

## PROTECTIVE EFFECT OF LIGNOCAINE AGAINST ACETYLCHOLINE AND BRADYKININ INDUCED TRACHEAL TISSUE CONTRACTION OF GUINEA PIGS IN VITRO

Fatima Qasim Malik, Bushra Tayyaba Khan\*, Mahjabeen Sharif\*

Yusra Medical and Dental College, Islamabad Pakistan, \*Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

### ABSTRACT

**Objective:** To evaluate the protective effect of lignocaine against acetylcholine and bradykinin induced airway contraction of isolated tracheal tissue of guinea pig in vitro.

**Study Design:** Laboratory based quasi experimental study.

**Place and Duration of Study:** Pharmacology department in collaboration with Physiology Department at Army Medical College, Rawalpindi, from Jan 2016 to Oct 2016.

**Methodology:** Effects of cumulative doses of acetylcholine (10<sup>-6</sup>-10<sup>-3</sup> M) and bradykinin (11 $\mu$ g -66  $\mu$ g) in the presence of fixed dose of lignocaine (1mM) were observed on isolated tracheal smooth muscle of guinea pig by constructing cumulative dose response curves. Isometric Force Transducer DT-475 (USA) attached to Power Lab 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 & acetylcholine and bradykinin pretreated with lignocaine were 0.025  $\pm$  0.0009 mV, 0.013  $\pm$  0.0007 mV, 0.013  $\pm$  0.0012 mV and 0.007  $\pm$  0.0002 mV respectively. So lignocaine significantly ameliorated acetylcholine and bradykinin induced contraction.

**Conclusion:** Lignocaine significantly inhibited the constrictor response of acetylcholine and bradykinin. The percent inhibition was more for acetylcholine than for bradykinin induced tracheal tissue contraction. So it is suggested that lignocaine may be used as spinal anesthesia in asthmatic patients undergoing surgeries due to its bronchodilatory effects.

**Keywords:** Acetylcholine, Bradykinin, Isolated Forced Transducer, Isolated Trachea, Lignocaine.

---

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

---

### 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<sup>1</sup>. According to Global Burden of Diseases Study published in 2012, the latest estimate of asthma prevalence was almost 334 million which is still increasing. The disease has immunological basis and is multifactorial<sup>2</sup>. 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<sup>3</sup>. Parasympathetic system provides the major innervation to the airways and acetylcholine is the main neurotransmitter. In inflammatory diseases of airways like asthma there is over activity of this system leading to broncho-constriction, vasodilatation and increased mucus secretion<sup>4</sup>. Bradykinin, one of the inflammatory mediators, has contribution in pathogenesis of allergic inflammatory conditions of airways like asthma<sup>5</sup>. Patients of asthma undergoing surgeries develops airway hyper-responsiveness secondary to endotracheal intubation, which comes out to be fatal sometimes. Endotracheal intubation should avoided in such patients<sup>6</sup>. Studies have shown that some local anesthetics in high thoracic and epidural anesthesia decrease bronchial reactivity in patients of airway allergic inflammatory diseases due to

---

**Correspondence:** Dr Fatima Qasim Malik, Senior Lecturer, Yusra Medical and Dental College, Islamabad Pakistan

Email: drfatimaqasim@yahoo.com

Received: 28 Nov 2017; revised received: 10 Mar 2019; accepted: 12 Jul 2019

their systemic effects<sup>7,8</sup>. So the present experimental study was undertaken to evaluate and compare the protective effects of lignocaine against acetylcholine and bradykinin mediated airway hyper responsiveness in guinea pig model as acetylcholine and bradykinin are two main mediators of asthma.

## METHODOLOGY

It was a laboratory based quasi experimental study. The study was conducted on isolated tracheal rings of 24 guinea pigs in Pharmacology and Physiology department, Army Medical College Rawalpindi from January 2016 to October 2016. Twenty four healthy Dunkin Hartely guinea pigs of either sex, weighing 400-600g, were included in this study through non-probability convenient sampling. Animals were randomly divided into four equal groups by random number table. Each group comprised of six animals (n=6). The drugs used in this study included lignocaine, acetylcholine and bradykinin.

