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Improved blind tracheal intubation in rats: a simple and secure approach

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ABSTRACT. Successful tracheal intubation is the prerequisite for open-chest models. Tracheal intubation in small animal such as the rat is often challenging due to the small size and special anatomy. We investigated whether endotracheal intubation can be performed safely and reliably in rats employing only gesture fixation and a catheter. Rats were randomly classified into three groups: Improved blind intubation (B group) was performed with gestures fixed intubation position. Transillumination intubation (T group) utilized light to locate the larynx. Incision intubation (I group) was intubated after trachea incision. The feasibility, difficulty, complications of the three groups were compared. B group was faster than the other two groups. Completion time of the operation was recorded as follows: B group: 35.00 ± 9.86 sec; T group: 57.12 ± 6.54 sec; I group: 184.33 ± 25.49 sec ($P \leq 0.001$). B group has fewer attempts than Group T ($P=0.001$). The operational success rates of all three groups (B group 14 (93.3%) vs. T group 12 (80.0%) vs. I group 13 (86.7%)) were similar ($P>0.05$). In terms of operation difficult and operational complications, the differences between the three groups were not significant. The rate of endometrial damage under microscope were no difference, too. The Improved blind endotracheal intubation is a simple method, with a comparable safety profile to that of the transillumination and incision intubaton.

KEY WORDS: laboratory animal, rat, tracheal intubation

Rats are standard experimental animals and are widely used in both fundamental, and translational experimentation to answer scientific questions. [13, 18, 20]. For example, in acute myocardial infarction, coronary microembolization, intratracheal administration, thymus surgery and other models that require thoracotomy, tracheal intubation is required to connect to the ventilator to maintain airway patency and blood oxygen exchange [10, 11, 23, 24]. Successful rat tracheal intubation is a prerequisite for artificial pulmonary ventilation. However, due to the rapid respiratory rate, narrow mouth, and high glottal position, it is difficult to expose the glottis during intubation [16]. Lack of exposure of glottis may easily lead to concomitant throat, laryngeal oedema, tracheal perforation, haemorrhage, and excessive secretion of obstruction of the trachea [5]. The structural complication impacts the operational procedure and mounts challenges to the operator. Over the years, several methods of endotracheal intubation, from simple to sophisticated, have been described before to improve the endotracheal intubation methodology, and scale up the intubation instruments in small animals like rats. However, it involves complex technical applications and requires specialized tools [2, 3, 15–17]. Regardless, the risk of perioperative animal death remains high. The purpose of this study was to improve the blinding method, use an easy-to-use technique and common equipment for endotracheal intubation and compare it with existing widely used methods.

MATERIALS AND METHODS

All animal procedures and the experimental protocols were approved by the Experimental Animal Ethics Committee of Guangxi Medical University and were carried out in accordance with the Guiding Opinions on the Treatment of Laboratory Animals and the Laboratory Animal-Guideline for Ethical Review of Animal Welfare (People's Republic of China national standard GB/T35892-2018).

Animal

All animals were purchased from the animal experimental center of the Guangxi medical university. A total of 200 to 250 g rats either sexes with an age range of eight-ten weeks old, and 45 Sprague-Dawley would be used as a control group in another thoracotomy model (coronary microembolization). Rats were housed in SPF-class animal rooms under ambient room temperature (22 ± 1°C),

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humidity (47 ± 2%), and in a 12-hr light cycle. The animals were randomly assigned into three groups: Improved blind intubation (B group), Transillumination intubation (T group), and Incision intubation (I group). Each group were assigned with fifteen animals.

Equipment and drugs

Following tools were used in this study. Surgical plates, rat surgical instruments, lighting equipment, clean gauze, iodine, 7F arterial sheath, 1% pentobarbital, 2% lidocaine (Hefeng Pharmaceutical Co., Ltd., Shanghai, China, Approval Number: H20023777) half-diluted to 1% with saline before use.

Endotracheal intubation

The animals were kept under fasting conditions, 12 hr before the surgical procedure. During fast, the animals were allowed to drink water for 4 hr. The animals were anesthetized by the intraperitoneal injection of 1% pentobarbital (40 $mg \cdot kg^{-1}$).The rats were lying on their backs. A small amount of 1% lidocaine was instilled from the unilateral nasal cavity and flowed through the pharynx to the throat for local anesthesia.

