**Journal of the American Association for Laboratory Animal Science**

Volume 58, Number 5, September 2019

**ORIGINAL RESEARCH**

***Husbandry***

**Mallien et al.** [**Effects of Soy in Laboratory Rodent Diets on the Basal, Affective, and Cognitive Behavior of C57BL/6 Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00001)**, pp. 532-541**

Domain 4

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Soy is a common source of protein in many commercial lab rodent diets. Soy also contains high amounts of isoflavones which are estrogenic. It is thought that these diets might affect estrogen-regulated systems including basal behavioral domains, affective behavior and cognition. This study evaluates C56BL/6 mice that receive a standard soy containing diet or a soy-free diet throughout their lifespan. Several different behavioral tests were performed: A nest test, novel cage test, open field-novel object test, elevated o-maze test, dark-light test, novel object recognition test, social memory test, forced swim test, hot-plate test, fear conditioning, and puzzle box test. Mouse serum samples were also analyzed for the presence of isoflavones which were detectable in all serum samples of mice that received the soy-free diet, but daidzein and genistein aglycone equivalent concentrations were 15-20 times lower than those in the standard group. The soy-free diet did not adversely affect the number or size of litters. In general, mice fed the standard diet weighed more than those given the soy-free diet, nesting behavior was not affected by sex or diet, and locomotion did not differ between treatment groups. Anxiety-sensitive parameters in the Elevated O-Maze test and the dark-light test remained unchanged, but despair behavior in the forced swim test was slightly decreased in soy-free-fed mice. Treatment with soy-free diet did not alter cognitive abilities in tests of fear conditioning, social memory, or novel object recognition. The soy-free diet did not alter functioning in the puzzle box test. Male mice fed the soy-free diet had the most frequent freezing behavior after fear conditioning. In all tests based on recognition, novelty was preferred by all groups. There were no sex or treatment-dependent effects on Social Memory and Novel Object Recognition. Dietary soy content did not influence executive functions or problem solving in the puzzle box test. A general improvement of responses over time indicated successful learning in all mice. However, female mice acquired the task more quickly than males and were able to solve the tasks in significantly less time. The study did not aim to detect which component of soy was likely to initiate possible changes. Whether to include or omit soy in lab animal diets continues to be debated.

QUESTIONS

1. T/F: Despair behavior in the forced swim test was slightly decreased in soy-free fed mice

2. T/F: Treatment with soy-free diet was shown to alter cognitive abilities in the novel object recognition test

3. Which statement most accurately describes the current recommendation on soy in lab animal diets:

a. Soy should be omitted from commercial lab rodent diets

b. Whether to include or omit soy continues to be debated

c. It is recommended to have soy in commercial lab rodent diets

d. It depends which component of soy is included in the diet.

ANSWERS

1. True

2. False -- Treatment with soy-fee diet did not alter cognitive abilities

3. b

***Management***

**Adams et al.** [**Effects of Pelleting, Irradiation, and Autoclaving of Rodent Feed on MPV and MNV Infectivity**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00002)**,** **pp. 542-550**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY:One of the potential sources for infectious agents into barrier rodent colonies is through feed, despite going through pelleting process. Murine norovirus and mouse parvovirus are stable in the environment and resistant to many forms of disinfection and could potentially survive through the pelleting process, and could partially explain the high prevalence in certain research colonies. Some studies have suggested that irradiation or autoclaving feed can decrease these occurrences, but no studies specifically have looked at the infectivity of MNV or MPV in the feed, the resistance of infectious particles in the standard pelleting process, or the effects of gamma irradiation and autoclaving. This study evaluates the effects of pelleting and irradiation on MNV and MPV infectivity in 4 to 6 week old, female, C57Bl/6 (MPV-resistant) and Swiss Webster (MPV-susceptible) mice (seronegative for both viruses in addition to other agents). Both viruses were propagated and infected into irradiated powdered chow and further processed (pelleted only, pelleted following irradiation, or pelleted following autoclaving). Mice were individually housed in sterilized microisolation caging using strict biocontainment practices. Mice were provided contaminated feed in a mason jar for 3 days and then provided standard irradiated chow until Day 28 (end of experiment). Mice were bled for serology against these agents before infection, and at days 7, 14, 21 and 28 post-infection. In summary, they found out that pelleting alone is insufficient to inactivate both viruses, and that contaminated pelleted feed subjected to gamma-irradiation (1/6 mice seroconverted after being fed pelleted feed with the highest infective dose) and autoclaving (no mice seroconverted at all doses) are beneficial for reducing viral loads in feed and reducing incidence of infection.