After approval from the institutional ethics committee, the guinea pigs were sacrificed by cervical dislocation<sup>9</sup>. A midline incision was given in the chest near trachea. The entire trachea from larynx to bronchi was dissected out. Serosa and loose connective tissue was removed from the trachea and then it was transferred to a dish containing Krebs Henseleit solution at 37°C. Epithelium was gently removed and the tracheal tube was cut into 2 to 3 mm wide rings each containing about 3 to 4 cartilages. Each small piece of tissue was opened by a longitudinal cut on the ventral side opposite to the smooth muscle, forming a tracheal chain with smooth muscle in the centre and cartilaginous portion of the rings on the both sides<sup>10</sup>. The tissue was transferred to isolated organ bath of 50 milliliter capacity containing Krebs solution at 37°C, provided with oxygen continuously. Krebs-Henseleit solution was used as nutrient solution which contained NaCl 11.82 mM, KCl 4.7 mM, MgSO<sub>4</sub>.7H<sub>2</sub>O 1.2mM, CaCl<sub>2</sub> 2.5 mM, KH<sub>2</sub>PO<sub>4</sub> 1.3 mM, NaHCO<sub>3</sub> 25.0 mM and dextrose 11.7 mM.

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) by means of a thread. Equilibration period of 15 minutes was allowed to the mounted tissue. During the equilibration, physiological solution in the organ bath was changed three or four times. The tracheal is muscle activity was recorded through Displacement Transducer. Dose response curves were constructed using Power Lab data acquisition unit (AHK/214 iworx)<sup>11</sup>.

### Experimental Groups

**Group I:** In group I, cumulative dose response curves were constructed using cumulative concentrations of acetylcholine ranging from 10<sup>-6</sup> to 10<sup>-3</sup> M. Next dose was added after attaining the maximum response with the previous dose. The effect was recorded through a Research Grade Isometric Force Transducer. After obtaining the maximal acetylcholine induced contraction, the tracheal strip was washed and allowed to relax passively. This group served as control group 1 for the study.

**Group II:** In group II, cumulative concentration curves were constructed by using various doses of bradykinin ranging from 11 µg to 66 µg. This group served as control group II.

**Group III:** In group III lignocaine was added to the organ bath in a concentration of 2mM. Cumulative concentrations of acetylcholine ranging from 10<sup>-6</sup> to 10<sup>-3</sup> M were added into the organ bath after 15 minutes in the presence of lignocaine. Cumulative concentration response curves pretreated with lignocaine were constructed.

**Group IV:** In group IV lignocaine was added to the organ bath in the same concentration as that in group III. After 15minutes, the successive doses of bradykinin ranging from 11 µg to 66 µg were added into the organbath in the presence of lignocaine. Dose response curves were constructed with bradykinin in the presence of lignocaine.

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. One way ANOVA and Post Hoc Tuckey Test using SPSS version 16 was used for comparisons of amplitude of contractions of four

directly increased the contractile response of tracheal tissue of guinea pigs (fig-1 & 2). Changes in amplitude of contraction were recorded in millivolts. Maximum amplitude of contraction in acetylcholine control group was  $0.025 \pm 0.0009$  mV and in bradykinin control group was  $0.013 \pm$

**Table-I: Comparison of group 1 (acetylcholine control) with group 3 (acetylcholine after pretreatment with fixed dose of lignocaine).**

Dose of acetylcholine ( $\mu$ g)	Amplitude of contraction of acetylcholine-control (Group 1) Mean $\pm$ SEM (mV) (n=6)	Amplitude of contraction of acetylcholine pretreated with lignocaine (Group 3) Mean $\pm$ SEM (mV) (n=6)	<i>p</i> -value between group 1 & 3	Percent response with acetylcholine (Group 1) (n=6)	Percent response of Acetylcholine pretreated with lignocaine (Group 3) (n=6)	Percent inhibition Between group 1 and 3
3	$0.007 \pm 0.0004$	$0.002 \pm 0.0004$	$<0.001^*$	28	10	65
6	$0.009 \pm 0.0002$	$0.003 \pm 0.0004$	$<0.001$	36	14	61
12	$0.011 \pm 0.0003$	$0.005 \pm 0.0007$	$<0.001$	44	22	50
24	$0.014 \pm 0.0004$	$0.007 \pm 0.0008$	$<0.001$	56	29	49
48	$0.018 \pm 0.0009$	$0.009 \pm 0.0008$	$<0.001$	72	36	50
96	$0.025 \pm 0.0009$	$0.013 \pm 0.0012$	$<0.001$	100	53	47

\*=significant ( $p < 0.05$ )

