Comparison of the beneficial and adverse effects of inhalable and injectable anaesthetics in animal models: a mini-review

KS Furtado*, FO Andrade

Abstract

Introduction

The use of anaesthetics to minimise distress suffered by animals during surgical procedures or euthanasia is vital. There are two major types of anaesthesia: inhalable (ether, halothane and the ‘flurane’ family) and injectable (barbiturates, steroids, dissociative agents, neuroleptanalgesics and propofol); both types have their own advantages and disadvantages. This mini-review focuses on discussing the most commonly used methods of anaesthesia in laboratory animals and on evaluating their advantages and disadvantages due to controversies about the best choice for anaesthesia.

Conclusion

Particularities of inhalable and injectable anaesthetics should be taken into consideration before using them. Thus, a proper evaluation of the protocol for anaesthesia should consider animal welfare, pharmacokinetics and adverse effects of the drug, which could affect the organ under study. In addition, the need for equipment and technical expertise, as well as the best route of administration, should be considered.

Introduction

Animal models are important to understand the pathophysiology of diseases, and consequently to define strategies for its prevention or treatment. The majority of in vivo studies require small animals, such as mice, rats, hamsters and gerbils, among others. Measures to minimise distress in animals should be taken before carrying out procedures, such as surgery and euthanasia, to prevent animal suffering and potential adverse effects on the study. In this context, the use of adequate anaesthesia and analgesia is crucial to animal welfare as well as to obtain reliable results.

There are two major types of anaesthesia: inhalable (volatile chemicals, including ether, halothane and the ‘flurane’ family) and injectable (barbiturates, steroids, dissociative agents, neuroleptanalgesics and propofol); both types have advantages and disadvantages that need to be carefully considered before using them. Therefore, this review focuses on briefly discussing the characteristics of the most commonly used anaesthetic agents in laboratory animals, due to controversies over the best choice for anaesthesia.

Discussion

Volatile inhalable anaesthetics

Even though the technology for injectable anaesthetics has advanced considerably, inhalable anaesthetics are still widely used, both in laboratory animals and in clinical studies. The effects of these anaesthetics can vary according to the species used, gender, age and physiological conditions. Their mechanism of sedation is thought to be through depression of spinal cord function, whereas amnesia is mediated in the cerebrum through increased activity of the inhibitory neuronal pathways dependent on the γ-aminobutyric acid type A (GABA-A) receptor and glycine, which inhibits the excitatory pathways dependent on the activation of neuronal nicotinic receptors for acetylcholine, serotonin and glutamate.

The most commonly used volatile inhalable anaesthetics are ether, halothane and members of the ‘flurane’ family (methoxyflurane, isoflurane, sevoflurane, desflurane and enfurane; Table 1), and they are used in many different species. Halothane and isoflurane are mostly used in laboratory animals, and they have similar physicochemical properties, including vapour pressure and molecular weight, although isoflurane has a lower coefficient of solubility in blood. This particular property confers isoflurane with a lower induction and recovery time than halothane.

Inhalable anaesthetics have many advantages, including rapid recovery, return from anaesthetic hypothermia, lower incidence of death, quick elimination through the lungs and relative technical ease of administration. However, some volatile anaesthetics, such as ethyl ether, can cause irritation in mucosal membranes and do not have a proper control of the dose administered. Further, the inhalable vapours need to reach a certain concentration within the pulmonary alveoli in order to cause anaesthesia. Since the loss of consciousness is not immediate, the animals can show adverse effects such as hypoxia and pungency (Table 2), as well as stress mediated by alterations in heart rate and increased activity of the sympathetic nervous system.

