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**Association of Primate Veterinarians. Association of Primate Veterinarians Cranial Implant Care for Nonhuman Primates in Biomedical Research, pp. 496-501**

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

Primary Species:  Macaques (*Macaca spp.*)

SUMMARY: The purpose of this article was to address the care of nonhuman primates involved in chronic cranial implants .There are many factors involved in successful implant procedure ranging from intrinsic factors of the animal as well as the implant itself and post-procedural care.

Animals that perform as expected and do not manifest abnormal behaviors or temperament should be considered for implantation. Social housing should not be excluded from animals with implants. Pre-surgical imaging with 3-D has provided an invaluable tool in placement of hardware. The use of piezoelectric drills has also made the procedure more precise and with less complications as high powered drills and hand drills.  Even alternatives to PMMA polymerization have been refined by the use of glass-PEEK coated with hydroxyapatite.

Maintaining post-surgical procedures have always been continual with chronic cranial implants.  Wound margin care and skin care require diligent care and persistence.  The author suggests using a 7-10 day rotation of different disinfectants to be effective against all possible pathogens. Cylinders should be cleaned regularly and the cap replaced.  Multiple cylinders should be cleaned separately with new instruments.  This article finds that using chlorhexidine for the inside of the cylinders to be contraindicated as chlorhexidine may have neurotoxic effects.

Granulation tissue can be removed using suction, "scraping", surgical debridement, or the use of 5-FU. These procedures may cause pain and an appropriate analgesic plan including local anesthesia.

Treating infections of cranial implants is more problematic with the formation of biofilm as disinfectant and antibiotics cannot easily access the site of infection. It is occasionally prudent to remove the animal from the study and reassess once the infection is over and the healing has occurred.

The article also reviewed suggestions for implants in NWMs.

No questions with answers were provided with this summary.

**ORIGINAL RESEARCH**

***Reproduction***

**Gomes et al. Transcutaneous Ultrasound Guided Intraovarian Injection in Rats (*Rattus norvegicus*), pp. 502-505**

Domain 3: Research

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Little information is available for percutaneous ovarian injections in rodents, felines and canine. In rats, the available volume for injection into the ovary is 150uL. This was determined through injecting methylene blue into the cranial pole of the kidney, in a single rat, until leakage was observed. It should be noted that smaller rats would have a smaller available volume for injection, while larger rats would have a larger available volume for injection. To visualize the ovary, a 13Hz multifrequency transducer with a dynamic range adjusted to 75 and focal zone of 1-1.6cm is used. An appropriate anesthetic plane is achieved through isoflurane inhalant anesthesia delivered through a nose cone. The ovary is localized through palpation of the caudal kidney while the rat is in lateral recumbency. Once the kidney is palpated, maintain its position digitally. Contact gel is applied to the area. Then the ultrasound probe is used to scan caudolaterally and caudomedially until the ovary is located. Once localized, restriction of the ovary is performed with digital pressure to allow for ovarian injection. The needle should be inserted cranially to the transducer and angled 45 degrees to the transducer. An increased ovarian length can be noted after successful injection. After the procedure, one of ten rats exhibited a score of one or greater on the rat grimace scale and tramadol was given at 10mg/kg SC.

QUESTIONS

1. T/F: The total available volume that can be given during percutaneous ovarian injection is 150uL, regardless of animal size.
2. The ovary is located \_\_\_\_\_\_\_ to the kidney:
   1. Cranial
   2. Lateral
   3. Medial
   4. Caudal
   5. Within

ANSWERS

1. False: Animal size will change the available volume that can be injected
2. D

***Husbandry***

**da Silva Medeiros Elidio et al. Housing Density and Aggression in Syrian Hamsters, pp. 506-509**

Domain 4: Animal Care

Secondary Species: Syrian hamster (*Mesocricetus auratus*)

SUMMARY: Syrian hamsters are territorial, aggressive, and solitary animals. Stress to the animals was managed by minimizing noise and avoiding quick and inattentive movements, rough handling of cage components, and external odors. In the cage, animals were approached slowly and allowed time to interact with the handler's hand. Sleeping animals were awakened to avoid frightening it. Animals were lifted with a “pinch” grip and transferred to clean cages along with the old nest. After cage change, group-housed females were more aggressive (intraspecific aggression or aggression during handling) than males, regardless of age, and individually housed females were less aggressive than females housed in groups of 4 per cage. Group-housed males achieved a nonaggressive level of interaction, such that individual housing of males was not necessary. Authors suggest that as best practices, male hamsters be housed in groups of 4 per cage and females housed individually.

