Combined retrograde–antegrade approach: a novel technique for endotracheal intubation in rats

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Endotracheal intubation in small animals is necessary for many different experiments. However, securing airway access can be difficult due to a variety of factors. We describe a modified approach for intubation in rats. Wistar rats (wt 150–300 g and age 5–7 weeks) were used. After intra-peritoneal anaesthesia, a guide wire was retrogradely inserted into the trachea, pulled out through the oral cavity and the intubation cannula introduced antegradely over it. The procedure was successful in all (15/15) animals. Time to secure access was 21.06 ± 6.31 min. Intubation was simple, safe and reliable for the entire duration of the actual experiment (2.67 ± 0.80 h) and performed with equal success by all operators. No intubation-related mortality was noted.

Keywords: Endotracheal intubation, experimental techniques, Wistar rats.

IN small laboratory animals, endotracheal intubation is often necessary for survival experiments. The procedure is easy to perform in larger animals, e.g. dogs and rabbits due to the applicability of a pediatric laryngoscope. In small laboratory animals such as rats, however, airway management is difficult due to a variety of factors including a small palate and oropharyngeal cavity, tiny larynx and epiglottis and rapidly moving vocal cords. Besides, no standard intubation equipment is commercially available for rats. For rodent intubation, several techniques have been described in the literature, i.e. intubation using the otoscope[1,2], blind oro-tracheal intubation[3], specially designed laryngoscope[4], insertion of specially designed oro-pharyngeal intubation wedge[5], fibre-optic light guide for illumination[6,7], video-endoscopic endotracheal intubation[8], transillumination method[9] and direct visualization of larynx using a infant laryngoscope blade[10–12]. Specially designed equipment is required in the above-mentioned methods that is not always easily available, is expensive and requires considerable practice for successful intubation. This study was conducted to develop an easy method of endotracheal intubation in the rat which requires simple material available to any research facility and improves access to the airway.

The study was carried out as part of a Department of Biotechnology funded research project involving role of mesenchymal stem cells in rat models of heart failure. The Institute Animal Ethics Committee reviewed and approved all procedures which were performed in the experimental animal laboratories.

Wistar rats of either sex, weighing an average of 150–300 g and of age 5–7 weeks, were used in the study. They were housed in temperature-controlled day–night cycled rooms. All animals were allowed to have 7 days acclimatization period before use. Animals were fed with commercial rodent food and fresh water. The animal caretaker and laboratory technicians involved had training and experience in the care of laboratory animals.

The equipment needed for the intubation procedure included an otoscope with a 5 × 62-mm plastic otoscope cone (Welch Allyn), 18 G intravenous cannula (BD Venflon), a short straight tip hydrophilic guide wire (Mini guidewire 0.038″, Terumo Corporation), anaesthetic drugs including ketamine and xylazine, polypropylene suture (6–0, 0.7 metric (Surgipro TM II)), retractor, scissors, forceps, blade (no. 15), needle (18 G, 80 × 38 mm, Dispo Van), betadine, 70% alcohol, etc. For ventilation, a small animal ventilator (model CIV-101, Columbus Instruments, Columbus, OH) was used.

Before intubation, each animal was anaesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg). The rat was then positioned on a clean, disinfected (70% alcohol) inclined wood plate. The upper incisors were fixed by a thread to the wood plate. The tongue was pushed to one side using non-traumatic forceps. The breathing rate and rhythm were carefully monitored visually during the whole procedure.

The hairs were removed with the help of an animal hair clipper from the neck of the animal. Position of the trachea was palpated by placing the finger on the skin overlying the larynx. A small incision (10 mm) was made on the ventral side of the neck in the midline, and tissue planes dissected with a sharp blade for visualization of the trachea. After incision, a small hole was created in the anterior wall of trachea with the help of a pointed needle (80 × 38 mm) (Figure 1). Thereafter, the soft end of the guide wire was inserted into the trachea. The mouth of the animal was opened wide and the approaching wire from below was directly visualized using the illuminated otoscope. This was necessary to prevent its inadvertent entry into the nasopharynx and injury to nasal structures. Once the wire tip was visualized in the lower oral cavity, it was manually held with forceps and pulled out through the mouth till only a segment of wire remained protruding through the tracheal opening (Figure 2). An intravenous cannula was passed over the guide wire from the mouth into the trachea using the Seldinger technique[13], till it just reached the external tracheal opening (Figure 3). The

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guide wire was then carefully removed and the cannula was connected to the tubes of the respirator, and the tubes were fixed to the table (Figure 4). Due to the direct visualization of intra-tracheal position of cannula, there was no need to observe for any indirect signs to confirm this location. The tracheal opening was then sutured using a polypropylene suture. The skin was subsequently closed (Figure 5). To prevent post-operative infection of the animal, an intraperitoneal injection of penicillin (1×10^6 μg/kg) was given at the completion of the procedure.

