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**OVERVIEW**

**Boivin et al. Review of CO2 as a Euthanasia Agent for Laboratory Rats and Mice, pp. 491-499**

Domain 2: Management of Pain and Distress

T1. Recognize pain and/or distress

T2. Minimize or eliminate pain and/or distress

T3. Euthanatize (Euthanize)

Primary Species: Mouse (*Mus musculus*) and Rat (*Rattus norvegicus*)

SUMMARY: In 2006, and again in 2013, a group of experts gathered in Newcastle, United Kingdom, to discuss the use of CO2 as an agent for rodent euthanasia. This review analyzes the Newcastle reports in light of new information, provides recommendations based on the findings from those new studies, and outlines areas requiring further investigation. Based on their review, the authors conclude that no suitable alternative agents to CO2 as a euthanasia agent are presently available, and recommend the continued humane use of CO2 for the euthanasia of laboratory rats and mice.

KEY POINTS

Criteria for the ideal euthanasia agent.

* Animal capture and restraint with minimum distress.
* Induction of immediate and permanent insensibility with no, or minimal, distress to the animal.
* Aesthetically acceptable to the public and to the person administering the procedure.
* Cost effective
* Easily administered by non-veterinary personnel without extensive training
* No or minimal risks to the person administering the procedure
* Causes no damage or residues that might interfere with examination or the future use of the carcass, including no residues that might create hazard to other animals following carcass disposal.

Three factors considered to make the use of CO2 less than optimal as a euthanasia agent.

* Aversion to CO2 gas
* Clinical reports of pain at high CO2 concentrations.
* Gasping associated with CO2 exposure

The 2006 Newcastle report represents a consensus opinion of 12 people representing research in CO2 euthanasia and experts in laboratory animal welfare and animal care.

The 2006 Newcastle committee report raised multiple issues, divided among four categories:

* First: problems perceived in using CO2 for euthanasia
* Lack of an ideal dose of CO2
* Exposing animals to concentrations of over 50% leads to mucosal pain prior to loss of consciousness,
* The expression of aversion and dyspnea with slow chamber-fill rates
* Second: when CO2 is used, the avoidance or minimization of pain and distress is more important than speed of euthanasia
* Third, the authors concluded that alternative gases may be useful, but information on agents such as argon, nitrogen and volatile anesthetic agents was insufficient to advocate for these agents as acceptable replacements for CO2 euthanasia
* Fourth, the authors of the 2006 Newcastle report concluded that additional research was required to address the many questions remaining regarding CO2 use for euthanasia of mice.

The 2013 Newcastle report recommendations for CO2 use were essentially the same as in 2006 except that

* The use of O2 with CO2 was now discouraged, given that combining the gases does not consistently improve animal wellbeing and can increase the time to unconsciousness and death.
* Further, the participants in the meeting acknowledged that all currently available euthanasia techniques will inevitably involve some degree of pain or distress or both, while also concluding that CO2 cannot be considered a humane method of euthanasia for rodents and that developing replacements is an essential goal.

Two issues raised by the two reports are: 1) Chamber Replacement Rate and 2) CO2 concentration

Evaluation of recent studies that were included in this paper’s comparison and evaluation of other euthanasia techniques for rodent euthanasia included consideration of the following

* Stress and pain assessment, with a critical concept for consideration involving the recognition that an animal must be conscious to experience stress, distress and/or pain.
* It is essential that scientists who are evaluating the wellbeing of animals during the euthanasia process ensure that they are familiar with the different stages of anesthesia and how those stages correlate to conscious and unconscious responses to noxious and distressing stimuli.
* CO2 causes pain at high concentrations
* Stress hormone response to euthanasia
* ACTH, norepinephrine, and epinephrine are better indicators of differences in stress during euthanasia than the use of corticosteroid levels. This is largely based on the more rapid production (and detection) of the first three, relative to that of corticosteroids, in response to stress/distress/pain.
* Behavioral evidence of stress or pain during euthanasia
* Aversion does not mean that the animals are in pain, distress, or in a negative affective state; it only represents that the animal does not want to be in the area because they do not like something and that they have the ability to exit the area or otherwise indicate their preference.
* Neural response to euthanasia
* Although often descriptive, the use of EEG as an objective measure of unconsciousness, or determination of state of consciousness, especially for decision making related to the humaneness of euthanasia methods, is an area of research that requires further exploration