In general, the effects of inhalable anaesthetics are variable, such as the following:

- The time for sedation depends on factors such as concentration and administration procedure.
Review

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All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

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Table 1 Comparison of the most commonly used volatile inhalable anaesthetics

<table>
<thead>
<tr>
<th>Ether</th>
<th>Halothane</th>
<th>Isoflurane</th>
<th>Enflurane</th>
<th>Sevoflurane</th>
<th>Metoxiflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly soluble, low induction; causes irritation in the eyes, nose and airways; risk of explosion</td>
<td>Rapid induction; efficacious for euthanasia; high potential for hepatotoxicity</td>
<td>Lower solubility than halothane, but faster induction; unpleasant smell; small hepatotoxicity</td>
<td>Lower potency than halothane; efficacious for euthanasia, linked to scattered convulsions; potential for hepatotoxicity</td>
<td>Less potent than halothane and isoflurane, as well as lower vapour pressure</td>
<td>Highly soluble; slow induction; potential for nephrotoxicity</td>
</tr>
</tbody>
</table>

Table 2 Advantages and disadvantages of the use of inhalable and injectable anaesthetics

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhalable anaesthetics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Useful for euthanasia and surgery of small animals</td>
<td>Adverse effects reported in small rodents and larger species; can cause stress</td>
<td></td>
</tr>
<tr>
<td>Can be administered by different methods and equipment</td>
<td>Prolonged loss of consciousness if apnoea occurs</td>
<td></td>
</tr>
<tr>
<td>Halothane and ‘fluranes’ are not explosive under room temperature</td>
<td>Ether is irritant, flammable and explosive</td>
<td></td>
</tr>
<tr>
<td>Fast recovery</td>
<td>Poor control over precise dose</td>
<td></td>
</tr>
<tr>
<td>Lower incidence of fatal events</td>
<td>Can cause hypoxia</td>
<td></td>
</tr>
<tr>
<td>Rapid elimination through the lungs</td>
<td>Can cause poignancy</td>
<td></td>
</tr>
<tr>
<td><strong>Injectable anaesthetics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Require lower doses than inhalable anaesthetics to induce anaesthesia</td>
<td>Shorter time of action, require repeated dosing, which can cause a long time of recovery</td>
<td></td>
</tr>
<tr>
<td>Do not require the use of vaporisers and ventilators</td>
<td>Administration via intraperitoneal, subcutaneous and intramuscular injections can cause unpredictable depth of anaesthesia</td>
<td></td>
</tr>
<tr>
<td>Promote quick loss of consciousness</td>
<td>Barbiturates have histotoxic effects and require intravenous administration</td>
<td></td>
</tr>
<tr>
<td>Control of cardiopulmonary function is more adequate</td>
<td>Some agents can cause apnoea, hypotension, hypertension or hypothermia</td>
<td></td>
</tr>
</tbody>
</table>

- The speed with which animals lose consciousness is faster at high concentrations of anaesthetics; however, exposure to high concentrations can cause adverse effects.
- These agents should be administered in their pure form, without contaminants, with the help of a cylinder or a commercial tank to facilitate the control of the concentration to be given.
- Most of the inhalable anaesthetics are occupationally hazardous to those manipulating them; for example, ether has a risk of explosion and halogenated agents can lead to narcosis.
- Pre-medication or non-inhalable anaesthetics should be used in diseased animals because disease states can reduce the velocity to reach the alveolar concentration required to cause sedation.
- Newborns are resistant to hypoxia and take longer to die due to overdosage of inhalable anaesthetics; therefore, if the goal is euthanasia, anaesthesia should be prolonged or an alternative method should be used.
- If the rapid flow of gases generates noise or air currents that are stressful to the animals, the equipment used should be calibrated properly to reduce noise and air currents.
- Inhalable anaesthetics should be administered in a place with appropriate accommodation for the animal, and this place should not be located near other animals to prevent lateral transmission of stress to animals that will undergo procedures later.
Inhalable anaesthetics also cause physiological alterations in both laboratory animals and humans. In particular, halothane and ‘fluranes’ lead to a reduction in mean arterial pressure. Further, they can alter heart rate and increase right atrial pressure depending on the dose administered.

In addition, volatile anaesthetics lead to a reduction in respiratory volume, as well as an increase in respiratory frequency, although the latter is insufficient to compensate for the loss in volume, which generates a depression in the minute-ventilation. Further, these anaesthetics increase the apnoeic threshold and reduce the ventilator response to hypoxia. However, these effects can be overcome through assisted or controlled ventilation using proper equipment during surgical procedures. In the brain, inhalable anaesthetics can cause a reduction in the overall cerebral metabolism, despite an increase in cerebral blood flow caused by reduced cerebrovascular resistance. In the kidney there is a reduction in glomerular filtration rate and effective renal plasma flow, and increases in filtration fraction as well as renal vascular resistance. It is important to stress that metabolism of sevoflurane generates fluoride ions, although fluoride levels in the kidney do not reach toxic concentrations under physiological conditions. In contrast, fluoride generated after metabolism of methoxiflurane can cause nephrotoxicity.