QUESTIONS

1. Which of the following correctly characterizes murine norovirus?

a. Enveloped, ssDNA virus

b. Enveloped, ssRNA virus

c. Non-enveloped, ssDNA virus

d. Non-enveloped, ssRNA virus

2. Which of the following correctly characterizes mouse parvovirus?

a. Enveloped, ssDNA virus

b. Enveloped, ssRNA virus

c. Non-enveloped, ssDNA virus

d. Non-enveloped, ssRNA virus

3. Which protein is used as a PCR target and antigen serology for MPV that is also conserved in MMV (Mice minute virus)?

a. VP1

b. VP2

c. VP3

d. Hemagglutinin

e. Neuraminadase

ANSWERS

1. d

2. c

3. b

**Kovach and Dash.** [**Using the Lean Six Sigma Methodology to Reduce Mouse Cage Sanitation Time for Animal Care and Use Programs**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00003)**, pp. 551-557**

Domain 4: Animal Care; K3 - Methods of sterilization, sanitation and decontamination

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The Lean Six Sigma method is a structured process improvement approach for systematically reducing waste and improving process performance, which was applied to an existing mouse cage sanitation process. The project involved mapping the existing process, determining a baseline measurement of current process performance, analyzing potential waste within the process and implementing countermeasures to make the organization’s routine operations (mouse cage changes) flow smoothly and efficiently.

Top causes of waste and implemented countermeasures included:

1. Clean towers not available— at least one clean tower was available on clean side before sanitation of a new dirty tower of mouse cages

2. Technicians not available— daily task schedule was developed for all technicians

3. Work performed in multiple places within clean cage wash—visual controls were used to designate and organize workspace in cage wash areas

Countermeasures improved average sanitation time of a single, full mouse cage tower (100 cages) from 94 min to 59 min. The described Lean Six Sigma method used in this study may be useful to provide guidance regarding how to conduct similar process improvement efforts within research institutions.

QUESTIONS

1. According to the AWA, primary enclosures for guinea pigs shall be cleaned and sanitized at least once every \_\_\_\_\_\_.

2. According to the AWA, food and water receptacles for dogs must be sanitized at least once every \_\_\_\_\_\_.

3. According to the AWA, primary enclosures of rabbits with solid floors shall have soiled litter removed and replaced with clean litter at least once every \_\_\_\_\_\_. Primary rabbit enclosures equipped with mesh floors shall have the trough/pan cleaned at least every \_\_\_\_\_.

4. According to *The Guide*, enclosures and accessories generally should be sanitized at least every \_\_\_\_\_ and bottles/sipper tubes usually require sanitation at least once every \_\_\_\_\_\_.

ANSWERS

1. 2 weeks

2. 2 weeks

3. Both are at least once each week

4. 2 weeks, week

**Moody et al.** [**Evaluation of Peroxides and Chlorine Oxides as Disinfectants for Chemical Sterilization of Gnotobiotic Rodent Isolators**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00004)**, pp. 558-568**

Primary Species – Mouse (Mus musculus)

SUMMARY: The use of gnotobiotic animals has expanded greatly in recent years, especially as the drive to elucidate the relationship of the microbiome to physical and mental health, chronic disease states, and as a way to create individually tailored medications for cancer chemotherapeutics. Keeping these animals clean requires great effort, from specialized housing units to meticulous training of personnel to finding appropriate sterilants that kill opportunistic pathogens but are safe to use around animals. Contamination can lead to losses of valuable animals, time, personnel efforts, and research opportunities. This group tested six different products typically used in home, industry, and hospital settings as chemical disinfectants, representing chlorine oxide-based and peroxide-based disinfectants. Their ability to chemically sterilize gnotobiotic isolators was tested on bacteria that had previously contaminated isolators at their facility; the chemical disinfectants were also evaluated based on their corrosiveness to the metal and plastic parts of the isolators. Overall, chlorine oxide-based disinfectants were more effective and less corrosive compared to the peroxide-based products, but no one product met all the criteria for a first choice disinfectant. This study highlights the importance of balancing the characteristics of all available disinfectants when selecting one for use.

