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A simple and safe method for tracheal intubation using a supraglottic intubation-aid device in mice[☆]

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ABSTRACT

Although mice are a commonly used animal species in experimental medicine, airway management of this species is not easy due to their small size. In order to develop a new method of tracheal intubation in mice, we produced a supraglottic intubation-aid conduit (SIAC) for mice, and tested the efficacy of this device in spontaneously breathing mice anesthetized with sevoflurane inhalation.

The success rate of tracheal intubation with the crude prototype of the SIAC was 50% and adverse effects on respiration and some trauma in the upper airway were occasionally observed. After refining the size and shape of the SIAC, the success rate of tracheal intubation with the refined prototype of the SIAC was 100% without any serious adverse effects.

This study showed that it is possible to produce a supraglottic airway device to aid tracheal intubation in mice and that the shape and size of the SIAC play a crucial role in successful tracheal intubation in mice.

1. Introduction

In the practice of clinical anesthesia, tracheal intubation under direct visualization of upper airway structure is the most common technique for obtaining the safe airway. Although mice are a commonly used animal species in experimental medicine, airway management of this species is not easy due to their small size. Since the pioneer work by Brown et al (1999), several methods for tracheal intubation in mice have been published (Vergari et al., 2003; Spoelstra et al., 2007; Hamacher et al., 2008; Ikeda et al., 2009; MacDonald et al., 2009; Konno et al., 2014; Thomas et al., 2014). Although these methods can be successfully used, there are still unsolved problems of cost and training because the tracheal intubation using these methods is performed essentially by direct or indirect vision of the upper airway structures. These procedures require special and expensive apparatuses such as a surgical microscope (Hamacher et al., 2008; Ikeda et al., 2009), a fiberoptic endoscope (Konno et al., 2014) or an arthroscope (Vergari et al., 2003) together with some training programs. We reasoned that the use of supraglottic airway devices would be the most efficient alternative to direct or indirect visualization of the larynx during tracheal intubation in mice since supraglottic airway devices including the classical laryngeal mask airway (LMA) (Brain, 1985), the intubating laryngeal mask (Brain et al., 1997), the i-gel (Sharma et al., 2007), and the air-Q® (Gaigon et al., 2011) have been used successfully for tracheal intubation in clinical practice for human patients. Although a new type of supraglottic airway device for animals (v-gel) having a soft, gel-like, non-inflatable cuff over the laryngeal area is available now (Crotaz, 2010), there is no v-gel available for mice and there has been no report of tracheal intubation using supraglottic airway devices in mice.

In this paper, we describe a new method of tracheal intubation using a supraglottic airway device which we call the "supraglottic intubation-aid conduit (SIAC)."

2. Materials and methods

The present experiments were performed under the "Guiding Principle for the Care and Use of animals in the field of Physiological Sciences" recommended by the Physiological Society of Japan. All the experimental protocols were approved by the Committee on Animal Research at the University of Chiba.

2.1. Preparation and procedures

The first step in this study is to produce a supraglottic airway device of our own design for mice through which a tracheal tube can be easily

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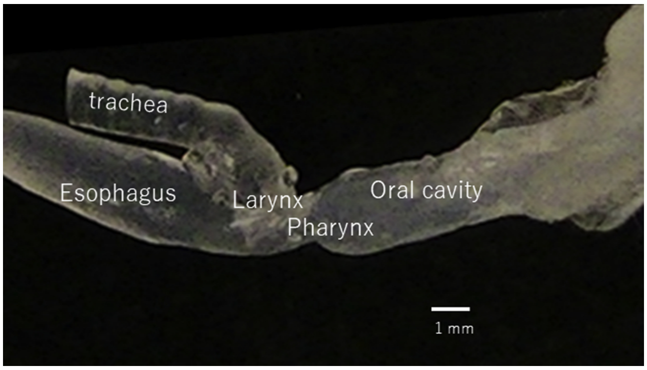

Fig. 1. A mold of the upper airway.

