

Use of Flavored Tablets of Gabapentin and Carprofen to Attenuate Postoperative Hypersensitivity in an Incisional Pain Model in Rats (*Rattus norvegicus*)

Brian P Zude,^{1,*} Katechan Jampachaisri,² and Cholawat Pacharinsak¹

Providing postoperative analgesia to rats by oral administration, compared with injections, reduces stress from frequent handling and is technically easier for investigators. The purpose of this study was to investigate whether bacon-flavored tablets containing gabapentin, carprofen or a combination of both drugs effectively attenuates postoperative mechanical and thermal hypersensitivity in a rat model of incisional pain. Forty-eight Sprague–Dawley rats were randomly assigned to 1 of 5 treatment groups: placebo tablet; a single, subcutaneous injection of buprenorphine sustained release at 1.2 mg/kg; gabapentin 90 mg/tablet; carprofen 5 mg/tablet; gabapentin 90 mg and carprofen 5 mg/tablet (gabapentin/carprofen). Tablets were given to rats on days -3, -2, -1, 0 (surgery), 1, and 2. Rats were anesthetized using isoflurane. A 1 cm skin incision was made aseptically on the plantar surface of the left hindpaw and closed by using suture. Mechanical (von Frey monofilament) and thermal (Hargreaves method) hypersensitivity were tested daily, and analyzed on days -1, 1, 2, and 3. The amount of tablet consumed was recorded daily; postoperatively rats consumed 101 to 133 mg/kg of gabapentin, 5.5 to 5.8 mg/kg of carprofen, and 86-137/1.9-3 mg/kg of gabapentin/carprofen, respectively. Both the gabapentin and carprofen groups displayed attenuated mechanical hypersensitivity on all 3 postsurgical days and decreased thermal hypersensitivity on Day 3. The gabapentin/carprofen group showed attenuated mechanical hypersensitivity on Day 2 and 3, but no significant reduction of thermal hypersensitivity. These data suggest that both gabapentin and carprofen, given orally by flavored tablet, effectively attenuate postoperative mechanical hypersensitivity for 3 d after surgery in a rat model of incisional pain.

Abbreviations: BupSR, buprenorphine Sustained Release; gaba/car, gabapentin with carprofen

DOI: 10.30802/AALAS-JAALAS-19-000093

The management of pain in animal patients is a complicated and challenging field within veterinary medicine. In laboratory animal medicine, postoperative pain is a common management issue. Effective pain management includes preemptive administration of analgesics prior to surgery or other invasive procedures, and a multimodal approach to analgesia during or after the procedure. These interventions improve animal recovery, expedite return to normal behaviors, and better manage physiologic status.⁴² Buprenorphine HCL and buprenorphine Sustained Release (BupSR), both partial μ -opioid receptor agonists, are commonly used analgesic drugs, typically administered every 6 to 12h¹¹ and 2 to 3 d⁹ respectively, due to their prolonged plasma half-lives.¹⁴ Although prior studies have shown that buprenorphine is an appropriate choice for management of mild to moderate pain,^{12,29} in some instances, opioids may be contraindicated due to their impact on immunologic or behavioral studies.^{5,7,37} Gabapentin, a structural analog of GABA (γ -aminobutyric acid),²⁴ is typically used as an antiepileptic drug, but has been studied for its analgesic potential in neuropathic pain models²⁴ and for surgical and inflammatory pain,^{8,25} when administered every 4 to 12 h.⁴³ Carprofen, typically

administered every 12 to 24 h^{13,32} is a nonsteroidal antiinflammatory drug (NSAID) that preferentially inhibits cyclooxygenase 2 secondarily to prostaglandin synthesis.^{6,21} Generally considered safe, NSAID have been reported to cause side effects, including gastrointestinal ulceration, renal dysfunction, and alterations to platelet function.²¹ In laboratory animals, NSAID are typically used for their antiinflammatory and analgesic properties.^{3,21} The potential for nonopioid analgesic action, paired with the fact neither are currently federally controlled substances, makes gabapentin and carprofen attractive choices for analgesia, particularly in the laboratory animal setting.

Important limitations to consider with any postoperative pain management modality are ease of use, ease of administration, and frequency of dosing. Although an ideal regimen may include injectable dosing every few hours, research personnel cannot always provide this level of care to a multicage project through the long hours of the night. Even well-trained laboratory staff may struggle with rodent restraint and injection-based dosing. Handling increases stress, which can have systemic physiologic impacts,^{1,10,16} so exploring alternative dosing methods that minimize animal handling are advantageous to animal welfare. Therefore, delivering analgesics by using mixed-drug tablets was introduced. This type of analgesic self-administration of buprenorphine has been used effectively in rodents.²⁷ To provide adequate analgesia with nonopioid analgesics while minimizing stress from frequent handling, this study aimed to

Received: 01 Jul 2019. Revision requested: 29 Jul 2019. Accepted: 24 Sept 2019.