QUESTIONS

1. Define ‘gnotobiotic.’
2. Which of these is not included in Shaedler’s altered flora (ASF)?
   1. Lactobacilli ASF 360 and ASF 361
   2. Bacteroides ASF 519
   3. Mucispirillum schaedleri
   4. EOS fusiform bacteria
   5. All are included in ASF
3. Name this piece of equipment:



ANSWERS

1. An environment (in this case, a mouse) in which all of the microorganisms are either known or excluded.
2. E – all of the listed bacteria are included in ASF
3. Flexible film isolator

**Mitchell et al. Low-cost, Small-scale Decontamination of Laboratory Equipment by Using Chlorine Dioxide Gas, pp. 569-576**

Domain 4: Animal Care

SUMMARY:Gaseous decontamination has proven to be effective against a broad range of microorganisms. Chlorine dioxide is a potent oxidizer and disrupts bacterial cell walls, and damages viral capsid proteins and viral RNA. As a decontaminant, the effectiveness of ClO2gas is directly correlated to relative humidity and contact time. ClO2gas decontamination is typically performed using large generators, but can also be used to decontaminate biological safety cabinets and small research equipment. For small-scale applications, the use of large generators may not be practical. The goal of this study was to create and validate an effective, small-scale method of ClO2gas decontamination that is affordable, efficient, safe, and reproducible.

Four different types of household toes with gasket-seal lid systems were used as exposure chambers. ClO2gas was generated by using two powdered reagents that were combined, shaken, then placed into the tote. To passively generate humidity, tap water was poured onto a dry kitchen sponge and placed inside the tote. For monitoring, holes were drilled in the lid and side of each tote, where the temperature, humidity, and gas sampling probes and tubing were placed. Adhesive putty was used to create seals around the probes and tubing. Different types of small lab equipment were placed into the totes and two types of endospore biological indicators (*Bacillus atrophaeus* and *Geobacillus stearothermophilus*) were taped to the equipment in different places.

The authors determined that a minimal dose of 71+/- 42 ppm-h of ClO2 gas at greater than 90% relative humidity for 15 hours inactivates both *Bacillus atrophaeus*and *Geobacillus stearothermophilus.*Findings suggest that ClO2gas inside the tote can achieve the minimal required dose for inactivation of the biological indicators, but that placement of the lab equipment within the tote may only disinfect an area as compared with sterilize. The authors suggest using a rack to lift materials off the bottom of the tote and positioning items loosely away from the sides and other materials to improve gas exposure.

When compared to a ClO2 gas generator, where a constant gas concentration can be maintained within the exposure chamber, only a finite quantity of ClO2gas is produced in this method described by the authors. The concentration of ClO2gas decreased gradually. This may be due to consumption of gas by the lab equipment or because the gas is highly water-soluble, and the increased surface area from the lab equipment on which condensation could form at high humidity was increased.

The authors also mentioned the use of a fume hood is necessary with this method, because there is no scavenging system. They recommend opening the lid for a minimum of two minutes prior to retrieving contents, to allow gas exhaust and prevent direct exposure of personnel to high gas concentrations.

QUESTIONS

1. ClO2 can be an occupational health concern. Why?

2. The vast majority of known lab rodent pathogens and opportunists do not form endospores and do not demonstrate noteworthy resistance to disinfection. According to the authors, what are the exceptions?

