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Prevention of Insulin Induced Airway Hyper-Reactivity with Montelukast on Isolated Tracheal Muscle of Guinea Pig

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ABSTRACT

Objectives: To explore the inhibitory effects of montelukast against insulin induced tracheal smooth muscle contraction of guinea pigs in vitro.

Background: Inhalational insulin was withdrawn from market in 2007 due to its potential to produce airway hyper-reactivity and bronchoconstriction. So we investigated the acute effects of insulin on airway reactivity and protective effects of montelukast against insulin induced airway hyper-responsiveness on isolated tracheal smooth muscle of guinea pig.

Study design: Experimental study.

Place and Duration of Study: This study was conducted in the Department of Pharmacology, Army Medical college Rawalpindi from December 2011 to June 2012.

Materials and Methods: Effects of increasing concentrations of histamine (10^{-8}- 10^{-3} M), insulin (10^{-8}- 10^{-3} M) and insulin pretreated with fixed dose of montelukast (10^{-5} M) were studied on isolated tracheal tissue of guinea pig. 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 amplitude of contraction with histamine was 92.5 ± 1.20 mm as compared to 35 ± 1.13 mm in insulin treated group. The maximum amplitude of contraction achieved with insulin in the presence of montelukast was 34.5 ± 1.024 mm.

Conclusion: Montelukast did not significantly inhibit the contractile response of insulin on isolated tracheal muscle of guinea pig, so pretreatment of inhaled insulin with montelukast has no clinical implication in amelioration of its respiratory adverse effects such as bronchoconstriction.

Key words: Histamine, inhalational insulin, oscillograph, tracheal muscle.

INTRODUCTION

Subcutaneous insulin is the mainstay for controlling blood glucose in diabetic patients[1]. Most of the diabetic patients defer to initiate subcutaneous insulin leading to sub-optimal glycemic control and eventually greater risk for short and long term complications of diabetes[2]. Non invasive, inhalational insulin is an attractive alternative to parenteral insulin[3]. Studies reveal that inhalational insulin thrice daily before meals can provide glycemic control comparable to conventional subcutaneous insulin regimen but with improved patient's compliance[4]. Its regular use is associated with fewer hypoglycemic episodes and less risk for weight gain as compared to subcutaneous insulin[5]. Unfortunately it was withdrawn from the market due to its respiratory adverse effects such as increased bronchial reactivity, cough, dyspnoea and bronchoconstriction[6]. Insulin has long been recognized as pro-inflammatory and pro-contractile hormone[7]. Various therapeutic strategies have been implicated to decrease the airway hyper-responsiveness mediated by inhaled insulin. Previous studies have shown that pretreatment with β_2 agonists elicited a significant protection against inhalational insulin induced bronchoconstriction[8], but protective effects of montelukast against increased airway reactivity due to inhaled insulin have never been evaluated. 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 get deposited in airway smooth muscle compartment in both cases[9]. It is well established that montelukast inhibits leukotriene D_4 induced airway hyper-responsiveness[10]. In a number of clinical experiments, montelukast also have shown to inhibit the airway inflammation[11]. Weak bronchodilatory effects of montelukast have also been demonstrated in guinea pig and rat models of asthma [12]. Due to these pharmacological effects of montelukast on human and guinea pig airways the current experimental study was planned to explore the efficacy of montelukast regarding its inhibitory effects on insulin mediated tracheal tissue contraction of guinea pig in vitro.

MATERIALS AND METHODS

Animals: The study was conducted on the isolated tracheal smooth muscle of 18 guinea pigs of Dunkin

Hartley variety (500 to 700 g)[13]. All the protocols described in this study were approved by Ethics committee of Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi.

Preparation of tissue and experimental setup: All guinea pigs were killed by cervical dislocation[14] . The trachea was dissected out and tracheal chain was prepared with smooth muscle in the centre and cartilaginous portions on both sides [15]. One end of the tracheal strip was attached to the hook of oxygen tube of tissue bath containing oxygenated Krebs-Henseleit solution at 37^o C, while the other end was connected to the Transducer (Harvard Model No 72-4494). Four channel oscillograph was used for recording tracheal contractions [16]**.**

Cumulative concentration response curve was obtained with increasing concentration of histamine **(**10^{-8} to 10^{-3}M) [17]. This group served as control (group I) and the effect of insulin on tracheal muscle was compared to it. In group II and III effects of varying concentration of insulin **(**10^{-8} to 10^{-3}M)[9] and insulin in the presence of fixed concentration of montelukast (10^{-5}M) [12] were studied by constructing cumulative concentration response curves.

