Jurnal Kedokteran Syiah Kuala
Volume 22, Number 2, Juni 2022
Pages: -

ISSN: 1412-1026
E-ISSN: 25500112
DOI: 10.24815/jks.v22i2.23561

Three-way stop cocks as breathing circuit for anesthetic procedures of animal study on Wistar rats

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Abstract General anesthesia procedures used in experimental animals are not limited to research in the field of anesthesia. Ventilators and breathing circuit systems for research involving anesthesia procedures in rats are limited in Indonesia. The limitations of ventilators and breathing circuit systems are one of the reasons for the lack of complex and advanced animal experimental studies in Indonesia. Therefore, the authors would like to discuss a general anesthesia procedure for intubation in rats using tools and materials that are readily available in the clinical setting. An endotracheal tube insertion procedure can use a Miller size 0 laryngoscope. The endotracheal tube uses a 16 G intravenous cannula in which the needle is replaced with a small wire. The 3-way stop cock system can be used as a replacement for the Mapleson E system for the breathing circuit system. The fresh gas flow (FGF) source will be connected to the angled-port, while the other two ports will be connected to the reservoir and the intravenous cannula to the experimental animal. We use FGF 3-5 times as much as minute ventilation and use of a reservoir capacity similar to tidal volume for spontaneous ventilation. Therefore, the oxygen flow rate is set to about 1-1.5 L / min. Reservoir is not required for controlled ventilation.

Keywords: anesthesia; animal model research; breathing circuit; rats; research laboratory

INTRODUCTION

General anesthesia procedures used in animals are not limited to research in the field of anesthesia. Research requiring the post-procedure recovery of animal model needs general anesthesia that maintains the physiological state. The role of ventilator equipped with precise breathing circuit becomes important in maintaining the physiological respiratory system animal model during the procedure.

The most commonly used animal model is a wistar rat. Ventilator and breathing circuit system for research involving anesthesia procedures in rat are not widely available in Indonesia. To the author's knowledge, it is only the Indonesian Medical Education and Research Institute (IMERI) that has anesthetic delivery system facility made by Harvard apparatus (http://imeri.fk.ui.ac.id/equipment/). However, its use is limited to delivering anesthetic gas through the gas chamber. The limitations of ventilator and breathing circuit system are one of the causes of limited complex and advanced animal research in Indonesia. Especially, the study requires the use of intubation procedures and the administration of artificial ventilation.

Therefore, the authors wanted to discuss general anesthesia procedure in rat using tools and materials that are easily available in the clinical sphere. The authors hope that the research in the field of anesthesia in Indonesia becoming more advanced on the state of using animal models.

MAIN SECTION

Wistar rats (Rattus norvegicus) are often used as animal models because the treatment is quite easy and does not cost a great deal.[1] Male adult rats weigh 300-500 grams, while female adults weigh 250-300 grams. Their respiratory rate ranges from 70-160 times per minute, depending on their activeness. A minute ventilation of this strain is reported to be about 303 ml/min or 83 ml/100 gr/min.[2] The tidal volume vary about 2.5 ml - 4.5 ml. Inspiration phase duration: expiration is reportedly close to 1:1.3.[3] Some studies made ventilator setting with

tidal volume of 6 ml, respiratory rate of 60 times per minute, and inspiration: expiration ratio of 1:1.[4] Previous studie used tidal volume setting of 3-5 ml and respiratory rate of 72 times per minute.[5]

Research could involve wistar rats as models in the field of anesthesia. For example, a study used a tibial nervus injury model in mice to examine the effect of dorsalis radix modulation on chronic pain.[6] The procedure of creating tibial nervus injuries and implantation of stimulation electrodes in the dorsalis ganglion requires a general anesthesia procedure using inhaled gas delivered through the nose cone or gas chamber. Another complex example is a study of the effect of various ventilation strategies to prevent lung damage in abdominal surgical animal models.[7] This study required general anesthesia procedures using intubation and muscle relaxant agents during abdominal surgical procedures.

General anesthesia induction can be done by administering either intravenous or inhalation anesthesia agents. Intravenous anesthesia agents are relatively easy to administer, but it is difficult to administer continuously if the injection area is covered in sterile cover during a surgical procedure.[8] This can be overcome by the administration of volatile gas of anesthesia which also offers a faster recovery period. However, breathing maintenance remains a priority during the procedure, especially for complex procedures, such as model creation through cardiac or brain surgery.

