	3.76	n.s
10^{-11}	37	0.63	34.87	0.69	5.75	<0.001
10^{-10}	38.12	0.54	35.25	0.55	7.52	<0.001
10^{-9}	38.37	0.67	35.37	0.77	7.81	<0.001
10^{-8}	39.25	0.52	36	0.59	8.28	<0.001
10^{-7}	39.37	0.62	36	0.62	8.55	<0.001
10^{-6}	39.25	0.61	36	0.59	8.28	<0.001
10^{-5}	39.12	0.83	35.25	0.75	9.89	<0.001
10^{-4}	38.5	0.73	34.62	0.65	10.05	<0.001
10^{-3}	37.75	0.64	33.75	0.64	10.59	<0.001

Table 4: Effects of Loratadine on Histamine induced Contractions in Isolated Trachea of Rabbit (Group-III): (Amplitude of contraction)

Drug concen-tration	Agonist		Antagonist		Agonist to antagonist	
gm/ml	Mean	SEM	Mean	SEM	%age	P-value
10^{-18}	2.00	0.00	1.87	0.12	6.5	n.s
10^{-17}	2.50	0.18	2.50	0.18	0.00	n.s
10^{-16}	3.62	0.18	3.62	0.18	0.00	n.s
10^{-15}	4.5	0.18	4.5	0.18	0.00	n.s
10^{-14}	5.25	0.16	5.25	0.16	0.00	n.s
10^{-13}	5.62	0.18	4.87	0.29	8.89	<0.001
10^{-12}	6.37	0.18	5.37	0.18	15.69	<0.001
10^{-11}	7.62	0.18	5.37	0.18	29.52	<0.001
10^{-10}	8.25	0.16	4.87	0.29	44.35	<0.001
10^{-9}	8.62	0.18	5.12	0.22	40.60	<0.001
10^{-8}	9.37	0.26	4.87	0.22	48.02	<0.001
10^{-7}	9.87	0.12	4.62	0.18	53.19	<0.001
10^{-6}	10.12	0.12	4.25	0.16	58.00	<0.001
10^{-5}	10.75	0.16	4.00	00	64.28	<0.001
10^{-4}	11.62	0.18	3.75	0.16	67.72	<0.001
10^{-3}	11.87	0.12	2.87	0.12	76.82	<0.001

Effects of Loratadine on Histamine Induced Contractions in Isolated Trachea of rabbit (Group-III):

Rate: Loratadine antagonized the rate of histamine induced contractions of tracheal smooth muscles from 0.85 to 3.76 % (non-significantly) at the concentrations 10^{-18} to 10^{-12} gm/ml and from 5.75 to

10.59 % (significantly p<0.001) at concentrations 10^{-11} to 10^{-3} gm/ml.

Amplitude: Loratadine antagonized the amplitude of histamine induced contractions of tracheal smooth muscles 6.5 % (non-significantly) at the concentrations 10^{-18} to 10^{-14} gm/ml and from 8.89 to 76.82 % (significantly p<0.001) at concentrations 10^{13} to 10^{-3} gm/ml.

DISCUSSION

In this invitro study we study and compare the antagonistic effects of first generation antihistamine Diphenhydramine hydrochloride with second generation antihistamine Loratadine. We found that first generation antihistamine Diphenhydramine hydrochloride had antagonized histamine induced contractions of isolated smooth muscles of trachea more significantly than second generation antihistamine Loratadine. Our results matches with the study of Dobashi k (1995)[18] who also found Diphenhydramine more potent inhibitor of antigen induced responses. Our results also match with research study results of Kay GG et al[8] who observed effects of first generation antihistamine more potent than second generation. That study was on allergic patients. Druce HM et al[12]did prove that classical antihistamine (H-1 receptor antagonists) are more potent than second or new generation antihistamines. Gozsy B and Kato L[2] have described mechanism of action of both types of antihistamines. They did pharmacokinetic analysis and proved that first generation antihistamines are more lipid soluble than new or second generation antihistamines. They also proved polarity of second generation , so this second generation have less central nervous system effects than first generation. Liu H observed antagonistic effects of antihistamines on muscarinic induced mucus cell ion transport and rank them on potency in order to Desloratadine > Cetirizine > Fexofenadine > Diphenhydramine > Loratadine[19]. The present study demonstrated on histamine induced contractions of isolated trachea; even that Diphenhydramine found more potent than Loratadine.

Sheffer AL and Samuel LL[9] researched on old generation and new generation antihistamines and proved that second generation have more effects than first generation. Their results are in contrast with our research results. They have not mentioned the exact mechanism of action of higher potency of second generation antihistamine. We assumed from comparison of their research and our research work that second generation antihistamines have more beneficial effects due to their non-sedative or less sedative effects. Individuals prefer to take less sedative antihistamine for rhinitis or anyother allergic condition to continue their daily life activities.

CONCLUSION

In this present invitro study, we had observed the effects of first generation anti-histamine Diphenhdydramine hydrochloride and second generation anti-histamine Loratadine on Histamine induced contractions of isolated tracheal smooth muscles. We found that Diphenhdydramine hydrochloride has more potent antagonistic action on histamine induced contractions of isolated tracheal smooth muscles than Loratadine.

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