

## IPRATROPIUM ATTENUATES INSULIN INDUCED TRACHEAL SMOOTH MUSCLE CONTRACTION OF GUINEA PIG IN VITRO

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### ABSTRACT

**Objective:** To evaluate the magnitude of insulin induced tracheal smooth muscle contraction of guinea pigs and exploration of protective effects of ipratropium against insulin induced increased airway reactivity of guinea pigs in vitro.

**Study Design:** Laboratory based randomized control trials.

**Place and Duration of Study:** Pharmacology department and Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi from December 2011 to July 2012.

**Material and Methods:** Effects of increasing concentrations of histamine (10<sup>-8</sup>- 10<sup>-3</sup> M), insulin (10<sup>-8</sup>- 10<sup>-3</sup> M) and insulin pretreated with ipratropium (10<sup>-6</sup> M), were studied on isolated tracheal tissue of guinea pig in vitro by constructing cumulative concentration response curves. The tracheal smooth muscle contractions were recorded with transducer on four channel oscillograph.

**Results:** Histamine and insulin produced a concentration dependent reversible contraction of isolated tracheal muscle of guinea pig. The mean  $\pm$  SEM of maximum amplitudes of contraction with histamine, insulin and insulin pretreated with ipratropium were 92.5  $\pm$  1.20 mm, 35  $\pm$  1.13 mm and 27.8 $\pm$  1.27 mm respectively. Ipratropium shifted the concentration response curve of insulin to the right and downwards.

**Conclusions:** Ipratropium significantly inhibited the contractile response of insulin on isolated tracheal muscle of guinea pig, so pretreatment of inhaled insulin with ipratropium may have clinical implication in amelioration of its potential respiratory adverse effects such as bronchoconstriction.

**Keywords:** Histamine, Inhaled insulin, Oscillograph, Tracheal muscle.

### INTRODUCTION

Subcutaneous insulin is the main stay for controlling blood glucose in diabetes. Non invasive, inhalational insulin is an attractive alternative to parenteral insulin for those patients who defer to initiate subcutaneous insulin<sup>7</sup>. Studies reveal that inhalational insulin thrice daily before meals can provide glycemic control comparable to conventional subcutaneous insulin but with improved patient's satisfaction and compliance<sup>3</sup>. Long term studies have also demonstrated a significant reduction in HbA1c with fewer hypoglycemic episodes and less risk for weight gain as compared to regular insulin<sup>6,13</sup>. Unfortunately it was withdrawn from the market due to its respiratory adverse effects such as increased bronchial reactivity, cough, dyspnoea

and bronchoconstriction<sup>14</sup>. The most likely mechanism of inhaled insulin induced bronchoconstriction is that insulin modulates the function of neuronal autoregulatory M2 receptors in airways which are responsible for inhibiting the release of acetylcholine (Ach). Insulin mediated airway hyper-responsiveness is possibly due to loss of M2 receptor function and subsequently increased release of acetylcholine is responsible for increased airway reactivity<sup>9</sup>. Previous studies have shown that pretreatment with  $\beta_2$  agonists elicited a significant protection against inhalational insulin induced bronchoconstriction but protective effects of ipratropium against increased airway reactivity due to inhaled insulin have never been evaluated<sup>11</sup>. Experimental and clinical evidences indicated that ipratropium offers protection against multiple diverse stimuli which increases the parasympathetic activity and contributes to bronchoconstriction<sup>10</sup>. Acetylcholine is the primary neurotransmitter which stimulates the

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muscarinic receptors and induces airway smooth muscle contraction<sup>16</sup>. Ipratropium inhibits the effect of acetylcholine by blocking the muscarinic receptors in respiratory passages (M1 to M5 subtypes). So it relaxes the airway smooth muscles and produces bronchodilatation<sup>4</sup>. Insulin induced isolated tracheal muscle contraction in guinea pig model described in the present study closely resembles the bronchoconstriction induced by pulmonary delivery of inhaled insulin as high concentration of insulin gets deposited in airway smooth muscle (ASM) compartment in both cases<sup>14</sup>.

So the current experimental study was planned to explore the acute effects of insulin on guinea pig airways and to evaluate the efficacy of ipratropium regarding its inhibitory effects on insulin mediated tracheal tissue contraction of guinea pig *in vitro*. Histamine is commonly used for provocative studies on airway smooth muscles<sup>1</sup>. So to evaluate the magnitude of insulin induced airway hyper-reactivity, histamine mediated airway hyper-responsiveness was taken as standard and was compared to insulin mediated tracheal smooth muscle contraction.

