Abstract

Objectives: Organ transplant in humans is an established therapy for a variety of end-stage organ diseases. However, due to organ shortages and lack of donors, the need for xenotransplant has gradually increased. Xenotransplantation has great potential to solve many of the problems facing organ transplantation. Pigs are being developed as xenogeneic organ donors for use in humans. In this study, we propose a novel and simple method for tracheal intubation in a swine model using neuromuscular blocking agents and laryngeal mask airway.

Material and Methods: Eight Yorkshire pigs were used for the 2 separate experiments, which were conducted 1 week apart. In the first experiment, an anesthesiologist with no previous comparable experience performed endotracheal intubation of pigs. One week later, using the same pig, a second experiment was performed by an experienced anesthesiologist.

Anesthesia was induced with intramuscular injection of a mixture of 1 mg/kg xylazine (Rompun, Bayer Korea Ltd., Seoul, Korea) and 7 mg/kg Zoletil (a mixture of tiletamine hydrochloride and zolazepam hydrochloride, Virbac Laboratory, Carros, France). The laryngeal mask was then placed, and 0.15 mg/kg vecuronium bromide was injected intravenously. Tracheal intubation was attempted after mask removal. The duration and number of intubation attempts were recorded, and the degree of intubation difficulty was scored.

Results: In all cases, the laryngeal mask was easily inserted, and endotracheal intubation was successfully completed. Oxygen saturation did not fall below 95%, and there were no hypoxemia episodes.

Degree of intubation difficulty and duration were not significantly different between the 2 anesthesiologists.

Conclusions: Tracheal intubation in our swine model was successfully performed using neuromuscular blocking agents and laryngeal masks without resulting in hypoxemia, with even anesthesiologists who are unfamiliar with endotracheal intubation of pigs easily able to do so using our protocol. Therefore, our protocol will enable all investigators to perform successful tracheal intubation in swine models.

Key words: Airway management, Laryngeal mask airway, Neuromuscular blocking agents, Pig

Introduction

Organ transplant is an established therapy for a variety of end-stage organ diseases.1 Because of increased organ transplants and the development of better surgical techniques, the number of patients on organ transplant wait lists is growing exponentially. However, a major current problem in organ transplant is the gap between the number of available transplantable organs and the number of patients waiting for an organ graft.1 Therefore, the need for xenotransplant has gradually increased.

Xenotransplant has great potential to solve many of the problems facing organ transplant, including supply shortage, donor-specific manipulation of the recipient's immune system, perhaps with permanent graft acceptance and minimal immunosuppression, and minimization of donor organ injury associated with brain death and procurement.2

Because of compatible size and the potential ready availability, pigs are being developed as xenogeneic organ donors for use in humans.2 In addition, since the 18th century, when the usefulness of swine models in the field of experimental biomedicine was first reported, pigs have been used as a standard experimental model3 for research in cardiovascular and gastrointestinal surgery and in
organ transplant. However, airway management of pigs under general anesthesia is technically demanding, and endotracheal intubation can be difficult and time consuming because pigs have a long oropharynx that does not open widely. Furthermore, their larynx slopes ventrally, making visualization of the larynx relatively difficult compared with humans. Even when it is performed by experienced operators, endotracheal intubation in pigs is not always successful. Therefore, considering the era of xenotransplantation, a safe swine protocol to prevent organ ischemia during the anesthesia induction period is needed.

In this study, we hypothesized that (1) neuromuscular blocking agents (NMBAs) may facilitate tracheal intubation of swine, especially for procedures conducted by inexperienced operators, and (2) any hypoxemia that may be developed with NMBAs use may be prevented by assisting ventilation with the laryngeal mask airway (LMA). Accordingly, the primary objective of our study was to test the hypothesis that endotracheal intubation in a swine model can be successfully achieved using NMBAs and LMA. The secondary end objective was to compare the required time to complete airway intubation in swine when performed by experienced versus inexperienced operators.

