

PROTECTIVE EFFECT OF SALBUTAMOL AND MONTELUKAST AGAINST INSULIN INDUCED AIRWAY HYPER-RESPONSIVENESS IN GUINEA PIG AIRWAYS IN VITRO

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ABSTRACT

Objective: To evaluate and compare the protective effects of salbutamol and montelukast in amelioration of insulin induced airway hyper-reactivity on isolated tracheal smooth muscle of guinea pig in vitro.

Study Design: Laboratory based randomized control trials (Experimental study).

Place and Duration of Study: Pharmacology Department and Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College Rawalpindi from January 2012 to September 2012.

Material and Methods: Effects of variable doses of insulin (10^{-7} - 10^{-3} M) and insulin pretreated with salbutamol (10^{-6} M) and montelukast (10^{-5} M) were observed on airways of guinea pig in vitro by constructing cumulative dose response curves. Four Channel Oscillograph and Transducer were used to record the tracheal smooth muscle contractions.

Results: Insulin induced a dose dependent reversible contraction of isolated tracheal muscle of guinea pig. The maximum response of insulin and insulin pretreated with salbutamol and montelukast were 35 ± 1.13 mm, 14.55 ± 0.62 mm and 34.5 ± 1.024 mm respectively. So salbutamol significantly inhibited the contractile response of insulin while montelukast failed to counteract the airway hyper-reactivity induced by insulin.

Conclusion: Salbutamol significantly inhibited the contractile response of insulin, while montelukast did not produce statistically significant effect on insulin induced airway hyper-reactivity. So it is suggested that pretreatment of inhaled insulin with salbutamol may be beneficial in inhibiting the broncho-constriction induced by insulin.

Keywords: Inhaled insulin, Oscillograph, Montelukast, Salbutamol, Tracheal muscle.

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INTRODUCTION

Predominant mode of insulin administration is by subcutaneous injection. Injection related anxiety leads to poor compliance and suboptimal glycemic control. So non-invasive inhalational insulin was approved in 2006 by United States Federal Drug Agency (US FDA)¹. Studies have shown that the therapeutic efficacy of inhalational insulin was comparable to subcutaneous insulin². Its use was associated with improved patient's compliance and less chances of weight gain. Studies have demonstrated that HbA_{1c} was significantly decreased with inhaled insulin³. But its use was limited due to its respiratory adverse effects such as cough and bronchoconstriction⁴. Insulin has

long been recognized as pro-inflammatory and pro-contractile hormone⁵. But conflicting studies are available with regards to the possible mechanism of insulin induced airway hyper-reactivity. Schaafsma and his colleagues suggested that insulin enhances the degranulation of mast cells so increased release of mediators like histamine and prostaglandins are responsible for airway hyper-reactivity⁶. In another study Belmonte and his co-workers revealed that insulin mediated airway hyper-responsiveness was due to vagal stimulation and increased cholinergic transmission of airways⁷. It is well established that salbutamol acts as a physiological antagonist and reverses the broncho-constriction irrespective of broncho-constrictor stimuli⁸. Recently montelukast has also been reported to possess the anti-inflammatory and weak bronchodilatory properties in guinea pig and rat models of

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asthma. Prophylactic use of montelukast decreases the symptoms of airway hyper-reactivity induced by variety of allergens and chemicals⁹. In view of above mentioned pharmacological effects of salbutamol and montelukast, the present experimental study was undertaken to evaluate and compare the inhibitory effects of salbutamol and montelukast against insulin mediated airway hyper-responsiveness in guinea pig in vitro.

MATERIAL AND METHODS

This laboratory based randomized control study was conducted on isolated tracheal rings of 18 guinea pigs in Pharmacology department and Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College Rawalpindi from January 2012 to September 2012. Eighteen healthy Dunkin Hartely guinea pigs of either gender weighing 500-700g were included through non-probability convenient sampling and were randomly divided into three equal groups using random number tables. Each group comprises of six animals (n=6).

midline incision was given to open the chest. The trachea was dissected out and was immersed in a dissecting dish containing oxygenated Krebs-Henseleit solution. After the removal of loose connective tissue, a midline incision was given to open the tracheal tube. Trachea was then cut into rings 2-3 mm wide, each containing about two cartilages. This tracheal ring was then placed in 50 ml tissue bath filled with krebs-Henseleit solution at 37°C. Tracheal tissue was connected to the oxygen tube of organ bath and the other end was tied to a Research Grade Isometric Force Displacement Transducer Harvard Model No 72-4494 (England) by means of a thread. Oxygen was provided to the tracheal tissue through oxygen cylinder and baseline tension was adjusted at 2 grams. The tracheal strip was allowed to equilibrate for 45 minutes, during this stabilizing period the nutrient solution was changed after every 15 minutes¹¹. The baseline tension of isolated tracheal tissue was maintained at 1 gram through out the experiment which was optimal for measuring the changes in the resting tension of tracheal muscles through Isometric

Table: Comparisons of Mean ± SEM of amplitudes of contractions and percent responses of isolated tracheal smooth muscle of guinea pig (n=6) to insulin control (group 1), with insulin pretreated with salbutamol (group 2) and montelukast (group 3).

