DETERMINATION OF RELAXANT EFFECT OF ROPIVACAINE AGAINST ACETYLCHOLINE AND BRADYKININ INDUCED AIRWAY CONTRACTION OF GUINEA PIGS

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

Margalla Institute of Health Sciences, Rawalpindi Pakistan, *Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To study the relaxant effect of ropivacaine against acetylcholine and bradykinin induced airway contraction of isolated tracheal tissue of guinea pig in vitro.

Study Design: Laboratory based experimental study.

Place and Duration of Study: Pharmacology department at Army Medical College, Rawalpindi, from Feb 2016 to Dec 2016.

Methodology: Response of variable doses of acetylcholine (10-6-10-3 M) and bradykinin (11µg-66 µg) in the presence of fixed dose of ropivacaine (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. For data analysis labchart software was used.

Results: Acetylcholine and bradykinin enhanced the tracheal muscle contractions of guinea pig. Amplitude of contraction with acetylcholine and bradykinin alone & acetylcholine and bradykinin pretreated with ropivacaine were 0.025 ± 0.0009 mV, 0.013 ± 0.0007 mV, 0.010 ± 0.0008 mV and 0.006 ± 0.0002 mV respectively. So ropivacaine significantly reversed acetylcholine and bradykinin induced contraction.

Conclusion: Ropivacaine significantly ameliorated the constrictor response of acetylcholine and bradykinin. The percent inhibition was more for acetylcholine than for bradykinin induced tracheal tissue contraction. Ropivacaine may be used as spinal anesthesia in asthmatic patients prior to surgeries as a bronchodilator.

Keywords: Acetylcholine, Bradykinin, Isolated force transducer DT-475, Ropivacaine.

INTRODUCTION

Asthma is a chronic inflammatory disease of airways characterized by airway hyper-responsiveness, bronchoconstriction, dyspnea and mucus secretion[1]. Mast cells, eosinophils and Tlymphocytes are mainly involved in pathogenesis of asthma. Rapid release of pro-inflammatory mediators like histamine, prostaglandins and bradykinin leads to airway smooth muscle contraction[2,3]. Bradykinin is implicated in the development of airway inflammation, airway hyperresponsiveness and remodeling[4]. Parasympathetic system is predominant in airways and acetylcholine is the main neurotransmitter. In asthma there is exaggerated response of this system that leads to increased airway reactivity, vasodilatation and increased mucus secreation[5].

Patients of asthma undergoing surgeries develops airway hyper-responsiveness secondary to endotracheal intubation which comes out to be fatal sometimes. Endotracheal intubation should be avoided in such patients[6]. Studies have suggested that some local anesthetics in high thoracic and epidural anesthesia decreases bronchial reactivity in patients of airway allergic inflammatory diseases due to their systemic effects[7]. Ropivacaine is an amide linked local anesthetic that is used for epidural, infilteration anesthesia and peripheral nerve block. Ropivacaine has an efficacy almost similar to that of bupivacaine. Ropivacaine is one of the least allergenic local anaesthetics that are used for spinal anaesthesia.

Correspondence: Dr Fatima Qasim Malik, Assistant Professor of Pharmacology & Therapeutics, Margalla Institute of Health Sciences, Rawalpindi Pakistan

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It is preferred over bupivacaine due to its reduced central nervous system toxicity and cardiotoxicity. The most common use of ropivacaine is in obstetrics. It is often co-administered with fentanyl for epidural analgesia in pregnant women during labour[8].

The relaxant effect of ropivacaine against inflammatory mediators like histamine, methacholine and carbachol has been studied[9], but to our knowledge protective effect of ropivacaine against bradykinin has never been explored. So this research project was carried out to evaluate the relaxant effect of ropivacaine against acetylchline and bradykinin mediated airway hyper responsiveness in guinea pig model as acetylcholine and bradykinin are two main mediators of asthma.

METHODOLOGY

This laboratory based experimental study were performed on isolated tracheal rings of twenty-four guinea pigs in Pharmacology department, Army Medical College Rawalpindi, from February 2016 to December 2016 after getting permission from Institutional Ethics Committee (certificate No. ERC/SA-16).

Twenty-four adult male Dunkin Hartely guinea pigs having weight 250-500g were included in this study through consecutive sampling technique. Guinea pigs were randomly allocated to four groups by random number table. Each group comprised of six guinea pigs (n=6). Ropivacaine, acetylcholine and bradykinin were used in this research study.

Guinea pigs were killed by a blow on the head[10]. Chest was incised and trachea was taken out and immersed in Krebs Henseleit solution at 37^{o} C. Trachea was divided into 3 to 4 mm wide rings each containing about 3 to 4 cartilages. A longitudinal cut was given on tracheal ring forming a tracheal chain with smooth muscle in the centre and cartilaginous portion of the rings on both sides. Tracheal ring was mounted with oxygen tube in organ bath filled with Krebs solution at 37^{o}C, provided with oxygen continuously[11].

