Comparison of Gel and Injection Delivered Carprofen for Post-Operative Pain Management in Mice

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INTRODUCTION

- Non-steroidal anti-inflammatory drugs remain a mainstay for the treatment of mild to moderate pain
  - This class of drugs is widely used in human, veterinary and laboratory animal applications to control post-surgical pain
- These drugs typically have short half-lives and require multiple administrations to maintain adequate analgesia
  - Labor and cost intensive
- Development of a gel formulated product is a viable approach for cost effectively delivering analgesics to rodents
  - Product can be opened and placed in the animals cage, allowing unrestricted access
  - The gel product can also provide the subjects hydration and nutritional needs
- Previous studies have shown that rodents will consume adequate amounts of hazelnut paste/Jell-O mixed with analgesics to relieve pain associated with laboratory procedures (Kalliokoski, et al. 2010)
- The overall aim of the current study was to use a proprietary MediGel™ product to deliver a commonly used NSAID (carprofen)

HYPOTHESIS AND SPECIFIC AIMS

- We hypothesize that the MediGel™ product will be a suitable carrier for delivering carprofen, thereby reducing pain behaviors in rodent models after a common surgical procedure (ovariectomy)
- The specific aims of our study were to:
  1. Induce a pain state (ovariectomy surgery) in rodents receiving control (vehicle) gels or gels that contain carprofen, and to compare antinociceptive effects to injectable carprofen
  2. Assess antinociceptive efficacy by determining abdominal tactile thresholds (von Frey assay) along with the assessment of locomotor activity and animal health (bodyweights and general observations)
  3. Conduct a pharmacokinetic (PK) analysis of mouse plasma samples at various time points post surgery from the gel and injection treated animals to compare the concentration of carprofen in the blood

METHODS

Subjects:
- Female C57BL/6J mice (Jackson Laboratories) weighing 15-25 g were used for all studies (3 mice per cage)
- Animals were housed in the University of New England Animal Care Facility under standard housing conditions with food and water available ad libitum with a 12 hr light:dark cycle (lights on 07:00)

Drug Treatments:
- Carprofen injection – 5 mg/kg, subcutaneous (s.c.) 30 min prior to surgery and every 24 hrs post surgery
- Carprofen gel – 5 mg/kg/day, 1-gel placed in each cage 24 hrs prior to surgery and replaced every 24 hrs

Ovariectomy Surgery:
- Mice were anesthetized with isoflurane and placed in ventral recumbency with the tail toward the surgeon
- A 70% EtOH wash was used to wet down the fur and a 1-1.5 cm dorsal midline incision was made caudal to the posterior border of the ribs
  - The fascia was cleared away using blunt dissection and underlying muscle wall was pierced on both sides 1 cm lateral to the spine to help locate the ovary, just beneath the muscle in the fat pad
- Forceps were then used to gently grasp the periovarian fat to lift and remove the ovary
- The uterine horn was then returned into the abdomen, and the process was repeated on the other side
  - The muscle incision was not sutured and the skin incision was closed using 9 mm wound clips

Abdominal von Frey Assay:
- Mice were placed in suspended plastic chambers with wire mesh flooring and allowed to habituate
- Mice were tested 5 times repeatedly, in ascending order, with the following filament sizes (0.16, 0.4, 1.0, 1.4, 2.0 and 4.0 grams)

Locomotor Assay:
- Locomotor chambers and TruScan software (Coulbourn Instruments) were used
- Activity was collected every 100 ms for a 30 min test session and averaged into 1-min bins

PK Evaluation:
- Blood was drawn via cardiac puncture and dispensed into EDTA treated microtainer tubes
- Blood samples were centrifuged for 10 min at 1200 rpm's
- Plasma was carefully pipetted off the top and placed in Eppendorf tubes and stored at -80° C

Analytical Analysis:
- UNE's Genomics, Analytics and Proteomics Core (GAPC) facility ran all analytical analysis
- Carprofen plasma concentrations were determined by an Agilent 1200 Series LC with an Agilent 6460 triple quadruple mass spectrometer with Jet Stream

EXPERIMENTAL DESIGN

- Separate groups of animals were used for behavioral testing and the blood draws/PK testing
- After a 5 day acclimation period to the animal facility, bodyweights and behavioral baselines (when applicable) were taken
- MediGel™ carprofen was given to the mice 24 hrs prior to ovariectomy and carprofen injection was administered 30 min prior to surgery and re-administered every 24 hrs to maintain adequate pain relief
- Behavioral testing occurred at baseline and then 24 and 72 hrs post surgery

Table 1. Treatment groups.