* Cardiovascular response to euthanasia
* The cardiovascular changes in these studies demonstrate that stress was associated with all of the procedures, and stress-related cardiovascular response levels did not differ as a result of the technique used.
* Examining Optimal CO2 Chamber Replacement Rates
* Rodents euthanized with slow CO2 CRR have a longer period of distress prior to unconsciousness
* Faster flow rates can be used without inducing pain.
* For many of the reviewed studies it was apparent that there are potential differences between strain, age, and technique used during the euthanasia process, thus potentially affecting the overall conclusions drawn from any individual study
* Use of O2 with CO2
* Based on the reviewed studies, the AVMA Panel on Euthanasia concluded that there appears to be no advantage to combining O2 with CO2 for euthanasia
* Alternative Gases and Halogenated Gases
* Concern regarding animals being hypoxic prior to unconsciousness led the AVMA Panel on Euthanasia to conclude neither nitrogen nor argon is an acceptable euthanasia method for rodents.
* Nitrous oxide has a higher human abuse potential than either CO2 or isoflurane alone. Further research on the potential animal welfare benefits are required prior to conclusions regarding the use of nitrous oxide for euthanasia of rodents
* The CO2 euthanasia process does not appear to elicit a response that is more stressful than that to isoflurane. In fact, some evidence suggests isoflurane prior to CO2 is more stressful
* Halogenated gas presents safety concerns for the personnel conducting the euthanasia
* There is a greater incidence of histologic damage in the lungs associated with CO2 euthanasia compared with isoflurane
* Embryonic, Fetal, and Neonatal Mice
* Reviewed studies added to the awareness that fetal and neonatal mice are resistant to the effects of CO2
* Future Directions and Gaps in Our Understanding
* Different responses to euthanasia techniques in association with sex, strain, group housing (empathy)

CONCLUSIONS

* We do not support a mandatory requirement to anesthetize mice with isoflurane prior to CO2 euthanasia.
* In addition, the use of alternative methods such as argon and nitrogen are not improvements in terms of euthanasia.
* If animals do not experience pain (mice are unconscious before CO2 reaches the pain-inducing level of 40%), we recommend using faster CO2 CRR to decrease the time mice are experiencing distress.
* Euthanasia should minimize pain and distress. According to current knowledge, the recommended use of CO2 does not lead to pain.
* In the absence of a better alternative agent, we recommend the continued humane use of CO2 for the euthanasia of laboratory rats and mice.
* We concur with the Newcastle reports that additional studies are required.

QUESTIONS

1. Which of the following is not an apparent disadvantage of the use of CO2 for rodent euthanasia?

a. Subject aversion to CO2 gas

b. Significant risk to the person administering the CO2

c. Clinical reports of pain at high CO2 concentrations.

d. Gasping associated with CO2 exposure

2. True or False: Addition of oxygen to CO2 provides significant advantages for CO2 euthanasia of rodents.

3. True or False: ACTH blood levels are a more sensitive method for assessing apparent stress/distress/pain response to a stimulus than corticosteroid measurement.

4. Which of the following apply to considerations for the CO2 flow rate (chamber replacement rate - CRR) as applied to euthanasia of mice and rats?

a. Rodents euthanized with slow CO2 CRR have a longer period of distress prior to unconsciousness

b. Faster flow rates can be used without inducing pain.

c. For many of the reviewed studies it was apparent that there are potential differences between strain, age, and technique used during the euthanasia process, thus potentially affecting the overall conclusions drawn from any individual study

d. All of the above

5. True or False: continued research has revealed that continued humane use of CO2 for the euthanasia of laboratory rats and mice still appears to be an appropriate and preferred technique.

ANSWERS

1. b

2. False

3. True

4. d

5. True

**Vemulapalli et al. Considerations When Writing and Reviewing a Higher Education Teaching Protocol Involving Animals, pp. 500-508**

Domain 5: Regulatory Responsibilities

Domain 6: Education

SUMMARY:This document discussed the use of animals in teaching protocols. Animal use in teaching ensures students receive adequate exposure and preparation for careers in biological sciences. Non-animal alternatives may not be fully suited to replace live experiences. Animal use cannot be completely eliminated, but how animals are used in teaching should be considered.

When reviewing protocols, IACUC should ask instructors to provide information regarding non-animal alternatives, the proposed animal use, and the safety of both students and animals. This document provides ways to assist investigators and IACUC reviewers to increase everyone’s understanding of how to replace, reduce, and refine animal use in teaching. The main recommendation was to create a questionnaire or supplementary set of questions specific to teaching protocols.

The following are examples, with questions varying based on the educational objectives:

1. Why do students need to learn the procedure on a live animal?

2.   Does the course build on animal experiences covered in previous courses?

3.  Will professional standards (VTNE exam, wildlife society, AVMA, etc.) be met by the animal use in this course?

4.   Will multiple or repeated procedures be performed on the same animal? What are the risks associated with repeat procedures, and how will these risks be managed?

QUESTIONS

1.  True or False: The Animal Welfare Act Regulations (AWAR) applies to animals used in research, tests, experiments, or teaching in K-12 education and higher education.

2.  Teaching protocols that receive support through Public Health Service (PHS) funds must adherence to which of the following:

a.   The PHS Policy

b.   Guide for the Care and Use of Laboratory Animals

c.   US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training

d.   All of the above

3.  True or False: In the United States, only institutions subject to the AWAR are required to report research or teaching animal use numbers.

ANSWERS

1. False: The AWAR explicitly excludes animals used in K-12 education. It does apply to animals used in higher education.

