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REVIEW

MODERN ASPECTS IN ANESTHESIA OF SMALL LABORATORY ANIMALS

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ABSTRACT

Experiments with small laboratory animals are required for better understanding of a disease flow, for studying the mechanisms of it's development and for the development of new therapeutic strategies. A significant part of experimental studies involve anesthesia. In this regard, the choice of the optimal anesthesia protocol is an important point of research, since an inadequate depth of anesthesia or the influence of undesirable factors can lead to death; the type, duration, and goals of the procedure should be also taken into account.

We have aimed to find out what has changed in anesthesia of laboratory animals lately, which drugs are currently relevant and what is the reason for their popularity.

Anesthesia of mice is challenging for several reasons: animal size, metabolic rate, and high risk of hypothermia and hypoglycemia. In addition, anesthetics affect physiological parameters and therefore even more affect the results of experiments. At the moment, there is a large list of drugs used in laboratory animals. Since they are divided into groups depending on the routes of administration, we selected the following drugs from a number of articles: injectable anesthetics (medetomidine, dexmedetomidine, zoletil-100, ketamine, xyla, propofol) and inhalation anesthetics (isoflurane, sevoflurane). Advantages and disadvantages of the drugs and their combinations were studied and described.

An analysis of the literature showed that injection anesthesia is considered the main method of anesthesia for experimental animals and is relatively well tolerated by animals; it also does not require additional bulky equipment and additional staff qualifications, there are antagonists for a number of drugs, and is also affordable.

In the majority of studies inhalation anesthesia was used in long-term complex manipulations/operations, since it is more manageable, agents require minimal metabolism, and in some cases do not require additional sedation.

Keywords:
anesthesia, analgesia, inhalation anesthesia, injection anesthesia, laboratory animals, antagonist, sedation

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ОБЗОР

СОВРЕМЕННЫЕ АСПЕКТЫ В ОБЛАСТИ АНЕСТЕЗИИ МЕЛКИХ ЛАБОРАТОРНЫХ ЖИВОТНЫХ

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РЕЗЮМЕ

Эксперименты с использованием лабораторных животных необходимы для лучшего понимания течения той или иной патологии, изучения механизмов ее развития и разработки новых терапевтических стратегий. Значительная часть экспериментальных исследований требует выполнения процедуры анестезии. В связи с этим выбор оптимального протокола по анестезии является важным пунктом исследований, так как недостаточная глубина наркоза и влияние нежелательных факторов на организм объекта могут привести к летальному исходу.

Для нас представляло интерес выяснить, что поменялось в области анестезии лабораторных животных за последнее время, какие препараты актуальны на данный момент и в чем причина их популярности.

Анестезия мышей является сложной задачей по нескольким причинам: размер животного, скорость метаболизма и высокий риск гипотермии и гипогликемии. Кроме того, анестетики действуют на физиологические параметры, что еще больше влияет на результаты экспериментов. На данный момент существует большой перечень препаратов, применяемых на лабораторных животных. Поскольку они подразделяются на группы в зависимости от путей введения, из ряда статей мы отобрали следующие препарата: инъекционные – «Медетомидин», «Дексмедетомидин», «Золетил-100», «Кетамин», «Ксила», «Пропофол» и ингаляционные – «Изофлуран», «Севофлуран». Изучили и описали преимущества и недостатки препаратов и их сочетаний.

Согласно анализу литературных источников, инъекционная анестезия считается основным методом наркоза для экспериментальных животных и сравнительно хорошо переносится животными, так же не требует дополнительной громоздкой аппаратуры, дополнительной квалификации сотрудников, на ряд препаратов существуют антагонисты, имеет доступную стоимость.

При длительных сложных манипуляциях/операциях в большинстве литературы использовали ингаляционный наркоз, так как он более управляем, агенты требуют минимального метаболизма, в ряде случаев не требует дополнительной седации.

Ключевые слова:
анестезия, анальгезия, ингаляционный наркоз, инъекционный наркоз, лабораторные животные, антагонист, седация

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RELEVANCE

A significant aspect for understanding the patterns of disease development and the development of new antitumor drugs are studies performed on laboratory animals [1]. The goal of the scientific direction for the creation of effective tumor models is the fullest reproduction in the body of experimental animals of human oncogenesis and related systemic changes, which leads researchers to a deeper study of anesthesiology [2].