Group	Assay	Sample Size (3 mice/cage)
Distilled Water (s.c.)	Bodyweight, Abdominal von Frey and Locomotor Activity	9
Carprofen (5mg/kg, s.c.)		9
MediGel (Placebo)		9
MediGel carprofen (5 mg/kg/day)		9
MediGel carprofen (5 mg/kg/day)	PK Blood Draw (t = 3 hrs)	3
Carprofen (5 mg/kg, s.c.)		3
MediGel carprofen (5 mg/kg/day)	PK Blood Draw (t = 24 hrs)	3
Carprofen (5 mg/kg, s.c.)		3
MediGel carprofen (5 mg/kg/day)	PK Blood Draw (t = 72 hrs)	3
Carprofen (5 mg/kg, s.c.)		3

TACTILE THRESHOLDS

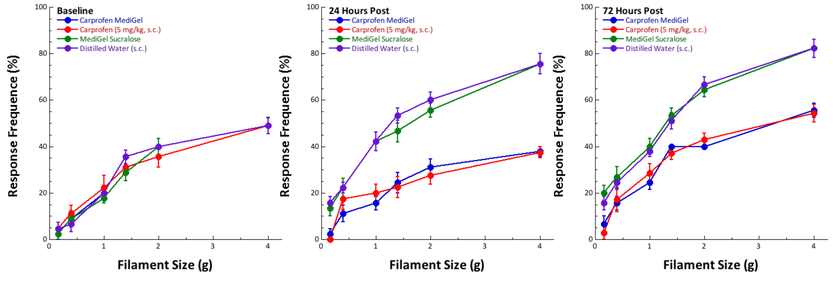

Figure 1. The figure depicts data from the mouse abdominal von Frey study. Mice were tested for tactile thresholds at various points pre (baseline–left) and post surgery (24 hrs-center and 72 hrs-right).

LOCOMOTOR ACTIVITY

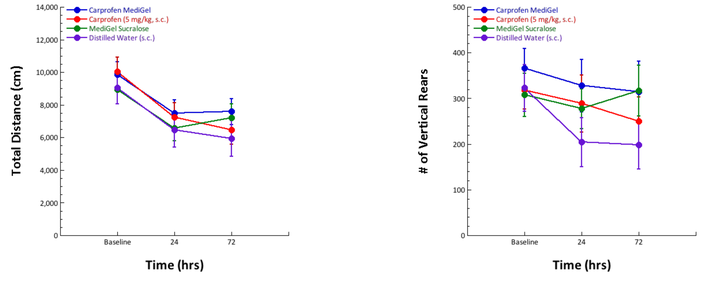

Figure 2. The figure depicts data from the locomotor assay. Mice were tested for the total distance traveled in 30 minutes (left) and the number of vertical plane entries (rears) on the right.

BODYWEIGHT ANALYSIS

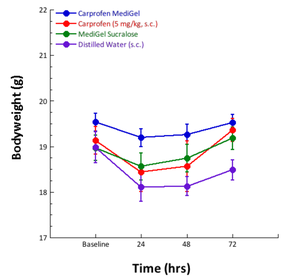

Figure 3. This figure assess bodyweights of the animals in the various treatment groups over the course of the experiment.

PHARMACOKINETIC ANAYSIS

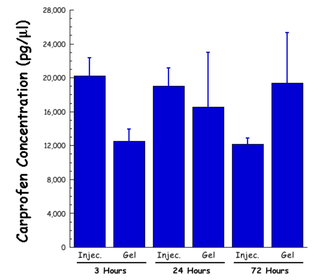

Figure 4. This figure shows data from the PK analysis of the concentration of carprofen that was detected in the blood at various time points post surgery.

DISCUSSION

- Treatment with either carprofen injections or gel formulation prevented the development of tactile allodynia following ovariectomy
  - The drug delivery appeared to have no effect on total distance traveled or vertical plane entries in the assessment of locomotor activity
- Bodyweight data was collected over the course of the experiment
  - The MediGel™ carprofen group did not lose as much weight as the other treatment groups following surgery
  - This could be attributed to starting the mice on the carprofen gel 24 hrs prior to surgery allowing for preemptive pain relief prior to and immediately following the ovariectomy surgery
- Pharmacokinetic (PK) analysis showed a constant level of carprofen in the blood in the MediGel™ carprofen group
  - The concentration of carprofen in the blood in the carprofen injection group appeared to decrease 72 hrs post surgery
- Collectively, the data support the use of MediGel™ carprofen as a post-operative analgesic for rodents
  - Provides equivalent efficacy compared to injectable carprofen and is more convenient to deliver

ACKNOWLEDGMENTS

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