QUESTIONS

1.   Give the scientific name for the following species:

a.  Golden (Syrian) hamster

b.   Chinese hamster

c.  European hamster

d.  Dwarf (Siberian, Djungarian) hamster

e.   Russian dwarf hamster

2.  Syrian hamsters have cheek pouches lacking which structure that allows for xenotransplants to survive

a.  Extensive vasculature

b.  Lymphatic structures

c.    Salivary ducts

d.   Basal epithelium

ANSWERS

1.   a.  *Mesocricetus auratus*

b.  *Cricetulus griseus*

c.   *Cricetus cricetus*

d.  *Phodopus sungorus*

e.  *Phodopus campbelli*

2.  b

**Winn et al. Testing Alternative Surface Disinfection Agents for Zebrafish (*Danio rerio*) Embryos, pp. 510-518**

Domain 4; K1

Primary Species: Zebrafish (*Danio rerio*)

SUMMARY: Reagent-grade sodium hypochlorite (bleach) is often used to disinfect zebrafish embryo surfaces because commercial-grade bleach has additional ingredients that may have adverse effects.  Bleach solutions <100ppm are commonly used to maximize embryo survival. In addition to these two disinfectants, chlorine dioxide and SCPP (potassium peroxymonosulfate), which are commonly used to disinfect equipment and have been shown to decrease bacterial loads on nets used in aquatics facilities, were tested as possible alternatives to reagent-grade bleach for surface disinfection of embryos. SCPP at 1% concentration has been shown to be effective at eliminating *P. neurophilia*spores, which are not eliminated by embryo bleaching and define SPF in zebrafish. 6hpf and 24hpf embryos were exposed to each of the four disinfectants at 50 and 100-ppm for 5 or 10 mins. The survival, hatching rate and defect rate did not differ significantly among age-matched controls, but the hatching rate after exposure to 50ppm SCPP was higher than those exposed to 50ppm reagent-grade bleach for 5 mins. SCPP may be a viable alternative to bleach for disinfection of zebrafish embryos, though further study of in vivo efficacy against common zebrafish pathogens is required. Chlorine dioxide at 50ppm or greater is not recommended as a zebrafish embryo surface disinfectant due to significant mortality among 6 and 24hpf embryos.

QUESTIONS

1. At which pH is bleach more germicidal?
2. 9.0
3. 7.0
4. 5.0
5. 3.0
6. Which surface disinfectant is not appropriate for 24hpf zebrafish embryos?
7. SCPP
8. Reagent grade bleach
9. Commercial grade bleach
10. Chlorine dioxide
11. True or False: Potassium peroxymonosulfate (SCPP) reduced bacterial light units on aquatic fishnets within 5 minutes.

ANSWERS

1. b
2. d
3. True

**Rice et al. Implementation of Manual and Automated Water Regulation for Rats (*Rattus norvegicus*) and Ferrets (*Mustela putorius*), pp. 519-528**

Domain 3: Research; Task 2: Advise and consult with investigators on matters related to their research

Primary Species: Rat (*Rattus norvegicus*)

Secondary Species: Ferret (*Mustela putorius furo*)

SUMMARY:  Water regulation is a procedure that allows animals to consume water volumes equivalent to ad libitum access, although that access is limited to specific time intervals (i.e., water is not available outside of the designated access periods).  While similar to water restriction in that free access for consumption is not available at all times, water regulation differs from water restriction in that water restriction limits an animal’s daily volume of water to less than ad libitum consumption.  Water regulation is often used in experimental research to motivate responses, with fluids used as reinforcers in behavioral assessments, thus the reliable performance required for most behavioral studies requires some level of restriction or regulation. In general, when fluids are the reward for performance of an action or task, animals will learn more rapidly, perform better, and work for longer periods of time if water is restricted or regulated. Water regulation has been successfully used with mice, rats, ferrets, and nonhuman primates.  Implementation details are sparse, but experience with these species has shown that animals will likely adapt to water regulation schedules more rapidly if they occur at the same time every day and the access period is signaled.  Species-specific requirements should be considered before implementing water regulation, but when properly conducted, chronic water regulation produces no adverse physiological facts and can be maintained for weeks at a time.  When implementing water regulation for daily behavioral testing, maximizing the period without water will ensure that water will be a potent reinforcer, although this requirement must be balanced with the health of the animals and logistical considerations.  During water regulation, researchers should monitor body weight and skin turgor to determine if supplemental fluid should be added or the schedule should be adjusted, e.g. provide longer access time.