On completion of the experiment and appearance of signs of adequate recovery, like toe pinch reflex, extubation was performed. The animal was then kept in a warm environment using bulb light for the next 60 min. The number of rats that died or experienced complications related to the intubation was recorded.

We were able to successfully intubate 15 out of the 15 rats (100%). Rat weight was 221.26 ± 39.19 g. After an initial short learning curve (the first two animals required five and three intubations, respectively), the intubation procedure took 21.06 ± 6.31 min. The average duration of intubation needed for completion of our main experiment was 2.67 ± 0.80 h. The technique was easy to learn, perform and teach. After being mastered by the first operator (surgeon) in the initial two rats, the subsequent intubation technique was successfully performed by each member of the team. No complications, including mortality, directly related to the intubation technique were noted.

Intubation is an essential pre-requisite in many animal experiments involving survival studies, especially surgical procedures. However, intubation can be a great challenge when dealing with small animals. Various authors have described several techniques for endotracheal intubation.

A technique of endotracheal intubation under direct vision of vocal cords and epiglottis was described by Kesel, requiring special equipment[14]. We believe using this special equipment is not reliable because it is risky and injurious for the oral cavity and/or upper airways of the animal.

Use of a surgical operating microscope was recommended by Pena and Cabrera for magnification and

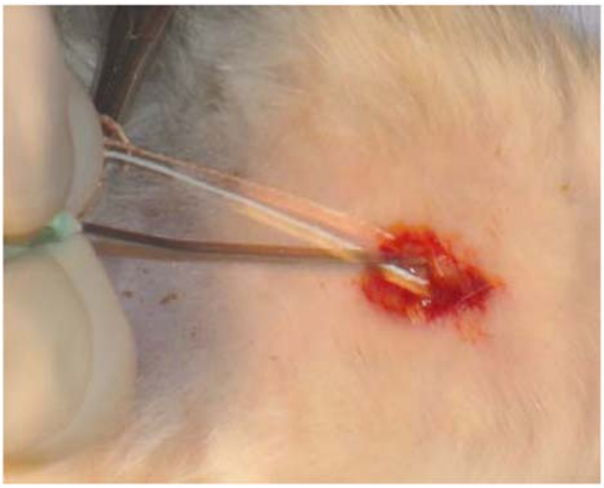

Figure 1. A hole is created in the anterior wall of the trachea with a thin pointed needle. Note the stabilization of trachea with a thread passed around it and pulled anteriorly.

Figure 2. After retrograde passage of a soft guidewire through the tracheal opening, it is pulled out through the oral cavity under direct visualization.

Figure 3. The cannula for intubation is introduced antegradely over the guidewire from the oral cavity, till the lower end of cannula reaches just proximal to the tracheal opening. The guidewire is removed from below.

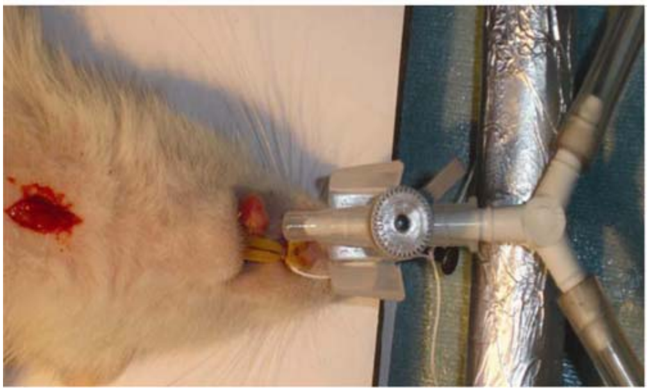

Figure 4. The cannula is connected to the tubes of the respirator and the tubes are fixed to the table.

Figure 5. As a final step, the tracheal opening and the skin incision are sutured.

illumination of the larynx to reduce the risk of mispositioning of the tube[15]. Although this allowed a controlled insertion of the tube with minimum risk, positioning of the microscope was very difficult.

Stark *et al.*[3] described a technique for blind oral tracheal intubation using a modified 16-gauge intravenous catheter. This technique has a $\simeq$ 90% success rate which requires 3–4 attempts to position the endotracheal tube correctly in trachea. It is however, very traumatic to the larynx and more attempts cause swelling, oedema and several other problems of the airway. In our opinion, it is not to be recommended for animals.

A fibre-optic light source was used by Thet[6] and Rivard *et al.*[9] for transillumination of the vocal cords but it was not easily available in all research laboratories and is very expensive ($400–500). Clary *et al.*[8] utilized video-endoscopy for the same purpose. Learning and handling of this equipment is however, complicated and it is not easily available.