One end of the tracheal strip was connected 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 given to the mounted tissue. During experiments, Kreb Henseleit solution in the organ bath was changed two or three times. Displacement Transducer was used to record changes in tracheal muscle tone. Dose response curves were constructed using Power Lab data acquisition unit (AHK/214 iworx)[12].

Group I (control group 1): In group I, cumulative dose response curves were constructed using cumulative concentrations of acetylcholine ranging from 10-6 to 10-3 M (3µg to 96 µg). After 15 minutes 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[13]. This group served as control group 1 for the study.

Group II (control group II): Variable doses of bradykinin ranging from 11µg to 66µg were poured on tracheal rings[14]. Starting with the lowest dose, 11 µg was added to the organ bath and its contractions were recorded on iWorx by Research Grade Isometric Force Transducer. After reaching plateau for this initial dose, the next dose of 22 µg was added cumulatively and tissue contractions were recorded. Similarly, successive doses of 33, 44, 55 and 66 µg were added and dose response curve were produced. This group served as control group II.

Group III (Acetylcholine pretreated with ropivacaine): In group III ropivacaine was added to the organ bath in a concentration of 1mm. Cumulative concentrations of acetylcholine ranging from 10-6 to 10-3 M (3µg to 96 µg) were added into the organ bath after 15 minutes in the presence of ropivacaine. Cumulative concentration response curves pretreated with ropivacaine were constructed[15].

Group IV (Bradykinin pretreated with ropivacaine): In group IV ropivacaine (1mm) was added to the organ bath. After 15 minutes, the successive doses of bradykinin ranging from 11 µg to 66 µg were added into the organ bath in the presence of ropivacaine[16]. Dose response curves were constructed with bradykinin in the presence of ropivacaine.

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. Value of $p \leq 0.05$ was taken as significant.

RESULTS

The study was conducted on 24 guinea pigs to observe the relaxant effect of ropivacaine against acetylcholine and bradykinin induced tracheal tissue contraction. Acetylcholine and bradykinin directly increased the constrictor 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 ± 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 ropivacaine from 0.025 ± 0.0009 mV to 0.010 ± 0.0008 mV in group III and 0.013 ± 0.0007 mV to 0.006 ± 0.0002 mV in group IV respectively (table-I). Our data showed statistically significant difference when independent sample

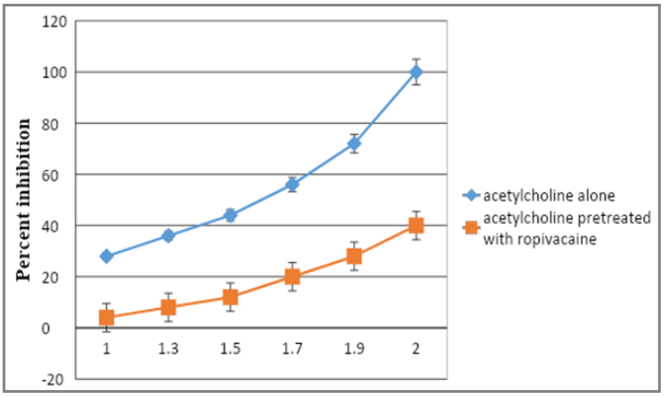

Figure-1: A comparison of dose response curve of group 1 (acetylcholine control) with group 3 (acetylcholine pretreated with ropivacaine).

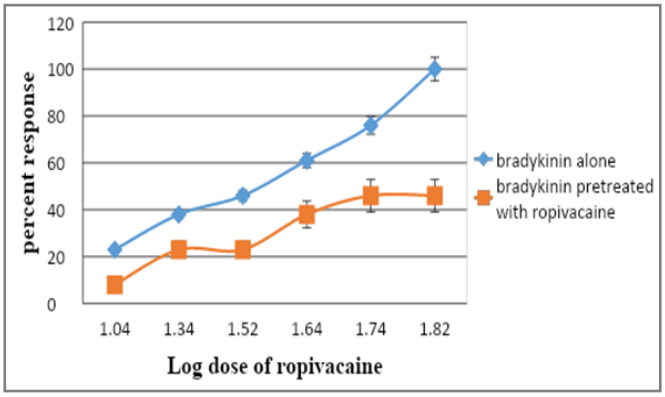

Figure-2: A comparison of dose response curve of group 2 (bradykinin control) with group 4 (bradykinin pretreated with ropivacaine).

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

Dose of acetylcholine (µg)	Amplitude of contraction of acetylcholine control (Group 1) Mean ± SEM (mV) (n=6)	Amplitude of contraction of acetylcholine pretreated with ropivacaine Group 3) Mean ± SEM (mV) (n=6)	*p*-value between Group 1 & 3	Percent response with acetylcholine (Group 1) (n=6)	Percent response of acetylcholine pretreated with ropivacaine (Group 3) (n=6)	Percent inhibition Between Group 1 and 3.
3	0.007 ± 0.0004	0.001 ± 0.0002	<0.001*	28	4	86
6	0.009 ± 0.0002	0.002 ± 0.0004	0.001*	36	8	78
12	0.011 ± 0.0003	0.004 ± 0.0005	0.001*	44	16	64
24	0.014 ± 0.0004	0.005 ± 0.0007	<0.001*	56	20	64
48	0.018 ± 0.0009	0.007 ± 0.0006	<0.001*	72	28	61
96	0.025 ± 0.0009	0.010 ± 0.0008	<0.001*	100	40	60

t-test was applied between group 1 (acetylcholine control) and 3 (acetylcholine pretreated with ropivacaine). The *p*-value between group 1 and 3 was significant with all doses of acetylcholine. Statistically significant difference was also observed between group 2 (bradykinin control) and group 4 (bradykinin pretreated with ropivacaine) when independent sample t test was applied between two groups (table-I & II).