placed into the trachea. Based on the information obtained from a textbook of anatomy for mice (Iwaki et al., 2001) and mice post-mortem studies in which molds of the upper airway were made using a silicon glue agent (1 component RTV, acid cure system, Shin-Etsu Co., Tokyo, Japan) (Fig. 1), the crude supraglottic airway device was fabricated. This supraglottic airway device for tracheal intubation (supraglottic intubation-aid conduit: SIAC) consists of two parts, i.e., a mask and an airway tube, like a laryngeal mask airway (Brain et al., 1985). The prototype of SIAC was made by us under a microscope. The distal part of a Tuohy epidural needle (B. Braun, 16 G) cut to a length of 20 mm was enclosed by a silicon catheter (Fuji systems, 8Fr). This silicon-enclosed pipe served as an airway tube. The distal end of the silicon catheter was cut on the slant and the mask part of the SIAC was constructed on the cut oblique section of the catheter using a silicon glue agent. The mask part of the SIAC for the mouse was designed by taking into consideration the fact that the mouse has a narrow and long oropharyngeal cavity. Therefore, the contour of the mask formed a long, narrow ellipse. In addition, the tip of the distal end of the tube was slightly curved since a tracheal tube was expected to move smoothly into the trachea from the distal end of the tube with an approach angle of 5–20 degrees when it was inserted from the proximal end of airway tube. Initially, we made 30 crude prototypes of the SIAC with different sizes, different shapes and different elevation angles. In the overall design, the mask part of the SIAC bears a resemblance to a wedge-shaped, elliptical doughnut when viewed from an oblique perspective (Fig. 2a). These crude prototypes of the SIAC were tested in anesthetized mice in the first study (Study I). Then, considering the results of Study I, we refined the sizes, shapes and approach angles of the SIACs and made 28 refined prototypes of the SIAC. We tested again the efficiency of these SIACs in the second study (Study II). In order to define the size and shape of the SIAC, the maximum length, the maximum width, the inner and outer cross sectional areas, the vertical thickness, and the wedge angle of the mask of each SIAC as well as the approach angle of the tracheal tube were measured (Fig. 2b-d). These measurements were done after obtaining photo images of the SIACs and analyzing these images using an image analysis software (ImageJ: National Institutes of Health, Bethesda, MD).

2.2. Anesthesia and tracheal intubation

Thirty-eight (15 males and 23 females) C57BL/6 inbred mice, aged 30–130 weeks and weighing between 25 and 40 g (Harlan, The Netherlands) were prepared for tracheal intubation trials. The animal was placed in a transparent plastic chamber (200 ml) and anesthetized with the gas mixture of 5% sevoflurane in O_2 delivered into the chamber at a flow rate of 0.5–1 L/min. Immediately after the animal was immobilized, the animal was taken out of the anesthesia induction chamber and transferred to a cylinder-shaped anesthesia facemask to obtain a relatively deep level of anesthesia with inhalation of 5% sevoflurane in O_2. When the level of anesthesia was relatively deep (defined as a spontaneous respiratory rate of less than 60 breaths/min), the anesthesia facemask was removed, and the intubation procedures were started. The intubation procedures were performed mainly by two authors of this paper (Y. J. & T. N.). Our procedure of tracheal intubation consisted of three steps. The first step was to insert an SIAC into the oropharyngeal cavity. Before insertion of the SIAC, each animal was positioned in the supine position with the head and neck extended and then the mouth was opened wide with tweezers. Then, a lubricated SIAC was inserted until either clear resistance was encountered or the distance from the top of the SIAC to the frontal incisors reached 18–19 mm. When spontaneous breathing stopped suddenly, and/or lingual cyanosis occurred, the SIAC was moved back 1 or 2 mm, and if spontaneous breathing recovered immediately with improvement of lingual cyanosis, the position of the SIAC was judged to be correct. The second step was to insert a tracheal tube through the proximal end of the SIAC conduit. Thus, either a 20-G or 22-G intravenous catheter as a tracheal tube was inserted through the conduit and advanced slowly. When clear resistance was felt, the position of the SIAC was moved slightly back and forth and tracheal intubation was tried again. The third step was a confirmation of tracheal intubation. The proper tracheal intubation was confirmed by inflating the lung with a 1ml-dropper (lung inflating bulb). All three of these steps have to be completed within two minutes, otherwise the animal awakens out of anesthesia which makes it impossible to continue the intubation procedures. For the insertion of each SIAC, the number of attempts required to achieve tracheal intubation was counted while the maximum number of intubation attempts was limited to three attempts. Failure to intubate was defined as the inability to place the tracheal tube into the trachea after three attempts or when the first or the second step of the intubation procedures caused irrecoverable damage to the animals.

