Mice Anesthesia, Analgesia, and Care, Part I: Anesthetic Considerations in Preclinical Resea

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Abstract

Animal experiments are necessary for a better understanding of diseases and for developing new therapeutic strategies. The mouse (*Mus musculus*) is currently the most popular laboratory animal in biomedical research. Experimental procedures on animals often require anesthesia and/or analgesia to obtain adequate immobilization and to reduce stress or pain. Mice anesthe is challenging for several reasons including the animals' size, metabolic rate, and the high risk of hypothermia and hypoglycemi Moreover, anesthetic agents influence physiological parameters, further interfering with experimental results. Small animal imag procedures are increasingly used in biomedical research both because the animals allow in vivo monitoring and because they a readily available for longitudinal and noninvasive studies as well as investigations into the evolution of diseases and the effects new therapies. Anesthesia must adapt to the imaging technique, the procedure length, and the aim of the study. The purpose o article is to review the existing literature on anesthetic protocols adopted in mice for molecular imaging studies and to report ou experience.

Key words: analgesia; anesthesia; chemical restraint; longitudinal studies; mice; preclinical research; small animal imaging

Introduction

Animal models are of paramount importance in biomedical research to improve in vivo understanding of the physiology of living organisms and the pathophysiology of most diseases and to provide fundamental information to address new treatment strateg Among small laboratory animals, mice (*Mus musculus*) are by far the most popular animal model in biomedical research becau of their biological similarity to man, reduced maintenance cost, easy handling, short reproduction rate, and, more recently, their outbreak in genetic manipulation studies. In fact, genetically engineered mice make up most of the current models of human diseases.

The use of mice in research has increased dramatically as a result of striking progress in the use of biomedical tools that have broadened medical knowledge and allowed new means of relatively noninvasive body exploration and treatment. Recently developed tools such as miniaturized imaging machinery as well as micro pumps, micro dialysis, and modern stereotaxic apparatuses have opened new mice research fields. Advances in anesthetic techniques have enabled investigators to overcon the small size of this species. Skilled techniques in anesthesia and analgesia have made it possible for scientists to avoid or at greatly lessen the animals' stress and suffering. Because animals do not voluntarily endure human manipulation, scientists are actively working to replace animals in medical research with the growing number of alternative methods such as ex vivo and in v studies, computer simulations, and artificial models. Such methods are also aimed at reducing the number of animals used for single experiment, restricting the authorization to operate on live animals, and refining the experiments through humane and ger

techniques. Nevertheless, in spite of these efforts, in vivo studies are still a necessity for understanding diseases and for developing new therapeutic strategies. Under these circumstances, it is of paramount importance to refine animal experiments to use highly sophisticated anesthetic procedures that include systemic and local analgesia, sedation, chemical restraint, and b trained and dedicated personnel. Because experimental procedures are stressful and potentially painful, it is compulsory to use sedation, analgesia, and sometimes general anesthesia for most animal experiments.

The individual distress and suffering of animals must be minimized not only for ethical reasons but also because of their potent adverse effects on experimental results. Therefore, safe and effective anesthetic and analgesic management is a crucial aspe the refinement of experimental methods, taking into account the specific influence of the chosen agents on physiological variab that are relevant for the results of a study. For these reasons, the choice of a suitable anesthetic procedure is a key factor in preclinical studies, and should comply also with the type and length of the procedure and the aim of the study.

Laboratory mice exhibit specific anatomic and physiologic peculiarities that influence the effects of anesthetic drugs. Due to the small body size, drug metabolism and excretion are extremely fast, reducing the half-life of injectable drugs and rendering the duration of anesthesia a more critical factor compared with larger species. For instance, a 1-hour procedure in a mouse require same metabolic cost as a 6-hour procedure in a cat. Moreover, the elevated body surface area of mice promotes heat loss and hypothermia, while their reduced glycogen reserve predisposes them to hypoglycemia. In addition, their high oxygen consumpt rate reduces the survival rate for hypoxemia. In fact, irreversible central nervous system (CNS[1]) damage occurs only a few sec after respiratory arrest in mice (Abou-Madi 2006).

Primary factors to take into account in choosing an anesthetic technique for mice are strain, age, weight, the disease model to b investigated, and the type of experimental procedure to be used. Small animal imaging procedures are being used increasingly biomedical research because of their potential utility in localizing physical and biochemical phenomena, and for allowing investigators to monitor in vivo, by serial and noninvasive means, the effects of pharmacological or gene therapies. Thus, imagi procedures pose their own anesthetic requirements based on the use of particular procedural techniques and on the duration o studies. In the text below, we review the existing literature on anesthetic protocols recommended for mice, and we report our ov experience in longitudinal imaging studies.

