

## Visible, Safe and Certain Endotracheal Intubation Using Endoscope System and Inhalation Anesthesia for Rats

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**ABSTRACT.** Anesthesia strongly influences laboratory animals, and it can also greatly affect the experimental data. Rats rank only second to mice in the number used in research fields, such as organ transplantation, regenerative medicine and imaging. Therefore, appropriate and effective anesthesia, including the protocol of the endotracheal intubation and inhalation anesthesia, is crucial. Hence, we evaluated these methods in this study. Twelve Wistar rats were intraperitoneally injected with M/M/B: 0.3/4/5, comprising of medetomidine, midazolam and butorphanol at a dose of 0.3 mg/kg + 4.0 mg/kg + 5.0 mg/kg body weight/rat, respectively. An endotracheal tube was then intubated into the trachea. After intubation, the rats were connected to the inhalation anesthesia circuit using isoflurane, and vital signs were measured until 30 min after connection. All intubations were successfully finished within 1 min, and the values of the vital signs were normal and stable. In addition, histopathological observation of the trachea and lungs showed no trauma. These results suggest that this visible endotracheal intubation method is simple, reliable, safe and favorable with regard to the rats' welfare.

**KEY WORDS:** 3Rs, anesthesia, endoscope, intubation, rat

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Laboratory animal science is generally performed considering the extrapolation of results to humans as the main purpose. But, in many laboratory experiments, the methods of general anesthesia are different than those in humans; inhalation anesthesia is predominantly used for humans and large experimental animals, whereas injectable anesthesia is mainly used for small experimental animals. Anesthesia strongly influences the body of laboratory animals, and it can also greatly affect the experimental data, particularly the vital signs, such as heart rate, breath rate, SpO<sub>2</sub> and blood pressure. Therefore, appropriate and effective anesthetic methods are important for reliable laboratory experimentation [9–11, 15, 26, 32]. Few studies have reported on the endotracheal intubation for small laboratory animals; mice [3, 5, 13, 20, 21, 26, 29, 30], rats [4, 12, 17, 24, 27] and guinea pigs [5]. However, endotracheal intubation is not easy in these small animals, and they require comparatively skillful techniques and special equipment. Furthermore, it is

difficult to confirm whether the intubation is successful or not. Therefore, we progressed further by focusing on clearly showing whether performing endotracheal intubation using the endoscopic technology and inhalation anesthesia is effective for mice. Our results indicate that this new endotracheal intubation method was simple, reliable and favorable with regard to animals' welfare [20]. However, some points requiring improvement were also observed during the process of the experiment.

Rats and mice, used for laboratory experiments, together form more than 90% of all mammalian species, and rats rank second only to mice in number used in biomedical research including imaging [1, 2, 25, 28] and long-time surgery [14, 16, 22, 33].

Therefore, in this experiment, we investigated the effectiveness of endotracheal intubation protocols using endoscopic technology and improvements of the inhalation anesthesia method for rats.

### MATERIALS AND METHODS

**Animals and housing conditions:** Six male Wistar rats [252.8 ± 11.0 g body weight (b.w.); mean ± standard deviation (SD)] and six females (183.7 ± 5.2 g b.w.) were purchased from Clea Japan Inc. (Tokyo, Japan). Animal care and experimental procedures were approved by the Kyoto Sangyo University Committee for Animal Care and Welfare.

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**Endotracheal Intubation:** Premedication and endotracheal intubation for rats were performed using similar materials and methods to those previously described in mice [20]. The protocol is briefly described below.

For the purpose of sedation, the rats were put into a chamber and exposed to a 5% concentration of isoflurane (Escain®, Mylan Seiyaku, Osaka, Japan) for 1 min, and room air was used as a carrier gas. After sedation and to intubate the endotracheal tube, a mixture, named “M/M/B: 0.3/4/5” and described by Kawai *et al.* [18] and Kiriwara *et al.* [19], was intraperitoneally injected as an anesthetic at a dose of 0.3 mg/kg b.w. of medetomidine (Domitor®, Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan), 4.0 mg/kg b.w. of midazolam (Dormicum®, Astellas Pharma Inc., Tokyo, Japan) and 5.0 mg/kg b.w. of butorphanol (Vetorphale®, Meiji Seika Kaisha, Ltd., Tokyo, Japan) as premedication. Atropine sulfate (Atropine sulfate Injection 0.5 mg, Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan) diluted up to 10 times with sterilized distilled water was also intraperitoneally injected at a dose of 0.04 mg/kg b.w. as a vagal block.