With the intent to identify and share details on how to successfully implement water regulation, this study evaluated implementation of water regulation schedules for rats and ferrets to ensure that both species would perform for fluids in behavioral assessments. Defining “successful implementation” as maintenance of appropriate weight gain and health status during toxicologic assessments, the authors placed rats (n = 510) and ferrets (n = 16) on water regulation manual and automated systems used for the rats, and an automated system for the ferrets. The methods were evaluated post hoc by comparing animal weights with normal growth curves, and evaluating health status (i.e., no dehydration, behavioral issues, or health concerns).  Rats can consume their total daily fluid requirements with only 10 to 15 min of water access, so the schedule of access provided under all methods should have been more than sufficient to maintain the health status of all rats, but results showed that the automated method was associated with greater intersubject variability and inadequate weight gain. Manual methods of water regulation were successful in rats by either moving the cage to prevent access to the drinking valve or by placing/removing water bottles, and both manual methods produced comparable and reliable weight gain.  Automated system control of water access to an entire rat rack was successful for most rats, but several rats failed to consume enough water even after 2 wk. of experience, and rats assigned to this system lost significantly more weight than rats assigned to the manual water regulation methods.  An automated system that controlled water access from water bottles was implemented for ferrets and was maintained for up to 30 wk., and results suggest that the ferrets consumed their daily water needs within 6 h, even when longer access periods were provided.  Retrospective comparison of body weights to standard growth curves for both species showed that all animals grew normally despite water regulation.  The water regulation schedules reported in the study were successful- implementation is easy to accomplish, can be used for extended time, and should be replicable even for laboratories with inexperienced personnel.  Differences in the systems and some species considerations provide insights into the key elements necessary for successful water regulation in rats and ferrets.

QUESTIONS

1. Both water regulation and water restriction limit opportunities for water consumption to defined time intervals... how do they differ?

2. Water regulation has been successfully used with each of the following species EXCEPT…?

a. Mice

b. Rats

c. Birds

d. Ferrets

e. Nonhuman primates

3. For this study of water regulation for rats and ferrets to ensure that both species would perform for fluids in behavioral assessments, which of the following statements is most correct?

a. Manual systems were used for both rats and ferrets

b. Manual and automated systems were used for rats, and an automated system was used for ferrets

c. Automated systems were used for rats, and both manual and automated systems were used for ferrets

d. Manual and automated systems were used for both rats and ferrets

4. T/F: Water regulation mimics the natural habitats of many animal species.

5. Animals will likely adapt to water regulation schedules more rapidly if access period(s) possess what two characteristics?

6. T/F: When properly conducted, chronic water regulation produces no adverse physiologic effects and can be maintained for weeks at a time.

ANSWERS

1. Water regulation allows the equivalent of ad libitum consumption within restricted time intervals, while water restriction does not

2. c. Birds

3. b. Manual and automated systems were used for rats, and an automated system was used for ferrets

4. True: Water is seldom always available (or accessible) in natural habitats of many animal species

5. (1) Access periods occur at the same time every day and (2) The start of the access period is signaled

6. True

***Management***

**Mocho et al. Assessment of Microbial Reduction by Cage Washing and Thermal Disinfection using Quantitative Biologic Indicators for Spores, Viruses, and Vegetative Bacteria, pp. 529-538**

Domain 4: Animal Care; K3. Methods of sterilization, sanitation, and decontamination; K4. Quality assurance techniques for animal care-related equipment (e.g., verification of effective cage sanitation) and supplies (e.g., water, food, bedding)

SUMMARY: Washing and disinfection of vivarium equipment, cages, bottles is a key technique of the biosecurity and rodent health surveillance program. In the current study, authors developed techniques (i.e., magnet attachments, quantitative biologic indicators (Q-BI), and measurement of thermal disinfection at equipment level) to evaluate the microbial decontamination achieved by a rodent equipment washer (Allentown CW-185) with and without thermal disinfection. Data indicated that 99% (only 3 magnets fell down out of 230) of the magnet attachments remained in place to carry Q-BI and temperature probes inside cages, water bottles or at equipment level throughout a cupboard washer chamber. Three different microorganisms *Bacillus atrophaeus*, *Enterococcus hirae* and minute virus of mice (MVM) were examined as Q-BI.

To simulate potential interference from biologic material and animal waste throughout cage processing, Q-BI contained test soil: bovine serum albumin with or without feces. As a quantitative indicator of microbial decontamination, the reduction factor (Fred) was calculated by evaluating microbial load of processed Q-BI with unprocessed controls. Authors detected variation between Q-BI varieties and assessed the washer’s ability to scale back microbial load. Fred data clearly demonstrated that the washing and thermal disinfection cycle may scale back a great deal of vegetative microorganism, virus and spores by 5 log10 CFU/TCID50 and beyond.