After confirmation of successful tracheal intubation, the animal was allowed either to breathe spontaneously through the T-piece anesthetic circuit or the animal was connected to a ventilator (SAR-830 ventilator, CWE Inc., Ardmore, PA, USA) and artificially ventilated with a rate of 150 bpm and tidal volume of 0.15-0.25 ml for 10–120 min while the anesthesia was maintained with 2–3% sevoflurane in O_2. Thereafter, inhalation of sevoflurane was discontinued and the animal was extubated and allowed to recover in a warm box. In some animals, tracheal intubation trials with different SIACs were repeated after six to eight weeks to verify if different SIACs yielded the same results. Some mice were sacrificed and a post-mortem examination was performed. All of the animals except those that were sacrificed for anatomical studies were observed for the following three days for signs of post-intubation complications including respiratory distress.

2.3. Statistical analysis

Commercial software was used for statistical analyses (SigmaPlot 11.2, Systat Software, Inc., San Jose, CA). The data are reported as mean ± SD, and statistical analysis was performed by the unpaired *t*-test when data were normally distributed. The nominal data were analyzed using a chi-square contingency table. When the total number of observations was too small for chi-square distribution, Fisher's exact test was used. $P < 0.05$ was considered significant.

3. Results

3.1. Study I

Of 30 crude prototypes of the SIAC in Study I, six SIAC prototypes were too large to place into the oropharyngeal cavity and thus tracheal intubation was not attempted with these SIACs. Tracheal intubation was attempted with the rest of the SIACs. However, soon we noticed that immediately after insertion, some of these SIACs which had a relatively long and large mask caused adverse responses such as a sudden cessation of spontaneous respiration and/or a sudden elicitation of

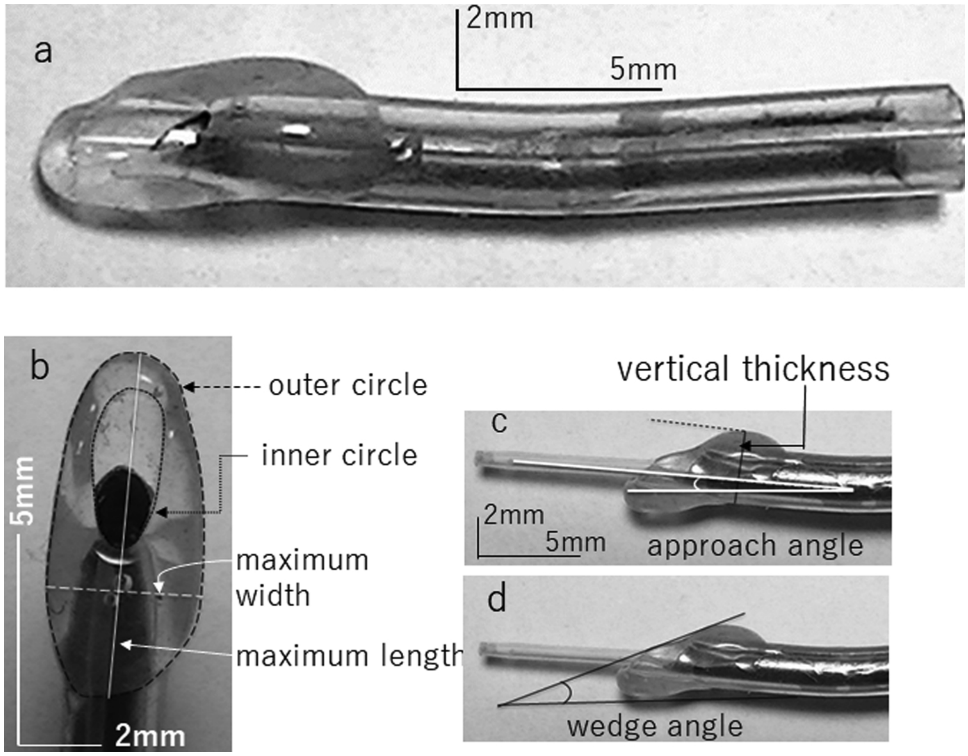

Fig. 2. SIAC crude prototype.
a: an oblique lateral view.
b-d: measurements of various parts of SIAC mask.
Outer cross sectional area is an area within the outer circle and inner cross sectional area is an area within the inner circle, respectively.