**Table-II: Comparison of group 2 (bradykinin control) with group 4 (bradykinin after pretreatment with fixed dose of lignocaine).**

Dose of bradykinin ( $\mu$ g)	Amplitude of contraction with Bradykinin control (Group 2) Mean $\pm$ SEM (mV) (n=6)	Amplitude of contraction with bradykinin pretreated with lignocaine Group 4 Mean $\pm$ SEM (mV) (n=6)	<i>A p</i> -value between group 2 and group 4	Percent response with bradykinin (Group 2) (n= 6)	Percent response of bradykinin pretreated with lignocaine (Group 4) (n=6)	Percent inhibition between group 2 and 4
11	$0.003 \pm 0.0003$	$0.001 \pm 0.0002$	$0.001^*$	23	10	57
22	$0.005 \pm 0.0003$	$0.002 \pm 0.0003$	$0.001^*$	38	17	55
33	$0.006 \pm 0.0003$	$0.003 \pm 0.0003$	$0.001^*$	46	25	45
44	$0.008 \pm 0.0004$	$0.005 \pm 0.0002$	$0.001^*$	61	39	36
55	$0.010 \pm 0.0008$	$0.006 \pm 0.0003$	$0.001^*$	76	46	39
66	$0.013 \pm 0.0007$	$0.007 \pm 0.0002$	$0.000^*$	100	52	48

\*=significant ( $p < 0.05$ )

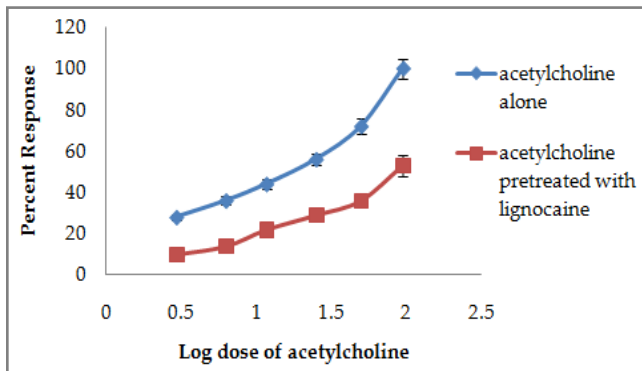
groups. Percentage responses for all the four groups were also calculated. Value of  $p < 0.05$  was taken as significant.

## RESULTS

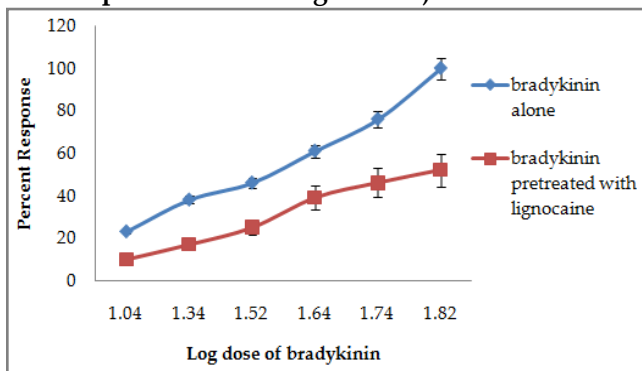
The study was conducted on 24 guinea pigs to observe protective effect of lignocaine against acetylcholine and bradykinin induced tracheal tissue contraction. Acetylcholine and bradykinin

$0.0007$  mV. This maximum response of acetylcholine was reduced in the presence of lignocaine from  $0.025 \pm 0.0009$  mV to  $0.013 \pm 0.0012$  mV in group III and  $0.013 \pm 0.0007$  mV to  $0.007 \pm 0.0002$  mV in group IV respectively (table-I). Our data showed statistically significant difference between group 1 (acetylcholine control) and 3 (acetylcholine pretreated with lignocaine). The *p*-value between group 1 and 3 was significant with

all doses of Ach. A  $p$ -value was 0.000 with 3 $\mu$ g, 24  $\mu$ g, 48 $\mu$ g, 96 $\mu$ g doses of Ach. A  $p$ -value for doses 6 $\mu$ g, 12 $\mu$ g was 0.001 which was highly significant. Statistically significant difference was also observed between group 2 (bradykinin control) and group 4 (bradykinin pretreated with lignocaine).



**Figure-1:** A Comparison of dose response curve of group 1 (acetylcholine control) with group 3 (acetylcholine pretreated with lignocaine).