Thermal disinfection was monitored with temperature probes linked to data loggers (DL). Study measured the period of exposure to temperatures above 82.2 °C, to calculate A0, the theoretical indicator for microbial lethality by thermal disinfection, and to evaluate whether or not the washer could pass the minimal quality standard of A0 = 600.

Temperature curves confirmed an A0 > 1000 persistently throughout all processed tools throughout thermal disinfection. In conclusion, authors recommended that when sterilization isn’t required, a cabinet washer with thermal disinfection could replace an autoclave which will be cost effective.

QUESTIONS

1.   In the current study which organism was not used for quantitative biologic indicators (Q-BI)?

* 1. *Bacillus atrophaeus*
  2. Geobacillus stearothermophilus
  3. Minute virus of mice (MVM)
  4. *Enterococcus hirae*

2.   The difference between the initial load and the remaining live microbes indicates the log10reduction called?

* 1. Measure of microbial lethality
  2. Theoretical evaluation of thermal disinfection efficacy (A0 value)
  3. Reduction Factor (Fred)
  4. Elimination efficiency

3.  In the current study, what was the recommended temperature for thermal disinfection for CW-185 ?

* 1. 70 °C
  2. 80 °C
  3. 82.2 °C
  4. 78.6 °C

4.   Thermal disinfection of bacteria, fungi, and heat-sensitive viruses require an A0 value (A0value estimates efficacy of thermal disinfection) of?

* 1. 600
  2. 1022
  3. 1230
  4. 1200

ANSWERS

1. b

2. c

3. c

4. a

***Anesthesia***

**Bennett et al. Comparison of Nociceptive Effects of Buprenorphine, Firocoxib, and Meloxicam in a Plantar Incision Model in Sprague-Dawley Rats, pp. 539-548**

Domain 2: Management of Pain and Distress

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: NSAIDs are commonly administered to rodents to treat postoperative pain and are preferred due to their dosing schedule of every 24 hours. This study aimed to evaluate the use of firocoxib, a COX-2 selective NSAID, compared with meloxicam and buprenorphine in a plantar incision pain model to elucidate if any of these drugs reduce allodynia and hyperalgesia. Allodynia was assessed using the Von Frey assay of mechanical stimuli, and hyperalgesia was assessed by using the Hargreaves assay of thermal stimuli. As of the date of publication of this study, there is no published dose of firocoxib in rats, however the medication has been assessed in a recent study in mice. The authors hypothesized that firocoxib would produce comparable antinociception in rats.

A preliminary pharmacokinetic study of firocoxib in rats was performed, revealing the median for its elimination half-life was 2.5 h (range 1.9-3.1 h) and maximum plasma firocoxib concentration occurred at 4-8 h in 3 of the 7 rats. In the next phase of the study, rats were placed under isoflurane anesthesia, the right hind paw was aseptically prepped, and a no. 11 blade was used to create a 1-cm longitudinal incision through the skin and fascia. The underlying plantaris muscle was elevated using curved forceps without disturbing the muscle origin or insertion. Skin was sutured with a single cruciate ligature of 6-0 nylon.

Rats were randomly assigned to 1 of 6 groups, n=12 per group (6 male, 6 female). Treatment groups included firocoxib 10 mg/kg SC q24h, firocoxib 20 mg/kg SC q24h, meloxicam 2 mg/kg SC q24h, buprenorphine 0.05 mg/kg SC q8h, saline SC q24h. One group of rats underwent anesthesia but not surgery. All first doses of the drugs were administered 30 minutes prior to surgery.

Behavioral testing for mechanical allodynia followed by thermal hypersensitivity at 24 h prior to surgery and at 20, 28, 44, and 52 h after surgery. Allodynia was measured with the Von Frey apparatus. The sensor tip was applied perpendicularly to the plantar aspect of the right hind paw about 3-4 mm medial to the incision. The paw withdrawal threshold (PWT) was determined as the force (in g) at which the rat withdrew its foot. The median of the 5 test values was subtracted from the median baseline values to obtain change in PWT, and allodynia was defined as a positive change in PWT.

Hyperalgesia was measured using a radiant heat stimulus applied to the plantar surface of the right hind paw from beneath a platform of glass. The paw withdrawal latency (PWL) was recorded as the duration in seconds of light exposure needed to evoke a brisk paw withdrawal. The median of 3 test values was subtracted from the median of baseline values to obtain a change in PWL, with a positive PWL indicating hyperalgesia. No treatment group in either assay at any time point was significantly different from its respective saline group, suggesting that medicated rats experienced allodynia and hyperalgesia that was similar to rats with surgery but no treatment. Recent literature suggests that higher doses of analgesics may be needed for comprehensive rodent analgesia, and the doses in this study may have been too low for this pain model.