Mice Anesthesia

Preanesthetic Care and Clinical Examination

Preanesthetic care reduces the incidence of complications that can occur in the course of anesthesia by ensuring the choice of most suitable technique and regimen. As a rule, animals purchased from external sources should be singly housed for 1 or 2 w both to acclimate the animals and to allow time before an experiment for animal care personnel to observe and evaluate the hea of the animals. Although the physical status of small rodents is documented when they are first inspected, subsequent signs of actual disease are often discovered incidentally later, during an experiment, during anesthesia, or even after anesthesia. For thi reason, it is mandatory to ascertain *before an experiment* the animals' behavioral patterns, body condition score, respiratory ra food and water intake, as well as defecation, urination, absence of skin lesions, eyes or nose discharges, or perineal soiling.

Personnel attending the mice must be trained to handle the animals gently but firmly because this handling has a strong influenc the animals' physiological functions. Physical restraint and manipulation can induce corticosteroid and epinephrine release, and stimulate cardiovascular and respiratory functions that increase glucose levels, body temperature, and anesthetic induction dos because they increase cardiac output (Hildebrandt et al. 2008). Stress can also negatively affect the quality of some molecular imaging procedures such as restraint distress during fluorodeoxyglucose (^{18}F-FGD[1]) injection, which can cause an evident upta of radiotracer in dorsal muscles, interfering with visualization of the heart, lungs, or metastatic chest lesions. Moreover, cardiovascular response to stress can upset the echocardiograph evaluation of left ventricle systolic function.

Preanesthetic fasting in mice is generally deemed unnecessary because the animals cannot vomit. Conversely, prolonged fast can cause hypoglycemia due to their low hepatic glycogen reserve (Rao and Verkman 2000). Some concern about anesthetizi mice that have a full stomach is linked to limited diaphragmatic excursion and to gastric blood pooling during digestion. Furthermore, in imaging studies a full stomach may interfere with adjacent structures. However, a fasting time of about 6 hours i suggested as an effective way to ensure uniformity in some studies such as positron emission tomography with ^{18}F-FGD (Hildebrandt et al. 2008). In addition, when optical imaging is performed, it is necessary to consider dietary composition becaus food components such as chlorophyll can be a source of background autofluorescence (McNally et al. 2006). Mice should alwa

have free access to water, even shortly before general anesthesia.

Preanesthetic Considerations

Anesthesia of small laboratory animals is particularly challenging due to the following problems that relate to their small body siz hypothermia, high metabolic rate, and lack of reliable clinical signs of respiratory and cardiovascular functions. Because hypothermia is potentially lethal, preservation of body heat is an integral part of anesthetic management. Core body temperature decreases suddenly after induction and continues to diminish during the course of prolonged general anesthesia, especially in small rodents. Therefore hypothermia must be prevented by providing heat through warming pads and infrared lamps. Hypothe can also negatively affect the quality of some molecular imaging procedures such as positron emission tomography with ^{18}F-FI increasing ^{18}F-FDG uptake by interscapular brown fat and meddling the visualization of neighbouring structures (Fueger et al. 2006).

In mice, strain, body weight, age, and sex add to the well-known anesthetic variability that exists among individuals of other spe As an example, mice less than 8 weeks of age metabolize anesthetics less efficiently than adults due to their immature liver enzymatic system and reduced homeostatic response. They also pose an increased risk of hypoglycemia, hydroelectrolytic an acid–base imbalances, and hypothermia due not only to their smaller size but also to the immaturity of their thermoregulatory centers (Paddleford 2000). Similar problems ensue in mice more than 18 months old, due to subclinical pathologies related to senescence such as hepatic, renal, or cardiac impairment.

The sex of mice influences the pharmacokinetics and metabolism of anesthetics probably due to differences in plasma corticosteroids, sexual hormones, or hepatic enzymes (Hildebrandt et al. 2008). As an example, a higher dose of ketamine is recommended for female mice compared with that for males. Obese mice present an altered biodistribution of lipophylic agent and a high incidence of hepatic dysfunctions and are therefore at high anesthetic risk because of hypoventilation and hypoxia. However, cachectic mice present low plasma protein binding and might hide renal, hepatic, or cardiac deficiencies. Several mu models that mimic human diseases such as obesity, diabetes mellitus, myocardial ischemia, and neoplasia require specific anesthetic protocols and pose increasing difficulties in serial studies. Furthermore, genome alterations of transgenic mice can influence the effects of anesthetic agents either accidentally (Quinlan et al. 1998) or deliberately, as reported by Xie and collea (2000), eliminating the anesthetic potential of tribromoethanol in VPSXR mice.

Knockout mice can withstand both obesity and altered sensitivity to anesthetics, as do knockout mice for the brain neuropeptide subtype receptor that is involved in learning and memory. Such mice display both obesity and reduced sensitivity to pentobarbi and to avertin (Kushi et al 1998; Naveilhan et al. 2001), whereas deletion of the Y2 receptor results in increased body weight an increased sensitivity to pentobarbital (Naveilhan et al. 2001). Due to the lack of an easy vascular access, we recommend giving mL subcutaneous (SC[1]) or intraperitoneal (IP[1]) fluid bolus to mice before anesthesia to allow for fluid loss and to expand the circulating volume.

