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EFFECT OF ISOFLURANE ANESTHESIA ON THE VITAL SIGNS MONITORING IN LABORATORY MICE

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Abstract

Inhalation anesthesia systems are used in laboratory animal experimentation due to their safety, easily adjustable dosage and rapid return to consciousness. Isoflurane is currently the most common volatile anesthetic used in mouse studies. The study aimed to investigate the influence of isoflurane on the vital functions during inhalation anesthesia in two mouse strains (inbred and outbred), suitable for blood collection and minor surgery. Heart rate, pulse distention, respiratory rate, peripheral arterial oxygen saturation and rectal temperature were measured during anesthesia and compared to the same parameters measured on awake animals. Results showed a decrease in the heart rate by 26% during 2% isoflurane anesthesia, while the breath rate decreased by 42%. Oxygen saturation remained at 95%–98% and the vascular distension caused by the pulse was relatively constant. Both groups showed a decrease in the rectal temperature by 1,6-2.2°C during anesthesia, with temperature values normalizing in 1.5-2 hours after anesthesia. The overall effects of isoflurane on mice vital signs were moderate, both induction and recovery from anesthesia proceeded quickly (1-4 minutes), with a rapid return of the animals to their normal state.

Key words*: inhalation anesthesia, isoflurane, vital signs, laboratory mouse.*

INTRODUCTION

Current attitudes toward animal research are best summarized by the "3Rs concept" (replacement, reduction and refinement). The concept of refinement aims to reduce to a minimum the pain, suffering and distress experienced by animals used for experimental purposes. Adopting best practice for commonly used procedures, such as injections, blood sampling and surgeries, can enormously improve animal welfare and can increase the reliability and validity of experimental results (Demers et al., 2006; Hubrecht & Carter, 2019).

When following the basic principles of laboratory animal welfare, the selection of an appropriate and effective anesthetic protocol for each individual animal is an essential part of laboratory animal experimentation (Aras et al., 2001; Richardson & Flecknell, 2005). General anesthesia may be used for surgical and non-surgical procedures and is also referred to as surgical anesthesia. Properly induced and maintained general anesthesia with effective monitoring is vital to maintaining animal welfare and creating reproducible studies.

Mouse anesthesia is challenging for several reasons including the animal size, metabolic rate, and the high risk of hypothermia and hypoglycemia. Moreover, anesthetic agents influence physiological parameters, further interfering with experimental results (Gargulio et al., 2012; Navaro et al., 2021). Laboratory mice are anesthetized by either inhalation of volatile anesthetics or injection of anesthetic drugs.

Inhalant anesthesia is highly demanded in rodents because the anesthetic depth can be easily controlled (Tsukamoto et al., 2015). Inhalant anesthetics available for use in laboratory animals include halothane, isoflurane, sevoflurane, and desflurane. Among them, isoflurane is most the common inhalant anesthetic used in mice (Gargulio et al., 2012; Richardson & Flecknell, 2005; Tsukamoto et al., 2015; Navaro et al., 2021). Isoflurane is

presently the animal inhalation anesthetic agent of choice for both short and lengthy procedures due to its short induction and recovery time and the reliability of its effects (Cesarovic et al., 2010). Isoflurane is also used for very short procedures because it enables mice manipulation and injection, blood collection, and minor surgical procedures (Gargulio et al., 2012).

The study aimed to investigate the influence of isoflurane on the vital functions during inhalation anesthesia in two mouse strains (inbred and outbred). Heart rate, pulse distention, respiratory rate, arterial oxygen saturation (SpO_2) and rectal temperature were measured during anesthesia and compared to the same parameters measured on awake animals. Also, a series of reflex measurements were carried out on each individual mouse to evaluate the depth of anesthesia.

MATERIALS AND METHODS

This study was carried out in compliance with the principles of ethics and in accordance with the provisions of EU Directive 63/2010 on compliance with the rules for the care, use and protection of animals used for scientific purposes.