Table 1
Comparison of size and shape data between the success group and the failure group of SIAC crude prototype.

	Success group	Failure group
Maximum width (mm)	3.3 ± 0.3*	3.7 ± 0.4
Maximum length (mm)	7.6 ± 1.8	8.5 ± 2.1
Inner cross-sectional area (mm^2)	3.4 ± 1.5*	6.6 ± 4.0
Outer cross-sectional area (mm^2)	25.2 ± 7.1*	33.6 ± 10.3
Vertical thickness (mm)	3.8 ± 0.3	4.1 ± 0.4
Approach angle (°)	10.8 ± 2.8*	8.8 ± 1.7
Wedge angle (°)	21.3 ± 5.7	21.8 ± 3.8

* $p < 0.05$.

ataxic respiration. In fact, two animals died after the insertion of the SIACs into the oropharyngeal cavity even though these SIACs were immediately removed from the oropharyngeal cavity. In Study I, the overall success rate of tracheal intubation was 50%. Of all of these successful intubations, 58% were successful with one attempt, 33% with two attempts, and 9% with three attempts. The main cause of failure appeared to be due to esophageal intubation but the cause of failure in two SIAC insertions was the death of animals. Then, the tested SIACs were assigned into two groups, i.e., the intubation success group and the intubation failure group. Table 1 summarizes the shapes and sizes of the two different SIAC groups. There are significant differences between the success group and the failure group in the maximum width, the inner cross sectional area of the mask, the outer cross sectional area of the mask and the approach angle of the tracheal tube. Table 2 shows the major complications observed during the tracheal intubation procedures. The SIACs in the failure group tended to be larger than those in the success group. These results suggest that the shape and size of the SIAC may play a crucial role in successful tracheal intubation in mice.

3.2. Study II

Bearing these results in mind, the shapes and sizes of the mask of the SIAC were redesigned and the refined prototypes of the SIAC were constructed so that a smooth insertion of the tracheal tube could be achieved without any adverse responses. The main improvements were as follows: 1) the width of the mask was narrowed, 2) the length of the mask was shortened, 3) the wedge angle and approach angle of the mask were increased, and 4) horizontal wings were added to the tube of the SIAC to stabilize its position in the oropharyngeal cavity. The sample size was calculated based on the results of Study I in which the success rate was 50% when 24 SIACs were tested. Assuming that the refinements of the SIAC would increase the success rate from 50% to 90% with an α risk of 0.05 and a power of 0.9, the sample size of 28 would be adequate to detect statistically significant results. In fact, after

Table 2
Complications observed during endotracheal intubation procedures.

	success group (n = 12)	failure group (n = 12)
upper airway mucosal trauma	0	2
respiratory arrest and/ataxic breathing	1	7*
death	0	2

$P < 0.05$, Fisher's exact test.

Table 3
Comparison of size and shape data between SIAC crude prototypes and SIAC refined prototypes.

	Crude prototype (n = 24)	Refined prototype (n = 28)
Maximum width (mm)	3.5 ± 0.4	3.1 ± 0.3$^{\#}$
Maximum length (mm)	8.0 ± 2.0	4.4 ± 0.8$^{\#}$
Inner cross-sectional area (mm^2)	5.0 ± 3.3	1.3 ± 0.4$^{\#}$
Outer cross-sectional area (mm^2)	29.4 ± 9.6	12.6 ± 1.9$^{\#}$
Vertical thickness (mm)	4.0 ± 0.5	3.3 ± 0.2*
Approach angle (°)	9.8 ± 2.5	11.3 ± 3.6
Wedge angle (°)	21.5 ± 4.7	43.2 ± 8.5$^{\#}$

* $p < 0.05$.
\# $p < 0.01$.

