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Original Article

Effects of Local Anesthetics on Smooth Muscle Tissue in Rat Trachea: An *In Vitro* Study

Ali Onur Erdem[1], Varlık K. Erel[2], Özlem Girit[3], Hasan Erdoğan[4], Sezen Özkısacık[1], Mesut Yazıcı[1]

[1]Department of Pediatric Surgery, Adnan Menderes University School of Medicine, Aydın, Turkey
[2]Department of Anaesthesiology, Adnan Menderes University School of Medicine, Aydın, Turkey
[3]Department of Biophysics, Adnan Menderes University School of Medicine, Aydın, Turkey
[4]Department of Internal Medicine, Adnan Menderes University School of Veterinary Medicine, Aydın, Turkey

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Abstract

OBJECTIVES: We evaluated the muscle responses of rat trachea to LA drugs, such as lidocaine and prilocaine, in terms of airway spasms.

MATERIALS AND METHODS: A total of 16 male rats were used. After ketamine anesthesia, the tracheal ring of each rat was removed and placed in the organ bath in the Krebs solution. The rat tracheal veins were randomly divided into two groups based on the LA applied at the basal tonus level: group 1 (n=8), lidocaine; group 2 (n=8), prilocaine. Second, the baths were washed. Supramaximal contraction was obtained by applying acetylcholine to the tracheal rings (n=16) at a basal tonus level. The rat tracheas with supramaximal contraction were randomly divided into two groups: group 3 (n=8), lidocaine; group 4 (n=8), prilocaine. The contraction responses of each group were recorded and statistically compared.

RESULTS: Lidocaine constituted a significant relaxation response in the tracheal tissue in both basal tonus and supramaximal tonus levels. Moreover, it was observed that the relaxation of lidocaine was higher in the supramaximal contraction than in the basal tonus tension level. However, for prilocaine, no significant change was observed in both tonus levels.

CONCLUSION: This study suggests that lidocaine as a LA drug should be preferred as the first choice in patients with respiratory risk, and that its use over prilocaine should be preferred, if supported by advanced clinical studies.

KEYWORDS: In vitro, local anesthetics, organ bath,trachea, prilocaine, lidocaine

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INTRODUCTION

Local anesthetics (LAs) have analgesic, antiarrhythmic, antibacterial/antifungal, antithrombotic, anticonvulsive, neuroprotective, and anti-inflammatory effects besides anesthetic effects. However, anesthetic, antiarrhythmic, and analgesic effects are most commonly used in the clinic. Especially, the antiarrhythmic effect of lidocaine has widespread clinical use. Considering the clinical benefits, side effects of some drugs can be reverted to the desired effect after some period of time [1-5].

Pain control is important to improve both clinical outcomes and patient comfort [6]. Local anesthesia is widely used for pediatric patients because it is a reliable, safe, and easy pain control method. Therefore, injecting an LA at the end of the surgery to relieve postoperative pain is a common practice [7, 8]. Lidocaine is often used as an LA because of its rapid onset of the sensory block and good efficacy. In clinical practice, lidocaine is often used in combination with another LA [9]. Such a combination prolongs the early onset of lidocaine and provides more exerts analgesic effects. In some studies, decreases in hemodynamic response were observed due to the direct application of lidocaine to the trachea before or during endotracheal intubation [10, 11]. In one of these studies, a decreased hemodynamic response was achieved with lidocaine application to the oropharyngeal region without laryngoscope assistance [12]. Thus, we conclude that lidocaine affects the trachea not only with its anesthetic effect but also through other mechanisms. Although routine local use of lidocaine is recommended during bronchoscopy, many studies showed better results in flexible bronchoscopy when combined with different narcotic agents [13]. We aimed to demonstrate the effects of lidocaine, which has been shown to have a positive effect on the respiratory system and trachea, on the tracheal muscle tissue in the organ bath with concrete data.

Prilocaine is another LA used recently in bronchoscopy. Some studies showed that benefits of prilocaine are equivalent to those of lidocaine in flexible bronchoscopy [14]. Studies showing the effect of prilocaine on the tracheal tissue are very limited; thus, lidocaine is used as an alternative to prilocaine. No clinical or experimental study has reported on the effect of prilocaine on the tracheal muscle tissue.

