A novel and simple method for endotracheal intubation of mice

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Summary

Endotracheal intubation in mice is necessary for experiments involving intratracheal instillation of various substances, repeated pulmonary function assessments and mechanical ventilation. Previously described methods for endotracheal intubation in mice require the use of injection anaesthesia to immobilize the animal during the intubation procedure or the use of a volatile anaesthetic prior to intubation for immobilization. With these methods, the control of anaesthetic depth during the intubation procedure is absent.

We describe a method for simple and rapid intratracheal intubation in mice for mechanical ventilation, using a self-built plastic support to facilitate the intubation procedure. General anaesthesia is maintained by means of inhalation through a non-rebreathing circuit connected to the plastic support. This set-up gives the operator control of anaesthetic depth and sufficient time to perform the intubation procedure. A purpose-made laryngoscopic blade is used to facilitate the intubation tube entering the trachea. The blade of the purpose-made laryngoscope is constructed as a retraction guide and is curved for easy handling. Under direct vision, the epiglottis is gently lifted by the laryngoscopic blade while the intubation tube is pushed into the trachea.

Following this novel intubation technique, we were able to mechanically ventilate mice for at least 2 h without severely disturbing blood gases. Histological evaluation of the lungs and microscopic evaluation of the trachea and larynx showed no signs of trauma related to the intubation technique or mechanical ventilation.

Keywords General anaesthesia; mechanical ventilation; endotracheal intubation; inhalation anaesthetics; laryngoscopic blade; mice

A method of endotracheal intubation in mice was first reported by Ho and Furst in 1973. They used the intubation method for intratracheal instillation of particulate suspensions into the lungs. The animal was anaesthetized with sodium pentobarbitone and placed in a purpose-made apparatus creating an angle in the neck with the head in an upward position. The intratracheal instillation was performed under a dissecting microscope with fibreoptic light for direct and illuminated vision of the larynx (Ho & Furst 1973). Alternative methods of endotracheal intubation for intratracheal instillations were published subsequently. Yap (1982) reported the use of a purpose-made laryngoscope. The animals

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were anaesthetized using sodium thiopentone and intubated using a pipette tip. The larynx was not visualized directly, increasing the risk of oesophageal intubation. Costa reported the use of a fibreoptic laryngoscope that allowed visualization of the epiglottis during intubation. Prior to intubation, the animal was anaesthetized by means of inhalation with enflurane, immobilizing the animal during the intubation procedure (Costa *et al.* 1986). Starcher and Williams (1989) used a restraining device in combination with direct visualization of the tracheal opening by putting a fibreoptic illuminator into the mouth of the animal. Mice were anaesthetized by means of inhalation with methoxyflurane prior to the intubation procedure. With both techniques, anaesthetic depth may not be maintained for a sufficient period of time to complete the whole intubation procedure. Otto-Verberne also restrained the animal and illuminated the larynx with a powerful external light source positioned on the neck. The animals were anaesthetized using CO_2 asphyxiation and intubated when starting to gasp for breath. Control of anaesthetic depth during the intubation procedure may be questioned using CO_2 ashyxiation (Otto-Verberne *et al.* 1992).

Brown used the intubation method for repeated pulmonary studies in mechanically ventilated mice. The animal was intubated using a purpose-made plastic support. A powerful external light source was used to illuminate the larynx and a custom-made laryngoscope was used to lift the lower jaw and displace the tongue of the animal. The tube was then inserted under direct vision into the trachea. The animal was anaesthetized with a mixture of etomidate and fentanyl (Brown *et al.* 1999). Deyo and Wei (1999) reported the technique for mechanical ventilation while general anaesthesia was maintained by means of inhalation with halothane. Prior to intubation, the animal was anaesthetized with halothane and a fibreoptic laryngoscope was used for direct visualization of the larynx. A pair of jeweller's loupes was used for a better view of the larynx.

Although the previously described techniques can be used successfully after induction of anaesthesia with injectable agents, considerable expertise is needed if they are to be used with inhalation anaesthetics. This is due to the interrupted supply of gas during intubation, leading to an untimely recovery of the animal if the procedure is not completed quickly. To circumvent these problems, we have devised a method for endotracheal intubation that is rapid and simple, while it allows anaesthesia to be maintained during the process of intubation.