It was apparent after behavioral testing that all the doses given to the rats after surgery were inadequate in providing antinociception, and so additional nociception testing was performed in animals without surgery. In these rats, no overall statistically significant association between mechanical allodynia and treatment group was found. However, firocoxib at 10 mg/kg was found to significantly increase hyperalgesia compared with buprenorphine, firocoxib 20 mg/kg, and meloxicam, and firocoxib 20 mg/kg significantly increased hyperalgesia compared with buprenorphine.

QUESTIONS

1. Which of the following drugs are considered selective COX-2 inhibitors?

a. Carprofen

b. Meloxicam

c. Aspirin

d. Firocoxib

2. Buprenorphine can be classified as a \_\_\_\_\_ opioid.

a. Kappa agonist/mu antagonist

b. Partial mu agonist

c. Full mu agonist

3. Which behavioral test below assesses allodynia in rodents?

a. Hargreaves assay

b. Tail flick test

c. Von Frey assay

ANSWERS

1. d

2. b

3. c

**Raulic et al. Development and Testing of a Sedation Scale for Use in Rabbits (*Oryctolagus cuniculus*), pp. 549-555**

Domain 1

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: Due to the difficulties with rabbit anesthesia, there is a need to have reliable methods of sedation.  However, an established sedation scale does not exit.  The authors of this study wanted to create a sedation scale that could be used for research purposes and for clinical practice.  The drugs tested for this study were midazolam, ketamine, dexmedetomidine, and alfaxalone, which are in commonly used for sedation in rabbits.  A total of 15 rabbits received IM injections of these drugs.  Following administration of the drugs the rabbits were assessed by blinded observers (six) using eight psychometric methods.  The methods evaluated by the observers were Posture, Palpebral reflex, Orbital tightening, Lateral recumbency, Loss of righting reflex, Placement of supraglottic airway device, Pinching of the toe, and General appearance/attitude.

The resulting sedation scale produced highly reliable and reproducible results.  For the individual evaluated methods there was good interrater reliability as well as very good intrarater reliability.  This indicated that the assessed level of sedation for each evaluated criteria was reliable between raters as well as within each rater.  Internal consistency was determined by calculating the Cronbach α for the scores assigned by the raters (median score between raters).  Any Cronback score over 0.75 is considered excellent with the score for this sedation scale scoring.

QUESTIONS

1. Which of the following drugs is a sedative and anesthetic that interacts with the GABA-receptor?
   1. Ketamine
   2. Dexmedetomidine
   3. Medetomidine
   4. Alfaxalone
2. Which of the following drugs is a sedative and anesthetic that is an Alpha-2 Adrenergic receptor agonist?
   1. Ketamine
   2. Dexmedetomidine
   3. Medetomidine
   4. Alfaxalone
3. In this recent publication, which of the following criteria are NOT seen as reliable indicators of sedation in rabbits?
   1. Ear position
   2. Toe Pinching
   3. Posture
   4. Orbital tightening

ANSWERS

1. d
2. b
3. a

**Abbo et al. Anesthetic Efficacy of Magnesium Chloride and Ethyl Alcohol in Temperate Octopus and Cuttlefish Species, pp. 556-567**

Domain 2: Management of pain and distress. Task 3: Administration  of Anesthesia

Tertiary Species: Invertebrates

SUMMARY: The study tested whether ethyl alcohol (EtOH) and magnesium chloride (MgCl2) were effective at reversibly depressing evoked activity in the pallial nerve in 2 temperate species of cephalopods (*Octopus bimaculoide* and *Sepia officinalis)*commonly used in research to study camouflage, behavior, and learning. Life-stages included: senescent (post-egg laying) females and lab-cultured juvenile (3-4 mo.) octopuses and senescent (~2 y) and subadult (~1 y) cuttlefish.

Historically, urethane (ethyl carbamate) and ethanol (EtOH) were commonly used anesthetics in cephalopods. Urethane is no longer used due to its carcinogenic and mutagenic effects on animals. Other anesthetic agents, including eugenol, 2-phenoxyethanol, isoeugenol, tricaine methanesulfonate) and hypothermia have been tried in cephalopods but MgCl2 and EtOH remain the most commonly used because of their availability, reliability, and ease of use.

In this study, neural signaling was assessed during induction and recovery by placing a hook electrode around the nerve-muscle bundle of the pallial nerve which innervates the mantle.

In the United States, cephalopods are not included in federal regulations that govern the use of animals in research laboratories but the Marine Biological Laboratory voluntarily upholds a Cephalopod Care Policy.

