The Effect of Lidocaine on Tracheal Smooth Muscle Tension in Guinea-pigs

Shin Ok Koh, M.D., Won Oak Kim, M.D., Hae Keum Kil, M.D.,
and Jong Rae Kim, M.D.

Department of Anesthesiology Yonsei University College of Medicine, Seoul, Korea

INTRODUCTION

Lidocaine is often administered intravenously to suppress those airway reflexes associated with tracheal intubation or tracheal suction in patients. In addition, intravenous lidocaine has spasmylytic effects against bronchospasm.

Effects of an airway relaxant and the mechanism of relaxation may include followings: interruption of reflex arcs, inhibition of the chemical mediator release and direct relaxation of airway smooth muscles as well as on vascular smooth muscles. The underlying mechanisms which are responsible for the direct effect of lidocaine on smooth muscles relaxation may be related to Ca²⁺ mobilization. However, there is little direct evidence to support this hypothesis with regard to airway or other smooth muscles.

The present study was designed to examine the effect of lidocaine on tracheal smooth muscles tension induced by carbachol, potent muscarinic receptor agonist and the mechanism involved in the inhibitory effect of lidocaine.
on tracheal smooth muscles pretreated with verapamil, an L-type voltage-operated calcium channel blocker, in guinea-pigs.

**MATERIALS AND METHODS**

1) **Animal preparation**

The study protocol was approved by the animal investigation committee at university. Male guinea-pigs weighing 350–450 grams each were anesthetized with ether inhalation. Explo-thoracotomy and neck dissection using a midline approach were done. The trachea was excised, cleaned of adhering adipose and connective tissue, and the trimmed trachea was opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis muscle. The opened trachea was cut into small segments each containing 3-4 cartilage rings.

2) **Perfusion**

Every preparation was mounted vertically in an organ bath which was filled with 30 ml of Krebs solution maintained at 37°C and aerated with 5% carbon dioxide in 95% oxygen. The modified Krebs solution contained the followings (mmol/liter): sodium chloride 120.7, potassium chloride 5.9, calcium chloride 2.5, magnesium chloride 1.2, sodium bicarbonate 15.5, sodium dihydrogen phosphate 1.2, and glucose 11.5. The upper end of the strip was connected by a small clip with cotton thread to a strain gauge transducer (Grass, Quincy), while the lower end of the strip was held vertically by a cotton thread and mounted in an organ bath.

Each preparation was set at 1.5 grams of resting tension and allowed to equilibrate for ninety minutes. Isometric tension was induced to increase muscle length to the maximum level. This was done with 10⁻⁶M carbamylcholine chloride (carbachol), (Aldrich Chemical Co., Milwaukee, WI), a stable and potent muscarinic receptor agonist. After a steady state was maintained (considered as 100% in each preparation), lidocaine hydrochloride, (Bulk Medicine and Pharmaceuticals, Hamburg, Germany) was cumulatively applied at a dose of 10⁻⁵ to 3 x 10⁻³ M while the specimens were kept at a steady state of tension (group 1). In group 2, after pretreatment with 10⁻⁵ M verapamil hydrochloride (Isotopin), (Sigma Chemicals, St. Louis, MO), an L-type voltage-operated calcium channel blocker, muscle tension was induced by 10⁻⁶ M carbachol and lidocaine was applied cumulatively at a dosage of 10⁻⁵ to 3 x 10⁻³ M. The changes in isometric tension of each sample was recorded at each concentration of lidocaine in group 1 and 2 on two channels of Grass model 7 polygraph recorder (Grass medical instrument polygraph, Quincy, USA) simultaneously via force transducers (FT-03) and calculated as % relaxation of initial tension induced carbachol.

Before end of the experiment, all tissue was washed repeatedly with fresh Krebs solution for 15 minutes. After washing out lidocaine with normal Krebs solution for 15 minutes, carbachol caused the same extent of response, reversing the effect of the lidocaine and the next experiment was proceeded.

3) **Data analysis**

The measured values were expressed as mean ± SE (n = number of observation). For each observation of group 1 and group 2, a strip from a different part of trachea tissue was used. One-way analysis of variance for repeated measurement was used to determine concentration-dependent effects. An unpaired t-test was done to compare the percent relaxation of tracheal muscle contraction and ED₅₀ and ED₉₀ between group 1 and group 2. A P value of less than 0.05 was considered to be significant in each case. The ED₅₀ and ED₉₀ values, the lidocaine concentration needed to decrease tension to 50% and 95% of the maximal response induced by 10⁻⁶ M carbachol, were calculated, using a four-parameter logistic equation (13).