**Materials and Methods**

This study was approved by the Institutional Animal Care and Use Committee (Internal Review Board No. KUIACUC-2012-125) of Korea University. Eight healthy Yorkshire pigs weighing 28 to 34 kg were used in this study.

The pigs were housed for at least 5 days in the laboratory animal research center of Korea University before the experiments. Room temperature was maintained within the range of 20°C to 22°C, and the relative humidity was approximately 40% to 70%. Lighting was both natural and artificial, with a 12:12-hour on/off cycle. The room was adjusted to make the animals comfortable. Food, but not water, was withheld for 12 hours before anesthesia.

For preanesthesia, pigs received an intramuscular injection of a mixture of 1 mg/kg Rompun (xylazine hydrochloride 23.32 mg/1 mL; Bayer Korea Ltd., Seoul, Korea) and 7 mg/kg Zoletil (a mixture of tiletamine hydrochloride 50 mg/1 mL plus zolazepam hydrochloride 50 mg/1 mL; Virbac Laboratory, Carros, France), after which the pigs were positioned in dorsal recumbency on an operating table with the neck extended. A pulse oximeter was taped to their tails, and electrocardiogram results were recorded. If oxygen saturation levels on pulse oximeter fell below 95%, we planned to insert the LMA. However, oxygen saturation during spontaneous ventilation did not fall below 95% in all pigs. The pigs were cannulated via the auricular vein and received a continuous intravenous infusion of Ringer lactate solution at 5 mL/kg/h throughout the surgical procedure. After local infiltration was completed using 40 mg lidocaine (lidocaine hydrochloride 2%, Daihan Pharm, Seoul, Korea) in the femoral region, the superficial femoral artery was catheterized for invasive blood pressure monitoring and arterial blood gas sampling.

During venous or arterial cannulation, pig responses were graded by the anesthesiologists using the following scale: 1, no response; 2, movement in only 1 section of the limb; 3, movement in more than 2 sections of the limb; and 4, generalized response (Table 1). If the response grade was more than 3, Zoletil (2 mg/kg) was injected intravenously.

Fifteen minutes after the administration of intramuscular preanesthetic medication, LMA was inserted (size 3 for up to 30 kg, size 4 for up to 43 kg). The LMA cuff was inflated with 20 mL of air. Pig lungs were spontaneously ventilated during LMA insertion, and the correct LMA position was confirmed by chest expansion and absence of air leakage around the cuff. Anesthesia was maintained with enflurane at an end-tidal concentration of 1.5%, with fraction of inspired oxygen of 0.5 (fresh gas flow; 2 L/min oxygen and 2 L/min nitrous oxide). Ten minutes after LMA insertion, 0.15 mg/kg vecuronium bromide (Vecron, Myungmoon Pharmaceutical Co. Ltd., Seoul, Korea) was injected intravenously for muscle relaxation, following which mechanical ventilation was initiated. Tidal volume and respiratory rates were set as 8 mL/kg and 15 breaths/minute. Two minutes later, LMA was removed, and the pig was intubated with a standard

<table>
<thead>
<tr>
<th>Item</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>No response</td>
<td>1</td>
</tr>
<tr>
<td>Movement in only 1 section of the limb</td>
<td>2</td>
</tr>
<tr>
<td>Movement in more than 2 sections of the limb</td>
<td>3</td>
</tr>
<tr>
<td>Generalized response</td>
<td>4</td>
</tr>
</tbody>
</table>

*Abbreviations: ASA, American Society of Anesthesiologist physical status; SD, standard deviation*
cuffed endotracheal tube (ETT; Portex, Smith-Medical, Watford, UK). The ETT size was determined by visual inspection of the larynx and confirmed by passage of the tube without resistance. We used ETT with a 6-mm internal diameter in all cases. The ETT cuff was inflated with the minimum amount of air that still allowed a small air leak. The correct ETT position was confirmed by direct visual control during laryngoscopy and bilateral chest auscultation. After vital signs had stabilized, total thyroidectomy was performed by the surgeon. Once the surgical procedure was completed, spontaneous ventilation was restored, and the trachea of pig was extubated. The pig was then transferred to a breeding cage. After 1 week, the pig was anesthetized using the same anesthesia protocol. The neck of the anesthetized swine was inspected by the surgeon for study purposes according to the surgical protocol. Pigs were killed after surgical experiments were completed.