Anesthesia protocol for electrode placement using EtOH: Subject was removed from its home tank and placed in a sedative bath of 1% EtOH and home tank water. After 5 minutes, if the animal was not adequately sedated for electrode placement, an additional 1% of EtOH was added. All animals were adequately sedated for electrode placement using 1-2% EtOH. After the electrode was placed on the pallial nerve, the water was changed completely to fresh seawater to allow the animal to recover from sedation.

Upon recovery, the experimental induction procedure began and EtOH was added to create a 0.5% EtOH bath. Each minute after EtOH administration, the animal’s skin was pinched w/ forceps to measure behavioral and neural responses. EtOH was added in 0.5% increments every 5 minutes until the animal was unresponsive. Once full anesthesia was achieved, the water was changed to fresh seawater and neural recording resumed until complete recovery.

Anesthesia protocol using MgCl2: To place the electrodes 1-2% EtOH was used. Using a stock solution of 7.5% MgCl2 and distilled water, premade baths were made comprising fresh seawater only, 1:3 MgCl2:seawater, 1:2 MgCl2:seawater, and 1:1 MgCl2:seawater. Once fully awake, the animal was placed in 1:3 MgCl2 bath. If still showing signs of consciousness or neural signaling after 5 minutes, it was placed into the 1:2 MgCl2 bath and subsequently, if awake after an additional 5 minutes, into the 1:1 MgCl2 bath. Recordings continued until the animal was fully anesthetized and then placed in fresh seawater until completely recovered.

Behavioral changes seen during induction and recovery: mantle relaxation, loss of sucker intensity, initial increased then slowed respiratory rate, loss of righting reflex, and pale body color (relaxation of skin chronophores)

Induction times were generally shorter for EtOH than MgCl2. Induction was longer in senescent adults compared with juveniles for both. Recovery times were generally longer for MgCl2 than EtOH. Recovery after MgCl2 was longer for cuttlefish senescent adults than subadults. Recovery after EtOH was similar between senescent adults and subadults.

While a delay was seen between the onset of behavioral signs of anesthesia (paralysis) and loss of neural signal (anesthesia), both MgCl2 and EtOH were effective at anesthetizing all animals. For the EtOH group, behavioral and neural measures of anesthesia were coupled more tightly than MgCl2. MgCl2 caused some signs of aversiveness (increased ventilation and activity during induction) in the octopuses, but EtOH did not. All animals were able to be anesthetized while continuing to breathe.

QUESTIONS

1.  In the United States, cephalopods are not included in federal regulations that govern the use of animals in research laboratories (T/F)

2. Most commonly used anesthetic agents in cephalopods:

* 1. Eugenol and 2-Phenoxyethanol
  2. EtOH and MgCl2
  3. EtOH and Isogenol
  4. Tricaine methanesulfonate (MS222) and Eugenol

3. Urethane is no longer used as an anesthetic because of:

a. Expense

b. Short shelf-life

c. Carcinogenic and mutagenic effects on lab animals

4.  *Octopus bimaculoide* and *Sepia officinalis*arecommonly used in research to study

a. Oncology

b. Immunology

c. Camouflage, behavior, and learning

5. T/F: Induction with MgCl2 caused some signs of aversiveness, but EtOH did not.

6. T/F: Induction times were generally shorter for EtOH and MgCl2.

7. T/F: Induction was longer for octopus senescent adults than juveniles for both EtOH and MgCl2.

8. T/F: Recovery times were generally longer for EtOH and MgCl2.

9. T/F: Recovery after MgCl2 was longer for cuttlefish senescent adults than subadults.

10. T/F: Recovery after EtOH was similar between cuttlefish senescent adults and subadults.

11. T/F: With MgCl2 induction, behavioral and neural measures of anesthesia were coupled more tightly than EtOH.

12. T/F: EtOH caused increased ventilation and activity during induction.

13. In this study, neural signaling was assessed by placing a hook electrode around the nerve-muscle bundle of the:

a. Brachial nerve

b. Axial nerve cord

c. Pallial nerve

ANSWERS

1. True

2. b

3. c

4. c

5. True

6. False

7. True

8. False

9. True

10. True

11. False

12. False

13. c

**Fabian et al. Pharmacokinetics of Single-Dose Intramuscular and Subcutaneous Injections of Buprenorphine in Common Marmosets (*Callithrix jacchus*), pp. 568-575**

Domain 2: Management of Pain and Distress; T2 (Minimize or eliminate pain and/or distress), K5 (pharmacological interventions for pain and distress and their effects on physiology, including age and species differences for such interventions, and depth and duration of analgesia provided by such interventions)