2.   d. All of the above

3.   True

**ORIGINAL RESEARCH**

***Biology***

**Nehete et al. Age- and Sex-associated Differences in Phenotypic and Functional Characteristics of Peripheral Blood Lymphocytes in Chimpanzees (*Pan troglodytes*), pp. 509-519**

SUMMARY: Chimpanzees share approximately 98% genetic sequence identity and are the closest phylogenetic relatives to humans. This has historically made chimpanzees valuable models for specific infectious disease research and vaccine development. However, in November 2015, the NIH started to phase out its funding of biomedical research involving chimpanzees and shortly thereafter made the decision to retire all NIH-supported chimpanzees to federal sanctuaries. Despite the use of chimpanzees to achieve advances in vaccine development, relatively little has been published regarding the normal levels and ranges for the phenotypes and functions of chimpanzee immune cells. In this study the authors collected blood from 94 clinically healthy chimpanzees and analyzed various immune parameters by using flow cytometry, immune function analysis and cytokine analysis.

The authors did not observe a difference in percentages of cell populations tested with age but did observe a lower percentage of NK cells in females than in males. The authors also noted an enhanced proliferative response in females to one of the mitogens tested. Additionally, decreased proliferation with increasing age was observed following stimulation with mitogens. The authors also identified significantly higher plasma levels of iL-6 in females relative to males but no difference in cytokine production following in vitro stimulation between male and female chimpanzees.

In contrast to published studies in rhesus, humans and other animals; these authors did not see age-associated differences in circulating white blood cells but did note slight differences in immunity and cytokine production. The observation of a smaller NK cell population in female chimpanzees compared to males agrees with published data demonstrating differences in lymphocyte subsets among sexes in humans and rhesus. According to previously published data, a key feature of NHP PBMC is the presence of prominent CD4+CD8+ T-cells, a finding that was supported by the current study. The authors of this study indicate this data is helpful in that it provides normal immune parameters in chimpanzees that will help better guide their continued care in sanctuaries and zoos.

QUESTIONS

1. A key feature of NHP PBMC’s is an overabundance of what cellular population?
	1. Monocytes
	2. CD4+CD8+
	3. CD4+CD8-
	4. CD4-CD8+
	5. NK cells
2. What year did the NIH suspend funding for biomedical research involving chimpanzees?
	1. 2015
	2. 2017
	3. 2010
	4. None of the above, the NIH still supports chimpanzee research

ANSWERS

1. b
2. a

***Reproduction***

**Woodward et al. Evaluation of a Zinc Gluconate Neutralized with Arginine Product as a Nonsurgical Method for Sterilization of Rhesus Macaques (*Macaca mulatta*), pp. 520-526**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Macaques (*Macaca* spp)

SUMMARY:Rhesus and cynomolgus macaques are the most common nonhuman primate species used in research.  Rhesus macaques are productive, so their breeding may need to be controlled as deemed necessary.  Ideal control methods would be effective, safe, inexpensive, noninvasive, easily administered, reversible, have minimal side effects, and maintain normal hormone levels.  Currently available contraception methods for macaques include invasive surgical intervention or hormonal therapy, which have the potential to affect subsequent research use.  The authors evaluated an FDA approved formulation of a zinc gluconate neutralized with arginine product as a nonsurgical alternative contraception method in male rhesus macaques.  Results included: scrotal enlargement was observed in 8 of 12 macaques; histological evaluations revealed necrosis in some areas of the seminiferous tubules and active spermatogenesis in 9 of 12 testes and sperm in 7 of 12 epididymides.  The authors concluded that chemical castration with the tested FDA approved formulation of zinc gluconate neutralized with arginine was not successful in rhesus macaques.

QUESTIONS

1. The majority of testosterone production takes place in which of the following?
2. Leydig cells
3. Epididymis
4. Sertoli cells
5. Seminiferous tubules
6. True or False: The testicular parenchyma is devoid of pain receptors.
7. Which of the following have been linked to decreased testosterone levels?
8. Bone loss
9. Periodontal pathology
10. Decreased salivary gland flow
11. Edentulism
12. All of the above
13. Which of the following is a well-documented adverse effect associated with vasectomies?
14. Testicular abscess
15. Spermatic granuloma
16. Testicular atrophy
17. None of the above

ANSWERS:

1. a
2. True
3. e
4. b

***Animal Health Surveillance***

**Marx et al. Results of Survey Regarding Prevalence of Adventitial Infections in Mice and Rats at Biomedical Research Facilities, pp. 527-533**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Mouse (*Mus musculus*) and Eat (*Rattus norvegicus*)

SUMMARY: This study represents a 2 years comprehensive report (from January 2014 to December 2015) of the methods used to detect and control infection agents as well as the prevalence of outbreaks in 63 NIH-funded rodent programs. Likewise, this survey was also used to further compare these results to similar reports published in 1998 and 2008. According to the data, 48% of the surveyed facilities were considered barriers, followed by 38% nonbarrier facilities, and 14% combined barrier-non-barrier facilities. Of those, 69% of these institutions housed mice in barrier housing, followed by 18% in nonbarrier facilities and 12% in barrier-non-barrier facilities. Among the different institutions surveyed, 95% of them still use dirty bedding sentinel animals and 98% of them still perform serological tests in the colony.