This experiment was conducted twice using the same pig. In the first experiment, an anesthesiologist with no previous experience in endotracheal intubation of pigs performed the endotracheal intubation under the supervision of an experienced anesthesiologist. One week later, a second experiment was performed by an anesthesiologist, considering that enough time had passed for any edema induced by the first experiment to subside.

The duration and the number of tracheal intubation attempts were recorded. The duration of each tracheal intubation attempt was defined as the time taken from deflation of the LMA cuff until connection of ETT to the ventilator and inflation of lungs. Each anesthesiologist scored the degree of difficulty using a 10-point visual analog scale, with 0 indicating extremely easy intubation and 10 indicating extremely difficult intubation. Arterial blood gas analysis was performed at 3 time points. Time point 1 was 15 minutes after the administration of preanesthetic medications, time point 2 was 10 minutes after LMA insertion, and time point 3 was 10 minutes after endotracheal intubation. All arterial blood samples were immediately analyzed, and the results were recorded.

Statistical analyses
Statistical data were collected and computed using the commercially available statistical and graphic software SigmaPlot 12.3 for Windows (Systat Software, Inc., Chicago, IL, USA). The duration of ETT intubation and rate of successful tracheal intubation attempts were evaluated and compared between the 2 experiments using the Mann-Whitney rank sum test. Partial pressures of oxygen in arterial blood were compared among time points 1, 2, and 3 using one-way analysis of variance. The results are expressed as means ± standard deviations, and a P value of < .05 was considered statistically significant.

Results
Our analyses included data from all 8 pigs (5 male and 3 female). The average weight of the pigs was 32.8 ± 2.2 kg. In pig 6, even though endotracheal intubation was successful, cardiac arrest occurred at the end of the surgical procedure (during total thyroidectomy). Electrocardiogram monitoring revealed ventricular tachycardia and a rapid decline in blood pressure. Despite cardiopulmonary resuscitation for 10 minutes, the pig expired, and we were not able to complete the second experiment in pig 6.

In all cases, LMA was easily inserted and ETT intubation was successfully completed. There were no differences in the degree of intubation difficulty and duration of ETT intubation (Table 2). In addition, the rate of successful tracheal intubation attempts showed no significant difference, regardless of operator experience (Figure 1 and Table 2).

Partial pressures of oxygen in arterial blood at time points 2 and 3 were significantly higher than that at time point 1, as shown in Table 3 (P < .001). However, there were no significant differences in
these pressures at time points 1, 2, and 3 between the 2 experiments, indicating no influence of operator experience (Table 3).

**Table 2. Demographic and Anesthetic Data for of Analyzed Pigs**

<table>
<thead>
<tr>
<th></th>
<th>Inexperienced Anesthetist</th>
<th>Experienced Anesthetist</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>32.86 ± 2.20</td>
<td>32.84 ± 2.22</td>
<td>.694</td>
</tr>
<tr>
<td>Duration of tracheal intubation, s</td>
<td>84.88 ± 102.70</td>
<td>96.43 ± 93.05</td>
<td>.955</td>
</tr>
<tr>
<td>Number of tracheal intubation attempts</td>
<td>1.25 ± 0.46</td>
<td>1.14 ± 0.38</td>
<td>.604</td>
</tr>
<tr>
<td>Degree of intubation difficulty</td>
<td>2.38 ± 1.51</td>
<td>3.29 ± 2.56</td>
<td>.875</td>
</tr>
<tr>
<td>Response grade</td>
<td>2.75 ± 1.04</td>
<td>1.86 ± 0.90</td>
<td>.220</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.