¹Department of Comparative Medicine, Stanford University, Stanford, California; ²Department of Mathematics, Naresuan University, Phitsanulok, Thailand

*Corresponding author. Email: bzude@stanford.edu

explore flavored tablet-based dosing of gabapentin, carprofen, and a combination of these drugs to minimize post-procedural pain in rats. We hypothesized that the non-opioid analgesia provided by gabapentin, carprofen or a combination in a flavored tablet would effectively attenuate hypersensitivity in an incisional pain model of rats.

Materials and Methods

Animals. Adult ($n = 48$; weight, 270 to 337 g) male Sprague-Dawley rats (*Rattus norvegicus*; Charles River, Wilmington, WA) were used for this study. Sentinel animals were free of Hantaan, Toolan H1, Kilham rat virus, Theiler virus, murine adenovirus types 1 and 2, pneumonia virus of mice, rat coronavirus, reovirus type 3, rat minute virus, rat parvovirus, Sendai virus, lymphocytic choriomeningitis virus, *Mycoplasma pulmonis*, and endo- and ectoparasites. Rats were singly housed in static microisolation cages (Allentown, Allentown, NJ) with ALPHA-dri paper bedding (Shepherd Specialty Papers, Milford, NJ). Rats were fed a commercial diet (Teklad Global 18% Protein Rodent Diet 2018, Harlan Laboratories, Madison, WI) during the entirety of the study. Ad libitum reverse osmosis purified water was accessible, and Fat Rat Huts (Bio-Serv no. K3365, Flemington, NJ) were available for enrichment. The housing room was on a 12:12h dark:light cycle with lights on at 7 AM, maintained at 70 to 74 °F (21 to 23 °C), and 30% to 70% relative humidity. Experimental procedures were conducted with approval by Stanford's Administrative Panel for Laboratory Animal Care. Stanford is an AAALAC International accredited facility, and all animals used were treated in accordance with the *Guide for the Care and Use of Laboratory Animals*.²⁰ All rats were weighed daily after completion of behavioral testing (at approximately 1100 h) from Day -3 to Day +3, and immediately prior to surgery, for a total of 7 d. At the study's conclusion, study rats were euthanized by carbon dioxide asphyxiation followed by bilateral thoracotomy as a secondary method of euthanasia.

Study design. In our experiment, rats were randomly assigned to groups in which they would receive 1 of 5 treatments: Group 1) placebo tablet ($n = 10$, Bio-Serv no. F05266, Flemington, NJ), Group 2) placebo tablet, and in addition dosed with buprenorphine Sustained Release (BupSR) at time of surgery ($n = 10$, 1.2 mg/kg subcutaneous buprenorphine SR-LAB, 1 mg/mL, ZooPharm, Windsor, CO), Group 3) gabapentin tablet ($n = 10$, 90 mg gabapentin, Bio-Serv no. F07617, Flemington, NJ), Group 4) carprofen tablet ($n = 10$, 2 mg Rimadyl, Bio-Serv no. MD 150 to 2, Flemington, NJ), or Group 5) gabapentin/carprofen (gaba/car) tablet ($n = 8$, 90 mg gabapentin and 2 mg Rimadyl, Bio-Serv no. F07618, Flemington, NJ). Dosages of each drug were tailored to achieve oral ingestion in amounts shown to produce effective analgesic levels in previous studies.^{8,13,14,18,23,31,33,38,44} In addition to their normal ad libitum chow pellets, bacon-flavored tablets (placebo or medicated) were provided daily during both the presurgical and postsurgical testing. Tablets were placed daily on enrichment surfaces (Fat Rat Huts), off the bedding, at approximately 1000 h every morning while behavioral testing or surgery was taking place. Any remnants of the previous day's tablet were removed, weighed and recorded when the new tablet was placed. Nine randomly selected rats covering all treatment groups were grossly necropsied after euthanasia, in consultation with a board-certified veterinary pathologist.