## MATERIAL AND METHODS

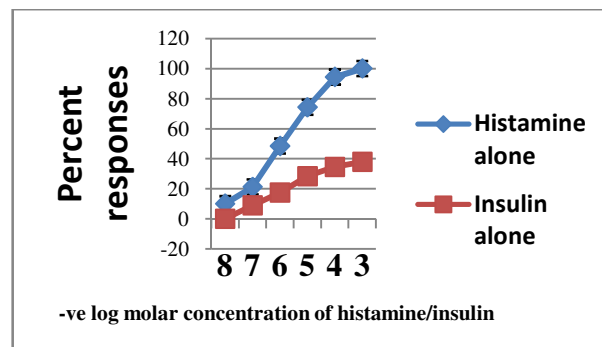
The current study was conducted in pharmacology department in collaboration with Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College Rawalpindi from December 2011 to July 2012.

Ipratropium (0.25mg/ml) was purchased from Chiesi Pharma. Regular human insulin (100 IU/ml) and histamine were obtained from Lilly Pharma and Sigma chemical Co. USA respectively.

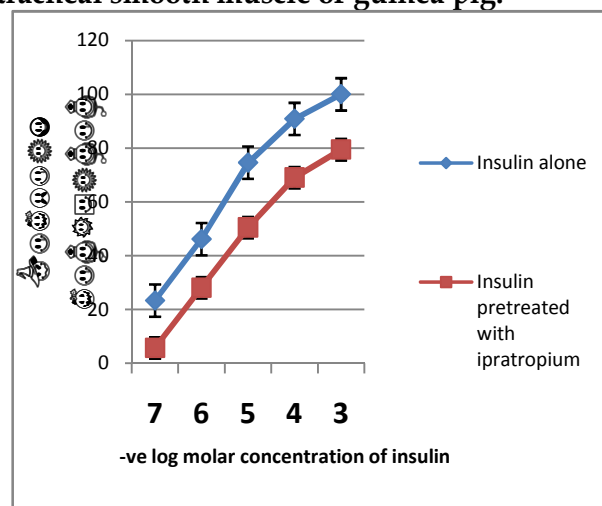
### Preparation of tissue and experimental setup

Eighteen guinea pigs were randomly divided into three groups after the approval of ethics committee of CREAM. They were killed by cervical dislocation<sup>2</sup>. The trachea was dissected out and tracheal chain was prepared with smooth

muscle in the centre and cartilaginous portions on both sides<sup>5</sup>. One end of the tracheal strip was



**Figure-1: Comparison of semi log concentration response curve of group 1 (histamine) and group 2 (insulin) on isolated tracheal smooth muscle of guinea pig.**



**Figure-2: Comparison of semi log concentration response curve of group 2 (insulin control) and group 3 (insulin after pretreatment with ipratropium) on isolated tracheal smooth muscle of guinea pig.**

attached to the hook of oxygen tube of tissue bath containing oxygenated Krebs-Henseleit solution at 37° C, while the other end was connected to a research grade isometric force displacement transducer (Harvard Model No 72-4494). Four channel oscillograph (Harvard Model No 50-9307) was used for recording tracheal contractions<sup>8</sup>.

Cumulative concentration response curve was obtained with increasing concentrations of histamine (10-8 to 10-3M)<sup>1</sup>. Histamine in a concentration of 10-8 M was added into the organ bath when the plateau was achieved then the next dose was added without washing the previous dose. When the maximal histamine induced contraction was obtained, the tracheal strip was washed three to four times and was allowed to relax passively. This group served as control (group I) and the effect of insulin on

guinea pig by using the same procedure as described for histamine. In group III ipratropium was added to the organ bath in a fixed concentration of 10-6 M<sup>16</sup>. After 15 minutes, the successive doses of insulin ranging from 10-7 to 10-3 M were added into the organ bath in the presence of ipratropium. Cumulative concentration response curves of insulin pretreated with ipratropium were constructed. Six experiments were performed in the same way to get six recordings in all the three groups.

**Table-1: Response of isolated tracheal muscle of guinea pig to histamine (group I) and insulin (group II).**

Concentration (M) of histamine/insulin	Amplitude of contraction with histamine (Mean $\pm$ S.E.M) (mm) (n=6)	Amplitude of contraction with insulin (Mean $\pm$ S.E.M) (mm) (n=6)	p-value between group 1 & 2	Percent response with histamine	Percent response with insulin
10-8	9.33 $\pm$ 1.33	0 $\pm$ 0	.000*	10.086	0
10-7	19.67 $\pm$ 1.081	8.167 $\pm$ 0.87	.000*	21.26	8.87
10-6	44.8 $\pm$ 1.68	16.16 $\pm$ 1.01	.000*	48.43	17.55
10-5	68.67 $\pm$ 2.106	26.1 $\pm$ 1.13	.000*	74.24	28.34
10-4	87.3 $\pm$ 1.33	31.8 $\pm$ 0.832	.001*	94.37	34.53
10-3	92.5 $\pm$ 1.20	35 $\pm$ 1.13	.001*	100	38

p value < 0.05 = Significant (\*)

**Table-2: Response of isolated tracheal muscle of guinea pig to insulin (group 2) and insulin pretreated with ipratropium (group-VI).**