**Table 3. Partial Pressure of Oxygen in Arterial Blood at 3 Time Points in the Endotracheal Intubation Groups (Inexperienced and Experienced Anesthetist)**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Partial Pressure of Oxygen in Arterial Blood, mm Hg</th>
<th>Inexperienced Anesthetist</th>
<th>Experienced Anesthetist</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time point 1</td>
<td>87.12 ± 32.582</td>
<td>82.643 ± 9.039</td>
<td>.536</td>
<td></td>
</tr>
<tr>
<td>Time point 2</td>
<td>244.025 ± 58.440</td>
<td>257.786 ± 48.748</td>
<td>.632</td>
<td></td>
</tr>
<tr>
<td>Time point 3</td>
<td>202.250 ± 53.360</td>
<td>211.700 ± 42.775</td>
<td>.714</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.

Discussion

To our knowledge, this is the first study to use NMBAs and LMA to facilitate tracheal intubation in a swine model. Our results showed that (1) there were no significant differences in the degree of intubation difficulty and (2) the duration and rate of endotracheal intubation attempts were similar regardless of operator experience.

In particular, the low score of the degree of intubation difficulty regardless of operator experience showed that NMBAs improved intubating conditions, which are influenced by the degree of muscle relaxation and facilitated endotracheal intubation. NMBAs, which are routinely used for intubation of humans in the operating room, are associated with improved intubating conditions and decreased procedure-related complications. Neuromuscular blocking agents are used for facilitating laryngoscopy and endotracheal intubation during general anesthesia in humans. According to Wilcox and associates, the use of NMBAs can decrease the incidence of hypoxemia and procedure-related complications and improve intubating conditions.

Furthermore, nonuse of NMBAs is associated with an increased risk of difficult tracheal intubation. Therefore, if NMBAs can be used during general anesthesia in swine, difficulties in intubation can be alleviated. Despite the advantages of NMBAs for facilitation of tracheal intubation in humans, these drugs have not been routinely used in pigs because a pig’s anatomic factors, such as the long oropharynx, makes manual mask ventilation in swine very difficult. To overcome difficulties in assisted mask ventilation and prevent hypoxemia, here, we used the LMA device.

Many reports have also strongly recommended LMA use for airway management in pigs because LMA is consistently easier to place and even inexperienced anesthesiologists can reliably secure the airway during spontaneous breathing in pigs. According to Fulkerson and associates, LMA was successfully used with mechanical ventilation in 40- to 50-kg swine for 1 hour, resulting in faster and technically easier maintenance of airways with LMA versus ETT. Wemyss-Holden and associates reported that most swine undergoing general anesthesia do not require endotracheal intubation with specific indications and that LMA use results in the maintenance of spontaneous breathing for up to 6 hours without complications. However, there are some limitations to the use of prolonged mechanical ventilation in pigs. Use of the LMA does not protect against pulmonary aspiration of gastric contents and coughing and laryngospasm occur frequently. In addition, long-duration use of and immobilization with LMA are difficult. Therefore, intubation with ETT is an effective method for providing long-term mechanical ventilation.

This study has a limitation in that the experiments were conducted twice in the same pig. Any airway edema induced by the first experiment may not have subsided, and complete healing of the surgical wound may not have occurred before initiation of the second experiment. In a second attempt of ETT intubation using pig 5, the duration of ETT intubation was 275 s (Figure 1). Despite the second experiment being performed by an experienced anesthesiologist, the duration of intubation attempts was longer and the degree of intubation was more difficult compared with those during the first experiment by the inexperienced anesthesiologist (Table 2).

In summary, this study demonstrated that tracheal intubation in the swine model can be successfully performed using NMBAs and LMA without resulting in hypoxemia, with even anesthesiologists who are
unfamiliar with endotracheal intubation of pigs easily able to do so using our protocol. Therefore, our protocol will enable all investigators to perform successful tracheal intubation in swine models.

References