

Acute effect of insulin on guinea pig airways and its amelioration by pre-treatment with salbutamol

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Abstract

Objective: To study the magnitude of insulin-mediated airway hyper-reactivity and to explore the protective effects of salbutamol in inhibiting the insulin-induced airway hyper-responsiveness on tracheal smooth muscle of guinea pigs in vitro.

Methods: The quasi-experimental study was conducted at the Pharmacology Department of Army Medical College, Rawalpindi, in collaboration with the Centre for Research in Experimental and Applied Medicine from December 2011 to July 2012. It used 18 healthy Dunkin Hartley guinea pigs of either gender. Effects of increasing concentrations of histamine (10-8-10-3M), insulin (10-8-10-3 M) and insulin pre-treated with salbutamol (10-6 M) were observed on isolated tracheal strip of guinea pig in vitro by constructing cumulative concentration response curves. The tracheal smooth muscle contractions were recorded with Transducer on Four Channel Oscillograph. Mean and standard error of mean were calculated. SPSS 16 was used for statistical analysis.

Results: Histamine and insulin produced a concentration-dependent reversible contraction of isolated tracheal muscle of guinea pig. The mean of maximum amplitudes of contraction with histamine, insulin and insulin pre-treated with salbutamol were 92.5 ± 1.20 mm, 35 ± 1.13 mm and 14.55 ± 0.62 mm respectively. Salbutamol shifted the concentration response curve of insulin to the right and downwards.

Conclusions: Salbutamol significantly reduced the insulin mediated airway hyper-reactivity in guinea pigs, suggesting that pre-treatment of inhaled insulin with salbutamol may have clinical implication in the amelioration of its potential respiratory adverse effects such as bronchoconstriction.

Keywords: Histamine, Inhaled insulin, Oscillograph, Tracheal muscle. (JPMA 64: 932; 2014)

Introduction

Insulin is administered through subcutaneous injections.¹ Non-invasive, inhalational insulin is an attractive alternative to parenteral insulin.² Clinical trials have shown that glucose-lowering activity of inhaled insulin is comparable to subcutaneous insulin, but with improved patient compliance.³ Its regular use is associated with fewer hypoglycaemic episodes and less risk for weight-gain as compared to subcutaneous insulin.⁴ Unfortunately, it was withdrawn from the market due to its respiratory adverse effects such as increased bronchial reactivity, cough, dyspnoea and bronchoconstriction. Insulin has long been recognised as a pro-inflammatory and pro-contractile hormone.⁵ The most likely mechanism of inhaled insulin-induced bronchoconstriction is that insulin modulates the mast cells degranulation and the subsequently increased release of histamine and contractile prostaglandins are responsible for allergic inflammation of airways.⁶ Some

experimental evidences also reveal that it is likely to be vagally mediated, and the increased release of acetylcholine is responsible for airway hyper-responsiveness.⁶

Extensive research has been done to ameliorate the airway hyper-responsiveness mediated by inhaled insulin. Experimental and clinical evidences have also shown that salbutamol prevents the allergen-induced bronchial reactivity by inhibiting the release of contractile prostaglandins and histamine from mast cells via β_2 receptors.⁷ Previous studies also reveal that salbutamol inhibits vagally-mediated cholinergic neurotransmission through heterogeneous β_2 receptors and inhibits acetylcholine release.⁷ Keeping in view these pharmacological effects of salbutamol, the present study was designed to evaluate the efficacy of salbutamol against insulin-mediated airway hyper-reactivity of guinea pig in vitro. Considerable evidences have suggested a causal relation between our experimental setup using isolated tracheal smooth muscle and pulmonary delivery of insulin as in both cases airway smooth muscle are directly exposed to high concentration of insulin.⁶

The objective of the study was to determine the

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magnitude of insulin-induced airway hyper-reactivity and its treatment with salbutamol. Histamine is commonly used for provocative studies on airway smooth muscles. Histamine-mediated enhanced myogenic tone of airways is taken as gold standard for in vitro studies.⁸ To evaluate the extent of insulin-induced airway hyper-reactivity, histamine-mediated airway hyper-responsiveness was taken as standard (control) in our study and was compared to insulin-mediated tracheal smooth muscle contraction.⁸