Animals, assigned to the improved blinded method were kept at supine position, then the highest point of the parietal bone of the animal was supported by using the middle finger of the left hand, so that the throat and trachea are at the same level. The larynx point was sensitized (On the median sagittal plane, the most prominent point of the laryngeal nodule forward) with the thumb, and the 7F arterial sheath was captured in the right hand, and migrated towards the larynx point at 4–5 cm (Fig. 1). Animals experienced a penetrating sensation when the throat were touched. After reaching the trachea, the sheath was in contact with the tracheal ring, and there was a special sense of friction. Then the sheath was connected to the ventilator and the thoracic region was visualised which was undulated, indicating that the intubation was successful. Transillumination intubation (T group) utilized light to locate the larynx. Incision intubation (I group) was intubated after trachea incision. The operational procedure for the T group, [1, 21] and I group referred to the methods of the predecessors. Before the formal implementation, each endotracheal intubation technology has been practiced. In the implementation, the three people took turns to perform each methods to reduce the difference caused by the technical proficiency.

Observation index

Following parameters were considered as the observation index.

1. Operational feasibility: (a) the time to complete the procedure, (b) success, (c) Repeat attempts, (d) Death. Intubation success is defined as survival and normal ventilation after intubation. If during the intubation process the rat has signs of life-threatening signs such as weak breathing or haemorrhage, stop the operation. 2. Operation difficulty: (a) strong vomiting reflex interrupts the intubation, (b) excessive salivation impedes the clear vision of the throat, (c) resistance of advancing limits the endotracheal intubation perform. 3. Operational complications: oesophageal injury, laryngeal injury, oral bleeding, surgery-related mortality to assess the safety, and 4. histological examination. Animals were euthanized after thoracotomy and trachea was exposed. The position of the endotracheal tube tip was identified using an inverted microscope at ×5 magnification. Histological examination of the tracheal tissue was performed using haematoxylin-eosin staining to observe the microscopic changes between the vocal cords and the position of the catheter tip.

Statistical analysis

All the statistical analyses were performed using SPSS 23.0. The measurement data were expressed as mean ± standard deviation and the differences between the three groups were compared by analysis of variance. The dose data were expressed as a percentage, and the χ^2 test was used to compare the differences. $P \leq 0.05$ was considered statistically significant.

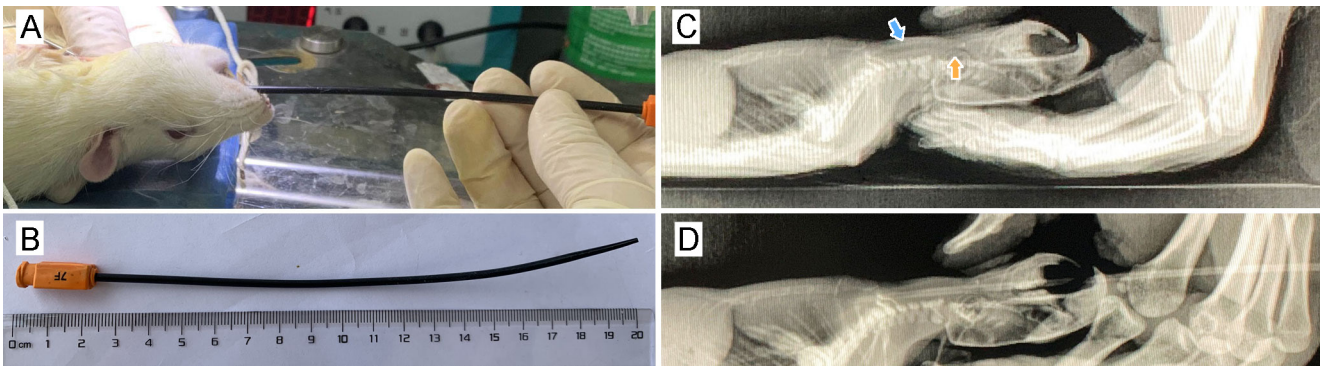

Fig. 1. Postural diagram of the improved blind endotracheal intubation A: The rats were placed in the supine position, then supported the highest point of the rat's parietal bone with the middle finger of the hand. The airway larynx of the rat was touched with the thumb to make the throat and the trachea at the same level. B: The 7F arterial sheath tube. C: Rats positon before tracheal intubation (X-ray perspective), orange arrow: throat, blue arrow: trachea .D: After endotracheal intubation (X-ray perspective).