Secondary Species: Marmosets/Tamarins (Callitrichidae)

SUMMARY:Buprenorphine HCl is commonly used for analgesia in marmosets, but little is published as far as dosing or duration of analgesia provided.  Doses ranging from 0.005 to 0.02 mg/kg have been proposed based on anecdotal information, however doses given at the higher end of that range (0.02mg/kg) have resulted in severe side effects for marmosets (ataxia, apnea, moderate sedation).  The goal of this study was to establish more concrete dosing for buprenorphine HCl in marmosets, which included dose (0.01mg/kg), duration of action (based on plasma concentrations >0.1ng/mL), and preferred route of administration (IM or SQ).  Blood was collected at 8-10 timepoints post injection (ranging from 15 minutes to 24 hours) using a hybrid sparse-serial sampling design (maintained up to 4 venipuncture events within 24 h, up to 3 home cage captures within 24 h, and up to 2 h out of the home cage per home cage capture).  Liquid chromatography–electrospray ionization–tandem mass spectrometry was used to determine plasma concentrations of buprenorphine HCl.  Based on the timepoint when the plasma concentrations passed the threshold, it was recommended that Buprenorphine HCl be administered as a dose of 0.01mg/kg every 4 (IM) to 6 (SQ) hours.

QUESTIONS

1. Buprenorphine is categorized as a \_\_\_\_\_\_\_\_\_\_\_ controlled substance:

a. Schedule I

b. Schedule II

c. Schedule III

d. Schedule IV

2. Which statement is FALSE regarding buprenorphine?

a. Buprenorphine is a partial μ- agonist (partial Mu-agonist)

b. Buprenorphine is a partial δ- agonist (partial Delta agonist)

c. Buprenorphine is a κ- antagonist (Kappa antagonist)

d. Buprenorphine is a nociceptin receptor agonist

3. What is the purpose of a hybrid sparse-serial sampling design with relationship to the marmoset?  Why is it helpful?

4. True or False:  Based on the provided data, buprenorphine at 0.01 mg/kg IM and SQ may be more appropriate for the provision of acute rather than long-term analgesia.

ANSWERS

1. c. Schedule III

2. b. Buprenorphine is a is a partial μ-agonist, δ- and κ-antagonist, and nociceptin receptor agonist

3. The purpose is to decrease the total blood collected from each individual animal in a 24-hour period while still allowing each animal to be used for all timepoints (by dividing the study into 2-3 study phases with a 2-3 week washout between phases).  It is important in marmosets since they are so small, and not amenable to multiple PK-related samplings without having multiple animals (to alternate sampling timepoints, as is done in sparse sampling when animals are bled once or at alternate timepoints then data are compiled into a composite profile).  A hybrid sparse-serial sampling design is helpful because it allows fewer animals to be used while still collecting all individual timepoints over a prolonged study period.

4. True

***Experimental Use***

**Pumphrey et al. Duration of Mydriasis Produced by 0.5% and 1% Tropicamide in Sprague-Dawley Rats, pp. 576-581**

Domain 1: Management of spontaneous and experimentally induced diseases and conditions

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY

Introduction: The objective of this article was to measure the duration of mydriasis induced by 2 different concentrations of commercially available tropicamide in albino rats. Tropicamide, a topical anticholinergic targeting muscarinic receptors, has been recommended as a mydriatic for ophthalmic exam in mammalian species based on its reliability, rapid onset of mydriasis, and relatively short duration of effect. It causes blurred vision and light sensitivity in the patient, which can alter behavior in lab animal species. It has been reported to persist for as little as 1 h in albino rodents, but no study has confirmed.

Methods: In the first phase of study, 7 mo. old female SD rats received complete ophthalmologic exam, with 1 drop of 1% tropicamide applied topically to one eye and 1 drop of balanced salt solution to the contralateral eye. Pupil diameter was measured at 20, 40, 60, 120, 180, 240, 300, 360 minutes under controlled lighting conditions in the same location within the animal housing facility. After 3 week washout period, the second phase was conducted. 1 drop of 0.5% tropicamide was applied to one eye and 1 drop of balanced salt solution to contralateral eye, with pupil diameter measurements at the same time previously described time points. Statistical analysis involved paired sample t-test to compare pupillary diameter between timepoints and treatment groups, significance p<0.05.

