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**OVERVIEW**

**Lerat et al. Pandemic Preparedness in Animal Facilities, pp. 244-248**

Domain 4: Animal Care

Domain 5: Regulatory Responsibilities

SUMMARY: COVID-19 has highlighted both the need for animal facility disaster plans to include pandemics, and their widespread absence from such. While literature primarily discusses acute events (i.e. loss of power, severe weather events), basic disaster planning concepts are readily applied to broader and more chronic situations including pandemics. Prior to implementation of any plan, resources should be adequately stocked for normal operations; stockpiling contributes to shortages, delays, and loss of useable space. A list of emergency personnel should be created, including those who regularly use the facility and are either proficient in animal care or may be trained. Finally, a plan for depopulation of animals with prioritization of irreplaceable lines should be created; this plan should acknowledge the financial, ethical, and psychological effects of depopulation. A graduated plan – i.e. where levels of staffing absence correlate to levels of facility operation – may serve as the best balance between maintaining animal welfare and continuing experimental progress. Despite these plans, cessation of all work may be necessary to prevent poor welfare due to overwhelming burden of animals per caretaker.

QUESTIONS

1. Which of the following engineering controls and practices should be considered when implementing social distancing recommendations?
   1. Air-changes per unit of time
   2. PPE usage
   3. Facility directionality/staff movement
   4. All of the above
2. T/F: The first objective of disaster planning is maintenance of experimental progress.

ANSWERS

1. d. Note these are considerations, not alternatives
2. False. First objective is personnel safety, second objective is animal welfare

**ORIGINAL RESEARCH**

***Reproduction***

**Estes et al. Behavioral and Reproductive Effects of Environmental Enrichment and *Pseudoloma neurophilia* infection on Adult Zebrafish (Danio rerio), pp. 249-258**

Domain 4

Primary Species: Zebrafish (*Danio rerio*)

SUMMARY: *Pseudoloma neurophilia*, an obligate intracellular parasite, is one of the most common pathogens in zebrafish laboratory settings. It results in spinal deformities, reduced growth rates, and decreased fecundity among other clinical signs. Due to its effects in zebrafish, it can alter experimental outcomes. The goal of the project was to identify the effects of *P. neurophilia* and environmental enrichment (EE) on AB zebrafish anxiety and reproduction. They hypothesized that anxiety would be higher with infected fish and decreased with group housing or EE. 300 fish were used in this study (M: 120 and F: 180). Behavioral testing consisted of the novel tank test and light dark test. Breeding was tested at 244 dpf. For the novel tank diving test: Pseudoloma free and infected fish had no significance in distance swam. Disease status or enrichment did not influence duration spent at the top of the tank. The authors found that the enrichment type had a significant effect on time spent in the dark half of the tank. During breeding, the infection status of the animals significantly affected breeding where negative fish spawned more eggs than infected fish. The main study findings included: Infected zebrafish had poor reproduction compared to non-infected fish, EE increased fish growth but did not affect fecundity, both infected and non-infected fish showed displays of anxiety during the tank diving test. The authors noted a decreased crossing between light and dark tanks by infected fish that they hypothesized was due to the physical changes produced by the disease (scoliosis/emaciation). The EE did not significantly affect breeding performance.

QUESTIONS

1. T/F: *Pseudoloma neurophilia* is transmitted vertically and horizontally.
2. *Pseudoloma neurophilia*are thought to display (select all that apply):
   1. Increased startle responses compared to negative tank mates
   2. Decreased freezing behavior
   3. Altered shoaling behavior
   4. None of the above
3. T/F Larval zebrafish appear to be more susceptible to *Pseudoloma neurophilia*which can compromise development and neurotoxicity studies.

ANSWERS

1. True
2. a and c
3. True

***Husbandry***

**Voros et al. Effects of Daytime Blue-Enriched LED Light on Physiologic Parameters of Three Common Mouse Strains Maintained on an IVC System, pp. 259-271**

Domain 3:Research

Primary Species:Mouse (*Mus musculus*)

SUMMARY: Effects of light quality and quantity on animal physiology are largely unknown. In recent years, light-emitting diode (LED) lighting has gained popularity in animal research facilities, not only due to energy conservation, but when enriched in the blue portion of the visible spectrum (465nm to 485 nm), has been shown to enhance animal health and wellbeing, as compared to cool white fluorescent (CWF) lighting.  In the current study, the authors sought to study strains of mice that produce circadian dark phase melatonin (C3H/HeNCrl) as compared to mice that do not (BALB/cAnNCrl and C57BL/6NCrl) in an effort to examine the influence of lighting type on a variety of parameters related to animal welfare. They hypothesized that mice exposed to blue-enriched LED light at day (bLAD), particularly C3H mice, would have positive physiologic and behavioral indices, such as lower body weight, lower cholesterol levels, lower indices of stress, more completely built nests, etc., than their CWF counterparts. Results revealed male C3H mice consumed more food and had lower cholesterol levels under bLAD conditions as compared with CWF conditions; female BALB mice had higher serum total protein under bLAD conditions than under CWF conditions; female B6 mice had higher phosphorus levels under bLAD conditions than under CWF conditions, and female C3H/HeNCrl mice had a higher neutrophil count under bLAD conditions as compared with CWF conditions. Despite the sex and strain associated differences, the changes were minimal and do not support the notion that one lighting condition is superior for the welfare of mice. Lighting conditions can influence research outcomes however, and should be considered when evaluating for example, body weight, food intake, water intake, and some hematology and biochemical values.

