Simplification of rat intubation on inclined metal plate

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ENDOTRACHEAL INTUBATION is often necessary during inhalation anesthesia to allow steady-state conditions for large operations and in vivo experiments in all fields of experimental surgery. In rats, placing an orotracheal tube is technically difficult primarily because of the small size of the subject and the lack of equipment specifically designed for this task. We describe a simple rat intubation technique in which the animal is suspended in dorsal recumbency on an inclined metal plate. The animal, anesthetized with ether, is fixed to a 70°-inclined metal plate in a dorsal position by means of a Mersilene ribbon hooked around the upper incisors. This method of positioning the animal is the most important step in the intubation process and further facilitates the technique already described by other authors. A human otoscope was used as a laryngoscope, intubation was performed using the Seldinger technique, and a 14-gauge intravenous catheter served as an endotracheal tube. This inexpensive technique is quickly learned and can be used in any laboratory. Safe and reliable airway management can thus be achieved, permitting in vivo examinations and operations.

endotracheal intubation; experimental techniques; laboratory animals

ENDOTRACHEAL INTUBATION is often necessary during inhalation anesthesia to allow steady-state conditions during in vivo experiments. On larger animals, such as rabbits and dogs, it is easy to perform with a standard pediatric laryngoscope. In small laboratory animals, such as rats, the anatomic situation with a small palate and oropharyngeal cavity, a tiny larynx and epiglottis, and vocal cords that move at high frequency, airway management can be difficult. In addition, no standard intubation equipment is available for rats.

Several methods of orotracheal intubation, from simple to sophisticated, have been described before. Blind oral tracheal intubation with the needle of an intravenous catheter, filed smooth, shortened, and bent according to the anatomy of the airway of the rats is described elsewhere (6). According to those authors, an average of three to four attempts is required to position the tube correctly. Illumination of the larynx by an extraluminal light source penetrating skin, soft tissue, and trachea is reported (10), as well as tracheal intubation under direct illumination of the vocal cords with a special fiberoptic laryngoscope or a fiberoptic light guide (2, 7). Direct laryngoscopy with a head-mounted, mirror-reflected, adjustable-focus light is reported (1) as well as the use of a specially designed oropharyngeal intubation wedge made by a 3-ml syringe and an ordinary table lamp (3), whereas Pena and Cabrera (5) used a surgical operating microscope that supplies both illumination and magnification of the larynx.

Although the method of orotracheal intubation we describe has been described in principle before by Weksler et al. (9), the special positioning of the rat in dorsal recumbency and a semisuspended position on an inclined metal plate makes the access to the upper airways easier and further facilitates the technique. The method we describe allows uncomplicated intubation of these small animals.

MATERIALS AND METHODS

Animals. The experiments described herein comply with the requirements of the German Animal Protection Act of 1986 and with the guidelines set by the Government Committee on Animal Experiments. All of the animals participating in this study were used for further metabolic examinations under inhalation anesthesia; no animals were used or killed for this study only. A total number of 80 rats (40 aged 20 wk, 40 aged 28 wk; Charles River Wiga, Sulzfeld, Germany) weighing 280–440 g were intubated before in vivo absorption studies of the small bowel were performed. A steady-state, body temperature, and a stable-acid/base status (blood gases) are mandatory prerequisites for performing such studies.

Equipment needed for the intubation. For the intubation, we used an inclined metal plate with a Mersilene ribbon (Ethicon, Hamburg, Germany), an otoscope with a 5 × 62-mm plastic otoscope cone (Riester, Jungingen, Germany), a guide wire, a standard 20-ml syringe, two 14-gauge (2.0 mm ID, 45 mm length) intravenous catheters (Abbott, Wiesbaden, Germany), ether (Chinosolfabrik, Riedel-e-Haen, Seelze, Germany), a 10.3-mm (7-Fr) human endotracheal tube (for mouth-to-nose ventilation in case of respiratory arrest), and a cotton tip (Fig. 1).

For ventilation, we used the Small Animal Ventilator KTR4 (Hugo Sachs Elektronik, March, Hugstetten, Germany) and for inhalation anesthesia we employed Foren (isoflurane; Abbott).

Animal preparation and intubation procedure. Before intubation, the animal is anesthetized with ether until the eyelid closure reflex is lost. Therefore, the animal is positioned in a glass chamber with a wire mesh at the bottom to avoid direct contact of the ether with the skin of the rat. Ether is poured into the closed chamber immediately before the animal is put into it.