Address for Correspondence: Ali Onur Erdem, Department of Pediatric Surgery, Adnan Menderes University School of Medicine, Aydın, Turkey
E-mail: aoerdem@adu.edu.tr
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In addition to the analgesic effects of these two LAs, which are becoming increasingly direct application to the respiratory tract, we aimed to demonstrate the muscle responses to normal and increased tonus values in the tracheal muscle tissue.

MATERIALS AND METHODS

The study was conducted in an electrophysiology research laboratory after getting approval from the Adnan Menderes University and approved by the Local ethics committee (13.06.2017/ 64583101/2017/067A). Sixteen adult, healthy, male Wistar-Albino rats weighing 250–300 g were used in the study. Before the experiment, the rats were kept in a wireframe for 12 hours of luminous light and 12 hours of the dark circadian rhythm at a controlled temperature of 20-25°C. Briefly, anesthesia was given with ketamine (50 mg/kg) and xylazine (3 mg/kg) injection intramuscularly. Muscular tissue samples obtained after the dissection and excision of the trachea of all ratswere immediately placed in the carbonized Krebs–Henseleit solution (118.3 mMNaCl, 4.7 mMKCl, 1.2 mM $MgSO_4$, 1.22 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$,and 11.1mM glucose, Sigma-Aldrich). Each ring-shaped muscular tissue was suspended by two stainless steel hooksin the organ bath filled with Krebs–Henseleit solution (May IOBS 99, Ankara, Turkey). The top hook was connected to the transducer (May GTA 0303 and Biopac Systems Inc. Model MP 100, USA) with an appropriate-sized thread, and the contractions were measured in milligrams. ACQ Knowledge was used as the computer software. Then, the standard equilibrium phase was applied to each muscular tissue sample. The tissue was first stretched by 4 g after a waiting period of 10 minutes, the tension was increased to 6 g, followed by another waiting period of 10 minutes. Finally, the tension was increased up to 8 g, followed by a waiting period of 30 minutes. After the contraction curve was stabilized, 0.1 mL of 10^{-4}M concentrated acetylcholine was administered using a micropipette, and the contraction response was observed. After reaching the peak contraction value and waiting for 5 minutes, the relaxation response was observed after administering 0.1 mL of 0.25 mg atropine using a micropipette. When the contraction curve flattened, the bath was washed twice with Krebs solution. We waited for approximately 30 minutes until the contraction value reached the baseline level.

The drugs and compounds (acetylcholine, atropine, prilocaine, and lidocaine) used in this study were obtained from Sigma-Aldrich.

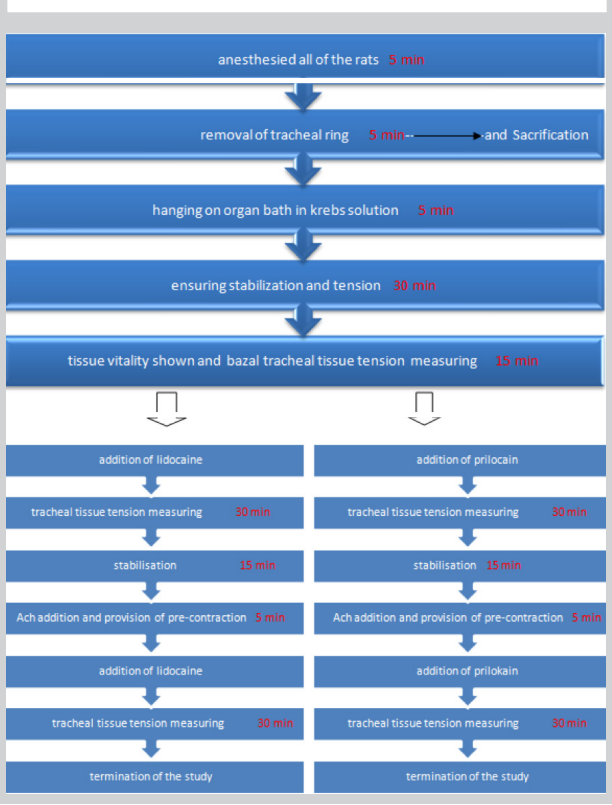
To the bath, 1 mg/mL of 10^{-1} M lidocaine was added for evaluating the effects of lidocaine on the muscle relaxation response. The bath was washed thrice with the Krebs solution after the contraction curve flattened (baseline level). We waited for 15 minutes to get a stable contraction curve. To the bath, 0.1 mL of 10^{-4} M concentrated acetylcholine was added with a micropipette to perform precontraction, and the contraction response was recorded. After reaching the supramaximal contraction, we waited for 5 minutes before adding 1 mg/mL of 10^{-1} M lidocaine, and the relaxation response in the supramaximal contraction was recorded.

