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A Comparative Study of the Beneficial Effects of Ipratropium and Beclomethasone against Insulin-Induced Tracheal Tissue Contraction in a Guinea Pig Model

Mahjabeen Sharif[a] Bushra Tayyaba Khan[a] Shoaib Bin Aleem[b]
Mohammad Asim Anwar[c]

Departments of [a]Pharmacology and Therapeutics and [b]Physiology, Army Medical College Rawalpindi, National University of Sciences and Technology, and [c]Pakistan Atomic Energy Commission General Hospital, Islamabad, Pakistan

Key Words
Airway hyper-reactivity · Inhaled insulin · Beclomethasone · Ipratropium · Tracheal muscle

Abstract
Objective: To evaluate the acute effects of insulin on airway reactivity and the protective effects of beclomethasone and ipratropium against insulin-induced airway hyperresponsiveness on isolated tracheal smooth muscle in a guinea pig model. ***Materials and Methods:*** The trachea of each guinea pig was excised; one end of the tracheal strip was attached to the hook of the oxygen tube of a tissue bath and the other end was connected to a research-grade isometric force displacement transducer. The effects of varying concentrations of insulin (10^{-7} to 10^{-3} M) and insulin pretreated with a fixed concentration of beclomethasone (10^{-6} M) and ipratropium (10^{-6} M) on the isolated tracheal tissue were studied by constructing cumulative concentration-response curves. Changes in tracheal smooth muscle contractions were recorded on a 4-channel oscillograph. ***Results:*** The means ± standard error of the mean of the maximum amplitude of contraction with increasing concentrations of insulin and of insulin pretreated with fixed concentrations of beclomethasone and ipratropium were 35 ± 1.13, 22 ± 1.15 and 27.8 ± 1.27 mm, respectively. ***Conclusion:*** The data showed that beclomethasone inhibited the contractile response of insulin to a greater extent than ipratropium. Thus we suggest that inhalational insulin pretreated with beclomethasone may be more efficacious than with ipratropium for the amelioration of potential respiratory adverse effects such as bronchoconstriction.

Introduction

Subcutaneous insulin is the mainstay for controlling blood glucose in diabetes. Non-invasive inhalational insulin is an attractive alternative to parenteral insulin for patients for whom subcutaneous insulin is not suitable [1]. Studies conducted on patients with type 1 and type 2 diabetes have revealed that inhalational insulin administered 3 times daily before meals can provide glycaemic control comparable to conventional subcutaneous insulin but with improved patient satisfaction and compli-

Dr. Mahjabeen Sharif, Assistant Professor
Department of Pharmacology and Therapeutics
Army Medical College Rawalpindi, National University of Sciences and Technology
Islamabad 44000 (Pakistan)
E-Mail mahjabeen30@hotmail.com

ance [2]. Long-term studies on these patients have also demonstrated a significant reduction in HbA_{1c} with fewer hypoglycaemic episodes and less risk for weight gain compared to injected insulin [3]. Unfortunately, inhalational insulin was withdrawn from the market due to its high cost [2]. Its use was also associated with an increased incidence of adverse respiratory effects such as cough, dyspnoea, increased bronchial reactivity and bronchoconstriction [4].

There are conflicting studies available on the possible mechanism of insulin-induced airway hyperreactivity [5]. The mechanism proposed by Terzano et al. [5] for inhalational insulin-induced bronchoconstriction is that insulin promotes the degranulation of mast cells, leading to an increased release of histamine and contractile prostaglandins, which mediates the allergic inflammation of airways. Belmonte et al. [6] suggested that inhaled insulin enhances the vagally mediated release of acetylcholine, resulting in airway hyperresponsiveness. Previous studies have shown that pretreatment with terbutaline, a β_2-agonist, elicited significant protection against inhalational insulin-induced bronchoconstriction [6, 7]. However, the protective effects of ipratropium and beclomethasone against increased airway reactivity from inhaled insulin have not been evaluated. Ipratropium, a synthetic quaternary anticholinergic agent, offers protection against multiple diverse stimuli by inhibiting the effect of acetylcholine on muscarinic receptors in the respiratory passages, and relaxes the airway smooth muscles and produces bronchodilatation [8]. Experimental evidence has shown that beclomethasone inhibits allergen-induced bronchial reactivity due to its ability to prevent the release of contractile prostaglandins and histamine from mast cells [9]. It has also been found to inhibit the vagally mediated contractile response in the smooth muscle of guinea pig airways [10]. Based on these pharmacological actions of ipratropium and beclomethasone, our experimental study was designed to explore and compare the efficacy of these drugs against insulin-mediated airway hyperreactivity on tracheal smooth muscle in a guinea pig model in vitro.