Statistical Analysis: The results were expressed as Means ± Standard Error of Means. The arithmetic means of amplitudes of contractions and SEMs were calculated using SPSS version 16. In order to find the significance of the difference between two observations 'student t test' was used

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 montelukast were 92.5 ±1.20 mm, 35 ± 1.13mm and 34.5 ± 1.024 mm respectively (Table 1 & 2). Maximum insulin induced contraction was 38 percent of histamine mediated contraction (Figure 1). The percentage responses for all the three groups were also calculated and compared (Table 1, 2 & 3). The shape of cumulative concentration response curve and amplitude of contraction remained almost the same for both groups so monteleukast did not significantly attenuate insulin induced hyper-reactivity (Figure 2).

The mean amplitude of responses, percent responses and percent deviations produced by each dose of histamine and insulin when compared between group I and 2 was found to be statistically significant (Table 1), while the percent responses and percent deviations produced by insulin and insulin pretreated with montelukast when compared between group 2 & 3 were found to be statistically insignificant (Table 2, 3, & Figure 2).

Table No.1: Comparison of amplitudes of contractions and percent responses of isolated tracheal muscle of guinea pig to histamine control (group 1) and insulin (group 2).

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	$p < 0.05$		$p < 0.05$	

Table No.2: Comparisons of amplitudes of contractions and percent responses of isolated tracheal muscle of guinea pig to insulin control (group 2) with insulin pretreated with montelukast (group 3).

Concentration of insulin (M)	Amplitude of contraction with insulin (n=6) (mean± S.E.M) (mm)	Amplitude of contraction with insulin pretreated with monteleukast (n=6) (mean ± S.E.M) (mm)	Percent response with insulin	Percent response with insulin pretreated with monteleukast
10^{-8}	0 ± 0	0 ± 0	0	0
10^{-7}	8.167 ± 0.87	7.83 ± 0.746	23.34	22.37
10^{-6}	16.16 ± 1.01	16 ± 1.045	46.17	45.71
10^{-5}	26.1 ± 1.13	26 ± 1.065	74.58	74.29
10^{-4}	31.8 ± 0.832	30.8 ± 1.04	90.86	88
10^{-3}	35 ± 1.13	34.5 ± 1.024	100	98.57
Mean	19.53 ± 0.828	5.25 ± 0.35	55.8	15.02
P value	Control	$P > 0.05$	Control	$P > 0.05$

Table No.3: Percent deviation between group 1 (histamine control) and group 2 (insulin) and between group 2 (insulin control) with group 3 (insulin pretreated with montelukast).

Concentration of Histamine/ Insulin (M)	Percent contraction		Percent deviation between group 1 & 2	Percent contraction		Percent deviation between group 2 & 3
	Group 1	Group 2		Group 2	Group 3	
10^{-8}	10.086	0	100	0	0	0
10^{-7}	21.26	8.87	58.28	23.34	22.37	4.16
10^{-6}	48.43	17.55	63.76	46.17	45.71	0.996
10^{-5}	74.24	28.34	61.8	74.58	74.29	0.389
10^{-4}	94.37	34.53	63.4	90.86	88	3.15
10^{-3}	100	38	62	100	98.57	1.43

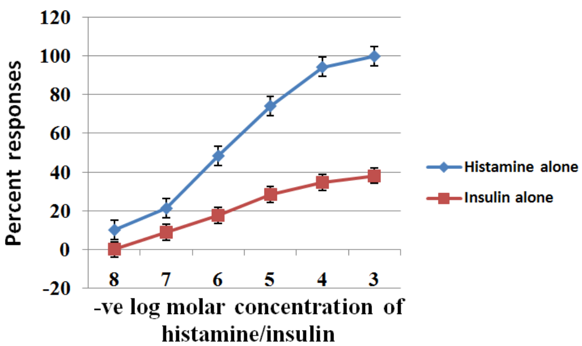

Figure Bo,1: Comparison of semi log concentration response curve of group 1 (histamine control) and group 2 (insulin) on isolated tracheal smooth muscle of guinea pig. Results are average of six separate experiments. Data is represented as mean ± standard error of means (SEM).

