

Determination of Beneficial Effects of Salbutamol and Ipratropium in Prevention of Insulin Induced Tracheal Smooth Muscle Contraction in Guinea Pig Model: A Comparative Study

¹Mahjabeen Sharif, ²Mubashir Sharif and ³Asma Shaukat

¹Department of Pharmacology and Therapeutics,
National University of Sciences and Technology (NUST) Islamabad,
Army Medical College Rawalpindi, Pakistan

²Combined Military Hospital (CMH) Sialkot, Sialkot Cantt, Pakistan

³Abbottabad International Medical College Abbottabad, Pakistan

Abstract: Aim use of inhalational insulin was limited due to its high cost and its potential to produce respiratory adverse effects. So current study was designed to evaluate the acute effects of insulin on airway reactivity and protective effects of salbutamol and ipratropium against insulin induced airway hyper-responsiveness on isolated tracheal smooth muscle of guinea pig. Material and methods: effects of variable doses of insulin (10^{-7} to 10^{-3} M), insulin pretreated with fixed concentration of salbutamol (10^{-7} M) and ipratropium (10^{-6} M) were studied on isolated tracheal tissue of guinea pig by constructing cumulative concentration response curves. Changes in tracheal smooth muscle contractions were recorded on four channel oscillograph. Results, the maximum amplitude of contraction with increasing concentrations of insulin, insulin pretreated with fixed concentration of salbutamol and ipratropium were 35 ± 1.13 mm, 14.55 ± 0.62 mm and 27.8 ± 1.27 mm respectively. Salbutamol inhibited the contractile response of insulin greater than ipratropium on isolated tracheal muscle of guinea pig. Conclusion maximum constrictor response of insulin in the presence of salbutamol and ipratropium was reduced by 41.57 and 79.42 percent respectively as compared with control group so we suggest that pretreatment of inhaled insulin with salbutamol may be preferred over ipratropium in amelioration of its potential respiratory adverse effects.

Key words: Airway-Hyper-Reactivity • Inhaled Insulin • Salbutamol • Ipratropium

INTRODUCTION

Subcutaneous insulin is the mainstay 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 regular insulin [3].

Unfortunately it was withdrawn from the market due to its respiratory adverse effects such as increased bronchial reactivity, cough, dyspnoea and bronchoconstriction [4]. Insulin has long been recognized as pro-inflammatory and pro-contractile hormone but conflicting studies are available with regards to its possible mechanism of action [5]. 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

Corresponding Author: Mahjabeen Sharif, Department of Pharmacology and Therapeutics,
National University of Sciences and Technology (NUST) Islamabad,
Army Medical College Rawalpindi, Pakistan. Tel: +92333-5077896.

responsible for increased airway reactivity [6]. The mechanism proposed by Terzano and his co-workers for inhaled insulin induced bronchoconstriction is that insulin promotes the mast cells degranulation leading to increase release of histamine and contractile prostaglandins, which mediate allergic inflammation of airways [7]. Previous studies demonstrate that salbutamol acts as a physiological antagonist and reverses the bronchoconstriction irrespective of bronchoconstrictor stimuli [8]. It is well established that salbutamol reduces the cholinergic neurotransmission in human and guinea pig airways by an action on presynaptic heterogeneous β_2 receptors to inhibit acetylcholine release [9]. Clinical and experimental evidences have shown that ipratropium offers protection against multiple diverse stimuli by inhibiting the effect of acetylcholine on muscarinic receptors in respiratory passages. So it relaxes the airway smooth muscles and produces bronchodilatation [10]. Based on these pharmacological effects of salbutamol and ipratropium, the current experimental study was designed to explore and compare the efficacy of salbutamol and ipratropium against insulin mediated tracheal tissue contraction of guinea pig *in vitro*.

MATERIAL AND METHODS

Eighteen guinea pigs procured from National Institute of Health (NIH) Islamabad were randomly divided into three groups. 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.

The guinea pigs were killed by cervical dislocation. The trachea was dissected out and tracheal chain was prepared with smooth muscle in the centre and cartilaginous portions on both sides. 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 Research Grade Isometric Force Displacement Transducer Harvard Model No 72-4494 (England). Four channel Oscillograph Harvard Model No 50-9307 (England) was used for recording the tracheal muscle contraction [11].

Group 1: Cumulative concentration response curve of insulin (10^{-7} to 10^{-3} M) on isolated tracheal muscle of guinea pig (n=6).

After the initial equilibration period, baseline tension was adjusted to 1 gm, a cumulative dose response curve of insulin was obtained using the concentrations ranging

from 10^{-7} to 10^{-3} M [4]. When the plateau was achieved with first dose of insulin, then the next dose was added without washing the previous dose. The tissue 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 passively. This group served as control and the effect of insulin pretreated with salbutamol and ipratropium on tracheal smooth muscle was compared to it.

Group 2: Cumulative concentration response curve of insulin in the presence of fixed concentration of salbutamol (10^{-7} M) on isolated tracheal muscle of guinea pig (n=6).

Salbutamol was added to the organ bath in a concentration of 10^{-7} M [12]. 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 salbutamol. Cumulative concentration response curves pretreated with salbutamol were constructed.

Group 3: Cumulative concentration response curve of insulin in the presence of fixed concentration of ipratropium (10^{-6} M) on isolated tracheal muscle of guinea pig (n=6).

Ipratropium was added to the organ bath in a concentration of 10^{-6} M [13]. 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. Concentration response curves pretreated with ipratropium were constructed.

Statistical Analysis: The results were expressed as Means \pm Standard Error of Means and statistically significant differences were assessed by one way ANOVA followed by Post Hoc Tuckey Test using SPSS version 16. The differences between the observations were considered as significant if *p* value was less than 0.05.