ANSWERS

1. Exposure to ClO2gas at high concentrations can cause irritation to the eyes, nose, and throat and may result in bronchitis and pulmonary edema. Due to this, it is important to ensure adequate ventilation both in the immediate area and adjacent spaces.

2. *Clostridium piliform* and *Syphacia* spp. eggs. They required a ClO2gas dose of 1440 ppm-h at 52% to 67% relative humidity to achieve complete inactivation.

***Anesthesia***

**Allen and Kendall.** [**Immunomodulation Associated with Sustained-release Buprenorphine in Female CD1 Mice Challenged with Ovalbumin**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00006)**, pp. 577-582**

Primary Species: Mouse (*Mus Musculus)*

Domain 2

SUMMARY:Opioids that act on the mu receptor, such as morphine and fentanyl, have an immunosuppressive effect through binding to opioid receptors on macrophages and activating the HPA axis. They reduce T and B cells, decrease NK cell activity, reduce antibody responses, and more. The immune effects of buprenorphine has been widely studied with varying results. The current study looked at the immune effects of sustained release buprenorphine by evaluating splenocyte cytokine responses and antibody production in ovalbumin-primed mice treated with SR Bup, Bup Hcl, SR vehicle, and saline. The ovalbumin antigen was combined with Freund complete adjuvant for the first immunization and Freund incomplete adjuvant was used for the booster at day 18 of treatment.

Results: In all treatment groups, IL10, TNF-alpha, and IFN-gamma increased in ovalbumin-stimulated splenocytes vs control. IL10 was significantly higher in SR Bup treated mice vs the other groups (saline and Bup HCl). The antibody response increased after vaccination but did not differ across treatment groups.

Conclusion:The immune effects of SR Bup vs Bup HCl, SR vehicle, or saline on adaptive and innate immunity are negligible.

QUESTIONS

1. Which selection is true regarding Freund's complete (FCA) and incomplete adjuvant (FIA)?

a. FCA contains mycobacterium and should be injected intradermally for best effects

b. FCA contains mycobacterium, usually *M. tuberculosis*, and FIA does not

c. FCA contains an oil in water emulsion and FIA does not

d. FCA can be used repeatedly to booster an initial vaccination as needed

2. T/F: The HPA axis has immunomodulatory properties since it stimulates production of immunosuppressive glucocorticoids

ANSWERS

1. b

2. T

**Bratcher et al.** [**Effects of Buprenorphine in a Preclinical Orthotopic Tumor Model of Ovarian Carcinoma in Female CB17 SCID Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00007)**, pp. 583-588**

Primary Species: Mouse (Mus musculus)

Domain 2: Management of Pain and Distress

SUMMARY

Background: Development of successful anticancer drugs relies on work done on cells in vitro and on animals studies done in vivo. Orthotopic cancer models involve implanting tumor cells over the organ or system where the tumor naturally occurs and allows evaluation of the tumor interactions with the local environment as well as distant metastases. Tumor cell implantation generally requires laparotomy or incisions through skin and muscle layers for access to the target organ and animals may experience pain or hyperalgesia postoperatively. Evidence in the literature suggests that repeated doses of NSAIDs and/or opioids can affect tumor cell growth. This has led to the withholding of analgesics in these studies, which raises ethical concerns. Surgical pain, stress, and anesthesia are also clinically relevant factors that can influence immunosuppression, inflammation, and sympathetic stimulation and may impact tumor growth and progression. It is important to evaluate the effects of analgesics on tumor models, as withholding analgesia in lab animals must be scientifically justified and approved by the Institutional Animal Care and Use Committee. This study examined the effects of buprenorphine on tumor growth using the OVCAR5 OT LMC cell line in mice.

