ORIGINAL ARTICLE

PROTECTIVE EFFECTS OF BECLOMETHASONE AGAINST INSULIN INDUCED AIRWAY HYPER-REACTIVITY IN GUINEA PIG AND EXPLORATION OF ITS MECHANISM OF ACTION

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Background: Inhalational insulin was withdrawn from market in 2007 due to its potential to produce airway hyper-reactivity and bronchoconstriction. So we explored the possible mechanism underlying the acute contractile effects of insulin and protective effects of beclomethasone against insulin induced airway hyper-responsiveness on isolated tracheal smooth muscle of guinea pig *in vitro*. **Methods:** This was a laboratory based experimental study. Effects of increasing concentrations of histamine (10^{-8}–10^{-3} M), insulin (10^{-8}–10^{-3} M), insulin pretreated with fixed concentration of indomethacin (10^{-6} M), and beclomethasone (10^{-6} 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±SEM of maximum amplitudes of contraction with histamine, insulin and insulin pretreated with indomethacin and beclomethasone were 92.5±1.20 mm, 35±1.13 mm, 14.55±0.62 mm and 22±1.154 mm respectively. Beclomethasone shifted the concentration response curve of insulin to the right and downwards. **Conclusion:** Beclomethasone significantly inhibited the contractile response of insulin which was presumably prostaglandin mediated. So pretreatment of inhaled insulin with beclomethasone may have clinical implication in amelioration of its potential respiratory adverse effects such as bronchoconstriction.

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

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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.[1] 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.[2] Long term studies have also demonstrated a significant reduction in HbA_{1c} with fewer hypoglycemic episodes and less risk for weight gain as compared to subcutaneous insulin.[3,4] Unfortunately it was withdrawn from the market due to its respiratory adverse effects such as increased bronchial reactivity, cough, dyspnoea and bronchoconstriction.[5] Conflicting studies are available with regards to the possible mechanisms of insulin mediated bronchoconstriction. The most likely mechanism is that insulin increases the synthesis and release of contractile prostaglandins and histamine from mast cells which in turn increases the inflammatory response of airway.[6,7] Some experimental evidences reveal that it is likely to be vagally mediated and increased release of acetylcholine is responsible for air-way hyper-responsiveness.[8]

Previous studies have shown that pretreatment with β_2 agonists elicited a significant protection against inhalational insulin induced bronchoconstriction but protective effects of beclomethasone against increased airway reactivity due to inhaled insulin have never been evaluated.[9] Experimental and clinical evidences indicated that beclomethasone prevents the allergen induced bronchial reactivity due to its ability to prevent the release of contractile prostaglandins and histamine from mast cells.[10] In several studies beclomethasone has also found to inhibit vagally mediated contractile response of guinea pig airways.[10] 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.[5] So the current experimental study was designed to explore the efficacy of beclomethasone against insulin mediated tracheal tissue contraction of guinea pig in vitro.

MATERIAL AND METHODS

This laboratory based experimental study was carried out in the Pharmacology department in collaboration

with Centre for Research in Experimental and Applied Medicine of Army Medical College Rawalpindi, Pakistan from December 2011 to July 2012. Twenty four guinea pigs were randomly divided into four groups after the approval of the ethics committee. They were killed by cervical dislocation.[11] Trachea was dissected out and tracheal chain was prepared with smooth muscle in the centre and cartilaginous portions on both sides.[12] One end of the tracheal strip was 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 the Transducer (Harvard Model No 72-4494). Four channel oscillograph was used for recording tracheal muscle contraction.[13]

Cumulative concentration response curve was obtained with increasing concentrations of histamine (**10^{-8}** to 10^{-3}M).[14] This group served as control (group I) and the effect of insulin (group II) on tracheal muscle was compared to it. In group II, III and IV effects of varying concentrations of insulin (**10^{-8}** to 10^{-3}M) and insulin in the presence of fixed concentration of indomethacin (10^{-6} M) and beclomethasone (10^{-6} M) respectively were studied by constructing cumulative concentration response curves.[15]

The results are expressed as Means±Standard Error of Means (SEM). The arithmetic means of amplitudes of contractions and SEMs were calculated using SPSS-16. In order to find the significance of the difference between two observations *t*-test was used at 5% level of significance.

