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**POSTION STATEMENTS**

**Association of Primate Veterinarians. Association of Primate Veterinarians’ Guidelines for the Judicious Use of Antimicrobials, pp. 601-606**

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

SUMMARY

Purpose:  Provide guidance regarding appropriate judicious use of antimicrobials in nonhuman primates.

Background:

Considerations for use of antimicrobials in NHP

1. Emphasize preventative strategies
2. Therapeutic alternatives
3. Behavioral management to minimize wounding
4. No routine group prophylactic use of antibiotics
5. Review and justification of use of antimicrobials important to treating refractory human infections
6. Antibiotic resistance to zoonotic agents increasingly hazardous
7. Bacterial colonization different from bacterial infection
8. Culture and sensitivity
9. Additional non-experimental variables

Guidelines

*Diarrhea*

* Prominent enteric pathogenic causes of diarrhea: Shigella flexneri, Yersinia enterocolitica, Yersinia pseudotuberculosis, Escherichia coli, Campylobacter jejuni, Klebsiella pneumoniae
* Fecal or rectal culture with sensitivity and/or PCR
* Alternatives include increased dietary fiber, coconut, vitamin B supplementation, probiotics, bismuth subsalicylate, fecal transplant, changes in diet

*Wounds*

* Identification and appropriate lavage can reduce contamination
* Consider NHP temperament and wound size, location and character when deciding whether or not to bandage.
* Topical treatments include hydrogel, honey, silver

*Perioperative Period*

* Consider type of surgical procedure, type of surgical wound, comorbidities of the NHP, postoperative husbandry conditions
* Prophylactic perioperative antimicrobials may be recommended for neurosurgical procedures or receiving implants.
* Topical antimicrobials should not be applied to surgical incisions.
* Both CDC and WHO recommend against additional doses of antimicrobials after wound closure
* Alternatives for NHPs who disturb surgical incision: prevent access, increase enrichment, ensure wound integrity
* If postop antimicrobials are chosen, use first generation cephalosporins as first choice.

*Outdoor and/or Group Housing*

* Outdoor-housed animals often relocated for treatment. Depending on culture and sensitivity, long-acting single-dose antimicrobial therapy may be used.
* Note that pharmacokinetics of antimicrobials is species-specific. Cefovecin does not last as long in NHPs as companion animals.

Research Indications and Alternatives to Antimicrobials

*Immunosuppression*

* Presumptive infections should be confirmed. Empirical therapy should be re-evaluated within 2-3 days and include de-escalation.

*Implanted Devices*

* Considerations include: location of implant, chronicity of implant, clinical signs presented
* Implants are at the most risk of contamination during surgical implantation.
* Implant-associated infections are unlikely to resolve with only antimicrobial therapy due to bio-film production.
* Treating infected implants is only recommended as a temporary measure until the implant can be removed or study endpoints achieved.
* Cranial implants are at high risk for mixed infections and adverse sequelae such as meningoencephalitis.
* Systemic antimicrobials should be reserved for treating infections with severely compromised dura or bone, animals with meningoencephalitis, animals with cephalic abscessation on imaging, and for a long duration (4-8 weeks)

*IACUC / Protocol Considerations*

* Antimicrobial therapy can add variables and affect reproducibility between studies and institutions.

*Decolonization of Bacterial Carriers*

* Individual animals should be tested for possible pathogens when there is concern.
* Routine treatment of subclinical carriers to standardize the microbiota or decolonize them should be avoided. Decolonization attempts may not last.
* Low resistance of bacterial enteric pathogens to commonly used antimicrobials

*Use of Important Human Antimicrobials*

* The World Health Organization’s Advisory Group on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR) maintains and categorizes antimicrobials
* AWARE guidelines group antibiotics into first or second choices (access), those to watch, and those to reserve.
* Highly resistant bacteria (ex. methicillin-resistant S. aureus, multi-drug resistant E. faecalis)
* When human-important antimicrobials are only treatment for a resistant infection, “the decision to use these drugs must prioritize the negative public health consequences over the research animal model”.

*Methods to Decrease Antimicrobial Use*

* CDC recommendations for human healthcare require antimicrobial stewardship by tracking infectious isolates and resistance patterns and success of antimicrobial treatment.
* It is recommended to collect samples for culture and sensitivity from all infected sites before starting antimicrobial therapy.
* If needed, screening programs in quarantine can allow identification of carriers and non-carriers and may allow some individuals to be disqualified for device implantation.

QUESTIONS

1. What is the most commonly used antimicrobial for perioperative prophylaxis?

1. Cefovecin
2. Cefpodoxime
3. Amoxicillin/Clavulonate
4. Cefazolin

2. What does de-escalation after empirical antimicrobial therapy include?

1. Expanding the antimicrobial spectrum
2. Minimizing the antimicrobial spectrum
3. Discontinuing current antimicrobial
4. a and c
5. b and c

3. When are telemetry devices at most risk of contamination?

1. Implantation
2. Removal
3. While being worn

ANSWERS

1. d
2. e
3. a

**Association of Primate Veterinarians. Association of Primate Veterinarians’ Guidelines for Post Research Retirement of Nonhuman Primates, 607-608**

Domain 5: Regulatory Responsibilities

SUMMARY: Rehoming of non-human primates requires significant planning and consideration. Institutions should develop local protocols, and reference to these guidelines may help inform decision making.