Materials and Methods:150 female CB17 SCID mice, aged 7-8 weeks old, were used in this study. All mice were randomly assigned to one of 3 treatment groups: 1) animals treated with control vehicle prior to surgery, 2) animals treated with buprenorphine 0.05 mg/kg SQ 30 min-1 h prior to surgery, or 3) animals treated with buprenorphine 0.05 mg/kg SQ 30 min-1 h prior to surgery and at 24h post-surgery. The surgical procedure was the same for all animals: under inhalation anesthesia an incision was created over the left renal area, the left ovary was exteriorized, 20uL (25,000 cells per uL) of tumor cell suspension was implanted in the ovarian bursa and the muscle and skin incision layers closed. Bioluminescence imaging (BLI) studies were conducted to determine tumor burden starting 1 week after surgery. For each group of 50 mice, the following parameters were assessed: tumor “take rate”, rate of tumor growth, and time to tumor endpoint. Endpoints for this study were defined as animals reaching 3 x 10^9 photons/s by using whole-body region of interest analysis, demonstrated distress, weight loss of 20%, and tumor size of 2-3g. Animals were evaluated daily for clinical signs of pain and distress.

Results: The tumor take rates for all experimental groups were 100%. Tumor growth rates increased over time for all groups and there was no difference in tumor growth rate between control and analgesia-treated groups. Unexpected deaths occurred 24-48 hours post-operatively in all groups, however only 1 mouse in the vehicle treated group died while 8 mice died in the two treatment groups (4 from each group). Due to these deaths, the investigators began administering the buprenorphine 30 min prior to surgery. There were no differences between groups in survival over the course of the model or beyond the 72-h postoperative period.

Discussion: Buprenorphine had no effect on tumor growth rate in this study, supporting the use of buprenorphine analgesia in this specific ovarian cancer model using the OVCAR5 OT LMT cell line. Endogenous opioids are expressed in tumor stroma and may exert a regulatory effect on adjacent tumor cells that express opioid receptors. It is unknown if exogenous opioids have an effect on tumor cells. The expression and function of the opioid receptor subtypes in the OVCAR5 OT LMC cell line are also unknown. There were 9 total deaths among 150 surgically implanted animals and although more of the deaths occurred in the buprenorphine-treated groups, statistical analysis revealed no significant difference in death among the 3 treatment groups. The investigator attributed these deaths to issues with hypothermia and has since instituted methods to help mice maintain body temperature during surgery. This study demonstrated that 1 or 2 doses of buprenorphine had no effect on tumor growth in a orthotopic ovarian cancer model in CB17 female mice, and provides more evidence that withholding of analgesics in orthotopic cancer models should be considered very carefully.

QUESTIONS

1. Which imaging modality is based on the sensitive detection of visible light produced during enzyme-mediated reactions in a living organism?
   1. PET
   2. MRI
   3. SPECT
   4. BLI
2. Which of the following statements reflects the view of the *Guide for the Care and Use of Laboratory Animals* with respect to pain and distress?
   1. The ability of animals to experience pain is limited to vertebrates.
   2. Pain is a stressor, and if not relieved, can lead to unacceptable levels of stress and distress in animals.
   3. Distress always induces an immediate and observable pathologic or behavioral alteration in animals.
   4. The proper use of analgesics in research animals is important but not an ethical or scientific imperative.
3. According to the American College of Laboratory Animal Medicine’s *Guidelines for the Assessment and Management of Pain in Rodents and Rabbits*, laparotomy is classified as:
   1. Causing moderate to severe pain
   2. Causing mild to moderate pain
   3. Causing minimal to mild pain
   4. Causing severe pain
4. Orthotopic transplantation is the transplant of tissue:
   1. from its normal anatomic site in the donor to its normal anatomic site in the recipient
   2. from one site to another on the same individual
   3. from its normal anatomic site in the donor to another anatomic site in the recipient
   4. between genetically dissimilar animals of the same species

ANSWERS

1. d
2. b
3. a
4. a

**Miller et al.** [**Pharmacokinetics and Safety of Intramuscular Meloxicam in Zebra Finches (*Taeniopygia guttata*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00008)**,** **pp. 589-593**

Domain 2: Management of Pain and Distress; T2 – Minimize or eliminate pain and/or distress

