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Literature Review

THREE-WAY STOPCOCK AS BREATHING CIRCUIT IN ANESTHETIC PROCEDURES ON WISTAR RATS AS ANIMAL MODELS IN RESEARCH

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ABSTRACT

Introduction: General anesthesia in experimental animals is not limited in the field of anesthesia research. In Indonesia, ventilators and breathing circuit systems utilized in research involving anesthesia in rats are not widely available. The limitations in using ventilators and breathing circuit systems in research are one of the reasons why Indonesia is lacking complex and advanced animal experimental studies. **Objective:** This study aimed to examine a general anesthesia procedure for intubation in rats using tools and materials commonly discovered in clinical settings. A search on the PubMed database using keywords consisting of animal study, rats, anesthesia, breathing circuit was performed. **Review:** An endotracheal tube insertion procedure may utilize a Miller size 0 laryngoscope, while the endotracheal tube may use a 16 G intravenous cannula in which the needle is replaced by a small wire. The 3-way stopcock system may be considered as a replacement for the Mapleson E system for the breathing circuit system. The Fresh Gas Flow (FGF) source needs to be connected to the angled port, while the other two ports are connected to the reservoir and the intravenous cannula which would be delivered to the experimental animals. FGF three to five times as much as the minute ventilation may be used and the use of a reservoir capacity is similar to the tidal volume of spontaneous ventilation. Therefore, the oxygen flow rate is set to approximately 1-1.5 L per minute. A reservoir is not required for controlled ventilation. **Conclusion:** The use of a 3-way stopcock as a non-rebreathing circuit system is effective because it utilizes the similar principle as Mapleson E. The ability to use common tools and materials for general anesthesia procedures would significantly boost research of animal models in Indonesia to a further level.

Keywords: Anesthesia; Animal Model Research; Breathing Circuit; Medicine; Rats; Research Laboratory

ABSTRAK

Pendahuluan: Prosedur anestesi umum yang digunakan pada hewan coba tidak terbatas pada penelitian di bidang anestesi. Ventilator dan sistem sirkuit pernapasan untuk penelitian yang melibatkan prosedur anestesi pada tikus terbatas di Indonesia. Keterbatasannya menjadi salah satu alasan kurangnya studi eksperimental hewan yang kompleks dan canggih di Indonesia. **Tujuan:** Penulis ingin membahas prosedur anestesi umum intubasi pada tikus menggunakan alat dan bahan yang sudah tersedia dalam praktik klinis sehari-hari. Kami melakukan pencarian di pangkalan data PubMed menggunakan kata kunci yang terdiri dari studi hewan, tikus, anestesi, sirkuit pernapasan. **Review:** Prosedur pemasangan selang endotrakeal dapat menggunakan laringoskop Miller ukuran 0. Selang endotrakeal menggunakan kanula intravena 16 G di mana jarum diganti dengan kawat kecil. Sistem *3-way stop cock* dapat digunakan sebagai pengganti sistem Mapleson E untuk sistem sirkuit pernapasan. Sumber aliran gas anyar (AGA) akan terhubung ke porta bersudut, sementara dua porta lainnya akan terhubung ke reservoar dan kanula intravena ke hewan eksperimental. Kami menggunakan AGA 3-5 kali lebih banyak dari ventilasi menit dan penggunaan kapasitas reservoar yang mirip dengan volume tidal untuk ventilasi spontan. Oleh karena itu, laju aliran oksigen diatur menjadi sekitar 1-1,5 L/menit. Reservoar tidak diperlukan untuk ventilasi terkontrol. **Kesimpulan:** Penggunaan *3-way stop cock* sebagai sistem sirkuit *non-rebreathing* menggunakan prinsip Mapleson E. Memanfaatkan ketersediaan alat dan bahan dalam praktik sehari-hari untuk prosedur anestesi umum akan membawa penelitian model hewan di Indonesia ke tingkat yang lebih lanjut.

Kata kunci: Anestesi; Penelitian Model Hewan; Sirkuit Pernapasan; Kedokteran; Tikus; Laboratorium Penelitian

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INTRODUCTION

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

Ventilators and breathing circuit systems for research involving anesthesia procedures in rats are not widely available in Indonesia. Indonesian Medical Education and Research Institute (IMERI) is possibly the only institution in Indonesian with an anesthetic delivery system facility manufactured by Harvard (http://imeri.fk.ui.ac.id/equipment/). However, the use is merely limited to delivering anesthetic gas through the gas chamber. The limitations of ventilator and breathing circuit system are one of the reasons why Indonesia is lacking in complex and advanced animal experimental studies. In addition, these studies require the use of intubation procedures and the administration of artificial ventilation.

