ORIGINAL RTICLE

ROLE OF NITRIC OXIDE IN THE EFFECT OF NEBIVOLOL ON ISOLATED TRACHEAL MUSCLE OF GUINEA PIG

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Background: The use of beta blockers is limited by their ability to produce bronchospasm in asthmatics. Third generation β-blockers like Nebivolol may show better tolerability because they may augment the release of nitric oxide (NO) from endothelial cells. However the involvement of NO in the respiratory effect of Nebivolol remains controversial. The present study, carried out on isolated tracheal muscle strips of guinea pigs, was designed to explore this controversy. **Method:** Varying concentration of histamine ranging from 10^{-7} M to 10^{-3} M were used to plot a concentration response curve on the isolated tracheal muscle strips of guinea pig and was used as a control. The same concentration response curve was plotted in presence of a fixed concentration of Nebivolol 10^{-6} M and then again in presence of a fixed concentration of L-Nitro Arginine Methyl Ester (L-NAME) 10^{-4} M and Nebivolol 10^{-6} M together in a series of experiments using six sets of isolated tracheal muscle strips in each case. **Results:** Nebivolol did not produce any significant shift in the concentration response curve while in the presence of L-NAME, Nebivolol shifted the histamine concentration response curve upwards and to the left. **Conclusion:** Nebivolol does not augment the histamine induced contraction of respiratory smooth muscle of guinea pig but in the presence of Nitric Oxide inhibitor L-NAME a significant augmentation of the same curve occurs, indicating a role of NO in the sparing of respiratory smooth muscle by Nebivolol.

Keywords: Nebivolol, L-NAME, Concentration response curve, Tracheal muscle

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INTRODUCTION

Pulmonary diseases with bronchial hyperactivity can be worsened or even precipitated by β_2 adrenoceptor blockage more commonly seen with non-selective β-blockers.[1] Nebivolol is a third generation β-blocker which may have advantage over classical β-lockers due to its sparing effect on tracheal muscle attributed to its ability to augment the release of NO from endothelial cells.[2,3] The potent effects of NO on vascular smooth muscle and its presence in major conducting airways raises the possibility that it could contribute to the regulation of airway smooth muscle tone.[4] However, the involvement of NO in the sparing effect of nebivolol on respiratory muscle is still controversial. Dal Negro *et al*, and Clini *et al* have reported in their *in vivo* study that single daily dose of nebivolol does not affect the production of exhaled NO in patients with mild to moderate asthma.[5,6] Still there are some studies which report that increase in NO release by nebivolol may contribute to its respiratory effects.[1,7] All the aforementioned review of literature therefore reveals the fact that there is no consensus on the role of NO in the respiratory effects of nebivolol and needs further elucidation. The present study was therefore aimed to explore the role of nitric oxide in modulating the effect of nebivolol on tracheal muscle of guinea pig.

MATERIAL AND METHODS

The present study has been conducted on the isolated tracheal smooth muscle of 24 guinea pigs (male and female) of Dunkin Hartley variety weighing 500 to 600 grams. Ethics Committee approval of the protocol was obtained. The animals were housed at animal house of Army Medical College, Rawalpindi at room temperature, and were given tap water *ad libitum* and were fed with a standard diet. Krebs Henseleit solution was used as the nutrient solution the composition of which per 1000 ml is: NaCl 118.2 mM, KCl 4.7 mM, $MgSO_4.7H_2O$ 1.2 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.3 mM, $NaHCO_3$ 25.0 mM, Dextrose 11.7 mM. Solutions of all drugs were prepared in the distilled water except for nebivolol the solution of which was prepared in Dimethyl sulphoxide since nebivolol is highly lipophilic and insoluble in water.[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. Its 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, i.e., 10^{-7} to 10^{-3} M with an interval of 10 minutes between each concentration. Six experiments were performed and the mean response for each concentration was worked out. A concentration response curve was obtained by plotting the percent contraction against the logarithm of concentrations.

In the second group tracheal muscle strips were pre-treated with fixed dose of nebivolol (10^{-6} M) for 15 minutes while in third group trachea was pre-treated with L-NAME (10^{-4} M) for 15 minutes and then the same procedure was followed for different concentrations of histamine.[10] In the fourth group the tracheal muscle was first pre-treated with fixed concentration of L-NAME for 15 minutes followed by nebivolol again for 15 minutes. Then the same procedure was followed. The results have been expressed as Mean±SEM using Microsoft Excel. The differences between the observations were considered significant if the *p*-value was less than 0.05 by using Student's *t*-test.

RESULTS

Group-1 was taken as the control group and percent response with 10^{-3} M in group-1 was taken as 100% and responses with other concentrations were compared with it (Table-1).

