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Respiratory face mask: a novel and cost-effective device for use during the application of myocardial ischemia in rats*

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Abstract: To shorten operation time and improve survival rate of rats with myocardial ischemia or myocardial infarction, we use a novel device comprised of a face mask and a head/neck retainer in this study. We report the basic design of the novel respiratory face mask (RFM) and evaluate its performance in a rat model of myocardial ischemia. The device is cost-effective and easier to handle than other devices, such as tracheal intubation. Compared with conventional tracheal intubation, we found that RFM shortens operation time significantly while keeping blood indices normal; the mean operation time for rats in the mask group was (32±3) min, and that for the intubation group was (45±7) min ($P<0.05$). Moreover, the size and shape of the RFM can be changed according to the body weight of rats. In conclusion, RFM is an appropriate device for the establishment of myocardial infarction or ischemia-reperfusion in rats.

Key words: Rats, Respiratory face mask (RFM), Myocardial ischemia, Myocardial infarction
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INTRODUCTION

A myocardial ischemia model is very valuable for ischemic heart disease research. Canines, pigs, rats, and mice have been used as animal models in this research area. Tracheal intubation is the primary technique used for artificial respiration during the preparation of myocardial infarction- and myocardial ischemia-reperfusion models in rats. It is categorized into invasive and noninvasive techniques. The invasive technique is subdivided into conventional tracheotomy (Selye *et al*., 1960; Liu *et al*., 2002; Chen *et al*., 2005; Imaizumi *et al*., 2008) and the newly reported forward or reverse intubation for tracheal puncture (Rivard *et al*., 2006; Zhao *et al*., 2006); however, all of these techniques destroy the normal laryngeal anatomic structure of the rat, with complications such as a low survival rate, high complexity of the intubation process, and a comparatively long preoperative period. In other reports (Li and Wang, 2006), only the trachea was exposed after separating the cervical muscles, and then positioning of the metal tube was performed; this method does not damage the trachea directly, but complicates the operation and sometimes accidentally injures the throat and trachea due to the use of hard metal materials. The noninvasive technique mainly refers to oral intubation (Rao *et al*., 2005; Sun *et al*., 2005), in which a light source must be used to localize the glottis. The drawbacks of the invasive and noninvasive tracheal intubation techniques include the following two aspects: (1) operators must be well trained, and must possess technical knowledge and skills; and (2) these techniques are not suitable for application during first-aid treatment.

In this report, we describe the design of a novel respiratory face mask (RFM) and evaluate its

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performance in a rat model of myocardial ischemia. The technical parameters are also optimized. We tested the RFM repeatedly in animal models, keeping in mind its potential future use in humans. This kind of mask is suitable for the establishment of oxygen supply for rat model of myocardial infarction or myocardial ischemia.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing (180±10) g from the Fourth Military Medical University, China, were used for all experiments. All procedures were approved by the University of Animal Care and Use Committee, and were in accordance with the regulations of the Animal Welfare Act of the Fourth Military Medical University and Health Guide for the Care and Use of Laboratory Animals.

Design and preparation of RFM

The RFM developed in this study can be divided into two parts: a respiratory mask and a head/neck fixator. The respiratory mask contains a face mask, a stator, a T-tube, and a flexible hose (Fig.1). The bell-shaped face mask (0.1~0.2 cm thick) is made from plexiglass. Masks can be prepared in different sizes and shapes according to the body weight of the rats; for example, a 4.0 cm mask aperture, 2.5 cm internal diameter, and 5 cm height are suitable for rats weighing 150~220 g. The circular stator (0.5 to 0.8 cm thick) is also made from plexiglass. In addition, two holes are provided in the stator to allow rubber bands to pass through. The stator is fixed to the mask using trichloromethane, and its aperture size matches the internal diameter of the mask. The T-tube and the flexible hose should not be too long. The head/neck fixator is made from hard iron wire with a diameter of 0.3 cm. The neck support with a saddle shape provides excellent stability; the fixator can be installed at the end of the surgical board, and screws are employed to rivet the fixator to the bottom of the surgical board (Fig.2).

After anesthesia, the rat neck was placed in the neck support of the fixator and suitably adjusted with the cervical supporter. The respiratory mask was placed on the rat's head and was stretched as much as possible until the mandible was totally enveloped during artificial respiration. The rubber band of the fixing board was fastened to both sides of the surgical board, and the respirator was turned on. If leakage of oxygen was observed from the mask aperture, cotton was used to seal the leakage. Organic solvents should be avoided for cleaning or wiping the fixing board while preparing and using the mask.