Surgery. Surgical plane of anesthesia was induced via 3% to 5% isoflurane in an induction chamber, followed by maintenance delivered by mask at 1% to 2% isoflurane and 100% oxygen. Sterile eye lubrication was applied, and rats were thermally supported via a circulating warm water blanket for

the duration of the surgery. A single dose of Cefazolin (20 mg/kg SC; GlaxoSmithKline, Research Triangle Park, NC) and warm 0.9% NaCl (10 mL/kg SC) were administered prior to the incision. The surgical procedure was performed as described previously.² In brief, each rat was placed in sternal recumbency, and the plantar surface of the left (ipsilateral) hindpaw was aseptically prepared and draped. Lack of withdrawal via toe pinch indicated surgical plane of anesthesia. Once achieved, a 1cm longitudinal full thickness skin incision was made on the plantar surface of the left hindpaw by use of a no. 15 blade, beginning approximately 0.5 cm from the tibiotarsus and extending 1 cm distally. Blunt dissection was used to identify the flexor digitorum brevis. It was then elevated, and incised longitudinally 0.5 cm through the muscle belly without disrupting the muscle attachments. Hemostasis was achieved as indicated by using gentle pressure of a sterile cotton applicator. The muscle was released, and the skin incision closed with a single interrupted horizontal mattress suture (5/0 polyglycolic Acid Suture, Henry Schein, Dublin, OH). Triple-antibiotic ointment (Taro Pharmaceuticals, Hawthorne, NY) was applied to the incision site. Rats were recovered in a heated cage and monitored continuously before being returned to their home cage.

Behavioral testing. Rats underwent behavioral testing of mechanical and thermal hypersensitivity for 3 d prior to surgery, from 0600 to 1000 h each day, and again daily for 3 d after surgery. Prior to each behavioral testing session, rats were given at least 15 min to acclimate in their testing environment before any testing would begin. Behavioral data from the first 2 d of testing (Day -3, Day -2) was not evaluated. The day prior to surgery (Day -1) was considered baseline. Von Frey testing was always performed prior to thermal testing, with an additional 15 min of acclimation in the thermal testing site prior to testing. All chambers were cleaned between usages, to minimize the stress associated with alterations in smell.

Response to mechanical stimuli. Rats were placed individually in a plastic chamber (20 × 12 × 8 cm) on an elevated wire-grid platform with 1 cm² perforations. A von Frey monofilament with a calibrated bending force (10 g, Aesthesio, DanMic Global, San Jose, CA) was tested to the plantar surface of both hindpaws perpendicularly for 10 trials. Each stimulus event was applied for 1 second, with a minimum of 2 seconds between tests to avoid injury or increased sensitivity, varying locations with each event, excluding the pads, toes, and heels. Withdrawal responses were measured as the number of times a rat lifted the paw completely off the grid due to stimulation. Mechanical hypersensitivity was defined as a significant increase in paw withdrawal frequency resulting from application of focal mechanical stimuli. Each rat's right (contralateral) hindpaw served as its control.

Response to thermal stimuli. Rats were placed individually and acclimated for 15 min in a plastic chamber (20 × 12 × 8 cm) on an elevated glass platform preheated to 30 °C. Radiant heat from a 50-W light bulb (Plantar Analgesia Meter, IITC Life Science, Woodland Hills, CA) was focused on the middle of the plantar surface of each hindpaw. 20 s was used as a maximum exposure time cutoff to prevent tissue injury. Each hindpaw was tested 4 times, as a testing group of 6 was rotated through, thus allowing at least 1 min cool down period between trials to minimize repeated-measures effects. Withdrawal latency was recorded as the mean of the last 3 trials. Thermal hypersensitivity was defined as a significant decrease in paw withdrawal latency resulting from the application of focal thermal stimuli. Each rat's right (contralateral) hindpaw served as its control.

