Biological Models Research and Technology
ISSN 9675-9225X (Online)

BMRT

RESEARCH ARTICLE

Oxygen supply as a refining technique for injectable anesthesia in laboratory rats

Jilma Alemán-Laporte[1,2*], Aline Magalhães Ambrósio[3], Dennis Albert Zanatto[1], Mariana de Souza Aranha Garcia-Gomes[1], Ana Tada Fonseca Brasil Antiorio[1], Luciana Ahlf Bandini[1], Pedro Kenzo Yamamoto[1], Denise Tabacchi Fantoni[3], Pedro Enrique Navas-Suarez[1], Gilbert Alvarado[1,4], Claudia Madalena Cabrera Mori[1]

[1]*Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo- USP, São Paulo, SP, Brasil.*
[2]*Centro de Investigación en Cirugía y Cáncer - CICICA, Universidad de Costa Rica, San José, Costa Rica.*
[3]*Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo- USP, São Paulo, SP, Brasil.*
[4]*Laboratorio de Patología Experimental y Comparada - LAPECOM, Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica.*

How to cite: Alemán-Laporte J, Ambrósio AM, Zanatto DA, et al. Oxygen supply as a refining technique for injectable anesthesia in laboratory rats. Bio M Res Tech. 2022;2:e00142021. https://doi.org/10.4322/2675-9225.00142021

Abstract

Well-controlled anesthesia is critical to reducing potential surgical complications and ensuring safe and successful procedures. Respiratory depression, inducing hypoxia, and hypercapnia are adverse effects of injectable anesthesia in laboratory rats. This study aimed to determine the effect of oxygen supply in laboratory rats anesthetized with the combination of ketamine (K) and xylazine (X) plus acepromazine (A) or methadone (Me). The results showed that oxygenation allowed adequate levels of SO_2 and paO_2, avoiding hypoxemia. However, all anesthetized rats showed respiratory acidosis with low pH and high $paCO_2$ levels, which was not reversed after oxygen administration. The acidosis could be related to hypoventilation due to respiratory depression induced by the XKMe association, as well as absorption atelectasis with the CO_2 accumulation during anesthesia. Despite respiratory acidosis, oxygen administration was beneficial for anesthetized rats preventing hypoxemia. This makes it possible to prevent all the metabolic alterations that cause cell death by hypoxia, improving the well-being of anesthetized rats, as well as the quality of the results obtained.

Keywords: blood gases, hypoxia, respiratory acidosis, welfare.

***Corresponding author:** jilma.aleman23@gmail.com
Competing interests: The authors have declared that no competing interests exist.
Received: October 08, 2021. Accepted: February 03, 2022.

INTRODUCTION

Experimental procedures on laboratory rats often require anesthesia. Although inhalation anesthesia have many advantages over injectable anesthesia such as easy control of the depth of anesthesia, minimal drug metabolism, and minimal recovery time; the lack of equipment, adequate facilities, or expertise may limit its use[1,2]. Therefore, injectable anesthesia (usually by intraperitoneal route) is the method of choice in small rodents. Some studies demonstrated the effects of injectable anesthetics on metabolic, hemodynamic, and cardiovascular parameters in laboratory rats[3-5]. One of the primary adverse effects of injectable anesthesia is respiratory rate depression, inducing hypoxia and hypercapnia, leading to a severe reduction of the blood oxygen saturation and alterations in the acid-base balance[6]. Hypoxia occurs due to inadequate supply or low concentration of oxygen in body tissues that may compromise cellular aerobic metabolism and lead to organ dysfunction. Hypercapnia is a condition of abnormally elevated blood CO_2 levels and might occur by lung disease, hypoventilation, or exposure to external sources of high concentrations of CO_2[7,8].

One of the most common injectable anesthesia protocol in rodents is the combination of ketamine and xylazine[9]. Other drugs such as acepromazine or opioid analgesics can be added to this protocol in order to offer the animal a balanced anesthesia that may allow better muscle relaxation or analgesia[10]. However, these drugs can decrease the oxygen supply to organs and tissues. Ketamine antagonizes glutamatergic N-methyl-D-aspartate receptors and hyperpolarization-activated cyclic nucleotide-gated channel 1 chloride ion channels, which both have a role in breathing control[11]. Xylazine activates noradrenergic 2-receptors, which inhibit respiratory neurons in the rostral ventrolateral medulla[12]. Opioids also causes respiratory depression attributed to opioid-mediated interaction with μ and δ receptors[13]. Thus, the combination of ketamine and xylazine affects the respiratory rate, especially when administered with opioids like methadone. This respiratory depression leads to hypoxia, hypercapnia, and acidosis, acting mainly in the central nervous system.