Materials and Methods

Our study was conducted at the Department of Pharmacology in collaboration with the Centre for Research in Experimental and Applied Medicine at the Army Medical College Rawalpindi, Pakistan. The Institutional Animal Ethics Committee approved the study. Eighteen guinea pigs procured from the National Institute of Health, Islamabad, Pakistan, were randomly divided into 3 groups of 6 animals each. The guinea pigs were killed by cervical dislocation. The trachea of each animal was removed by dissection, and the tracheal chain was prepared with the smooth muscle in the centre and the cartilaginous portions on both sides [11]. One end of the tracheal strip was attached to the hook of the oxygen tube of a tissue bath containing oxygenated Krebs-Henseleit solution at 37°C and the other end was connected to a research-grade isometric force displacement transducer (Model No. 72-4494, Harvard Apparatus, Kent, UK). A 4-channel oscillograph (Model No. 50-9307, Harvard Apparatus) was used for recording the contraction of the tracheal muscle.

Group 1. Concentrations of insulin only were administered; these ranged from 10^{-7} to 10^{-3} M [4]. When the plateau was achieved with the first dose, then the next dose was added without washing. The tissue contractions were recorded on an oscillograph. When the maximal insulin-induced contraction was obtained, the tracheal strip was washed 3–4 times and was allowed to relax passively. The cumulative concentration-response curve of insulin on the isolated tracheal muscle was obtained (n = 6). This group served as a control against which the effect of insulin pretreated with ipratropium and beclomethasone on tracheal smooth muscle was compared.

Group 2. Beclomethasone was added to the organ bath at a concentration of 10^{-6} M [12]. After 15 min, successive doses of insulin ranging from 10^{-7} to 10^{-3} M were added to the organ bath. A cumulative concentration response curve of insulin in the presence of beclomethasone on the isolated tracheal muscle was obtained (n = 6).

Group 3. Ipratropium was added to the organ bath at a concentration of 10^{-6} M [13]. After 15 min, successive doses of insulin ranging from 10^{-7} to 10^{-3} M were added to the organ bath. A cumulative concentration-response curve of insulin in the presence of ipratropium on the isolated tracheal muscle was obtained (n = 6).

Percent responses for all 3 groups were calculated, taking the response with 10^{-3} M as 100% and using the formula:

Percent response = observed response/absolute response × 100.

Statistical Analysis

The results are expressed as means ± standard error of the mean (SEM) and statistically significant differences were assessed by 1-way ANOVA followed by the post hoc Tukey test using SPSS v16. The differences between the observations were considered as significant if the p value was <0.05.

Results

The insulin produced a dose-dependent reversible contraction of guinea pig tracheal smooth muscle (fig. 1). The tracheal smooth muscle contraction measured by taking the amplitude of contraction with an insulin concentration of 10^{-3} M was 35 ± 1.13 mm. This contraction was significantly reduced in the beclomethasone- and ipratropium-treated groups to 22 ± 1.154 and 27.8 ±1.27 mm, respectively (table 1). When comparing groups 1, 2

Table 1. Comparisons of means of amplitudes of contractions and percent responses of isolated tracheal smooth muscle of guinea pig to insulin only (group 1; n = 6) and to insulin pretreated with beclomethasone (group 2; n = 6) and ipratropium (group 3; n = 6)

Concentration of insulin	Amplitude of contraction with			p value	Percent response with		
	insulin only (control), mm	insulin pretreated with beclomethasone, mm	insulin pretreated with ipratropium, mm		insulin only (control)	insulin pretreated with beclomethasone	insulin pretreated with ipratropium
10^{-7} M	8.167±0.87	0±0	2±0.73	0.000*	23.34	0	5.71
10^{-6} M	16.16±1.01	5.167±0.83	9.83±1.33	0.000*	46.17	14.77	28.09
10^{-5} M	26.1±1.13	12.33±1.08	17.66±0.76	0.000*	74.58	35.23	50.46
10^{-4} M	31.8±0.83	18.17±1.04	24.16±1.72	0.000*	90.86	51.91	69.02
10^{-3} M	35±1.13	22±1.15	27.8±1.27	0.001*	100	62.86	79.42

The values for the amplitude of contraction are means ± SEM. * Significant difference.