QUESTIONS

1.  This anatomic location in the hypothalamus regulates the daily dark phase pineal production of melatonin, resulting in high dark phase and low light phase levels.

2.  Strains such as C3H, CBA and FVB are genetically predisposed to retinal degeneration and extensively used as a model for retinitis pigmentosa due to homozygosity for this allele.

3.  What is the recommended range of light to prevent phototoxic retinopathy in sensitive species?

ANSWERS

1.   Suprachiasmatic nucleus

2.   *rd*1

3.  130 to 325 lux

**Russell et al. Effects of Laboratory Housing Conditions on Core Temperature and Locomotor Activity in Mice, pp. 272-280**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Aim is to study how common laboratory housing conditions affect core temperature and locomotor activity in mice, authors of this study conducted a retrospective analysis of data derived from approximately 10 years of studies conducted at the University of Michigan (U of M) and the University of Arkansas for Medical Sciences (UAMS) involving radiotelemetry in mice. Using data collected during studies of behavioral pharmacology and the neuropharmacology of drugs of abuse, Russell et al., identified “non-injection control sessions” in which core temperature and locomotor activity were monitored across various housing conditions for at least 24 h. The resulting data set represented more than 20,000 samples of core temperature and motor activity across a broad range of conditions that are likely to be encountered in standard research settings, allowing a thorough analysis of the contributions of ambient temperature, cage density, bedding and nesting materials, and running wheel access on core temperature and locomotor activity in mice. All mice were of the same strain (NIH Swiss mice) and sex (male) and were of similar age. Results of this study presented evidence that core temperature in the laboratory mouse is relatively resistant to environmental conditions and the standard husbandry practices likely to be encountered in a research setting. In contrast, locomotor activity was quite sensitive to the simultaneous provision of fresh bedding and nesting material in the form of cotton squares. These common husbandry practices induced significant and prolonged stimulation of motor behavior, hyperthermic effects, and a disruption of circadian rhythms. Some of these effects may be related to the novelty-induced exploratory behavior and hyperthermia.

QUESTIONS

* 1. The ambient temperature range in which thermoregulation occurs without the need to increase metabolic heat production or activate evaporative heat loss mechanisms is called the thermoneutral zone (TNZ) and is bounded by the lower and upper critical temperatures (LCTs and UCTs). The TNZ for mice ranges between?

a. 24°C - 28°C

b. 26°C - 34°C

c. 26°C - 30°C

d. 24°C - 30°C

2.   Recommended dry-bulb macroenvironmental temperature for mice is?

a. 20°C - 24°C

b. 20°C - 26°C

c. 24°C - 28°C

d. 26°C - 34°C

3.   As per CDC and NIH, average rectal or intraperitoneal temperature for a mouse is?

a. 39.2 ± 0.7°C

b. 36.8 ± 0.2°C

c. 36.5 ± 1.3°C

d. 36.7 ± 0.9°C

4.   The process of inserting a rectal probe in mice rapidly raises rectal temperature by about?

a. 1°C - 2°C

b. 1.5°C - 2°C

c. 2.0°C - 2.5°C

d. 0.5°C - 1.5°C

5.   While studying the effect of ambient temperature, mice housed at what temperature (in °C)exhibited least variation (difference between highest and lowest mean temperature)?

a. 20

b. 26

c. 23

d. 28

6.  In the current study, the group of mice housed at what ambient temperature exhibited greatest nocturnal increase in locomotor activity?

a. 20

b. 26

c. 23

d. 28

7.   In the current study, simultaneously providing fresh bedding and a cotton square significantly increased core temperature above the species-typical range. This apparent hyperthermic effect was on the order of 1 °C, and lasted for approximately 2.5 h. Even when temperatures returned to the species-typical range, mice housed with new bedding and a cotton square exhibited higher temperatures than those in all other bedding conditions until approximately how many hours into the study?

a. 3.5

b. 2.5

c. 4.5

d. 4.0

e. 2.0

8.  While studying the effects of bedding and nesting material on locomotor activity, a clear effect of bedding condition on motor activity was observed. In which group of mice the highest locomotor activity was observed

a. Old bedding

b. New bedding

c. Old bedding plus cotton square (nest)

d. New Bedding plus cotton square (nest)

ANSWERS

1. b

2. b

3. c

4. d

5. d

6. a

7. c

8. d

**Allen et al. Assessing Accumulation of Organic Material on Rodent Cage Accessories, pp. 281-288**

Domain 4: Animal Care

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

SUMMARY: Changing and sanitization of cages and cage components is among the most labor-intensive activities in the research animal facility, and therefore, one of the costliest. These husbandry practices can also have direct effects on research animals and may create experimental confounds. The Guide provides specific recommendations for the sanitization of both rodent cages and cage accessories. The Guide also acknowledges that the increased use of individually ventilated cages (IVC) has led to investigations of the maintenance of a suitable microenvironment with extended sanitization intervals. Accrediting and regulatory agencies indicate that institutions may use site-specific and data-driven approaches to determine the ideal frequencies for sanitization of cages and accessories. The author’s goal in this study was to make evidence based decisions on their current cage changing practices and determine best interval for sanitation of wire bar inserts and filter top lids. (Adenosine triphosphate) ATP evaluation was chosen to evaluate surface contamination and sanitization efficacy.