According to a previous study carried out in our laboratory in which we used the same protocols chosen for this study; it was evidenced that both methods generated a respiratory depression with low levels of oxygen saturations when animals were not oxygenated. Some authors concluded that the administration of oxygen is critical to rodents under injectable anesthesia[3,14,15] due to the high death rate possibly associated with overwhelming hypoventilation adverse effects of the anesthesia on some individuals[6]. For this reason, the present study aimed to determine the effect of the administration of oxygen on blood gasometry of rats that were anesthetized using the combination of ketamine and xylazine with acepromazine or methadone.

MATERIALS AND METHODS

Animals

Twenty-four SPF Wistar-Han rats (*Rattus norvegicus*), 12 females and 12 males aged from 8 to 12 weeks (250-350g) were used. Animals were obtained from the animal facility of the Institute of Biomedical Science of the University of São Paulo and free of *Mycoplasma pulmonis, Pasteurella pneumotropica, Bordetella bronchiseptica, Helicobacter* spp., *Klebsiella oxytoca, Klebsiella pneumoniae, Pasteurella multocida, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus β-hemolytic* spp., *Streptococcus pneumoniae*, *Salmonella* spp, Kilham Rat Virus, Rat Pneumonia Virus, Reovirus, as well ecto and endo parasites. A maximum of four animals was housed per cage on corn cob bedding in polypropylene open cages (41x34x16cm) changed once a week. Paper rolls were added as environmental enrichment. All the animals were maintained under climate-controlled conditions of 12/12h light/dark cycle, temperature range from 22°C to 24°C, relative humidity of 45% to 65%, ad libitum access to drinking water, and a standard food-pellet diet (Nuvital®-Quimtia, PR, Brazil).

Before beginning the experiments, the animals were housed in the animal facilities of the Department of Pathology of the School of Veterinary Medicine and Animal Science, University of São Paulo, where they stayed for at least one week to adapt. The experiment was always performed from 1:00 PM to 3:00 PM to avoid any interference with circadian rhythm.

All animal procedures followed the guidelines of the Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil (Authorization no.9635260116).

Sample size calculation

A power calculation was determined by a total power of 0.84 and a two-sided significance of 0.05. The variability of the oxygen saturation was estimated based on the results obtained in a previous study[16]which presented the same treatments[16]; thus, 9% was determined as the maximum standard deviation. The minimum size effect was based on this parameter with a difference of 11%. Thereby, we justified a sample size of 12 animals based on a calculated n of 10.49. The previous study demonstrated no significant differences between sexes, so we decided to use six females and six males in each treatment. We confirmed again that there were no differences in the results between sexes in this study.

Anesthetic procedure

Animals were randomly assigned to two different groups composed by six animals of each sex and were submitted to the following protocols: 1) XKA: Xylazine 7.5 mg/kg (Anasedan®-Ceva, 20 mg/ml, SP, Brazil) + Ketamine 60 mg/kg (Dopalen®-Ceva, 100 mg/ml, SP, Brazil), + Acepromazine 2 mg/kg (Acepran®-Vetnil, 2 mg/kg, SP, Brazil), 2) XKMe: Xylazine 5 mg/kg + Ketamine 60 mg/kg + Methadone 5 mg/kg (Mytedom®-Cristália, 10 mg/ml, SP, Brazil). The anesthetic protocol was administered by the intraperitoneal route and the doses were based on a previous study[16]. After losing the righting reflex, the rats were laid on dorsal recumbency on a preheated thermal blanket to minimize the decrease in body temperature (between 35°C-37°C). Sterile ocular lubricant (Vidisic® Gel-Bauch+Lomb, 2 mg/kg, SP, Brazil) was administered to both eyes.

Arterial blood gas analysis

Immediately after each rat had lost the pedal reflex, an incision was made in the abdomen to place a 24G catheter into the abdominal aorta to collect blood (Figure 1). The first sample was collected with the animal breathing room air. After, a cap was placed on the catheter, and the arterial lumen was occluded with a clamp to block the blood outflow. Then, 70% oxygen was administered using a nose cone for one minute with a flow rate of 0.5 L/min. Promptly, a second blood sample was collected. Both samples were collected with 1ml heparin syringes (BD A-LineTM, PL, UK) and placed in a cooler at 4 °C (samples did not contact the ice). Then, they were immediately transported to be analyzed by an automated arterial blood gas system (Cobas b 121 System, Roche Diagnostics Limited, MA, IN). SO_2, paO_2, $paCO_2$, pH, and $cHCO_3$ were measured.

