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TERMINAL BLOOD COLLECTION IN RATS AND MICE: FACILITATION OF CARDIAC PUNCTURE TECHNIQUE BY ANAESTHESIA PROCEDURES

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ABSTRACT

Rodent families such as rats and mice are the most common lower rank animals preferred by researchers due to the many beneficial factors they bear for research investigations. One of the methods mostly used by researchers in the Institute for Medical Research (IMR) for their studies is the cardiac puncture technique facilitated by anaesthesia. This method requires general anaesthesia due to the pain that the procedure elicits in the rats and mice. During intracardiac phlebotomy, new and inexperienced researchers commonly encounter the death of animals due to injury of the thoracic cavity from multiple stabs of needles. Improper anaesthesia techniques may also cause failure in blood collection or an insufficient volume of blood obtained, which may affect data analysis and interpretation later. The anaesthesia drug of choice is either isoflurane for gas anaesthetic or a combination of Ketamine-xylazine via the intraperitoneal cavity. It is strongly suggested to use isoflurane gas in toxicity research as it does not cause any organ abnormalities. Thus, the aim of this article is to emphasise the protocol, anaesthesia drug of choice, benefits and drawbacks of the cardiac puncture method for researchers in IMR.

KEYWORDS: Rats and mice, Cardiac puncture, Institute for Medical Research

INTRODUCTION

Rats and mice are mammals in the family Rodentia. The unique features of rodents are distinguished by the presence of continuously growing pairs of incisors in the upper and lower jaws (1). Both rats and mice are small mammals that have always been the choice to be used in studies and many research investigations (2). Several reasons why researchers rely on these small mammals include the size, ease of maintenance requirements, adaptation to the environment as well as quick reproduction (2). Mice and rats' genetic, biological and behavioural characteristics that closely resemble those of human beings make them the best test systems to represent human body reactions (2).

The importance of gathering raw data from these animals has always been emphasised and blood samples are among the most frequently collected and easily accessible data. Various methods can be used in order to collect blood from these animal families. For instance, retro-orbital sinus bleeding, initial tail snip and intracardiac bleeding techniques require the facilitation of general anaesthesia (3). The most popular approach employed by researchers in IMR is to collect large amount of blood via cardiac puncture, which is believed to be the easiest and fastest method of bleeding. Though this technique of phlebotomy appears to be a simple method, it requires ethical considerations to ensure that animal welfare is not compromised. As such, the application of the Animal Care and Use Committee (ACUC) under the Ministry of Health has made compulsory for all animal research to ensure that all procedures are complying with the ethical requirements.

The appropriate technique and a competent handler to perform blood collection are needed to certify that the pain, distress, or discomfort generated from the sampling is minimal. Furthermore, sampling techniques and routes must be chosen properly to assist in the collection of blood depending on the amount required. A standard and proper protocol must be established to guide researchers and handlers to apply an appropriate procedure for these animals. Thus, in this article, the types of drugs and methods of administration to be applied to the rats and mice prior to cardiac puncture will be explained thoroughly. Since this phlebotomy method is a blind stick, it is necessary for the handler to acquire proper training and ample experience to perform the technique well.

New and inexperienced researchers in animal work are required to acquire proper training on methods of handling and techniques for intracardiac phlebotomy. Even if the procedure seems simple, the most common mistake observed are when the needle is inserted too deep into the thoracic cavity; which could cause the needle to either miss or stab through the heart. Consequently, this leads to the death of animals without being able to collect blood samples for data analysis. This article intends to guide and explain the basic techniques available to researchers during hands-on procedure. Moreover, the types of drugs used and the method of administration to be applied to rats and mice prior to the procedure will also provide some idea for the preparations required before cardiac puncture. The aim of this article is to explain the standard protocol of terminal blood collection via intracardiac for researchers in IMR, its advantages, drawbacks, and the range of chemical restraints used in laboratory rodents; especially rats and mice.

MATERIALS AND METHODS

Anesthesia Procedures

In order to perform cardiac puncture, the rodents must be properly anaesthetized prior to the procedure using either injectable or gas anaesthetic agents. For the terminal blood collection technique, it is required for the laboratory rodent to reach a deep anaesthetic plane due to the painful procedure applied. The most reliable method to assess whether it was deeply anaesthetized is via the absence of pedal withdrawal or with a tail pinch reflex in rats and mice (4). Moreover, the mucous membrane must be ensured to be pink in colour, not purple or bluish. A drug combination of ketamine and xylazine can be used to achieve general anaesthesia in these laboratory rodents. Besides, usage of inhalant anaesthetic agents such as isoflurane with the assistance of vaporisers has to be done in 2 stages of anaesthesia, which are the induction using chamber and the maintenance of anaesthesia by mask or nose cone (Table 1).