Each animal considered for retirement should be evaluated by a veterinarian to determine

clinical suitability and a behavioral specialist to determine behavioral suitability, as well as consideration of the type of research performed. In particular, extra caution should be used for animals:

* That participated in infectious disease studies
* With implanted devices
* Animals with chronic clinical conditions or compromised immune systems
* Geriatric animals

The potential retirement facility should also be evaluated. Factors for evaluation include:

* Veterinary care
* Specific housing for animals
* Behavioral management program
* Employee experience
* Regulatory oversight
* Facilities and security
* Biosecurity
* Social outreach and social media

QUESTIONS

1. What is an anthropozoonotic disease?

2. What risks are associated with long term use of external retirement facilities, even after pre-screening?

3. What types of studies may be performed on retired animals?

ANSWERS

1. Pathogens from in humans that are capable of being transmitted to non-human animals.

2. Staff changes, owner changes, management changes, financial problems

3. Primate aging, management of obesity, impact of physical exercise on general health and reversibility of certain health conditions, social interactions, innovative housing and enrichment arrangements, and psychological aspects of resources provisioned.

**ORIGINAL RESEARCH**

***Biology***

**Smyth et al. Point-of-Care Glucose and Lipid Profile Measures Using a Human Point-of-Care Device in Mouse Models of Type 2 Diabetes Mellitus, Aging, and Alzheimer Disease, pp. 609-615**

Domain 1: Management of Spontaneous and Inducible Diseases

Primary Species: Mouse (*Mus musculus)*

SUMMARY: Point of care (POC) technology has helped streamline treatments in patients and reduce lab costs. Unfortunately, there are few resources available on their use in preclinical animal models. The authors set out to compare human Cardiocheck POC to veterinary laboratory chemical analyzer (LCA) within Type 2 diabetes mellitus, aging and Alzheimer mouse models in the hopes that human POC devices can be used for reliable data collection. 4 groups of mice were used for this study 1) 15 diet-induced obesity (DIO) C57BL/6J mice (a model of T2DM), tested at 10 to 21 week old; 2) 16 C57 controls age-matched to the DIO mice, tested at 12 to 21 week old; 3) 5 5XFAD mice (complete nomenclature is B6SJL‐Tg [APPSwFlLon,PSEN1\*M 146L\*L286V]6799Vas/Mmjax) on a C57 background (model of AD) tested at 16 week old; and 4) 4 aged C57 mice tested at 28 to 60 week old. Animals were anesthetized and samples were removed by terminal cardiac puncture to obtain 200-300 ul of blood. Samples were tested on the POC glucose devise immediately after obtaining the sample, a lipid profile was performed, and serum was obtained for Glucose SL Assay Cholesterol-SL Assay, N-geneous LDL Cholesterol Assay, and Triglyceride-SL Assay for comparison. The LCA glucose, total cholesterol and HDL results were higher than the POC device. The AD mice had normal glucose values but aberrant lipid profiles. When comparing human to rodent normal ranges, it is important to note that often the rodent “normal” may fall out of range for the human normal and therefore the device may not be able to accurately read some parameters. The results from this paper demonstrated that the Cardiocheck POC device can be used to identify patterns of glucose and lipid levels within the rodent models tested. The authors hope that these devices may become widely accepted. However, the device is not suitable for cholesterol and triglycerides.

QUESTIONS (True or False)

1. Point of care testing requires only a small amount of blood (5-40 ul).
2. Diabetic induced obesity C57/B6J mice typically have a fasting blood glucose greater than 240 mg/dl.

ANSWERS

1. True
2. True

**Stokol et al. Hematologic and Biochemical Reference Intervals and Urinary Test Results for Wild-caught Adult Southern Giant Pouched Rats (*Cricetomys ansorgei*), pp. 616-629**

**Domain 3:** Research

**Tertiary Species:** Other Rodents

**SUMMARY**: Adult Southern Giant Pouched (SGP) rats were captured in the field of Tanzania and transported to the US for a long-term project unrelated to the current study. Due to lack of published clinical pathologic data for this species, the objective of the study was to establish reference intervals, using the American Society of Veterinary Clinical Pathology guidelines. Samples were collected from 60 anesthetized rats, via the coccygeal artery, for hematology and biochemistry analyses, along with cytochemical staining reactions of peripheral blood leukocytes and urine via cystocentesis. Lymphocytes were the dominant leukocyte in peripheral blood and granular lymphocytes were identified in most animals. Male rats had significantly higher RBC, absolute reticulocyte counts, and MCV than did female rats. Minor sex-associated differences in urea nitrogen concentration and GGT activity were noted. Leukocytes showed unique cytochemical staining characteristics. Small amounts of protein and bilirubin were found in the urine of rats of both sexes and of female rats, respectively, particularly in concentrated urine. Results obtained will provide a useful resource for investigators or veterinarians working with these animals and will help to confirm their health or identify underlying disease.