Percentage responses and percentage inhibitions for all the four groups were also calculated. Percent inhibitions were calculated by substracting percent response of acetylcholine pretreated with ropivacaine from percent response with acetylcholine divided by percent response with acetylcholine multiplied by 100. The mean percent inhibition of acetylcholine pretreated with ropivacaine was 36 percent and for brady-kinin pretreated group was 38 percent (table-I & II). Mean percent inhibition was more for acetylcholine treated group as compared to bradykinin treated group.

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

Dose of bradykinin (µg)	Amplitude of contraction with brady-kinin control (Group 2) Mean ± SEM (mV) (n=6).	Amplitude of contrction with bradykinin pretreated with ropivacaine (Group 4) Mean ± SEM (mV) (n=6)	*p*-value between group 2 and group 4	Percent response with bradykinin (Group 2) (n=6)	Percent response of bradykinin pretreated with ropivacaine (Group 4) (n=6)	Percent inhibition between group 2 and 4
11	0.003 ± 0.0003	0.001 ± 0.0002	0.001*	23	8	65
22	0.005 ± 0.0003	0.003 ± 0.0003	0.001*	38	23	39
33	0.006 ± 0.0003	0.003 ± 0.0003	0.001*	46	23	50
44	0.008 ± 0.0004	0.005 ± 0.0002	0.001*	61	38	38
55	0.010 ± 0.0008	0.006 ± 0.0003	0.001*	76	46	39
66	0.013 ± 0.0007	0.006 ± 0.0002	<0.001*	100	46	54

DISCUSSION

Our results are consistent with findings of a study by Mikami in which maximum contraction of Ach was achieved at 10 -6 M 17. In another study done by Kieffer and his colleagues, Ach showed maximum contraction in a dose of 20 µM on mouse trachea[18].

Maximum contraction of tracheal tissue was 0.013 ± 0.0007 mV at a dose of 66 µg which is 52 percent of maximum contraction by Ach. 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 µg of bradykinin and reached its maximum at 77µg[19].

In third set of experiments, effect of Ach was observed on tracheal smooth muscle pretreated with 1mM concentration of ropivacaine. A dose response curve was plotted by using variable doses of Ach. Ropivacaine produced a significant reduction in response which was reduced from 0.025 ± 0.0009 mV of Ach control group to 0.010 ± 0.0008 mV of ropivacaine pretreated group. Ropivacaine inhibited the response of Ach control group to 58 percent. Our results are consistent with a study done by Gao and his co-workers in which he compared the effect of ropivacaine with calcium blocking agents on isolated rabbits tracheal tissue against Ach mediated contraction. He concluded that ropivacaine has an inhibitory effect on Ach induced tracheal tissue contraction[20]. Another study conducted on isolated tracheal tissue revealed that ropivacaine attenuates the contractile effect of Ach in a dose dependant manner. This relaxant effect is probably due to inhibitory action of ropivacaine on neuronal function and a decrease in the calcium influx[21].

In group 4, bupivacaine significantly reduced bradykinin induced tracheal contraction from 0.013 ± 0.0007 mV to 0.006 ± 0.0002 mV shifting the dose response curve to right and downwards. Comparisons of mean values of contrac-

tile responses and mean percent responses between group 2 (bradykinin alone) and group 4 (bradykinin pretreated with ropivacaine) were found to be significant. The mean percent inhibition with ropivacaine was 48 percent. A study conducted on isolated tracheal tissue revealed that ropivacaine attenuated the contractile effect of histamine in a dose dependant manner[22]. The relaxant effect of ropivacaine has been studied against inflammatory mediators of asthma like histamine, and acetylcholine but to our knowledge it has never been studied against bradykinin. So ropivacaine can serve as a treatment option in patients of airway hyperreactivity undergoing endotracheal intubations, bronchoscopies and surgeries.

The concentration response curve of Ach pretreated with ropivacaine when compared with dose response curves of bradykinin pretreated with ropivacaine, it was observed that ropivacaine inhibited the effect of acetylcholine (60%) more than that of bradykinin (54%) and percent inhibition of ropivacaine was more against acetylcholine 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. So ropivacaine can serve as a treatment option in patients of airway hyper-reactivity undergoing endotracheal intubations, bronchoscopies and surgeries.

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CONCLUSION

Our study revealed a significant ameliorating effect of ropivacaine against acetylcholine and bradykinin mediated tracheal tissue contraction. So we suggest that ropivacaine can be used as spinal anesthesia in patients of asthma prior to surgeries as a bronchodilator.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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