Premedication

Preanesthetic medication by tranquillizers and analgesics is generally administered to reduce apprehension, favor stress-free induction and recovery, reduce doses and side effects of other anesthetic agents, and achieve pre-emptive analgesia. Howeve mice a “single shot” anesthetic protocol is advisable to minimize the stress caused by multiple injections. Atropine (0.04 mg/kg IP, or intramuscular [IM[1]]) has been recommended before induction of anesthesia to reduce bronchial and salivary secretions a to protect the heart from vagal inhibition (Flecknell 1989). Atropine can be mixed with other hydrosoluble agents or administere minutes before induction by the IM route. Nevertheless, Zuurbier and colleagues (2002) reported that the addition of atropine to ketamine/medetomidine anesthesia does not prevent alpha-2-generated bradycardia.

Anesthetic Regimen

Anesthesia in laboratory animals is a state of unconsciousness, analgesia, muscle relaxation, and a-reflexia (Kohn et al. 1997). Anesthetic regimens can be of two kinds: injectable or inhaled, according to the nature of the administered drugs. A main indica for general anesthesia in imaging procedures is the need for a constant immobility, avoiding movement artifacts. Anesthetic de in mice can be clinically monitored by observing the loss of the righting and palpebral reflexes, and by assessing muscular tone response to painful stimulation, and rate and depth of respiration. An ideal anesthetic agent for mice should be easy to adminis produce a fast and adequate immobilization, have limited side effects, and be reversible and safe for animals and operators.

Unfortunately such an anesthetic is not available, and the best drug selection is highly variable according to different experimen circumstances.

Induction of general anesthesia in mice can be achieved by a variety of drugs and techniques (Flecknell 1989). The most commonly used anesthetics in mice include the injectable agents avertin, pentobarbital, and ketamine, which are often combine with other agents such as acepromazine, xylazine, diazepam, several narcotic analgesics, and the inhalation agents halothane, isoflurane, and sevoflurane. Compared with injectable techniques, inhalation anesthesia provides greater safety, particularly for prolonged procedures, due to a lesser cardiovascular depression, a reduced impact on liver and kidney functions, and becaus promotes rapid recovery and allows quick adjustments and easy maintenance of a steady anesthetic depth. However, inhalant agents foster respiratory depression (particularly in the presence of respiratory diseases), myocardial depression, vasodilation, hypotension (Paddleford 2000), exhibiting weak analgesic effects. Compared with injectable agents, the modern inhalation anesthetics require complex and expensive equipment such as precision vaporizers and flowmeters, specific breathing system and efficient scavenging systems to prevent pollution.

In spite of some obvious advantages, injectable anesthetics also present disadvantages such as difficulty in choosing an initial dose, impossibility of removal from the patient once injected, and no chance of accurately modulating the depth of prolonged anesthesia. Furthermore, they can cause prolonged recovery, pronounced alterations of heart and respiratory rate, and risks in combination with misplaced injection as for abdominal organs by the IP route. On these grounds, reversible agents with high therapeutic index are preferable. Moreover, in line with “balanced anesthesia” principles, a combination of injectable agents sho be chosen to minimize the adverse effects of each one.

Injectable Anesthesia

In mice, injectable anesthetics can best be administered via IP, IM, and IV routes. The SC route is unpredictable for anesthetic induction because of its variable and slow absorption rate. The injection volume should be carefully considered according to the available route: adequate volumes by the IP route range from 0.1 to 1mL, by the IV route from 0.05 to 0.2 mL, and by the IM ro not to exceed 0.05 mL in adult mice (Flecknell 1989). One advantage of some injectable anesthetic agents is their reversibility specific antagonists such as yohimbine and atipamezole for alpha-2 agonists, flumazenil for benzodiazepines, and naloxone fo opioids.

Barbiturates

Barbiturates are γ-aminobutyric acid (GABA[1])-mimetic drugs that inhibit the release of norepinephrine and glutamate. The most popular two barbiturates are pentobarbital, a short-acting oxybarbiturate (sleep time of 60-120 minutes), and thiopental, an ultrashort-acting thiobarbiturate (sleep time of 10-20 minutes) (Table 1). Although pentobarbital can be administered by the IM r only IV or IP administration is recommended for thiopental because of its high histotoxicity, which causes significant tissue dam (van Zutphen et al. 1993).