Prilocaine evaluation was conducted by adding 0.5 cc (1.1 mg) of pure form of prilocaine, and the relaxation response was observed. The bath was washed thrice with the Krebs solution after the contraction curve flattened (baseline level). We waited for 15 minutes to obtain a stable contraction curve. To the bath, 0.1 mL of 10^{-4} M concentrated acetylcholine was added using a micropipette to perform precontraction and the contraction response was recorded. After supramaximal contraction was attained, we waited for 5 minutes and then added 0.5 cc (1.1 mg) of prilocaine to the bath and recorded the relaxation response in the supramaximal contraction (Table 1).

Statistical Analyses

The normality of data was assessed with the Kolmogorov–Smirnov analysis and logarithmic transformation was applied to the non-normally distributed data. The data showed a normal distribution after the transformation. All data were compared using the GraphPad Prism Program (7.00 for Macintosh, California, USA) with the help of Welch's Correction and unpaired *t*-test. In all analyses, $p<0.050$ was considered statistically significant.

Figure 1. A time-line diagram to illustrate study design



MAIN POINTS

- In our organ bath study, Lidocaine causes relaxation in rat trachea during a organ bath.
- In our organ bath study, Prilocaine has no effect on rat trachea tone.
- In our organ bath study, The relaxation responses taken with lidocaine in higher tonus values are higher than basal tonus values.

RESULTS

The difference between pre- and post-medication values was found to be statistically significant for lidocaine when lidocaine and prilocaine were applied on tissue samples in the basal tension in the organ bath, but there was no statistically significant difference for prilocaine. Thus, it was concluded that lidocaine caused relaxation in the tissue ($p<0.05$) (Figure 2-4). The difference between pre- and post-medication levels for lidocaine was statistically significant when lidocaine and prilocaine were applied to tissue samples precontracted with Ach in the organ bath. However, there was no statistical difference for prilocaine; therefore, it has been concluded that lidocaine also causes relaxation in the precontracted tissue ($p<0.05$) (Figure 3-5).

When the relaxation response in the basal tension tissue and precontracted tissue was compared, a statistically significant difference was seen for lidocaine ($p<0.050$). Lidocaine produced more relaxation response than the normal tension tissue in the precontracted tissue (Figure 6).

Lidocaine showed significant relaxation response in a tracheal muscle tissue in both basal and increased tonus values. Prilocaine showed no effect on the basal or precontracted trachea tissue.

DISCUSSION

Lidocaine is commonly used in flexible bronchoscopy applications. It has been shown that applying lidocaine to the patients before or during the procedure positively contributes to the process [15, 16]. Whether this benefit is due to the anesthetic and analgesic characteristics of lidocaine or is the