**Figure-2:** A Comparison of dose response curve of group 2 (bradykinin control) with group 4 (bradykinin pretreated with lignocaine).

The  $p$ -value between group 2 and 4 was 0.001 for all doses of bradykinin except for 66 $\mu$ g dose for which  $p$ -value between group 2 and 4 was 0.000 which was highly significant (table-I & II).

Percentage responses and percentage inhibitions for the groups were also calculated. The mean percent inhibition of acetylcholine pretreated with lignocaine was 54 percent and for bradykinin pretreated group was 47 percent (table-I & II). So we conclude that lignocaine significantly attenuated acetylcholine and bradykinin induced tracheal smooth muscle contraction.

## DISCUSSION

Lignocaine is the most commonly used amide-type of local anesthetic. In addition to its anaesthetic effects, lignocaine has important anti-arrhythmic, analgesic, antinociceptive, immunomodulating and anti-inflammatory properties<sup>12</sup>. The current study was undertaken to evaluate the relaxant effect of lignocaine against acetylcholine and bradykinin mediated airway hyper-responsiveness on isolated tracheal tissue of guinea pig in vitro. Acetylcholine reversibly increased the contraction of guinea pig tracheal smooth muscles. Acetylcholine is the major neurotransmitter in airways which produces contraction of smooth muscles through M3 receptors via quantal and non quantal release<sup>13</sup>.

Lignocaine significantly reduced the contractile responses of acetylcholine and bradykinin. The mean values of responses as well as mean percentage responses when compared between group 1 and 3 were found to be significant. The maximum response was reduced to  $0.013 \pm 0.0012$  mV with lignocaine as compared to acetylcholine control group which was  $0.025 \pm 0.0009$  mV. Our results are in accordance with the observations of a study conducted by Kao and his colleagues on isolated tracheal smooth muscle of rats.

It was observed that lignocaine in a dose of 10-3 M decreased methacholine and acetylcholine induced tracheal smooth muscle contraction. Using the same dose, lignocaine also inhibited electrical field stimulation spike contraction of isolated tracheal muscle. The study indicated that lignocaine could cause bronchodilatation by blocking parasympathetic tone, antagonizing the effect of cholinergic receptors and by decreasing the influx of  $Ca^{++}$  through L-type calcium channels<sup>14</sup>.

Our study is in agreement with human trials in which when salbutamol was combined with lignocaine its bronchodilatory effect was potentiated. Possible mechanisms involved in bronchodilatory effect of lidocaine were also evaluated in this study. It was concluded that relaxant effect of lignocaine may be partially due



to increase release of nitric oxide, blockage of  $Ca^{++}$  influx to the respiratory muscles and due to increased cAMP<sup>15</sup>.

Bradykinin also produced a dose dependant reversible contraction of tracheal smooth muscles but to a lower extent than produced by acetylcholine. Bradykinin mediates its effect via B1 and B2 receptors. Both G-protein coupled receptors mediate airway inflammation and airway hyperresponsiveness in asthmatics and in other inflammatory airway diseases. Noor and his co-workers reported the 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  $\mu$ g of bradykinin and reached its maximum at 77 $\mu$ g<sup>16</sup>. Mulrennan also reported similar effects of bradykinin on isolated trachea at concentration range of 10-11 M to 10-5 M<sup>17</sup>. Zhang and his colleagues described that there is up regulation of bradykinin receptors which leads to hyperresponsiveness in asthmatic individuals<sup>18</sup>.

In group 4, lignocaine significantly reduced bradykinin induced tracheal contraction from  $0.013 \pm 0.0007$  mV to  $0.007 \pm 0.0002$ mV shifting the dose response curve to right and downwards. Comparisons of mean values of contractile responses and mean percent responses between group 2 (bradykinin alone) and group 4 (bradykinin pretreated with lignocaine) were found to be significant. The relaxant effect of lignocaine has been studied against other inflammatory mediators of asthma like histamine and acetylcholine but to our knowledge it has never been studied against bradykinin. So lignocaine can serve as a treatment option in patients of airway hyper-reactivity undergoing endotracheal intubations, bronchoscopies and surgeries.

Percent inhibitions obtained with acetylcholine pretreated with lignocaine group were compared with those of bradykinin treated groups, it was observed that percent inhibition of lignocaine 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.

## ACKNOWLEDGEMENT

We are grateful to National University of Medical Sciences (NUMS) Rawalpindi for providing financial support for this research project.