Clinical signs and characteristics of the samples collected

During blood sample collection color of blood and the presence of sings of cyanosis were reported.

A-a gradient

The following equation calculated A-a gradient:

$$\text{A-a gradient} = [FiO_2 (P_{atm} - PH_2O)] - (paCO_2/0.8) - paO_2 \quad (1)$$

Where FiO_2 was 0.21 (to non-oxygenated animals) and 0.7 (to oxygenated animals), P_{atm} was 700 mmHg (atmospheric pressure of São Paulo, Brazil with an elevation of 700 meters over the sea 14 and PH_2O was 47.

paO2/FiO2 ratio

This value was calculated by dividing the paO_2 of each animal by 0.21 (non-oxygenated animals) or 0.7 (oxygenated animals).

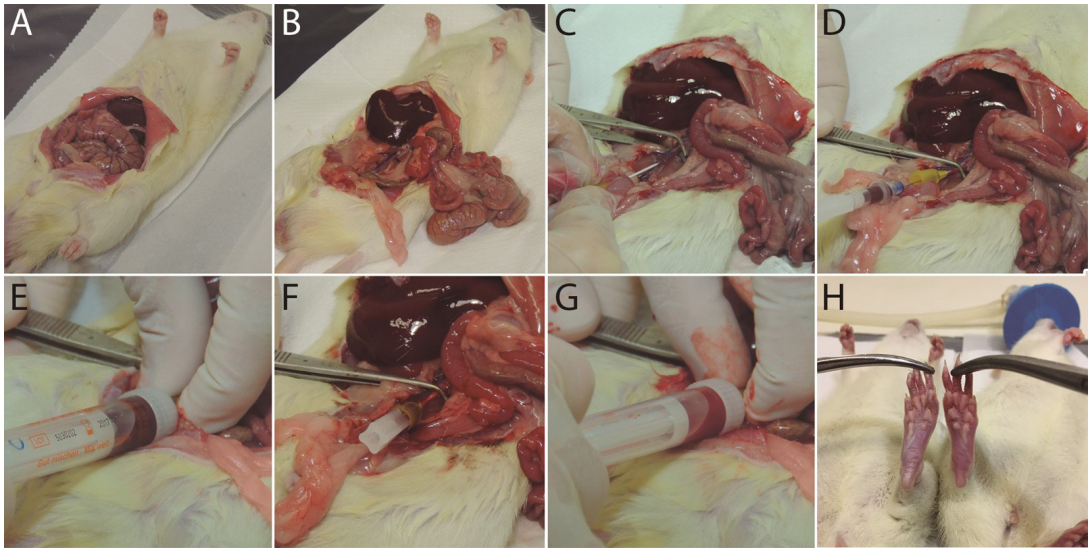

Figure 1. Blood sampling from the abdominal aorta of anesthetized rats and clinical signs before and after oxygenation. A) After the rat lost the pedal reflex, an incision in the middle line was made to expose abdominal organs. B) Organs were removed from the abdominal cavity to expose the abdominal aorta. C) A 24G catheter was introduced in the abdominal aorta, and an elbow head surgical tweezer, placed around the artery. D) When the catheter was already in place, the tweezer was closed, and the cap was removed. E) The first blood sample was collected with rat breathing room air (FiO_2: 21%) with a 1 ml heparinized syringe. F) Rats received a 70% oxygen supply for 1 minute; meanwhile, the cap was placed in the catheter and the tweezer was closed to avoid hemorrhage. G) Second blood sampling was collected. Blood is brighter than the first sample after oxygenation. H) Comparison of skin coloration of two rats, one non-oxygenated (left) showing cyanosis (bluish color to the paw skin) and other oxygenated (right) not presenting signs of cyanosis.

Histopathology

After blood sampling, animals were euthanized by decapitation. Lungs were collected and placed in 10% formalin for histopathological analysis. Tissues were embedded in paraffin blocks and sliced in 5µm sections using a microtome. Sections were floated in a water bath, adhered to standard glass slides, and allowed to dry at room temperature. Slides were then stained with hematoxylin and eosin for further analysis and examined under the microscope to document pathological changes.

Inclusion and exclusion criteria

Only healthy SPF Wistar-Han rats were included. Animals that had an error in all parameters of the arterial blood gas analyses would be excluded. However, no animals were excluded.