**RESULTS**

1) **Effect of lidocaine on muscle tension induced by carbachol**

In group 1, 4.9 ± 0.5, 17.5 ± 1.4, 37.5 ± 2.7 and 60.6 ± 2.5, 78.3 ± 2.0% of maximal muscle contraction induced
Fig. 1. Effect of lidocaine on tension induced by carbachol. This figure represents the time course of the effect of cumulatively applied lidocaine \((10^{-5} \sim 3 \times 10^{-3} \text{ M})\) on tension induced by \(10^{-6} \text{ M}\) carbachol. Upper trace(A): Muscle contraction was induced by \(10^{-6} \text{ M}\) carbachol after pretreating the muscle strip with \(10^{-5} \text{ M}\) verapamil (Group 2). Lower trace(B): Muscle contraction was induced by \(10^{-6} \text{ M}\) carbachol (Group 1).

Table 1. Relaxation Effect of Lidocaine on Airway Tension Induced by Carbachol

<table>
<thead>
<tr>
<th>Lidocaine concentration(M)</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^{-5})</td>
<td>4.9±0.5⁹</td>
<td>7.3±0.8</td>
</tr>
<tr>
<td>(10^{-4})</td>
<td>17.5±1.4⁹</td>
<td>27.9±1.9</td>
</tr>
<tr>
<td>(3 \times 10^{-4})</td>
<td>37.5±2.7⁹</td>
<td>53.2±2.7</td>
</tr>
<tr>
<td>(10^{-3})</td>
<td>60.6±2.5⁹</td>
<td>77.2±2.2</td>
</tr>
<tr>
<td>(3 \times 10^{-3})</td>
<td>78.3±2.0⁹</td>
<td>93.9±1.3</td>
</tr>
</tbody>
</table>

Values are mean±SE (%) (n=18, means number of observations from 18 animals). Group 1 refers to that group of guinea-pigs in which the muscle tension was induced by \(10^{-6} \text{ M}\) carbachol. Group 2 refers to that group of guinea-pigs in which muscle tension was induced by \(10^{-5} \text{ M}\) carbachol after pretreatment with \(10^{-5} \text{ M}\) verapamil. *p<0.05 versus Group 2.

Fig. 2. This figure represents the effect of lidocaine on tension induced by carbachol. Note the representative time course of the effect of cumulatively applied lidocaine \((10^{-5} \sim 3 \times 10^{-3} \text{ M})\) on tension induced by \(10^{-6} \text{ M}\) carbachol.

Group 1: The concentration dependent effect of lidocaine on elevated tension as induced by \(10^{-6} \text{ M}\) carbachol.

Group 2: The concentration dependent effect of lidocaine on elevated tension as induced by \(10^{-6} \text{ M}\) carbachol after pretreating the muscle strip with \(10^{-5} \text{ M}\) verapamil.

Table 2. Values of \(ED_{50}\) and \(ED_{95}\) of Group 1 and Group 2

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ED_{50})</td>
<td>1.21±0.10⁹</td>
<td>0.74±0.05</td>
</tr>
<tr>
<td>(ED_{95})</td>
<td>2.45±0.08⁹</td>
<td>1.85±0.06</td>
</tr>
</tbody>
</table>

Values are \((\text{mean±SE}) \times 10^{-3} \text{ M}\). Group 1 refers to those guinea-pigs in which contraction was induced by \(10^{-6} \text{ M}\) carbachol. Group 2 refers to those guinea-pigs in which contraction was induced by \(10^{-5} \text{ M}\) carbachol after pretreatment with \(10^{-5} \text{ M}\) verapamil. \(ED_{50}\) and \(ED_{95}\) refer to the lidocaine concentration that decreased tension to 50 and 95 percent of the maximal response induced by \(10^{-6} \text{ M}\) carbachol. *p < 0.05, versus Group 2.

by \(10^{-6} \text{ M}\) carbachol was decreased at a concentration of \(10^{-5}, 10^{-4}, 3 \times 10^{-4}, \text{ and } 10^{-3}, 3 \times 10^{-3} \text{ M}\) lidocaine in a concentration - dependent manner (p<0.05) (Table 1, Figure 1 B). In group 2, \(7.3±0.8, 27.9±1.9, 53.2±2.7 \text{ and } 77.2±2.2, 93.9±1.3\%\) of maximal muscle contraction induced by \(10^{-6} \text{ M}\) carbachol was decreased at a concentration of \(10^{-5}, 10^{-4}, 3 \times 10^{-4} \text{ and } 10^{-3}, 3 \times 10^{-3} \text{ M}\) lidocaine in a concentration dependent manner (p<0.05) (Table 1, Figure 1 A). At the same concentrations of lidocaine, \(10^{-5} \sim 3 \times 10^{-3} \text{ M},\) the percent of depression
was greater in group 2 than that of group 1 (p<0.05) (Table 1, Fig. 2).

2) Values of \( ED_{50} \) and \( ED_{95} \) of group 1 and group 2

The \( ED_{50} \) values in group 1 and 2 were \((1.21 \pm 0.10) \times 10^{-3} \) M and \((0.74 \pm 0.05) \times 10^{-3} \) M. The \( ED_{95} \) values in group 1 and 2 were \((2.45 \pm 0.08) \times 10^{-3} \) M and \((1.85 \pm 0.06) \times 10^{-3} \) M. The values of \( ED_{50} \) and \( ED_{95} \) were greater in group 1 than those of group 2 (p<0.05) (Table 2).