Inhalable anaesthetics cause a reduction in hepatic blood flow after the anaesthesia is administered, as well as reversible alterations in hepatic functions. In addition, studies suggest that halothane, which is metabolised to halogenates, is associated with hepatocyte lesions caused by binding of its halogenated metabolites to cytochrome P450 enzymes. Oxidative metabolism involving cytochrome P450 enzymes during exposure to halothane is similar to what is observed during exposure to enflurane, isoflurane and desflurane. Thus, regarding expression of antigens, we observe the following: halothane > enflurane > isoflurane. On the other hand, sevoflurane is not metabolised into halogenates, but into hexafluorisopropanol. It is important to stress that ether, used mainly for anaesthesia and/or euthanasia of rats and mice, has been replaced by other anaesthetics because it is inflammable and causes discomfort to animals. However, Zhang et al. reported that volatile anaesthetics, including ether and isoflurane, have a smaller impact on the receptors located in the membrane of erythrocytes when compared to sodium pentobarbital and chloralose, maintaining a stable immune function in mice, whereas many other anaesthetics can lead to immunosuppression.

Injectable anaesthetics

Use of inhalable anaesthetics at high doses can cause progressive cardiopulmonary depression, which is a major concern. Thus, non-inhalable anaesthetics can be used to reduce inhalation agent requirements resulting in less cardiovascular depression. The use of injectable anaesthetics has been growing since the past decade, as these agents can directly target the vascular system and reach the central nervous system quickly, where they promote rapid loss of consciousness. Further, intravenous injection does not require special equipment, such as vapourisers and ventilators, and does not overload the pulmonary system. When administered correctly, injectable anaesthetics reduce distress in animals (Table 2).

Injectable anaesthetics can be administered via different routes, such as subcutaneous (s.c.), intraperitoneal (i.p.), intravenous (i.v.) or intramuscular (i.m.). The i.m. route is not recommended for smaller animals, for example mice. The routes differ according to the ease of administration, the velocity of drug absorption and the volume that can be injected. The easiest administration route is s.c.; however, the absorption rate is slow and variable. The i.p. route is most commonly used for injectable drugs in rodents due to the large surfactant area of the peritoneum, which allows for better physiologic buffering of the anaesthetic, although it requires a longer time for recovery. Although i.v. allows administration of substances with the most predictable and fastest action, this technique requires expertise.

Injectable anaesthetics (Table 3) can be further divided into barbiturates, steroid anaesthetics, dissociatives, neuroleptanalgesics and other hypnotics such as disopropylphenols. Barbiturates are positive allosteric modulators of GABA chloride channels, which ultimately inhibit excitatory neurotransmission elicited by norepinephrine and glutamate release. The loss of consciousness caused by barbiturates is quick and smooth, although when administered i.p. they can cause perioperative irritation, which can reduce the absorption of the drug and cause pain after injection. In general, barbiturates do not exert analgesic efficacy. Pentobarbital and thiopental are the most commonly used barbiturates. Pentobarbital is used in rodents due to its accessibility, long shelf-life, lower cost and quick action. Also, it is not linked to irritation events. However, it can lead to respiratory depression, reduction in stroke volume and hypotension. In addition, it causes brain and global body hypothermia during general anaesthesia due to reduction in brain metabolic activity.

Thiopental is considered a short-action anaesthetic, which produces a smooth and rapid sedation and can be used in all species. However, it...
Steroid anaesthetics
This class of anaesthetics, represented by alfaxalone, is a neuroactive steroid that acts on GABA-A receptors promoting unconsciousness and muscle relaxation. The advantages of this agent are that it has low toxicity, good overall safety, rapid elimination by liver metabolism and non-irritant. Alfaxalone has a poor solubility in water and some formulations of this anaesthetic agent can lead to adverse effects, including histamine release caused by the solubilising agent. Also, it may cause moderate hypotension.