Materials and Methods

The quasi-experimental study was conducted at the Pharmacology Department in collaboration with the Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, from December 2011 to July 2012. A total of 18 healthy Dunkin Hartley 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 numbers table. After approval from the institutional ethics committee, the animals were killed by cervical dislocation.⁸ The trachea was dissected out and the tracheal chain was prepared with smooth muscle in the centre and cartilaginous portions on both sides. The tracheal strip was attached to the hook of oxygen tube of tissue bath containing oxygenated krebs-Henseleit solution at 37°C.⁹ Tracheal contractions were recorded with Research Grade Isometric Force Displacement Transducer (Harvard Model No 72-4494) on four-channel oscillograph (Harvard Model No 50-9307).¹⁰

In group 1, cumulative dose-response curves of histamine were constructed with varying concentrations (10-8 to 10-3 M).¹¹ When maximum response with 10-8 M concentration was obtained, the subsequent doses were added without washing the previous dose. Four-channel oscillograph was used for recording tracheal muscle

contraction. This group served as control group 1 and dose-response curve of histamine was compared with that of insulin to determine the magnitude of insulin-mediated contraction, because in research studies histamine-mediated airway smooth muscle contraction is commonly taken as standard (100 percent) Ref Needed.⁸

In group 2, cumulative concentration-response curve of insulin was obtained using varying concentrations ranging from 10-8 to 10-3 M and the same procedure was repeated as described for histamine.⁶

In group 3, salbutamol sulphate (10-7 M) was added to the organ bath. After 15 minutes, cumulative dose-response curve was constructed by using varying concentrations of insulin (10-7 to 10-3 M).¹²

The arithmetic means of amplitudes of contractions and standard errors of mean (SEMs) were calculated using SPSS version 16. Student t test was used to determine the difference between two observations and p value of less than 0.05 was considered significant.

Results

Insulin-induced contraction of the tracheal smooth muscle was evident at a concentration of 10-7 M. However, a significant enhancement of insulin-induced contractions was observed at 10-5M, 10-4 M and 10-3 M (Figure).

Changes in tracheal smooth muscle contractions were measured by taking the amplitudes of tracheal smooth muscle contraction. Amplitudes of contraction with maximum dose of histamine and insulin (10-3 M) were 92.5±1.20mm and 35±1.13mm respectively (Table-1). Histamine and insulin significantly enhanced the myogenic airway smooth muscle tone. To evaluate the extent of insulin-induced airway hyper responsiveness, the percentage responses for groups 1 and 2 were also calculated. Maximum constrictor response of insulin was

Table-1: Comparison of responses of isolated tracheal muscle of guinea pig between histamine (group I) and insulin (group II).

Concentration (M) of histamine/insulin	Amplitude of Contraction with histamine (Mean ± SD) (mm) (n=6)	Amplitude of Contraction with insulin (Mean ± SD) (mm) (n=6)	P value between group 1 & 2	Percent response with histamine	Percent response with insulin
10-8	9.33±3.265	0±0	0.000*	10.086	0
10-7	19.67±2.65	8.167±2.14	0.000*	21.26	8.87
10-6	44.8±4.12	16.16±2.48	0.000*	48.43	17.55
10-5	68.67±5.16	26.1±2.78	0.000*	74.24	28.34
10-4	87.3±3.266	31.8±2.04	0.001*	94.37	34.53
10-3	92.5±2.949	35±2.76	0.001*	100	38

p-value < 0.05 = Significant (*)

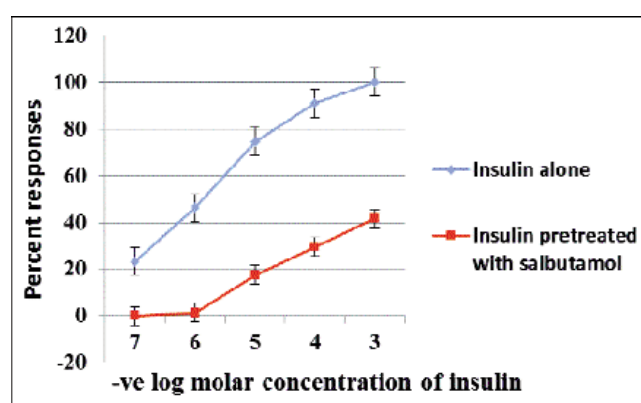
SD: Standard Deviation.

