



Efficacy Studies of Gel-Delivered Analgesics in Rodent Models of Pain



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INTRODUCTION

- Non-steroidal anti-inflammatory and opioid drugs remain a mainstay for the treatment of mild to severe pain
 - These drug classes are 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 proprietary MediGel™ products to deliver a NSAID (carprofen) or an opioid (buprenorphine)

HYPOTHESIS AND SPECIFIC AIMS

- We hypothesize that MediGel™ products will be suitable carriers for delivering carprofen and buprenorphine, thereby reducing pain behaviors in rodent models of inflammatory and post-surgical pain
- The specific aims of our study were to:
 - Estimate concentrations of analgesics in the gel product that will result in sufficient in vivo blood/tissue concentrations that reduce pain behaviors
 - Induce pain states (CFA paw inflammation or paw incision) in rodents receiving control (vehicle) gels or gels that contain carprofen or buprenorphine, and compare antinociceptive effects to conventional injections of these drugs
 - Assess antinociceptive efficacy by determining tactile thresholds (von Frey assay) and thermal latencies (Hargreaves test), along with paw volume and animal health (bodyweights and general observations)

METHODS

- Subjects:**
- Male ICR mice weighing 25-35 grams were used for Complete Freund's Adjuvant studies (group housed, 4 mice/cage)
 - Male SASCO Sprague Dawley rats (Charles River Laboratories) weighing 250-350 grams were used for all plantar incision studies (individually housed)
 - 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.) every 12 hrs
- Carprofen gel – 10 mg/kg dose dissolved in a sucralose MediGel™
- Buprenorphine injection – 0.05 mg/kg, s.c. every 12 hrs
- Buprenorphine gel – 0.6 mg/kg dose mixed into a hazelnut MediGel™

Complete Freund's Adjuvant (CFA):

- 1.paw injection of CFA was administered to all mice at a volume of 20 µl

Plantar Incision Surgery:

- Rats were anesthetized with isoflurane during the surgical procedure
- A 1 cm incision was made on the plantar surface of the left hind paw
- Once the plantaris muscle was located, a forceps was placed between the muscle and bone and a 1 cm slit was made

von Frey Assay:

- Animals were tested using the up down method (Dixon, et al. 1980), mice had a starting filament of 2.44 or 0.04 g, rats had a starting filament of 4.31 or 2 g

Thermal (Hargreaves) Assay:

- Animals were tested using a Plantar IR device (Ugo Basile), IR intensity ~ 28 (mouse) and ~ 40 (rats)

Paw Volume Assay:

- Animals were tested using a Plethysmometer (Ugo Basile)

EXPERIMENTAL DESIGN

- Animals were received into the animal facility and immediately randomized into groups listed below (Table 1)
- Baseline assessments were taken for all animals in the von Frey, thermal and paw volume assays (Day 7) and were followed by an injection or access to a gel delivered analgesic (Day 7 PM)
- An injection (mice) or surgical procedure (rats) was performed and all animals were re-tested at the following time points: 8, 24 and 48 hours post-injury (Days 8-10)
- Bodyweights and gel consumption were measured from arrival into the animal facility to completion of the study (7 AM and 5 PM daily)

Table 1. Treatment groups and experimental protocol by day.

Habituation Days 1-6	Baseline Day 7	Injection/Surgery Day 8 (t = 0 hrs)	Testing Days 8-10
Standard Food/Water	Standard Food/Water	Standard Food/Water	Standard Food/Water
Standard Food/Water	Standard Food/Water Injectable Analgesic PM	Injectable Analgesic	Injectable Analgesic
Control Gel	Control Gel	Control Gel	Control Gel
Control Gel	Control Gel Analgesic Gel PM	Analgesic Gel	Analgesic Gel

