ORIGINAL ARTICLE

EFFECT OF NEBIVOLOL ON TONE OF TRACHEAL MUSCLE OF GUINEA PIG

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Background: The use of β-blockers is limited by adverse effects such as bronchospasm in asthmatics. Third generation β-blockers such as nebivolol may show better tolerability in asthmatic subjects because they lack β-blocker induced bronchoconstriction. **Methods:** Effects of nebivolol on the tracheal muscle strips prepared from ovalbumin-sensitised guinea pigs of both sexes were studied. Two sets of experiments were designed after dividing the animals randomly into two groups. Using oxygenated Krebs-Henseleit solution as the nutrient medium, the trachealis muscle activity was measured with isometric force displacement transducer and recorded on 4-channel Oscillograph. **Result:** Nebivolol 10^{-6} M did not produce significant effect on contractions evoked by histamine in concentrations ranging from 10^{-7} M to 10^{-3} M. The mean of amplitude of contraction for different concentrations of histamine were calculated and compared with the group treated with histamine only. Mean of amplitude of contraction, percent responses and percent deviations when compared with the control group were insignificant (p>0.05). **Conclusion:** Nebivolol did not affect the tone of airway smooth muscle in ovalbumin-sensitised guinea pigs. Nebivolol may be considered safe in patients with airway disease however, further clinical evaluation and exploratory work is required.

Keywords: Nebivolol, Histamine, Chronic Obstructive Airway Disease, Tracheal muscle

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INTRODUCTION

Beta adrenoceptor antagonists are one of the most effective drugs in treatment of cardiovascular as well as non-cardiovascular diseases.[1]The blockage of β_2 receptors in bronchial smooth muscle has little effect on lung function in normal subjects, but can lead to life-threatening bronchoconstriction in patients with asthma or chronic obstructive airway disease (COAD).This is more commonly seen with non-selective β-blockers.[2] So new drugs like nebivolol were developed to overcome such adverse events. nebivolol is a potent and selective β_1 receptor blocker, devoid of intrinsic sympathomimetic activity. It is useful in hypertension by modulating NO-release in blood vessels and causing vasodilation. Moreover nebivolol due to its dual action leads to a peculiar haemodynamic profile.[3] In some studies it has also shown an improved tolerability profile, with respect to adverse events such as bronchospasm in patients with chronic obstructive airway disease (COAD) compared to classical β-antagonists; in fact it has been shown to have relaxant effect on tracheal muscle.[4] However there are other studies which report that nebivolol has sparing effect, not the relaxant effect on airway smooth muscle in asthma patients.[5,6] The review of literature also reveals that the effect of nebivolol has not been studied on histamine-induced tracheal muscle contraction in *in vitro* studies. So the current study was aimed at determining the effect of nebivolol on histamine-induced contraction in tracheal muscle of guinea pig (which was ovalbumin-sensitised so as to create an animal model of asthma).[7]

MATERIAL AND METHODS

The present study has been conducted on the tracheal smooth muscle of guinea pigs of Dunkin Hartley variety weighing 500–600 gm. Ethics Committee approval of the protocol was obtained. The animals were given tap water *ad libitum* and were fed with a standard diet. Guinea pigs were sensitised to ovalbumin so as to create animal model of asthma and divided randomly into two groups. Development of sensitivity was confirmed by demonstration of Schultz-Dale reaction.[7] Krebs Henseleit solution was used as the nutrient solution the composition of which per 1000 ml was: NaCl 118.2 mM KCl 4.7 mM, $MgSO_4.7H_2O$ 1.2 mM, $CaCl_2$ 2.5mM, KH_2PO_4 1.3 mM, $NaHCO_3$ 25.0 mM, Dextrose 11.7mM. Solutions of all drugs were prepared in distilled water except nebivolol which was prepared in dimethyl sulphoxide.[8]

The trachea was obtained from guinea pigs and preserved in Kreb's solution. Rings, 2–3 mm wide were formed from it and cut into strips by a longitudinal cut on the ventral side opposite to the smooth muscle. The strip was then suspended in a tissue bath of 50 ml capacity, containing Kreb's solution at 37 ºC and was aerated with oxygen continuously. It's one end was attached to the oxygen tube while the other end was connected to an isometric force displacement transducer. The tissue was equilibrated for 45 minutes against an imposed

tension of two grams. A tension of one gram was applied to the tracheal strip continuously throughout the experiments.[9] The trachealis muscle activity was recorded through the transducer on 4-channel oscillograph by adding different concentrations of histamine at an interval of ten minutes between each concentration. Six experiments were performed in this group and the mean response for each concentration was worked out. A concentration response curve was obtained by plotting the per cent contraction against the logarithm of concentrations.

In the second group tracheal muscle strips were pretreated with fixed dose of nebivolol, i.e., 10^{-6} M which was left in contact with the tissue for 15 minutes.[10] Same procedure was then followed for different concentrations of histamine.

The results have been expressed as Mean±SEM using Microsoft Excel. The difference between the observations was considered significant if the *p*-value was less than 0.05 by using Student's *t*-test.

RESULTS

In a series of six experiments, the Mean±SEM values of the responses and the per cent responses to different concentrations of histamine are shown in the tables. Percent response with 10-3 M was taken as 100% and responses with other concentrations were compared with it.

The mean values of responses produced by different concentrations of histamine when compared between Group 1 and Group 2 were found statistically insignificant ($p>0.05$). The mean per cent deviations calculated for each dose of histamine used in Group 1 and Group 2 were 2.61, 4.48, 5.3, 4.6 and 3.75 per cent respectively. The mean deviation was 4.1%.