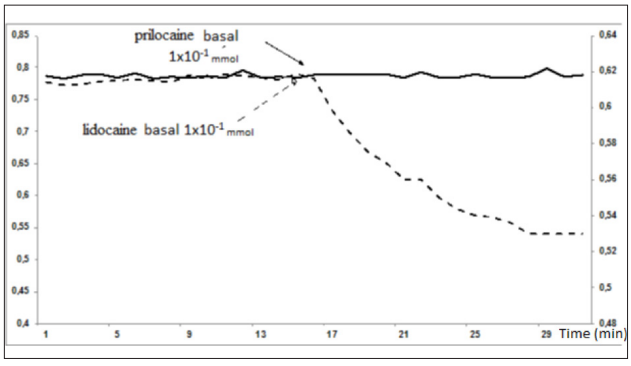


Figure 2. Basal lidocaine and prilocaine max and mean graphic

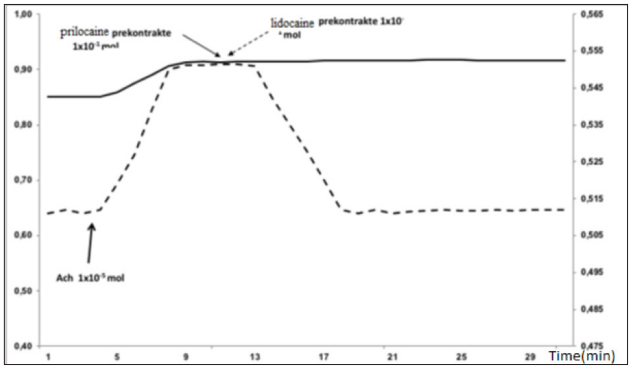


Figure 3. Precontracted lidocaine and prilocaine max and mean graphic

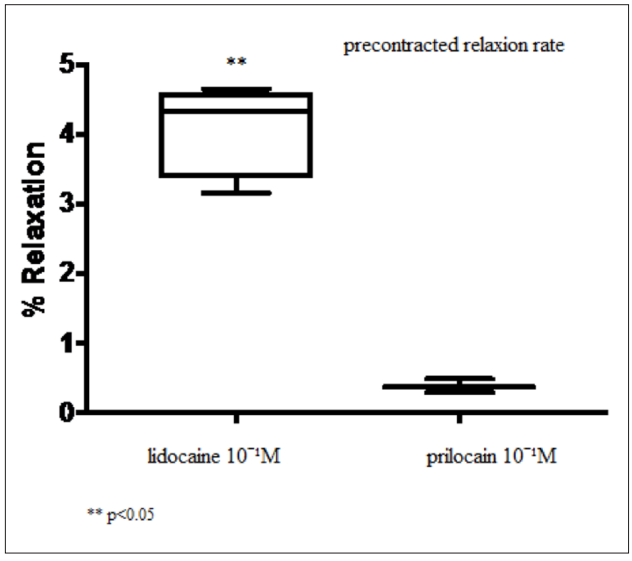


Figure 5. Precontracted lidocaine and prilocaine max relaxasyon graphic

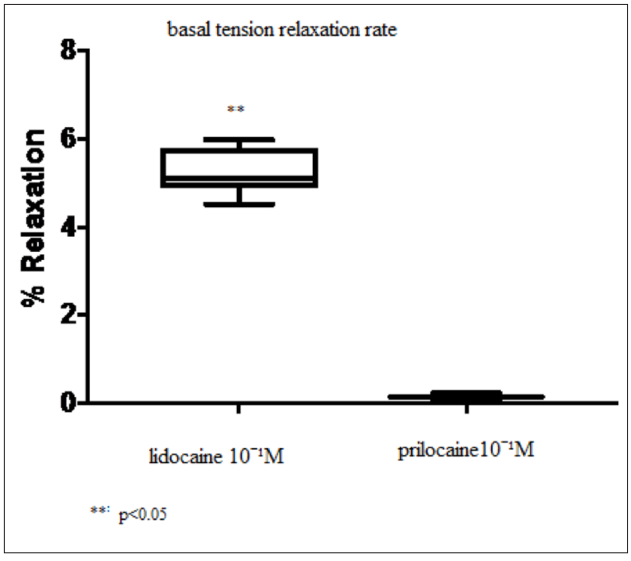


Figure 4. Basal lidocaine and prilocaine mean relaxasyon graphic

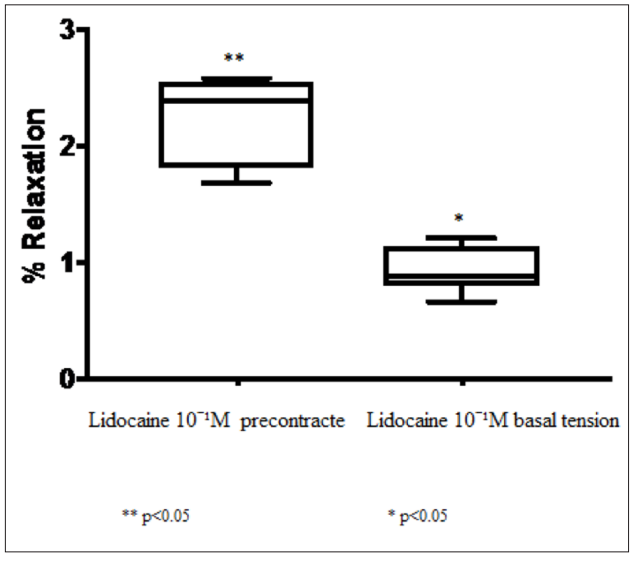


Figure 6. Basal lidocaine and prilocaine max relaxasyon graphic

result of the muscle response generated by the local effect in the trachea tissue is a question that needs to be answered. Since clinical studies are affected by many different systemic mechanisms, it is difficult to answer this question. In our experimental organ bath study, we measured the specific response of a tracheal muscle tissue to lidocaine in the basal and supramaximal contractions. The muscle responses obtained with lidocaine were with a higher relaxation response, especially in the supramaximal contraction.