Blood collection technique by cardiac puncture

a) Needle size

The proper size of the needle is determined based on puncture sites and the size of the animal. The appropriate size for intracardiac blood withdrawal on rats is 21-23G, while, a 23G needle between 0.5 – 1.5 inches in length is preferred for mice (for diaphragm approach).

b) Syringe size

The choice of syringe size is influenced by blood withdrawal volume and animal size. For intracardiac blood collection, a 1 – 3 mL syringe is sufficient for mice,whereas, in rats, a 5 mL syringe is suitable.

c) Protocol

The predetermination of the blood sample volume can be done by calculating the total blood volume of the rodent used. As an illustration of the intracardiac phlebotomy method, a male Sprague Dawley rat weighing 250 g was facilitated by isoflurane gas for anaesthesia during this procedure. A reference on the calculation of maximum sample volume in a rat or mice is shown in Table 2.

A detailed protocol of intracardiac phlebotomy was explained as below:

1. Prepare suitable needle sizes and syringes for blood withdrawal.
2. Place a deeply anaesthetized rodent in dorsal recumbency on a flat surface.
3. Place the index finger of the non-dominant hand on the chest of the rodent and find the strongest heart pulsation.
4. Insert the needle through the desired approach site, either from;
   i. the left side of the chest (Figure 1)
   ii. the diaphragm from the xiphoid cartilage (Figure 2)
   iii. the top of the ribs (between the two ribs) (Figure 3)
5. Observe for a drop of blood to enter the needle hub, as an indicator of accurate needle insertion into the heart.
6. Pull the syringe plunger slowly and steadily without moving the syringe to withdraw blood.
7. Withdraw the maximum amount of desired blood from the rodent.
8. Disconnect the syringe from the needle once it is full and re-connect a new empty syringe to the needle.
9. Use a new syringe to collect more blood, if desired.
10. Carefully transfer the withdrawn blood into the desired blood tubes.

Table 1. Anesthesia methods and dosages for mice and rat

ANIMAL SPECIES	ANESTHESIA METHOD	DOSAGE AND METHOD
MICE / RAT	Inhalation anaesthesia *lubricate the eye with eye ointment to prevent dry eye	1. Induction with induction chamber: 4% Isoflurane 1-2 L/mol flow rate of O^2 (Flow rate of O^2 follows the same volume of chamber size) 2. Observe animal until it has lost righting reflex and respiratory patterns started to slow down 3. Turn isoflurane vaporiser dial to 0% 4. Turn the valve so that the nose cone valve is 'on' and turn 'off' the valve flowing to the chamber 5. Maintenance using nose cone: 1.5-3% Isoflurane
MICE	Injectable anaesthesia	**Ketamine/Xylazine** 75-100 mg/kg ketamine, IP 10 mg/kg xylazine, IP
RAT	Injectable anaesthesia	**Ketamine/Xylazine** 80-100 mg/kg ketamine, IP 10 mg/kg xylazine, IP

Table 2. Total blood volume calculation and maximum sample volume in rat and mouse

Species	Range of weight (g)	Blood volume (mL/kg)	Total blood volume (TBV), normal adult (mL)	Maximum sample volume (mL)
Rat	250 - 500	54 - 70	Male 29-33 Female 16-19	Male 13-15 Female 7.5 - 9
Mouse	18 - 40	58.5	Male 1.5 - 2.4 Female 1.0 - 2.4	Male 0.8 - 1.4 Female 0.6 - 1.4

11. Euthanise the animal immediately after the procedure.

RESULTS AND DISCUSSION

There are several routes of blood sampling in mice and rats; however, each method holds its own advantages and disadvantages. This includes the parameters of clinical pathology (5), stress level, activity and tissue damage in an animal after a phlebotomy procedure (6). Basically, to draw a high amount of blood for several laboratory procedures, one of the most preferred phlebotomies is through the intracardiac as heart muscles are less collapsible compared to vein blood collection (5). Moreover, intracardiac phlebotomy offers good consistency in terms of the values and variations in clinical pathology results (5).

In general, rats with an average body weight of 300 g have a total blood volume of 21 mL, of which approximately 13-15 mL of blood per rat could be collected through intracardiac phlebotomy. The handler must be aware that the collection of a certain amount of blood should be performed with a single stab, avoiding the need for repetition that could cause blood leakage to pool within the thoracic cavity, leading to death. The death of an animal happens to be one of the main concerns in phlebotomy procedures that lead to the loss of samples or data collection.