Concentration of insulin (M)	Amplitude of contraction with insulin (mean $\pm$ S.E.M) (mm) n=6	Amplitude of contraction with insulin pretreated with ipratropium (mean $\pm$ S.E.M) (mm) n=6	p value between group 2&3	Percent response with insulin	Percent response with insulin pretreated with ipratropium
10-7	8.167 $\pm$ 0.87	2 $\pm$ 0.73	0.004*	23.34	5.71
10-6	16.16 $\pm$ 1.01	9.83 $\pm$ 1.33	0.007*	46.17	28.09
10-5	26.1 $\pm$ 1.13	17.66 $\pm$ 0.76	0.000*	74.58	50.46
10-4	31.8 $\pm$ 0.832	24.16 $\pm$ 1.72	0.003*	90.86	69.02
10-3	35 $\pm$ 1.13	27.8 $\pm$ 1.27	0.004*	100	79.42

p value < 0.05 = Significant (\*)

tracheal muscle was compared to it because in research studies histamine mediated airway smooth muscle contraction is commonly taken as standard (100 percent). In group II effect of varying concentrations of insulin (10-8 to 10-3M)<sup>14</sup> were studied on isolated tracheal muscle of

### Statistical Analysis

The results were expressed as means  $\pm$  standard error of means and statistically significant differences were assessed by "student t-test" using SPSS version 16. The differences

between the observations were considered as significant if *p* value was less than 0.05.

## RESULTS

In a series of six experiments for each group, histamine and insulin produced a dose dependent reversible contraction of tracheal chain of guinea pig. Maximum mean amplitudes of contraction with histamine, insulin and insulin pretreated with ipratropium were  $92.5 \pm 1.20$  mm,  $35 \pm 1.13$  mm and  $27.8 \pm 1.27$  mm respectively. The percentage responses for all the three groups were also calculated (Table 1 and 2). Maximum insulin induced contraction was 38 percent of histamine mediated contraction (Figure-1). Insulin concentration response curves in the presence of ipratropium were shifted to the right and downwards indicating a profound inhibitory effect on air-way hyper-reactivity induced by insulin (Figure-2). The mean amplitude of responses produced by each dose of insulin and insulin pretreated with ipratropium when compared between group II and III were found to be statistically significant (Table-2 and Figure-2).

## DISCUSSION

The present study was carried out to evaluate the beneficial effects of ipratropium regarding its ability to attenuate the insulin induced contractions of isolated tracheal smooth muscle of guinea pig. Histamine and insulin produced a concentration dependent, reversible contraction of tracheal smooth muscle. These findings were consistent with the results of Schaafsma and his coworkers who also reported the acute contractile effects of insulin on tracheal preparations of guinea pig in vitro<sup>14</sup>.

The maximum insulin induced tracheal tissue contraction was 38 percent of histamine mediated contraction which is consistent with the findings of Schaafsma and his colleagues who demonstrated that insulin induced tracheal muscle contraction was 33 percent of histamine mediated contraction, using the same experimental setup<sup>14</sup>. Our findings are in accordance with in vivo studies in which

treatment of diabetic rats with insulin resulted in M2 receptor dysfunction that leads to increased airway hyper-responsiveness and eosinophilia after allergen challenge<sup>12</sup>. Consistent with these findings Terzano and his coworkers reported that low levels of insulin resulted in decreased M2 muscarinic receptor sensitivity and subsequently decreased airway reactivity in diabetes induced rat model<sup>15</sup>.

Ipratropium shifted the concentration response curve of insulin downwards and to the right with percent response of 79.42 percent of the insulin control. The mean values of amplitudes of contractions when compared between insulin control group and ipratropium pretreated group, were found to be statistically significant. Since insulin mediated hyper-reactivity is likely to be vagally mediated in guinea pigs and rats, ipratropium may afford protection against insulin induced tracheal contraction due to its ability to inhibit the reflex acetylcholine induced bronchoconstriction mediated by multiple diverse stimuli<sup>10</sup>. Moreover blockage of M3 receptors by ipratropium in airways counteract the enhanced Ach release that would result from dysfunction of inhibitory M2 receptors induced by insulin<sup>4</sup>.

This in vitro study provides the first evidence that ipratropium can significantly inhibit the contractile response of insulin on guinea pig airways. Insulin induced isolated tracheal muscle contraction in guinea pig model described in the present study closely resembles the bronchoconstriction induced by pulmonary delivery of inhaled insulin as airway smooth muscles are directly exposed to high concentration of insulin in both cases<sup>14</sup>. So pretreatment with ipratropium may be considered as an attractive option for diabetic patients encountering respiratory adverse effects with inhaled insulin therapy.

## CONCLUSIONS

Insulin has acute contractile effects on tracheal smooth muscle of guinea pigs which

were significantly inhibited in the presence of ipratropium. So ipratropium can become useful therapeutic agent for attenuation of bronchoconstriction mediated by inhaled insulin therapy in asthmatic patients.

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