Currently, various versions of anesthesia protocols are widely used in practice, depending on the tasks and the type of laboratory animals used: white mice, white rats, various types of hamsters, guinea pigs, rabbits, etc. [3]. One of the most frequently used types of experimental animals are mice [3; 4].

The provision of anesthesia and anesthesia for these animals is an important practice in the laboratory, which is necessary for performing invasive procedures, achieving prolonged immobility, for sensitive imaging techniques and pain relief [4; 5]. Thus, the safe and effective use of anesthesia is an important aspect of improving experimental methods, taking into account the specific effect of the selected agents on the physiological parameters that are relevant to the results of the study. For these reasons, the choice of an appropriate anesthetic procedure is an important factor in experimental studies and should correspond to the type and duration of the procedure, as well as their purpose [3].

Laboratory mice have specific physiological and anatomical features that affect the effect of sedatives. Due to their small size and metabolism, the excretion of drugs from the body occurs very quickly. These features bring their own nuances to the procedure of introduction into anesthesia [6].

Also, when choosing anesthesia, the following factors should be taken into account: the weight and age of the animal, the model of the disease being investigated and the type of procedure [7].

At the moment, there is a large list of anesthetics used on laboratory animals. They are grouped depending on the routes of administration: inhalation – based on the introduction of drugs into the body in the form of steam or gas through the respiratory tract ("Isoflurane", "Sevoflurane", etc.) and non–inhalation – based on the introduction of anesthetics parenterally ("Propofol", "Xyla", "Medetomidine", "Zoletil" etc.) [8; 9].

It was interesting to find out what has changed in the field of anesthesia of laboratory animals, in particular mice, which drugs are relevant at the moment and what is the reason for their frequent use.

In this regard, the purpose of our review is to analyze and systematize the data obtained from scientific articles on anesthetics.

Literature search methods

We conducted a systematic search in the PubMed database, using the keywords "anesthesia, laboratory, mice, inhalation, injection" and e-Library: "anesthesia, laboratory mice, experiment". For 10 years, 4180 results were found by search queries in the PubMed database, by keywords, and 2812 articles were found in the e-Library.

The exclusion criteria were:

1. studies in which there was no name of the anesthetic;
2. studies in which laboratory animals were not mice;
3. studies that did not describe the condition of animals when anesthetics were administered, and there were no data on the condition of animals after manipulations under the influence of anesthetics
4. studies for which there was only a summary in the public domain;
5. articles that can only be accessed on a paid basis;
6. studies that did not describe side effects on the animal's body.

As a result of the work done, we selected 106 articles on inhalation and 251 on injection anesthesia in laboratory mice and 46 publications were analyzed in detail.

Analysis of research results

Mice are one of the most widely used types of experimental animals. A lot of manipulations are performed on these rodents, which require either a mild sedative effect or deep anesthesia [10]. In the publications we have studied in recent years, the method of "balanced anesthesia" is indicated, which is the introduction of a mixture of sedatives, analgesics and anesthetics to create high-quality anesthesia. It also allows the use of lower doses than if each component was used in a single mode [7; 11]. This practice has the advantage of synergy and avoids undesirable effects observed when using increased

doses of individual components [11]. Depending on the route of administration, anesthetics can be divided into inhalation – based on the introduction of drugs into the body in the form of steam or gas through the respiratory tract ("Isoflurane", "Sevoflurane", etc.) and non–inhalation – based on the introduction of anesthetics parenterally ("Propofol", "Xyla", "Medetomidine", "Zoletil", etc.)

Injection anesthesia

According to the analysis of literature sources, injection anesthesia is considered the main method of anesthesia when conducting experimental procedures in small rodents [12]. This type of anesthesia has its advantages, since it can be used in almost any conditions, does not require additional bulky equipment, and is relatively well tolerated by animals. But there are also disadvantages, these include: poor handling, the possibility of developing cardiac and respiratory complications a few hours after anesthesia [13–15]. Doses of anesthetics for injectable agents depend on the types of animals used, routes of administration, age, gender, stress, body condition, environment, experimental setup, previous drug treatment and the required level of anesthesia. During the initial period of use, it is important to closely monitor the animals and make any adjustments necessary for future use [14].