120 cages of sentinel mice and rats were housed in static microisolator or IVC Allentown caging. Mice were housed 3 animals per cage; rats 2 per cage. ATP samples were taken from a 4x4cm area on each wire bar insert and filter top lid on days 0 (prior to housing animals), 7,  14,30, 60, 90, and 180 (filter top lid only) after cage change.

Mean organic debris accumulation on wire bar inserts, demonstrated by ATP relative light units (RLU) values, was higher in static cages than in IVC; the same was found for filter tops. A large difference was detected between the mouse static filter top group as compared with the other filter top groups. The authors suggest many factors may have influenced these findings, which include: higher airflow in IVC resulting in animals spending more time in nesting material, where reduced animal activity may reduce aerosolization of particulate matter and reduced debris accumulation of cage accessories in IVC; higher airflow in IVC may result in increased aerosolization of debris and increased accumulation on cage accessories vs high airflow causing a cleansing effect and reducing adherence of debris to cage accessories; the number of animals per cage, where more animals and activity contribute to aerosolization of particulate matter;

The authors found that the mean RLU value for mouse static wire bar group was slightly above 100,000 at 14 days, whereas mouse IVC and rat static and IVC cage components were below 100,000 at 14 days. Mouse static filter top values exceeded 100,000 at 60, 90, and 180 days. The values in all of the other filter top groups never exceeded 100,000. Based on the findings, the authors recommend 100,000 RLU could be considered an actionable value. The authors changed their institutional protocol to the following: static and IVC wire bar inserts changed every 14 days, which can be extended up to 90 days for IVC mouse and rat cages; mouse static filter top lids to be changed every 30 days, and static rat and IVC mouse/rat filter top lids to be changed up to as infrequently as every 180 days.

QUESTIONS

1.  In general, according to the Guide, rodent enclosures and accessories, such as tops, should be sanitized at what frequency?

2.   What is ATP? How does ATP testing work?

ANSWERS

1.  At least once every two weeks.

2.  ATP is a molecule found in all living and recently deceased organisms and its presence can be used as an indicator of the presence of biologic residues or contamination on surfaces. A surface is swabbed, and the sample is exposed to an ATP releasing agent that lyses cells. The sample is then exposed to an ATP activated light producing substrate, an enzyme, and oxygen. The amount of light produced during the enzymatic reaction is directly associated with the amount of ATP present and the light emitted can be quantified in relative light units (RLU).

**Blevins et al. Effects of Oxygen Supplementation on Injectable and Inhalant Anesthesia on C57BL/6 Mice, pp. 289-297**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Common anesthetics such as ketamine, xylazine, and isoflurane can cause respiratory depression, hypoxemia, and hypercapnia. This often goes unnoticed due to the rodent's small size and specialized equipment needed. Anesthetic groups were low-dose ketamine/xylazine (100/8 mg/kg), medium-dose ketamine/xylazine/acepromazine (100/10/1 mg/kg), and high-dose ketamine/xylazine/acepromazine (150/12/3 mg/kg). A crossover design was used in which each mouse was anesthetized twice at the same dose group (above) once with and once without oxygen supplementation (either medical air or room air). The study showed that mice anesthetized with injectable and inhalant anesthesia are profoundly hypoxic, as measured by pulse oximetry, when supplemental oxygen is not provided. Anesthetized mice that did not receive supplemental oxygen all became hypoxic and this was prevented in mice that received oxygen. At the high injectable dose of anesthetics, all mice not receiving oxygen supplementation died while all mice receiving oxygen supplementation survived. Providing supplemental oxygen also decreased the duration of the surgical plane of anesthesia. Also the MAC for isoflurane was not affected. Major conclusions indicate that oxygen supplementation is recommended for all anesthetized mice.

QUESTIONS

1. What is the mechanism of action of Ketamine?

a.   GABA agonist

b.   GABA antagonist

c.   NMDA agonist

d.  NMDA antagonist

2. What is the mechanism of action of Xylazine?

a.   GABA agonist

b.  GABA antagonist

c.   a-2 agonist

d.   a-2 antagonist

ANSWERS

1. d

2. c

**Frederickson et al. Comparison of Juvenile Feed Protocols on Growth and Spawning in Zebrafish, pp. 298-305**

Domain 4: Animal Care

Primary Species:  Zebrafish (*Danio rerio*)

SUMMARY: Zebrafish are an increasingly important animal model, but their care has not been fully standardized. Most facilities still use live feed, particularly artemia, at least during some life stages, which can be inconsistent and labor-intensive. This study sought to compare two feeding protocols for juvenile zebrafish (30-75 dpf): FKA (3:1 mixture of fish flake and freeze-dried krill twice daily and live artemia twice daily) and GMA (Gemma Micro 300 feed once per day and artemia once per day). The GMA-fed juveniles showed better reproductive performance initially, but both feeding approaches yielded comparable lifetime results: no difference in mortality or fecundity by 2 years of age. Since many zebrafish studies rely on manipulation of the embryo, early increases in reproductive performance are important for researchers. GMA also represented a labor savings once proper equipment was in place, but the diet and equipment were more expensive than FKA.

