2014 OSU ASLAP Summer Student

Extern: Amanda Darbyshire, ASLAP Foundation Summer Fellowship

- Currently a student at The Ohio State University, Columbus OH
- Projected graduation 2016

Mentor: Dr Dondrae Coble, DVM, DACLAM, Director Experimental Surgery Core ULAR, Assistant Professor Clinical, Veterinary Preventive Medicine

Dr Coble is Clinical Veterinarian for OSU facilities and as the Director of the Experimental Surgery Core which provides clinical and didactic support for the Laboratory Animal Residency Program. He is a member of ASLAP and AALAS.

Research Project: “An Assessment of the Safety of Recuvyra following Topical Administration in Mice”
An Assessment of the Safety of Recuvyra following Topical Administration in Mice
Amanda Darbyshire
August 5, 2014

Objective
- Hypothesis: Topical administration of Recuvyra is safe for use in mice.
- Most currently used pain relievers require frequent dosing
  - Time commitment
  - Potentially stressful to postoperative mice
  - May allow for a drop in analgesic efficacy
- Recuvyra may allow multiple days of uninterrupted analgesia after one application

Current Analgesic Use in Mice
- Buprenorphine
  - Q12h SC
  - Partial mu agonist
  - Most common opioid used in mice
- Tramadol
  - Q24h SC
  - Weak mu opioid
- Meloxicam
  - Q24h SC or PO
  - NSAID, COX-2 inhibitor
  - May provide better analgesia than Buprenorphine

Current Analgesic Use in Mice
- Buprenorphine, tramadol, and various NSAIDs can be added to drinking water for continuous administration
  - Mice must consume water to receive analgesia
  - Weight loss and dehydration
- Buprenorphine in food?

What is Recuvyra?
- Transdermal solution of fentanyl (50mg/ml)
- Approved for control of postoperative pain in dogs
  - One application provides analgesia for 4 days
- 1000x more potent than injectable Fentanyl in humans!

Benefits of Recuvyra
- One application!
- No need for CRI, or multiple injections
- Provides prolonged analgesia
- Avoids first-pass metabolism in intestines and liver
Other Forms of Fentanyl

- Dermal Patch
  - Provides pain relief for 2–3 days
  - Affected by heat and blood flow rate
  - Cannot be accurately dosed for use in rodents

- Injectable
  - Short-acting
  - Used as a CRI or perioperative injection

Advantages of Recuvyra vs Patch

- Approved for use in dogs
- No need to clip hair
- No worries about adhesion to skin
- Not altered by temperature
- No need for re-administration
- No potential for abuse by clients

Administration

- Dosed at 2.7mg/kg 2–4 hours prior to surgery in dogs
- Applied topically to dorsal scapular area
- Wear gloves, eye protection, and a lab coat during application

Mechanism of Action

- Rapid dermal absorption and sequestration in stratum corneum
- Absorbed into the bloodstream through the stratum corneum
- Activates \( \mu \) (mu) opioid receptors

Metabolism and Excretion

- Cytochrome P450 metabolism
  - Biotransformation of other drugs via oxidation
- \( P \)-glycoprotein membrane efflux transporter
  - \( P \)-glycoprotein deficient mice may have prolonged and increased analgesic effect
- Excreted in feces and urine

Storage and Cost

- Supplied in 10ml vials (50mg/ml)
- \$300/kit
  - Includes Recuvyra, needleless adaptor, 15 3ml syringes, 15 applicator tips
- Store at 20–25°C (68–77°F) for 30 days once open
Safety
- Class II opioid
- Avoid contact with application site for 72 hours
- Antidote is naltrexone or naloxone
- Must take online training course prior to use
  - http://www.recuvyratraining.com

Side Effects in Dogs
- GI stasis
- Pronounced sedation
- Decreased food and water intake
- Hypothermia
- Bloody stool
- Diarrhea

Contraindications
- Do not apply to broken or diseased skin
- Do not administer to animals that are hypovolemic, debilitated, or suspected to have paralytic ileus
- Do not administer to animals that will experience mild to absent pain
- Do not administer a second dose for the same procedure
- Do not administer other opioids within 7 days