Table-2: Comparison of responses of isolated tracheal muscle of guinea pig between insulin (group 2) and insulin pre-treated with salbutamol (group 3).

Concentration of insulin (M)	Amplitude of contraction with insulin (mean $\pm$ SD) (mm) n=6	Amplitude of contraction with insulin pre-treated with salbutamol (mean $\pm$ SD) (mm) n=6	p-value between group 2&3	Percent response with insulin	Percent response with insulin pre-treated with salbutamol
10 ⁻⁷	8.167 $\pm$ 2.14	0 $\pm$ 0	.004*	23.34	0
10 ⁻⁶	16.16 $\pm$ 2.48	0.5 $\pm$ 0.837	.007*	46.17	1.43
10 ⁻⁵	26.1 $\pm$ 2.78	6.17 $\pm$ 1.169	0.000*	74.58	17.62
10 ⁻⁴	31.8 $\pm$ 2.04	10.33 $\pm$ 1.63	.003*	90.86	29.5
10 ⁻³	35 $\pm$ 2.76	14.55 $\pm$ 1.52	.004*	100	41.57

p-value < 0.05 = Significant (*)

SD: Standard Deviation.

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

38 percent of maximal histamine response.

The insulin-induced tracheal smooth muscle contraction was significantly reduced in salbutamol-treated group from 35 ± 1.13 mm to 14.55 ± 0.62 mm. The means of amplitudes of contractions with varying doses of insulin when compared between group 2 and 3 were found to be statistically significant (Table-2). Maximum constrictor response of insulin in the presence of salbutamol (group 3) was reduced by 41.57 percent compared with insulin group. Insulin concentration response curve in the presence of salbutamol was shifted to the right and downwards, indicating a profound inhibitory effect of salbutamol on airway hyper-reactivity induced by insulin.

Discussion

The study was conducted to observe the protective effects of salbutamol against insulin-mediated airway hyper-reactivity on tracheal muscle of guinea pig in vitro. Histamine and insulin increased the airway reactivity in a

dose-dependent manner. The magnitude of insulin-mediated airway hyper-reactivity was evaluated by comparing it with histamine-mediated tracheal tissue contraction as histamine-provoked airway hyper-responsiveness is taken as standard (control) for experimental studies. The maximum insulin-induced tracheal tissue contraction was 38 percent of histamine-mediated contraction (control) in contrast to 33 percent contraction which was reported in an in vitro study using the same experimental setup.⁶ Our results are also in accordance with in vivo studies in which airway reactivity of diabetic rats was increased after the administration of insulin due to the increased release of prostaglandins and histamine from mast cells.¹³

Next, we studied the effect of salbutamol in the amelioration of insulin-induced tracheal contractions. The maximum percent response of insulin in the presence of salbutamol was reduced to 41.57 percent of insulin control. So salbutamol significantly ameliorated insulin-mediated airway hyper-reactivity. The protection offered by salbutamol is presumably through the inhibition of release of contractile prostaglandins and histamine from mast cells of isolated tracheal strip.⁷ The beneficial effects of salbutamol may be due to the inhibition of cholinergic neurotransmission through heterogenous β_2 receptors and subsequently, decreased release of acetylcholine leads to decreased airway reactivity.¹⁴ Our observations are also supported by clinical trials in which pre-treatment of inhaled insulin with terbutaline enhanced the absorption of inhaled insulin due to the inhibition of bronchoconstriction mediated by insulin in human volunteers.¹⁵

The current study provides a clue that salbutamol can attenuate the pro-contractile effect of insulin. So pre-treatment with salbutamol may ameliorate respiratory adverse effects of inhaled insulin therapy in diabetic patients.

Conclusion

Insulin can mediate reversible contraction of isolated tracheal smooth muscle of guinea pig which was significantly inhibited by pre-treatment with salbutamol. As such, salbutamol can become a useful therapeutic agent for the amelioration of insulin-induced airway hyper-reactivity and bronchoconstriction.

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