For the administration of these drugs, one of the methods is used: intravenous, intramuscular, intraperitoneal [15].

The analysis of the literature showed that the most common drugs in the articles are: "Medetomidine", "Dexmedetomidine", "Zoletil-100", "Ketamine", "Xyla", "Propofol" and their combinations. In this review, we have reviewed several of the most common drugs and their combinations.

Drugs:

"Medetomidine"

It stimulates peripheral and central α2-adrenoreceptors, has a selective effect, which reduces the drug load on the animal's body. It is one of the most popular drugs because of its high α2 selectivity against receptors. "Medetomidine" is a sedative and analgesic. Its main side effects include bradycardia, hypotension, respiratory depression, hypothermia and diuresis. To stop the effect of "Medetomidine" on the animal's body, drugs containing such an active substance as atypamezole hydrochloride are used in practice. Due to the antagonistic effect on α2 receptors, the release of norepinephrine is suppressed and, as a result, the effects caused by Medetomidine are stopped [16].

"Dexmedetomidine"

"Dexmedetomidine" has been identified as the active enantiomer, aka the mirror drug "Medetomidine".

"Dexmedetomidine" is an α2-adrenergic receptor agonist similar to "Medetomidine", but it lacks the pharmacologically inactive enantiomer of levomedetomidine. "Dexmedetomidine" has a stronger anesthetic effect than "Medetomidine" and is 40 times stronger than "Xyla" [7; 17]. "Dexmedetomidine" provides excellent muscle relaxation and pain relief during surgical interventions. In addition, the administration of the drug with the active substance – atypamezole hydrochloride, provides rapid elimination of the anesthetic effect and leads to rapid recovery of physiological parameters [17].

"Ketamine"

"Ketamine" causes a state of "dissociative anesthesia", in which deep anesthesia, sedation and muscle rigidity (stage of catalepsy) are observed. It does not depress the central nervous system (CNS), so reflexes remain intact. Side effects are as follows: the eyes remain open, so it is additionally recommended to use eye ointment; the presence of spontaneous movements and muscle tension, which causes an initial increase in blood pressure [18]. Unlike other anesthetics, Ketamine does not inhibit breathing or cardiac output. Also, this drug, used in mono mode, does not provide sufficient anesthesia. But the combination with "Xyla", "Medetomidine" or "Diazepam" creates an effective anesthesia [19].

"Propofol"

The drug is an isopropylphenyl compound available for intravenous administration. Propofol exerts its influence on the central nervous system by modulating gamma-aminobutyric acid (GABA) channels through sites other than barbiturates, steroids or benzodiazepines. It quickly causes loss of consciousness, recovery is faster and more complete with minimal residual effects, has good potential as an anesthesia regimen for functional studies. However, it does not cause analgesia, so when performing painful manipulations, additional analgesic drugs should be used. Cerebral blood flow, perfusion pressure and intracranial pressure decrease after administration of Propofol. It is a powerful respiratory depressant, so apnea often occurs during induction if the drug is not administered slowly [15].

Table 1. Drugs and their combinations for anesthesia

Drug and/or drug combination	Indication	Adverse effects	Method of drug administration	Specific antagonist	References
Medetomidine	Sedation	Bradycardia, hypotension, respiratory depression, hypothermia and diuresis	IM; SC	Atipamezole	[6; 11; 16]
Dexmedetomidine	Sedation	No information was found in the studied articles	IM; SC	Atipamezol	[17; 23]
Ketamine	Anesthesia	Skeletal muscle tone, risk of depression or respiratory arrest.	IM	No information was found in the studied articles	[7; 13; 18]
Propofol	anesthesia	No information was found in the studied articles	IV slowly	No information was found in the studied articles	[13; 14; 19]
Zoletil 100 + Xyla	Sedation + anesthesia	Transient hypertension, prolonged hypotension, a decrease in cardiac output by 30–50 %, diuresis, hypothermia, hyperglycemia, cerebral hypoxia and a decrease in intestinal motility lasting for several hours	IM	No information was found in the studied articles	[15; 20–23]
Alfaxalon + Xyla	Sedation + anesthesia	Hypotension and hypoventilation have a significant effect on the cardiovascular system, manifested by a low pulse rate.	IM	Atipamezol	[24–26]
Ketamine + Xyla	Sedation + anesthesia	Hypotension and hypoventilation have a significant effect on the cardiovascular system, manifested by a low pulse rate.	IM	Atipamezol	[16; 20; 27–29]
Isoflurane	Inhalation anesthesia	Circadian rhythm disorders, as well as hypothermia and hypoglycemia	Inhalation	No information was found in the studied articles	[30; 32–36]
Sevoflurane	Inhalation anesthesia	Circadian rhythm disorders, as well as hypothermia and hypoglycemia	Inhalation	No information was found in the studied articles	[13; 31; 37–39]