The rat is then positioned on an inclined metal plate and suspended by a Mersilene ribbon hooked around the upper incisors (Figs. 2 and 3), the person intubating the animal standing behind the inclined metal plate. The hypersalivation induced by ether inhalation makes it necessary to clear the airways by aspiration with a 20-ml syringe and a 14-gauge intravenous catheter. The animal’s mouth is opened with the aid of a cotton tip and the tongue pushed to one side.

Intubation is performed in three steps. The vocal cords are visualized by inserting the otoscope into the oral cavity while pushing the tip of the epiglottis forward with the tip of the otoscope. Under direct vision of the upper airways, a 20-cm-long, 1-mm-thick guide wire is introduced through the vocal cords via the inside of the otoscope cone through the mouth while the vocal cords are visualized. After ensuring that the guide wire is correctly positioned, a 14-gauge intravenous catheter is passed over it and advanced using the Seldinger technique. The guide wire is carefully removed and the catheter placed in the trachea. The intubated rat is then placed on a prewarmed operating table. The catheter itself is not affixed to the rat’s mouth; it is connected to the tube of the respirator, and this tube is fixed to the
table with a special clamp. As soon as the respirator system is connected, condensation in the translucent tube and regular, symmetrical expansion and collapse of the thorax confirms the correct position of the tube. The settings of the ventilator (Small Animal Ventilator KTR4) we use are as follows: respiration rate 53/min, inspiration phase of ventilation cycle 30%, plateau 0%, mean respiratory pressure 9–12 mmHg (pressure-controlled mode, inspiratory tidal volume of ~2 ml/kg body wt). Isoflurane at 5.0% is administered initially, and after 3–5 min it can be reduced to 2.5–3.0% in oxygen as carrier gas. The ventilator is connected to a scavenger system; we used a surge tank and applied a negative pressure of a −10-cm water column to prevent the loss of isoflurane into the room. The respirator settings above were tested and optimized for our metabolic studies in prior experiments.

The technique described is quickly mastered, can be performed within 3–5 min, and permits the undisturbed performance of operations (transplantations) and/or in vivo examinations.

RESULTS

We intubated 80 laboratory rats with our technique; after an initial short learning curve (5–10 intubations under assistance of an experienced veterinarian) the whole procedure took 3–5 min. The rat is positioned on an inclined metal plate and suspended by a Mersilene ribbon hooked around the upper incisors (Figs. 2 and 3), the person intubating the animal standing behind the inclined metal plate. In the beginning, it is difficult to visualize the rapidly moving vocal cords and position the guide wire fast enough before the animal awakens. For novices it took up to six attempts and 15–20 min to position the guide wire and the tube correctly. For experienced persons, however, it took one attempt, i.e., 3–5 min to anesthetize, intubate, and connect the animal to the respirator.

We had three initial (4.7%) mispositionings of the tube in the esophagus. After we noticed this, we ventilated the animal with a human endotracheal tube with an internal diameter of

Fig. 1. Intubation equipment: otoscope, guide wire, abbcath, syringe, cotton tip. The experiments described comply with the requirements of the German Animal Protection Act of 1986 and with the guidelines set by the Government Committee on Animal Experimentation. All animals participating in this study were used under inhalation anesthesia; no animals were killed for this study.

Fig. 2. Positioning of the rat in a semisuspended dorsal recumbency position on the inclined metal plate. The Mersilene ribbon is hooked around the upper incisors.
10.3 mm (7 Fr), anesthetized it again with ether, and repeated the whole intubation procedure. One rat died due to a mispositioning of the tube that went unnoticed.

Regular, symmetrical expansion of the thorax and condensation in the translucent tube confirmed its correct position in the trachea. Of course, this is only a minimal assessment by current anesthetic standards. One could envision a rat breathing spontaneously and therefore having regular thoracic expansion even though the tube is positioned in the esophagus by mistake, but the animal will wake up quickly if no inhalative narotic gas goes into the lungs. Additional methods that can be applied to rodents include using the handle of a scalpel at the tip of the orotracheal tube to observe condensation on the cool steel. A more definitive assessment can involve the use of a mainstream capnograph to register exhaled CO₂.