Table 1: Recommended dosages for barbiturates

Drugs	Dosage	Comments	References
Pentobarbital	50-90 mg/kg IP	Cardiorespiratory depression, hypotension; long duration	Flecknell (1989), Kohn et al. (1997), Hildebrand et al. (2008)
Thiopental	30-40 mg/kg IP	Cardiorespiratory depression, hypotension; short duration	Flecknell (1989), Hildebrand et al. (2008)

Significant differences in sleep time and loss of righting reflex between the long-sleep (LS) and short-sleep (SS) selected lines mice have been well-established after pentobarbital administration (Bennett 2000; Christensen et al. 1996). In studies of the ef of 60 mg/kg of pentobarbital in 23 inbred strains, investigators reported the following: DBA mice showed the longest sleeping t and NZW the shortest; C57Bl/6 mice slept significantly longer than BALB/c; and ob/ob mice showed shorter pentobarbital slee times than +/ob ones (Kohn et al 1997). Due to the restricted therapeutic index of barbiturates, their use is often confined to nonsurvival procedures or to record some neurophysiologic signals such as visual or auditory evoked responses (University of California San Francisco, Institutional Animal Care and Use Committee). Their main adverse effects are respiratory and cardiovascular depression. In some European countries, barbiturates are controlled substances, therefore their detention and u are strictly regulated.

Tribromoethanol (TBE[1])

Commercially named Avertin, TBE is a popular injectable anesthetic for mice that offers the advantage of not being a controlled substance. It should be administered at a dosage of 240 mg/kg IP to provide good muscular relaxation and short-lasting anesth (15-30 minutes). TBE causes moderate cardiopulmonary depression and hyperglycemia. The effects of TBE are somewhat unpredictable in mice less than 16 days old, in obesity or diabetes mouse models (db/db o ob/ob), and in strains with genetic predisposition to hyperglycemia such as C57Bl/6J. Its effects are also variable among individuals, different strains, and stock c solution used (Zeller et al. 1997).

Since 1980, several studies have reported that TBE can cause necrosis of abdominal skeletal muscular fibers, and at high dos and after reiterated injections, it can induce peritonitis, abdominal adhesions, and intestinal ileus (Zeller et al. 1997). TBE decay the presence of heat or light, producing toxic by-products that are known to be both nephrotoxic and hepatotoxic. In fact, administration of degraded TBE solutions has been associated with delayed death up to 24 hours after surgery (Table 2). On th grounds, Swiss and Dutch animal ethics committees strongly disapprove of the use of TBE, while in the United States the use c TBE is limited to terminal procedures and is banned for embryo-transfer procedures or serial studies (Arras et al. 2001; Meyer Fish 2005).

Table 2: Recommended dosages for tribromoethanol

Drugs	Dosage	Comments	References
Tribromoethanol	240 mg/kg IP	Peritonitis, intestinal ileus peritoneal inflammation, adhesions, mortality after the second administration	Zeller et al. (1997) Flecknell (1989) Arras et al. (2001) Meyer and Fish (2005)

Dissociative Anesthetics

Dissociative anesthetics produce catalepsy, analgesia, amnesia, and immobilization characterized by CNS activation and sympathetic stimulation (Paddleford 2000). Dissociative anesthetics include ketamine and tiletamine, both of which have a wide margin of safety and a pronounced analgesic effect that prevents spinal sensitization (wind-up) by inhibiting N-methyl-D-asparta receptors (Table 3). They can induce light respiratory depression while preserving cardiovascular function. Their peculiar anest

effects reflected in the term *dissociative* are consistent with increased muscular tone and rigidity, persistence of some cranial nerve reflexes, and electroencephalogram activation patterns. Dissociative anesthesia produces a sympathetic stimulation that increases the plasmatic levels of norepinephrine and could interfere with studies involving the sympathetic nervous system. The can increase intracranial blood flow and pressure although in mice, unlike other species, they show no proconvulsant activity. Th use of the S (+) isomer of ketamine has been reported to produce a shorter sleeping time and fewer side effects (ataxia, tail flicking, hyperactivity) compared with the commonly used racemic mixture R (-) ketamine. Ketamine is often combined with othe anesthetic agents such as phenothiazines, α2 agonists, and benzodiazepines to improve the quality of anesthesia while reducir side effects (Kiliç and Henke 2004). Inasmuch as dissociative anesthetics allow open eyes, personnel should protect the anima corneas with an eye ointment to prevent corneal drying and damage.

Ketamine is a controlled substance in some European countries, therefore its use is limited by the same strict rules enforced fo opioids, barbiturates, and other drugs of abuse. Tiletamine is a parent compound that is about 10 times more potent than ketam and it is commericially available in combination with the benzodiazepine zolazepam in products called Zoletil® or Telazol®. The combination tiletamine-zolazepam by itself provides profound dissociative anesthesia without muscle rigidity and proconvulsant effects. In combination with xylazine, its anesthetic power is enhanced, fitting the needs of surgical anesthesia. Unfortunately Zoletil® is not a suitable anesthetic agent for mice due to its long-lasting effects, delayed recovery, and severe cardiorespirator depression.