**QUESTIONS**

1.       Identify the species of Giant African Pouched rats (note: images did not come from article):



2. All of the following rodents are suspected of being potential reservoirs of monkeypox, except:

a. Prairie dogs (Cynomys sp.)

b. Dormice (Graphiurus sp.)

c. Kangaroo rats (Dipodomys sp.)

d. Gambian giant pouched rats (Cricetomys sp.)

e. Rope squirrels (Funisciurus sp.)

**ANSWERS**

1.   a. Cricetomys gambianus (known as the Gambian Pouched Rat)

b. Cricetomys ansorgei (known as the Ansorgei Pouched Rat)

c. Cricetomys emini (known as the Emin’s Pouched Rat)

d. Cricetomys kivuensis (known as the Kivu Pouched Rat)

2. c

***Husbandry***

**Hicks et al. Effects of Maternal Fenbendazole on Litter Size, Survival Rate, and Weaning Weight in C57BL/6J Mice, pp. 630-636**

Domain 1

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Benzimidazoles, particularly fenbendazole, are commonly used as prophylactic or therapeutic agents against pinworm murine parasites. The purpose of this study was to investigate the effects of fenbendazole on mouse fecundity, with a secondary aim of elucidating differences between continuous treatment and alternate-week treatment. Experienced C57BL/6J male breeders were paired with virgin females and allocated to one of 5 groups: control (standard 5053 diet only), continuous fenbendazole feed until weaning, continuous fenbendazole feeding until birth, alternate-week fenbendazole feeding until weaning, or alternate-week fenbendazole feeding until birth. The number of pups at birth, pup weight at weaning, and pup survival to weaning were compared between groups.

There were no differences in the number of pups at birth, pup weight at weaning, and pup survival to weaning between groups. Based on these findings, fenbendazole does not appear to significantly impact fecundity in C57BL/6J mice. This is in contrast to another anthelmintic, ivermectin, which has been documented to adversely impact reproduction in mice. The impact of pinworm infection itself was not evaluated.

QUESTIONS

1. Which of the following is not a natural pinworm of mice?
   1. *Syphacia obvelata*
   2. *Syphacia muris*
   3. *Auspiculuris tetraptera*
   4. *Enterobius vermicularis*
   5. All of the above have mice as a natural host
2. What is the mechanism of action of benzimidazoles?
   1. Inhibition of microtubule polymerization by binding to β-tubulin
   2. Opening of glutamate-gated chloride channels causing hyperpolarization
   3. Agonists at nicotinic acetylcholine receptors leading to neuromuscular depolarizing blockade
   4. Tegumental damage and paralytic muscular contraction
   5. Inhibition of 45-pS channels, leading to a flaccid paralysis
3. Which of the following describes the effect of infection with Kilham rat virus on reproduction in rats?
   1. Infertility
   2. Fetal resorption
   3. Abortion
   4. Reduced litter sizes
   5. All of the above

ANSWERS

1. d

2. a

3. e

**Klug et al. Effects of Stocking Density on Stress Response and Susceptibility to Infectious Hematopoietic Necrosis Virus in Rainbow Trout, pp. 637-645**

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

Tertiary Species: Other Fish

SUMMARY: This article looked at stocking density and stress and its relationship to Infectious Hematopoietic Necrosis (IHN) disease in rainbow trout.  The study looked at two stocking densities at high density (HD – 20 fish/1L) and low density (LD – 20 fish/5L) and chronic stress response.  The study also looked at cortisol concentrations in water and serum and WBC counts as indicators of the stress response.

Results showed no difference between HD or LD in serum cortisol levels or water cortisol levels over time. Results showed a difference in HD vs LD in N:L (neutrophil:lymphocyte) ratio as well as HD vs LD and acclimated vs not acclimated prior to virus exposure, but this finding did not correlate with mortality.  The HDna (high density acclimated) fish had the highest percent mortality (82 ±-6%) followed by HDna, LDna (both at 74%) and LDa (65%). Although, analysis did not find this to be significant, there was a slight increase in mortality in the non-acclimated vs the acclimated group.

In summary, this article revealed that these animals did not have a significant difference in stress response in HD vs LD. They also conclude that density did not have a factor in IHNV mortality.

QUESTIONS

1.  What is the scientific name for the rainbow trout?

a.  *Oncorhynchus mykiss*

b.   *Carassius auratus*

c.  *Danio rerio*

d.  *Oryzias latipes*

2.   What are the common names of the other species?

3.  Infectious hematopoietic necrosis virus (IHNV) is what type of virus?

a. Iridovirus

b.  Birnavirus

c.  Rhabdovirus

d.  Paramyxovirus

ANSWERS

1.  a

2.  What are the common names of the other species?

a.   Rainbow trout

b.  Goldfish

c.   Zebrafish

d.  Medaka

3.  c

***Management***

**Thurston et al. Compassion Fatigue in Laboratory Animal Personnel during the COVID-19 Pandemic, pp. 646-654**

SUMMARY: Compassion fatigue is a widely recognized problem in human healthcare and other care related professions. CF is a serious issue in laboratory animal care and if left unchecked, can have a devastating and distressing effect emotionally. CF has been linked to high staff turnover, employee absenteeism, diminished productivity , negativity etc. during the COVID-19 pandemic laboratory animal research institutions was compelled to implement new work practices in order to maintain essential animal care operations such as culling animal colonies which can increase CF risks. CF is also associated with 2 other related disorder: burnout and secondary traumatic stress (STS). One study reported that 87% of North American veterinarians have experienced CF and given the elevated risk of suicide in veterinary profession, CF and STS should not be taken lightly.