Endotracheal intubation of a 16G and 2 inch long indwelling needle (outer diameter: 1.70 mm, inner diameter: 1.30 mm; SR-FF1651, Terumo Corporation, Tokyo, Japan) was performed using the endoscope system, “Tesala AE-C1” (AVS Co., Ltd., Tokyo, Japan, <http://www.avs.co.jp/animal/tesala/tesala3.html>). The endoscope probe AE-F07070 was used for capturing images and as a substitute stylet.

After the intraperitoneal injection of both M/M/B: 0.3/4/5 and diluted atropine sulfate, the sedated rats were placed on the intubation stand, and then, a laryngoscope was used to guide the insertion of the endotracheal tube. Before intubation, to avoid trauma, it was necessary to confirm if the vocal cords were open using the endoscope. When the vocal cords were closed by the epiglottis, the rat's glottis was opened by pressing the chest and/or by lifting up the epiglottis, or by pushing the soft plate gently using the tip of the probe. After successful intubation, only the probe was removed, and the tube was fixed to a position on the upper jaw using a 3–0 silk suture.

**Inhalation anesthesia for the rat using a ventilator:** Narcobit II (Natsume Seisakusho, Co., Ltd., Tokyo, Japan) was used both as a vaporizer and as an anesthetic circuit, and SLA Ventilator (Natsume Seisakusho, Co., Ltd.) was used as a ventilator. Room air was used as a carrier gas, and the ventilator was set as follows:  $250 \pm 10$  ml flow volume/min for male and  $190 \pm 10$  ml flow volume/min for female, 70 breaths/min and 50% I/I + E (I: intake, E: exhaust). Airway pressure in the anesthetic circuit was continuously monitored, and the alarm was set to sound when the pressure exceeded 1,600 pa [2, 7, 13, 21, 31]. Vital signs were continuously measured and recorded using the MouseOx Plus® (Starr Life Sciences Corp. Pittsburgh, PA, U.S.A.). A foot clip sensor was placed over the dorsal artery of foot, and the values of SpO<sub>2</sub>, heart rate (HR, beat/min) and respiratory rate (RR, breath/min) were measured and recorded. A probe was used to measure the temperature of the rectum, and the value of the body temperature (BT) was also measured and recorded. Inhalation anesthesia was carried out using isoflurane. To control the

spontaneous breathing of the rats, isoflurane was maintained at 3.0% concentration for males and at 2.5% for females following the connection of the anesthetic circuit for a period of 5 min. Subsequently, the concentration of isoflurane was decreased to 2.5% for males and 2.0% for females until the anesthesia was stopped [10, 11, 15, 32]. Atipamezole (antisedan®, Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan) is an antagonist to medetomidine and was intraperitoneally injected at a dose of 1.5 mg/kg b.w. immediately after the connection with the anesthetic circuit. During the anesthesia, all rats were warmed on a hot plate.

**Evaluations of the endotracheal intubation and inhalation anesthesia:** After the anesthesia, the inner surfaces of the trachea were observed using the endoscope. After 5% isoflurane anesthesia for euthanasia, the tracheas and lungs were macroscopically observed at autopsy. These organs were collected and fixed for 24 hr in 4% paraformaldehyde/phosphate buffer solution. They were dehydrated in graded alcohol and embedded in the PathoPrep 568 (Wako Pure Chemical Industries, Ltd., Osaka, Japan). For the histological analysis, the sections were stained with hematoxylin and eosin solution and evaluated using a light microscope.

**Statistical analysis:** Statistical analysis was conducted using the JMP software (SAS Institute Inc., Cary, NC, U.S.A.). Differences on SpO<sub>2</sub>, HR, RR and gender between male and female were analyzed using the unpaired student's *t*. A *P* value less than 0.05 was considered to be statistically significant.

## RESULTS

The endoscopic figures of the rats' endotracheal intubation process from the TESALA AC-1 are shown in Fig. 1. During the endotracheal intubation, the probe covered with the endotracheal tube was inserted into the larynx, and the images of a closed or opened epiglottis were shown on the monitor of the TESALA AE-C1 system (Fig. 1A and B). The opened epiglottis could be confirmed by moving the vocal cord according to the breath, making it easier to insert the tube into the trachea (Fig. 1B and C). When the epiglottis was closed (Fig. 1A), it was difficult to insert the tube. However, it could be opened by pressing the rat's chest and/or by lifting up the epiglottis, and/or by pushing the soft plate gently using the tip of the probe.

