European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2004; 8: 103-106

A new method of orotracheal intubation in mice

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Abstract. – **A new method of orotracheal intubation in mice is described.**

After intraperitoneal induction of anaesthesia, 36 male animals, belonging to common laboratory strains, have been intubated with the aid of a straight, small bore arthroscope, connected to a video-camera. After the insertion of a guide wire of appropriate size across the vocal cords, a polyethylene (PE) cannula has been introduced over it as an endotracheal tube.

Success rate has been 100% both in first intubations and in re-intubations; all procedures have been performed in a mean time of about 3 min.

Post-mortem examination of mice did not show any significant damage to upper airway mucosae related to the technique.

Key Words:

Tracheal Intubation, Video-assisted Endoscopy, Mice.

Introduction

Controlled pulmonary ventilation in mice, maybe the most common laboratory rodent species, has been always a challenge for investigators, especially when animals are scheduled to undergo repeated studies.

Mice are small (body weight of adult specimens usually does not exceed 30 g), and upper airway anatomy does not help in performing a safe and reliable tracheal intubation: incisors are relatively large, opening of mandible is restricted, the glottis is placed very anterior when the neck is extended, and beside technical difficulties there is the risk of bleeding, pharynx oedema and laryngeal perforation. Moreover, several strains of transgenic laboratory mice are expensive, and loosing animals during the first steps of investigation because of a poor airway control may represent a significant additional cost. Many methods of tracheal intubation have been proposed for small rodents, and some will be discussed later in this paper.

We present a video-assisted endoscopic method of tracheal intubation in mice using a small bore, 0° arthroscope connected to a monitor for the visualization of the glottis, a polyethylene (PE) tube as a cannula for intubation and a guide wire to facilitate its introduction across the vocal cords.

We are using this technique with more than satisfactory results in a still in progress study on new generation volatile anaesthetic agents. The study design includes repeated accurate measurements of several laboratory values over an eight-week period. This technique is rapid, safe, and there is no need of a specifically skilled operator, but only a good knowledge of mice upper airway anatomy. Mice, on the contrary of more invasive airway control techniques, can be re-intubated without any major complication.

Materials and Methods

Up to may 2004, we used 36 male animals, aged 8-10 weeks, weight 25-28 g, belonging to laboratory mice strains CD-1 (50%) and C57BL/6 (50%) (Charles River, Calco, Italy). All mice were maintained under conventional housing and feeding conditions in accordance

Figure 1. The Olympus mod. A 7002 straight, small bore (OD = 1.7 mm, length = 58 mm) arthroscope with its connection to the light source.

Figure 2. The arthroscope is inserted into the mouth of an anaesthetized mouse in order to allow larynx aditus visualization.

with EC directives. Study design conforms to the Guide for the Care and Use of Laboratory Animals (NIH 85-23, revised 1996).

Anaesthesia was obtained administering intraperitoneally a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg). Absence of both swallowing and righting reflexes has been assumed as a criterion of deep anaes-

Figure 3. After visualization of the aditus, the 1.1 mm OD PE cannula is inserted over the guide wire as an endotracheal tube into the trachea.