Animals, materials and methods

Animals

Male C57BL/6 inbred mice, aged 6–10 weeks and weighing between 25 and 30 g (Harlan, The Netherlands) were used. All animals were kept in type 3 makrolon cages (Tecnhilab-BMI, The Netherlands) filled with woodchips (Abedd LTE E-001, Austria) in groups of 2–6 animals. The animals were subjected to a 12 h dark/12 h light cycle, with water and food (Hope Farms, The Netherlands) given *ad libitum* and housed in a room at 19–24°C and 47–63% relative humidity. Animal treatment and care were provided in accordance with institutional guidelines. All procedures used in this study were approved by the Animal Ethics Commission of the University of Amsterdam and in accordance with the Dutch Inspection Authorities.

Laryngoscope construction

The purpose-made laryngoscopic blade was constructed from a dental spatula (D/E No. 6; Dental Union BV, The Netherlands). The blade was modified as a retraction guide and slightly curved (Figure 1). The retraction guide consists of half a hollow tube. Note that the retraction guide construction will depend on whether the worker is left or right handed.

Plastic support

A plastic support (Plexiglas) was constructed to fixate the animal during the intubation

Figure 1 Purpose-made laryngoscopic blade with retraction guide

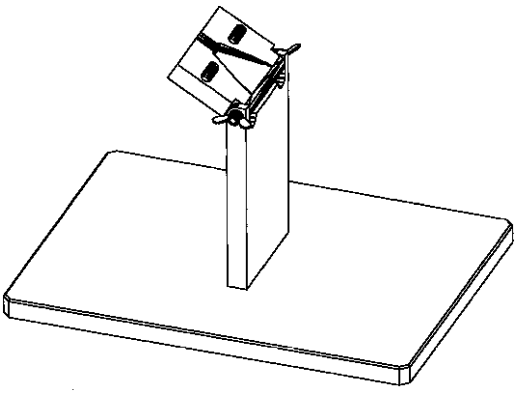

Figure 2 Purpose-made plastic support

Figure 3 Positioning of the mouse on the plastic support while general anaesthesia is maintained by means of inhalation

procedure (Figure 2). A 45-degree angle was created in the neck of the animal while general anaesthesia was maintained by an open breathing circuit connected to the support. A 1 mL syringe was used as a facemask and waste gases were scavenged by a suction arm connected to a central suction system. Once the animal was fixated, a fibreoptic arm of a strong halogen light source (Schott KL1500 Elektronic, Germany) was positioned over the neck of the animal (Figure 3).

Intubation tube

An intravenous catheter (Neoflon outer sheath, 24 gauge, 19 mm length; Becton Dickinson, Sweden) was used as the endotracheal tube. To prevent air leakage around the tube, a piece of silicon tubing (0.9 mm optical density (OD), 0.6 mm inner diameter (ID) and 4.0 mm length) was placed around the catheter to act as a cuff. The cuff was placed approximately 2 mm from the tip. The catheter had a round moulded tip to prevent damage to the soft tissue by sharp edges. This was achieved by briefly placing the tip in an open flame.

Anaesthesia

Prior to intubation, the animal was anaesthetized with a mixture of 5% isoflurane (Forene, The Netherlands) in O_2 in an anaesthetic chamber. Loss of the pedal reflex was used as an index of onset of surgical anaesthesia. The animal was placed on the plastic support and anaesthesia was maintained with a mixture of 2–3% isoflurane in oxygen. The concentration of isoflurane was adjusted to abolish coughing and swallowing reflexes during the intubation procedure. Oxygen (100%) was used as a carrier gas to ensure proper oxygenation during the intubation procedure. After intubation, the animal was connected to a mouse ventilator (Minivent Type 845; Hugo Sacks Elektronic-Harvard, Germany) and ventilated with 35% O_2 and 63–64% N_2 with 1–2% isoflurane at a rate of 120 breaths/min, with an

inspiratory–expiratory fraction of 1 : 1 and a tidal volume of 0.2 mL.

Endotracheal intubation procedure

The animal was placed on the plastic support and the neck was suspended at an angle of 45 degrees backwards and the animal's upper teeth were connected to a piece of wire. The animal's nose was positioned in the facemask – 1 mL syringe – by adjusting the length of the piece of wire. This position was maintained during the whole intubation procedure. The fibreoptic arm of a strong external halogen light source was positioned at the front neck of the animal (Figure 3), illuminating the larynx and making the vocal cords clearly visible. The laryngoscopic blade was used to lift the lower jaw and displace the tongue.