The use of nasal speculum as a rodent laryngoscope for rat intubation was reported by Gustafsson *et al.*[16] with 92.3% success rate. They used a blunt wire as a stylet for a 14-gauge intravenous catheter to intubate rats. Similarly, Alzaben *et al.*[17] used the blunt, plastic-covered stylet that was introduced between the vocal cords as a guide for a 16-gauge intravenous catheter which was used as an endotracheal tube. The major difference from the technique described by Gustafsson *et al.* was the use of an otoscope as a light source by Alzaben *et al.* The success rate of Alzaben *et al.* was higher (98.7%) than that reported by Gustafsson *et al.* (92.3%). The former technique allowed a better field of light source and vision for accurate positioning of stylet between the vocal cords. Limitations of using the nasal speculum include tachycardia and limb movement during its insertion and opening of the oropharyngeal cavity.

Kastl *et al.*[1] described orotracheal intubation of rats using an otoscope cone and a guide wire while the rat was in dorsal recumbency on an inclined metal plate. Posthumous examination of the upper airways of their experiments, however, showed mild tracheolaryngeal erythema and oedema of the vocal cords. This may affect recovery from anaesthesia and breathing of the animal.

All these techniques are antegrade and involve direct visualization of vocal cords for successful intubation. Repeated attempts at direct antegrade intubation may cause severe oedema and consequent swelling of the upper airways. Therefore, these methods may cause injury to the animal and, in our opinion, may not be very reliable. Tracheostomy may be a reliable alternative method for guaranteeing undisturbed intubation. However, retaining the tracheostomy has some disadvantages including possible infection at the tracheostomy site and bleeding. Animals may also have resulting difficulty in swallowing and movement.

In the beginning, we had difficulty with positioning of the animal and visualizing the vocal cords for correct placement of the guide wire. Elevated position of the plate was found to be better for this experiment.

Our initial attempts at antegrade intubation using the pediatric otoscope cone were not successful as the small field of vision hindered us from properly visualizing the vocal cords. Keeping the otoscope cone wedged into the trachea for prolonged periods of time to visualize the cords also led to animal mortality in some of the cases. This also caused inadvertent entry of the wire into the oesophagus on some occasions. The team therefore, relied upon the interventional radiology expertise of one of the operators to perform a combined antegrade–retrograde approach. This consisted of an initial limited tracheostomy with retrograde guide wire introduction and the subsequent procedure being completed by the antegrade approach. It ensured 100% reliability in achieving endotracheal access, without the need for observing the described indirect signs of successful intubation needed with the other techniques. This technique was reasonably quickly mastered and easy to teach to other investigators. It did, however, rely upon skillful tracheostomy and subsequent skin suturing by the operator. No complications were noted in our study.

With our modification of the traditional technique, endotracheal intubation in rats could be safely performed with high success rate, which makes it highly suitable for basic cardiovascular research. Our intubation method requires no special equipment, needs little training to master and thus far has not been seen to be associated with any complications.

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Distribution, abundance and conservation of primates in the Highwavy Mountains of Western Ghats, Tamil Nadu, India and conservation prospects for lion-tailed macaques

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In the present study, we surveyed the primate species in the Highwavy Mountains of Tamil Nadu, India. Five primate species, including Nilgiri langur, Hanuman langur, bonnet macaque, lion-tailed macaque and slender loris were recorded in the region. Coffee and cardamom plantations in the hill system still hold the population of endangered lion-tailed macaque and Nilgiri langur. However, tea plantations act as a barrier for the movement of primate groups between the forest patches. The disturbance in the hill system and its consequence may be a reason for the increased group size of lion-tailed macaques. The inclusion of the lion-tailed macaque occurring areas to the newly declared Megamalai Wildlife Sanctuary in the Highwavy Mountains is recommended.

Keywords: Distribution, Highwavys, lion-tailed macaque, Megamalai, primates.

THE forests of the Western Ghats harbour a large number of flora and fauna. Due to its high biodiversity, the hill system has been recognized as one of the global biodiversity hotspots[1]. Nevertheless because of high human density[2] and high anthropogenic pressure, the rate of forest loss is alarming and these forests are considered to be one of the world's most endangered forests[3]. Developmental activities such as construction of dams, roads and power lines, converting the forests for commercial plantations such as coffee, tea and eucalyptus, and exploitation of trees for decades to cater to the wood industry led to a sharp decline of forest cover and resulted in fragmentation[4,5]. As a consequence of this, populations of many species have become fragmented. In addition, hunting of wild animals by humans has resulted in local extinction of some species[6,7]. Nevertheless, protected area network was created during 1972 to conserve the flora and fauna of the country (Wildlife Protection Act 1972, ref. 8). Although some parts of the Western Ghats were declared as

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