ORIGINAL ARTICLE

EFFECT OF BUPIVACAINE AGAINST ACETYLCHOLINE AND BRADYKININ INDUCED TRACHEAL TISSUE CONTRACTION OF GUINEA PIGS *IN VITRO*

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Background: Some inhaled local anaesthetics are effective in preventing airway constriction provoked by various stimuli. The objective of this study was to evaluate the protective effect of bupivacaine against acetylcholine (Ach) and bradykinin induced airway contraction of isolated tracheal tissue of guinea pig *in vitro*. **Methods:** It was a laboratory based randomized control trial, carried out in Pharmacology Department of Army Medical College Rawalpindi from January to December 2016. Effects of cumulative doses of acetylcholine (3–96 µg) and bradykinin (11–66 µg) in the presence of fixed dose of bupivacaine (2 mM) were observed on isolated tracheal smooth muscle of guinea pig by constructing cumulative dose response curves. Isometric Force Transducer DT-475 (USA) attached to PowerLab® Data Acquisition Unit, was used to record the tracheal smooth muscle contractions. **Results:** Acetylcholine and bradykinin reversibly increased the tracheal muscle contractions of guinea pig. Maximum amplitude of contraction with acetylcholine and bradykinin alone and acetylcholine and bradykinin pretreated with lignocaine were 0.025±0.0009 mV, 0.013±0.0007 mV, 0.009±0.0005 mV, and 0.006±0.0006 mV respectively. **Conclusion:** Bupivacaine significantly inhibited the constrictor response of acetylcholine and bradykinin. The percent inhibition was more for acetylcholine than for bradykinin induced tracheal tissue contraction.

Keywords: Bupivacaine, Acetylcholine, Bradykinin, Isometric Force Transducer, Isolated Trachea

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INTRODUCTION

Asthma is one of the most prevalent chronic airway diseases that is characterized by varying levels of bronchoconstriction, airway hyper-responsiveness, mucus secretion and chronic inflammation, resulting in airway dysfunction.[1] The disease has immunological basis and is multifactorial.[2] Large number of inflammatory cells are involved in the pathogenesis such as eosinophils, mast cells and $CD4^+$ T lymphocytes that release mediators like histamine, prostaglandin and bradykinin, ultimately causing the symptoms of asthma.[3] Parasympathetic system provides the major innervation to the airways and acetylcholine (Ach) is the main neurotransmitter. In inflammatory diseases of airways like asthma there is over activity of this system leading to bronchoconstriction, vasodilatation and increased mucus secretion.[4] Bradykinin, one of the inflammatory mediators, has contribution in pathogenesis of allergic inflammatory conditions of airways like asthma.[5] Patients of asthma undergoing surgeries develop airway hyper-responsiveness secondary to endotrcheal intubation which comes out to be fatal sometimes. Endotracheal intubation should be avoided in such patients.[6] Studies have suggested that local anaesthetics can reduce respiratory reflexes. Studies have suggested that some local anaesthetics in high thoracic and epideural anaesthesia decrease bronchial reactivity in patients of airway allergic inflammatory diseases due to their systemic effects.[7,8] Bupivacaine is an amide linked local anaesthetic that is used for epidural, infiltration anaesthesia and peripheral nerve block. The relaxant effect of bupivacaine against inflammatory mediators like histamine, methacholine and carbachol has been studied[9,10] but, to our knowledge, protective effect of bupivacaine topically applied to trachea against bradykinin has never been explored.

MATERIAL AND METHODS

This laboratory based randomized control trial was conducted on isolated tracheal rings of 24 guinea pigs in Pharmacology Department, Army Medical College Rawalpindi from January to December 2016. Twenty-four Dunkin Hartely guinea pigs aged between 6–8 weeks weighing 400−600 g were indiscriminately assigned into four groups having equal number (n=6) of animals. All guinea pigs underwent allocation concealment via stratified randomization technique.

All the protocols described in the study were approved by Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College Rawalpindi. After sacrificing guinea pigs, trachea was dissected out and was cut into 2 to 3 mm wide rings each containing 3–4 cartilages. Tracheal tissue was transferred to organ bath containing Krebs Henseliet solution at 37 ℃ provided with oxygen continuously.[11] One end of the tracheal strip was attached to the oxygen tube in tissue bath and the other end was attached to a Research Grade Isometric Force Transducer DT-475 (USA). Trachealis muscle activity was recorded through Displacement Transducer. Dose response curves were

constructed using PowerLab® Data Acquisition Unit (AHK/214 iworx).[12]

Group I (Control group 1): Cumulative dose response curves were constructed using cumulative concentrations of acetylcholine ranging from 3 µg to 96 µg. Effect was recorded through a Research Grade Isometric Force Transducer.[13]

Group II (Control group II): Cumulative concentration response curves were constructed by using cumulative doses of bradykinin ranging from 11 µg to 66 µg.[14]

Group III (Ach pretreated with bupivacaine): Bupivacaine (2 mM) was added in organ bath. After 15 minutes cumulative concentrations of acetylcholine (3 µg to 96 µg) were added into the organ bath. Cumulative concentration response curves pre-treated with bupivacaine were constructed.[15]

Group IV (Bradykinin pre-treated with bupivacaine): Bupivacaine (2 mM) was added to the organ bath. After 15 minutes, the successive doses of bradykinin (11 µg to 66 µg) were added into the organ bath.[16]

Dose response curves were constructed with bradykinin in the presence of bupivacaine. The data was taken as an average of six observations of isolated tracheal rings in each group. Mean and standard error of means were calculated. Independent sample *t*-test was applied to compare the amplitudes of contraction between group I and III and between group II and IV. Percentage responses for all the four groups were also calculated, and $p<0.05$ was taken as significant.