The study involved an online survey to 200 personnel involved with animal research to gauge CF and if it increased during the pandemic. The survey examined professional quality of life, self-assessed levels of CF, institutional changes, changes in animal welfare, and institutional measures to alleviate CF.

Result: A total of 86% of respondents was shown to have CF during their careers and 41% experienced CF for the first time, new CF symptoms or worsening CF symptoms during the pandemic. In addition, 90% of participants who reported a CF during the pandemic also reported and effect on their personal and professional lives. Most reported work stressors during the pandemic ranges from 26-74%. Starting from the highest: worrying about own health and the health of their loved ones by being an essential worker, loss of face-to-face interactions with coworkers, worrying about job security and potential job loss, feeling helpless, lack of communications from leadership, euthanasia of animals, unpredictable work schedule, and concerns about the impact of COVID-19 on the health or welfare of the animals they care for.

Some research showed that young female veterinarians are at high risk for mental health difficulties and other previous work also showed that women were more depressed during the COVID-19 pandemic. Although this current study was demographically limited, other surveys such as the Canadian population have also yielded similar findings regarding CF. the result from this current study demonstrated that CF is an ongoing, pervasive problem among lab animal personnel. One of the most significant outcome of the survey was that they did not feel as valued or supported as other essential personnel during the pandemic, largely given to the nature of animal research and confidentiality as opposed to other healthcare essential workers with open supporting messages from the public (i.e.: heroes work here). Finding ways to publicly promote the vital work performed by laboratory animal care workers could decrease the CF experienced. A detailed guide for building CF program can be further looked into and workplaces should implement or expand programs to build and support emotional resilience and compassion satisfaction to laboratory animal personnel. A CF program should be continuously evolving to meet the needs of the staff

QUESTIONS

1. What does ProQOL Scale stands for and its mechanism?

2. What does CF? CS? stands for

3. What is the difference between burnout and STS

ANSWERS

1. ProQOL – Professional Quality-of-Life Scale. This is a validated scale designed to assess and score burnout, STS, and CS.

2. (CF) Compassion fatigue. A state of physical and emotional exhaustion that can affect care givers and those related professions overtime, depleting the feelings of care and empathy that drew them to these professions (CS) Compassion satisfaction. The feeling of fulfillment and gratification that a person gets from their work, often from caring laboratory animals.

3. Burnout results from a person’s long term, unrelieved exposure to occupational, mental or physical exhaustion and that stress occurs gradually overtime. Whereas (STS) Secondary traumatic stress, Is a syndrome also called as “vicarious trauma” in which persons experiences stress by witnessing distress, such as animals may experience during research and although the exposure to trauma is indirect, the effect can be profound and in some cases even leading to posttraumatic stress disorder.

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***Health Surveillance***

**Garcia et al. The Effects of Water Volume and Bacterial Concentration on the Water Filtration Assay Used in Zebrafish Health Surveillance, pp. 655-660**

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

Primary Species:  Zebrafish (*Danio rerio*)

SUMMARY: Various microbes in zebrafish system water can be detected using a PCR filter assay in which water is filtered from a zebrafish system. This study looks atdifferent volumes of *Edwardsiella ictaluri, Aeromonas hydrophilia,*and*Mycobacterium marinum* in stock solutions with known concentrations filtered using positive pressure to determine probability of obtaining a positive result. In this study, the probability of detecting 2 of the 3 test organisms (*E. ictaluri* and *M. marinum*) was affected by the volume of water assessed whereas the probably of obtaining a positive result was less affected by testing larger volumes of water when *A. hydrophilia* was the organism. In general, results support filtering relatively large volumes of water (≥3L) before the filter is sent for PCR screening.

QUESTIONS:

1.   Which of the following is the most likely etiology for a zebrafish presenting with granulomas in the kidney?

a.  *Edwardsiella ictaluri*

b.  *Aeromonas hydrophilia*

c.  *Mycobacterium marinum*

d.   *Pseudoloma tomentosa*

2.        Which of the following agent(s) is/are Gram positive in their staining characteristics?

a.  *Edwardsiella ictaluri*

b.  *Aeromonas hydrophilia*

c.  *Mycobacterium marinum*

d.   *Pseudoloma tomentosa*

ANSWERS

1.       c

2.       c

***Anesthesia/Analgesia***

**Myers et al. Serum Buprenorphine Concentrations and Behavioral Activity in Mice After a Single Subcutaneous Injection of Simbadol, Buprenorphine SR-LAB, or Standard Buprenorphine, pp. 661-666**

Domain: 4 Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY

Introduction: Buprenorphine hydrochloride is a partial mu opioid agonist and partial k-opioid antagonist that has been show to provide effective analgesia in mice when a serum concentration of 0.5-1 ng/mL is reached. Repeated injections of buprenorphine HCL every 4 to 6 hours are required to provide continuous analgesic coverage nor animals undergoing major survival surgery, which can require repeated handling and lead to stress. Sustained release formulations of buprenorphine such as Buprenorphine SR and Simbadol have been developed to minimize stress. Buprenorphine SR is viscous, concentrated and cannot be diluted so although it can provide continuous analgesia for mice for 48 to 72 hours, accurate dosing may be challenging. Simbadol is a high concentration formulation of buprenorphine that is labeled for use in cats. It is less viscous than Buprenorphine SR allowing for more accurate dosing and a recent study has shown that it can provide sustained plasma concentrations and prolonged analgesia for >24 hours.