Results: Pupillary dilation sufficient to allow thorough posterior segment evaluation was achieved in all eyes treated with both concentrations of tropicamide. The maximum pupillary diameter after treatment with 0.5% tropicamide was 4.17 ± 0.22 mm at 40 min, while maximum pupillary diameter after treatment with 1% tropicamide was 4.28 mm at both 20 and 40 min. In all eyes treated with 0.5% tropicamide, the mean pupillary diameter was significantly different from baseline mean pupillary diameter for that eye at all timepoints up to 300 min. This was significantly different than the concurrent mean pupillary diameter in the control eye at all timepoints. Application of either 0.5% or 1% tropicamide also produced a transient visually apparent and statistically significant mild to moderate mydriasis in the contralateral (balanced salt solution) eye.

Discussion: In conclusion, both 0.5% and 1% tropicamide provided adequate mydriasis for ophthalmic exam in this group of female Sprague–Dawley rats. The duration of action was at least 5hr (300 min) for 0.5% tropicamide and 6hr (360 min) for 1% tropicamide, and both concentrations produced contralateral mydriasis.

QUESTION

1.   What anatomical site can be used for blood collection in the rat eye? The mouse eye?

ANSWER

1.  Rat: retro-orbital venous plexus; mouse: retro-orbital sinus

**Dixon et al. Mid-Tibiofibular Amputation as a Method of Terminal Blood Collection in *Xenopus laevis*, pp. 582-586**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions (K9- Diagnostic procedures)

Domain 2: Management of Pain and Distress (K7- Euthanasia)

Secondary Species: African clawed frog (*Xenopus laevis* and *Xenopus tropicalis*)

SUMMARY: The current accepted method of terminal blood collection in the African clawed frog (*Xenopus laevis*) is cardiocentesis.  However, due to their small size and the limited volumes of blood that can be collected, a new technique, the mid-tibiofibular amputation, was investigated to determine if it was comparable to current procedures.  The leg amputation method produced significantly higher mean blood mass than cardiocentesis.  The total mean percentage body weight was 3.7% with leg amputation as compared to 1.7% with cardiocentesis.  The leg amputation method was faster than cardiocentesis with the leg amputation technique requiring an average of 3.07 minutes compared to 8.27 minutes for the cardiocentesis.  There was no significant differences in serum chemistry and blood counts between the collection methods indicating that the leg amputation technique did not contaminate the blood sample with lymph, bone, or tissue debris. The results suggest leg amputation is a rapid terminal blood collection technique for *Xenopus laevis* which will provide large volume of blood for analysis; however, caution should be used if the samples need to be sterile as sterility of the samples was not considered as a part of this study.

QUESTIONS

1. What are the published volumes of blood that can be collected by cardiocentesis in a 110 g adult female *X. laevis*?

2. The density of whole blood is estimated to be \_\_\_\_\_ for quick assessment of intraoperative blood loss?

3. The heart rate of amphibians is temperature dependent; therefore, blood collection time will increase/decrease for frogs reared in cold water.

ANSWERS

1. 2-7 mls

2. 1 g/ml

3. Increase

**Tournade et al. Effects of Tinidazole on Food Intake in Chinchillas (*Chinchilla lanigera*), pp. 587-591**

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

Tertiary Species: Other Rodents

SUMMARY:Tinidizole (20mg/kg PO q12h for 5 days) did not significantly reduce food intake in healthy chinchillas, and is potentially clinically safer than metronidazole. Chinchillas are susceptible to typically subclinical, but zoonotic Giardia infections as well as anaerobic bacterial infections contributing to periodontal disease. Current recommended treatments of metronidazole at 20mg/kg PO q12h resulted in profound reduced food intake which can be detrimental in these hindgut fermenters. Tinidazole has been safely and effectively used in a variety of veterinary species, and is considered a top choice for the treatment of new and refractory parasitic infections in humans. This study examined the effects of 2 single high doses (100mg/kg and 200mg/kg PO), and repeated dosing regimen (20mg/kg PO q12h for 5 days) of a compounded tinidazole suspension on feed intake in healthy chinchillas. The study was blinded, and in a cross-over design with a 14 day “washout” period. Feed intake reduction was transient and dose-dependent in the single high-dose groups. There was no significant change in feed intake in the repeated dosing regimen. No other adverse effects were noted in any of the regimens. Pharmacokinetic and efficacy studies would need to be performed in the future.

QUESTIONS

1. What class of antibiotics do both tinidazole and metronidazole fall under?
   1. Aminoglycoside
   2. Nitroimidazole
   3. Fluroquinolone
   4. Sulfonamide
2. What are the potential clinical implications of reduced food intake in hindgut fermenters like rabbits and chinchillas?

ANSWERS

1. b. Nitroimidazole
2. Hypomobility of the GIT, improper nutrient production through cecal fermentation, hepatic lipidosis, ketoacidosis, malocclusion