In their in vitro experiment with human trachea, Rogliani et al. [17] showed that 1 mg/mL lidocaine applied to the tracheal muscle tissue in the organ bath test produced a relaxation effect. While they showed that the lidocaine effect increased dose dependently, they also checked the tracheal muscle tissue response in the basal tonus value. Their work supports our study.

Some studies in the literature show that lidocaine suppresses coughing [18, 19]. This effect has been demonstrated in the basal tonus values of human tracheal muscle in vitro, particularly by providing trachea muscle relaxation [17]. In addition to these data, we have also shown that lidocaine provides a relaxation response in both basal tonus and supramaximal tonus levels. Only lidocaine is recommended for in vitro examination of ciliary activity of the tracheal mucosa with LAs because of its low toxicity and efficacy [20].

Many clinical studies have been performed with prilocaine, which is increasingly used in bronchoscopy applications and has been presented as an alternative to lidocaine. Thornton explained that the safety margin of prilocaine used during bronchoscopy is wider and it can be used safely [21]. However, there is no study experimentally showing its effect on the tracheal muscle tissue. We measured the responses of the tracheal muscle tissue at both basal tonus and supramaximal tonus levels; we did not find any muscle response to prilocaine in the tracheal muscle tissue. Thus, we have shown in our experimental study that the clinically obtained data are not due to muscle response. Therefore, we conclude that the specific tissue response we obtained in our experimental study will provide insights to the subsequent studies in explaining the physiology of in vivo responses achieved in clinical use.

There are only a few studies in the literature on the effect of prilocaine on the tracheal tissue. Although prilocainehas been recommended for clinical trials due to its wider safety margins and safe dose range, it has side effects such as methemoglobinemia, especially in pediatric patients [22]. The superiority of lidocaine overprilocaine has also been shown in a study conducted on pig trachea for comparing the effects of lidocaine and prilocaine. We were able to demonstrate the effect of lidocaine on the tracheal muscle tissue in parallel with the data from the other study [23].

The most important limitation of this experimental study is the inability to predict the tissue response when so many other factors, in vivo, are evaluated simultaneously. Another limitation of our study is that we did not know, at the tissue level, the exact effect of anesthetic drugs and the interaction of the anesthetic drugs with the two different agents, although the effects of the tissue were equal for each group.

During rigid bronchoscopy for the removal of foreign bodies from children, lidocaine and prilocaine were used together and the superiority of lidocaine over the control group was demonstrated in a clinical study [24]. Our study data show that, in addition to the local analgesic effects of lidocaine, its muscle relaxation effects might be effective in achieving this superiority.

Local administration of lidocaine and prilocaine before or during general anesthesia is thought to contribute to anesthesia, especially by suppressing the cough reflex or by reducing local pain sensation [25-28]. On the basis of our study, we think that lidocaine, which is used locally on the trachea, also contributes to tracheal muscle relaxation in addition to anesthesia.

LAs stabilize the neuronal membrane by inhibiting the ionic currents necessary to initiate and conduct stimulation in the nerves, thereby eliciting a local anesthetic effect. Systemically, both lidocaine and prilocaine are thought to produce a systemic response with direct effects of epinephrine on the beta-adrenergic receptor stimulating effect [29-31]. We think that the data obtained in our study will contribute to the explanation of the clinical pharmacology of both lidocaine and prilocaine.

All airway-related initiatives are highly risky. Even the smallest support obtainable at this point can be life-saving. On the basis of our experimental study data, we believe that LA application during the airway-related intervention and the use of lidocaine, in particular, can be a support with a muscle relaxation response. We believe that the data we have obtained experimentally should be supported by other clinical studies before clinical use.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Adnan Menderes University (Date: 13.06.2017/Number: 64583101/2017/067).

Informed Consent: N/A.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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