For mice, the pathogen (viral and parasite) exclusion lists were consistent between institutions (mouse adenovirus 1 and 2, polyoma virus, K virus, MHV, EDIM, MPV, MMV, pneumonia virus of mice, Sendai virus, ectromelia virus, Reovirus 3, lymphocytic choriomeningitis virus, TMEV, *Aspiculuris tetraptera*, *Syphacia obvelata, Syphacia muris, Myobia musculi, Radfordia spp, and Mycopts spp., and Mycoplasma pulmonis*). *Corynobacterium bovis and Demodex musculi* were excluded in only 36% of the institutions but several expressed increased concerns for the presence of these organisms. Moreover, roughly ~12.5% of institutions completely excluded *Helicobactor*spp. and Norovirus from their facilities. Within the past 2 years, institutions reported the largest rate of viral outbreak for MPV (43), followed by EDIM (16), MHV (13), MVM (4) and finally TMEV (1). The largest bacterial outbreak rate in mice within the 2 years period was reported *for Helicobacter* (14), followed by *C. bovis* (5), *P. pneumotropica (4), Klebsiella oxytoca (2) and finally P. aeruginosa, S. xylosus and Staphylococcus spp* (1 each). Finally, within the past 2 years, institutions reported the largest rate of mice parasitic outbreak for fur mite (34), followed by pinworms (23).

For rats, greater than 95% of institutions excluded sialodacryoadenitis virus, rat parvovirus, rat virus (Kilham rat virus), Toolan H1 virus, lymphocytic choriomeningitis virus, Sendai virus, pneumonia virus of mice, rat minute virus, *Aspiculuris tetraptera, Syphacia obvelata, Syphacia muris, Myobia musculi, Radfordia spp. and Myocoptes spp*. In addition, 75% of 95% of institutions excluded rat rotavirus, rat theilovirus, hantavirus, *Mycoplasma pulmonis, Clostridium piliforme, Salmonella spp, Streptobacillus moniliformis*, cilia-associated respiratory bacillus, *Giardia spp., Psorergates simplex, Rodentolepis nana, Hymenolepis diminuta, Taenia taeniformis, Polyplax spinulosa, and Encephalilitozoan cuniculi*. Within the 2-year period, institutions reported the following outbreaks in this species: parvovirus (2), rat theilovirus (2), pinworms (3), fur mites (2) and *E. cuniculi* (1).

Compared with similar surveys performed in 1998 and 2008, the rate of viral outbreaks in mice colonies decreased, particularly in barrier facilities, whereas the prevalence of parasitic outbreaks remained constant. The overall low rate of viral infections relative to the total population of mice living in barrier facilities suggest that diverse biosecurity measures are effective at reducing viral outbreaks in murine colonies but apparently are less effective against parasitic pathogens. Furthermore, even though fenbendazole-based therapies appear to be effective in reducing the pinworm burden in the colonies, fur mite detection using current paradigm (dirty bedding sentinels) appears less effective. For that reason, 50% of the institutions surveyed also indicated the adoption of PCR-based methods to detect these organisms.

QUESTIONS

1. The most common methods to detect adventitial infections in mice in the US are?

a.  Dirty bedding sentinels and serology

b.  Dirty bedding sentinels and PCR

c.  Serology and PCR

d. Necropsy and PCR

2. According to a recent Institutional survey, the virus responsible for the longest outbreak history (~ 20 years) in mice housed in biomedical research facilities in the US include which of the following?

a.   Mouse parvovirus

b.  Mouse hepatitis virus

c.   Norovirus

d. Sendai virus

e. All of the above

3. Compared with similar surveys performed in 1998 and 2008, the rate of viral outbreaks in mice colonies \_\_\_\_\_\_\_\_\_\_\_\_\_\_, particularly in barrier facilities, whereas the prevalence of parasitic outbreaks \_\_\_\_\_\_\_\_\_\_\_\_\_\_

a.  Increased; decreased

b.  Decreased, increased

c.   Increased; remained constant

d.  Decreased; remained constant

ANSWERS

1. a

2.  a

3.  d

***Anesthesia***

**Kick et al. Pharmacokinetic Profiles of Nalbuphine after Intraperitoneal and Subcutaneous Administration to C57BL/6 Mice, pp. 534-538**

Domain 2: Management of Pain and Distress; Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY:  Research activities with mice often warrant use of analgesic agents, and depending on what’s being done with/to the mice, opioids are go-to medications for most pain management plans.  A significant issue with opioids is that most are controlled substances that require both state and federal registration and use tracking. Nalbuphine -a synthetic agonist–antagonist opioid that acts at both μ and κ receptors to alleviate both somatic and visceral pain- is a potentially effective opioid analgesic for mice that is not currently classified as a controlled substance, but little is known about its suitability as an analgesic for mice, and standard dosage regimens have not been developed for mice. Compared with other opioids, nalbuphine has fewer side effects and is relatively inexpensive. It also does not antagonize prostaglandins, thereby allowing parturition to proceed successfully, suggesting that it might have some utility in managing dystocia.