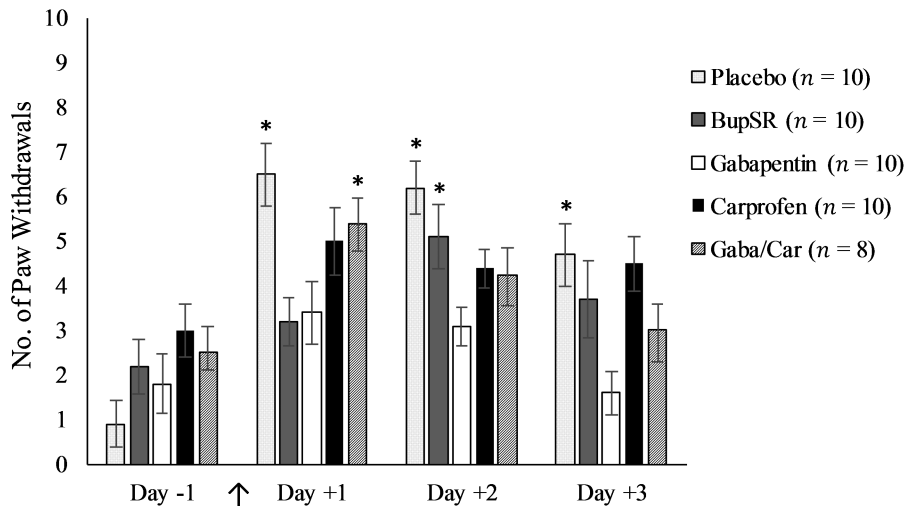


Figure 1. Mechanical hypersensitivity (no. of paw withdrawals; mean \pm SEM) of the ipsilateral hindpaw. \uparrow , Indicates the day of surgery (Day 0). *, Value is significantly (P less than 0.05) different from Day -1 (baseline) value for the same treatment group.

Tablet consumption. Remaining tablet remnants were weighed when they were replaced daily, allowing calculation of the amount of drug ingested by comparing to the tablets' baseline weight. Tablets were produced under strict SOPs and quality assurance testing by the manufacturer and were assumed homogenous for drug content, allowing for this calculation. In addition, comparing this value to the individual rats' daily weight measurement allowed for calculated mg/kg ingested. Group averages could then be compared.

Statistical analyses. Data sets were analyzed by using repeated-measures ANOVA with Bonferroni correction for multiple comparisons (R Development Core Team, 2015) to examine differences in withdrawal responses within groups over time. Datum was expressed as mean \pm SEM. A P value of less than 0.05 was considered significant.

Results

Responses to mechanical sensitivity tests. Mechanical sensitivity was not significantly different among groups before treatment (Day -1). For ipsilateral (surgical) hindpaws (Figure 1), the mechanical hypersensitivity of rats in the placebo group on Day +1, Day +2 and Day +3 was significantly higher than that on Day -1. The mechanical hypersensitivity of rats in the BupSR group was not significantly higher on Day +1 and Day +3 but was higher on Day +2 when compared with Day -1. The mechanical hypersensitivity of rats in either of the gabapentin or carprofen groups on Day +1, Day +2 and Day +3 was not significantly higher than that on Day -1. For the gaba/car group, the mechanical hypersensitivity of rats was not significantly higher on Day +2 and Day +3, but was higher on Day +1 than Day -1. In the contralateral hindpaws, mechanical hypersensitivity of rats did not differ between time points in all treatment groups (data sets not shown).

Responses to thermal sensitivity tests. Thermal sensitivity was not significantly different among groups before treatment (Day -1). For ipsilateral hindpaws (Figure 2), the thermal hypersensitivity of rats in the placebo group on Day +1, Day +2 and Day +3 was significantly lower than that on Day -1. The thermal hypersensitivity of rats in the BupSR group was significantly lower on Day +1, Day +2, and Day +3 compared with Day -1. The thermal hypersensitivity of rats in both the gabapentin and the carprofen groups on Day +1 and Day +2,

but not Day +3, was significantly lower than that on Day -1. For the gaba/car group, the thermal hypersensitivity of rats on Day +1, Day +2, and Day +3 was significantly lower than Day -1. In the contralateral hindpaws, the thermal hypersensitivity of rats did not differ between time points in all treatment groups (data sets not shown).

Tablet consumption. The average tablet consumption is shown (Table 1 and 2). The average tablet consumption for the placebo group was 90%. The average of tablet consumption for the other groups are: gabapentin 48%, carprofen 91%, gaba/car 42%. Ingestion of gabapentin ranged from 101 to 161 mg/kg, carprofen ranged from 5.5 to 5.8 mg/kg, and gaba/car 86 to 138/1.9 to 3.1 mg/kg respectively.

Body weight. The weights of all rats in all treatment groups increased from the baseline day (Day -1) through the last day (Day +3) of the experiment, confirming expected growth over the duration of the experiment (data not shown). The gains were not statistically different between groups.

Necropsy. At the completion of the study, 9 rats were grossly necropsied (2 rats from each study group, 1 control) in consultation with a boarded veterinary pathologist. No gross lesions or overt gastric ulceration was observed in any animal.

Discussion

This study demonstrates that a formulated flavored tablet is a viable option when considering alternative delivery systems to administer pain-relieving medications to rats subjected to an incisional injury. Both thermal and mechanical hypersensitivity were significantly different after surgery in the placebo group, indicating the presence of pain. Our data show that a significant reduction in mechanical hypersensitivity can be achieved by providing gabapentin or carprofen via the oral route.