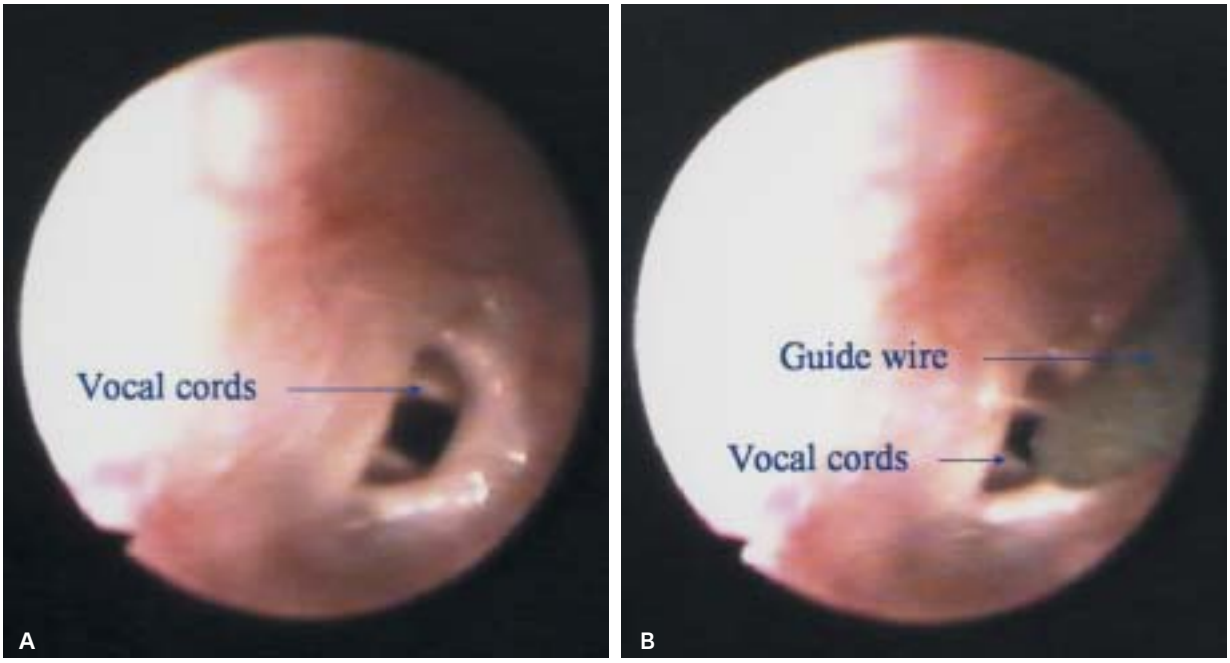

Figure 4. A-B, These images, taken with the aid of a videocamera, show the larynx aditus before and after the insertion of the guide wire. Note that the latter is a specifically designed guide for a very thin 24 G arterial cannula.

thesia. A warmed normal saline pad was placed under supine anaesthetized mice, with head and neck extended, in order to maintain a constant physiologic body temperature.

A straight, small bore arthroscope [outer diameter (OD) = 1.7 mm, 0°, length = 58 mm] (Figure 1), connected to a light source and a videocamera (all instruments and devices have been supplied by Olympus Italian branch), was inserted to the left of incisors down to the base of the tongue to allow visualization of the larynx aditus and rhythmic opening of the vocal cords during spontaneous respiration (Figure 2). Secretions, if present, were removed by a 1.1 mm OD PE cannula, connected to a syringe.

A soft-tip, stiff 0.4 mm OD guide-wire (Arrow Int, PA, USA) was inserted 3-4 mm across the vocal cords during inspiration in order to facilitate the introduction of a 30 mm long, 0.70 OD PE cannula over the guide (Figure 3). Figures 4a-b show the larynx aditus as it appears on the monitor before (Figure 4a) and after (Figure 4b) the insertion of the guide wire. After removing the guide-wire, the animal was connected (Figure 5) to a specifically designed pulmonary ventilator (Rodent Ventilator mod. 683, Harvard, USA) that was set, according to Brown et al.[1], at a tidal volume of 200 µl and at a respiratory rate of 110 breaths/min with a O_2/air 50% mixture and a flow rate of 1 l/min.

Figure 5. The mouse as it finally appears connected to the pulmonary ventilator.

After completing the scheduled steps of the study, and after animals involved in have been sacrificed with an intraperitoneal overdose of Tanax, a post-mortem examination has been carried out on 10 of them, in order to detect any possible injury and/or lesion of the upper airway directly connected to the intubating procedure.

Results

Despite the anterior location of the glottis, the straight fibre-optic arthroscope allowed good intubating conditions; the success rate, evaluated by the direct visualization and video recording of the introduction of the tip across the vocal cords, was 100% with a mean time of 3 ± 0.4 min. No additional difficulty was observed when mice were re-intubated during the following 8 weeks of the study, and the success rate was 100% again, with similar intubating times.

No major complications resulted. Moderate mucosal bleeding (enough to dirty but not to darken the lens of the arthroscope) has delayed the procedure in three cases, but did not compromise the success of intubation.

Post-mortem examination showed only superficial abrasions without mucosal oedema.

Discussion

Tracheal intubation in laboratory animals is indicated in studies where controlled pulmonary ventilation is necessary and in longitudinal studies requiring recovery from repeated general anaesthesia. Moreover, tracheal intubation allows several procedures, such as the instillation of a variety of substances in the lung, pulmonary function measurements and other evaluations, e.g. the use of radiological imaging.

On most medium-sized animals, such as rabbits and dogs, tracheal intubation can quite easily be performed with infant-sized laryngoscope and endotracheal tubes; in smaller animals, such as mice, hamsters, rats and guinea pigs, the technique is more difficult, both because of their size and their anatomic peculiarities.

Ethical concerns of several researchers and animal rights advocates[2] have caused a strong reduction of studies involving primate species and even dogs and cats. Therefore, it is not surprising that the appearance of smaller size laboratory animals in scientific experiments is increasing.

As stated by Jou et al[3], it seems reasonable to focus some research on techniques and devices introducing simplification and speed in modern animal studies.

Many methods of tracheal intubation in smaller size mammalians have already been described in the literature, and a variety of devices, often self-made, have been proposed; for a selected list of references see Jou et al again.

Surgical methods, both tracheostomy and exposure of the larynx by dissection[4], are invasive, the postoperative recovery may be too long for the design of the study, and scares and retractions, becoming from surgical approach, do not usually allow repeated procedures. Furthermore, driving the tracheal cannula into the aditus in a blind way, as described when larynx is surgically exposed, increases the risk of taking a false path.

In our study, we do not perform invasive procedures, all materials are commercially available and there is no need of specific training, but a good knowledge of animal anatomic peculiarities is enough for the purpose.

The method of video-assisted intubation of mice we have herein described is rapid, reliable and safe; above all, it is suitable for repeated tracheal intubations.

The cost may be high, but we do not think it is prohibitive.

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