Using 8-10-week-old male C57BL/6 mice, authors compared the pharmacokinetic profiles of 10 mg/kg nalbuphine administered via subcutaneous (SC) or intraperitoneal (IP) dosing routes.  Nalbuphine injectable (10 mg/mL) diluted 1:10 in sterile saline was administered at a 10 mg/kg dose using a tuberculin syringe with a 25-guage needle- the 10 mg/kg dose was chosen because it was within the 0.44-41.8 mg/kg range reported in existing literature, and was well under the reported 1200 mg/kg SC toxicity levels.  The plasma therapeutic drug level in humans is reportedly 20 ng/mL, and authors used this level as an initial benchmark for mice in the absence of published data suggesting otherwise.  In the tested mice, plasma concentrations rose well above 20 ng/mL at the 5-min time point and dropped to 13.3 and 10.6 ng/mL for the SC and IP routes, respectively, by 2 h after administration, suggesting that nalbuphine would have a rapid onset and a duration of clinical action lasting approximately 2 h.  The SC route achieved peak plasma levels faster than the IP route and sustained higher levels for a longer period, and plasma concentrations for both routes were below the level of detection by 12 h.

These results suggest that nalbuphine is absorbed in and eliminated quickly from mice, making it a possible candidate for acute pain management, lasting approximately 2 h by both routes of injection.  Plasma concentrations differed significantly between SC and IP nalbuphine at several time points prior to 2 h, but levels were well above 20 ng/mL for both routes, making it unlikely that the route of administration would have any biologic significance in terms of nalbuphine’s clinical effectiveness as an acute analgesic.  In mice, analgesic durations have been reported of 6 to 8 h for buprenorphine HCl, 4 to 5 h for carprofen, 2 to 3 h for morphine, and 1 to 2 h for butorphanol.  Nalbuphine’s duration of effect is ~ on par with butorphanol’s, making nalbuphine suitable only for managing acute pain (e.g., pre- or postop analgesia, dystocia, short-lasting GI pain, etc.) if used as a sole agent. Combining nalbuphine with another analgesic agent that have a later onset (e.g., carprofen) might provide enhanced antinociceptive effect for a longer duration.

This study did not examine the clinical efficacy of nalbuphine as an analgesic, but the pharmacokinetic profiles generally agree with another study reporting 2 h of analgesia in rats given nalbuphine intramuscularly when challenged with a cold ethanol tail-flick test. Future studies of the clinical efficacy of nalbuphine are necessary to determine the most appropriate dosing regimen.

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QUESTIONS

1. Which of the following statements about nalbuphine is NOT true?

a.  Nalbuphine is a synthetic opioid that acts at both μ and κ receptors to alleviate both somatic and visceral pain

b. Nalbuphine is not currently classified as a controlled substance

c.  Nalbuphine is absorbed in quickly and eliminated slowly from mice

d. Nalbuphine is relatively inexpensive and has fewer side effects than other opioids

e. Nalbuphine does not antagonize prostaglandins and might have some utility in managing dystocia in mice

2.  According to this study, nalbuphine’s duration of effect in mice appears to most closely resemble which analgesic?

a.  Buprenorphine

b. Carprofen

c.  Morphine

d. Butorphanol

e. Meloxicam

3. T/F: Based on this study, the route of administration -IP versus SC- would not have any biologic significance in terms of nalbuphine’s clinical effectiveness as an acute analgesic in mice?

4.  T/F: If used as a sole analgesic agent in mice, nalbuphine would likely be suitable for managing only acute pain such as experienced with pre- or postoperative analgesia or dystocia?

ANSWERS

1.   c. nalbuphine is absorbed in quickly and eliminated slowly from mice (it is absorbed AND eliminated quickly in mice)

2.  d. butorphanol

3.  True

4.  True

**Wright et al. Pharmacokinetics of Single-dose Subcutaneous Meloxicam Injections in Black-tailed Prairie Dogs (*Cynomys ludovicianus*), pp. 539-543**

Domain 2: Management of Pain and Distress; K5

Tertiary Species: Other Rodents

SUMMARY: Meloxicam, a NSAID that preferentially inhibits the COX2 isoenzyme, is frequently used in a variety of species where pharmacokinetic and pharmacodynamics data are sparse.  The previous pharmacokinetic profile of meloxicam in prairie dogs was examined using a single subcutaneous bolus at a low dose (0.2mg/kg) or high dose (4mg/kg) and found to be inadequately low and too high, respectively.  The present study investigated pharmacokinetics of meloxicam after a single 1 mg/kg subcutaneous dose administered to 8, male, 6mth old black-tailed prairie dogs.  Animals were induced in an induction chamber with 5% isoflurane at 3L/min oxygen and maintained with 2-3% isoflurane at 2 L/min oxygen Venous blood (0.5 mL) was drawn using a 25ga needle on a 1 ml syringe prewashed with heparin for baseline blood sampling (time 0hr), with meloxicam immediately administered either IV (n=2) or SQ (n=6).  Subsequent venous blood samples (0.5 ml) were collected at 0.5, 1, 2, 4, 8, 12, 24 hr after meloxicam administration.  All sampling was performed in gas-anesthetized prairie dogs.  Noncompartmental pharmacokinetic parameters of meloxicam were estimated with computer program.