My Study

Administration of Recuvyra to Mice
- Male, C57BL/6 mice, 8 weeks of age
- Singly housed
- Administered Recuvyra to dorsal tail base with a pipette while anesthetized (5 minutes) with Isoflurane
Pilot Study
- 3 Male C57BL/6 mice
  - Low dose, (20mg/kg)
  - Middle dose, (200mg/kg)
  - High dose, (500mg/kg)
- Middle and high dose mice were euthanized the day after application due to adverse effects
- Doses were lowered for safety assessment study

Safety Assessment Study
- Groups (n=5)
  - Low dose, 2.5µl (5mg/kg)
  - Middle dose, 6.3µl (12.5mg/kg)
  - High dose, 10µl (20mg/kg)
  - Control, 2.5µl (saline)
- Mice were dosed on Day 1 and followed through Day 5

Parameters Assessed During Study
- Survival
- Body weight
  - Day 1–5
- Behavior and nociception
  - Nest Complexity Scoring
    - Day 1–5
  - Time-to-integrate-to-nest test (TINT)
    - Days 2–5
  - Open field test
    - Days 1–5
  - Tail-flick test
    - Days 1–5

Nest Complexity Scoring
- Purpose: Nest building is a sign of health and welfare in mice
- Assessment: Nests are scored 7–9 hours after Recuvyra application, and at 24, 48, 72, and 96 hour time points
  - By scoring initially at 7–9 hours post-application, nest scoring can be a short-term indicator of impairment

1
>90% of the Nestlet is intact

2
50–90% of the Nestlet remains intact
The Nestlet is mostly shredded but there is no identifiable nest: < 50% of the Nestlet remains intact

> 90% of the Nestlet is torn up into a flat nest with walls higher than mouse body height on less than 50% of its circumference

> 90% of the Nestlet is torn up, the nest is a crater, with walls higher than mouse body height on more than 50% of its circumference

Purpose: Mice should be motivated to quickly perform nesting behavior when given fresh nesting material
- Most mice integrate within 2 minutes
- Singly housed mice are more likely to test negative
- Mice must have a nest complexity score of 2 or greater to test

Assessment:
- Nest complexity is scored at 24, 48, 72, and 96 hour time points
- ¼ Nestlet is then added to each cage
- If Nestlet is moved from the original position within 10 minutes, the test is positive

Performing TINT
Purpose: Assesses emotionality in a novel environment
- Mice should eagerly explore a new environment and exhibit thigmotaxis
- Locomotor activity
- Anxiety behavior

Assessment: Mouse is placed in the center square of the chamber and allowed to explore for 5 minutes
- A video camera records data

Center square duration
- Exploration
- Anxiety

Rearing
- Locomotion
- Exploration
- Anxiety

Defecation
- Anxiety
- Emotionality
Purpose: Assesses nociception by application of radiant heat to the tail
- Measures the reflex latency of the withdrawal reaction
- Increased latency suggests analgesia

Assessment:
- The mouse is restrained and a point at the middle of the tail is marked
- The marked area is heated with the apparatus until the tail is withdrawn, or 15 seconds
- The latency is recorded to the nearest 0.1s
- The test is repeated in areas proximal and distal to the initial site with 5 minutes between each reading, and the latency times are averaged

Liver Values Increased
- ALT 303U/L (17–77U/L)
- AST 1296U/L (54–298U/L)
- ALP 114U/L (35–96U/L)

Phosphorus Increased
- 20.9mg/dL (5.7–9.2mg/dL)

One mouse in the high dose group was euthanized on Day 1 due to adverse effects
- Liver Values Increased
  - ALT 303U/L (17–77U/L)
  - AST 1296U/L (54–298U/L)
  - ALP 114U/L (35–96U/L)
- Phosphorus Increased
  - 20.9mg/dL (5.7–9.2mg/dL)

Four mice were euthanized on Day 4 due to tail necrosis
Six mice were euthanized on Day 5 after completion of the study due to tail necrosis
Mice Removed From Study

- Necrosis of Tail from Days 4 and 5

Body Weight

- Graph showing body weight over days with different doses (Control, Low, Middle, High).

