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Review

Anesthesia protocols in laboratory animals used for scientific purposes

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Summary. *Background:* A suitable, effective and free of complications anesthetic protocol is very important in experimental studies on animal models since it could bias the outcome of a trial. To date there is no universally accepted protocol for induction, maintenance and recovery from anesthesia. The endotracheal intubation with the use of inhalation anesthesia is used very especially in the from of large size laboratory animals, because it is a secure and easy control mode. However, it is not common for small laboratory animals because of the high technical skills required. *Aim:* The aim of this paper is a review of the main methods of induction of anesthesia in laboratory animals. *Materials and methods:* We performed an electronic search of MEDLINE (PubMed interface), ISI Web of Science and Scopus using the keywords "anesthesia" and "animal (s)" or "protocol (s)" or "surgery", without the data or the language restriction. We consider only the most common laboratory animals (rats, mice, rabbits, pigs). We identify all the scientific articles that refer to the use of anesthetics for studies on laboratory animals in all areas: experimental surgery, CT, MRI, PET. All documents identified the search criteria are subject to review only by identifying relevant studies. *Conclusions:* There is a strong need for application of existing guidelines for research on experimental animals; specific guidelines for anesthesia and euthanasia should be considered and reported in future studies to ensure comparability and quality of animal experiments. (www.actabiomedica.it)

Key words: experimental surgery, laboratory animals, induction and maintenance of anesthesia, inhalation anesthetic, anesthetical drugs

Introduction

Laboratory animals are sometimes used in experimental clinical studies such as pre-marketing of a drug or a medical-surgical device or in regenerative medicine and surgery. The anesthesia protocols influence the survival of laboratory animals and can also greatly affect the experimental data results. To date, there is no anesthetic protocol widely used for single laboratory animal species. The murine species (rats and mice) is the most used model in various research fields, such as for organ transplantation, regenerative medicine and imaging. The pigs are animals that are used for the search, since their cardiorespiratory physiology is very similar to humans (1). The pig animal model, however, is extremely sensitive, so the primary objective is therefore to provide a quiet environment without causing anguish and stress and it should be also adequately sedated for transport also ensuring normothermia (2). The lagomorphs model is instead an animal model of medium size useful, for example, in studies in which the murine model is too small and pig model too big. Four aspects are of paramount importance for a correct management of the trial: a correct inhalation anesthetic, effective anesthesia, the duration of the entire experimental procedure and a correct protocol of endotracheal intubation (3).

In general, anesthesia can affect some physiological parameters, such as pressure, blood oxygen saturation, cerebral blood flow and many other factors that may affect the postoperative follow-up. The majority of anesthetic agents decrease the cerebral metabolism and they often affect the neurotransmission of nerve impulses, for which, the body temperature and other physiological parameters, should be monitored during anesthesia (4).

Methods

Search strategy

We performed an electronic search of MEDLINE (PubMed interface), ISI Web of Science and Scopus using the keywords "anesthesia" and "animal (s)" or "protocol (s)" or "surgery" in

"Title/Abstract/Keywords", without the data or the language restriction, to identify all the scientific articles that refer to the use of anesthetics for studies on laboratory animals in all areas: experimental surgery, CT, MRI, PET. All documents identified the search criteria are subject to review only by identifying relevant studies.

Overall, 27 publications were identified, 8 of them have been excluded according to our study criteria. Each experimental study on animal model we tested was approved by the "Organismo Preposto al Benessere Animale" (OPBA), as required by current regulations. In total they have been taken into account and analyzed 19 scientific studies regarding the use of anesthesia in laboratory animals for different surgical procedures and not.

Premedications

The anesthesia is commonly used in laboratory animals, and can be induced by different methods depending on the type of study and the type of animal taken into account.

Konno et al., for the sedation in rats, used a closed glass chamber, where inside is fed a mixture of isoflurane (Escain®) at a concentration of 5% with airflow used as a carrier gas for 1 min (5).

After sedation and intubation of the subject, it is used by Konno et al. a mixture, called «M / M / B: 0.3 / 5.4» described by Kawai et al (6) e Kirihara et al. (7) as an anesthetic injected intraperitoneally at a dose of 0,3 mg/kg b.w. of medetomidine (Domitor®), 4,0 mg/kg b.w. of midazolam (Dormicum®) e 5.0 mg/kg b.w. of butorphanol (Vetorphale®) as premedication (5).

Hedenqvist et al. suggest to use sufentanil-midazolam combination as premedication in rabbits (8) and medetomidine like anesthesia protocols in small laboratory animals. Parenteral anesthetic combinations such as ketamine and xylazine are suggest like the agents of choice for anesthesia in the rabbit, because they are effective, easily administered and inexpensive (9). The ketamine/xylazine/acepromazine combination is also a useful regimen for normovolemic animals when anesthetic duration greater than that produced by ketamine/xylazine alone is required (9).