A single subcutaneous dose of meloxicam (1 mg/kg) quickly reaches CMAX (mean, 4.07 ug/ml) and AUCINFlevels over 24hr that are higher than those achieved in various other species receiving what is considered a therapeutic dose, suggesting that a 1 mg/kg dose may be excessive for administration every 24hr; however, without pharmacodynamics studies, the effective concentration of meloxicam in prairie dogs remains unclear.  Subcutaneous injections of meloxicam in prairie dogs showed good bioavailability (82%) and was eliminated more slowly than in other rodent species.  Fractional clearance identified in the present study was 1.68, or 2x faster than in prairie dogs given 0.2 and 4 mg/kg SC in a previous study; this difference may be due to the inclusion of females or the use of mature adult (7y old) prairie dogs in the previous study.  Future pharmacokinetic research should evaluate doses of meloxicam between 0.2 and 1 mg/kg for use in prairie dogs, as well as the extent of animal age and sex characteristic effects on pharmacokinetic profile.

QUESTIONS

1. Which factor was NOT a likely component of varying clearance rates between the current study and the previous study assessing meloxicam pharmacokinetics in prairie dogs at 0.2 mg/kg and 4 mg/kg SC dosing?

a.   Inclusion of females in the previous study

b.  Use of mature adults in the previous study

c.  Use of gas anesthesia for sampling in the present study

2. Which species has been shown to demonstrate sex-associated differences in meloxicam pharmacokinetics?

a. Rat

b.   Rabbit

c.  Cat

d.   Dog

3. True/False – The authors of the current study are confident that a 1 mg/kg subcutaneous dose of meloxicam is appropriate for therapeutic effectiveness in prairie dogs for analgesic control.

ANSWERS

1. c.  The authors felt it unlikely that anesthetic events appreciably altered the clearance rates of meloxicam and thus the results of the current study due to similar rates of elimination in the early sampling period (when animals were anesthetized and sampled more frequently), and late in the sampling period (when anesthesia and sampling occurred much less frequently)

2. a.  Rat – One study showed that male rats as compared with female rats, had significantly lower mean AUCINF, mean residence time, and mean terminal half-life.  In another study, single high-dose administration of meloxicam achieved an LD50 of 83.5mg/kg in female rates compared with greater than 200mg/kg in male rates.

3. False – Clinical efficacy and safety of meloxicam in prairie dogs was not a purpose of this study and was not examined, and the results of the present study suggest that a 1 mg/kg dose may be excessive for administration every 24 hours in prairie dogs.

**Barnett et al. Comparison of 6 Injectable Anesthetic Regimens and Isoflurane in Gray Short-tailed Opossums (*Monodelphis domestica*), pp. 544-549**

Domain 2: Management of Pain and Distress

SUMMARY:The gray short-tailed opossum is widely used in laboratory animal research in areas of developmental biology, oncology, immunology, and comparative biology; and is the most widely used marsupial in lab animal research. Gray short-tailed opossums are susceptible to a variety of cancers and are uniquely suited to study developmental biology due to their immaturity at birth. Although these animals are used frequently in biomedical research, little has been published regarding safety and efficacy of various anesthetic cocktails. The goal of this study was to characterize different anesthetic cocktails, namely ketamine/xylazine and ketamine/dexmedetomidine, at different concentrations and compare them against isoflurane gas anesthesia. The researchers found that ketamine/xylazine and ketamine/dexmedetomidine did provide a surgical plane of anesthesia at higher doses, however both drug cocktails showed side effects of bradycardia and bradypnea, in addition to lowered SpO2 in the ketamine/dexmedetomidine group. Due to the side effects and higher dosage necessary to achieve a surgical plane of anesthesia, the authors recommended the use of isoflurane gas anesthesia over injectable methods tried in this project for the gray short-tailed opossum.