Tertiary Species: Other Birds

SUMMARY: Meloxicam is a commonly used NSAID in several bird species, but its use had not been characterized in zebra finches (or any passerine species). This study examined the maximal concentration and elimination half-life of meloxicam administered to healthy zebra finches using two doses: 1 mg/kg and 2 mg/kg. Another goal of this study was to determine the safety of IM meloxicam in zebra finches. To that end, the birds were injected every 12 hours for a total of 8 doses with either saline or meloxicam at 1 mg/kg or 2 mg/kg. Plasma was collected for biochemical analysis and complete necropsies were performed by a veterinary pathologist. Neither the maximal concentration nor the elimination half-life differed significantly between dose groups, however the maximal concentration of the 2 mg/kg group was higher compared to the 1 mg/kg group. The time above target concentration, 3500 ng/ml, was 9.5 hours at 2 mg/kg, and 5.5 hours at 1 mg/kg. There were no significant differences between the three groups (saline control, 1 mg/kg meloxicam and 2/mg/kg meloxicam) for Hct, biochemical analytes, or histopathology scores from pectoral muscle, kidney, or GI tissues. The authors conclude that IM meloxicam at 1-2 mg/kg is safe in zebra finches and that it should be dosed every 12 hours or more frequently. Further study investigating pharmacodynamics and efficacy is warranted.

QUESTIONS

1.   What are two adverse effects associated with administration of NSAIDs?

2.  Which bird(s) in this image would be considered the heteromorphic sex (ZW) based on phenotype alone?

3.   A small amount of blood may be collected from which location in a zebra finch?

a.  Left jugular vein

b. Right jugular vein

c.  Lateral saphenous vein

d.   Cranial vena cava

ANSWERS

1.  Renal toxicity, GI erosions/ulcers, bleeding disorders

2.   d. female; a. is a male, b. and c. are juveniles)

3. b

***Experimental Use***

**Kawano et al.** [**Sterility and Stability of Diluted Meloxicam in Compounded Multi-dose Vial after 365 Days**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00009)**, pp. 594-596**

Domain 2: Management of Pain and Distress

SUMMARY:Meloxicam is a commonly used NSAID in veterinary medicine. Since it is formulated for companion animals, it usually has to be diluted and the shelf life when diluted is unknown.  In this article meloxicam was diluted to a concentration of 0.5 mg/mL in 30 mL amber multi dose vials. The vials were punctured daily for 30 days. Sterility and stability of the compound was tested. There was no bacterial contamination in any vials at any of the 0, 10, 20, and 30 day time points tested. There was no significant difference of mean concentration of the drug after 30 days of puncturing and at 365 days of storage.  In conclusion, diluted meloxicam in a multi dose vial that is punctured daily for 30 days can remain stable and sterile for 365 days.

QUESTIONS

1. Which of the following NSAIDs is NOT a COX-2 Selective Inhibitor?
   1. Meloxicam
   2. Robenacoxib
   3. Flunixin
   4. Firocoxib
2. T/F: Inhibition of COX1 comes with several unwanted side effects and therefore COX2 inhibitors are preferred in rodents

ANSWERS

1. c

2.  True

**CASE REPORTS**

**Page et al. Lack of Absorption of a Sustained-release Buprenorphine Formulation Administered Subcutaneously to Athymic Nude Rats, pp. 597-600**

Primary Species: Rat (*Rattus norvegicus*)

Domain 2: Management of Pain and Distress

SUMMARY: Forty-five athymic nude rats were treated with 0.6 mg/kg (0.11ml/rat) subcutaneous buprenorphine SR LAB once in addition to 5 days of oral ibuprofen for analgesia following implantation with a triple negative type of breast cancer. Eight rats did not survive until study endpoint, but 37/45 presented at the date of euthanasia 3 months after implantation with subcutaneous nodules over the shoulders. These appeared to be cystic structures lined by fibrous connective tissue filled with pink, proteinaceous fluid and mildly infiltrated with lymphocytes, plasma cells, and macrophages. On chromatographic analysis, the cysts were found to contain buprenorphine. The authors postulate that cell-mediated (T cell) immunity is required to dissolve the polymer vehicle used for sustained-release buprenorphine. Based on the size and apparent demarcation of the cysts found to contain buprenorphine, it seems the buprenorphine was not released, though blood levels were not measured at or immediately following the time of treatment.