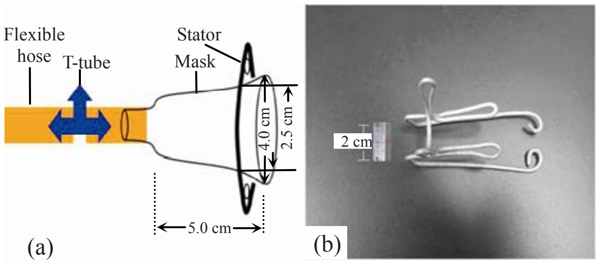

Fig.1 Schematic diagram of the RFM. (a) Respiratory mask. This model is suitable for rats weighing 150~250 g; (b) Prototype of the head and neck retainer. It is installed at the end of the surgical board. There are two circles at the end of the fixator where two screws are used to rivet the fixator to the surgical board

Fig.2 Rat fixed with the respiratory face mask

Experimental procedure

Myocardial infarction was induced by left coronary artery ligation. Rats were allocated into either the mask group (n=10) or the intubation group (n=10). The rats were anesthetized with pentobarbital sodium (40 mg/kg i.p.). When respiration was necessary, the mask was placed on the rat's face or oral intubation was performed with a 18G IV catheter, and ventilation was performed with a respirator (Medical Instrument Corporation of Zhejiang University, China) at 60 breaths per min and a tidal volume of 7 ml per breath in the mask group or 5 ml per breath in the intubation group. The chest and the fourth intercostal muscles were separated bluntly, the heart was externalized by distracting the third and fourth ribs with an eye speculum, and the proximal left coronary artery was ligated under a stereomicroscope. The chest was closed while the lungs were inflated to minimize pneumothorax. After the experiments, three different parameters were assessed: (1) blood gas analysis, conducted with blood samples obtained from the femoral artery at baseline, 10 min after thoractomy, and 15 min after spontaneous respiration; (2) operation time, calculated from anesthetic onset until the respirator was detached and autonomous respiration was resumed; and (3) intraoperative status.

Data analysis

Data were presented as mean±*SD*. Statistical significance was evaluated with Student's *t*-test. The difference was statistically significant when $P<0.05$.

RESULTS

The success ratios of intubation and modeling in the rats were 100% without intraoperative and postoperative death among rats from the two groups. Arterial blood gas indices of rats in the mask group were within the normal ranges at the different time intervals; there were no statistical differences in the indices between the mask and intubation groups (Table 1). Compared with rats in the intubation group, rats in the mask group had fewer intraoperative oral secretions, and after removal from the respirator, their autonomous respiration resumed. The mean operation time for rats in the mask group was (32±3) min, and that for the intubation group was (45±7) min ($P<0.05$). In the intubation group, one rat developed glottic edema, and four cases developed cannula and trachea adhesion, which is more likely to occur in dry climates and which delayed the detachment of the respirator from the rats and increased the operation time. After the operation, no anomalies, such as apathetic activity or a reduction in food consumption, were observed in either of the groups.

DISCUSSION

There have been reports regarding improvement of the 60 ml syringe (Wilson and Hatchell, 1991) and 50 ml centrifuge tube (Li *et al*., 2001) in respiration masks for rats, but they have been restricted to short-term experimental studies on the administration

Table 1 Arterial blood gas and acid-base parameters

Parameters	Value	
	Intubation group (n=10)	Mask group (n=10)
pH		
Baseline	7.35±0.03	7.34±0.02
10 min after thoracotomy	7.34±0.05	7.33±0.04
15 min after spontaneous respiration	7.32±0.03	7.33±0.05
PaO_2 (mmHg)		
Baseline	94.9±2.2	93.8±2.0
10 min after thoracotomy	92.8±2.1	92.1±2.7
15 min after spontaneous respiration	90.5±2.3	91.3±2.0
SaO_2 (%)		
Baseline	94.1±2.5	93.0±3.1
10 min after thoracotomy	91.6±2.3	91.4±2.5
15 min after spontaneous respiration	88.9±3.2	90.2±2.7
$PaCO_2$ (mmHg)		
Baseline	33.8±3.1	34.4±2.8
10 min after thoracotomy	34.7±2.3	35.1±3.5
15 min after spontaneous respiration	35.0±2.6	35.8±2.6
HCO_3^- concentration (mmol/L)		
Baseline	22.4±3.6	22.1±3.9
10 min after thoracotomy	22.1±3.8	21.8±3.7
15 min after spontaneous respiration	21.2±3.4	21.2±3.8

PaO_2: partial pressure of oxygen in artery; SaO_2: oxygen percent saturation in artery; $PaCO_2$: partial pressure of carbon dioxide in artery. $^{*}P<0.05$, $^{**}P<0.01$ vs the intubation group of anesthesia or inhalational gas (McBean *et al*., 1995; Dalton *et al*., 1996; Ayoub *et al*., 2001; Braun *et al*., 2001; Wood *et al*., 2001; Belayev *et al*., 2003). There have been no reports on the subject of an artificial respiration mask for the establishment of myocardial ischemia model in rats.

The RFM device developed in this study is cost-effective, easy to fabricate, simple to use, reproducible, and safe, and the components are readily available. The primary advantages of this device are that the operator needs no specific technical training and that the time taken to operate is decreased. In addition to these advantages, the head/neck fixator and fixing board fasten the mask stably to the head and face. Nevertheless, the mask has a disadvantage: some gas gets into esophagus, which, however, must be little. If oxygen is administered with the mask applied while establishing ischemia-reperfusion models, abdominal pressure will increase as ventilation time increases, but this will not influence autonomous respiration or the spontaneous activities of the rats. Abdominal pressure often returns to normal values within a postoperative period of 4~6 h.

In conclusion, it was found that the RFM device fabricated in this study maintained normal blood oxygen saturation levels and partial pressure of oxygen, had no airway stimulation, shortened operation time, and improved survival rate. Hence, it is an appropriate device for the establishment of myocardial infarction or ischemia-reperfusion models in rats.

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