Table 3: Recommended dosages for dissociative anesthetics

Drugs	Dosage	Comments	References
Ketamine	80-100mg/kg IP	Only sedation	Xu et al. (2007)
Tiletamine + zolazepam	40/80 mg/kg IP	Only sedation	Flecknell (1989)

Alpha-2 Adrenergic Agonists

The alpha-2 adrenergic agonists are a class of tranquilizers that include molecules with different selectivity for alpha adrenergic receptors. These drugs produce species-dependent dose-related CNS depression, and are powerful sedatives, hypnotics, an analgesics. Among the advantages offered by these molecules is the availability of receptor-specific antagonists such as yoim or atipamezole. Peculiar side effects in combination with the administration of alpha-2 agonists include hypertension/hypotensio increased peripheral vascular resistance, decreased cardiac output, increased central venous pressure, respiratory depressior hyperglycemia, glycosuria and enhanced diuresis. Reflex bradicardya and second degree atrioventricular heart block may occu a consequence of hypertension and cannot be prevented by administering anticholinergics. The alpha-2 agonists used in mice include xylazine, medetomidine and dexmedetomidine.

The combination of ketamine and xylazine is still the most widely used ketamine combination in mice, providing good immobiliz with some degree of analgesia. Several different dosage combinations of the ketamine/xylazine mixture have been reported fo mice in the medical literature (Table 4), varying from 65/4 to 100/10 mg/kg. The large variability of the recommended dosages depends on differences related to strain, sex, age, and type of experimental procedure. As an example, echocardiography can performed with lower doses compared with invasive and painful procedures, which require higher dosages. Medetomidine is a newer compound that is about 10 times more powerful than xylazine and that exhibits a high selectivity for alpha-2 receptors. It provides the specific antagonist “Atipamezole.” In combination with ketamine, medetomidine prolongs mice anesthesia time (a 50 minutes), and recovery time is proportional to sleeping time. Moreover, the compound produces greater bradicardia and bradypnea compared with equipotent doses of xylazine (personal observation). Recently, Wells and colleagues (2009) reporte that in male mice, anesthesia following 0.5 mg/kg of medetomidine and 50 mg/kg of ketamine created a potential risk of “obstructive uropathy” due to the formation of a seminal coagulum, whereas such a complication was not observed with a dose 100 mg/kg of ketamine and 10 mg/kg of xylazine. The complication has been observed in our laboratory as well. The ketamine-medetomidine mixture is less commonly used than the ketamine-xylazine mixture, which is reserved for painful or short-term

procedures that require good immobilization (e.g., dual energy x-ray absorptiometry [DEXA] or computed tomography [CT]). Dosages reported in the literature vary between 40 and 80 mg/kg for ketamine and 0.25 to 1 mg/kg for medetomidine.

Dexmedetomidine is the newest of the alpha-2 agonist compounds that produces enhanced sedative and analgesic effects. It the pharmacologically active dextro-isomer of medetomidine, which possesses a higher affinity for α2 receptors. Dexmedetom also is reversed by Atipamezole. Although not supported by some reports in the literature, empirical doses of dexmedetomidin 0.5 to 1 mg/kg have been reported for mice anesthesia in combination with 50 to 75 mg/kg IP of ketamine (www.utsouthwestern.edu/).

Table 4: Recommended dosages for combinations with ketamine and α2 agonists

Drugs	Dosage	Comments	Referen
Ketamine + xylazine	100/10 mg/kg IP 65/4 mg/kg IP 100/5 mg/kg IP 100/1.25 mg/kg IP	α2 agonist reversible with atipamezole 1mg/kg IP; to prolong anesthesia can be administered ¼ – ½ of the dosage used of ketamine	Fleckn (1989 Buitrago (2008 Chari et (2001 Roth et (2001 Janseer al. (2004 Schaefe al. (200
Ketamine + medetomidine	75/1 mg/kg IP female 75/1 mg/kg IP male 50/1 mg/kg IP	α2 agonist reversible with atipamezole 1mg/kg IP moderate respiratory depression	Voipio e (1988 Fleckn (1989 Taylor e (2000 Cruz et (1998
S-Ketamine + medetomidine racemic ketamine + medetomidine	75 mg/kg + 0.25 mg/kg 50 mg/kg + 0.25 mg/kg	α2 agonist reversible with atipamezole 1mg/kg IP; action more short and awakening more fast than racemic ketamine	Kilic ar Henke (2004
Ketamine + dexmedetomidine	50-75/0.5-1 mg/kg IP	α2 agonist reversible with atipamezole 1mg/kg IP moderate respiratory depression	UT Southwes

Phenothiazine Tranquillizers

Several molecules of the phenothiazine class of tranquilizers are used for mice restraint and anesthesia. Acepromazine, which among the most powerful and widely used compounds of this class of drugs in animals, produces minimal depression on the respiratory system and antiemetic, antihistaminic, and antiarrhythmogenic effects. Related compounds are promazine, chlorpromazine, and propionylpromazine.

