Original Article

Insulin Induced Airway Hyper-Responsiveness

Attenuation of Insulin Induced Airway Hyper-Responsiveness with Anti-Inflammatory Drugs in Guinea Pig Airways

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ABSTRACT

Objective: Inhalational insulin was withdrawn from market because it enhances airway reactivity in human beings. So the present study was designed to observe the acute effects of insulin on airway reactivity of guinea pigs and to explore the inhibitory effects of sodium cromoglycate, beclomethasone and montelukast against insulin induced airway hyper-reactivity on isolated tracheal smooth muscle of guinea pig in vitro.

Study Design: The quasi experimental study.

Place and Duration of Study: This study was conducted at the Pharmacology Department, Army Medical College Rawalpindi from February 2012 to October 2012.

Materials and Methods: Effects of variable doses of insulin (10^{-7}- 10^{-3} M) and insulin pretreated with fixed dose of sodium cromoglycate (10^{-6} M), beclomethasone (10^{-6} M) and montelukast (10^{-5} M) were studied on isolated tracheal tissue of guinea pig by constructing cumulative dose response curves. Transducer and Four Channel Oscillograph were used to record the changes in resting tension of guinea pig airways.

Results: Beclomethasone attenuated the contractile response of insulin greater than sodium cromoglycate and montelukast.

Conclusions: Beclomethasone was more efficacious than sodium cromoglycate and montelukast in amelioration of insulin induced tracheal tissue contraction. So we infer that pretreatment of inhaled insulin with beclomethasone may be preferred over sodium cromoglycate and montelukast in reducing its airway hyper-responsiveness.

Key Words: Inhaled insulin, beclomethasone, sodium cromoglycate, montelukast, oscillograph.

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INTRODUCTION

Subcutaneous insulin is the mainstay for controlling blood glucose in diabetes.[1] Inhalational insulin had been available in market for diabetic patients who defer to take subcutaneous insulin due to needle phobia.[2] Studies have shown that use of inhalational insulin tends to normalize the blood glucose level[3] It significantly decreased HbA_{1c} and caused far less hypoglycemia and less tendency for weight gain.[4] But inhaled insulin was banned due to its high cost and increased bronchial hyper-reactivity, cough, and dyspnea.[5] The proposed mechanism of insulin airway hyper-reactivity is that insulin stimulates the release of histamine from mast cells which enhances airway hyper-responsiveness.[6,7]

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Cromoglycate sodium has been used for prevention of asthma, exerts anti-inflammatory effects in respiratory tract.[8] Recently montelukast has also been reported to possess anti-inflammatory and weak bronchodilatory properties in guinea pig and rat models of asthma. [9,10] Experimental and clinical evidences have also shown that beclomethasone prevents the allergen induced bronchial hyper-reactivity as it prevents the contractile prostaglandins and histamine release from mast cells.[11,12] Keeping in view these pharmacological effects of sodium cromoglycate, beclomethasone and montelukast, the current experimental study was designed to explore and compare the effects of these drugs to attenuate insulin induced airway hyper-reactivity in guinea pigs.

MATERIALS AND METHODS

The current study was carried out in the Department of Pharmacology Army Medical College Rawalpindi, from February 2012 to October 2012.

Twenty four guinea pigs were included in the study through non-probabilty convenient sampling and were divided into four groups. Each group has six animals. The Institutional Animal Ethics Commitee approved the study. The guinea pigs were sacrificed. The trachea was dissected out. Four to five tracheal chains were made from one trachea of guinea pig.[13,14] Tracheal strip was

connected to oxygen tube of tissue bath having krebs-Henseleit solution at 37° C and Research Grade Isometric Force Displacement Transducer Harvard Model No 72-4494 (England). Changes in the contractions of tracheal muscles were recorded on Four Channel Oscillograph Harvard Model No 50-9307.[15]

Experimental groups: In group 1, cumulative dose response curves of insulin were obtained by using the doses ranging from of 10^{-7} to 10^{-3}M.[16] When the plateau was obtained with first dose (10^{-7}M) of insulin, then the next dose (10^{-6} M) was poured without washing the previous dose. Oscillograph was used to record the changes in contractions of tracheal muscles. Maximum insulin induced contraction was obtained with 10^{-3}M dose of insulin. This group served as control group. In group 2, 10^{-6} M sodium cromoglycate was poured on the tracheal tissue.[17] 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 sodium cromoglycate. Cumulative dose response curves pretreated with sodium cromoglycate were constructed. In group 3 and 4 cumulative dose response curves of insulin pretreated with fixed dose (10^{-6} M) of beclomethasone[18] and montelukast (10^{-5} M) were constructed by using the same procedure as described for group 1.[19]

Statistical Analysis: One way ANOVA followed by Post Hoc Tuckey Test using SPSS version 16 was used to find differences between amplitudes of contractions of four experimental groups. *p* value of less than 0.05 was taken significant.

RESULTS

Insulin significantly enhanced the contraction of tracheal smooth muscle (Figure 1). Changes in tracheal smooth muscle contractions were measured by taking the amplitude of contraction. Maximum response with 10^{-3} M dose of insulin was 35 ± 1.13mm. So insulin directly increased the tone of tracheal muscle. Amplitude of contractions obtained with insulin pretreated with cromoglycate sodium, beclomethasone and montelukast were 27.8 ± 1.27 mm, 22 ± 1.154 mm and 34.5 ± 1.024 mm respectively. There was statistically significant difference in constrictor response of insulin between group 1, 2 & 3, while difference between the amplitude of contraction between group 1 and 4 was insignificant (Table 1).