Endpoints

Dyspnea or severe hypothermia (temperature under 35°C) during anesthesia were considered as endpoints. However, no animals presented these clinical signs.

Statistical analysis

The analysis was blinded to avoid investigator's bias. Unpaired t-test with Welch's correction was used to analyze statistical differences between gender in all arterial blood gases parameters for each treatment. Effect of oxygen supply in arterial blood gases was analyzed with a two-way analysis of variance (ANOVA) followed by Sidak's multiple comparison test. Results were considered significant at $p < 0.05$.

Histopathological findings were presented as the frequency in each protocol group. We used McNemar's chi-squared test to analyze the association of the anesthetic protocols with the histopathological findings. Statistical analysis was performed with GraphPad Prism 8.2.1 software (GraphPad Software, Inc., 7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA).

RESULTS

Differences between sexes

No significant differences were evident between the sexes. For this reason, data from females and males of each treatment were combined.

Arterial blood gas analysis

The rats submitted to the XKMe protocol had a low pH (<7.35) before oxygenation (p=0.0275) when compared with the XKA protocol. Oxygen supply caused a reduction of pH levels (p<0.0001) in both protocols (Figure 2A).

Rats anesthetized with XKA and XKMe showed increased levels of SO_2 and paO_2 (p<0.0001) after oxygen supply (Figure 2B and 2C). Before receiving oxygen, SO_2 levels were lower than 90% in most animals. The lower values (< 80%) were observed in XKMe anesthetized rats without oxygen (p<0.0001) (Figure 2B). Non-oxygenated animals of both protocols had low paO_2 levels - below 80 mmHg (p<0.0001) - compared with oxygenated ones. Animals anesthetized with XKMe presented the lowest values of paO_2 (<60 mmHg). Non-oxygenated animals showed high $paCO_2$ values (>40 mmHg). These values increased after oxygenation in rats anesthetized with XKA (p=0.0015) and XKMe (p=0.0003) (Figure 2D). Animals submitted to XKMe protocol presented higher paCO2 levels than those submitted to XKA protocol before (p=0.0035) and after (p=0.0005) oxygenation (Figure 2D). In the $cHCO_3$, no effect was observed at the time of oxygen administration. However, it was significantly lower in the XKA before oxygenation (p=0.0119) compared with the XKMe protocol. (Figure 2E).

paO_2/FiO_2 ratio

The rats submitted to the XKA protocol did not present hypoxemia before or after the oxygenation (paO_2/FiO_2 >300 mmHg). In contrast, rats submitted to the XKMe protocol showed a reduction in this relation before oxygenation (p=0.00326) compared with the XKA protocol, which improved with oxygen administration (Figure 2F). paO_2/FiO_2 was higher in animals submitted to the XKA (p=0.0062) and XKMe (p=0.0024) protocols after oxygen supply (Figure 2F).

A-a gradient

All rats from both protocols presented values lower than 10 mmHg before oxygenation. After the administration of 70% oxygen, its level increased significantly in the animals submitted to the XKMe protocol (p=0.0116) (Figure 2G).

Clinical signs and characteristics of the samples collected

During the blood sampling, 100% of the samples showed a color difference before and after oxygenation. They were dark red before oxygenation and bright red after it. Likewise, it was evident that all animals presented cyanosis, before being oxygenated (Figure 1).

Histopathology

No macroscopic changes were observed in the lungs. Atelectasis, alveolar hemorrhage, congestion, and edema were the microscopic findings of both anesthetic protocols (Table 1). No significant results were observed in the statistical analysis.

DISCUSSION

In the search for new techniques for refining anesthetic procedures in laboratory animals, oxygen support during injectable anesthesia is recommended to minimize potential intraoperative complications and ensure success in surgical interventions. In the present study, independently of the anesthetic protocol used, the animals developed respiratory acidosis despite oxygenation. That