The study was also approved by the Ethics Committee of "Cantacuzino" National Medico-Military Institute for Research and Development and approved by the competent authority.

10 NMRI female mice (outbred strain) and 10 C57BL/6 female mice (inbred strain), 12 weeks old were used in the study. The animals were provided by Băneasa SFP (Specific Pathogen Free) Animal Facility area for rats and mice of "Cantacuzino" National Medico-Military Institute for Research and Development, Bucharest.

All aspects related to animal housing and care were undertaken in accordance with the national and international regulations concerning animal testing. The animals were acclimated to the facility for 1 week before experimentation and housed under standard conditions, 10 mice per cage, 18-24℃ temperature, 35-75% humidity and light controlled conditions (12 h/12 h light and dark cycles).

For the vital signs monitoring we used MouseOx Plus Pulse Oximeter for Mice and Rats (Starr Life Sciences Corporation, USA). Heart rate, arterial oxygen saturation (SpO2), pulse distention, breath rate, and temperature were evaluated for each group (Figure 1). Animals were monitored by placing the collar sensor on the back of the neck and the rectal sensor for temperature measurement. Previously, animal hair was removed from the cervical dorsal region for a better signal detection. The awake animals were easily restrained in this position and the vital signs were recorded on the MouseOx Plus integrated software (Figure 2).

Figure 1. MouseOx Plus pulse oximeter used in mice for noninvasive physiological vital signs monitoring

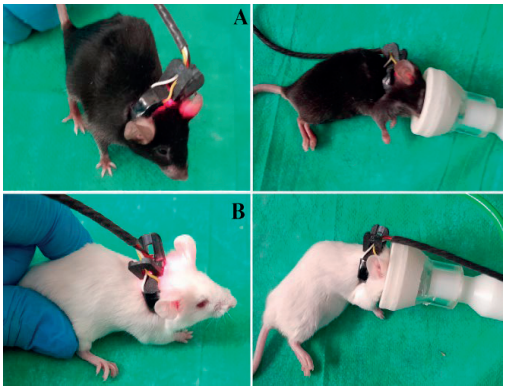

Figure 2. Vital signs monitoring on awake/ anesthetized mice: A- C57BL/6; B- NMRI

Figure 3. Ugo Basile Veterinary anesthesia workstation for small animals

Isoflurane anesthesia (Anesteran, Rompharm, Romania) was administered using an available rodent inhalant anesthesia system (Ugo Basile Veterinary anesthesia workstation 21100, Italy) which has a digital vaporizer and internal air-flow pump, an induction chamber and a nose-cone, connected to an evacuation tubing (Figure 3). The mask incorporates a latex diaphragm, which holds the rodent nose, keeping the animal in correct position and ensuring a continuous positive flow of fresh oxygen and anesthetic.

The animal was placed into the induction chamber and the O_2 flow meter was turned on to a flow rate of 1 L/min. Isoflurane exposure can be aversive so for induction gas concentration was set at 0.5% flow rate and slowly increased up to 5%. The induction time was defined as the duration between the loss of righting reflex and the commencement of surgical anesthesia.

Once loss of the postural reaction and righting reflex was confirmed, the mouse was rapidly transferred to the nose mask. Collar sensor and temperature sensor were placed on the right position and measurements were recorded for a 20 minutes period. Anesthesia was maintained with 2% isoflurane concentration at a flow rate of 1 L/min and the animal was placed on a nylon pad to maintain a constant surface temperature underneath.

A series of observations and reflex measurements were carried out on each individual mouse to evaluate the depth of anesthesia. Tail pinch, pedal withdrawal in the forelimbs/hind limbs, and abdominal skin pinch were applied at 5 min intervals by using blunt forceps containing a spacer between its arms. The reflex tests were registered as positive or negative, whether any motor response was observable or not. To reduce sources of variation in response to the stimuli, all reflex tests were carried out and assessed by the same operator. After 20 minutes aneshesia, isoflurane was turned off and each animal was monitored continuously while keeeping the nose-cone on oxygen flow running for another 2-3 minutes. To assess mice recovery after discontinuing inhalation system, the vital signs were monitored another 10 minutes and the rectal temperature was measured every 15 minutes for the next 120 minutes.