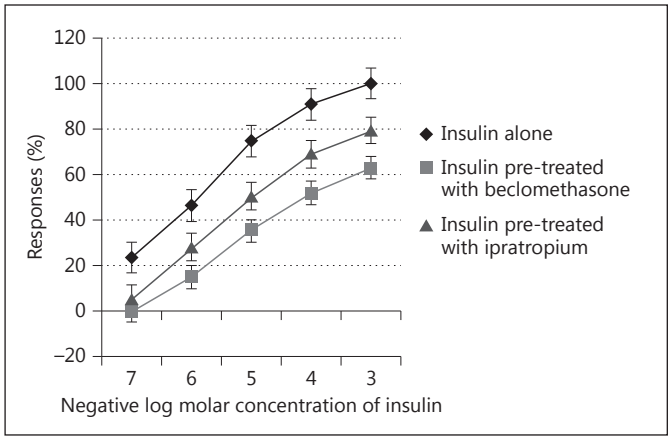

Fig. 1. Comparison of a semi-log concentration-response curve of group 1 (control) with group 2 (insulin after pretreatment with beclomethasone) and group 3 (insulin after pretreatment with ipratropium) on the isolated tracheal smooth muscle of the guinea pig. Results are an average of 6 separate experiments. Data are represented as means ± SEM.

and 3, the means of amplitudes of contraction with varying doses of insulin (10^{-7} to 10^{-3} M) were found to be statistically significant (table 1).

The percentage of maximum constrictor response of insulin in the presence of beclomethasone and ipratropium was reduced by 62.86 and 79.42%, respectively, compared to that in the controls (table 1). This meant that beclomethasone was 25% more efficacious than ipratropium in reducing the insulin-induced airway hyperreactivity. The insulin concentration-response curve in the presence of beclomethasone was shifted to the right and downwards more than with ipratropium (fig. 1).

Discussion

Our study demonstrated that insulin within a concentration range of 10^{-7} to 10^{-3} M induced contraction in the airway smooth muscle of guinea pigs. These contractions were reversible and sustained in nature. Schaafsma et al. [4] also reported the acute contractile effect of insulin on the isolated tracheal preparation of guinea pigs, but the concentration of insulin that they used was in the range of 10^{-10} to 10^{-5} M. Our observations are also supported by in vivo studies in which treatment of diabetic rats with insulin resulted in airway hyperreactivity and inflammation. This enhanced airway reactivity was due to the release of inflammatory mediators from mast cells under the influence of insulin [14].

Beclomethasone reduced the insulin-induced airway hyperreactivity significantly. Since insulin is a pro-inflammatory and pro-contractile hormone [15], the potential protective effect of beclomethasone against insulin-induced tracheal muscle contraction was presumably through its anti-inflammatory effects and its ability to prevent the release of contractile prostaglandins and histamine which, in turn, inhibited the airway hyperresponsiveness mediated by insulin [16]. In a recent study, it was observed that prolonged exposure to insulin induced a hypercontractile phenotype in isolated bovine tracheal muscle which, in turn, increased the airway reactivity. This increased airway hyperresponsiveness might be due to the mitogenic potential of insulin. It was significantly inhibited in the presence of beclomethasone due to the ability of the latter to inhibit the proliferation of bovine tracheal muscle [16]. We therefore consider that beclomethasone can offer long-term protection for the airway smooth muscles of diabetic patients who regularly use inhalation-

al insulin. Further studies are warranted to establish these effects of beclomethasone on human airways.

Ipratropium also had a profound inhibitory effect on insulin-mediated tracheal tissue contraction. Since insulin-mediated airway hyperreactivity is likely to be vagally mediated in guinea pigs and rats [6], ipratropium could have afforded protection against insulin-induced tracheal contraction due to its ability to inhibit the reflex acetylcholine-induced bronchoconstriction by blocking the muscarinic receptors (M_1–M_5) in airway smooth muscles [17]. Moreover, blockage of M_3 receptors by ipratropium in airways counteracts the enhanced acetylcholine release that would result from the dysfunction of the inhibitory M_2 receptors induced by insulin [6].

The inhibition effect of beclomethasone was greater than that of ipratropium. Hence, beclomethasone is adjudged to be more efficacious than ipratropium in reducing the contractile response mediated by insulin. A probable explanation could be due to beclomethasone's broad anti-inflammatory efficacy, i.e. its ability to prevent the release of inflammatory mediators from mast cells and its inhibition of vagally mediated airway hyperresponsiveness [9].

Conclusion

Ipratropium and beclomethasone significantly inhibited the contractile response of insulin. However, beclomethasone was more efficacious than ipratropium in the amelioration of insulin-induced tracheal tissue contraction. Therefore, we suggest that pretreatment with ipratropium and beclomethasone can be considered as an attractive option for diabetic patients encountering adverse respiratory effects with inhaled insulin therapy. Further clinical trials are warranted to confirm whether the protection offered by ipratropium and beclomethasone in the guinea pig model can translate to the human airways.

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