QUESTIONS

1. Which is not an important measure of zebrafish reproductive performance?
   1. Spawning success
   2. Fertilization rate
   3. Clutch size
   4. Gestation length
2. At what age are zebrafish considered juveniles?
   1. 20-60dpf
   2. 10-55dpf
   3. 30-75dpf
   4. 75-100dpf
3. How many times per day are juvenile zebrafish normally fed?
   1. 1
   2. 2
   3. 3
   4. 4
   5. 5

ANSWERS

1. d

2. c

3. d

***Health Surveillance***

**Hanson et al. PCR Testing on Media Placed in Soiled Bedding as a Method for Mouse Colony Health Surveillance, pp. 306-310**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Traditional rodent colony health surveillance has required the use of live sentinel animals. Recent improvements to the process have shifted focus from the use of live animals to environmental sampling. Plenum sampling has become a common site for environmental sample collection; however, various rack designs and use of static caging make sampling from the plenum less than ideal.

The current study sought to identify an alternative sampling strategy that would simplify sample collection from both IVC and static cage types. The authors placed flocked swabs (‘passive swabs’) and filter media in experimental IVC and static cages.  Over 90 days, soiled bedding was collected from colony mouse cages during routine cage change and placed in the experimental cages. Colony mouse cages contained mice that tested positive for mouse norovirus (MNV), *Helicobacter*spp., and *Radfordia affinis*. At the end of 90 days, an ‘active’ flocked swab was used to stir the soiled bedding within the experimental cage. All samples collected were tested by PCR for MNV, *Helicobacter*spp., and fur mites.

Passive swabs performed the best with 100% of samples yielding positive results for all 3 agents from both caging types. Active swabs were equally effective in static caging with 100% detection rate for all 3 agents. Active swabs in IVC cages showed 100% detection rate for *Helicobacter* spp. and fur mites, but only 80% for MNV.  The filter media was the least effective in both cage types for MNV; 80% detection in IVC cages and 60% in static cages. However, filter media showed 100% detection for *Helicobacter spp.* and fur mites in both cage types.

This study showed filter media and flocked swabs allow successful detection of MNV, *Helicobacter* spp. and fur mites in both static and IVC cage types - a potential alternative for environmental sampling.

QUESTIONS

1. Which of the following characteristics describe *Radfordia*?
   1. Oval body with “Boxing gloves” tarsal suckers
   2. Oval body with two claes at the terminal tarsal structure
   3. Elongated profile with two claws at the terminal tarsal structure
   4. Elongated profile with a single claw on the second pair of legs
2. Which agents is not readily transmitted through soiled bedding?
   1. Murine norovirus (MNV)
   2. Mouse hepatitis virus (MHV)
   3. Mouse parvovirus (MPV)
   4. Sendai virus

ANSWERS

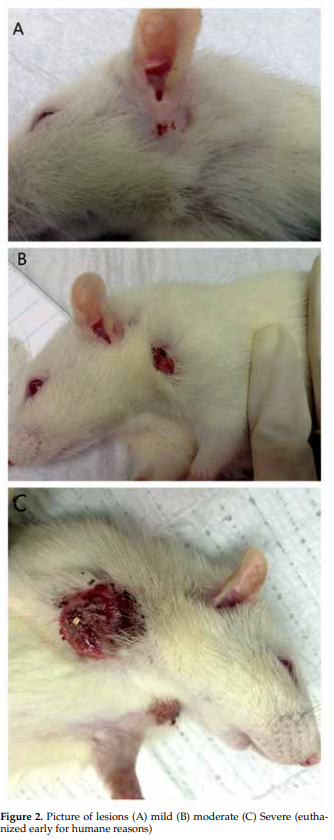
1. c
2. d

**Skiles et al. Evaluation of Treatment Options for Ulcerative Dermatitis in the P Rat, pp. 311-318**

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

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Recent studies have shown that nail trims are the most successful intervention in mice for ulcerative dermatitis but no studies have evaluated this in rats. P rats are rotational outbred adult rats that are phenotypically selected from a Wistar foundation stock to prefer drinking alcohol. These rats are used to study ethanol seeking behaviors and addiction. One breeding facility has observed a 10% incidence of ulcerative dermatitis in these rats. Additional environmental enrichment, oral and topical treatments, or mechanically trimming the animals’ nails have all been suggested as treatment modalities. This study evaluated the following treatments: topical TAO 3x/week, antiseptic wound powder 3x/week, trimming hind nails once every 2 weeks under anesthesia, shacks changed weekly, pumice stones (referred to as lava ledges) changed weekly, and a no treatment control group. Rats were singly housed. 66 rats (42F, 24M) were entered into the study from the breeding colony as soon as UD was diagnosed (clinical enrollment occurred over 4 years). Nail trimming was added as a treatment group only after 90% of the rats were assigned to the other treatment groups. Lesions were photographed and surface area was measured under isoflurane anesthesia. Wounds were measured at least every 2 wks. Study endpoints included healing of wounds, reaching humane endpoints, or at the end of 12 wks. Histopathology and scoring of the lesions were performed. No evidence was found on histology of pumice stone ingestion. The nail trim treatment group had significantly improved healing compared with the other groups. No significant improvements were found compared with the control group for pumice stones, TAO, or wound powder. No significant difference was found for total inflammation scores between any of the groups. Nail trims are the recommended treatment of UD in mice and P rats. Environmental enrichment may help prevent development of UD rather than treat animals with lesions already present.