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 amplitudes of contraction with histamine, insulin and insulin pretreated with indomethacin and beclomethasone were 92.5±1.20 mm, 35±1.13mm, 14.55±0.62 mm and 22±1.154 mm respectively. Maximum insulin induced contraction was 38 percent of histamine mediated contraction (Table-1 and Figure-1). The percentage responses for all the four groups were also calculated and compared (Table 1, 2 & 3). Insulin concentration response curve in the presence of indomethacin (prostaglandin synthesis inhibitor) was shifted to the right and downwards indicating a pivotal role of prostaglandins in insulin induced airway hyper-reactivity (Figure-2). Beclomethasone had a profound inhibitory effect on airway hyper-reactivity induced by insulin (Figure-2).

The mean amplitude of responses, percent responses and percent deviations when compared between group I and II, group II and III and group II and IV were found to be statistically significant (p<0.05) (Table-1,2 & 3).

Table-1: Comparison of amplitudes of contractions and percent responses of isolated tracheal muscle of guinea pig to histamine control (group I) and insulin (group II).

Concentration (M) of histamine/insulin	Amplitude of Contraction with histamine (n=6) (Mean±S.E.M) (mm)	Amplitude of Contraction with insulin (n=6) (Mean±S.E.M) (mm)	Percent response with histamine	Percent response with insulin
10^{-8}	9.33±1.33	0±0	10.086	0
10^{-7}	19.67±1.081	8.167±0.87	21.26	8.87
10^{-6}	44.8±1.68	16.16±1.01	48.43	17.55
10^{-5}	68.67±2.106	26.1±1.13	74.24	28.34
10^{-4}	87.3±1.33	31.8±0.832	94.37	34.53
10^{-3}	92.5±1.20	35±1.13	100	38
Mean	53.7±1.454	19.53±0.828	58.06	21.21
p-value	<0.05		<0.05	

Table-2: Comparisons of amplitudes of contractions and percent responses of isolated tracheal muscle of guinea pig to insulin control (group I) with insulin pretreated with Indomethacin (group III) and beclomethasone (group IV).

Concentration of insulin (M)	Amplitude of contraction with insulin (n=6) (mean±S.E.M)	Amplitude of contraction with insulin pretreated with Indomethacin (n=6) (mean±S.E.M) (mm)	Amplitude of contraction with insulin pretreated with beclomethasone (mean±S.E.M) (mm)	Percent response with insulin	Percent response with insulin pretreated with indomethacin	Percent response with insulin pretreated with beclomethasone
10^{-8}	0±0	0±0	0±0	0	0	0
10^{-7}	8.167±0.87	0±0	0±0	23.34	0	0
10^{-6}	16.16±1.01	0.5±0.34	5.167±0.83	46.17	1.43	14.77
10^{-5}	26.1±1.13	6.17±0.477	12.33±1.08	74.58	17.62	35.23
10^{-4}	31.8±0.832	10.33±0.67	18.17±1.045	90.86	29.5	51.91
10^{-3}	35±1.13	14.55±0.62	22±1.154	100	41.57	62.86
Mean	19.53±0.828	5.25±0.35	9.6±0.684	55.8	15.02	27.4
p-value	Control	<0.05	<0.05	Control	<0.05	<0.05

Table-3: Percent deviation between group I (histamine control) and group II (insulin) and between group II (insulin control) with group III (insulin pretreated with indomethacin) and group IV (insulin pretreated with beclomethasone).

Concentration of Histamine/Insulin (M)	Percent contraction		Percent deviation between group 1 & 2	Percent contraction		Percent deviation between group 2 & 3	Percent contraction		Percent deviation between group 2 & 4
	Group 1	Group 2		Group 2	Group 3		Group 2	Group 4	
10^{-8}	10.086	0	100	0	0	0	O	0	0
10^{-7}	21.26	8.87	58.28	23.34	0	100	23.34	0	100
10^{-6}	48.43	17.55	63.76	46.17	1.43	96.90	46.17	14.77	68
10^{-5}	74.24	28.34	61.8	74.58	17.62	76.37	74.58	35.23	52.76
10^{-4}	94.37	34.53	63.4	90.86	29.5	67.53	90.86	51.91	42.8
10^{-3}	100	38	62	100	41.57	58.43	100	62.86	37.14

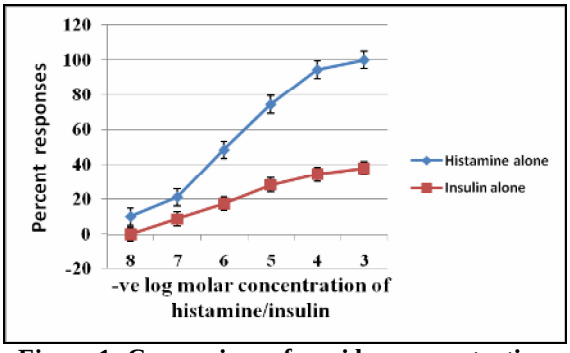

Figure 1: Comparison of semi log concentration response curve of group I, (Histamine control) and group II (insulin) on isolated tracheal smooth muscle of guinea pig. Results are average of six separate experiments. Data is represented as mean±standard error of means (SEM).