After the endotracheal tube was intubated through the opened glottis, the inner lining of the trachea could be viewed as the shape of bellows (Fig. 1C and D). Furthermore, the smooth and flat image indicated an incorrect intubation into the esophagus (Fig. 1E).

Results of the endotracheal intubation are shown in Table 1. All rats intraperitoneally injected with M/M/B: 0.3/4/5 and diluted atropine were successfully treated with the endotracheal intubation without respiratory trouble and connected to the anesthetic circuit. The time for successful intubation was 12–45 sec (mean  $\pm$  SD of all rats, males and females were  $22.1 \pm 10.8$  sec,  $28.0 \pm 12.6$  sec and  $16.2 \pm 3.7$  sec, respectively). There was almost significant difference in the time taken for successful intubation between males and

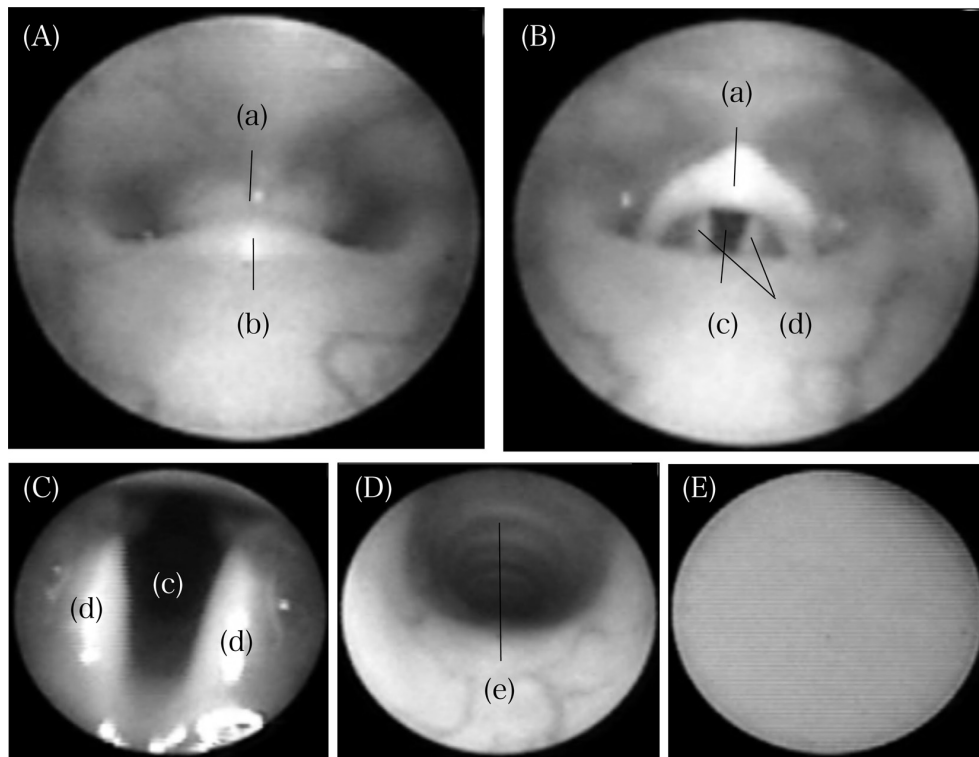


Fig. 1. Images of the oral cavity, trachea and esophagus from TESALA AE-C1 in rats.

(A) Posterosuperior view of the larynx with closed epiglottis<distal> (B) Posterosuperior view of larynx with opened epiglottis<distal> (C) Posterosuperior view of larynx with opened epiglottis<proximal> (D) Inside view of the trachea (E). View of incorrect intubation into the esophagus (a) epiglottis, (b) soft plate, (c) glottis, (d) arytenoid cartilage and (e) epiglottis. (A) was common before the endotracheal intubation, (B) must be observed before tracheal intubation to avoid trauma, (C) was the view around the glottis with the epiglottis open and (D) was inside the trachea and inner lining of the trachea. These should be confirmed before intubation for a simple, reliable, safe and favorable method with regard to the animals' welfare. (E) was confirmed when the probe was mis-inserted into the esophagus.