RESULTS AND DISCUSSIONS

During the study, there were no fatal events and all mice returned successfully and rapidly to their normal state (1-4 minutes). Vital signs were first measured on awake animals and then in 5 minutes from the isoflurane anesthesia being installed. Values are given as means for each group and significant differences are analyzed using an unpaired Student's T-test. P-values <0.05 were considered statistically significant.

Results showed a decreased heart rate by 24.74% in NMRI group and 28.5% in C57BL/6 group during anesthesia and a return to the initial heart rate level within 8-10 minutes after discontinuing inhalation system (Figure 4). Respiratory rate significantly decreased in the first 5 minutes after gas exposure, by 39.2% in NMRI group and 53.12% in C57BL/6 group. However, breath rate returned to the initial values in 5-7 minutes after turning off the anesthetic gas. (Figure 5). SpO_2 recorded a slight decrease during anesthesia but none of the animals showed an O_2 saturation under 95.8% (Figure 6). Under stable anesthesia, the pulse distension was relatively constant for

both groups, with a slight decrease in 5 minutes after isoflurane exposure (Figure 7).

Temperature measurement during anesthesia showed a mean decrease by 1.8°C for NMRI mice and by 2.2°C for C57BL/6 mice. After anesthesia, rectal temperature returned to the initial level within 97 minutes for NMRI mice and 123 minutes for C57BL/6 mice (Figure 8).

Finally, we evaluated the anesthetic depth. When a reflex reaction to stimulation was observed, the score was 0 and when no reaction to stimulation was observed, the score was 1. Each measurement was scored and noted, and the anesthetic depth was calculated by total score for each mouse. A score of 3 or more was defined as surgical anesthesia. Under 2% isoflurane maintenance, none of the mice showed a motor response to testing of the pedal withdrawal reflex, tail pinch or abdominal skin pinch. Based on these tests, all of the animals monitored in both groups achieved surgical anesthesia.