The goal of this study was to determine if Simbadol can maintain serum concentrations of buprenorphine at 1 ng/mL over a 24 hour period in both male and female mice. The serum level of this drug (Simbadol) was compared with buprenorphine SR-Lab and buprenorphine HCL.

Methods: 48 mice were divided into 3 treatment groups (buprenorphine SR-LAB, buprenorphine HCL and Simbadol) and were injected with 1 mg/kg SQ with one of the buprenorphine products. Blood was obtained via cardiocentesis at the following time points: 1, 4, 8, 12, 16 hours after administration of the buprenorphine product and the mice selected for that timepoint were then euthanized by CO2 and cervical dislocation. Serum concentrations for each time point were quantified by LC-MS/MS. One mouse from each group was placed in an open field test after injection and activity was tracked for 5 minute intervals for 40 minutes after injection

Results: All groups had similar buprenorphine curves including rates of decline. The standard buprenorphine group had mean concentrations less than 1 ng/mL by 12 hours and the Simbadol group’s mean concentration was less than 1 ng/mL by 16 hours. The Buprenorphine SR-LAB group had a concentration above the therapeutic threshold throughout the 24 hours. Behavioral changes noted in mice included mild ataxia, staub tail reaction and tiptoe gait. All resolved within 1 hour after injection. Increased open field activity occurred in female mice receiving the Simbadol and standard buprenorphine as well as in male mice receiving Simbadol.

Conclusion: Overall, Simbadol does not provide sustained serum concentrations of buprenorphine in mice and Buprenorphine SR-LAB is recommended when longer term analgesia is required.

QUESTIONS

1.  According to a recent journal article which of the following medications can provide sustained serum concentrations of buprenorphine in mice for longer than 24 hours?

a. Simbadol

b. Buprenorphine HCL

c. Buprenorphine SR-LAB

d. Meloxicam

2. Which of the following behavioral changes may be seen after injection of buprenorphine in mice?

a. Increased urination

b. Rectal prolapse

c. Corneal ulceration

d. Increased activity and mild ataxia

3.  According to the Controlled Substances Act, which schedule substance is buprenorphine?

a. Schedule I: no currently accepted medical use

b. Schedule III: potential for abuse with moderate or low physical dependence

c. Schedule IV: low potential for abuse

d. Schedule V: low potential for abuse / contains only a limited quantity of narcotics in the formulation

ANSWERS:

1. c. Buprenorphine SR-LAB
2. d. Increased activity and mild ataxia
3. b. Schedule III: potential for abuse with moderate or low physical dependence

**Houston et al. Pharmacokinetics and Efficacy of a Long-lasting, Highly Concentrated Buprenorphine Solution in Rats, pp. 667-674**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

Summary:Buprenorphine (Bup) is one of the analgesics commonly used for the management of postoperative pain in research animals. However, published dosing regimen in rats suggest that Buprenorphine do not maintain a therapeutic level between dosing intervals, which may result in inadequate analgesia. On the other hand, Sustained released (SR) Bup maintains therapeutic levels over time more effectively and decreases the handling for repeated injections; still, SR-Bup can be difficult to obtain due to state laws directed at the opioid crisis. An FDA-approved, long lasting, highly concentrated (LHC) veterinary formulation of Bup provides 24 h of analgesia in cats and it seems to be a good option for prolonged postoperative analgesia avoiding the need for compounding pharmacy. The study aimed to determine the pharmacokinetics of LHC-Bup in outbred male and female Sprague-Dawley rats to learn if plasma levels could be maintained over the therapeutic threshold for 24 h. The analgesic efficacy was evaluated using the paw withdrawal analgesiometric test and a laparotomy model to determine if LHC-Bup is a viable alternative for treating postoperative pain in rats.

For the pharmacokinetics study of LHC-Bup, female and male rats (N=20) were administered with 0.5 mg/kg, SC. Then, blood was collected before dosing (baseline), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, and 72-h after administration; liquid chromatography tandem mass spectrometry was used to analyze blood samples. For the paw withdrawal assay, the Randall-Selitto analgesiometer was applied with gradually increasing mechanical force on the palmer aspect of the paw of each rat assigned in two different groups, injected SC with 0.5 mg/kg LHC-Bup and injected SC with saline. Laparotomy for ovariectomy or orchiectomy was performed in rats to evaluate efficacy of LHC-Bup, then rats were treated with 0.5 mg/kg LHC-Bup or saline; a third group included rats that received anesthesia without surgery and treated with LHC-Bup, and a final group included rats that had anesthesia, no surgery and saline. Additionally, behavioral assessment was performed in these rats; the frequency of grooming, wound licking, rearing, ataxia, hunched posture and coprophagy was recorded during a 5-min observation period. A score system was developed.