The phenothiazine tranquillizers produce no analgesic effects, therefore they should not be used for painful procedures unless analgesia is provided by other means. Their main undesirable effects are cardiovascular depression and hypotension due to th alpha adrenergic blocking action, which limits their use in pediatric or geriatric mice. Furthermore, because they decrease seizu threshold, they are not recommended in subjects with CNS lesions. In mice, acepromazine is used to potentiate and prolong ketamine and xylazine anesthesia (Arras et al. 2001) (Table 5).

Table 5: Recommended dosages for combinations with ketamine and phenotiazine

Drugs	Dosage	Comments	References
Ketamine + acepromazine	100/5 mg/kg IP	Sedation	Flecknell (1989
Ketamine + xylazine + acepromazine	100/10/3 mg/kg IP	Surgical anesthesia	Arras et al. (2001) Buitrago et al. (2008)

Benzodiazepines

Benzodiazepines are graded as hypnotics, central muscle relaxants, and “minor tranquilizers” because of their limited sedative action. They include the lipo-soluble main compound diazepam, the hydro-soluble midazolam, zolazepam, and flunitrazepam, a many other molecules that produce mild CNS depression through their agonist activity at GABA-a-type receptors. Benzodiazep can be reversed by the GABA receptor antagonist Flumazenil. They exert minor cardiovascular and respiratory depressant effe and their dosage is limited by their ceiling effect. Among laboratory animals, benzodiazepines display significant species variati in that they cause minimal sedation in some species but marked sedation in rabbits and rodents (Flecknell 1989).

Midazolam has shorter onset and duration compared with diazepam. Because they both cause minimal cardiopulmonary depression, they are recommended for old animals and diseased patients including cardiovascular and metabolically comprom patients. They are very popular and are compatible with the majority of anesthetic agents (Table 6).

Table 6: Recommended dosages for combinations with ketamine and benzodiazepines

Drugs	Dosage	Comments	References
Ketamine + diazepam	100/5 mg/kg IP	Immobilization	Flecknell (1989)
Ketamine + midazolam	100/5 mg/kg IP 100/3 mg/kg IP	Immobilization	Flecknell (1989)

	50/3 mg/kg IP		Schaefer et al. (2005) Roth et al. (2001)

Zolazepam is commercially available only in combination with the dissociative agent Tiletamine (see above) as an anesthetic compound called Zoletil® or Telazol®. This compound is recommended mainly for anesthetizing rats and guinea pigs, and its u in mice can produce severe cardiorespiratory depression (Flecknell 1989; Gardner et al. 1995).

Opioids

A wide range of opioid analgesics is available for use in animals with different analgesic potency, duration, and effects on body systems (Table 7). Opioids are usually classified as agonists, partial agonists, mixed agonist-antagonists, and antagonists. Ago such as morphine, meperidine, and fentanyl as well as partial agonists such as pentazocine and buprenorphine are excellent analgesics with different power and duration. Pure agonists can cause some undesirable side effects (e.g., respiratory depress sedation, bradycardia, and peripheral vasodilation induced by histamine release, especially at higher doses) such as those that might be administered when using opioids as a part of a balanced anesthetic regimen. Opioid effects can be antagonized by naloxone (Buitrago et al. 2008; Flecknell 1989).

Table 7: Recommended dosages for opioids

Drugs	Dosage	Comments	Refere
Morphine	10 mg/kg SC	2-4 hourly	Fleck (198 Fleck (199
Meperidine	20 mg/kg SC	2-3 hourly	Fleck (198
Fentanyl	0.06 mg/kg SC	Short-acting synthetic opiate (30 minutes); widely used during surgical procedures.	Fleck (198
Buprenorphine	2 mg/kg SC	12 hourly	Fleck (198 Fleck (199
Naloxone	0.01-0.05 mg/kg IV, IM		Fleck (198

Neuroleptoanalgesia

Neuroleptoanalgesia is carried out by combining a narcotic analgesic with a tranquilizer to produce a state of deep sedation with profound analgesia sufficient to perform short painful procedures. According to De Castro and Mundeleer (1959), some

neuroleptoanalgesic compounds are marketed as premixed combinations. For example, Leptofen®, Innovar®, and Innovar Ve combine at different concentrations but with a fixed ratio of 50:1. The butirrophenone Droperidol combines with the opioid Fent or the compound Hypnorm®, with the same ratio of 10 mg/mL of Fluanison with 0.2 mg/mL of Fentanyl. Currently the term *neuroleptoanalgesia* is used for many different combinations of any opioid with several kinds of tranquilizers (Table 8). The us Innovar in mice is limited because it can produce tissue necrosis at the injection site (Flecknell 1989). Fentanyl (0.05 mg/kg) ca also be combined with midazolam (5 mg/kg) and with medetomidine (0.5 mg/kg) to perform surgery in mice, the advantage of t combination being that at any time each or all of the agents can be reversed by naloxone, flumazenil, and atipamezole, respecti (Thal and Plesnila 2007). The main side effects related to the use of opioids are respiratory depression and bradycardia.