RESULTS

Feasibility and safety assessment

Three techniques were applied to 15 B group, 15 T group, 15 I group. A total of 45 animals were used. There were 14 rats in B group, 12 in T group, and 13 in I group survived and successfully intubated. There were similar rate of success in the three group B/T/I (93.3% vs. 80.0% vs. 86.7%). There were no death in group B, but one rat was not been intubated due to obvious intubation resistance and weak breathing. One rat died of laryngeal injury excessive bleeding and two rats were not been intubated for strong vomiting reflex and excessive salivation in T group. Two rats in I group died of suffocation due to too long intubation time. The operation time was 35.00 ± 9.86 sec for B group, 57.12 ± 6.54 sec for T group and 184.33 ± 25.49 sec for I group ($P \leq 0.001$). The number of attempts in B group and I group were less than T group ($P=0.001$). No difference in difficulties and complications among the three groups (Table 1).

Histological assessment

At the autopsy, the tip positions of the tracheal ducts of the three groups of animals were located 15 mm from the tracheal carina. We could not visualize any significant tracheal mucosal damage. Microscopic examination of the tracheal tube near the end of the tracheal tube, some case showed that the endotracheal membrane was partially broken, and the cilia were damaged. Also, the submucosal congestion and oedema, inflammatory cell infiltration, and the severity of tissue changes were noticed. The incidence of microscopic tissue damage is no statistical difference (B group 4 (26.7%); T group 5 (33.3%); I group 3 (20.0%), $P>0.05$) (Fig. 2).

DISCUSSION

The tracheal intubation method is divided into invasive and non-invasive method [4, 16, 22]. The tracheal intubation was applied both invasively and non-invasively in two separate groups. In the animal model experiments of acute diseases, tracheotomy cannulation can be used. In a previous report, Locali [9] and other anesthetized rats, making a small incision in the neck midline at a distance of 8 mm, the fascia was separated, free of thyroid and the vascular clamp was used to separate the anterior cervical muscle. It is also possible to expose the white, annular cartilage trachea. Using a sharp knife, a small opening (1 mm) was introduced in front of the trachea and gently feed the catheter into the catheter. This method can ensure the accuracy of the position of the cannula, and the damage of the oesophagus, throat and mouth caused by the intubation can be avoided. In our experiments, there were two dead animals in I group, one animal died from injury and vascular bleeding during tracheal detachment, and the other died of prolonged tracheal detachment and inability to suffocate in time. This method requires the operator to be familiar with the anatomy of the neck and skilled in using surgical instruments. Improper operation may cause serious complications. Moreover, it is necessary to separate the tissue and incision of the trachea cartilage during intubation, which has a greater impact on the anatomy of the animal's airway. Structural changes are more prone to breathing difficulties after extubation. In chronic disease model experiments, if the incision is too large, it may cause difficulty in airway recovery and increase the chance of postoperative infection. These non-experimental factors may affect the experimental results. Therefore, tracheotomy is not suitable for chronic disease models and airway models.

Previously, many studies have reported the use of non-invasive methods such as the illumination method, and the blind method [2, 11, 16].