QUESTION

1. What is the etiology of ulcerative dermatitis?

ANSWER

1. The etiology is not known; however, P Rats and C57BL/6J mice are frequently used in addition studies, suggesting a possible correlation between addiction and self-mutilation and dermatitis. In humans, links have been found between addiction and compulsive behavior such as trichotillomania and skin picking.

***Anesthesia***

**Rousseau-Blass et al. A Pharmacokinetic-Pharmacodynamic Study of Intravenous Midazolam and Flumazenil in Adult New Zealand White-Californian Rabbits (*Oryctolagus cuniculus*), pp. 319-328**

Domain3: Research; K1. biomethodology techniques (e.g., collection of blood and other body fluids and tissues; handling and restraint; administration of compounds and treatments); K9. principles of experimental design and statistics including scientific method

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: Benzodiazepines enhance GABA affinity for the GABAa receptor resulting in sedation, anxiolysis, and muscle relaxation with minimal cardiovascular depression. Flumazenil is a selective GABAa antagonist that acts via competitive inhibition of the benzodiazepine binding site on the GABAa receptor. This was a prospective, randomized, blinded, crossover trial with 2 treatment arms separated by a 2 week washout period. Rabbits were assigned to receive midazolam followed by either flumazenil or saline; and then received the opposite treatment in the second arm of the study. Time from midazolam injection to head down, lateral recumbency, and loss of righting reflex (LORR) were recorded. The delay between treatment injection (FLU/SAL) and return to righting reflex was recorded. Sedation levels were assessed using a modified version of the sedation scale (Wagner, 2017). Arterial blood samples from the ear were collected for the PK study.

No correlation was detected between the initial concentration of midazolam and the time to LORR or between the initial midazolam concentration and the plasma concentration of midazolam when LORR occurred. The return to righting reflex was 25 times faster in the FLU group than the SAL group and occurred at a significantly higher midazolam concentration. No differences between groups were detected in mean arterial blood pressure at any time point.

Metabolism of midazolam by the liver leads to the production of an active metabolite, 1-OH-midazolam. Although this metabolite has approximately half the activity of the parent drug and has a longer half-life, it is unlikely to have contributed to midazolam duration of action at the plasma concentrations reached in rabbits after IV administration, because the concentrations of midazolam were much greater than that of the metabolite. FLU had no effect on the PK of midazolam or 1-OH-midazolam.

The delayed time to return of lateral recumbency after FLU administration (as compared with SAL) suggests FLU initially prevented the effects of midazolam, but eventually dropped below a concentration that was effective. FLU (0.05 mg/kg, IV) antagonizes the sedative effects of midazolam administered intravenously at 1.2 mg/kg in rabbits. However, a risk of re-sedation at approximately 20 min after FLU injection warrants continued monitoring in the

recovery phase.

QUESTIONS

1. Periasnesthetic mortality, with the majority of deaths occurring during the recovery period, remains higher in which of the following species:

a. Dog

b. Rabbit

c. Cat

d. Ferrets

2.  The \_\_\_\_\_  is a phenomenon in which a drug gets metabolized at a specific location in the body that results in a reduced concentration of the active drug upon reaching its site of action or the systemic circulation.

a. Portal shunt

b. Partition coefficient

c. Blood-gas partition

d. First pass effect

ANSWERS

1. b

2. d

**Haertel et al. Predictors of Subcutaneous Injection Site Reactions to Sustained-Release Buprenorphine in Rhesus Macaques (*Macaca mulatta*), pp. 329-336**

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

Domain 2: Management of Pain and Distress

Primary Species: Macaques(*Macaca spp.*)

SUMMARY: A retrospective review of subcutaneous injection site reactions to sustained-release buprenorphine hydrochloride (Buprenorphine SR) in Rhesus macaques. Comparison of the occurrence of subcutaneous injection reactions between 3 mg/ml and 10 mg/ml formulations showed that when using the 10 mg/ml concentration there was 5 times less likely incidence of injection site reactions.

Subcutaneous administration of sustained-release buprenorphine hydrochloride delivers buprenorphine within 1 hour of administration and provides 5 days of analgesia. It is a combination of buprenorphine in a biodegradable polymer deliver system (sustained-release matrix, DL-lactiode-co-caprolactone copolymers with N-methyl-2-pyrrolidone (NMP) as a solvent). It is hydrophobic, water-insoluble, and precipitates in body fluids forming a gel depot for sustained release of the drug occurring by hydrolysis or immune cell degradation. The copolymer remains in the body subcutaneously for up to 2 years.

Foreign-body reactions begin when immune cells internalize antigens then process and present them via MHC glycoproteins. Macaques have MHC class I and II glycoprotein alleles that interact with T-cells.

In this retrospective study, the volume of buprenorphine SR injected subcutaneously ranged from 0.1 ml to 1.4 ml and the dose was 0.2 mg/kg for both 3 mg/ml and 10 mg/ml formulations.

Subcutaneous reactions were defined as subcutaneous swelling greater than 0.5 cm in diameter at the injection site. Reactions were characterized microscopically as pyogranulomatous inflammation surrounding an unidentified clear material. Histological reports also described multinucleated giant cells.

2 best predictors of reactions were concluded to be body weight (mean weight: 7.6 kg); suggesting a dose related response (heavier animals need more buprenorphine and thus receive more sustained release matrix) and macaques with the MHC allele Mamu-B\*29.