Dose of insulin (M)	Amplitude of contraction (Group 1) (mm) (n=6)	Amplitude of contraction (Group 2) (mm) (n=6)	Amplitude of contraction (Group 3) (mm) (n=6)	p-value between group 1 & 2	p-value between group 1 & 3	Percent response (Group 1) (n=6)	Percent response (Group 2) (n=6)	Percent response (Group 3) (n=6)
10-7	8.167 ± 0.87	0 ± 0	7.83± 0.746	< 0.001*	0.839 NS	23.34	0	22.37
10-6	16.16 ± 1.01	0.5 ± 0.34	16 ± 1.045	< 0.001*	0.991 NS	46.17	1.43	45.71
10-5	26.1 ± 1.13	6.17 ± 0.477	26 ± 1.065	< 0.001*	0.991 NS	74.58	17.62	74.29
10-4	31.8 ± 0.832	10.33 ± 0.67	30.8 ± 1.04	< 0.001*	0.697 NS	90.86	29.5	88
10-3	35 ± 1.13	14.55 ± 0.62	34.5± 1.024	< 0.001*	0.927 NS	100	41.57	98.57

*= Significant (p<0.05)
NS = Not significant (p>0.05)

The drugs which were used in the study included salbutamol sulphate, montelukast and regular human insulin. All the eighteen guinea pigs were sacrificed by cervical dislocation¹⁰. A

Force Displacement Transducer on Four Channel Oscillograph Harvard Model No 50-9307¹².

In group I, cumulative dose response curve of insulin was constructed using the

concentrations ranging from of 10^{-7} to 10^{-3} M. When the maximum amplitude was obtained with first dose of insulin, then the next dose was added without washing the previous dose. The tracheal contractions were recorded on Oscillograph. When the maximal insulin induced contraction was obtained, the tracheal strip was washed three to four times and was allowed to relax passively⁶. This group served as control and the effect of insulin pretreated with salbutamol and montelukast on tracheal smooth muscle was compared to it. In group II salbutamol was added to the organ bath in a concentration of 10^{-7} M. After 15 minutes, variable doses of insulin ranging from 10^{-7} to 10^{-3} M were added into the organ bath in the presence of salbutamol¹³.

animals in each group. One Way ANOVA and Post Hoc Tuckey Test using SPSS version 16 was used for comparison amongst all the three groups. Percentage responses for all the three groups were calculated. Means \pm Standard Error of Means of amplitude of contractions were also calculated and compared. Value of $p < 0.05$ was taken as significant.

RESULTS

Insulin induced a dose dependent contraction of tracheal strips of guinea pigs (fig). Alterations in airway reactivity were assessed by taking the amplitudes of contractions. Maximum amplitude of contraction in control group with 10^{-3} M concentration of insulin was 35 ± 1.13 mm. Insulin directly increased the myogenic tone of

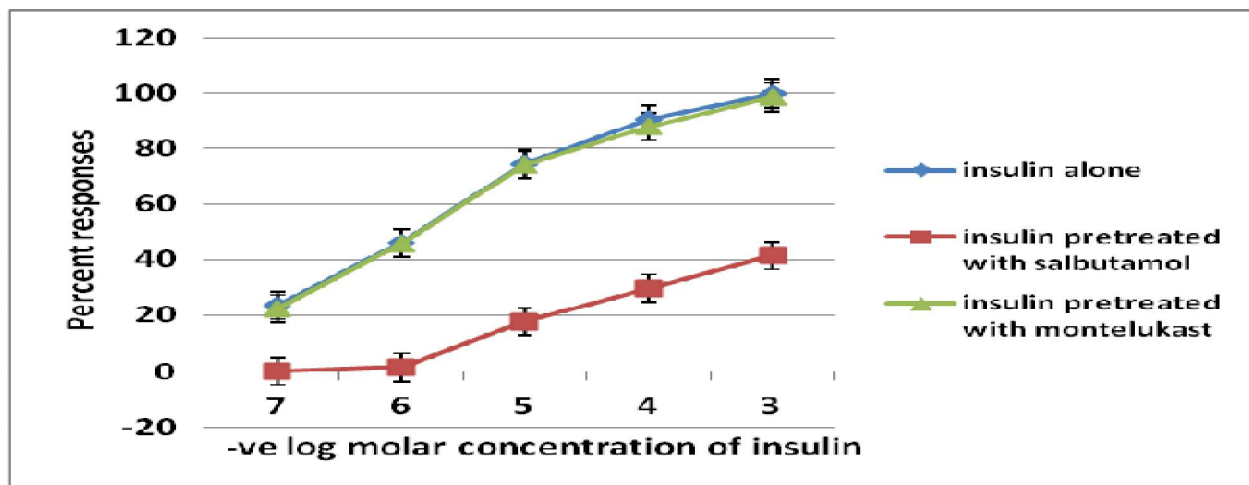


Figure: Comparison of semi log concentration response curve of group 1 (insulin control) with group 2 (insulin after pretreatment with salbutamol) and group 3 (insulin after pretreatment with montelukast) on isolated tracheal smooth muscle of guinea pig. Results are average of six separate experiments (n=6). Data is represented as mean \pm standard error of means (SEM).