Illumination method

In a previous study, Cambron *et al*. have used the transmitted light cannula method: that was placed a 50 W power source at a distance of 5 cm from the skin of the rat neck, and the light can pass through neck skin and tracheal walls were exposed. Using

Table 1. Characteristics of three kinds of rat tracheal intubation

Item	B group (n=15)	T group (n=15)	I group (n=15)	*P* value
Body weight, g	225.27 ± 26.02	225.8 ± 21.16	223.29 ± 18.85	NS
Success, n	14 (93.3%)	12 (80.0%)	13 (86.7%)	NS
Operational feasibility				
Time to complete, second	35.00 ± 9.86	57.12 ± 6.54	184.33 ± 25.49	0.000
Repeat attempts	0 (0–1)	2 (1–4)	0 (0–1)	0.001
Death, n	0 (0%)	1 (10%)	2 (20%)	NS
Operation difficulty and complications				
Strong vomiting reflex	2 (13.3%)	4 (26.7%)	0 (0%)	NS
Excessive salivation	3 (20%)	4 (26.7%)	1 (6.7%)	NS
Resistance of advancing	2 (13.3%)	4 (26.7%)	2 (13.3%)	NS
Esophageal injury	1 (6.7%)	3 (20%)	0 (0%)	NS
Laryngeal injury	1 (6.7%)	1 (6.7%)	0 (0%)	NS
Oral bleeding	0 (0%)	1 (6.7%)	0 (0%)	NS

Data are mean ± SD, number (proportion). NS, not significant.

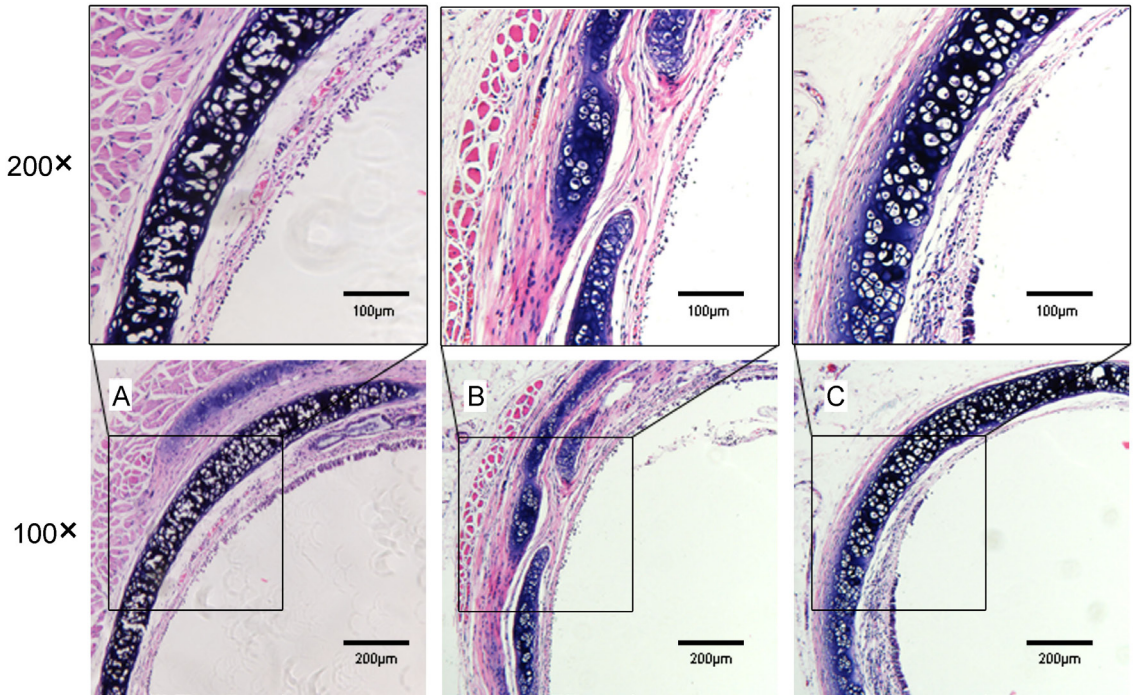

Fig. 2. Histological examination (haematoxylin-eosin staining) of the site around the tip of the tracheal tube. A: Improved blind intubaion, B: Transillumination intubation, C: Incision intubation. Microscopic examination of the tracheal tube near the end of the tracheal tube showed that the endotracheal membrane was partially broken, and the cilia were damaged. The submucosal congestion, oedema, inflammatory cell infiltration, and the severity of tissue changes were observed.