Therefore, this study aimed to examine general anesthesia procedures in rats using tools and materials that are easily available in the clinical settings. It is hoped that, through this study, future research in the field of anesthesia in Indonesia may further advance in using animal models.

A search in PubMed database using keywords consisting of animal study, rats, anesthesia, breathing circuit was performed. References of various studies were also evaluated to identify additional relevant examination.

LITERATURE REVIEW

Wistar rats (Rattus norvegicus) are frequently used as animal models since the required treatment is relatively simple and economical. Being an animal model of cardiovascular pathology research contributes to understanding and providing treatment to a broad range of medical conditions. Specifically, in the context of acute myocardial infarction (AMI), rat models have been commonly used in studies of pathogenesis, investigations, and novel therapies, although there were often difficulties in translating experimental findings to clinical benefits. However, in recent years there have been two important changes in clinical approaches to AMI. First, there has been an increasing recognition of the pathophysiology in human AMI which has occurred at numerous levels, not merely within the epicardial coronary artery, but also within the microvasculature and the myocardium. Second, contemporary treatments are shifting from the thrombolytic dissolution of epicardial coronary thrombus to direct mechanical approaches using angioplasty and stents. These changes in the understanding of AMI present implications for the relevance of these animal models. The following discussions will review and examine the current rat models of AMI, place them in a clinical context, observe their advantages and limitations, as well as outline possible future developments, providing an overview of the place of these important models of AMI (1). Male adult rats approximately weigh 300-500 grams, while female adults approximately weigh 250-300 grams. Their respiratory rate ranges from 7 to 160 times per minute, depending on their agility. A minute ventilation of the strain is

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reported to be approximately 303 ml/min or 83 ml/100 gr/min (2). The tidal volume varies between 2.5 ml and 4.5 ml. Inspiration expiration ratio is reportedly close to 1:1.3 (3). Several studies created ventilator settings with a tidal volume of 6 ml, respiratory rate of 60 times per minute, and inspiration:expiration ratio of 1:1 (4). Previous study utilized tidal volume settings of 3-5 ml and respiratory rate of 72 times per minute (5).

Research may utilize Wistar rats as animal models in the anesthesia field. For example, a study used a tibial nerve injury model of mice to examine the effect of dorsalis radix modulation on chronic pain (6). The procedure of creating tibial nerve injuries and implantation of stimulation electrodes in the dorsalis ganglion required a general anesthesia procedure using inhaled gas delivered through the nose cone or gas chamber. Another advanced example is a study towards the efficacy 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 may be carried out by administering either intravenous or inhalation of anesthesia agents. Intravenous anesthesia agents are relatively simple to administer, but difficult to administer continuously if the injection area is covered in sterile cover during a surgical procedure (8). This may 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 devices in Indonesia has served as an obstacle in the advancement of animal model study using general anesthesia procedures. 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 nosecone is not able to maintain the patency of the airway during the procedure.

The intubation procedure is an alternative that requires the rats to be extubated and regain consciousness, which differs from tracheotomy. Intravenous cannula without needles may be used as endotracheal tubes. The narrowest passage of the larynx is reportedly located at the glottis with a diameter of approximately 1.5 mm (9). Therefore, an intravenous cannula with the size of 16G and an internal diameter of 1.4 mm may be selected. Needles on intravenous cannula are replaced with small wires to avoid the risk of trauma. The larynx is located approximately 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 wide 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 the glottis or approximately 52 mm from the hard palate arch, hence insertion using an intravenous cannula of 16 G with a length of 45 mm would provide sufficient depth without the risk of exceeding the carina position.

The insertion of an endotracheal tube may utilize Miller's laryngoscope size 0 (see figure 1) (10). This laryngoscopy was selected since anesthesiologists had become accustomed to the tool. The aid 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

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palate when the tongue has been pressed by the laryngoscopy blade towards the ventral. When inserting an intravenous cannula, the glottis will be exposed when the cannula is inserted by slightly pressing the hard palate.