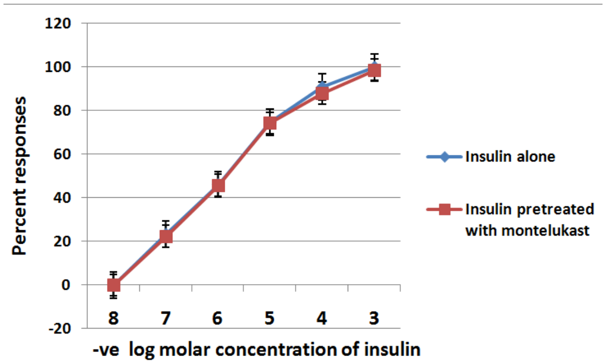

Figure No.2: Comparison of semi log concentration response curve of group 2 (insulin control) and group 3 (insulin after pretreatment with montelukast) on isolated tracheal smooth muscle of guinea pig. Results are average of six separate experiments. Data is represented as mean ± standard error of means (SEM).

DISCUSSION

The present study was carried out to evaluate the beneficial effects of montelukast against insulin induced contraction of isolated tracheal smooth muscle of guinea pig. In the first and second set of experiments, histamine and insulin produced a concentration dependent, reversible contraction of tracheal smooth muscle. The mean ± SEM of maximum amplitude of contraction with histamine (control) was 92.5 ± 1.20 mm as compared to 35 ± 1.13 mm in insulin treated group, difference being statistically significant ($p < 0.05$). The maximum insulin induced tracheal tissue contraction was 38 percent of histamine mediated contraction. These findings were consistent with the results of Schaafsma and his coworkers who also reported the acute contractile effects of insulin that were 33 percent of histamine (control) mediated contraction on tracheal preparation of guinea pig [9]. The mean percent deviation between the two groups was 68.2 percent. Similar findings have been reported in other in vivo studies. An ovalbumin challenged diabetic rats when treated with insulin, the airway inflammation to antigen provocation was aggravated due to its pro-inflammatory effects[18].

When the isolated tracheal muscle was pretreated with fixed concentration of montelukast (10^{-5} M), it did not produce statistically significant effect on insulin induced contraction of tracheal smooth muscle. The maximum amplitude of contraction was 35 ± 1.13 mm as compared to 34.5 ± 1.024 mm in insulin control group. The mean percent response remained 98.57 percent of insulin control group that was statistically insignificant ($p > 0.05$). The percent deviation between the two groups was 2.025 percent, so the ability of montelukast to attenuate insulin induced tracheal contraction was almost negligible.

Previous studies have shown that montelukast acts as an anti-bronchoconstrictor drug with limited efficacy as it can antagonize only one of the several bronchoconstrictor mediators [19]. In the current study, montelukast failed to inhibit the insulin induced tracheal muscle contraction suggesting that leukotrienes may not be involved in insulin mediated tracheal contraction. Insulin mediated airway contraction is likely to be mediated by the release of contractile Prostaglandins, histamine and acetylcholine [9,20], so our findings are in accordance with the results of a study in which montelukast failed to counteract the isolated tracheal tissue contraction of guinea pig induced by Ach, histamine, arachidonic acid, PGD_2 and $PGF_{2\alpha}$ [21].

The results of this study indicate that insulin is able to induce the airway smooth muscle contraction. This in vitro study provides the first evidence that montelukast cannot significantly inhibit the contractile response of insulin on guinea pig airways, so pretreatment with montelukast has no therapeutic implication for diabetic patients encountering respiratory adverse effects with inhaled insulin therapy. But further clinical trials are warranted to confirm whether these effects of montelukast, observed on guinea pig airway smooth muscle can translate to human airways.

CONCLUSION

Montelukast did not significantly inhibit the contractile response of insulin on isolated tracheal muscle of guinea pig, so pretreatment of inhaled insulin with montelukast has no clinical implication in amelioration of its respiratory adverse effects such as broncho constriction.

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