ORIGINAL ARTICLE

Comparing the Inhibitory Effects of Diphenhydramine Hydrochloride with Cetirizine Dihydrochloride 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 Cetirizine dihydrochloride on isolated trachea of rabbit.

Design: Comparative controlled invitro 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 were used. Fresh Kreb's nutritional solution was prepared for each subject. Tracheal smooth muscles were exposed to standard dilution of histamine, and then they were challenged with serial dilutions (10^{-18} to 10^{-3} gm/ml) of Diphenhydramine hydrochloride and Cetirizine dihydrochloride separately, and effects 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 Cetirizine dihydrochloride inhibit the rate of histamine induced contractions from 0.85 to 12.33 % and amplitude from 0.0 to 82.69 %, as concentration of drugs increased.

Conclusion: Cetirizine found more potent antagonistic effects on rate of histamine induced cotractions of isolated tracheal smooth muscles than Diphenhdydramine hydrochloride.While Diphenhdydramine hydrochloride has more potent antagonistic action on amplitudes of histamine induced contractions of isolated tracheal smooth muscles than Cetirizine.

Keywords: Histamine, Diphenhydramine hydrochloride, Cetirizine dihydrochloride and Rabbit's 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[1,2].

Histamine is generally considered as the principle mediator of acute inflammatory process and allergic reaction[3], in both the upper and lower respiratory airways[4]. It has important role in gastric acid secretion and function as neurotransmitter and neuromodulator[5]. It is found in all tissues, but high amount in lungs, skin, gastrointestinal tract, mast cells and basophils[6]. It is also found in animals, in plants, as a component of venoms and secretions from insect stings[7]. 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[8].

Anti-histamines are the classic H_1 receptors mediated response blockers and competitively blocks the receptor mediated response of target tissues[9]. Difference 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[10].

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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. 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 [5].

Cetirizine Dihydrochloride: It is a potent, non-sedative, long-acting H_1 receptor antagonist belongs to second generation antihistamines [8, 11]. It is a member of piperizine group of H_1 antagonists[12], minimally metabolized, so it may be anti-histamine of choice for patients with hepatic dysfunction and can also be used in elderly patients without dosage reduction. It has a low rate of penetration of the blood brain barrier and is without any significant anti-cholinergic and anti-serotonin effects, used in the treatment of allergy[13].

MATERIAL AND METHODS

All experimental works were carried out for the period of six months, in the Department of Pharmacology and Therapeutics, Basic Medical Science Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi. 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. In this vitro 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 tissue. 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 10-12 bubbles of oxygen per minutes and temperature was equilibrate in Kreb's bicarbonate solution for 90 minutes. Bath solution was changed after every 15 minutes. The drug 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 was recorded on 7B 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 contractions of 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 response were recorded. From these responses, standard concentration of histamine dihydrochloride (10^{-3} gm/ml) was selected, that 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 (10^{-3} gm/ml) 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 Cetirizine Dihydrochloride (from 10^{-18} to 10^{-3} gm/ml) in the presence of selected standard concentration of histamine dihydrochloride (10^{-3} gm/ml) 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

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 0.17 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.

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

Rate: Cetirizine Dihydrochloride antagonized the rate of histamine induced contractions of isolated tracheal smooth muscles from 0.85 to 3.70 % (non-significantly) at the concentrations 10^{-18} to 10^{-12} gm/ml and from 7.78 to 12.33 % (significantly $p<0.001$) at concentrations 10^{-11} to 10^{-3} gm/ml.

Amplitude: Cetirizine Dihydrochloride antagonized the amplitude of histamine induced contractions of isolated tracheal smooth muscles 0.00 % (non-significantly) at the concentrations 10^{-18} to 10^{-13} gm/ml and from 13.33 to 82.69 % (significantly $p<0.001$) at concentrations 10^{-14} to 10^{-3} gm/ml.

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

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

Drug concentration	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

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

Drug concentration	Agonist		Antagonist		Agonist to antagonist	
gm/ml	Mean	SEM	Mean	SEM	%age	P-value
10^{-18}	29.37	0.46	29.62	0.49	0.85	n.s
10^{-17}	30.87	0.47	30.87	0.47	0.00	n.s
10^{-16}	32.37	0.56	32.12	0.47	0.77	n.s
10^{-15}	35.5	0.46	33.25	0.49	1.33	n.s
10^{-14}	34.5	0.46	34.12	0.44	2.72	n.s
10^{-13}	35.37	0.46	35.00	0.46	3.46	n.s
10^{-12}	36.37	0.41	35.25	0.52	3.70	<0.01
10^{-11}	36.37	0.47	34	0.70	7.78	<0.001
10^{-10}	37.12	0.54	34.62	0.49	9.42	<0.001
10^{-9}	38.00	0.42	35	0.46	7.89	<0.001
10^{-8}	39.00	0.53	35.25	0.70	9.19	<0.001
10^{-7}	39.37	0.53	35.75	0.75	9.19	<0.001
10^{-6}	39.5	0.62	35.37	0.62	10.45	<0.001
10^{-5}	39.25	0.61	35.37	0.82	9.85	<0.001
10^{-4}	39	0.42	34.37	0.59	11.87	<0.001
10^{-3}	38.5	0.46	33.75	0.61	12.33	<0.001

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

Conc:	Agonist		Antagonist		Agonist to antagonist	
gm/ml	Mean	SEM	Mean	SEM	%age	P-value
10^{-18}	2.12	0.12	2.12	0.12	0.00	n.s
10^{-17}	2..87	0.12	2.87	0.12	0.00	n.s
10^{-16}	4.12	0.22	4.12	0.12	0.00	n.s
10^{-15}	5.37	0.18	5.37	0.22	0.00	n.s
10^{-14}	6.12	0.22	6.12	0.18	0.00	n.s
10^{-13}	6.87	0.22	6.87	0.22	0.00	n.s
10^{-12}	7.5	0.18	6.5	0.22	13.33	n.s
10^{-11}	8.25	0.25	5.75	0.18	30.30	<0.001
10^{-10}	9.12	0.22	5.62	0.16	38.04	<0.001
10^{-9}	9.62	0.18	5.25	0.18	45.42	<0.001
10^{-8}	10.37	0.18	4.5	0.16	56.60	<0.001
10^{-7}	11.12	0.12	4.12	0.18	62.94	<0.001
10^{-6}	11.87	0.12	3.87	0.12	67.79	<0.001
10^{-5}	12.00	0.00	3.37	0.12	71.91	<0.001
10^{-4}	12.00	0.00	2.62	0.18	77.5	<0.001
10^{-3}	12.25	0.16	2.12	0.12	82.69	<0.001

DISCUSSION

In the present in vitro study, we have observed the effects of first generation anti-histamine Diphenhdydramine hydrochloride and second generation anti-histamine Cetirizine Dihydrochloride on Histamine induced contractions of isolated tracheal smooth muscles. We found that Cetirizine Dihydrochloride has more potent antagonistic action on rate of histamine induced contractions of isolated tracheal smooth muscles than Diphenhdydramine hydrochloride, while Diphenhdydramine hydrochloride found more potent antagonist on the amplitudes of histamine induced contractions of isolated tracheal smooth muscles than Cetirizine Dihydrochloride .These results confirm the findings of previous studies like study of Liu H, who 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[14]. Our results also match with research study results of Sheffer AL and Samuel LL (1990) researched on old generation and new generation antihistamines and proved that second generation has more effects than first generation[10]. In our study Diphenhydramine inhibit the histamine induced amplitude of tracheal contraction 100%, which matches with the study of Dobashi K1995[15].

CONCLUSION

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

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