Nest Complexity

- Graph showing nest complexity over days with different doses (Control, Low, Middle, High).

TINT

- 60% of the middle dose group had not built a nest on day 1.
- 25% of the high dose group had not built a nest on day 1.
- 20% of the middle dose group had not built a nest on day 2.
- These mice were not tested.

Tail-Flick

- Graph showing tail-flick times with different doses (Control, Low, Middle, High).
All mice survived dosing with Recuvra, except one from the high dose group. Mice initially lost weight, but regained it by the end of the study. Nest complexity decreased with dosing initially, and then returned to normal. Mice were less likely to integrate new nesting material after dosing. Mice initially had higher tail-flick latencies according to dose, which gradually decreased over 4 days. Mice showed less exploratory behavior initially.

**Limitations of Study**

- This study did not include a surgical pain model, however it is important because the clinical safety of opioids may be greater in the presence of pain.
  - Humans in severe pain may tolerate doses 3-4 times higher.
  - The adverse effects of opioids can be antagonized by pain.
- Number of mice
- Tail-flick assay

**Conclusions**

- The optimal dose of 12.5mg/kg is safe for use in mice, however mice are depressed initially.
- One application lasts multiple days.
- No break in analgesic efficacy.
- Less handling of post-operative mice.
- Convenient for staff.
What's Next?

- Repeat the study with more mice
- Recuvyra in mice using a surgical pain model to determine analgesic efficacy
- Pharmacokinetic fentanyl study
- Test blood for signs of organ toxicity

Acknowledgements

- Dr. Dondrae Coble
- Dr. Matthew Hogan
- Dr. Valerie Bergdall
- Dr. Hickman-Davis
- Dr. Popovich’s Lab
- Dr. Nelson’s Lab
- ULAR Staff–Psychology
- QA Staff

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Recuvyra Pamphlet


2014 OSU CASS Summer Student

Extern: Kelsey Cornelius Cass Summer Fellowship

• Currently a student at The Ohio State University, Columbus OH
• Projected graduation 2016

Mentor: Stephanie Lewis, DVM, MS, DACLAM, Director, Large Animal Quality Assurance and Clinical Medicine, Principle Investigator Training, ULAR, Associate Professor Clinical, Veterinary Preventive Medicine

Dr. Lewis is a Clinical Veterinarian for OSU facilities and as the Director of Large Animal Clinical Medicine provides clinical and didactic support for the Laboratory Animal Residency Program. She is the faculty advisor and student liaison for ASLAP, a member of AALAS and the OSU Institutional Biosafety Committee, and serves on the IACUC at OSU and the Veterinary Technician Institute at Bradford School, Columbus, Ohio.

Research Project: “Nest location preference in mice housed in individually ventilated and static caging systems”
Nest location preference in mice housed in individually ventilated and static caging systems
Kelsey C. Cornelius
Stephanie Lewis, DVM, MS, DACLAM
Tara Martin, DVM
Candace Hendrick

Agenda
• Background
• Materials & Methods
• Results
• Discussion
• Future Directions

Mice In Research
• Most commonly used lab animal model
• Many studies observe mice for changes in behavior
• Important to define normal behavior
• Ex of normal behavior: building nests

Why do Mice Build Nests?
• Help rear young, better breeding performance
• Less stress
• Less aggressive, less cannibalism
• Greater weight gain, Increase feed efficiency
• Eye irritation in hairless strains

Nest Side Effects
More uniform data!

Why do Mice Build Nests?
• We can evaluate nests
  – Behavioral, neuro studies
  – Pain or distress in mice
• How evaluate:
  – Present or absent
  – Walls
  – Similar to wild mice nests

How do Nests Help Researchers?
Factors Influence Nest Quality

• More recently….
  – Pain, Distress = WELFARE!
• But also!
  – Environment (macro and micro): housing (temperature, air flow), etc
  – Genetics
  – Sex
  – Age
  – Nest materials
  – Research side effects
  – Breeding, Pups

Temperature Influences Nests

Fig 1. Affect of Temperature on Nest Score (Gaskill 2013)