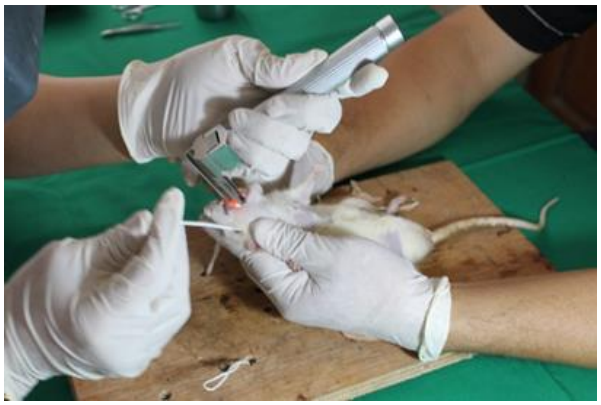

Figure 1. Intubation using Miller laryngoscope size 0

Following intubation, an intravenous cannula need to 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. A three-way stopcock system may be used as a replacement for the Mapleson E system. The three-way stopcock would function as a t-piece. The fresh gas flow (FGF) source is subsequently 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 would cause atmospheric gases to be inhaled. The 3-way stopcock shape with a 90-degree angle carries an advantage. If the FGF port is angled towards the endotracheal tube, continuous positive pressure would form and increase resistance. If the angle leads to the reservoir, a venturi effect may form causing sub-atmospheric pressure.

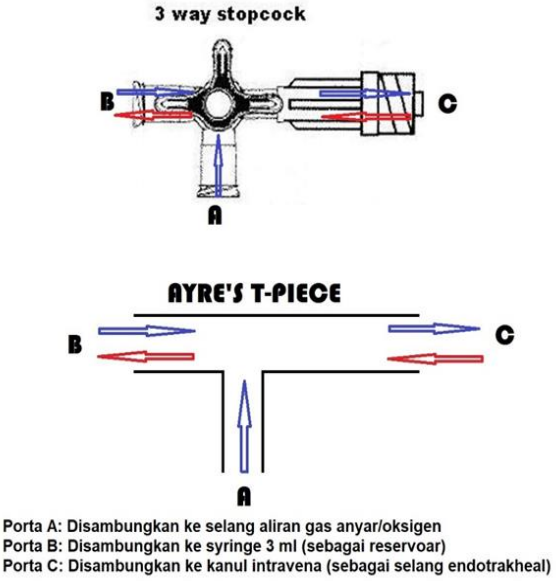

Figure 2. The adoption of Ayre's t-piece principle on 3-way stop cock

To understand the breathing system using Mapleson E, the respiration phase needs to be divided into 3 parts, namely: the inspiration phase, the expiration phase, and the final pause of the expiration (11). When the inspiration for spontaneous ventilation, the FGF would 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 would flow into the reservoir arm to be disposed into the atmosphere. At the end of the expiratory phase, the FGF would rinse the remaining gas and fill the reservoir arm.

The use of a 3-way stopcock would result in an additional dead space of fewer 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 a nosecone is used, the dead space would increase significantly, hence the volume of inhaled air increases as well.

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Reservoir uses a 3 ml syringe without a plunger. According to the principle of Mapleson E, the use of a reservoir with a minimum volume of tidal volume would minimize the inhalation of atmospheric gases when inspiring at spontaneous ventilation. The 3 ml syringe was selected since the diameter is not large enough to prevent the mix of atmospheric gases and exhalation gases in the reservoir.

In the use of Mapleson E, the FGF rate need to exceed PIFR to ensure that no dilution of anesthetic gas due to inhalation of atmospheric gases occurs and prevents 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 need to equally ensure rinsing mixed air in the reservoir during the expiation phase. An oxygen flow speed of 6 L/min would be equivalent to PIFR, ensuring no anesthesia gas dilution and rebreathing. However, gas wastage may become 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 rats, the FGF rate required more than minute ventilation to ensure the rinsing of 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). FGF rate of 3-5 times a minute ventilation and reservoir as much tidal volume for spontaneous ventilation may be used. Therefore, the oxygen flow rate is set to be approximately 1-1.5 L/min.

The anaesthesia gas delivery system may 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 may 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 at 1:1. The reservoir is not required on controlled ventilation because atmospheric gas inspiration and re-inhalation do not occur, as has been carried out by Nugroho et al (15).