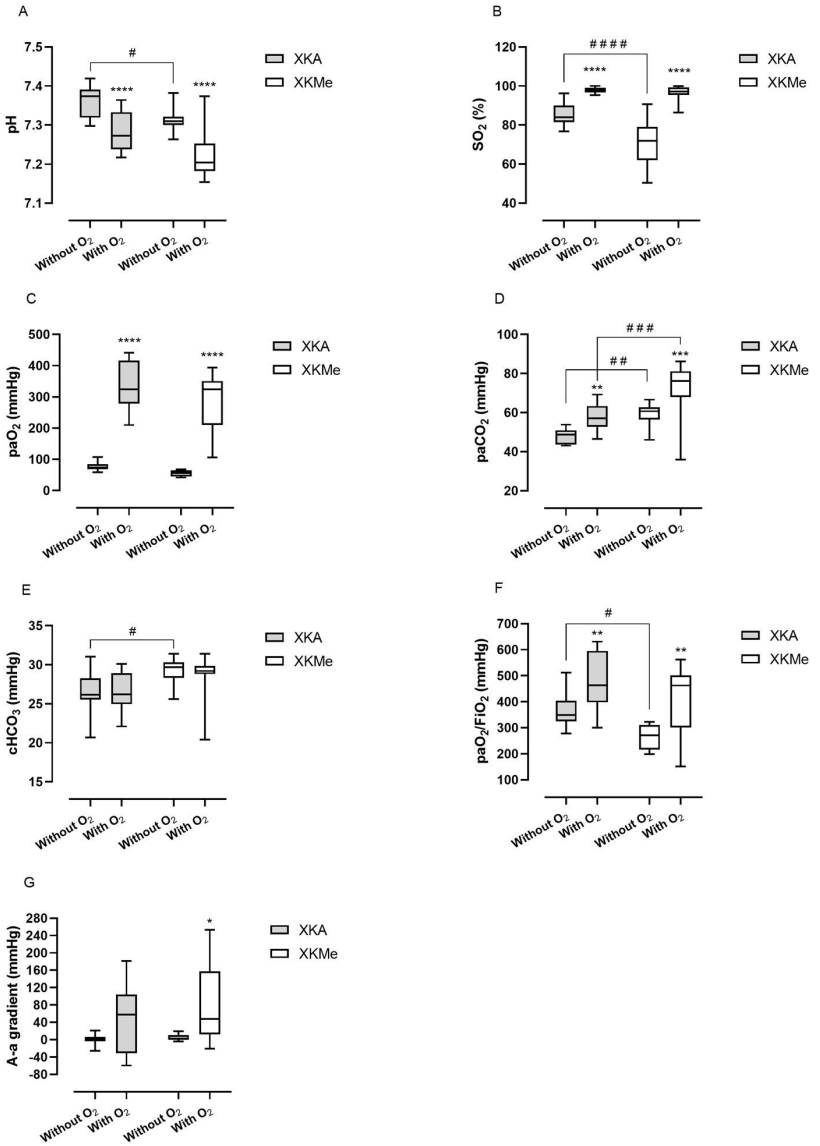

Figure 2. Effects of oxygen on blood-gas values in rats submitted to two anesthetic protocols (X: Xylazine, K: Ketamine, A: Acepromazine, Me: Methadone). A) pH, B) $paCO_2$, C) SO_2, D) paO_2, E) $cHCO_3$, F) $paCO_2/FiO_2$ ratio, G) A-a gradient. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, comparisons: XKA without O_2 (FiO_2: 21%) × with O_2 (FiO_2: 70%); XKMe without O_2 (FiO_2: 21%) × with O_2 (FiO_2: 70%). #p<0.05, # #p<0.01, # # #p<0.001, # # # #p<0.0001, comparisons: XKA × XKMe without O_2; XKA × XKMe with O_2.

Table 1. Histopathological findings in rats submitted to different anesthetic protocols (X: Xylazine, K: Ketamine, A: Acepromazine, Me: Methadone) McNemar's chi-squared test showed no statistical differences between protocols (p>0.05)

Histopathological findings		XKA	XKMe
Atelectasis	Mild	3/12	3/12
	Moderate	6/12	3/12
	Severe	1/12	1/12
Congestion	Mild	4/12	4/12
	Moderate	4/12	6/12
	Severe	1/12	0/12
Alveolar hemorrhage	Mild	1/12	1/12
	Moderate	2/12	1/12
	Severe	1/12	3/12
Edema	Mild	5/12	4/12
	Moderate	1/12	1/12
	Severe	0/12	0/12

may be related to respiratory depression (hinders adequate ventilation) and to absorption atelectasis (leads to CO_2 retention). However, the immediate improvement observed in SO_2, paO_2, and clinical signs (such as the resolution of cyanosis) in all the anesthetic protocols used in this study reinforces the importance of oxygenating the rats during injectable anesthetic procedures.