RESULTS

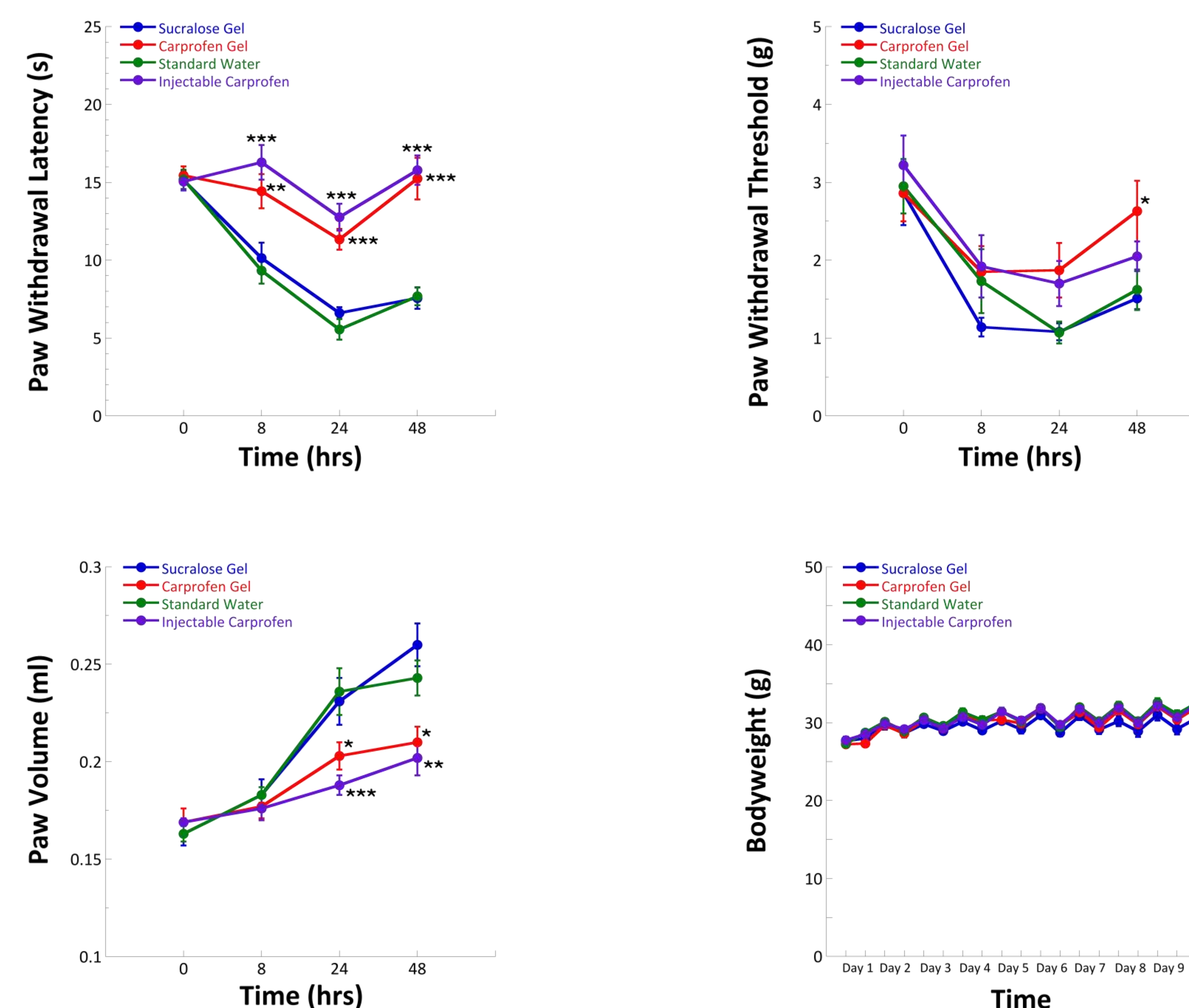


Figure 1. The figure depicts data from the mouse CFA study. Mice were tested for thermal latencies (top left panel), tactile thresholds (top right panel) and paw volumes (bottom left panel). Bodyweights were recorded twice daily for each group (bottom right panel).