Since this method of phlebotomy is a blind stick, it is necessary for the handler to acquire proper training and sufficient experience to perform the technique well. The most common mistake is when the needle is inserted too deep into the thoracic cavity; causing the needle to either miss heart or stab through the heart. As a result, little or no blood sample is collected, yet the test system may die of respiratory failure as a result of loss of negative pressure in the thoracic cavity due to the puncture injury made by needle stab.

A 23G hypodermic needle is deemed suitable for use with rats and mice with an average bodyweight of 300 g and 25 g, respectively. However, the use of larger gauge needles such as 21G, may also benefit this procedure as the sample can be collected faster due to the larger size of the needle bore. Smaller needle bores will result in longer times for blood draw, which may affect sample quality as the injury resulting from the needle stab will basically stimulate haemostasis thus leading to a blood clot in the needle hub. The handler must also not neglect the importance of mixing the blood with anticoagulants in order to collect plasma since insufficient mixing will result in an unusable sample. Thus, by ensuring method of drawing blood is done efficiently and carefully, this problem can be prevented (7).

Additionally, researchers must always be mindful that intracardiac procedures will cause trauma to the heart. Arora (8) found several pathological alterations post intracardiac procedure, which are haemopericardium, ventricular haemorrhage, formation of fibrous tissues in the myocardium and other complications. Thus, researchers will have to consider the artefacts that arise due to the traumatic injury of the myocardium, if the heart is included for tissue evaluation in research investigations.

Another method of cardiac puncture is done by exposing the heart through a surgical procedure and performing a right ventricular cardiac puncture, which allows for a more accurate and consistent blood draw. Conversely, the technique that is explained in this protocol does not involve cutting into the chest of an animal and is said to be a less violent method (9). This study found most respondents preferred cardiac exsanguination as a method of euthanasia due to the fact that the method was less bloody and messy, with the benefit of a high amount of blood for sample analysis.

Terminal blood collection through the intracardiac is a method where a maximum amount of blood is withdrawn from an animal. In a study