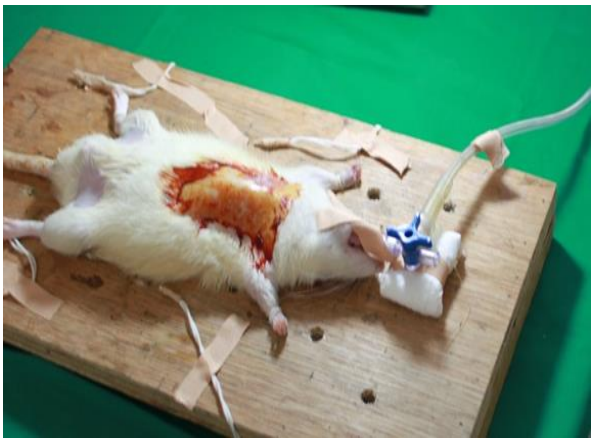

Figure 3. The application of 3-way stop cock on controlled-ventilation general anesthesia procedure

The breathing system using a 3-way stopcock presents several drawbacks. The controlled ventilation would 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 could not be well controlled, hance may injure the lungs of animal models. Estimation of flow rate settings was 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

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analysis for this method to be developed into a standard procedure. The study of resistance formed during the expiration phase is also required.

CONCLUSION

The use of a 3-way stopcock as a non-rebreathing circuit system is effective since it employs similar principle as Mapleson E. The ability to use common tools and materials for general anesthesia procedures would bring research of animal models in Indonesia to a further level.

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REFERENCES

1. Bhindi R, Witting P, McMahon A, Khachigian L, Lowe H. Rat models of myocardial infarction. Pathogenetic insights and clinical relevance. Thromb Haemost. 2006;96(5):602–10.
2. Snow SJ, McGee MA, Henriquez A, Richards JE, Schladweiler MC, Ledbetter AD, et al. Respiratory effects and systemic stress response following acute acrolein inhalation in rats. Toxicol Sci. 2017;158(2):454–64.
3. Whitehead GS, Kimmel EC, Reboulet JE, Still KR. Pulmonary Function in Normal Rats. 1999;(TOXDET 99-5). Available from: http://www.stormingmedia.us/56/5687/A568763.html
4. Breuer T, Bleilevens C, Rossaint R, Marx G, Gehrenkemper J, Dierksen H, et al. Dexmedetomidine Impairs Diaphragm Function and Increases Oxidative Stress but Does Not Aggravate Diaphragmatic Atrophy in Mechanically Ventilated Rats. Anesthesiology. 2018;128(4):784–95.
5. Proctor E, Fernando AR. Brit. J. Anaesth. (1973), 45, 139 ORO-ENDOTRACHEAL INTUBATION IN THE RAT. 1973;
6. Yu G, Segel I, Zhang Z, Hogan Q, Pan B. Dorsal root ganglion stimulation alleviates pain-related behaviors in rats with nerve injury and osteoarthritis. Anesthesiology. 2020;133(2):408–25.
7. Maia LDA, Samary CS, Oliveira M V., Santos CL, Huhle R, Capelozzi VL, et al. Impact of Different Ventilation Strategies on Driving Pressure, Mechanical Power, and Biological Markers during Open Abdominal Surgery in Rats. Anesth Analg. 2017;125(4):1364–74.
8. Rivard A, Simura K, Mohammed S, Magembe A, Pearson H, Hallman M, et al. Rat intubation and ventilation for surgical research. J Investig Surg. 2006;19(4):267–74.
9. Stark RA, Nahrwold ML, Cohen PJ. Blind oral tracheal intubation of rats. J Appl Physiol Respir Environ Exerc Physiol. 1981;51(5):1355–6.
10. Schaefer CF, Brackett DJ, Downs P, Tompkins P, Wilson MF. Laryngoscopic endotracheal intubation of rats for inhalation anesthesia. J Appl Physiol Respir Environ Exerc Physiol. 1984;56(2):533–5.
11. Kaul TK, Mittal G. Mapleson's breathing systems. Indian J Anaesth. 2013;57(5):507–15.
12. Inkster JS. The T-piece technique in anaesthesia: An investigation into the

Available at https://e-journal.unair.ac.id/IJAR | **DOI:** https://doi.org/10.20473/ijar.V4I12022.55-61

inspired gas concentrations. Br J Anaesth. 1956;28(11):512–9.
13. Harrison GA. Ayre's t-piece: A review of its modifications. Br J Anaesth [Internet]. 1964;36(2):115–20. Available from: http://dx.doi.org/10.1093/bja/36.2.115
14. Butterworth, J. F., Mackey, D.C., & Wasnick JD. Analgesic agents. In: Clinical anesthesiology. 5 th. New York: Mc Graw hill education; 2013.
15. Nugroho J, Yuniarti WM, Wardhana A, Ghea C. Modification on acute myocardial infarction model through left anterior descending coronary artery ligation: An experimental study on rats (Rattus norvegicus) using readily available materials. Vet World. 2019;12(9):1448–53.

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