Table 1. Result of the rat tracheal intubation using TESALA

ID	Body Weight (g)	Sex	Time until success of intubation (sec)	Existence of troubles after intubation
M1	237	♂	27	Success of the tracheal intubation without trouble
M2	259	♂	45	Success of the tracheal intubation without trouble
M3	260	♂	22	Success of the tracheal intubation without trouble
M4	254	♂	41	Success of the tracheal intubation without trouble
M5	242	♂	12	Success of the tracheal intubation without trouble
M6	265	♂	21	Success of the tracheal intubation without trouble
F1	181	♀	12	Success of the tracheal intubation without trouble
F2	178	♀	20	Success of the tracheal intubation without trouble
F3	178	♀	20	Success of the tracheal intubation without trouble
F4	189	♀	12	Success of the tracheal intubation without trouble
F5	188	♀	15	Success of the tracheal intubation without trouble
F6	188	♀	18	Success of the tracheal intubation without trouble

M/M/B: 0.3/4/5 was composed of medetomidine (0.3 mg/kg) + midazolam (4.0 mg/kg) + butorphanol (5.0 mg/kg), respectively. M/M/B: 0.3/4/5 was intraperitoneally injected as a premedication. Atropine sulfate, diluted up to 10 times with sterilized distilled water, was also intraperitoneally injected at a dose of 0.04 mg/kg body weight (b.w.) for the vagal block. Time taken for successful intubation was 12–45 sec [mean  $\pm$  standard deviation (SD) of all rats, males and females: 22.1  $\pm$  10.8 sec, 28.0  $\pm$  12.6 sec and 16.2  $\pm$  3.7 sec, respectively). No respiratory trouble occurred during the endotracheal intubation of the rats.