RESULTS

Acute effects of insulin were studied on isolated tracheal smooth muscles of guinea pig. Insulin induced a dose dependent reversible contraction of tracheal smooth muscle (Figure 1). Changes in tracheal smooth muscle contractions were measured by taking the amplitude of contraction. Maximum amplitude of contraction with 10^{-3} M concentration of insulin was 35 ± 1.13 mm. So insulin

Table 1: Comparisons of means of amplitudes of contractions and percent responses of isolated tracheal smooth muscle of guinea pig to insulin control (Group 1), with insulin pretreated with salbutamol (Group 2) and ipratropium (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 salbutamol (n=6)(mean ± S.E.M) (mm)	Amplitude of contraction with insulin pretreated with ipratropium (n=6) (mean ± S.E.M) (mm)	p-value	Percent response with insulin	Percent response with insulin pretreated with salbutamol	Percent response with insulin pretreated with ipratropium
10 ⁻⁷	8.167 ± 0.87	0 ± 0	2 ± 0.73	.000*	23.34	0	5.71
10 ⁻⁶	16.16 ± 1.01	0.5 ± 0.34	9.83 ± 1.33	.000*	46.17	1.43	28.09
10 ⁻⁵	26.1 ± 1.13	6.17 ± 0.477	17.66 ± 0.76	.000*	74.58	17.62	50.46
10 ⁻⁴	31.8 ± 0.832	10.33 ± 0.67	24.16 ± 1.72	.000*	90.86	29.5	69.02
10 ⁻³	35 ± 1.13	14.55 ± 0.62	27.8 ± 1.27	.001*	100	41.57	79.42

P value < 0.05 = Significant (*)

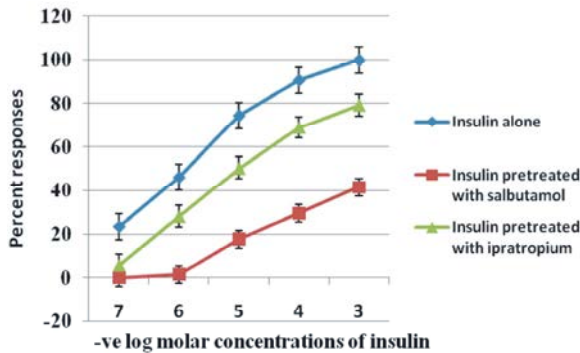


Fig. 1: Comparison of semi log concentration response curve of group 1 (Insulin control) with group 2 (Insulin after pretreatment with salbutamol) and group 3 (Insulin after pretreatment with ipratropium) on isolated tracheal smooth muscle of guinea pig. Results are average of six separate experiments. Data is represented as mean ± standard error of means (SEM)

directly enhanced the myogenic airway smooth muscle tone. This insulin induced tracheal smooth muscle contraction was significantly reduced in salbutamol and ipratropium treated groups from 35 ± 1.13mm (Control) to 14.55 ± 0.62 mm and 27.8 ± 1.27mm respectively (Table 1). The means of amplitudes of contraction with varying doses of insulin (10⁻⁷ -10⁻³M) when compared between group 1, 2 and 3, were found to be statistically significant (Table 1).

The percentage responses for all the three groups were also calculated. Our data showed that maximum constrictor response of insulin in the presence of salbutamol and ipratropium was reduced by 41.57 and 79.42 percent respectively as compared with control group (Table 1).

Insulin concentration response curve in the presence of salbutamol was shifted to the right and downwards more than ipratropium (Figure 1). Although salbutamol and ipratropium both had a profound inhibitory effect on airway hyper-reactivity induced by insulin yet salbutamol is more efficacious than ipratropium (Figure 1).

DISCUSSION

In the present study, we used an *in vitro* model of guinea pig trachea to observe the acute effects of insulin and protective role of salbutamol and ipratropium against insulin induced airway hyper-reactivity. Insulin directly enhanced the airway smooth muscle tone. Schaafsma and his colleagues also reported the acute contractile effect of insulin due to increase release of contractile prostaglandins from mast cells of isolated tracheal smooth muscles of guinea pigs [4].

Our findings are also supported by *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 [14].

Salbutamol significantly reduced the insulin induced airway hyper-reactivity. This potential protective effect of 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]. The beneficial effect may also be ascribed due to its ability to reduce the cholinergic neurotransmission in airway smooth muscles by an action on presynaptic heterogenous β₂ receptors to inhibit acetylcholine release [16]. Our findings are in agreement with the clinical observations in which inhalation of albuterol 30 minutes before the administration of inhaled insulin increased the absorption of inhaled insulin due to reduction of bronchoconstriction induced by inhaled insulin in asthmatic patients [17].

Ipratropium also inhibited the insulin induced tracheal smooth muscle contraction by shifting the concentration response curve to the right and downwards. Since insulin mediated hyper-reactivity is likely to be vagally mediated in guinea pigs and rats [6], ipratropium may afford protection against insulin induced tracheal contraction due to its ability to inhibit the reflex acetylcholine induced bronchoconstriction by blocking the muscarinic receptors (M₁ - M₅) in airway smooth muscles [10].

The concentration response curve obtained with ipratropium was compared to the curve of salbutamol, it was observed that ipratropium inhibited the effects of insulin but less than that of salbutamol. So salbutamol is more efficacious than ipratropium in amelioration of insulin induced airway hyper-reactivity. It may be due to its rapid bronchodilatory effects, its ability to prevent the release of inflammatory mediators from mast cells and inhibition of vagally mediated airway hyper-responsiveness [16]. 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 [4]. So we suggest that pretreatment with salbutamol may be preferred over ipratropium for counteracting the respiratory adverse effects with inhaled insulin therapy.

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