The percentage responses for all the four groups were also calculated. Maximum contraction of insulin treated with sodium cromoglycate and beclomethasone was attenuated by 79.42 and 62.86 percent respectively as compared with control group (Table 2). The mean percent response in the presence of montelukast remained 98.57 percent of control group (Table 2).

Although beclomethasone and cromoglycate sodium both significantly ameliorated insulin induced airway hyper-reactivity yet beclomethasone is more efficacious than sodium cromoglycate (Figure 1), while montelukast failed to counteract insulin induced smooth muscle contraction (Table 1).

Table No.1: Comparisons of means of amplitudes of contractions of isolated tracheal smooth muscle of guinea pig to insulin control (group 1), with insulin pretreated with cromoglycate sodium (group 2), beclomethasone (group 3) and montelukast (group 4)

Concentration of insulin (M)	Amplitude of contraction with insulin (n=6) (Mean ± S.E.M) (mm)	Amplitude of contraction with insulin pretreated with Sodium cromoglycate (n=6) (Mean±S.E.M)(mm)	Amplitude of contraction with insulin pretreated with beclomethasone (n=6) (Mean± S.E.M) (mm)	Amplitude of contraction with insulin pretreated with montelukast (n=6) (Mean± S.E.M) (mm)
10^{-7}	8.167 ± 0.87	2 ± 0.73	0 ± 0	7.83±0.746
10^{-6}	16.16 ± 1.01	9.83 ± 1.33	5.167 ± 0.83	16±1.045
10^{-5}	26.1 ± 1.13	17.66 ± 0.76	12.33 ± 1.08	26±1.065
10^{-4}	31.8 ± 0.832	24.16 ± 1.72	18.17 ± 1.045	30.8±1.04
10^{-3}	35 ± 1.13	27.8 ± 1.27	22 ± 1.154	34.5±1.024
p-value	0.000*	0.000*	0.000*	0.99#

P value < 0.05 = Significant (*) *P* value>0.05= insignificant (#)

Table No.2: Percent responses of isolated tracheal muscle of guinea pig to insulin (group 1), insulin pretreated with cromoglycate sodium (group 2), beclomethasone (group 3) and montelukast (group 4)

Concentration of insulin (M)	Percent response with insulin	Percent response with insulin pretreated with sodium cromoglycate	Percent response with insulin pretreated with beclomethasone	Percent response with insulin pretreated with montelukast
10^{-7}	23.34	5.71	0	22.37
10^{-6}	46.17	28.09	14.77	45.71
10^{-5}	74.58	50.46	35.23	74.29
10^{-4}	90.86	69.02	51.91	88
10^{-3}	100	79.42	62.86	98.57

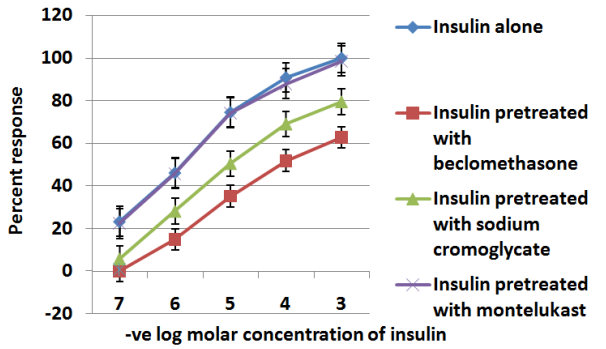

Figure No.1: Comparison of concentration response curve of group 1 with group 2, 3 and 4.

DISCUSSION

The current study was conducted to evaluate the inhibitory effects of cromoglycate sodium, beclomethasone and montelukast against insulin induced tracheal tissue contraction. Insulin reversibly enhanced the airway reactivity of guinea pigs. Our findings were consistent with a study in which isolated tracheal tissue of diabetic rat when pretreated with insulin, airway hyper-responsiveness was aggravated due to increase release of contractile prostaglandins on isolated tracheal smooth muscle of rat.[20] In group 2, cromoglycate sodium significantly reduced the insulin mediated airway smooth muscle contraction. Inhibition of mast cell degranulation and prevention of release of inflammatory mediators by cromoglycate sodium probably contribute to its beneficial effects.[21] These findings were consistent with clinical studies in which cromoglycate sodium significantly attenuated the contractile response to several kinds of allergens.[22] Beclomethasone also significantly inhibited the insulin induced tracheal smooth muscle contraction. Since insulin is a pro-inflammatory and pro-contractile hormone, potential protective effects of beclomethasone may be due to its anti-inflammatory effects and its ability to prevent the release of prostaglandins and histamine, which ameliorated airway hyper-responsiveness mediated by insulin. [23,24] In fourth group, montelukast did not significantly reduce insulin induced airway hyper-reactivity. The dose response curve of beclomethasone was compared to the curve of cromoglycate sodium and montelukast, it was concluded that beclomethasone attenuated the effects of insulin but greater than that of cromoglycate sodium and montelukast. So beclomethasone is more efficacious than cromoglycate sodium and montelukast in inhibiting the contractile response of insulin.

CONCLUSION

Insulin reversibly enhanced guinea pigs airway smooth muscle contraction. Beclomethasone was more efficacious than cromoglycate sodium and montelukast in reducing insulin induced tracheal tissue contraction. So we suggest that diabetic patients taking inhalational insulin may be pretreated with inhaled beclomethasone rather than cromoglycate sodium or montelukast to attenuate its respiratory adverse effects.

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Author's Contribution:

Concept & Design of Study:	Mahjabeen Sharif
Drafting:	Bushra Tayyaba Khan
Data Analysis:	Muhammad Asim Anwar
Revisiting Critically:	Bushra Tayyaba Khan, Muhammad Asim Anwar
Final Approval of version:	Mahjabeen Sharif

Conflict of Interest: The study has no conflict of interest to declare by any author.

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