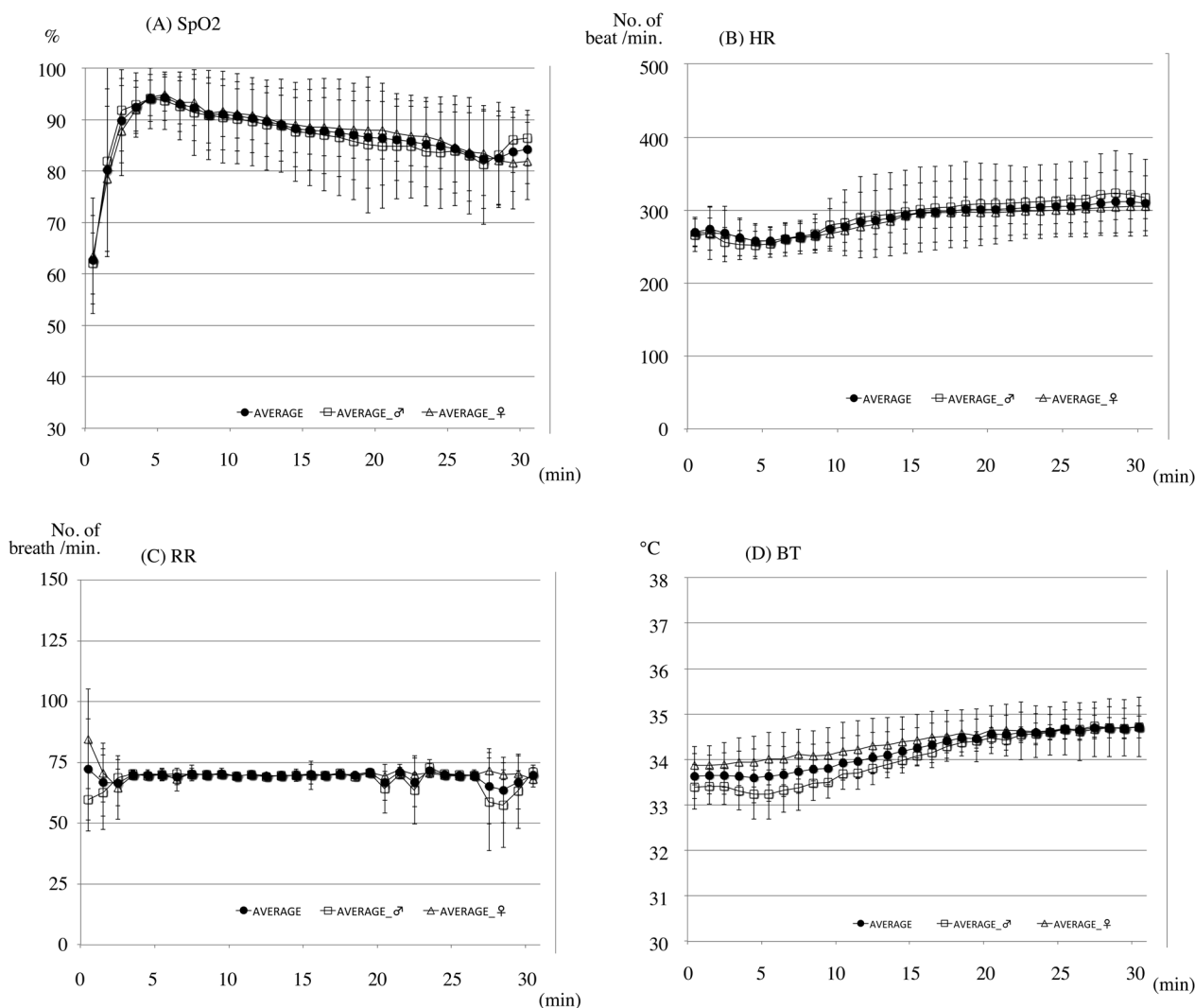


Fig. 2. Vital signs during inhalation anesthesia of endotracheal-intubated rats intraperitoneally injected with M/M/B: 0.3/4/5 and atropine sulfate.

(A) SpO<sub>2</sub>; arterial oxygen saturation (%); The values of SpO<sub>2</sub> reached up to 90% or more within 3 min after the connection of the anesthetic circuit. (B) HR; heart rate (beat/min); The values of HR were stable ( $288.6 \pm 18.1$  beat/min). (C) RR; respiratory rate (breath/min); The values of RR were stable ( $69.2 \pm 1.9$  breath/min). (D) BT; body temperature (°C); Although there was a difference between males and females in the values of BT after the connection of the anesthetic circuit, the difference decreased with time.

females ( $P=0.052$ ).

The results of vital signs measured are shown in Fig. 2. In all rats, the SpO<sub>2</sub> values reached up to 90% or more within 3 min after the connection to the anesthetic circuit was established; the values then gradually reduced over time until 30 min. The values of both HR and RR were stable for 30 min after the connection to the circuit ( $288.6 \pm 18.1$  beat/min and  $69.2 \pm 1.9$  breath/min). The values of RR were almost the same as the preset value (70 breath/min) set by the ventilator. Although a difference between male and female BT values was observed, the difference almost disappeared at 20 min or more after the connection to the anesthetic circuit. No difference on SpO<sub>2</sub>, HR and RR between male and female was found.

No trauma was observed in the inner surface of the trachea using the endoscope (Fig. 1D). In addition, no problem was observed in the macroscopic observation of both the tracheal and pulmonary tissues at autopsy. Histopathological evaluation of the tissues also revealed no problems, such as the pleural effusion, pulmonary edema, emphysema and dilatation of the tracheobronchial tree. The alveoli, respiratory bronchus and bronchioles of the mechanically ventilated rats did not show signs related to the intubation technique or mechanical ventilation (Fig. 3).