## CONCLUSION

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

## CONFLICT OF INTEREST

No conflict of interest to be declared by any author regarding this study.

## REFERENCES

1. Qi X, Gurung P, Malireddi R, Karmaus P, Sharma D, Vogel P, et al. Critical role of caspase-8-mediated IL-1 signaling in promoting Th2 responses during asthma pathogenesis. *Mucosal Immunol*; 29(4): 818-24.
2. Fahy JV. Type 2 inflammation in asthma [mdash] present in most, absent in many. *Nature Reviews Immunol* 2015; 15(1): 57-65.
3. Doeing DC, Solway J. Airway smooth muscle in the pathophysiology and treatment of asthma. *J Appl Physiol* 2013; 114(7): 834-43.
4. Muroi Y, Han L, Canning B, Gao P, Udem B, Zacccone E. Parainfluenza 3 virus induces increased cough sensitivity, bdnf expression, and vagal sensory TRPV1 expression in the airways. C33 immune pathology of viral infections. *Am Thoracic Soc* 2016;26 (2):148-51
5. Maurer M, Bader M, Bas M, Bossi F, Cicardi M, Cugno M, et al. New topics in bradykinin research. *Allergy* 2011; 66(11): 1397-406.
6. Regli A, von Ungern-Sternberg BS. Anesthesia and ventilation strategies in children with asthma: part I-preoperative assessment. *Current Opinion in Anesthesio* 2014; 27(3): 288-94.
7. Nafe LA, Guntur VP, Dodam JR, Lee-Fowler TM, Cohn LA, Reiner CR. Nebulized lidocaine blunts airway hyper-responsiveness in experimental feline asthma. *J Feline Medicine Surg* 2013; 15(8): 712-6.
8. Serra MF, Neves JS, Couto GC, Cotias AC, Pão CR. JM25-1, a Lidocaine Analog Combining Airway Relaxant and Anti-inflammatory Properties Implications for New Bronchospasm Therapy. *J American Soc Anesthesiol* 2016; 124(1): 109-20.
9. Wang C, Qiu W, Zheng Y, Li H, Li Y, Feng B, et al. Extra-neuronal monoamine transporter mediates the permissive action of cortisol in the guinea pig trachea: Possible involvement of tracheal chondrocytes. *PLoS one* 2013; 8(10): 76191-99.

10. Sharif M, Khan BT, Bakhtiar S, Anwar MA. Comparative study of protective effects of salbutamol and beclomethasone against insulin induced airway hyper-reactivity on isolated tracheal smooth muscle of Guinea Pig. *Int J Prod Res* 2015; 14(2): 567-71.
  11. Aleem SB, Hussain MM, Farooq Y. Serum levo-carnitine levels and skeletal muscle functions in type 2 diabetes mellitus in rodents. *J Coll Physician Surg Pak* 2013; 23(2): 132-6.
  12. Shipton EA. New formulations of local anaesthetics-part I. *Anesthesiology research and practice* 2012; 34: 546409.
  13. Delventhal L, Wiegand S, Kummer W, Nassenstein C. Characterization of cellular sources for non-quantal ACH release in mouse airways. *Pneumologie* 2016; 70(7): 47-52.
  14. Kao CH, Chu YH, Wang HW. Effects of lidocaine on rat's isolated tracheal smooth muscle. *J Eur Federation Oto-Rhino-Laryngol Societ* 2010; 267(5): 817-20.
  15. Mikami M, Zhang Y, Kim B, Worgall TS, Groeben H, Emala CW. Dexmedetomidine's inhibitory effects on acetylcholine release from cholinergic nerves in guinea pig trachea: A mechanism that accounts for its clinical benefit during airway irritation. *BMC Anesthesiol* 2017; 17(1): 52.
  16. Noor A, Najmi MH, Bukhtiar S. Effect of montelukast on bradykinin-induced contraction of isolated tracheal smooth muscle of guinea pig. *Indian J Pharma* 2011; 43(4): 445.
  17. Mulrennan SA, Redington AE. Nitric oxide synthase inhibition. *Treatments in respiratory Medicine* 2004; 3(2): 79-88.
  18. Zhang Y, Cardell LO, Edvinsson L, Xu CB. MAPK/NF- $\kappa$ B-dependent upregulation of kinin receptors mediates airway hyperreactivity: A new perspective for the treatment. *Pharmacol Res* 2013; 71(1): 9-18.
-