Dissociatives
Dissociative anaesthetics reduce synaptic excitation by inhibiting glutamate actions in the brain, the major excitatory neurotransmitter, through blockade of N-methyl-D-aspartate (NMDA) receptors. In addition, dissociatives can block muscarinic acetylcholine receptors, potentiate the effects of GABA synaptic inhibition and weakly activate µ-opioid receptors. Examples of dissociative anaesthetics include ketamine, tiletamine and phencyclidine, ketamine being the most commonly used in experimental models using rodents.

Ketamine has a wide safety margin and produces profound analgesia and amnesia without depression of cardiovascular function. On the other hand, it can increase heart rate, consequently increasing cardiac output and blood pressure. When ketamine is used alone, it produces muscle rigidity resulting in incomplete anaesthesia. Therefore, it is usually administered in combination with α-2 adrenergic agonists like xylazine, which is a powerful sedative, hypnotic and analgesic drug.

Neuroleptanalgesics
Neuroleptanalgesics are a combination of a potent opioid and a neuroleptic agent that inhibit pain perception and promote sedation. However, these agents can cause respiratory depression, poor muscle relaxation, hypotension and bradycardia. They should be administered with a fast-acting benzodiazepine (such as midazolam or diazepam) in order to promote proper muscle relaxation. When neuroleptanalgesics are used alone, they can lead to adverse effects, including nausea, excitement and poor degree of muscle relaxation. Their effects can be inhibited by administration of µ-opioid receptor antagonists. For rodents, the use of fentanyl/fluanisone and midazolam is the combination of choice. This combination produces a good surgical anaesthesia coupled with proper muscle relaxation. Compared to pentobarbital, these agents show better tissue perfusion and lower serum corticosterone levels. However, they can induce respiratory depression and skin twitch latency.

Propofol
Propofol is a commonly used intravenous general anaesthetic, and is structurally distinct from other hypnotics such as barbiturates. It has sedative-hypnotic effects that induce loss of consciousness reliably and rapidly. This agent causes sedation by activating GABA neurotransmission through GABA-A receptors, leading to inhibition of excitatory neural activity. However, propofol has lower analgesic activity, which requires the use of an adjunctive analgesic agent.

The advantages of propofol are that it is rapidly metabolised and is associated with rapid recovery after a bolus dosage or continuous infusion, even after long periods of anaesthesia administration. Furthermore, it was suggested that propofol has neuroprotective effects, reduces intracranial pressure and shows potent antioxidant and anti-inflammatory properties. On the other hand, this agent can induce apnoea, reduction in systemic blood pressure and hyper-algesia. Other adverse effects of propofol have been reported, including bradycardia and airway obstruction.

Conclusion
There is plenty of discussion regarding the procedures utilised to cause anaesthesia and/or euthanasia of animals, either domestic or laboratory.
Selection of the proper method requires knowledge of not only exact mechanisms of action but also animal welfare. Thus, many different parameters should be taken into account to choose the adequate method of anaesthesia for a particular experiment, including cost, ease of administration and possible off-target effects of the administered substances within the organ systems to be studied.

Inhalable anaesthetics have many advantages over injectable anaesthetics, particularly for use in laboratory animals. Anaesthesia with isoflurane, one of the most commonly used volatile anaesthetics, has rapid induction, easy control over administration and lower percentage of possible complications. Similar to isoflurane, other inhalable anaesthetics have a larger margin of safety when compared to injectable anaesthetics. However, it is important to note that ether, despite its advantages, has been gradually replaced by other non-inflammable inhalable anaesthetics, such as isoflurane and halothane.

On the other hand, use of injectable anaesthetics allows for a lower dosage of administration, have little or no respiratory depression and do not require the use of specialised equipment (vaporisers and respirators). In addition, they can be used in combination with inhalable anaesthetics, resulting in administration of lower doses of both agents and, consequently, lower incidence of adverse effects resulting from exposure to anaesthetic agents.

Abbreviations list
GABA-A, γ-aminobutyric acid type A; i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; NMDA, N-methyl-D-aspartate; s.c., subcutaneous.

References
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