QUESTIONS

1. Gray short-tailed opossums are susceptible to which cancer after exposure to UV radiation?

a.  Squamous cell carcinoma

b. Basal cell carcinoma

c.   Malignant melanoma

d.   Lymphoma

2. The breeding cycle of gray short-tailed opossums is:

a.  Seasonal breeders- breeding in the Spring

b. Seasonal breeders- breeding in the Fall

c.  Induced ovulators, nonseasonal

d.  Nonseasonal breeders

3. T/F: Gray short-tailed opossums are *not* amenable to xenograft transplantation of tumors.

ANSWERS

1.  c

2.   d

3.  False; gray short-tailed opossums are amenable to xenograft transplantation during the period of early development

**Domain 2:** Management of Pain and Distress; T3. Administration of anesthesia

**Tertiary Species:** Other Mammals

**SUMMARY**: The gray short-tailed opossum is the most commonly used marsupial species in biomedical research, and has been used in the areas of developmental biology, neurobiology, oncology, immunology, and comparative biology.  Gas anesthesia appears to be the most commonly used general anesthesia in studies involving surgical manipulations in opossums.  This study compared six different combinations of intraperitoneal ketamine/xylazine and ketamine/dexmedetomidine to determine the best anesthetic range and characterize physiologic responses to the drugs.  They also compared these drug combinations with isoflurane gas anesthesia.  The combinations of ketamine/xylazine and ketamine/dexmedetomidine both provided effective surgical anesthesia, but the most effective combination was ketamine 100 mg/kg with dexmedetomidine 0.1 mg/kg (K100D0.1).  All injectable treatments that induced a surgical plane of anesthesia caused bradycardia, so anticholinergic agents like atropine or glycopyrrolate should be considered.  Respiratory depression was also observed in all anesthetic regimens, including isoflurane.  Isoflurane produced the most consistent and reliable anesthesia, so would be preferable should a choice between injectable and gas anesthesia delivered with a precision vaporizer be presented.  Apnea, respiratory depression, and decreases in oxygen saturation was observed in all treatments involving xylazine, so ketamine/dexmedetomidine was recommended over ketamine/xylazine.  Lower doses of ketamine/dexmedetomidine and ketamine/xylazine than presented in this study (K100D0.05 and K40X5) would be unsuitable for surgical anesthesia.

QUESTIONS

1.  What is the scientific name of the gray short-tailed opossum?

a.  *Oryzomys palustris*

b.  *Monodelphis domestica*

c.  *Odocoileus virginianus*

d.  *Didelphis virginiana*

2.  Name two cancers opossums are susceptible to and can be used as a model for human disease.

3.  Which form of anesthesia is the most commonly used in opossums?

a. Inhalant anesthesia

b. Alfaxalone

c. Ketamine/xylazine

d. Ketamine/dexmedetomidine

4.  Which of the following is a characteristic of marsupials and has resulted in extensive research use in the areas of developmental biology and neurobiology?

a. Cheek pouches

b. Choriovitelline placenta

c. Vomeronasal organ

d. Developmental immaturity at birth

ANSWERS

1.  b

2.  Malignant melanoma and inherited corneal cancer

3. a

4. d

**Jen et al. Pharmacokinetics of a Transdermal Fentanyl Solution in Suffolk Sheep (*Ovis aries*), pp. 550-557**

Domain 2: Management of Pain and Distress

Secondary Species: Sheep (*Ovis aries*)

SUMMARY: Appropriate pain control, including the use of analgesics and anesthetics, is a central tenant in laboratory animal medicine. It is required by Federal regulations such as the Animal Welfare Act, as well as by the *Guide for the Care and Use for Laboratory Animals*. Furthermore, numerous studies have shown that animals receiving proper pain control yield more scientifically-sound data than those who don't. Post-operative pain control in larger species where multiple injections over a given length of time may be improbable tend to utilize transdermal fentanyl patches. They are advantageous due to their ease of application, long duration of action, and simple reversal of the drug by removing the patch. However, disadvantages include a long time to maximal effective plasma concentration, necessitating placing the patch prior to the day of surgery; variable absorption due to improper placement on the skin or the patch falling off; and the possibility of an animal ingesting the patch. This study evaluated a long-acting transdermal fentanyl solution (TFS) recently found to provide therapeutic levels of fentanyl in dogs for up to 96 h. The TFS is touted as being quick-drying and fast-acting, lacking first-pass metabolism, and having a longer duration of action. The study's goal was to find a TFS dose that would provide a minimal effective concentration for analgesic efficacy of 0.5 ng/mL in the sheep, would provide a faster T-max than the patch, and elicit the fewest adverse effects. They evaluated dosages of 2.7 mg/kg, 1.7 mg/kg, and 0.5 mg/kg. Although the TFS was superior to the transdermal patch in providing a greater maximal concentration and shorter time to T-max, the half-life between the two products were similar. In addition, severe adverse effects were noted at the two higher doses of TFS, and mild to moderate adverse effects were noted at the lowest dose. With these results in mind, further study is strongly recommended before using the TFS in sheep instead of the transdermal patch.

QUESTIONS

1. Name the agent used to reverse opioid effects.

2. Describe fentanyl in terms of the following: selective vs. non-selective, type of receptor, and potency compared to morphine.