The parameters respiratory rate, depth of anesthesia, symmetrical expansion of the thorax, movement of whiskers, pupils, color of tongue, and tonicity of the muscles were monitored throughout the whole experiment.

Neither laryngospasm nor tracheal or bronchial perforation were observed. Pathological examination of the upper airways at the end of the experiment showed mild tracheolaryngeal erythema and edema of the vocal cords in all animals; in two animals, minimal bleeding of the hard palate was observed. This can be of consequence for the animals; it might affect recovery from anesthesia and cause difficulty in breathing.

**DISCUSSION**

Various authors have described other methods for endotracheal intubation of rats (1, 2, 5, 7, 8). In 1964, Kesel (4) described a technique of endotracheal intubation under direct vision of the epiglottis and the vocal cords requiring special equipment, which, in our opinion, risks injuring the animal’s oral cavity and/or upper airways. We would therefore not recommend using this method.

A technique of blind oral-tracheal intubation with a success rate of ~90% is described by Stark et al. (6), which, however, sometimes requires repeated (3–4) attempts to position the endotracheal tube correctly in the trachea. The needle of a 16-gauge intravenous catheter was filed smooth, shortened, and bent at a 145° angle. The authors write that “a slight resistance is encountered as the catheter passes through the vocal cords” and that “less resistance will be felt if the catheter enters the esophagus...” This seems to be very traumatic to the larynx. Repeated attempts at intubation may cause severe edema and
consecutive swelling of the upper airways. This method, which allows little control, may injure the animal and, we believe, is not reliable enough.

Minitracheostomy is a reliable but time-consuming method of guaranteeing undisturbed intubation and ventilation in laboratory animals that will be euthanized at the end of the experiment. For experiments involving long-term follow-up, however, it cannot be recommended.

Pena and Cabrera (5) recommended the use of a surgical operating microscope to supply both illumination and magnification of the larynx to facilitate the insertion and reduce the risk of mispositioning of the tube. This necessitates a precise and, in our opinion, difficult positioning of the microscope but allows a controlled insertion of the tube with a low risk of traumatizing the airways.

Thet (7) used a fiberscope light guide for illumination of the vocal cords which, according to our opinion, is rather expensive ($400–$500) but worth trying when a fiberscope light guide is available in the laboratory.

Although the method of orotracheal intubation we describe herein has been described in principle before (8, 9), the special positioning of the rat in dorsal recumbent, semisuspended position on an inclined metal plate further facilitates the technique, since the animal can now be fixed without extra assistance. Weksler et al. (9) recommend intubating the animals in the prone position, using the same equipment as we do (oscope, Seldinger technique). The particular anatomy of the rat, i.e., a small palate, a small oropharyngeal cavity, a tiny larynx and epiglottis, and vocal cords that move at high frequency with an opening diameter of 1.5 mm (6), makes airway management difficult, especially if the animal is in the horizontal position. Placement of the animal on an inclined metal plate permits direct access to the kinked trachea.

In the beginning, we intubated the rats while they were lying flat in dorsal recumbency on the operating table. However, we had difficulties positioning the animals and visualizing the larynx and the vocal cords to allow placement of the guide wire before the animal recovered from anesthesia. The use of the inclined plate provided superior conditions.

It is very important to practice handling of the small instruments before intubating the animals and to become familiar with the Seldinger technique to keep the learning curve short and to avoid injuring the airways.

In our experiments, posthumous examinations of the upper airways (the animals were killed for other reasons) showed mild tracheolaryngeal erythema and edema of the vocal cords, which can be of major consequence for the animals: it might affect recovery from anesthesia and cause difficulty in breathing by inspiratory and expiratory stridor. This is probably attributable to the diameter of the tube and not to the technique itself. One could consider using a smaller tube, e.g., 1.7 mm internal diameter (16 gauge) to reduce upper-airway trauma, as described by Weksler et al. (9).

The method of rat intubation described by Jou et al. (3), using a specially designed intubation wedge made from a 3-ml plastic syringe can also be performed more easily in animals positioned on an inclined plate in the way we describe (3).

In conclusion, the foregoing is a simple and reliable method of intubating small laboratory animals that is inexpensive and can be performed with standard laboratory equipment. It permits uncomplicated, controlled ventilation and inhalation anesthesia even for periods of several hours. The method is quickly mastered, and after an initial learning process, the complication rate is low.

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GRANTS

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REFERENCES