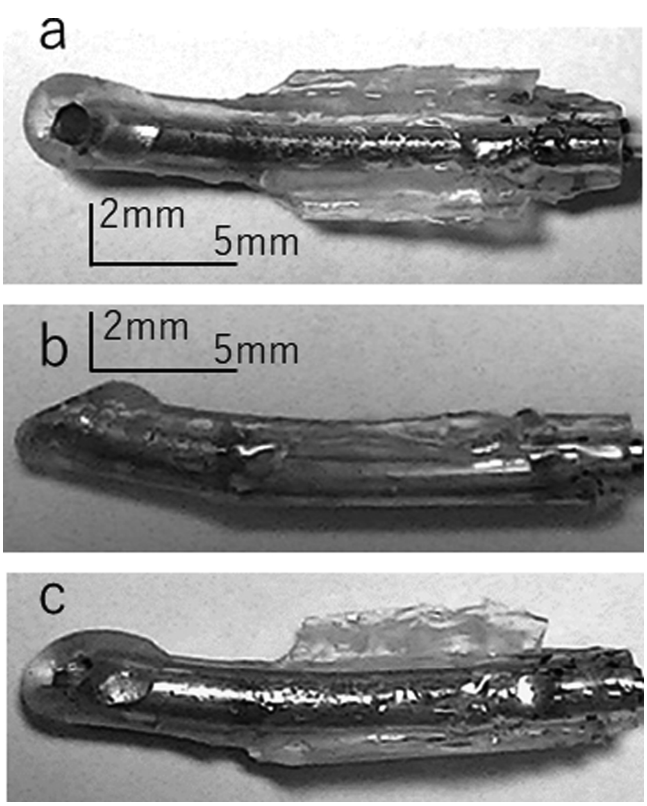

Fig. 3. SIAC refined prototype.
a.overhead view; b. lateral view; c. oblique view.

the refinements of the SIAC, the success rate for tracheal intubation increased dramatically from 50% to 100%. Of these successful intubations, 82% were successful with one attempt and 18% with two attempts. The size and shape of the refined prototypes of the SIAC are shown in Table 3 and in Fig. 3. Compared to the size and shape of the crude SIAC prototype, the mask size of the refined SIAC prototype is much smaller and the shape is more rounded.

Two mice died on the third day after intubation in Study I. Since both mice were quite old (125 weeks and 130 weeks, respectively), it was not clear whether the tracheal intubation or their age was involved in their deaths. In addition in Study I, in two different mice traces of blood were observed on the masks of the SIACs. This was probably due to slight mucosal bleeding of the upper airway. These mice started to squeak immediately after the experiments and showed a loss of appetite and a slight weight loss. However, these symptoms subsided in a few days. No major complications were observed after Study II and no animal showed any signs of respiratory distress.

4. Discussion

4.1. Major findings

We demonstrated that the tracheal intubation without the need of direct visualization of the larynx in mice can be performed easily, rapidly, and safely using a proper size of an SIAC. The success rate of tracheal intubation with the refined prototypes of the SIAC was 100%. Although we did not measure the exact duration of the intubation procedures, the successful intubation was done within one minute after the start of intubation procedure in most of the trials. This duration is within the allowable range since it takes usually two minutes for mice anesthetized with only 5% sevoflurane to awaken from the anesthetic. The duration of time required for tracheal intubation may not be an important issue when the mice are anesthetized with intraperitoneally-injected anesthetic agents such as ketamine, xylazine, medetomidine or midazoram (Brown et al., 1999; Vergari et al., 2003; Hamacher et al., 2008; Ikeda et al., 2009 ; MacDonald et al., 2009; Thomas et al., 2014). However, the use of various injected anesthetic agents can be a confounding variable in pharmacological or physiological studies in which interactions between injected and inhaled agents or residual effects of injected agents may influence the study results. In this study, we anesthetized animals only with inhalation of sevoflurane. Although the use of relatively older inhalational anesthetics such as halothane and isoflurane has been reported (Spoelstra et al., 2007; Hamacher et al., 2008), the solubility of sevoflurane in blood is much lower than those of isoflurane and halothane and thus sevoflurane causes more rapid induction and faster recovery from anesthesia compared to halothane and isoflurane (Forman and Ishizawa, 2014).