Cumulative concentration response curves pretreated with salbutamol were constructed. In group III montelukast was added to the organ bath in a concentration of 10^{-5} M. 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 montelukast¹⁴. Concentration response curves pretreated with montelukast were constructed.

The data were taken as average of observations of isolated tracheal rings from six

guinea pig airways and was inhibited in salbutamol and montelukast treated groups from 35 ± 1.13 mm (control) to 14.55 ± 0.62 mm and 34.5 ± 1.024 mm respectively (table). One Way Anova was applied to compare the amplitude of contraction of all the three groups. P-values for all three groups were found to be less than 0.001. Then Post Hoc Tukey Test was applied which showed statistically significant difference between group 1 and 2 and between group 1 and 3 it was insignificant (table).

To evaluate the protective effect of salbutamol concentration response curves were plotted for insulin in the presence of fixed concentration of salbutamol. The amplitude of contraction in the presence of salbutamol was significantly decreased. The percent response in the presence of salbutamol was reduced to 41.57 percent of insulin control. The mean values of responses when compared to insulin control group were found to be statistically significant. So salbutamol significantly inhibited the insulin induced airway smooth muscle contraction.

The percentage responses for all the three groups were also calculated. The maximum percent response of insulin in group 2 and 3 pretreated with salbutamol and montelukast were reduced by 41.57 and 98.57 percent respectively as compared with 100 percent of control group (table).

There was a significant rightward shift of insulin curves in the presence of salbutamol which indicates antagonistic effects of salbutamol against insulin induced airway hyper-reactivity. However montelukast failed to counteract airway hyper-reactivity and there was no significant rightward shift of insulin curve in the presence of montelukast. The shape and amplitude of dose response curves remained almost the same for both the groups (fig).

DISCUSSION

The current study was undertaken to evaluate and compare the protective effects of salbutamol and montelukast against insulin induced airway hyper-reactivity. Insulin produced a dose dependent, reversible contraction of tracheal smooth muscle. These findings were in accordance with results of Schaafsma and his colleagues who reported the acute contractile effect of insulin due to increased release of contractile prostaglandins in isolated tracheal smooth muscle of guinea pig⁶.

The protection offered by salbutamol against insulin mediated tracheal contraction is presumably through its ability to prevent the release of inflammatory mediators from tracheal

strip of guinea pig^{15,16}. The beneficial effect may also be ascribed to its ability to reduce the cholinergic neurotransmission in airway smooth muscles by an action on presynaptic heterogeneous β_2 receptors to inhibit acetylcholine release¹⁷. Our findings are in agreement with the clinical observations in which inhalation of albuterol 30 minutes before the administration of inhaled insulin caused reduction of bronchoconstriction in asthmatic patients¹⁸.

In third group, concentration response curves were plotted for insulin on isolated tracheal strips pretreated with montelukast. Montelukast did not significantly attenuate the insulin mediated airway smooth muscles suggesting that leukotrienes may have no role in insulin mediated tracheal contraction. Previous studies have shown that montelukast decreases bronchoconstriction and has with limited efficacy as it can antagonize only one of the several bronchoconstrictor mediators¹⁹. Consistent with our results is another study in which montelukast failed to counteract the tracheal muscle contraction of guinea pig induced by insulin, Ach, histamine, arachidonic acid and $\text{PGF}_{2\alpha}$ ²⁰.

The concentration response curve obtained with salbutamol was compared to the curve of montelukast, it was observed that salbutamol inhibited the effects of insulin but much greater than that of montelukast. Comparison of the curves for group 2 and 3 showed that salbutamol was much more effective in reducing the contractile response induced by insulin as compared to montelukast. A probable explanation could be due to its ability to prevent the release of inflammatory mediators from mast cells and inhibition of vagally mediated airway hyper-responsiveness¹⁶.

CONCLUSION

Salbutamol significantly ameliorated the insulin induced tracheal tissue contraction as compared to montelukast.

So we suggest that the diabetic patients taking inhalational insulin may be pretreated with inhaled salbutamol to ameliorate its

potential respiratory adverse effects. But further clinical trials are warranted to confirm whether the protection offered by salbutamol against insulin effect can translate to human airways.

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CONFLICT OF INTEREST

This study has no conflict of interest to declare by any authors.

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