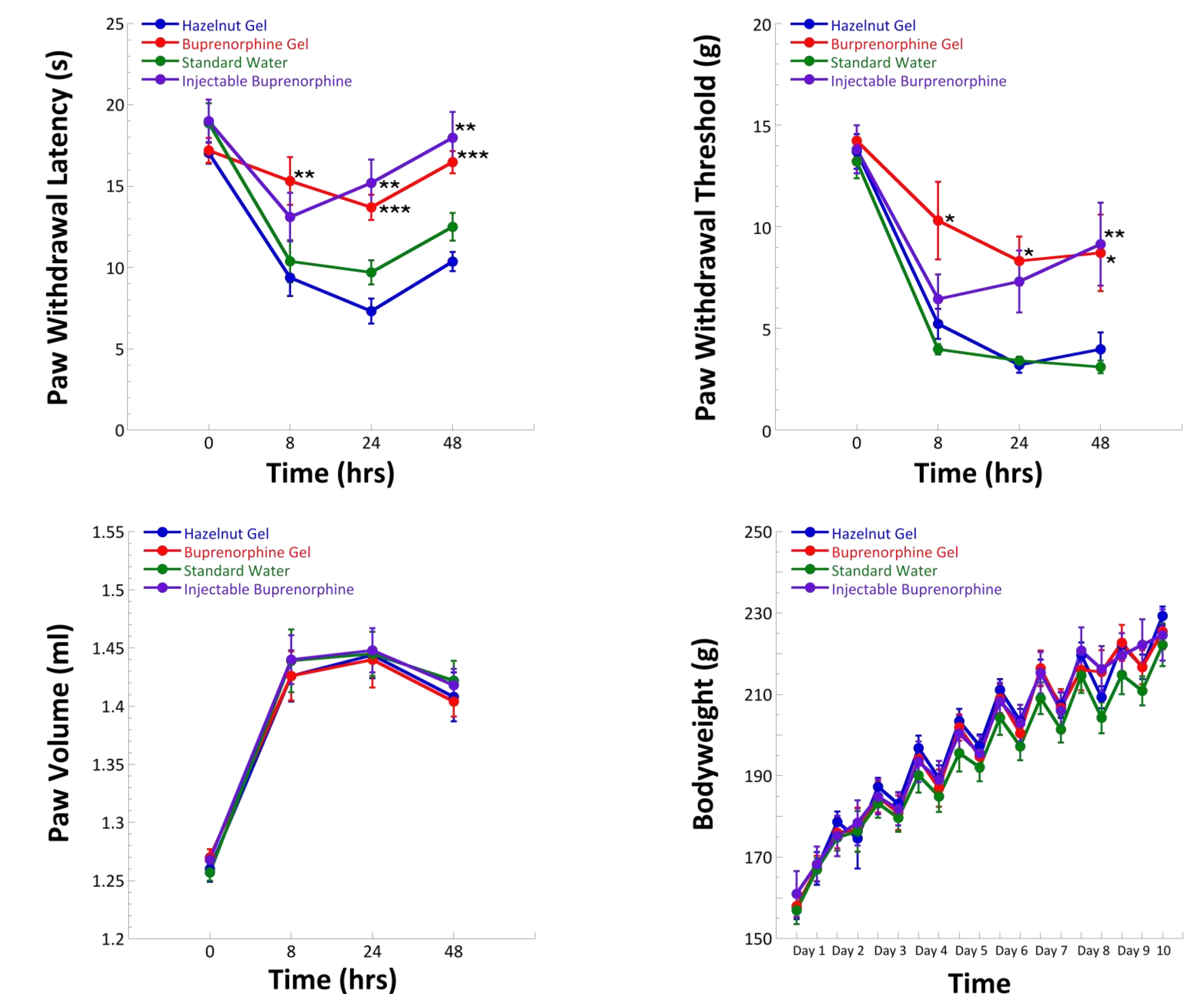


Figure 2. This figure depicts data from the rat plantar incision study. Rats were tested for thermal latencies (top left panel), tactile thresholds (top right panel) and paw volumes (bottom left panel). Bodyweights were recorded twice daily for each group (bottom right panel).

DISCUSSION

- Treatment with either carprofen injections or gel formulation prevented the development of thermal hyperalgesia associated with the CFA injection
 - The drug delivery also attenuated tactile allodynia and edema/increased paw volume associated with CFA at 24 and 48 hours post-injury
- Buprenorphine formulated in the hazelnut MediGel™ prevented the development of thermal hyperalgesia and tactile allodynia associated with the plantar incision surgery
- Injectable buprenorphine prevented the development of thermal hyperalgesia and showed a trend in reversal of tactile allodynia at 12 and 24 hrs post surgery with a significant reduction in allodynia 48 hrs post-injury
- The sucralose MediGel™ product was an effective drug delivery system that also provided adequate hydration for the group housed animals
 - Comparable level of antinociception compared to injections
 - Equivalent changes in bodyweights between gel and conventional water groups
- Hazelnut MediGel™ proved to be an effective way to deliver buprenorphine
 - There were no observed difference in bodyweight with the animals that received the hazelnut in addition to standard food and water
 - The flavoring presumably masked the bitter taste associated with buprenorphine

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