The absence of advanced anesthesia machines is an obstacle to research in animal model study using general anesthesia procedures in Indonesia. General anesthesia is commonly administered through the anesthetic gas delivery system through a non-rebreathing breathing circuit connected to the rat's airway using a nosecone. However, the use of the nosecone is not able to maintain the patency of the airway.

The intubation procedure is an option in research that requires rats to be extubated and regain consciousness, not a tracheotomy. Intravenous cannula without needles can be used as endotracheal tube. The narrowest passage of the larynx is reportedly located at glottis with a diameter of about 1.5 mm.[9] Therefore, we chose an intravenous cannula with size of 16G and an internal diameter of 1.4 mm. Needles on intravenous cannula are replaced with small wire in order to avoid the risk of trauma. The larynx is located about 1.75 cm from the hard palate arch by forming a 35-degree angle on the sagittal field connecting the hard palate arch and mandibles as high as inferior incisor teeth when the mouth is open. Therefore, a small wire, serving as a stylet, is bent by 145 degrees at a point of 1.75 cm from the end to facilitate the insertion into the larynx. Carina is located about 35 mm from glottis or about 52 mm from the hard palate arch so that insertion using an intravenous cannula of 16 G with a length of 45 mm will provide sufficient depth without the risk of exceeding the carina position.

The insertion of an endotracheal tube can use Miller's laryngoscope size 0 (see figure 1).[10] The use of this laryngoscopy was chosen because anesthesiologists had become accustomed to the use of the tool. The help of an assistant is necessary to hold the upper incisor teeth with a thread and pull the tongue towards the ventral using the other hand. Glottis is usually covered by a soft palate when the tongue has been pressed by the laryngoscopy blade towards the ventral. When inserting intravenous cannula, glottis will be exposed when the cannula is inserted by slightly pressing the hard palate.

Figure 1. Intubation using Miller laryngoscope size 0

After intubation, intravenous cannula should be connected to the breathing circuit system. The main problem is the availability of anesthesia delivery systems or breathing circuits that are easily available in daily practice. Three-way stop cock system can be used as a replacement for the Mapleson E system. The three-way stop cock will function like a t-piece. The fresh gas flow (FGF) source is later connected in the angled port, while the other two ports are connected to the reservoir and intravenous cannula to the animal model (see figure 2). This aims to maintain positive pressure during expiration through the administration of FGF and avoid the formation of venturi

effects which will cause atmospheric gases to inhale. The 3-way stop cock shape with a 90-degree angle has an advantage. If the FGF port is angled towards the endotracheal tube, continuous positive pressure will form and increase resistance. If the angle leads to the reservoir, a venturi effect can form causing sub-atmospheric pressure.

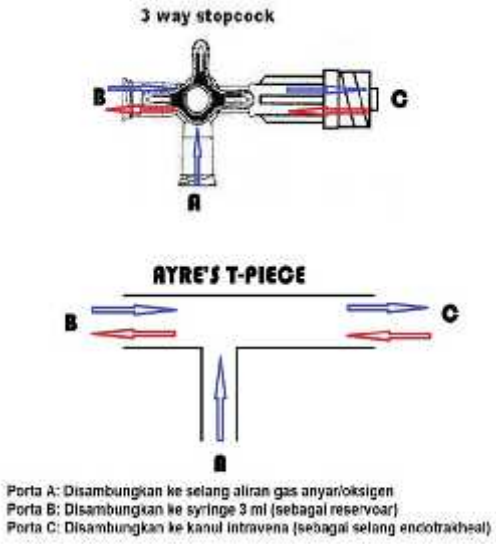

Figure 2. The adoption of Ayre's t-piece principle on 3-way stop cock

To understand the breathing system using Mapleson E, we must divide the respiration phase into 3 parts, namely: the inspiration phase, the expiration and the final pause of the expiration.[11] When the inspiration for spontaneous ventilation, the FGF will be entirely inhaled if the FGF exceeds the peak inspiratory flow rate (PIFR). If the FGF rate is less than PIFR, the inhaled gas is a mixture of FGF-derived gas and gas found in the expiratory arm. During the expiration phase, FGF and exhalation gas will flow into the reservoir arm to be disposed into the atmosphere. Then at the end of expiratory phase, the FGF will rinse the remaining gas and fill the reservoir arm.