QUESTIONS

1. What portion of the immune system is thought to contribute to breakdown of the vehicle for buprenorphine SR?

1. B cell immunity
2. T cells
3. Complement
4. Macrophages

2. True or False: buprenorphine SR provides reliable analgesia in Sprague-Dawley rats.

1. True or False: buprenorphine provides reliable analgesia in immunodeficient rats.

ANSWERS

1. b

2. True

3. True

**Rodriguez et al.** [**Hydromorphone-induced Neurostimulation in a Yorkshire Swine (*Sus* *scrofa*) after Myocardial Infarction Surgery**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000005/art00011)**, pp. 601-605**

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

Primary Species: Pig (*Sus scrofa*)

SUMMARY: A 3-month-old castrated male Yorkshire-cross swine was used in a project where researchers aimed to develop an intervention treatment for myocardial infarction. In order to perform this experiment, the pig underwent a survival surgery to create a 20-25% free-wall infarction of the ventricular myocardium by ligating the left cranial descending artery. 30 min before the infarction, the pig was given amiodarone, lidocaine, magnesium sulfate, and calcium to reduce the likelihood of postinfarction arrhythmias. For analgesia during the immediate postoperative period, 100ug fentanyl patch, meloxicam (at time of induction), intracostal bupivacaine nerve block (surgical site before closure), and hydromorphone were given. After the surgery, the pig was monitored hourly for 5 hours and then every 3 to 4 hours through 23 hours postoperatively; after that, monitoring occurred at least 5 times daily.

During the first checkpoint, the pig showed decreased SpO2 at 92% (room air) which was attributed to pulmonary edema secondary to myocardial infarction and a single dose of furosemide was given. For approximately 48 hours postoperatively, the pig was reluctant to move and had mild proprioceptive deficits of the right pelvic limb which was attributed to the prolonged positioning intraoperatively. The pig also had intermittent bouts of predominantly ventricular arrhythmias that was treated with amiodarone (common with this model after myocardial infarction surgery). During an echocardiogram performed the day after surgery, scant pericardial and pleural effusion were noted, and the cardiac contractility was deemed adequate at this time.

Early on postoperative day 3, the pig was profoundly mentally depressed and unresponsive. An irregular cardiac rhythm was detected on auscultation and the SpO2 decreased to 92%. Because of these issues, euthanasia was suggested, and the pig was given a single dose of hydromorphone to ensure it was not in pain while they prepared for euthanasia. Surprisingly, less than an hour after the hydromorphone was given, the pig’s clinical signs improved considerably and the decision to euthanize was reversed. The pig was continued on a regimen of hydromorphone every 4 hours for the next 12 hours. The pig continued to experience pain and discomfort that was treated accordingly and on day 9 postoperatively, the pig was anesthetized for a terminal imaging procedure.

Hydromorphone is a semisynthetic morphine derivative that is active primarily at the μ and κ opioid receptors. This case shows that hydromorphone potentially can have a positive stimulatory effect in Yorkshire swine; the positive outcomes included an increase in normal porcine activity with no or minimal increases in pathologic activities. Based on the dramatic improvement in the pig’s mentation after hydromorphone administration, the researchers believed that hydromorphone induced neural stimulation (which was documented in other species) played an important role in this response. The mechanism for the stimulatory effect is unknown but may be associated with metabolism of hydromorphone to hydromorpone-3-gluconuride that can pass through the blood brain barrier and evoke excitatory behavior. However, because of the potential effects on respiratory function with opioids, it is important to monitor the animals and provide reversal (naloxone) if undesired side effects occur.

QUESTIONS

1. Which vein in the pig drains the intercostal vessels into the coronary sinus?

2. What are the receptors that hydromorphone acts primarily on?

3. What is the metabolite of hydromorphone that can cross the blood brain barrier and can elicit excitatory behavior?

ANSWERS

1. Left azygous (hemiazygous) vein

2. μ and κ (mu and kappa)

3. Hydromorphone-3-gluconuride