Re et al. creating a mixture of lidocaine, ketamine (10) and an opioid (0.6 mg ketamine /kg/h and lidocaine 3 mg/kg/h combined with morphine 0.24 mg/kg/ or fentanyl 0.0045 mg/kg/h) administered during premedication, they have noticed no significant decrease in the minimum alveolar concentration of volatile anesthetics administered in pig models (11). However, the effectiveness of this combination shows marked variations and opioids are likely to be less effective in pigs than in other species (11).

Induction and maintenance of anesthesia

The induction of anesthesia in small animals is carried out, in most cases, using anesthetic gas.

According Risling et al. the open-drop delivery of isoflurane or sevoflurane is an effective tool to anesthetize mice and small animals. The volatile concentration needed to induce anesthesia in mice following the application of 0.5 ml of anesthetic in an induction chamber volumes of 725 mL to 87.6 kPa and 20˚C, measured by a gas analyzer of precision. Anesthesia was induced with isoflurane at concentrations of 6,80±0,57% after 35.70±6.95 s while using sevoflurane induction is significantly longer (45.50±9,96 s) and requires concentrations of gas greater than (7.41±00:57%). Animals taken into the study had a rapid recovery both by using isoflurane than with sevoflurane (12).

The twelve Wistar rats studied by Konno et al., after premedication and induction of anesthesia intraperitoneally, were intubated with endotracheal tube and connected to the circuit for inhalation anesthesia with isoflurane maintained at a concentration 3.0% for males and 2.5% for females for a period of 5 min. Subsequently, the concentration of isoflurane is reduced to 2.5% for males and 2.0% for females up to the interruption of anesthesia (13).

During anesthesia, all rats should be heated on a hot plate. All intubations have ended successfully within 1 minute, and the values of vital signs measured up to 30 minutes after the monitoring were normal and stable. Moreover, the histopathological observation of the trachea and the lungs carried by Konno et al. showed no trauma despite endotracheal intubation is not easy in small animals and requires technical skill and special equipment (13). These results suggest that endotracheal intubation is a reliable, safe and environment with regard to the welfare of rats (14).

On a study reported by Imai et al. of 8 experimental models lagomorphs (white New Zealand rabbits), it was used an anesthetic gas line that provides for the administration of halothane or isofluorane. In this study it is seen that it is preferable to use the halothane as it gives a less respiratory depression during anesthesia than using isofluorane (15, 16).

Hedenqvist et al. have evaluated the possibility of finding an alternative to using isoflurane to maintain anesthesia in rabbits (8). In the study published in 2015 they have made 18 compared Himalaya rabbits divided into two groups of equal number: they were premedicated with 0.1 mg kg (-1) medetomidine and 5 mg/kg of carprofen subcutaneously, followed by the induction of intravenous sufentanil (2.3 mg mL) and midazolam (0.45 mg mL). After endotracheal intubation, anesthesia was maintained with sufentanil-midazolam in 9 subjects and Sevoflurane in the remaining 9. There were no significant differences between the two groups. In rabbits treated with sevoflurane, however, mean arterial pressure decreased in the pre-surgical phase, the heart rate increased by 25% during and after surgery, and body weight decreased by 4% after surgery (8).

For bigger animal models such as the pig, Pehböck et al. recommend starting the anesthesia by injection of ketamine and propofol followed by endotracheal intubation during spontaneous breathing (3). It is therefore necessary the presence of a specialist in anesthesia for a correct management of the airway of the animal in order to avoid dangerous complications such as death. The vascular access can be provided by a cut-down (skin incision for insertion needle-venous cannula) or ultrasound-guided techniques in the groin or in the neck region (17).

Jalde et al. of nine pigs premedicated with a bolus intramuscular injection of ketamine 10 mg/kg intravenous dose of propofol 2mg/kg injected prior to intubation have noted that very high doses of propofol caused sudden arrhythmias and refractory with circulatory collapse in some animals in the studio. Therefore, it is recommended, according to the authors, infusing low doses of ketamine intravenously in order to reduce the total amount of propofol. Anesthesia was maintained with sevoflurane which promotes a low Vt (Tidal volume) and less influence of propofol the neuro-ventilatory efficiency (18).

After intubation, maintenance anesthesia is performed by Pehböck et al. on the pig model with morphine or piritramide, propofol and rocuronium (3). Normothermia (38.5˚C) must be guaranteed (19).

Mikkelsen et al. use propofol and remifentanil infusion but have noticed, especially after a single bolus of remifentanil, a lowering of cerebral oxygenation levels although within normal limits (20).