"Xyla" + "Zoletil-100"

The drug "Xyla" is used both as a sedative and as a component of balanced combinations of anesthesia. It is believed that the sedative effect of α2-adrenoreceptor agonists is carried out by stimulating the subtypes of α2-adrenoreceptors in the blue spot of the brain stem, which reduces the release of norepinephrine [20]. Although newer drugs "Medetomidine" and "Dexmedetomidine" have a higher specificity to the α2-adrenergic receptor, "Xyla" is still widely used in veterinary medicine [21]. Side effects of this drug are: transient hypertension, prolonged hypotension, a decrease in cardiac output by 30–50 %, diuresis, hypothermia, hyperglycemia, cerebral hypoxia and a decrease in intestinal motility lasting for several hours [22].

The main advantage of α2-adrenergic receptors is the availability of antagonists. Effective relief of xylazine sedation by an antagonist leads to a rapid awakening of the object, as well as to the leveling of most of the previously listed side effects. When deciding on the administration of an antagonist, the researcher responsible for anesthesia should take into account some factors: the antagonist should not be administered until the need for sedative, anxiolytic and analgesic effects of the agonist disappears [23].

To enhance the anesthetic effect, the drug "Xyla" is combined with "Zoletil 100". "Zoletil 100" is used for general anesthesia of animals, it consists of two components: tiletamine and zolazepam. Tiletamine is a general anesthetic of dissociative action, causing a pronounced analgesic effect, but insufficient muscle relaxation. Zolazepam inhibits subcortical areas of the brain, causing anxiolytic and sedative effects, relaxes striated muscles. Zolazepam enhances the effect of tiletamine, and also prevents seizures caused by tiletamine, improves muscle relaxation and accelerates recovery after anesthesia [7].

"Alfaxalon" + " Xyla"

When searching for articles on this drug, we found that "Alfaxalon" was evaluated as an anesthetic in mice.

Alfaxalon is a neuroactive steroid that acts as a GABA agonist. The early version of "Alfaxalon" was insoluble in water, and polyoxyl 35 castor oil was added to it to increase solubility. However, this composition of this drug, which was used as a veterinary anesthetic, was discontinued because the solubilizing agent induced the release of histamine, which contributed to the appearance of anaphylactic reactions [24; 25].

Subsequently, the composition of the drug was changed using 2-hydroxypropyl-β-cyclodextrin as a solubilizing agent, which eliminated these side effects. After the changes, "Alfaxalon" began to gain great popularity in veterinary medicine as a sedative and a component of intravenous general anesthesia in various animal species [24; 26].

One of the studies showed that "Alfaxalon" in combination with "Xyla" can be administered intraperitoneally [25]. The authors found that "Alfaxalon" + "Xyla" provides a longer duration of anesthesia during prolonged manipulations than "Ketamine" + "Xyla". After analyzing this study, the following conclusions can be made: intraperitoneal administration of "Alfaxalon" + "Xyla" provided effective immobilization and anesthesia, which may be suitable for orthopedic operations, imaging or other invasive procedures [25].

"Ketamine" + "Xyla"

Ketamine is used as an additional anesthetic due to its limited ability to provide adequate relaxation of skeletal muscles. In addition, the use of a combination of anesthetics with "Xyla" as an anesthetic in rodents has limitations, including a long induction time and weak local tolerance with intramuscular administration [20; 27].