Table-1: Effect of histamine on ovalbumin sensitised isolated tracheal muscle of guinea pig (Group-1)

Histamine Concentration (M)	Amplitude of Contraction (Mean±SEM)	Percent Response
10^{-7}	12.50±1.76	15.63
10^{-6}	33.16±1.57	41.46
10^{-5}	52.66±1.60	65. 83
10^{-4}	68.0±2.78	85
10^{-3}	80.0±2.56	100

Table-2: Effect of fixed dose of nebivolol (10^{-6} M) on concentration response curve of histamine in ovalbumin-sensitised tracheal muscle of guinea pig (Group-2)

Concentration Histamine (M)	Amplitude of Contraction (Mean±SEM)	Percent Response
10^{-7}	12.83±1.01	16.03
10^{-6}	34.66±1.02	43.32
10^{-5}	55.50±1.89	69.37
10^{-4}	71.16±2.54	88.95
10^{-3}	83.00±2.42	103.75

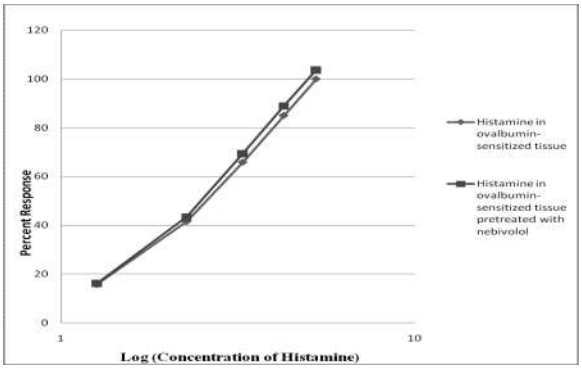

Figure-1: Log concentration-response curves of histamine in tracheal muscle in Group1 and Group2

DISCUSSION

The risk of developing bronchospasm following administration of β blockers is particularly high in asthmatic patients. This can probably be explained by simultaneous blockade of β_1 and β_2 receptors, which inhibits the increased sympathetic drive to bronchial smooth muscle.[11]

From our study, it is inferred that nebivolol has sparing effect on tracheal muscle of ovalbumin sensitized guinea pigs. This is because nebivolol *per se* does not have any type of action on respiratory smooth muscle. These findings support the results of *in vivo* study by D'Agostino *et al*[6] whereby nebivolol, either acutely or chronically administered, did not affect airway responsiveness to inhaled histamine in rabbits. In a study conducted by De Clerck *et al*[7], changes in heart rate and broncho-constrictor response to histamine were analysed in anaesthetised guinea-pigs after administration of propranolol, atenolol and nebivolol. It was reported that nebivolol decreased heart rate without significantly increasing pulmonary reactivity to histamine. However these findings oppose the findings of *in vivo* study by Cazzola *et al.*[12] wherein effects of celiprolol and nebivolol were strikingly similar. In that study spirometric indices were measured after the intake of celiprolol and nebivolol by patients with mild asthma and it was reported that both the drugs had negative effect on these indices with insignificant difference between their effects. While our previous study on respiratory effects of celiprolol concluded that celiprolol opposed histamine induced contractions in tracheal muscle of guinea pigs thereby showing that effects of nebivolol and celiprolol are different, while celiprolol produced bronchodilation, nebivolol had sparing effect.[13]

There may be many possible mechanisms which can explain the sparing effect of nebivolol. First mechanism is related to its β_1 selectivity. It is the most selective β_1-adrenoceptor antagonist currently available for clinical use.[14] Beta 1 receptor selectivity is an important determinant of less incidence of

bronchoconstriction seen with cardio-selective β blockers but even they do increase airway hyper-responsiveness, though to a lesser extent.[11] So the different effect of nebivolol cannot be fully explained by its β_1 selectivity.

Another possible mechanism is that the effect of nebivolol may be because of partial agonist activity at β_2 receptors but several studies have shown that nebivolol lacks partial agonist activity at β_2 receptors.[15] Therefore; this mechanism does not seem to be plausible.

The finding that nebivolol did not augment airway responsiveness to histamine suggest that blockade of sympathetic nervous system does not fully explain the increase of airway responsiveness to β-blockers. The lack of effect of nebivolol on airway responsiveness to histamine suggest that other regulatory mechanisms may be involved, e.g. nitric oxide and relaxant prostaglandins since nebivolol has been reported to modulate the endogenous production of nitric oxide[4]. Our previous two studies evaluated the roles of nitric oxide and relaxant prostaglandins in the sparing effect of nebivolol on respiratory muscle. It was concluded that while nebivolol modulated endogenous production of nitric oxide, it had no role in prostaglandin release.[16,17] Considering the potential role of nitric oxide in the control of airways, this mechanism may be responsible for nebivlol's sparing effect on airways.

CONCLUSION

Nebivolol had no significant effect on histamine-induced contractions isolated tracheal muscle strips of guinea pigs. Thus nebivolol may be considered as safe beta blocker in hypertensive and heart failure patients with concurrent asthma. However clinical trials are required to establish its safety in asthmatic patients.

AUTHORS CONTRIBUTION

The work is a product of intellectual thinking and experience of all the authors. All the members have contributed in various degrees to the research concept, experimental design, analytical procedures adopted, interpretation of data as well as drafting and final publication of the article. AS: Principle author, conducted the study, did write-up ST, MS, MHN: helped in data collection analysis and literature search

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