The blade was then pushed further in the direction of the larynx and the epiglottis was gently lifted. Keeping the laryngoscope in this position (Figure 4), the catheter was pushed under the retraction guide of the blade into the trachea, passing between the vocal cords.

The animal was removed from the plastic support and the catheter was connected to the ventilator. Temperature was kept at 37°C using a temperature-controlled heating pad with rectal temperature monitoring and an infrared lamp. The whole procedure of intubation from the placement on the plastic support to the connection to the ventilator was achieved in less than 2 min. The ease of intubation and efficiency of ventilation was assessed in 20 mice, which were then used in an unrelated experiment, evaluating the effect of blood replacement after bleeding.

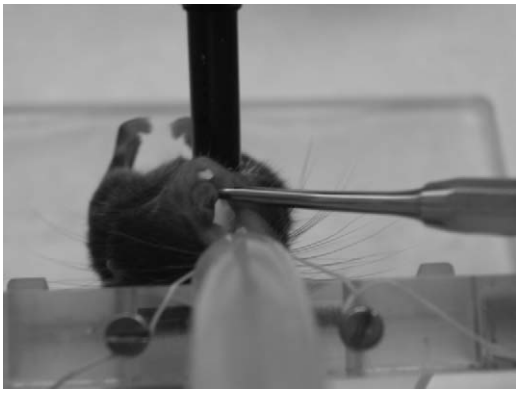

Figure 4 Displacement of the tongue

Surgical procedure

The depth of anaesthesia was checked by testing the pedal reflex before proceeding with surgery. The carotid artery was catheterized with polyethylene tubing (0.61 mm OD and 0.28 ID) and mean arterial blood pressure (MAP) and heart rate (HR) were recorded using a heparinized saline-filled catheter. The catheter was connected to a blood pressure transducer (Truwave PX-600F; Baxter), which was sampled at 1 kHz, and subsequently displayed and stored at 0.5 Hz using LabView version 5.1 (National Instruments). The jugular vein was catheterized with silicon tubing (0.6 mm OD, 0.3 mm ID) filled with heparinized saline connected to a 1 mL syringe.

Bleeding experiment

Thirty minutes after the jugular vein was catheterized, 15% of the circulating blood volume (0.35 mL blood for a 30 g mouse) was withdrawn from the jugular vein catheter. The effect on HR (beats/min) and MAP (mmHg) was continuously monitored for 1 h after blood withdrawal. The animals were mechanically ventilated for at least 2 h including the time to catheterize the carotid artery and the jugular vein. The experiment ended by taking a 0.2 mL blood sample from the carotid catheter for analysis of blood gases (pO_2 and pCO_2), blood acidity (pH) and haemoglobin (tHb) concentration (ABL550). The animals were killed by a KCl infusion through the jugular vein resulting in a cardiac arrest.

Histological and microscopic evaluation

Evaluation of the intubation technique was performed in an unrelated study. One group of three animals was intubated as previously described and mechanically ventilated for 30 min. After 30 min, the animals were killed by an intraperitoneal injection of 0.3 g sodium pentobarbitone/kg body weight

(Euthesate, Ceva Sante Animale BV Naaldwijk, The Netherlands). Under a dissecting microscope, the larynx and trachea were exposed and assessed for trauma. The right lateral lung lobe was removed for histological evaluation. The lungs of mechanically ventilated animals were compared to a control group of three male C57BL/6 inbred mice aged 6–10 weeks and weighing 25–30 g (Harlan, The Netherlands).

Lung processing

Immediately after removal of the lungs, lobes were fixated for 24 h in 2% glutaraldehyde. The lungs were dehydrated in graded alcohol and embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany). For the histological analysis, semi-thin sections (2 μm) were stained with toluidine blue and evaluated by light microscope.

Statistics

Blood pressure data and blood values are presented as mean ± SD. Statistical analysis included a multivariate analysis of variance (ANOVA), with a Scheffe *post hoc* testing to evaluate difference among means. A value of $P < 0.05$ was considered statistically significant.