Alternatives to 3 mg/ml buprenorphine SR should be used when choosing a pain-relieving drug for larger macaques and for macaques known to express the MHC allele Mamu-B\*29. Dose-related adverse drug responses to copolymers can be mitigated by using more concentrated formulations which would have less copolymer per milliliter, such as 10 mg/ml.  Another alternative suggested was Simbadol, an FDA-approved highly concentrated buprenorphine solution which does not contain copolymers and delivers buprenorphine for 48-72 hours in macaques.

QUESTIONS

1.Two predictors of subcutaneous reactions to Buprenorphine SR:

a. Outdoor housing and number of injections

b. Age and sex

c. Body weight and MHC allele Mamu-B\*29

2. Buprenorphine SR concentrations of 3 mg/ml and 10 mg/ml showed equal occurrences of subcutaneous reactions:

a. True

b. False

c. Sometimes

3. Higher concentrations of Buprenorphine SR contain:

a. More copolymer per milliliter

b. Less copolymer per milliliter

c. Same amount of copolymer per milliliter

4. Simbadol is a viable alternative to Buprenorphine SR because it contains

a. More copolymer

b. Less copolymer

c. No copolymer

5. Buprenorphine SR provides:

a. 3 days of analgesia

b. 4 days of analgesia

c. 5 days of analgesia

6. Simbadol provides:

1. 3 days of analgesia
2. 4 days of analgesia
3. 5 days of analgesia

ANSWERS

1. c

2. b

3. b

4. c

5. c

6. a

**Killoran et al. Rapid Recovery and Short Duration Anesthesia after Low Dose Ketamine and High Dose Dexmedetomidine in Rhesus Macaques (*Macaca mulatta*), pp. 337-340**

Domain 2: Management of Pain and Distress

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: While ketamine is a standard drug for macaque anesthesia, this anesthesia is characterized by muscle tremors and preserved airway reflexes. Tolerance can also develop. Dexmedetomidine is an alpha2 selective agonist that provides good muscle relaxation and is reversible, but anesthesia cannot be accomplished with this drug alone. This study compared the anesthesia/recovery of male and female rhesus macaques <15yo when low dose ketamine was used with high dose dexmedetomidine, versus high dose ketamine alone.

Thirty-six animals with average reactions to past anesthesia were chosen. Either 10mg/kg ketamine or 1.5mg/kg ketamine + 0.03mg/kg ketamine was administered. Time to sedation (able to remove from cage) and time to recovery (time from return to cage to access to quarter of cage) were recorded. Heart rate, O2 saturation, and response to toe pinch were measured every 5 minutes. Animals were returned to cages after 30 or 45 minutes. Dexmedetomidine was reversed with atipamezole at time of return to cage.

Time to sedation did not differ. Time to recovery was faster with ketamine + dexmedetomidine (one third of the time of ketamine alone). Ketamine + dexmedetomidine provided at least 30 minutes of anesthesia/analgesia to toe pinch stimulus. Ketamine + dexmedetomidine caused bradycardia but it was never profound and did not adversely affect O2 saturation.

In conclusion, the low dose ketamine + high dose dexmedetomidine with atipamezole reversal resulted in a significantly shorter recovery time than high dose ketamine. This combo also provided at least 30 minutes of restraint and analgesia adequate for mild to moderately painful procedures. Potentially adverse effects of alpha2 agonists such as dexmedetomidine include derangement of fetal blood pressure, derangement of conduction within the heart, induction of peripheral vasoconstriction, and inhibition of insulin secretion. For these reasons, this drug should be avoided in pregnant animals, and animals with heart conditions, diabetes, or hypertension.

QUESTIONS

1. Which of the following is a potentially adverse effect of alpha2 agonists?

a. Peripheral vasoconstriction

b. Tachycardia

c. Derangement of fetal blood pressure

d. a and c

2. T/F:. Low dose ketamine + low dose dexmedetomidine resulted in significantly shorter recovery from anesthesia than high dose ketamine alone.

3. Name 3 disadvantages of using ketamine for macaque anesthesia.

ANSWERS

1. d

2. True

3.  Preserved airway reflexes, muscle tremors, tolerance can develop

***Experimental Use***

**Xu et al. Effects of Analgesics on Tumor Growth in Mouse Models of Prostate Cancer Bone Metastasis, pp. 341-348**

Domain 2

Primary Species - Mouse (*Mus musculus*)

SUMMARY: Administration of analgesics in rodent models of neoplasia may impact tumor growth kinetics and metastasis in a model-dependent manner. This study evaluated the impact of 2 analgesics, carprofen and buprenorphine, on tumor growth in 2 murine models of prostate cancer bone metastasis. C57BL/6 and SCID mice were administered either saline, 5 mg/kg carprofen, or 0.1 mg/kg buprenorphine followed by intratibial injection of either prostate cancer tumor cells or saline. C57BL/6 mice were allografted with murine cancer cells, and SCID mice were xenografted with human cancer cells. Bioluminescent imaging, radiographs, nociception (von Frey), locomotion (distance traveled), and weight were assessed weekly in all animals until the terminal 28 d endpoint.