RESULTS

Acetylcholine and bradykinin directly increased the constrictor response of tracheal tissue of guinea pigs. Changes in amplitude of contraction were recorded in millivolts. Maximum amplitude of contraction in acetylcholine control group was 0.025±0.0009 mV and in bradykinin control group was 0.013±0.0007 mV. This maximum response of acetylcholine was reduced in the presence of bupivacaine to 0.009±0.0005 mV in group III and to 0.006±0.0006 mV in group IV respectively (Table-1, 2).

Our data showed statistically significant difference when independent sample *t*-test was applied between group 1 (acetylcholine control) and 3 (acetylcholine pre-treated with bupivacaine). The *p* value between group 1 and 3 was significant with all doses of Ach. Statistically significant difference was also observed between group 2 (bradykinin control) and group 4 (bradykinin pretreated with bupivacaine) when independent sample *t*-test was applied between two groups (Table-1, 2).

The mean percent inhibition of acetylcholine pre-treated with bupivacaine was 36% and for bradykinin pre-treated group was 38% (Table-1, 2). Bupivacaine significantly attenuated acetylcholine and bradykinin induced tracheal smooth muscle contraction.

Table-1: Comparison of amplitude of contraction between Group 1 and Group 3 (mV, Mean±SEM)

Dose of acetylcholine (µg)	Amplitude of contraction (Group 1)	Amplitude of contraction (Group 3)	*p*-value between group 1 and 3	Percent response (Group 1)	Percent response (Group 3)	Percent inhibition between group 1 and 3
3	0.007±0.0004	0.00±0.0002	0.000*	28	4	86
6	0.009±0.0002	0.002±0.0003	0.001*	36	8	78
12	0.011±0.0003	0.004±0.0005	0.001*	44	16	73
24	0.014±0.0004	0.005±0.0004	0.000*	56	20	64
48	0.018±0.0009	0.007±0.0004	0.000*	72	28	61
96	0.025±0.0009	0.009±0.0005	0.000*	100	36	64

*Highly Significant

Table-2: Comparison of amplitude of contraction between group 2 and group 4 (mV, Mean±SEM)

Dose of bradykinin (µg)	Amplitude of contraction (Group 2)	Amplitude of contraction (Group 4)	*p*-value between group 2 and group 4	Percent response (Group 2)	Percent response (Group 4)	Percent inhibition between group 2 and 4
11	0.003±0.0003	0.001±0.0002	0.001*	23	7	70
22	0.005±0.0003	0.003±0.0002	0.001*	38	15	61
33	0.006±0.0003	0.003±0.0004	0.001*	46	23	50
44	0.008±0.0004	0.005±0.0003	0.001*	61	23	62
55	0.010±0.0008	0.006±0.0006	0.001*	76	31	59
6	0.013±0.0007	0.006±0.0006	0.000*	100	38	62

*Highly Significant

DISCUSSION

Acetylcholine has acute, concentration dependent, contractile effect on tracheal muscle of guinea pigs. Our results were consistent with findings of a study by Mikami[17] in which maximum contraction of Ach was achieved at 10^{-6} M. In another study done by Kieffer *et al*, Ach showed maximum contraction in a dose of 20 µM on mouse trachea.[18]

Bradykinin produced dose dependent reversible contraction of tracheal smooth muscle but to a lower extent than produced by Ach. Noor *et al*[19] have reported similar contractile effects of bradykinin on isolated tracheal tissue of guinea pigs. Significant contractions of smooth muscle of trachea were observed at a dose of 11 µg of bradykinin and reached its maximum at 77 µg.

Bupivacaine significantly reduced the contractile responses of acetylcholine. Our results are in accordance with work of Chang *et al* on isolated

tracheal smooth muscle of rats. They observed that bupivacaine decreased methacholine induced tracheal smooth muscle contraction. Bupivacaine also inhibited electrical field stimulation spike contraction of isolated tracheal muscle.[20] Zhao *et al* suggested that bupivacaine can cause bronchodilatation by blocking parasympathetic tone, antagonizing the effect of cholinergic receptors, by releasing nitric oxide and by decreasing the influx of Ca^{++} through L-type calcium channels and due to increased cAMP.[21]

Bupivacaine also significantly ameliorated bradykinin induced tracheal contraction. Comparisons of mean values of contractile responses and mean percent responses between group 2 (bradykinin alone) and group 4 (bradykinin pretreated with bupuvacaine) were found to be significant. The relaxant effect of bupivacaine has been studied against other inflammatory mediators of asthma like histamine and acetylcholine but it has not been studied against bradykinin. Bupivacaine can serve as a treatment option in patients of airway hyper-reactivity undergoing endotracheal intubations, bronchoscopies and surgeries.

It was observed that percent inhibition of bupivacaine was more against acetylcholine mediated tracheal tissue contraction as compared to bradykinin induced contraction. This may be due to the fact that acetylcholine is the main mediator of asthma and the main neurotransmitter in airways.

CONCLUSION

Our study revealed a significant ameliorating effect of bupivacaine against acetylcholine and bradykinin mediated tracheal tissue contraction. Bupivacaine can be used as spinal anaesthesia in patients of asthma and other airway inflammatory diseases undergoing general anaesthesia and surgical procedures due to its bronchodilatory effect.

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