Results

Figure 5 shows the effects of removing 15% of the circulating blood volume. The average MAP and HR at the beginning of the experiment were, respectively, 79.8 ± 7.81 mmHg and 543 ± 40.2 beats/min ($n = 7$). At the end of the experiment, MAP was decreased significantly to 50.6 ± 6.90 mmHg. The acute haemorrhagic shock after removal of 15% of the circulating blood volume gave an overall decrease in MAP of 28.9 ± 7.56 mmHg. No significant changes were measured in HR after blood removal. After having ventilated the animal for 2 h – at the end of the experiment – blood analysis showed that the tHb (g%) was 11.5 ± 1.0, pO_2 and pCO_2 were, respectively, 185 ± 24 and 33.1 ± 4.3 mmHg and the pH was 7.25 ± 0.06.

The microscopic exposure of the larynx and upper part of the trachea showed no disruption of structures. No fresh bleeding or remains of bleeding were observed while dissecting the larynx and trachea. Figure 6 clearly shows the intact trachea and epiglottis.

Histological evaluation of the lung morphology showed no differences between control mice ($n = 3$) and ventilated mice ($n = 3$). The alveoli and respiratory bronchioles of the mechanically ventilated mice did not show signs of injury or infiltration with inflammatory cells (Figure 7) and morphology was comparable with the control group (Figure 8).

Discussion

The technique of endotracheal intubation described here can be used for a number of applications where the absence of trauma is

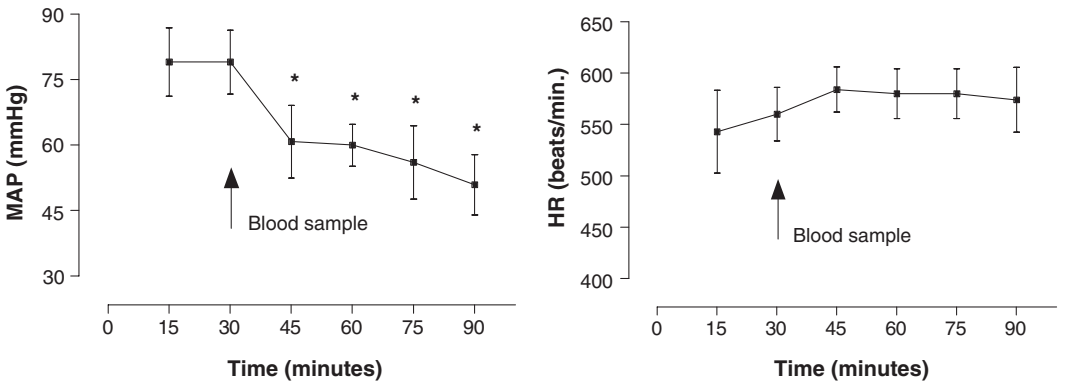

Figure 5 The effects of removing 15% of the circulating blood volume

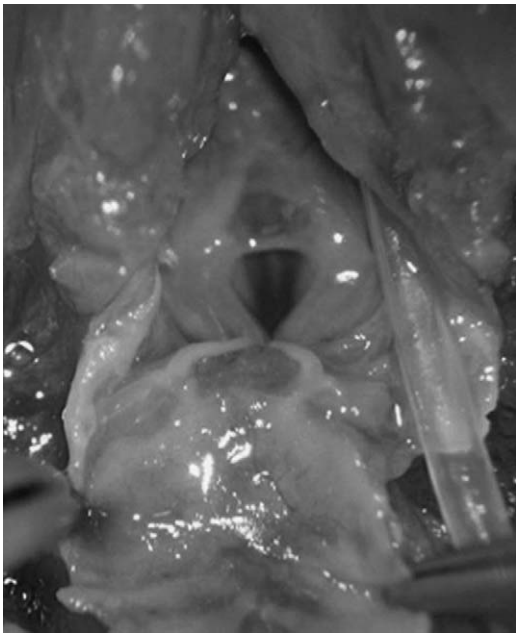

Figure 6 The intact trachea and epiglottis

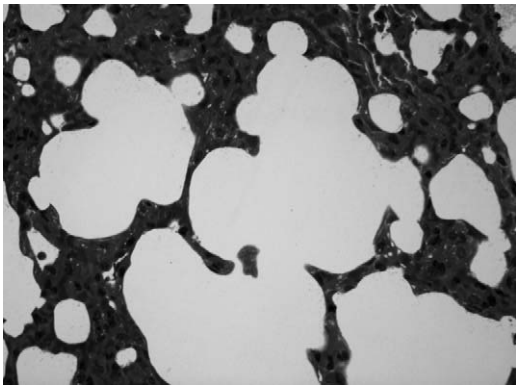

Figure 7 The alveoli and respiratory bronchioles of the mechanically ventilated mice did not show signs of injury or infiltration with inflammatory cells

important (Flecknell 1993, Berul *et al*. 1996, Deyo & Wei 1999, Schwarte *et al*. 2000, Zuurbier *et al*. 2002). Furthermore, respiratory support by means of controlled ventilation can be advantageous during anaesthesia since most anaesthetic agents have a depressing effect on respiratory function. Unlike larger species such as dogs and cats, mice are rarely intubated because of the technical difficulties involved (Flecknell 1996). This can have serious