Plasma samples from rats after receiving LHC-Bup revealed a peak concentration of Bup 15 min after administration of 90 ng/ml in males and 34 ng/ml in female rats with therapeutic levels above the 1 ng/ml for over 12 h in male rats and for at least 24 h in female rats. These findings suggest that male rats will require more dosing than female rats. On the analgesiometer assay, rats treated with LHC-Bup showed a higher threshold for at least 12 h in males and at least 24 h in females after dosing, an indication that LHC-Bup provided analgesia for extended period as compared with the shorter acting Bup-HCL. Fewer pain behaviors were occurred with LHC-Bup treatment for at least 12 h after surgery, in both sexes as compared with saline treated rats. In conclusion, LHC-Bup can be used in rats with minimal side effects, and the analgesic response occurs in a sex dependent manner; male rats may require more frequent administration of LHC-Bup than do females to achieve continues analgesia. Also, LHC-Bup provides analgesia in both male and female rats for the initial 12 h after surgery.

QUESTIONS

1. Which of the following behavioral methods evaluate stimuli-evoked nociception?

a.  Grimace scale test

b.  Von Frey test

c.  Randall-Selitto

d.  b & c

e.   All of the above

2. According to this study, which of the following statements is false:

a.  Signs of pain were most evident in the first 12 hours after surgery, suggesting that this is the most critical period for providing analgesia.

b. Male rats with LHC-Bup maintain a therapeutic plasma level for at least 24 hours.

c.  The analgesic response of LHC-Bup occurs in a sex dependent.

d. Increased coprophagy in LHC-Bup treated rats was recognized as a side effect.

ANSWERS

1.      d

2.      b

**Bowling et al. Effects of a Heated Anesthesia Breathing Circuit on Body Temperature in Anesthetized Rhesus Macaques (*Macaca mulatta*), pp. 675-680**

Domain 2

Primary Species:  Macaques (*Macaca spp.*)

SUMMARY: Heat loss during anesthetic events, especially those over 1 hour in macaques, is a considerable concern for the wellbeing of the animals.  The loss of heat during surgery can cause alterations in the physiology of the animal which ultimately introduces confounding variables into experiments.  The four main methods of heat loss during anesthesia are radiation, conduction, convection, and evaporation.  Steps can be taken to reduce the loss of heat under anesthesia such as forced warm air, hot packs, or heated tables.  This study investigated the efficacy of a heated anesthesia breathing circuit that warms inspired anesthetic gas to mitigate the evaporative mechanism of heat loss and its effects on body temperature in anesthetized rhesus macaques as compared with forced air warming alone.  The study involved 10 male rhesus macaques who all underwent two anesthetic events that were two hours long each, one with the heated anesthetic gas and one without.  Body temperatures were monitored throughout the two anesthetic events.

Animals exposed to the heated breathing circuit returned to baseline body temperature faster than those with forced warm air alone.  As measured via esophageal and rectal temperatures the animals on the heated circuit reached their low temperature followed by a temperature increase faster than the control animals.   The authors speculate that the esophageal temperature improvements in the animals with the heated breathing circuit might have been influenced by the proximity of the warmed trachea to the esophageal temperature probe.  Still, the findings of this study suggest that warmed anesthetic gas (104 F) improves the body temperature of NHPs undergoing an anesthetic procedure, especially when paired with forced warm air.

QUESTIONS

1. Which of the following is NOT considered a method of heat loss for an animal undergoing a two hour anesthetic event?
   1. Convection
   2. Conduction
   3. Urine Loss
   4. Radiation
   5. Evaporation
2. Which of the following is known to be altered by hypothermia in rhesus macaques?
   1. Gut microbiota shift
   2. EEG pulses
   3. Sense of smell post-anesthesia
   4. Drug pharmacokinetics
3. What is normal body temperature range for an adult Rhesus Macaque?
   1. 37-39°C
   2. 34-36°C
   3. 35-37°C
   4. 36-38°C

ANSWERS

1. c
2. d
3. a

**Hausmann et al. Analgesic Efficacy of Tramadol and Morphine in White’s Tree Frogs (*Litoria caerulea*), pp. 681-686**

Domain 3: Research; Task 3: Design and conduct research

Tertiary Species: Other Amphibians

SUMMARY: To date, studies of antinociceptive efficacy have been reported for only a few amphibians species, primarily the northern leopard frog (*Rana pipiens*) and other laboratories species. Additional validated models are needed for evaluation of pain and analgesic efficacy in amphibians. Few studies were aimed at determining effective clinical antinociception in amphibian species. Currently, opioids are the foundation of antinociceptive research and pain management in amphibians. They have a well-developed endogenous opioid system.

This study hypothesized that oral tramadol and the positive control of subcutaneous morphine would provide dose-dependent antinociception in White’s tree frogs, measured as a significant increase in hindlimb withdrawal latency after exposure to noxious thermal stimuli delivered by using a standard Hargreaves apparatus, and that neither drug would have clinically relevant adverse effects. Two randomized, placebo-controlled, complete crossover studies were preformed, with oral tramadol (n=12) and with SC morphine (n=12).