Table 8: Recommended dosages for neuroleptoanalgesics

Drugs	Dosage	Comments	References
Fentanyl + fluanisone	0,105/ 3.333 mg/kg IP	Immobilization	Flecknell (1989)
Fentanyl+ fluanisone + midazolam	0.105/3.333/5 mg/kg IP	Immobilization	Zuurbier et al. (2002)
Fentanyl + midazolam + medetomidine	0.05/5/0.5 mg/kg IP	Surgical anesthesia	Thal and Plesnila (2007)

Muscle Relaxants

Neuromuscular blocking drugs produce paralysis of skeletal muscles and are used to aid stable mechanical ventilation by block respiratory movements, or to provide more suitable conditions for surgery. In rats, the use of pancuronium (2 mg/kg), a blocking agent that acts by competing with acetylcholine for receptor sites at the neuromuscular junction, has been reported. Its action c be reversed by neostigmine, which blocks the activity of enzymes that break down acetylcholine (Flecknell 1989). Administratio pancuronium (1 mg/kg) is also reported in mice (Walker et al. 1999).

Inhalation Anesthesia

Inhalation anesthetics offers a wide margin of safety and allows the maintenance of a constant plane of anesthesia compared w injectable ones. Absorption and elimination of inhalation anesthetics occur through the lungs and allow rapid induction and reco

Inhalation anesthetics are administered by anesthesia machines and delivered via a breathing system such as a simple Ayre's T piece up to a complex automatic ventilator. The basic anesthesia machine consists of a source of oxygen, a flowmeter, a preci vaporizer, a breathing circuit, and a scavenging system. In small animals, inhalation anesthesia can be easily induced by placing animal in an "induction chamber" and maintaining the desired depth with a face mask. The non-rebreathing "Bain circuit" is commonly used in rodent anesthesia. To perform thoracic surgery, to improve gas exchanges, or to control breathing motion d imaging of the chest, artificial ventilation is needed. The anesthetic potency of inhalation agents is indicated by their minimum alveolar concentration value, which, like toxic dose 50 (TD_{50}), measures the alveolar concentration needed to abolish the respo to a standardized painful stimulus in 50% of a population. The lower the minimum alveolar concentration value, the more potent the anesthetic. The concentration of an inhalation agent for anesthetic induction and maintenance is usually expressed as the percent value of inspired gas mixture (Kohn et al. 1997). Currently the most popular inhalation anesthetics for laboratory animals include nitrous oxide and the halogenated compounds halothane, isoflurane, and sevoflurane (Table 9).

Table 9: Recommended dosages for inhalation anesthetics

Drugs	MAC (%)	Concentration for Induction (%)	Concentration for Maintainance (%)

Halothane	0.95	4-5% + 0.8-1 L/min	1-2% + 0.8-1 L/min
Isoflurane	1.38	4-5% + 0.8-1 L/min	1-3% + 0.8-1 L/min
Sevoflurane		Individualized based on the mouse response + 0.8-1 L/min	Individualized based on the mouse response + 0 L/min

Nitrous Oxide

Nitrous oxide is a compressed gas that displays good analgesic qualities with minimal depressant effects on respiratory and cardiovascular functions. However, it has very low anesthetic potency, which can be used to speed up induction and to reduce t required concentration of other agents, thereby reducing the degree of cardiovascular and respiratory depression at a given de of anesthesia. It is usually administered 60 to 75% in a mixture with oxygen. After nitrous oxide inhalation anesthesia, 100% oxy must be delivered for a few more minutes because the anesthetic agent leaving peripheral tissues floods alveolar spaces caus transient dilution hypoxia in the immediate postanesthetic period (Flecknell 1989).

Halothane

Halothane is a potent volatile anesthetic agent that produces good surgical anesthesia and muscle relaxation. Unfortunately, it is also a potent cardiovascular and respiratory depressant; it sensitizes the heart to catecholamines and can produce immune depression after repeated exposition. About 20% of inhaled halothane is metabolized by the liver (Paddleford 2000). After repeated and prolonged expositions, halothane has been shown to be mutagenic and hepatotoxic in humans (Kohn et al. 1997)

Isoflurane

Isoflurane is presently the animal inhalation anesthetic agent of choice for both short and lengthy procedures due to its short induction and recovery time and the reliability of its effects. It does not sensitize the myocardium to catecholamines, and it spar cardiac output more than other volatile agents. Indeed, isoflurane can cause a more severe respiratory depression compared v halothane and a dose-dependent hypotension. Isoflurane is also used for very short procedures because it enables mice manipulation and injection, blood collection, and minor surgical procedures. Side effects of isoflurane include induction and emergency “delirium,” immune depression, delayed growth, and cleft palate in litters whose mothers have been exposed to this anesthetic (Kohn et al. 1997; Mazze et al. 1985).