All C57BL/6 mice grafted with murine prostate cancer cell line RM-1 reached experimental endpoint early (d14) due to pathologic tibial fractures. For this group, tumor measurements did not differ significantly between treatment groups. Bioluminescent imaging of tumors demonstrated higher tumor burden in analgesic treated groups compared to control, but no significant difference between individual treatment groups. Bone involvement was higher in the carprofen treated group on day 7, but not thereafter. There were no consistent differences in welfare indicators. In SCID mice, tumor burden and bone involvement were not significantly different between groups except for a single time point at day 14. Again, there were no consistent differences in welfare indicators.

This study suggests that there was no consistent, significant impact of a single administration of analgesic on tumor development in 2 mouse models of prostate cancer bone metastasis. There were a few sporadic and minor differences between groups in some evaluated parameters, however, there were no consistent, repeatable differences that persisted over the course of the study. Welfare tests of nociception and locomotion did not demonstrate clear benefits of analgesia; however, these were assessed at a relatively late (24h) time point.  The authors recommend administration of analgesia for intratibial injection of prostate cancer cells as there were no significant demonstrable impacts of analgesia on tumor growth kinetics.

QUESTIONS

1. Which of the following correctly defines an allograft?
   1. Seeding of tumor cell lines into the into the relevant organ of tumor origin in an animal model
   2. Transplantation of tissues to a recipient from a genetically non-identical donor of the same species
   3. Cell type that is derived from a species that is different from the recipient of the specimen
   4. Transplantation of tissues from one part of the body to another in the same person
2. Which of the following is not a limitation of the welfare indicators used in this study?
   1. They were conducted in the presence of humans and in an unfamiliar environment, which may have led to increased anxiety and exploratory behavior.
   2. The Von Frey analgesiometric assay may be more useful for assessing pain at 3-6 h post-insult, and not as useful at 24 h post-insult.
   3. The tumor cell line was not screened for rodent viruses other than mycoplasma.
   4. Interpretation of body weight in tumor models is challenging because mice can lose weight due to poor body condition or gain weight due to tumor growth
3. Subcutaneous administration of compounds results in \_\_\_\_\_\_\_\_\_\_ systemic absorption compared to intramuscular administration.
   1. Equivalent
   2. Faster
   3. Variable
   4. Slower

ANSWERS

1. b

2. c

3. d

**Rusch et al. Effects of Three Consecutive Days of Morphine or Methadone Administration on Analgesia and Open-Field Activity in Mice with Ehrlich Carcinoma, pp. 349-356**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Ehrlich carcinoma, a transplantable tumor inoculated subcutaneously or intraperitoneally, is considered to cause pain yet is widely used in rodents to evaluate therapeutic drugs on the inhibition of tumor growth. In that sense, pain recognition and management are an important component of the experimental protocol to ensure the welfare of research animals. The use of opioid analgesics, such as morphine and methadone, is well known as the most effective treatment of cancer pain; however, opioids tend to alter locomotor activity and exploratory behavior in mice. This study evaluated the analgesic effect of morphine and methadone in BALB/c mice with Ehrlich carcinoma; plus, their influence on behavior.

BALB/c mice were inoculated intraperitoneally with 2 x 106 Ehrlich tumor cells and seven days after, mice were sort in 7 different groups: (MO5) morphine 5mg/kg, (MO7.5) morphine 7.5 mg/kg, (MO10) morphine 10mg/kg, (ME2.85) methadone 2.85mg/kg, (ME4.3) methadone 4.3mg/kg, (ME5.7) methadone 5.7 mg/kg and (Saline) 0.9% saline. Drugs were administered via SQ every 6 hours for 3 days. Additionally, mouse grimace scale (MGS) and open-field activity was assessed in mice. For MGS, mice were recorded for 3 to 5 min without human presence on day 0 (before tumor inoculation) and after tumor inoculations on days 7, 8 and 9 at different time points (0, 40, 90, 150, 240, 360 min after drug injection); for each time point orbital tightening, cheek bulge, nose bulge, ear position and whisker position were scored from 0 to 2. In the open field test, total distance traveled (cm/5 min), average speed (cm/s), frequency of animals rearing and self-grooming behavior was evaluated on day 0 (baseline, before tumor inoculation), then, subsequently on day 7, 8 and 9 at different time points (40, 90, 150, 240 and 360 min after drug injection).

All mice developed Ehrlich ascitic carcinoma 7 days post inoculation; in general, lower locomotor activity and grooming was observed at day 7 and worsened as the experimental period progressed. Mice in the saline group had higher MGS scores consistent with pain on day 8. Morphine reduced the MGS scores for 150 min after the first administration and could promote analgesia for up to 240 min after serial administration at doses of 7.5 and 10 mg/Kg. However, methadone did not provide equivalent analgesia. After morphine administration, locomotor activity in the open field test increased in a dose dependent manner but this was not observed for the methadone groups. Although activity increased significantly after methadone administration, the effect was not dose dependent. Moreover, locomotor activity was higher on days 8 and 9 than on day 7 for both intermediate and the highest morphine and methadone doses. Morphine treated mice showed a lower rearing frequency and higher average speed, and consequently, an increased horizontal distance traveled. Finally, the frequency of self-grooming decreased after the administration of both morphine and methadone.

In conclusion, morphine should be still considered the drug of choice for pain management in mice with Ehrlich ascitic carcinoma; other findings in this study corroborate the alteration of behavior in mice, previously reported, after the administration of morphine and methadone such as an increase of locomotion activity, and decrease in self-grooming and rearing frequency in mice.