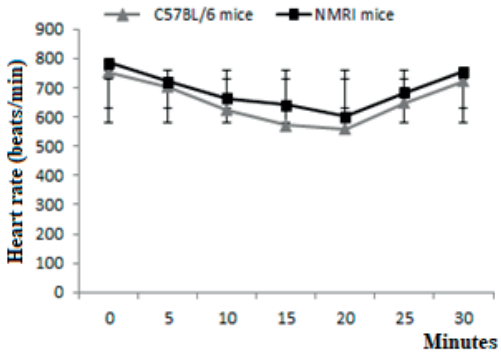

Figure 4. Heart rate measurement (beats/min) over time

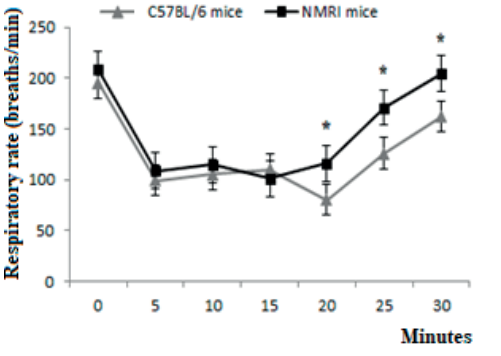

Figure 5. Respiratory rate measurement (breaths/min) over time

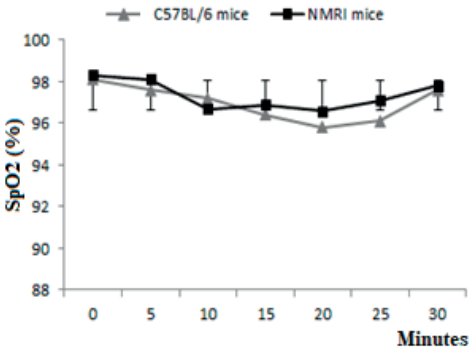

Figure 6. SPO2 (%) measurement over time

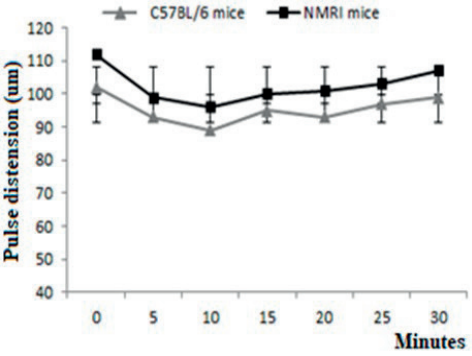

Figure 7. Pulse distention (µm) measurement over time

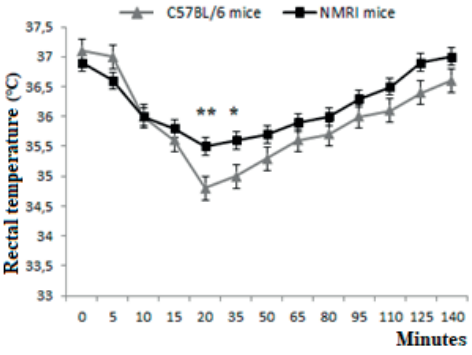

Figure 8. Temperature measurement (°C) during anesthesia and recovery

Concern regarding animal welfare and the quality of data obtained from research projects using animals is increasing.

A suitable, effective and free of complications anesthetic protocol is very important in experimental animal studies although there is no universally accepted protocol for induction, maintenance and recovery from anesthesia (Cicero et al., 2018).

The main factor in selecting an appropriate anesthesia is the required level of anesthetic depth that varies depending on the experimental procedure. Another important factor is the potential for cardiorespiratory adverse reactions. (Arunachalam & Sasidharan, 2021; Tsukamoto et al., 2015). Cardiovascular and respiratory function can be assessed by monitoring vital signs, which include the heart rate, respiratory rate, pulse distention, and arterial oxygen saturation (SPO2).

As mentioned previously, laboratory mice exhibit specific anatomic and physiologic peculiarities that influence the effects of anesthetic drugs. Due to their small body size, drug metabolism and excretion are extremely fast, reducing the half-life of injectable drugs and rendering the duration of anesthesia a more critical factor (Gargulio et al., 2012). Moreover, when compared with larger species, the higher ratio of body surface area to body volume in mice promotes heat loss and hypothermia, while their reduced glycogen reserve predisposes them to hypoglycemia. In addition, their high oxygen consumption rate reduces the survival rate for hypoxemia (Navaro et al., 2021). Small body size of mice also causes physical problems. For example, endotracheal intubation and ventilation of this species are demanding tasks, and many veterinary anesthesia monitoring devices could not be used for mice anesthesia (Ahmadi et al., 2022).

The most commonly utilized inhalant is isoflurane, although sevoflurane has also been used successfully in mice studies (Navaro et al., 2021; Richardson & Flecknell, 2005; Lipiski et al., 2017; Cesaronic et al., 2010).

For surgical intervention and short-term experimentation, isoflurane is the most frequently used anesthetic in mice studies, in part because of its moderate cardio-depressive effects in comparison to the injectable agents - pentobarbital/urethane or ketamine/xylazine mixture (Constantinides et al., 2021).

Isoflurane does not sensitize the myocardium to catecholamines, and it spares cardiac output more than other volatile agents. Indeed, isoflurane can cause a more severe respiratory depression compared with halothane and a dose-dependent hypotension (Gargulio et al.,2012). Isoflurane is poorly soluble in blood and undergo minimal metabolism. As a result, recovery after anesthetic discontinuation is rapid, even in animals with significant hepatic or renal impairment (Lee-Parritz, 2013).