Respiratory acidosis was a complication observed in all cases, occurring when blood pH falls to values below 7.35; and CO_2 levels increase to values above 60 mmHg (hypercapnia). That was observed in most animals before oxygenation and worsened after it[1]. Hypoventilation can lead to respiratory acidosis as a result of low gas exchange in the alveoli: obstructions in the respiratory tract, pneumonia, pneumothorax, emphysema, neuromuscular disorders, respiratory diseases, central nervous system depressants (respiratory center), or excessive CO_2 inhalation. Acidosis can also be related to hyperoxia because of the administration of high oxygen concentrations (over 70% in our study)[17]. Acidosis occurred in this condition because of the absorption atelectasis, considering that oxygen is highly soluble in blood and for this reason, oxygen diffuses quickly across the alveoli into the bloodstream, accelerating the rate of alveolar collapse[17]. Oxygen in lower concentrations is less soluble because it is mixed with other gases, such as nitrogen, that help the alveoli to preserve their patency and reduces the speed of their collapse[17,18].

The A-a gradient is the difference between the alveolar partial oxygen pressure and the arterial partial oxygen pressure[19]. This parameter should be calculated when a ventilation-perfusion mismatch is suspected[17]. In room air, the usual A-a gradient is lower than 10 mmHg; values over 20 mmHg are considered an oxygenation deficiency. The A-a gradient increases 5 to 7 mmHg when FiO_2 increments 10%[20]. Most animals presented mild to moderate atelectasis in the histopathology analysis. However, we observed normal A-a gradient values for animals that breathed room air (FiO_2 = 21%), which suggests that atelectasis occurred after oxygenation. Considering that 70% FiO_2 is attained when the A-a gradient is about 50-70 mmHg, only the rats under the XKMe protocol had an altered oxygen exchange. However, hypercapnia and a decrease in blood pH occurred mainly before oxygenation. That indicates that the depressant effect of opioids and xylazine in the central nervous system reduced CO_2 elimination, particularly in the XKMe protocol. In other species, like humans, low FiO_2 (30%-35%) prevents hypoxia and hypercapnia[21]. In our study, we did not measure different percentages of FiO_2, so it would be interesting to test if lower FiO_2 reduces CO_2 accumulation.

In this study, the non-oxygenated animals showed moderate hypoxemia demonstrated by unsatisfactory levels of SO_2 and paO_2.[22] The lack of oxygen in the blood cells causes hypoxemia which can be related to respiratory depression, more intense in the first 10 minutes after administering anesthesia[16]. Nevertheless, oxygenation reverted it and was reflected by the better clinical condition of the animals. On the other hand, low values of the paO_2/FiO_2 ratio (an indicator of gas exchange) in non-oxygenated rats of the XKMe protocol revealed that this anesthetic protocol requires oxygenation[23]. It can cause mild to severe hypoxemia, which can affect the results or lead to death. Oxygen supply increased the paO_2/FiO_2 ratio in all cases, showing that it is crucial for hypoxemia correction.

Some authors had reported the benefits of oxygenating rats during anesthesia. Mechelinck et al. (2019)[15] and Fornari et al. (2012)[7] demonstrated that supplying oxygen increased the survival rate of rats anesthetized with injectable anesthesia. Duggan et al. (2005)[18] showed that, despite absorption atelectasis, oxygenation increased FiO_2. Oxygen support combined with a FiO_2 from 24% to 100% in rats anesthetized with KX reduced mortality to zero, improved systemic oxygen tension and cardiac function and decreased pulmonary prostaglandin F2 concentrations.

The changes in the color of blood samples, skin, and mucosa before and after oxygenation were clinical signs of the benefits of oxygen supply. When hemoglobin loses its oxygen, blood turns to dark bluish-red color[24]. Cyanosis occurs when poorly oxygenated blood circulates in the superficial capillaries and venules, granting the skin and mucous membranes a blue coloration. Cyanosis can develop when SO_2 is lower than 85%. When it happens, the paO_2 should be lower than 60 mmHg (minimum value corresponding to the reference range), possibly resulting in a substantial drop in paO_2 that can lead to hypoxemia[25]. Thus, visually controlling the color of the mucosa and monitoring the oxygen saturation with a pulse oximeter are critical parameters to monitor during anesthesia. Pulse oximeters are relatively accurate devices to measure normal oxygen levels but become more inaccurate when saturation falls below 80%. Nevertheless, it is valuable for clinical evaluation since a reduction in oxygen saturation during anesthesia can be immediately detected and corrected[1].