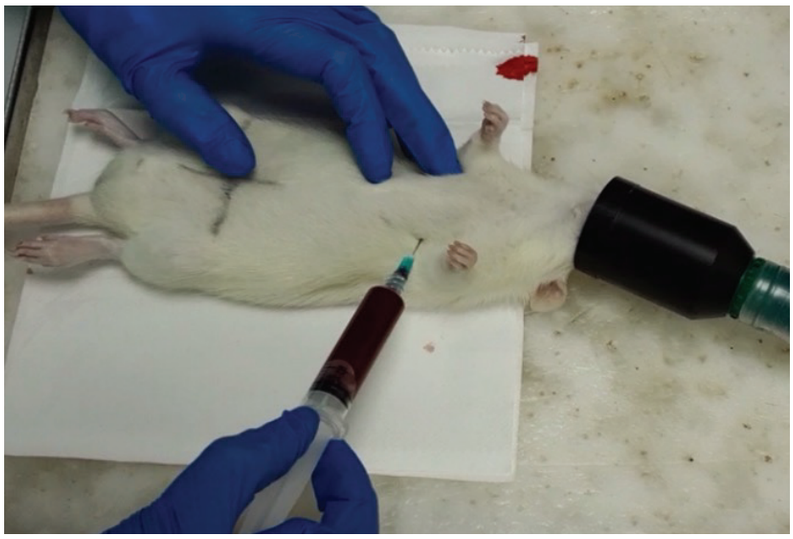

Figure 1. Blood sample collection via the left side of the chest

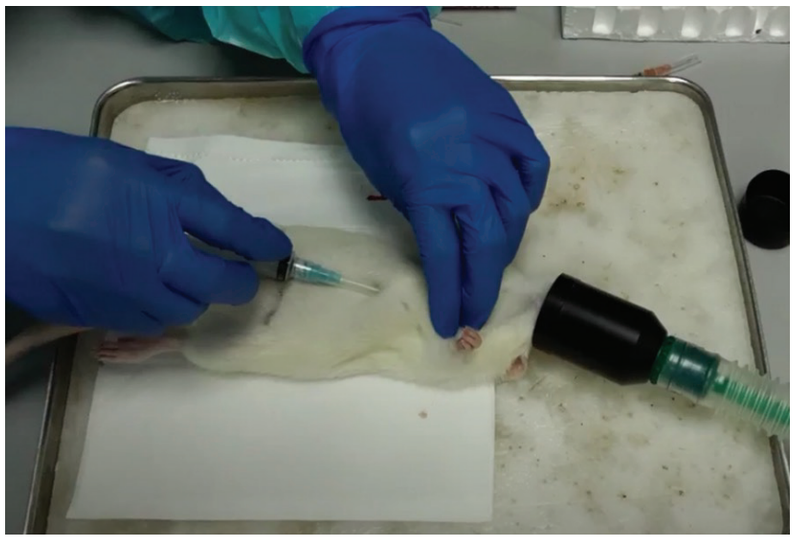

Figure 2. Blood sample collection via diaphragm from the xiphoid cartilage

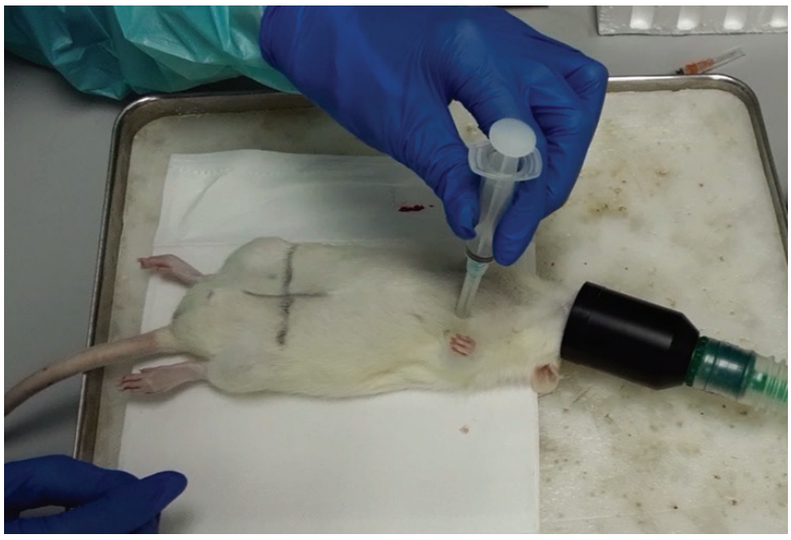

Figure 3. Blood sample collection from the top of the ribs (between the two ribs)

that requires a high amount of blood, intracardiac phlebotomy offers a good harvest where almost half of the total blood volume in an animal can be collected (3). However, animals undergoing this procedure must be deeply anaesthetised to ensure that the animal suffers as little as possible as a result of the painful procedure. According to American Veterinary Medical Association (AVMA) (10) Guidelines for the Euthanasia of Animals, euthanasia by means of exsanguination is allowed in a condition that the animal is completely anaesthetised. Besides, the guidelines also mentioned that an ideal euthanasia will result in a rapid loss of consciousness followed by cardiac or respiratory distress, together with eventual loss of brain function with minimal distress and anxiety effect on the animal. Furthermore, according to Williams et al. (11), most literatures have suggested that humane euthanasia by exsanguination during cardiac puncture is possible provided the animal is under sedation.

Isoflurane has been commonly used by most researchers as an inhalant anaesthetic during *in vivo* research, since it provides rapid induction and recovery from anaesthesia apart from the minimal biotransformation (12). Furthermore, due to its minimal effect on liver microsomal enzymes, isoflurane is recommended for use in inducing anaesthesia in animal studies involving drug metabolism and toxicology (13). A report from a previous study on the usage of isoflurane also showed that there is only an increase in the serum calcium ion concentrations in haematology and blood chemical results, which shows that it is good for toxicity studies (14). In addition, Nagate et al. (14) also performed an animal necropsy study where they found that isoflurane did not impart any abnormalities in the test system's main organs and tissues, particularly the lung and trachea, which are exposed directly to the drug. Hence, it can be concluded that the use of isoflurane is suitable, especially in toxicity research. Another type of anaesthesia commonly administered is the combination of Ketamine and Xylazine. The combination of these two drugs provides a relatively safe anaesthesia method through a single intraperitoneal injection (15).

CONCLUSION

Researchers should always follow a proper standard procedure in order to collect blood samples from laboratory animals, such as rats and mice. The aim is to minimise stress, pain and any other physiological reactions to human manipulations that are commonly reflected in samples collected. Even though the intracardiac phlebotomy method is approved for euthanasia, the application of general anaesthesia is necessary to ensure the animal is in a deep stage of anaesthesia due to cardiac puncture, which is a painful process. Moreover, the assistance of this phlebotomy method by the use of anaesthesia like isoflurane is highly suggested, particularly in toxicology research.

ETHICAL APPROVAL

The use of rodents in this manuscript was approved by the Animal Care and Use Committee (ACUC), Ministry of Health, with approval number ACUC/KKM/01(03/2018)(04).

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