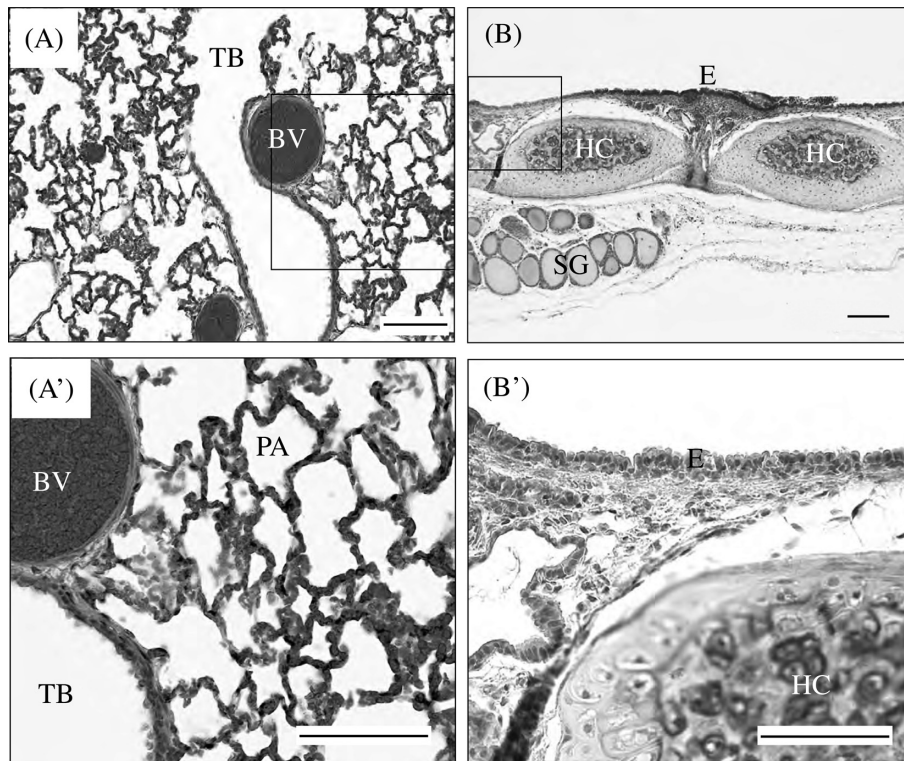


Fig. 3. Histopathological evaluation of the alveolar and tracheal tissues of the intubated rat. (A) and (A'): alveolar tissue of the rat, low and high magnification. (B) and (B'): tracheal tissue of the rat, low and high magnification. (A') and (B') are the magnified square domains in (A) or (B). TB: terminal bronchiole, PA: pulmonary alveolus, BV: blood vessel, HC: hyaline cartilage, E: epithelium, SG: seromucous gland. The alveoli, respiratory bronchus and bronchioles of the mechanically ventilated rats did not show signs of trauma related to the intubation technique or mechanical ventilation. Alveolar and tracheal tissues showed normal morphology. Pleural effusions, pulmonary edema, emphysema and dilatation of the tracheobronchial tree were not observed. Hematoxylin and eosin stain, scale bar=100  $\mu$ m

## DISCUSSION

We have previously showed that mouse endotracheal intubation using endoscopic technology was simple, reliable, safe and favorable with regard to animal welfare [20]. However, we also observed some points that could be more practically improved. Although rats rank second only to mice in number used in biomedical research and share many of the research purposes and the attributes of mice, they are commonly used in research fields, such as an organ transplantation [14, 16, 22, 23, 33], regenerative medicine [22, 23, 33] and imaging [1, 2, 25, 28], because rats' body size is larger than that of mice. Therefore, in this experiment, we investigated both the effectiveness of endotracheal intubation in rats using endoscopic technology and improvements of inhalation anesthesia using isoflurane for rats.

The reason for using the M/M/B: 0.3/4/5 of the same composition as the mice was to achieve endotracheal intubation as rapidly and precisely as possible. Apprehensions on the use of medetomidine, which suppresses breathing in mice, were groundless with no unexpected fluctuations in vital signs being observed following the use of M/M/B: 0.3/4/5

[10, 32]. Successful endotracheal intubation was achieved in a time equivalent to or a little faster than the results in mice. In addition, no intubation damage was macroscopically, endoscopically and histopathologically observed. Atipamezole is an antagonist to medetomidine. Therefore, this can reverse rat very rapidly and also has few side effects [10, 11, 15, 32]. When not neutralizing M/M/B, SpO<sub>2</sub> value does not fully rise (the data is not shown). Therefore, it was intraperitoneally injected at a dose of 1.5 mg/kg b.w immediately after the connection with the anesthetic circuit. Great fluctuation or the unexpected value caused using M/M/B: 0.3/4/5 was not observed on the vital sign. These results suggest that M/M/B: 0.3/4/5 is suitable as a premedication for the endotracheal intubation of rats, and atipamezole, a medetomidine antagonist, may greatly contribute to the stability of vital signs.

In general anesthesia for human, except for some exceptions, pure oxygen is not used, and partial pressure of oxygen is mainly used at 30–40% in recent years [31]. However, the anesthesia device with the function to regulate the partial pressure of oxygen is expensive. Therefore, we changed the carrier gas from pure oxygen (100%) to room air (20%), which might also make the values of SpO<sub>2</sub>, HR, RR and BT

stable. Therefore, room air might be able to be called the carrier gas candidate now, and the further examination is required on the partial pressure of oxygen.

Macroscopic observations were performed at the autopsy, and the results indicated no abnormal findings in the tracheas or lungs. In addition, histopathological observation of the respiratory organs of the mechanically ventilated rats did not reveal signs of trauma related to the intubation technique or mechanical ventilation. These results suggest that the equipments and their settings used in this experiment were practical and safe for rats, although the protocols corresponding to various situations, including open heart surgery, long-term surgery and imaging, should be necessary [6–8].

In this experiment, we demonstrated an easy, quick and safe endotracheal intubation method for rats using an endoscope system with M/M/B: 0.3/4/5 as premedication and inhalation anesthesia using isoflurane and room air. The widespread use of this method would benefit not only research data but also animal welfare.

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