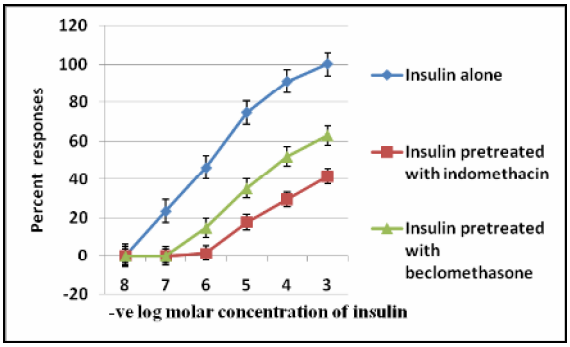

Figure 2: Comparison of semi log concentration response curve of group II (Insulin control) with group III (insulin pretreated with indomethacin) and group IV (insulin after pretreatment with beclomethasone) on isolated tracheal smooth muscle of guinea pig. Results are average of six separate experiments. Data is represented as mean±standard error of means (SEM).

DISCUSSION

The present study was carried out to evaluate the protective effects of beclomethasone against insulin induced tracheal tissue contraction and explored one possible mechanism that could underlie the acute contractile effect of insulin on isolated tracheal muscle of guinea pig. Histamine and insulin produced a concentration dependent, reversible contraction of tracheal smooth muscle. Our findings are in accordance with the results of a study in which acute contractile effect of insulin was observed on tracheal preparations of guinea pig *in vitro*.[5] The maximum insulin induced tracheal tissue contraction was 38% 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% of histamine mediated contraction, using the same experimental setup.[5] The mean percent deviation between the two groups was 68.2%. Our findings are in accordance with *in vivo* studies in which ovalbumin challenged diabetic rats when treated with insulin, the airway inflammation, and reactivity was aggravated.[15]

Tracheal muscle when pretreated with cyclo-oxygenase inhibitor indomethacin, concentration response curve of insulin was shifted downwards and to the right with percent response of 41.57% of the insulin control suggesting a pivotal role of contractile prostaglandins in insulin mediated airway hyperreactivity. Our findings are in accordance with results of a study in which insulin induced tracheal muscle contraction was strongly inhibited in the presence of indomethacin (Prostaglandin synthesis inhibitor), FP and EP_1 receptor blockers.[5]

Beclomethasone inhibited the insulin induced tracheal smooth muscle contraction by shifting the concentration response curve to the right and downwards. The percent response of insulin in the presence of beclomethasone was 62.86% of insulin control. The mean values of amplitudes of contractions and mean percent responses when compared between two groups, were found to be statistically significant (p<0.05). Since insulin is a pro-inflammatory and procontractile hormone, the potential protective effects of beclomethasone against insulin induced tracheal muscle contraction is presumably through its anti-inflammatory effects and its ability to prevent the release of prostaglandins and histamine which in turn inhibit airway hyper-

responsiveness mediated by insulin.[16,17] Our findings are in agreement with a study in which prolong exposure to insulin induced a hypercontractile phenotype in isolated bovine tracheal muscle which in turn increased the airway reactivity. This increased airway hyper-responsiveness was significantly inhibited in the presence of beclomethasone due to its ability to inhibit the proliferation of bovine tracheal muscle.[7]

This *in vitro* study provides the first evidence that beclomethasone 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.[18] So pretreatment with beclomethasone may be considered as an attractive option for diabetic patients encountering respiratory adverse effects with inhaled insulin therapy.

CONCLUSION

Insulin induces airway smooth muscle contraction presumably through the production of prostaglandins which were significantly inhibited in the presence of beclomethasone. So beclomethasone can become useful therapeutic agent for attenuation of bronchoconstriction mediated by inhaled insulin therapy in diabetic patients**.**

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