4.2. Adverse and beneficial effects in the use of the SIAC

The use of the SIAC was not without potential hazards. In fact, two animals died during insertion of crude SIAC prototypes due to a sudden cessation of spontaneous breathing including tongue cyanosis. Although the mechanisms of this sudden death are unknown, we speculate that the sudden cessation of spontaneous breathing might be due to a reflex type of response since a slight backward movement of the SIAC or the removal of the SIAC from the oropharyngeal cavity usually resulted in an immediate improvement of the situation. With regard to this, several studies have shown that distension of the esophagus in anesthetized animals causes various reflexes to occur such as the esophago-glottal closure reflex and the diaphragm inhibitory reflex (DeTroyer and Rousso, 1982; Cherniack et al., 1984; Shaker et al., 1992, 1994). The sudden cessation of spontaneous breathing observed in our study might be related to the exaggeration of these reflexes in mice. It is also worthwhile to note that the size of the mask of the SIAC plays a crucial role in eliciting these reflex types of responses since the mask size in the failure SIAC group was bigger than that in the success SIAC group (Table 1).

In contrast with most of the previous studies of tracheal intubation of mice in which tracheal intubation was conducted while the animals were suspended on a tilted plastic support (Brown et al., 1999; Spoelstra et al., 2007; Ikeda et al., 2009; MacDonald et al., 2009) or hung perpendicularly (Hamacher et al., 2008; Thomas et al., 2014), in this study tracheal intubation was conducted in the supine position. The supine position has advantages over the suspended position with respect to monitoring of vital signs during the intubation procedure. Certainly, the sudden cessation of spontaneous breathing can be detected immediately in the supine position when it occurs during the intubation procedure.

4.3. Refinements and future perspectives of the SIAC

The general wedge-shape of the crude SIAC prototype somewhat resembles that of the v-gel for small animals such as the cat and rabbit although the size of the mouse is much smaller than the size of these animals. However, unlike the v-gel for small animals, the refined SIAC does not have a tight esophageal seal. Although the SIAC with a non-inflatable silicon cuff was designed anatomically to fit perilaryngeal structures, we did not seek an effective seal against the larynx and the esophagus because we believed that unlike various supraglottic airway devices, the SIAC does not necessarily need to form a tight seal around the glottic inlet and the esophagus, but still provides a clear airway for intubation. Most of the supraglottic airway devices use either an inflatable cuff or a non-inflatable soft cuff to wedge into the upper esophagus to obtain an efficient seal and a good stability. However, due to its small size it seems to be unrealistic and unnecessary for the mask of the SIAC to incorporate such an inflatable cuff.

We also reasoned that a deep insertion of the SIAC may stimulate the sensory receptors of the upper esophagus and may thereby frequently cause adverse reflex effects in mice. This suggests that a very tight seal of the cervical esophagus may not be desirable in mice. Accordingly, the distal section of the mask of the refined SIAC has a blunted tip with a steep wedge angle rather than a sharp and prolonged tip with a gentle wedge angle which prevents the tip of the SIAC from wedging tightly into the cervical esophagus. Thus, compared with the prolonged face of the crude SIAC, the refined SIAC has a more rounded face. It is worthwhile to mention that the crude SIAC was designed anatomically to fit not only the peripharyngeal structures but also the hypopharyngeal and the upper esophageal structures whereas the refined SIAC was designed anatomically to fit mainly the peripharyngeal structures. It may be possible that if we are able to refine the design of the SIAC further in order to obtain an effective seal against the larynx and the esophagus, the SIAC could be used as an intubation laryngeal mask airway. However, in order to get an effective seal around perilaryngeal structures, either a special type of tiny inflatable cuff or a non-inflatable soft cuff made of materials giving a gas-tight seal such as is used in i-gel or v-gel is definitely needed.

4.4. Conclusion

In summary, we described in detail a new method of tracheal intubation in mice using a SIAC. We showed that this new method is very simple, safe, and effective. In addition, this new method has an advantage over previously reported methods of tracheal intubation in mice in terms of cost.

Conflict of interests

The authors state that there are no conflicts of interest regarding this work.

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