Temperature Influences Nests

Fig 2. Effects of different factors on thermoneutral zone (Gordon 1998)

Housing Influences Nests

• Little published data:
  – does cage ventilation affect nest complexity, nest location?
• Types of housing: Static caging (SC) or individually ventilated caging (IVC)
• Static caging relies on air flow reaching cage to provide ventilation

Housing Influences Nests

• IVCs: air flow enters cage through port, exits through filter lid
  – We control air changes per hour

Housing Influences Nests

• IVC Benefits
  – stocking density
  – contamination
  – cage Δ freq
  – humidity, CO₂, NH₃
  – Protection
  – Sterile

Housing Influences Nests

• IVC Cons
  – stress?
Housing Influences Nests

• Nests counteract negative effects of high intracage ventilation rates (Baumans 2001)
• Less stress at lower rates (30 vs 60 ACH)

Housing Influences Nests

• IVC more air changes/hr than static
• What does the Guide say about air changes/hour?
• Why do we keep IVCs at ~35 ACH?
  – At least 30 ACH to keep cage free of NH₃ (Reeb-Whitaker et al., 2001)

Housing Influences Nests: Our Hypothesis

• Mice in IVCs will build more complex nests at the front of the cage, furthest from air intake

Why do we care?

• Potential confounding effect in research
• Husbandry
• Determine comfort level in IVCs
• Baseline for behavioral, neurologic studies
  – Recognize deviations from normal behavior

The Nest Study

1. Comparison of IVC and static housing
   – BALB/c, C57BL/6 mice
   – 14 cages (n= 56)
   – Male, Female
2. Survey of IVC housed mice in BRT
   – 1769 cages (n = ~8500)
   – Male, Female, Breeding
**Laboratory Animal Care and Use Program and Facilities**

**Compare IVC and static housing**

- **IVCs**
  - Average ACH: 34 (20-40 range)

- **Static Caging**
  - Average ACH: 16-19 (Reeb et al. 1997)

**Data Collection**

- Nest complexity, location
- Enrichment Type
- Temperature
- Air changes per hour (ACH)

**Materials**

- Enviro-Gard™ Cage Monitor Control Unit
- iPad
- Books on Tape
- Nestlets (5 cm square)
- Thermometers

**Enrichment Options**
Nest Location

- Cage dimensions
  29.8 cm x 15.6 cm x 14.1 cm

Nest Scoring

- 0 = untouched
- 1 = dispersed
- 2 = flat
- 3 = cup
- 4 = half dome
- 5 = full dome
- IE = In Enrichment

Naturalistic nest scoring system from Hess et al., 2008
Laboratory Animal Care and Use Program and Facilities

4 = ½ dome

5 = full dome

IE = In Enrichment

Results

BRT Survey Results

The majority of mice cages surveyed in the BRT were located in the back third of the individually ventilated cage.

The majority of cages surveyed had nest scores of 2 (flat nests) or greater.

What about single housed mice?
The majority of nests in all 3 groups (males, females, or breeders) had a score of 2 (flat nests).

Bottles were the most common enrichment device found in the BRT.

~10% of cages with enrichment had nests inside the enrichment.

Mean nest scores were significantly higher for IVCs than static.

Better to use cage wires with less of a slope?
- Mice in IVCs build nests in back of cage
- More room for enrichment, higher nest
**Discussion**

- Provide alternative nesting material?
  - Average nest score all mice in BRT: 2.34
- Need baseline nesting data for different mice
  - Lots of variables!

**Ways to Improve Study**

- Allow mice to acclimate to static cages
- Nestlets in center of all cages
- Score all nests at same time: start of light phase next morning (6 am)
- Pictures of nests then blind scoring by 2 scorers
- Limit mouse disturbances

**Future Studies**

- Vary time of nest scoring
- Temperature monitoring
- Vary amount/type of nesting material
- Change feed hoppers
- Determine lighting effects

**Challenges**

**Questions?**
Special Thanks!

- Stephanie Lewis*
- Judy Hickman-Davis
- Tara Martin*
- Matt Hogan
- Candace Hendrick*
- Pete McKinley
- Charlie Martin
- Mike Rowley

*My study team

References