DISCUSSION

Increase in intracellular \( Ca^{2+} \) can be triggered by two mechanisms: ① via the release of \( Ca^{2+} \) from intracellular stores, especially the sarcoplasmic reticulum and ② the entry of extracellular \( Ca^{2+} \) through voltage-operated or receptor-operated channels. It is well known that smooth muscle contraction is maintained by a \( Ca^{2+} \) influx through voltage-operated and receptor-operated channels\(^{14}\).

Although it is generally accepted that smooth muscle contraction is primarily regulated by \( Ca^{2+} \), smooth muscle contractile forces do not simply depend on \( Ca^{2+} \). Morgan and Morgan\(^{15}\) first showed that agonists can increase the effectiveness of intracellular \( Ca^{2+} \) on the contractile apparatus.

Our results indicated that lidocaine decreased the muscle tension induced by carbachol when administered in a range of concentration of \( 10^{-5} \) to \( 3 \times 10^{-3} \) M in a concentration-dependent manner. Verapamil, an L-type voltage-operated calcium channel-blocker, decreased muscle contraction induced by \( 10^{-6} \) M carbachol almost to the resting level and appears to enhance the the inhibitory effect of lidocaine. This implied that the increase of \( Ca^{2+} \) through voltage operated calcium channels may play an important and perhaps even a controlling role in the maintenance of smooth muscle contraction. Therefore lidocaine may decrease \( Ca^{2+} \) by regulating at least voltage-operated calcium channels\(^{16}\).

Hay and Wadsworth\(^{17}\) state that the inhibitory action of lidocaine on KCl responses in rat vas deferens was reversed by raising the extracellular \( Ca^{2+} \) concentration. Spedding and Berg\(^{18}\) report that drugs such as lidocaine can also interact with calcium channels as deduced from similar observations in guinea-pig taenia. Our study also indicated this.

Carbachol activated \( Ca^{2+} \)-nondependent muscle contraction; Carbachol, a muscarinic receptor agonist, activated phospholipase C via the G-protein linked to it. The phospholipid component of the cell membranes was broken down into second messengers, inositol 1, 4, 3-triphosphate and diacylglycerol (DAG). Carbachol induced a sustained increase in the DAG content of intact tracheal smooth muscles\(^{19}\). The only well-characterized effect of DAG was the activation of phospholipid / \( Ca^{2+} \)-dependent PKC.

Under basal conditions, PKC is thought to be located mainly in cytosol and to be deactivated, but after carbachol stimulation, PKC is rapidly translocated to the membrane, where it begins to associate with membrane phospholipid DAG\(^{20}\). It has been shown that PKC plays an important role in tracheal smooth muscle contraction, which is independent of \( Ca^{2+} \)\(^{16}\).

The \( ED_{50} \) and \( ED_{95} \) values, the lidocaine concentration that decreased tension to 50 % and 95 % of the maximal response were \((1.21 \pm 0.10) \times 10^{-3} \) M and \((2.45 \pm 0.08) \times 10^{-3} \) M, higher in group 1 (this contraction was induced by \( 10^{-6} \) M carbachol) than those of group 2 (this contraction was induced by \( 10^{-6} \) M carbachol after pretreating the muscle strip with \( 10^{-3} \) M verapamil). This was supported by the data of Table 1 and Figure 2, also, showing that the relaxation percent of muscle contraction was greater in group 2 at the same concentrations of lidocaine. \( ED_{50} \) values for \( Ca^{2+} \) and tension induced by 40 mM K+ in adult pigs were approximately \( 3.4 \times 10^{-4} \) M and \( 1.2 \times 10^{-4} \) M\(^{11}\). If tension was induced by \( 10^{-6} \) M acetylcholine, the \( ED_{50} \) values for \( Ca^{2+} \) and tension were approximately \( 2.8 \times 10^{-4} \) M and \( 1.5 \times 10^{-4} \) M. Differences relating to the \( ED_{50} \) values between our data and that of Kai's group may have been due to differences in species, or to the fact that the muscle contraction was induced by such different agents from carbachol as.
potassium or acetylcholine. Another handicap to our study was that we did not measure the intracellular calcium concentration simultaneously with muscle tension as in the Kai et al. group study.

In conclusion, lidocaine decreased the tracheal smooth muscle contraction induced by carbachol in a dose dependent manner. Verapamil, an L-type voltage-operated calcium channel blocker, antagonized tracheal muscle contraction induced by carbachol and thus appears to enhance the inhibitory effect of lidocaine. However, we did not measure intracellular calcium simultaneously with muscle tension and cannot explain the exact intracellular mechanism of the inhibitory effect of lidocaine on airway tension of guinea-pigs.

ACKNOWLEDGMENT

The authors would like to thank Mr. Tim Cornish (Office of Biomedical Research Publications, Yonsei University College of Medicine) for English language revision.

REFERENCES