3. What property of fentanyl results in greater tissue distribution and allows access to CNS binding sites?

ANSWERS

1. Naloxone - FDA approved pure opioid antagonist

2. Selective mu-opioid receptor with 50-100x the potency of morphine

3. High lipophilicity

***Experimental Use***

**Sanders et al. Pharmacokinetics of Single-bolus Subcutaneous Cefovecin in C57BL/6 Mice, pp. 558-561**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Dermatitis is a common clinical problem in mice, particularly in C57BL/6 and those on that background. Treatment is generally unsuccessful, and mice are often euthanized as a result of this condition. In dogs and cats, Cefovecin, a semi-synthetic cephalosporin antibiotic, is
effectively used to treat similar dermatitides by single-bolus dosing. In those species, the half-life of Cefovecin is 7-14 days. In dogs and cats this long half-life is due to high affinity for plasma proteins and slow renal excretion. Pharmacokinetic studies have been completed in multiple other laboratory animal species, including multiple species of non-human primates, but not in mice.

Mice were dosed with the recommended dose (8mg/kg) and a dose 5x higher (40mg/kg) and blood was collected at time points ranging from 5 minutes to 24 hours. Cefovecin was extracted from plasma sample using a solid-phase extraction method and analyzed using reversed phase
HPLC. Cefovecin follows first-order elimination kinetics, with exponential decrease on a linear plot of plasma concentration compared with time. The Cmax (maximal concentration) increased in line with the dosage (5-fold increase in 40mg/kg dose vs 5mg/kg). In dogs and cats,
Cefovecin follows nonlinear kinetics because plasma concentrations do not increase with the dose. Half-life for both time points in mice was similar at about one hour for both doses, suggesting elimination mechanisms were not saturated. Like dogs and cats, Cefovecin in mice
is primarily renally excreted. However, the elimination rate constant in mice is much higher than in those species. Cefovecin is mainly confined to the intravascular fluid in mice. The half-life and first order kinetics of Cefovecin do not make it a good single-bolus option for treatment of C57BL/6 mice.

QUESTIONS

1. How long does cefovecin maintain effective plasma concentrations in dogs and cats?

a. 7-14 days

b. 14-21 days

c. 3-5 days

d. 1-2 days

e. 12 hours

2. Does cefovecin maintain plasma concentrations high enough for single-bolus therapeutic use in C57Bl/6?

a. Yes

b. No

3. What was the approximate half-life of cefovecin after single-bolus dosing in C57Bl/6?

a. 30 minutes

b. 1 hour

c. 4 hours

d. 12 hours

ANSWERS

1. a

2. b

3. b

**Skorupski et al. Quantification of Induced Hypothermia from Aseptic Scrub Applications during Rodent Surgery Preparations, pp. 562-569**

Domain 2: Management of pain and distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Common surgical preparation disinfectants used in rodent medicine include povidone-iodine (P-I). 70% isopropyl alcohol (IPA) is predicted to have an aversive evaporative cooling effect and in some institutions alcohol has been avoided and preference given to sterile water or saline. Forty-eight male mice of various ages (5-11mo), backgrounds (B6 and Tph1-/-) and body weights (31-45g) were assigned to different groups each of which underwent skin preparation with different solutions.

Skin application of IPA resulted in the steepest drop in core and surface body temperatures in anesthetized mice, but mice recovered within minutes after application to near baseline core body temperature levels and surface temperatures exceeded those of control animals. Mice that had skin prepared using P-I combined with saline had core body and surface temperatures that did not recover during the period of anesthesia. Alcohol as part of a skin preparation protocol is a viable alternative to saline.

QUESTIONS

1. Prolonged hypothermia following surgical skin preparation was associated with the applications of which of the following? (Two correct answers):

a. Povidone-iodine alternating with 70% isopropyl alcohol

b. Povidone-iodine alternating with sterile 0.9% saline

c. Povidone-iodine alternating with warmed (37Â°C) sterile 0.9% saline

d. 70% isopropyl alcohol applied 3 times

e. Povidone-iodine applied 3 times

2. Skin preparation solutions decrease the body temperature through which of the following? (Two correct answers):

a. Evaporative cooling effects

b. Vasoconstrictive responses at the skin level

c. Retention or pooling of the solutions

d. Direct effects on central vasomotor control mechanisms after skin absorption

e. Limited applications of small volumes of skin preparation solutions

ANSWERS

1. b and c

2. a and c

**Okada et al. An Efficient, Simple, and Noninvasive Procedure for Genotyping Aquatic and Nonaquatic Laboratory Animals, pp. 570-573**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

T3. Diagnose Disease or Condition as Appropriate

K9. Diagnostic Procedures

SUMMARY: Various animal models are indispensable in biomedical research. Increasing awareness and regulations have prompted the adaptation of more humane approaches in the use of laboratory animals. With the development of easier and faster methodologies to generate genetically altered animals, convenient and humane methods to genotype these animals are important for research involving such animals. The authors report that skin swabbing is a simple and noninvasive method for extracting genomic DNA from mice and frogs for genotyping. The authors demonstrated that this method was highly reliable and suitable for both immature and adult animals. The advantage of swabbing was that it is a simpler and more humane approach for genotyping vertebrate animals.  See Figures 2, and 3.

QUESTION

1. Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a segment of what?

a.  mRNA

b.  DNA

c.  RNA

d.  dDNA

ANSWER

* 1. b. DNA