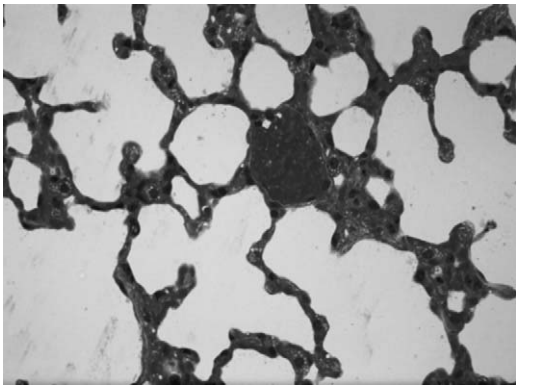

Figure 8 Morphology of the lungs in the mechanically ventilated mice was comparable with the control group

physiological consequences. Data obtained in our laboratory has shown that mice breathing spontaneously during anaesthesia with ketamine/medetomidine had severely disturbed blood parameters (tHb 13.4 ± 0.42 g%, pO_2 107 ± 24.7 mmHg, pCO_2 97.0 ± 24.7 mmHg and pH 7.00 ± 0.05). This was also the case in CD-1 mice breathing spontaneously while under anaesthesia with chloral hydrate and xylazine (Dalkara *et al*. 1995). The observed arterial pO_2 and pCO_2 of the mechanically ventilated mice in this study had values similar to mice that underwent 40 min of open chest operation and were mechanically ventilated (Guo *et al*. 1998).

Tracheostomy is often used to achieve mechanical ventilation in models where the animal is killed at the end of the experiment (Kuwaki *et al*. 1996, Georgakopoulos *et al*. 1998, Kubota *et al*. 1998). The intubation technique reported here can be carried out by experienced operators in about the same time it takes to complete tracheostomy, but without causing unnecessary trauma.

The use of an agent such as isoflurane allows the depth of anaesthesia to be controlled during intubation, abolishing coughing and swallowing reflexes during intubation. Since anaesthesia is continuosly maintained, inexperienced operators can take the time necessary to achieve intubation.

Our method of intubating and mechnically ventilating the animal showed no

cause of any trauma to the larynx, trachea or lungs. Our ventilating parameters – tidal volume and breathing frequency – did not differ from other studies related to ventilation-induced lung injury and inflammation (Gurkan *et al.* 2003, Wilson *et al.* 2003). However, we did not use positive end expiratory pressure (PEEP). In our set-up, this could easily be achieved by putting the expiratory tube of the Minivent in a 2.5 cm deep column of water, thus providing 2.5 cm H_2O pressure.

Based on a report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement, the maximum of chronic blood removal in mice is 15% of the circulating blood volume (BVA/FRAME/RSPCA/UFAW, 1993). The reported data show a significant decreased MAP of 28.9 ± 7.56 mmHg in the mechanically ventilated anaesthetized mouse after blood removal.

Conclusion

The described method for endotracheal intubation for controlled ventilation in mice can be used for maintaining general anaesthesia. The control of anaesthetic depth during the intubation procedure is advantageous, abolishing coughing and swallowing reflexes. The use of a laryngoscopic blade for direct vision on the larynx is essential. The retraction guide of the blade will help to guide the intubation tube into the trachea. This method may be suitable for other applications such as instillations and repeated pulmonary function assessment. The use of invasive tracheostomy for controlled ventilation can be replaced by this method of endotracheal intubation preventing unnecessary trauma. Although the animals underwent bleeding, the pO_2 and pCO_2 values were similar to values reported in literature without severe haemorrhagic shock, indicating proper ventilation. The technique is suited for general anaesthesia by means of controlled ventilation and methods where the absence of trauma is important. Histological evaluation of the lungs and microscopical evaluation of the trachea and larynx showed no signs of injury related to the intubation technique.

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