Table-1: Comparison of Group 1 with Group 2

Concentration of histamine (M)	Mean (mm)		SEM		SD		*P*
	Gp1	Gp2	Gp1	Gp2	Gp1	Gp2	
10^{-3}	79.33	79	0.98	1.23	2.42	3.03	0.782
10^{-4}	66.66	68	1.74	2.01	4.27	4.93	0.400
10^{-5}	51.33	53.16	1.56	2.16	3.82	5.30	0.13
10^{-6}	32.66	32.83	1.54	2.13	3.77	5.23	0.901
10^{-7}	11.5	12.16	0.61	1.07	1.51	2.63	0.618

Table-2: Comparison of Group 1 with Group 3

Concentration of histamine (M)	Group-1 (histamine) % response	Group-3 (Hist+L-NAME) % response	*p*
10^{-7}	14.5	14.5	>0.05
10^{-6}	41.18	39	>0.05
10^{-5}	64.71	65.4	>0.05
10^{-4}	84.03	84.25	>0.05
10^{-3}	100	99.5	>0.05

Table-3: Comparison of Group 2 with Group 4

Concentration of histamine (M)	Mean (mm)		SEM		SD		*p*
	Gp2	Gp4	Gp2	Gp4	Gp2	Gp4	
10^{-3}	79	85	1.23	1.69	3.03	4.14	0.02
10^{-4}	68	73.5	2.01	1.66	4.93	4.08	0.04
10^{-5}	53.16	62.83	2.16	2.83	5.30	6.94	0.04
10^{-6}	32.83	40.66	2.13	1.83	5.23	4.50	0.01
10^{-7}	12.16	15.83	1.07	1.22	2.63	2.99	0.04

DISCUSSION

From the above findings, it is inferred that nebivolol has no significant effect on histamine-induced contractions of tracheal smooth muscle. These findings support the results of *in vivo* study whereby nebivolol, both acutely or chronically administered, did not affect airway responsiveness to inhaled histamine in rabbits.[7] Similar findings have been reported in other *in vivo* studies. In a study conducted by De Clerck *et al*, it was reported that nebivolol decreased heart rate without significantly increasing pulmonary reactivity to histamine.[11]

In this study some aspects concerned with the mechanisms that may be responsible for the lack of bronchoconstrictor effect of nebivolol on tracheal smooth muscle were explored. There may be many possible mechanisms which can explain the sparing effect of nebivolol. It is the most selective β_1-adrenoceptor antagonist currently available for clinical use; its β_1 selectivity is 3.5 times more than bisoprolol which was previously considered as the most cardioselective β-blocker. Beta 1 receptor selectivity is an important determinant of less incidence of bronchoconstriction and other adverse effects seen with cardioselective β-blockers.[3] However several *in vivo* and *in vitro* studies have shown that cardioselective blockers such as atenolol and metoprolol do increase airway hyperresponsiveness, though to a lesser extent. De Clerck *et al* compared the bronchoconstrictor effects of atenolol, nebivolol and propranolol in guinea pigs and they reported that bronchoconstriction was greatest with propranolol followed by atenolol while nebivolol had sparing effect.[11] So the different effect of nebivolol cannot be fully explained by its β_1 selectivity.[7] 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.[12] Therefore, this mechanism does not seem to be plausible.

Nebivolol has been reported to modulate the endogenous production of NO.[1] Nitric oxide is an important endogenous bronchodilator and is generated by a family of NO synthase isoforms in the airways.[13] Considering the potential role of endogenous NO in the control of airways, its role was evaluated in the effects of nebivolol. For that purpose, L-NAME which is a competitive inhibitor of nitric oxide synthase was used. In one group effect of histamine was studied on tracheal muscle strips pretreated with fixed concentrations of L-NAME (10^{-4} M) and its curve was compared with curve of control group. The difference was statistically insignificant indicating the absence of any effect of L-NAME on histamine induced contraction of tracheal muscle. In another group, the isolated tracheal muscle of guinea pig was pretreated with fixed concentrations of L-NAME (10^{-4} M) and nebivolol (10^{-6} M) respectively and then the effects of histamine were studied on this tissue model. At all concentrations of histamine contraction of tracheal muscle was augmented and the *p*-value was <0.05. The above mentioned augmented response of tracheal muscle to nebivolol in the presence of L-NAME can be explained on the basis of

involvement of NO. L-NAME by inhibiting the activity of enzyme NO synthase decreased the synthesis of NO induced by nebivolol thereby preventing its modulatory effect on airway smooth muscle thus increasing its contraction.

Nebivolol appears to interact with the endothelial NO pathway in two complementary ways: it increases NO synthase (NOS) activity and it possess a complementary antioxidant activity, through which the pathological free radical induced depression of intracellular NO levels can be prevented, thereby increasing the amount of NO by reducing its oxidative inactivation.[14,15] Nitric oxide that is released may interfere with the cholinergic neurotransmission either by functional antagonism on airway smooth muscle or via pre-junctional inhibition of release of acetylcholine from cholinergic nerve terminals. These findings suggest that NO indeed has some role in the sparing effect of nebivolol on the airways. This may be due to the reason that nebivolol induced-bronchoconstriction is counter balanced by the release of NO by nebivolol which causes bronchodilation resulting in the overall sparing effect of nebivolol on the airway smooth muscle. The NO-mediated inhibition of the acetylcholine-dependent bronchoconstriction may thus contribute to explain the differences between nebivolol and other β-blockers on the airway responsiveness.

CONCLUSION

NO may be responsible for sparing effect of nebivolol on airway smooth muscle. This may be due to the reason that nebivolol like classical β-blockers induces bronchospasm which is counter balanced by the relaxant effect of nitric oxide released by nebivolol thus lacking the net effect on airway smooth muscle.

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