Our study results showed an important respiratory depression (more pronounced for C57BL/6 group) while SPO_2 remained relatively stable under 2% isoflurane anesthesia. The heart rate recorded a moderate decrease during anesthesia with a rapid return to the initial level and slight variation of the pulse distension.

Tsukamoto et al. concluded that the heart rate decrease with isoflurane was lower than that observed in the injectable anesthetics (pentobarbital monoanesthesia; ketamine and xylazine combined; medetomidine, midazolam, and butorphanol combined), suggesting that isoflurane produces the smallest cardiac influence. However, compared to the injectable anesthetics, isoflurane administration showed a prominent decreased breath rate, respiratory depression being the major adverse effect.

Constantinides et al. compares the stability of heart rate (HR), and body temperature under isoflurane different concentrations and observed that the most stable blood pressure and heart rate accompanied an inhalant anesthetic dose level of 1.5%.

Ewald et al. showed that under stable anesthesia the vascular distension caused by the pulse is relatively constant and is of higher amplitude than the vascular distension caused by breathing while chronic exposure to excess isoflurane (2.5%) results in a decline in the pulse distension.

Picard et al. evaluated the effects of isoflurane dose on myocardial function in a murine model and concluded that light isoflurane anesthesia is preferable for hemodynamic analysis or anesthesia in a cardiac disorder model.

Various mice studies investigated the minimum alveolar concentrations, defined as the minimum concentration of inhaled anesthetic at which 50% of the animals fail to respond with purposeful movements to the testing of reflexes (Aranake et al., 2013).

The minimum alveolar concentration for isoflurane is estimated to be 1.4% to 2.0% depending on the severity of the noxious stimulus, where the stimulus is applied, and animal-specific factors such as mouse strain,

underling health conditions, age, gender, etc. (Aranake et al., 2013; Sonner et al., 2000).
Cesaronic et al. compares the effect of isoflurane and sevoflurane in laboratory mice anesthesia and established mean minimum alveolar concentration as 1.85% for isoflurane and 3.25% for sevoflurane in C57BL/6J mice. The most prominent side-effect they observed during anesthesia was respiratory depression with hypercapnia, acidosis and a marked decrease in respiration rate.
Hypothermia is one of the most common complications during anesthesia, and mice are particularly susceptible because of their high surface area to mass ratio (Navaro et al., 2021; Buitrago et al., 2008). Hypothermia has several negative consequences, including cardiac arrhythmias, hypercoagulability, increased susceptibility to infection, delayed recovery and decreased minimum alveolar concentrations for inhalant anesthetics. (Flecknell, 2016; Gargiulo et al., 2012). Baseline body temperature measured rectally for C57BL/6 mice at the start of isoflurane anesthetic exposure was previously found to be 36-37°C with a 2°C decrease in body temperature during prolonged anesthesia (Caro et al., 2013). Therefore, hypothermia must be prevented and controlled by providing heat through warming pads and infrared lamps.
An important factor that should be considered when using isoflurane is the lack of analgesic properties provided by inhalant anesthetics. During anesthesia, isoflurane provides unconsciousness so pain perception is absent but during recovery an effective analgesic protocol should be considered depending on the experimental procedure. (Navaro et al., 2021; Flecknell, 2016).

CONCLUSIONS

Safe and effective anesthetic management is a crucial aspect for the refinement of animal experimental methods.
Isoflurane concentration investigated in this study is assumed for surgical anesthesia in both mouse strains, with moderate overall effects on the vital signs and a rapid return of the animals to their normal state.
NMRI strain showed a lower decrease of the respiratory rate and rectal temperature during anesthesia compared to C57BL/6 mice. Heart rate and O_2 saturation showed similar changes during isoflurane anesthesia for both tested groups.
Additional studies are needed to determine the influence of isoflurane on vital signs across several concentrations and for long term anesthesia.

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