There are few centers that perform a check of the subject during the study procedures in laboratory animals. According to the study carried out by Uhlig et al. no control during anesthesia were described in 439 cases out of 732 (60.0%) of interventions involving the use of anesthesia. In the remaining procedures 293/732 (40.0%) involving anesthesia, the use of monitoring techniques have been described during the only anesthesia 114/293 (38.9%), the experimental procedures 26/293 (8,9%) or in both cases in 113/293 (38.6%) of the interventions. In 40/293 interventions (13.7%) is no monitoring was specified (21).

Post-anaesthesia monitoring

Post-anesthetic monitoring is very important in the recovery phase of the laboratory animals. It is im-

portant to control the side effects that might be from general data, such as heart rate, body temperature and the concentration of gases and electrolytes in the blood, as well as it is important to assess the reflex responses. According Fleischmann et al. mice should be awakened in their cages and evaluate the heart rate, body temperature and the degree of pain for at least 24 hours (22).

There are many methods tried to assess pain in rats that holds the account or vital signs and can rely on an accurate animal inspected (23). Arterial blood gases exam, recommended postoperatively, can reveal acidosis, hypoxia, hypercapnia and an increased concentration of glucose (22). To induce waking in rats, as well as set a pain relief, it is appropriate to use anesthetic antagonists to ensure a faster awakening. Fleischmann, using naloxone-flumazenil-atipamezole, noted that rats regained consciousness after 110±18 s and are quickly returned to the physiological basal values. Without antagonist instead mice showed marked hypothermia (22±1.9°C) and bradycardia (119±69 beats/min) to several hours (22). The effect of anesthesia induced in rabbits is antagonizzabile in 25-25 min with rapid animal's recovery time. Fundamental becomes the monitoring of body temperature, heart rate and oxygen saturation in the blood according to Flecknell studies (24).

In bigger animals models, such as pigs, it is good to have an adequate observation center for a few days in the frame of reference so that you can transfer the animal immediately after surgery and avoid a second sedation for transportation. The recovery phase in the large animals is slower and requires support and continuous monitoring in the later stages upon awakening (25).

Discussion

Anesthetic agents which are most frequently used (ketamine, propofol, isoflurane/halothane) to induce and maintain anesthesia in laboratory animals influence the carbon dioxide tension in arterial blood (PaCO2) or exhaled (as ETCO2) and can cause respiratory acidosis. They must therefore be carefully monitored all the vital parameters of the animal and restoring fluid and electrolyte balance in the event that it were altered.

Ketamine typically increases cerebral blood flow and indirect sympathetic mimetic effects on the metabolism of the brain (26) increasing the plasma concentration of norepinephrine and, being an antagonist of NMDA receptors, it can also determine neuronal damage known as Olney lesions (27). All these combination of drugs used in rabbits xylazine-ethyl-(1-methyl-propyl) malonyl-thio-urea salt (EMTU), ketamine-EMTU, xylazine-pentobarbital, xylazine-acepromazine-ketamine (XAK), ketamine-chloral hydrate and ketamine-xylazine can induce a depression of respiratory rate. Although rectal temperature values were reduced to some degree in each group, the most profound hypothermia was induced by XAK (28). Propofol is a short-act anesthetic drug that readily crosses the blood-brain barrier; its effect starts after a minute. It is rapidly cleared from plasma, and the consciousness returns more quickly with propofol that with other anesthetic drugs. Propofol allows a better cerebral autoregulation most other anesthetic agents (29). Isoflurane and halothane allow ga ood control of anesthesia duration and deepness (30). The anesthesia can also affect the blood glucose levels and lipid concentration that may indirectly affect brain metabolism (31).

The cerebral metabolism may also be affected by changes in body temperature, and in particular that hypothermia is common during prolonged anesthesia in small animals. Hyperglycemia, for example, can greatly increase the risk of global cerebral ischemia (32) since the fluctuations in blood glucose levels can greatly affect brain function by modulating the mechanisms and neuroprotective properties of the blood-brain barrier. Blood glucose levels should be monitored carefully during maintenance of anesthesia in order to avoid both hyper- and hypoglycemia (4). Medetomidine commonly used to sedate laboratory animals can cause hypotension and respiratory depression, especially at low doses, while not reduce cerebral blood flow (33). The drugs mainly used in different anesthesia protocols in the literature can cause numerous side effects that could change the success of a clinical trial and damages the animal model "quod vitam". Monitoring of vital signs and animal welfare must be safeguarded during all study procedures (34).

Conclusions

This systematic review revealed insufficient reporting of methods of anesthesia in experimental studies. The studies are always with a low number of laboratory animals. In addition, this review shows that there is a strong need for guidelines in research on experimental animals; specific guidelines for anesthesia and euthanasia should be considered and reported in future studies to ensure comparability and quality of animal experiments. This is very important to translate experimental results in (future) clinical applications.

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