Xylazine could affect lung histopathological findings. The alpha$_2$ adrenoreceptor agonist can cause an acute pulmonary venous spasm, followed by severe pulmonary congestion[26]. Consequently, the microvascular pressure rises considerably, rupturing alveolar-capillary walls and resulting in extensive diapedesis of red cells into the alveolar lumen[26]. Xylazine can also increase permeability in the pulmonary vascular endothelium resulting from an endothelium injury and consequent lung edema[27]. All these alterations can cause respiratory distress in the animal, which can lead to hypoxemia.

Based on our results, we recommend providing oxygen supply to rats when using injectable anesthesia, especially when combined with opioids. Also, animals should be healthy and not present any respiratory system compromise (preferably using SPF animals). Nose cones are the best choice to ventilate rats because tracheal intubation or tracheostomy is more invasive and can damage or destroy the anatomic laryngeal structure, leading to complications - mortality, a more complex intubation process, and increased post-operatory recovery period[28]. Continuous monitoring of the animal's parameters is necessary, primarily measuring SO_2 and CO_2. Monitoring these parameters could be by pulse oximetry and capnography, when gasometry is not possible.

In conclusion, the present study demonstrated that oxygen supply restored hypoxemia produced by respiratory depression during anesthesia. Administering oxygen has an immediate effect that increases its saturation and paO_2, keeping the animal stable during anesthetic procedures and, consequently, improving recovery and reducing mortality risk. However, respiratory acidosis can occur in high concentrations of FiO_2. Moreover, additional studies are required using lower FiO_2 concentrations to establish the best choice for rats.

ACKNOWLEDGEMENTS

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors thank the anonymous reviewers for their thoughtful comments that improved the manuscript. We want to thank Nelzinho for the technical support in the animal facility during the development of my experiment.

Financial support

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001", segundo a PORTARIA nº 206, DE 4 DE SETEMBRO DE 2018.

REFERENCES

1. Flecknell P. Laboratory animal anesthesia. 3rd ed. Newcastle: Elsevier Inc; 2009. p. 79-108. http://dx.doi.org/10.1016/B978-0-12-369376-1.00003-4.
2. Tranquilli WJ, Thurmon JC, Grimm KA. Lumb & Jones' veterinary anesthesia and analgesia. 4th ed. Iowa: Blackwell Publishing; 2015. p. 1055.
3. Godoy Fernandes AL. Guia de pneumologia. 1st ed. São Paulo: Manole; 2004. p. 100.
4. Molina A, Moyano M, Serrano-Rodriguez J, Ayala N, Lora AJ, Serrano-Caballero JM. Analyses of anaesthesia with ketamine combined with different sedatives in rats. Vet Med. 2015;60(7):368-75. http://dx.doi.org/10.17221/8384-VETMED.
5. Picollo C, Serra AJ, Levy RF, Antonio EL, Santos L, Tucci PJF. Hemodynamic and thermoregulatory effects of xylazine-ketamine mixture persist even after the anesthetic stage in rats. Arq Bras Med Vet Zootec. 2012;64(4):860-4. http://dx.doi.org/10.1590/S0102-09352012000400011.
6. Arras M, Autenried P, Rettich A, Spaeni D, Rülicke T. Optimization of intraperitoneal injection anesthesia in mice: drugs, dosages, adverse effects, and anesthesia depth. Comp Med. 2001;51(5):443-56. PMid:11924805.
7. Fornari RV, Wichmann R, Atsak P, et al. Rodent stereotaxic surgery and animal welfare outcome improvements for behavioral neuroscience. J Vis Exp. 2012;59(59):e3528. http://dx.doi.org/10.3791/3528. PMid:22314779.
8. Ganesh T, Estrada M, Duffin J, Cheng HL. T2* and T1 assessment of abdominal tissue response to graded hypoxia and hypercapnia using a controlled gas mixing circuit for small animals. J Magn Reson Imaging. 2016;44(2):305-16. http://dx.doi.org/10.1002/jmri.25169. PMid:26872559.
9. Stokes EL, Flecknell PA, Richardson CA. Reported analgesic and anaesthetic administration to rodents undergoing experimental surgical procedures. Lab Anim. 2009;43(2):149-54. http://dx.doi.org/10.1258/la.2008.008020. PMid:19116297.