This study is the first to systematically evaluate tramadol as a possible analgesic drug in White’s tree frogs. No significant differences in hindlimb withdrawal latencies were detected between saline and either oral tramadol or subcutaneous morphine at any dose tested. This outcome may be due to experimental methodologic factors or species differences. Other antinociceptive models, such as noxious mechanical stimuli or a surgical model, might be a more ecologically appropriate method to detect analgesic drug efficacy in tree frogs. These negative results are unexpected given the known presence of endogenous μ-agonists and -opioid receptors in some amphibian species. Tramadol with its μ-agonist properties (similar to morphine) should also have been an ideal candidate to produce an analgesic effect, but this was not observed.

No adverse effects or signs of sedation were observed with either tramadol or morphine. No plantar foot lesions were observed at any time point during or after the study and no deaths occurring during or after these studies. Oral tramadol change in the heat rate of the frogs implied drug absorption despite the lack of sedation or thermal antinociception. The depressive effect on heart rate occurred within 1 hr. of administration, supporting the hypothesis that oral absorption is relatively rapid and can induce a biologic effect.

The results of the current study indicates that White’s tree frogs respond appropriately when placed in the Hargraves apparatus with regard to reliably withdrawing a hind limb in response to a noxious stimulus. Tramadol and morphine appeared safe but did not produce statistically significant changes in hindlimb withdrawal latencies. However, tramadol did reduce heart rate, implying that the drug or a metabolite were biologically active. These results provide a starting point for comparisons with nonopioids and use of different nociceptive models, such as other behavioral and surgical models in order to determine the most clinically effective analgesic medication and dosage.

QUESTIONS (True or False)

1. Amphibians have a well-developed endogenous opioid system
2. Amphibians have documented presence of µ-, ĸ- and delta-opioid receptors
3. The relative potency of various opioid-receptor-specific drugs are similar between amphibians and mammals

ANSWERS

1. True
2. True
3. True

**Martinelli et al. Comparison of Thermal and Mechanical Noxious Stimuli for Testing Analgesics in White’s Tree Frogs (*Litoria caerulea*) and Northern Leopard Frogs (*Lithobates pipiens*), pp. 687-691**

Domain 2: Management of pain and distress; Task 1: Recognize pain and/or distress; Task 2: Minimize and eliminate pain and/or distress

Tertiary Species: Other Amphibians

SUMMARY: This study evaluated 2 models of nociception, thermal (Hargreaves test) and mechanical (von Frey test) with White’s Tree Frogs (*Litoria caerulea,*WTF) and Northern Leopard Frogs (*Lithobates pipiens,*NLF) using either morphine sulfate at 50 mg/kg or saline. Also, NLF  were evaluated after injection of morphine at 2 doses (50 mg/kg or 100 mg/kg) as compared with saline using von Frey filaments as a mechanical stimulus.

Historically, 2 tests that have been used in amphibian research: the acetic acid test (AAT) and the noxious thermal limb withdrawal test using the Hargreaves apparatus. The AAT causes tissue damage and inflammation and has been associated with infection, sepsis, and death so von Frey filaments (vFF), a mechanical nociceptive test commonly used in mammals, was evaluated as an alternative.

For the Hargreaves test, the temperature of the thermal stimulus was ~45 degrees C and was alternated between right and left hind limbs.

For the modified von Frey test, a 4.74 vFF (6.0 g) was applied to the caudal ventrum or plantar surface of the hindlimb.

After obtaining baseline data with the modified vFF or the thermal withdrawal test, the frogs were injected with either saline or morphine at 2 doses (50 mg/kg or 100 mg/kg) SC on the dorsum in the cranial half of the body and vFF sensitivity data was collected 1, 3, and 6 hours after the injection

No statistically significant differences in hind limb withdrawal latencies were found between morphine at 50 mg/kg and saline treated groups using the Hargreaves test or vFF test in either WTF or NLF.

For the vFF test alone, NLF that received morphine at 100 mg/kg had significantly longer response times at 1 and 3 hours compared to baseline than those that received 50 mg/kg; demonstrating a significant dose dependent anti-nociceptive response to morphine.

Amphibians may respond more consistently to mechanical noxious stimuli, using a modified von Frey test, than with the thermal noxious stimuli of the Hargreaves test.

QUESTIONS

1. Historically, 2 tests that have been used in amphibian research

a. The acetic acid test (AAT)

b. The Hargreaves test

c. The von Frey Filament test

2. T/F: The AAT causes tissue damage and inflammation and has been associated with infection, sepsis, and death.

3. T/F: Amphibians may respond more consistently to mechanical noxious stimuli, using a modified von Frey test, than with the thermal noxious stimuli of the Hargreaves test.

4. T/F: During the modified von Frey test, the temperature of the thermal stimulus was ~45 degrees C and was alternated between right and left hind limbs.

5. T/F: During the Hargreaves test, a 4.74 vFF (6.0 g) is applied to the caudal ventrum or plantar surface of the hind limb.

6. T/F: NLF that received morphine at 100 mg/kg demonstrated a significant dose dependent anti-nociceptive response than those that received 50 mg/kg.