The use of a 3-way stop cock will result in an additional dead space of less than 0.1 ml. This additional dead space is located from the end of the port connected to the intravenous cannula to the stopcock. This is calculated at a minimum. If using a nosecone, the dead space will increase quite a lot, so the volume of air inhaled again increases as well.

Reservoir uses a 3 ml syringe without a plunger. As per the principle of mapleson E, the use of reservoir with a minimum volume of tidal volume will minimize the inhalation of atmospheric gases when inspiring at spontaneous ventilation. The 3 ml syringe is chosen because the diameter is not large so as to prevent the mix of atmospheric gases and exhalation gases in the reservoir.

In the use of mapleson E, the FGF rate should exceed PIFR to ensure that there is no dilution of anesthetic gas due to inhalation of atmospheric gases and also prevent the rebreathing of gases in the expiratory arm. A study reported that mice had PIFR of up to 100 ml/s or 6 L/min.[3] The reservoir decreased the need for FGF rate to prevent inhalation of atmospheric gases during spontaneous ventilation inspiration. However, the FGF rate should also ensure rinsing mixed air in the reservoir during the expiation phase. An oxygen flow speed of 6 L/min will be equivalent to PIFR, ensuring no anesthesia gas dilution and rebreathing. However, gas wastage will be an issue.

Optimal FGF rate determination on spontaneous ventilation depends on respiratory rate, minute ventilation and expiratory arm capacity.[12] Since the pause of end-expiration must have been very short on the spontaneous ventilation of rat, the FGF rate required more than a minute ventilation to ensure rinsing the exhalation gas before the start of the inspiration phase. The FGF rate should be at least a minimum rate of 2.5-4 times when using a tidal volume-sized expiratory arm.[13] We can use the use of FGF rate 3-5 times the minute ventilation and the use of reservoir as much tidal volume for spontaneous ventilation. Therefore, the oxygen flow rate is set to about 1-1.5 L/min.

The anesthesia gas delivery system can use a simple anesthesia machine consisting of oxygen sources, pressure regulators, flowmeter, evaporator containing volatile anesthetic gases, port to the nonrebreathing system.[14] Controlled ventilation can be performed by closing the reservoir tip using the finger with a ratio of the duration of the inspiration phase and expiration in rats generally around 1:1. Reservoir is not required on controlled ventilation because atmospheric gas inspiration and re-inhalation do not occur, as has been done by Nugroho et al.[15]

The breathing system using 3-way stop cock has some drawbacks. The controlled ventilation will become more difficult by manual ventilation to ensure the long accuracy of the inspiration and expiration phases. The closure of the reservoir tips every half second is certainly difficult to achieve constantly. Intrapulmonary pressure cannot be well controlled, so may injure the lungs of animal models. Estimation of flow rate settings were extrapolated from studies of flow rates in humans. This certainly does not take into account the duration of each respiration phase. The risk of rebreathing and waste of gas needs to be further studied using gas analysis in order for this method to be developed to be a standard procedure. The study of resistance formed during the expiration phase is also required.

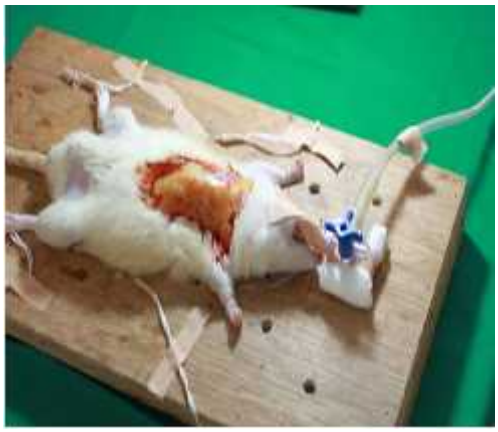

Figure 3. The application of 3-way stop cock on controlled-ventilation general anesthesia procedure

CONCLUSION

The use of 3-way stop cock as a non-rebreathing circuit system use mapleson E principle. The availability of tools and materials for general anesthesia procedures using those available in the daily practice will take animal model research in Indonesia to a further level.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interests.

ACKNOWLEDGMENTS

This work did not receive specific funding, but was performed as part of Ardyan Wardhana employment at the Faculty of Medicine, Universitas Surabaya, East Java, Indonesia.

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