QUESTIONS

1.  T/F: Rearing frequency is triggered and modulated by the hippocampus; reduced rearing occurs when the environment is deemed dangerous or unfamiliar inhibiting the exploratory drive in situations of stress, anxiety and ataxia.

2.   Why self-grooming behavior in animals, especially rodents, is important?

ANSWERS

1.  True

2.  Self-grooming is an important behavior associated with hygiene maintenance, thermoregulation, social communication and excitement in rodents.

**Krueger et al. Effects of Pair Housing on Patency of Jugular Catheters in Rats (*Rattus norvegicus*), pp. 357-364**

Domain: 3

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: The development and refinement of appropriate practices to socially house rats with chronic implants are important factors in optimizing animal wellbeing and successful research outcomes. Although social isolation may introduce an experimental variable and adversely affect research outcomes, a limitation of pair housing in the research setting is concern that a cage mate may damage an implanted device. Limited information is available about the incidence of cage mate-related catheter complications and the methods used to socially house rats with chronic vascular implants.

This study is about whether social housing was a feasible experimental refinement for rats participating in an opioid self-administration and reinstatement study. The 2 goals of the current study were to determine the effects of social housing on catheter patency (experiment 1) and on catheter patency of rats participating in an opioid self-administration and cue-induced reinstatement study (Experiment 2).

Opportunities for refinement were identified throughout the study, one of which was an additional aim for Experiment 2. The study examined the use of a ‘buddy barrier’ for pair-housed rats participating in the opioid self-administration study.

Experiment 1: effect of social housing in long-term catheter patency: there was no statistical difference in catheter loss and catheter patency between single-housed rats and pair-housed rats. The catheter type (plastic or metal) had no significant effect on overall patency rate. The level of veterinary clinical intervention required in pair-housed rats was not significantly different than of single-housed rats.

Experiment 2: effect of social housing on catheter patency of rats participating in an opioid self-administration paradigm: pair housed animals were successfully maintained on an infusion study.

Overall, results demonstrated that rats with chronic vascular implants could safely be housed in pairs.

When analyzing possible factors affecting patency rates, one of the study’s most robust findings was a difference between rat stocks. Heterogenous Stock (HS) rats had a significantly higher proportion of catheters remain patent for the duration of the experiment than did Sprague-Dawley (SD) rats. Strain or stock of rats appears to affect the long-term patency of vascular catheters to a greater extent than housing conditions, perhaps due to differences in behavior and /or the foreign body immune response displayed by different rat stocks.

To maximize the time of healing post-surgery, a ‘buddy barrier’ was placed in the cage for 5 days immediately after surgery. This barrier allowed the animals to remain in stable pairs with tactile, olfactory, auditory and visual contact while reducing play behavior that had the potential to disrupt the healing process.

Compared with single housing, pair housing allows social contact and may increase the overall wellbeing of rats under study.

QUESTIONS (True or False)

1. Rats are a naturally social species and social housing in the laboratory does not impede critical behaviors such as feeding.

2. Single housing may significantly affect the interpretation of study results if not properly accounted for

ANSWERS

1. True

2. True

**Hankenson et al. Using Waterless Alcohol-based Antiseptic for Skin Preparation and Active Thermal Support in Laboratory Rats, pp. 365-373**

Domain 3: Research

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Skin preparation for veterinary patients consists of hair removal and cleaning of the surgical site by multiple applications of a relevant agent and liquid rinses.

Prior to a cranial surgical procedure, 12 male and 12 female BDNF (SD-Bdnfem1Sage) rats 6 to 8 weeks old were implanted with a subcutaneous temperature transponder while anaesthetized with isoflurane. After a 5–6-day recovery period, rats were randomly assigned to 4 prewarming treatment groups – no prewarming (control), warm-water blanket (WWB) under cage, WWB under cage and IP warm fluids, and IP warm fluids – and anaesthetized with isoflurane for a stereotaxic cranial surgical procedure. After clipping the fur, the skin was cleaned using a waterless alcohol-based (WAB) antiseptic – 2 applications with 1 minute contact time. Swabbing was done post-clipping and post-cleaning. A midline cranial incision exposing the cranium was closed using wound clips. Swabbing was done after skin closure. A WWB provided an external source of warmth and temperature was additionally monitored using a rectal thermometer. Pre- and post-op analgesia was provided, and respiration was monitored during anesthesia.

A one-way ANOVA was used to compare the prewarming treatment groups and recovery times and the relationship between recovery times and body weights. There was no significant difference intraoperatively in body temperatures and recovery times across the groups. Heavier animals recovered significantly faster than lighter animals. Nine/24 rats had no bacterial growth post-cleaning and post-skin closure. 15 rats showed dramatic fall in the CFUs detected post-clipping and post-cleaning/post-skin closure.

Rats were housed in microisolation cages under standard housing conditions.

QUESTIONS

1. The authors found that the average difference in temperature readings from core measurements and subcutaneous transponders was approximately:

a. 1°C

b. 0.2°C

c. 0.7°C

d. 0.4°C

2. The authors cultured all EXCEPT ONE of the following bacterial species from the skin swabs. The species not cultured was:

a. *Staphylococcus aureus*

b. *Escherichia coli*

c. *Pseudomonas aeruginosa*

d. *Enterococcus gallinarum*

ANSWERS

1. d

2. c