ANSWERS

1. a and b

2. True

3. True

4. False

5. False

6. True

***Experimental Use***

**Mitchell et al. Evaluation of Pain and Distress and Therapeutic Interventions for Rectal Prolapse in Mice to Reduce Early Study Removal, pp. 692-699**

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

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Rectal prolapse (RP) is a common condition in lab mice that is perceived to be painful. Institutional practices of treatment may consist of no treatment, lubrication, reduction with dextrose or with surgery, or euthanasia. In humans, a prolapsed rectum is reported to be benign and nonpainful, though patients elect surgical correction due to the effects of incontinence, constipation, discharge, and tenesmus. In mice, causes for RP include stress, pregnancy and parturition, age, genetics, study manipulations, or infectious pathogens. Mice of some strains or genetic backgrounds are more likely to develop RP, such as transgenic knockout mice deficient in urokinase type plasminogen activator, nonmuscle myosin II cKO, and IL10 KO strains. Study manipulations and inducible models of colitis or gastrointestinal neoplasia are also associated with a higher likelihood of developing a RP. Infectious pathogens such as Helicobacter spp., Citrobacter rodentium, and the murine pinworms Syphacia obvelata and Aspiculuris tetraptera, are also commonly associated with RP. The objective of the current study was to clinically assess spontaneous RP in mice currently assigned to ongoing research protocols for pain and distress and to compare treatment options intended to maintain healthy RP mucosa. The hypothesis was that both topical treatments would maintain the RP mucosa better than no treatment.

120 mice with spontaneous RP were identified. The mouse was assigned a base score with the following criteria: mm measurement of prolapse length, gross mucosal health, pain/distress, and BCS. Feces was tested for Helicobacter and pinworms. Mice were assigned to the following treatment groups: petroleum jelly, lidocaine jelly, no treatment. Treatment took place three times weekly. Mice received a weekly score. Histopathology was assessed when mice were euthanized. None were euthanized due to RP euthanasia criteria.

Each treatment group had an increase in RP size over time. The rate of RP growth in the lidocaine jelly treatment group was about half that of the no treatment group. Body condition score was higher in the lidocaine jelly group than the no treatment group. Gross mucosal health appearance and pain/distress scores did not differ. 48% of breeding mice were able to produce further litters. 96% of mice tested positive for a Helicobacter species. None were positive for pinworms. No histopathological differences were found between treatment groups.

The current study concludes that no treatment is necessary for RP to maintain mucosal health and that the condition appears to be nonpainful.

QUESTIONS

1. Which pathogen is NOT associated with a higher likelihood of developing RP?

a.   Helicobacter spp.

b. Mouse parvovirus

c. Citrobacter rodentium

d. Syphacia obvelata

2. T/F: Lidocaine jelly treatment of RP led to slower prolapse growth rates than no treatment, but there was no change in gross mucosal health appearance or pain/distress scores.

3. What are the life cycle lengths of Syphacia obvelata and Aspiculuris tetraptera, respectively?

ANSWERS

1. b

2. True

3. 11-15d; 23-25d

**Holdridge et al. The Effectiveness of Hot Bead Sterilizers in Maintaining Sterile Surgical Instrument Tips across Sequential Mouse Surgeries, pp. 700-708**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Aseptic technique involves multiple steps: 1) cleaning & sterilization of surgical instruments, 2) reduction of bacterial load on patient using antiseptics, 3) maintenance of sterile field, 4) proper surgeon prep/protective equipment, & 5) sanitization of procedure area. Failure to apply proper aseptic technique can result in infections causing abnormal physiology and behavior, reducing animal welfare and affecting research. “Tips-only” surgery is a technique in which the tips of the surgical instruments is sterilized using hot bead sterilizers. Only the tip of the instrument can be used to manipulate the surgical field. A total of 104 surgeries were performed in 3 series: under strict aseptic technique (45 animals), with deliberate fur contamination (45 animals), and with deliberate contamination of instruments with cecal contents (14 animals). Surgical instruments were steam autoclaved prior to surgeries. Instruments were swabbed prior to, or following hot bead sterilization, or before and after sterilization, for bacterial culture or ATP analysis. Surgical instruments were cleaned following each procedure before placing in the sterilizer. Bead temperature was maintained at 500 F. ATP measurement showed no relationship between ATP and culture. ATP analysis was therefore discontinued. The probability that contaminated instrument tips were effectively sterilized, decreased by 11.5% after each surgery. Excluding the cecal contamination series resulted in lower probability of failure (4%) after each surgery. Twenty-five percent of instruments in the cecal contamination series were not sterile. Hot bead sterilization is not recommended for surgical procedures involving gross contamination of surgical instruments. Up to 5 consecutive surgeries can be performed, if there is minimal contamination of instruments, with >80% probability that instruments will be sterile.

QUESTIONS

1. Which device is used to quantify ATP levels in a biological sample?
2. Which of the following is NOT required for rodent survival surgery?
   1. Designated procedure area free of clutter and sanitizable
   2. Sterile surgical gloves, gown, mask
   3. Maintenance of a sterile field
   4. Proper patient preparation using antiseptics to reduce bacterial load

ANSWERS:

1. Luminometer
2. b. Sterile surgical gloves are not required but are recommended by ACLAM guidelines on rodent surgery