10. Welberg LAM, Kinkead B, Thrivikraman KV, Huerkamp MJ, Nemeroff CB, Plotsky PM. Ketamine–Xylazine–Acepromazine anesthesia and postoperative recovery in rats. J Am Assoc Lab Anim Sci. 2006;45(2):13-20. PMid:16542037.
11. Chen X, Shu S, Bayliss DA. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. J Neurosci. 2009;29(3):600-9. http://dx.doi.org/10.1523/JNEUROSCI.3481-08.2009. PMid:19158287.
12. Karbing DS, Kjærgaard S, Smith BW, et al. Variation in the PaO2/FiO2 ratio with FiO2: mathematical and experimental description, and clinical relevance. Crit Care. 2007;11(6):R118. http://dx.doi.org/10.1186/cc6174. PMid:17988390.
13. Chevillard L, Mégarbane B, Baud FJ, et al. Mechanisms of respiratory insufficiency induced by methadone overdose in rats. Addict Biol. 2010;15(1):62-80. http://dx.doi.org/10.1111/j.1369-1600.2009.00184.x. PMid:20002023.
14. Hajighahramani S, Vesal N. Evaluation of several drug combinations for intraperitoneal anaesthesia in adult male rats. Majallah-i Tahqiqat-i Dampizishki-i Iran. 2007;8:106-15.
15. Mechelinck M, Kupp C, Krüger JC, et al. Oxygen inhalation improves postoperative survival in ketamine-xylazine anaesthetised rats: an observational study. PLoS One. 2019;14(12): e0226430. http://dx.doi.org/10.1371/journal.pone.0226430. PMid:31834913.
16. Alemán-Laporte J, Bandini LA, Garcia-Gomes MSA, et al. Combination of ketamine and xylazine with opioids and acepromazine in rats: physiological changes and their analgesic effect analysed by ultrasonic vocalization. Lab Anim. 2020;54(2):171-82. http://dx.doi.org/10.1177/0023677219850211. PMid:31142228.
17. Wilding LA, Hampel JA, Khoury BM, et al. Benefits of 21% oxygen compared with 100% oxygen for delivery of isoflurane to mice (Mus musculus) and rats (Rattus norvegicus). J Am Assoc Lab Anim Sci. 2017;56(2):148-54. PMid:28315643.
18. Duggan M, McNamara PJ, Engelberts D, et al. Oxygen attenuates atelectasis-induced injury in the in vivo rat lung. Anesthesiology. 2005;103(3):522-31. http://dx.doi.org/10.1097/00000542-200509000-00015. PMid:16129977.
19. Silverstein D, Hopper K. Small animal critical care medicine. 2nd ed. St. Louis: Elsevier; 2014. p. 77-92.
20. Mythen M, Burdett E, Stephens RC, et al. Anaesthesiology e-book: Churchill's ready reference. 1st ed. Aberdeen: Churchill Livingstone; 2010.p. 8-36.
21. Agrawal J, Rajput A. Intraoperative fraction of inspired oxygen : an enigma to be unravelled. Anaesth Crit Care Med J. 2019;4(4):000163. http://dx.doi.org/10.23880/ACCMJ-16000163.
22. Duke-Novakovski T. Basics of monitoring equipment. Can Vet J. 2017;58(11):1200-8. PMid:29089659.
23. Heinzman DM. Cyanosis. In Zaoutus LB, Chiang VW, editors. Comprehensive pediatric hospital medicine. 1st ed. Philadelphia: Elsevier; 2005. p. 145–148.
24. Damjanov I. Pathology secrets. 3rd ed. Barcelona: Mosby, 2008, p. 138.
25. Hedenqvist P, Roughan JV, Flecknell PA. Effects of repeated anaesthesia with ketamine/medetomidine and of pre-anaesthetic administration of buprenorphine in rats. Lab Anim. 2000;34(2):207-11. http://dx.doi.org/10.1258/002367700780457536. PMid:10817461.
26. Bacon PJ, Jones JG, Taylor P, Stewart S, Wilson-Nunn D, Kerr M. Impairment of gas exchange due to alveolar oedema during xylazine sedation in sheep; absence of a free radical mediated inflammatory mechanism. Res Vet Sci. 1998;65(1):71-5. http://dx.doi.org/10.1016/S0034-5288(98)90030-3. PMid:9769076.
27. Amouzadeh HR, Sangiah S, Qualls CW Jr, Cowell RL, Mauromoustakos A. Xylazine-induced pulmonary edema in rats. Toxicol Appl Pharmacol. 1991;108(3):417-27. http://dx.doi.org/10.1016/0041-008X(91)90088-V. PMid:1902333.
28. Yuan Y, Li F, Wang Y, et al. Respiratory face mask: a novel and cost-effective device for use during the application of myocardial ischemia in rats. J Zhejiang Univ Sci B. 2009;10(5):391-4. http://dx.doi.org/10.1631/jzus.B0820216. PMid:19434766.