Analysis of the literature data showed that the combination of "Ketamine" + "Xyla" is one of the most frequently used schemes for anesthesia of mice. Its main disadvantage is a gentle dose – effect curve, which leads to unpredictable consequences. It is noteworthy that comparable dosage regimens of "Ketamine" + "Xyla" in mice can give a variety of results, ranging from surgical anesthesia to death [16; 28].

Combinations of "Ketamine" + "Xyla" can affect the hemodynamics of the brain, causing a decrease in cerebral blood flow and affecting brain oxygenation, which can have mixed effects when visualizing perfusion [28; 29]. In addition, the combination of "Ketamine" with "Xyla" has a significant effect on the cardiovascular system, manifested by a low pulse rate and hypotension [29].

Inhalation anesthesia

This type of anesthesia is based on the introduction of anesthetics into the body in the form of steam or gas through the respiratory tract. The saturation of the body with anesthetics occurs due to their diffusion through the alveoli and depends on the concentration, type of anesthetics, their solubility in blood and tissues, the state of blood circulation and respiratory system [30].

Gas anesthesia has many advantages, including: 1) increased control over the depth of anesthesia; 2) minimization of research variability due to the presence of agents (for example, "Isoflurane") that require minimal metabolism; 3) reduction of cardiopulmonary depression, which leads to increased safety during induction and reduced recovery time [31].

From the proposed spectrum of inhalation anesthesia, we selected the following substances: "Isoflurane" and "Sevoflurane". This is due to the frequency of occurrence of these anesthetics in the literature.

"Isoflurane"

"Isoflurane" is a common inhalation anesthetic in laboratory animal medicine and veterinary practice, although the exact mechanism of its action is complex and not fully understood, it is widely used due to the fact that it is minimally metabolized (< 0.17 %) in the liver and, therefore, less toxic to animal metabolism compared to with injectable anesthetics [32].

"Isoflurane" causes moderate depression of the respiratory and cardiovascular systems, but supports better cardiac function than the combination of "Ketamine" and "Xyla" [33]. However, in addition to these benefits, it has also been found that "Isoflurane" causes reversible object recognition memory deficits, impaired learning function, circadian rhythm disorders, as well as hypothermia and hypoglycemia, which can negatively affect the recovery period. In mice and rats, repeated administration of "Isoflurane" causes a pronounced rejection than a single administration [34–36].

"Sevoflurane"

Sevoflurane was first synthesized in the late 1960s, but was not approved for medical use until 1990 due to concerns about the decomposition of sodium lime and the release of fluorine ions during metabolism, which can also cause nephrotoxicity [37]. After a while, it was proved that none of these problems has clinical significance for humans or animals [38–39]. It is less soluble than Isoflurane, which means that induction and recovery occur even faster. "Sevoflurane" is better tolerated by induction through a facial mask and a camera, since it has low acuity and low irritant effect on the respiratory tract [40]. "Sevoflurane", depending on the dose, causes reversible loss of consciousness and pain sensitivity, suppression of voluntary motor activity, decreased autonomic reflexes, as well as sedation of the respiratory and cardiovascular systems.

CONCLUSION

According to the literature data, the range of topical anesthetics has increased significantly over the past 10 years. Specific antagonists are used for a number of injectable drugs. Analysis of the literature data has shown that inhalation anesthesia is the preferred option for prolonged, complex manipulations/operations, since it is more manageable, agents require minimal metabolism, as a rule, does not require additional sedation with third-party drugs.

But it is also worth considering a number of disadvantages in this method: the cost of equipment, the need for a cleaning system for the removal (withdrawal) of exhaust gases and protection of personnel (oversaturation of employees with gas leads to dizziness, loss of consciousness, tachycardia poisoning, etc.), as well as to work on this equipment requires qualified team and annual maintenance, which carries additional financial costs.

For minimally invasive manipulations, the use of injectable anesthesia is recommended. This type of anesthesia has a number of advantages: the use of the drug and its combination in any conditions, does not require additional cumbersome equipment, staff qualifications, antagonists are used, affordable cost.

There are also disadvantages: poor handling, difficulty in selecting the dosage and administration of the drug due to the small weight of the animal.

All of the above indicates the dynamics of development in the field of anesthesia protocols of laboratory animals.

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