the small tongue depressor with a 145° angle at the front, the tongue was pushed, the epiglottis and vocal cord movements are clearly visible under the transmitted light, allowing the cannula to be viewed under light vision [1]. The original intention of the illumination method is to change the position and improve the instrument to achieve intubation when the glottis can be viewed directly to improve the success rate and safety. Practitioners of the illumination method have continuously improved various equipment for this purpose, from the position plate during intubation [15], the device that can open the mouth as much as possible [6, 8, 19], the electronic device that illuminates the glottis [12, 16], and the device to avoid damaging the throat during intubation [15]. Whether the glottis can be fully exposed is the key to the success of intubation, so it is generally necessary to maintain special posture equipment and special intubation equipment to expose the glottis. Live animals experience various reactions as we operate, and the proper use of anesthetic drugs is also important.

If the degree of anesthesia is insufficient and the tongue is pulled too much, it makes the animal resistant, which eventually turns the vocal cords to be easily twisted and closed, making the glottic positioning difficult, and intubation difficult to succeed. Also, excessive anesthesia may cause significant respiratory depression and increase mortality [7].One animal died in the T group, because the tongue was pulled excessively, the glottis was closed, the glottis was not clear, the intubation was delayed, and the rat died of asphyxia. Our experience is that although the light can make the glottis faintly visible, the throat of the rat is small. When the rat is breathing, the thorax undulates and the glottis opens and closes continuously, so the glottis is difficult to locate. Only by fixing the rat in a special position [15], a special device opens the mouth [6], fixes the tongue [8], and the glottic exposure is better. However, electronic imaging equipment could not be obtained, and the success rate was not as high as in other literatures. Intubation under direct vision is a very ideal state, but due to the limitations of the equipment, the promotion is limited.

Blind method

In a previous study, Stark *et al*. mentioned used the blind intubation method [14]. In the same study the success rate of tracheal intubation in rats was very high in a supine position and reached up to 90%. However, usually it takes 3 to 5 min to complete, and repetition is often required. Trial insertion of three to four times, or even six to eight times. The successful feeling of intubation can be measured by the resistance of the catheter when entering the trachea through the vocal cords, while it was free of resistance when entering into the oesophagus. Repeated trial insertion in the early blind intubation could easily cause throat injury and vocal cord edema, and strong mechanical stimulation can cause excessive secretion of the airway, affecting ventilation [14]. The following improvements were made to our blind method: 1. we used local anesthetic drugs: lidocaine to anesthesize the throat locally. It did not increase the dose of general anesthesia and anaesthesia the throat to reduce vomiting caused by severe vomiting and excessive secretions. Based on the anatomical structure of the rat, the animal was supported by hand to form a position favorable for intubation: the rat was placed in a supine position, and the operator supported the parietal bone of the rat with his

right hand. The head was tilted back so that the three parts of the mouth, pharynx and throat were pulled horizontally, and the thumb was placed on the throat to guide the forward direction of the intubation.

Compared with the T group and I group, the B group has a shorter operation time in rats, which may be more advantageous in avoiding asphyxia caused by delayed intubation, and in terms of difficulty in intubation and complications of intubation which are not significantly different from the other two methods. The incidence of microscopic tissue damage (B group 4 (26.7%); T group 5 (33.3%); I group 3 (20.0%), $P>0.05$) is slightly higher than previously reported in the literature [15]. The reason may be that the tip of the catheter is not smooth enough. Due to the lack of direct vision, the blind method requires higher smoothness of the tube end, which is also one of the concerns of the blind method. Because of the lack of direct vision, the blind method requires higher smoothness of the tube end, and the smooth end of the tube can reduce the damage of the intima. This is also one of the concerns of the blind method. We plan to improve tracheal intubation with a smoother tip in the next step.

Compared with the transillumination intubation and incision intubation, the improved blind intubation has shorter operating time in rats, and its security is comparable to the other two methods. We found the improved blind endotracheal intubation technique was simple and required less tools, and is feasible to implement in general laboratories.

In this study, we proposed a new blind tracheal intubation technology, which is improved and easy to implement, and its safety is comparable to the other two common technology. This technique can be considered as one of the preferred technology to quickly establish a safe airway in rat experiments.

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