Sevoflurane

Sevoflurane can provide an even faster anesthetic induction and recovery compared with isoflurane, and it maintains heart rate constant values. Like isoflurane, it can induce respiratory depression and hypotension in a dose-dependent manner. Its high co does limit the use of sevoflurane in laboratory animals (Kohn et al. 1997).

Anesthetic Monitoring and Physical Management

During sedation and anesthesia, it is imperative to carefully monitor and support mice body temperature, heart and respiratory r mucous membranes, and the degree of CNS depression. After induction, animal care personnel should position the animal on heated platform or use a heating lamp to maintain body temperature above 95 to 99°F. They should measure core temperature an esophageal or rectal probe, monitoring tissue oxygenation by pulse-oxymetry, and hearth rate and rhythm by electrocardiogr Personnel should gently tape down the animal’s limbs and monitor the delivery of oxygen by a nose cone placed over the muzz Finally, they should apply an ocular lubricant to prevent corneal desiccation. Anesthesia is considered adequate when the anima stays still quietly, is unresponsive to external stimuli, and has constant heart and respiratory rates. In mice the absence of the palpebral reflex suggests a fair anesthetic depth.

Analgesia and Recovery Care

Sometimes imaging procedures entail slightly painful or invasive procedures such as intracavitary or intravascular injections, blo vessel catheterization, or endocavitary probe penetration. Under these circumstances, it is important to adopt an adequate analgesic protocol, possibly in line with “pre-emptive analgesia.” To alleviate acute postoperative pain (Table 10) after assessir degree, appropriate analgesic medication (either opioids or nonsteroidal anti-inflammatory drugs) or local anesthesia can be us successfully in mice.

Table 10: Recommended dosages for analgesics

Drugs	Dosage
Meloxicam (NSAID)*	1 mg/kg SC, PO 30 min presurgery and q24h postsurgery
Carprofen (NSAID)	5 mg/kg SC PO q24h
Ketoprofen (NSAID)	2-5 mg/kg SC q12-24h
Buprenorphine (opioid)	0.05-0.1 mg/kg SC q12h
Tramadole (opioid)	10-30 mg/kg IP or 1mL 5% solution in 150 mL of water
Lidocaine (local anesthetic drugs)	1-4 mg/kg or 0.4 mL/kg of a 1% solution

*NSAID: Non-Steroidal Anti-Inflammatory Drug

Opioids are indicated in animals with moderate or severe pain. Opioids have a relatively short duration of action in small rodents to their faster metabolic rates compared with larger species. As a result, buprenorphine, which has a prolonged duration of acti most rodents, is preferred in mice. As an alternative or an adjunct to opioid administration, the use of nonsteroidal anti-inflamma drugs is also recommended in mice. Local anesthetics can be used to provide mice surgical analgesia as well, granting high qu pre- and postoperative analgesia by nerve blocks, central analgesia, infiltration of the surgical field, or topical application of lido or prilocaine cream (Flecknell 1989; Flecknell 1998). Ossibuprocaine eye ointment is effective for most painful eye procedures is critically important to prevent surgical field infections by maintaining aseptic surgical conditions in combination with the use of local or systemic antibiotics (Table 11).

Table 11: Recommended dosages for antibiotics

Drugs	Dosage
Enrofloxacin	5-20 mg/kg q24h SC or 50-200 mg/L in drinking water
Thrimetroprim-sulphonamides	15-30 mg/kg PO, SC, IM q12h

The safest systemic antibiotics for mice that are not harmful to their symbiotic intestinal bacterial population include fluorochinol and the thrimetroprim–sulphonamides combination. Gentamicine (5-10 mg/kg) can be used SC (Quesenberry and Carpenter 2 or, in our experience, topically; however, antibiotic administration should be delayed until complete anesthetic recovery takes pl because of hypotension and prolonged anesthesia due to their calcium blocking action. A warm, dry, and quiet environment witl oxygen-enriched atmosphere is recommended during recovery to reduce shivering and hypoxemia. Fluid therapy is a strong support for a fast and complete anesthetic recovery. Administering by the SC or IP routes 1.2 mL of 0.9% saline or of half-stre dextrose/saline solution for a 20-g mouse, divided in two doses daily, will prevent dehydration.

Conclusion

Mice anesthesia demands a deep knowledge of the physiology and pharmacology of this species. According to the aims of experimental procedure, anesthetic protocol should be tailored by the use of tranquilizers, injectable/inhalation anesthetics and analgesics. Patient monitoring and post-operative care reduce the rate of complications, improving animal welfare and experim results.

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