Currently, BupSR is the standard of care for postoperative analgesia in laboratory rodents because it has been highly tested with consistent results for attenuating mild to moderate pain with a relatively long duration of action (2 to 3 d) after a single injection.^{14,33} However administration requires hands-on injectable dosing of a federally controlled opioid,³⁷ thereby increasing potential animal stress, requiring higher trained individuals to administer, and increasing the potential to affect immunologic or behavioral results and potentially impacting research outcomes.^{5,7,37} Although injectable BupSR maintained

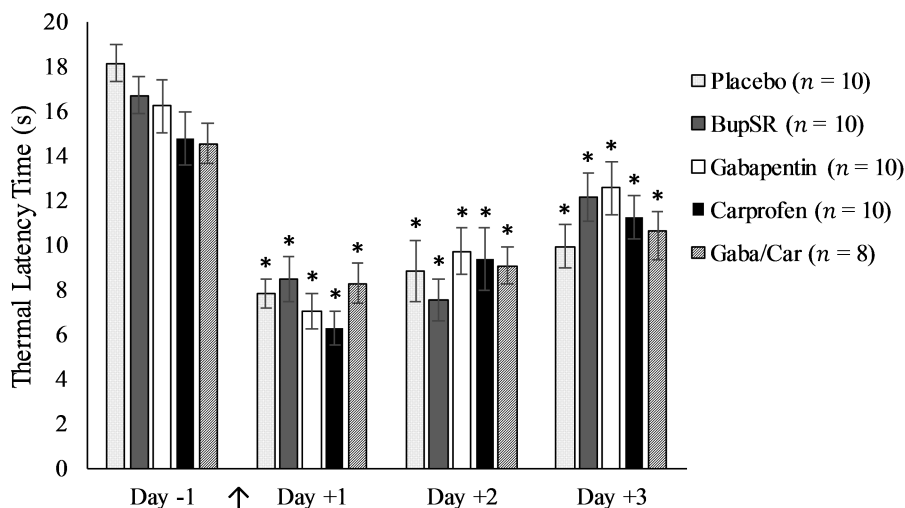


Figure 2. Thermal hypersensitivity (measured as latency [s] to withdrawal; mean \pm SEM) of the ipsilateral hindpaw. \uparrow , Indicates the day of surgery (Day 0). *, Value is significantly (P less than 0.05) different from Day -1 (baseline) value for the same treatment group.

Table 1. Daily percentage of tablet ingested.

	Day -3	Day -2	Day -1	Surgery Day	Day +1	Day +2	Average
Placebo ($n = 10$)	90.0%	90.0%	90.0%	90.0%	90.0%	90.0%	90.0%
BupSR ($n = 10$)	78.4%	76.2%	79.3%	57.9%	84.1%	85.3%	76.9%
Gabapentin ($n = 10$)	47.8%	47.2%	58.0%	37.9%	47.1%	51.7%	48.3%
Carprofen ($n = 10$)	90.3%	90.7%	90.0%	91.6%	94.0%	91.5%	91.4%
Gaba/Car ($n = 8$)	40.9%	48.2%	43.0%	31.8%	38.7%	51.2%	42.3%

Data are shown as average ingestion of the presented flavored tablet, over an approximately 24h period by each treatment group.

the highest degree of attenuation of mechanical hypersensitivity in our study, it failed to mitigate hypersensitivity on Day +2 post surgery. This could be due to ineffective slow-release functionality, or hypersensitivity due to systemic opioid rebound.^{11,17,28,39} Buprenorphine HCL (0.1 mg/kg SQ once) has been reported to be less effective in pain relief in later recovery periods.¹¹ Although in our previous studies, BupSR attenuated both mechanical and thermal hypersensitivity for 4 d,⁹ BupSR did not attenuate thermal hypersensitivity in this current study. This may be explained by studies showing buprenorphine can have a variable effect.³⁰ In a plantar incision model, doses of morphine (a full μ -agonist) needed to attenuate mechanical hypersensitivity (1.5 mg/kg SC) was lower than that in thermal hypersensitivity (1.8 mg/kg SC).⁴⁵ Similarly, the dose of BupSR required to attenuate thermal hypersensitivity may be higher than that required to attenuate mechanical hypersensitivity, accounting for the lack of thermal hypersensitivity attenuation we observed.

In this current study, gabapentin effectively attenuated mechanical, but not thermal, hypersensitivity for all 3 d at doses of 101 to 161 mg/kg from Day -1 to Day +2, slightly above the oral doses of gabapentin (100mg/kg)^{8,18,31,38} that were previously found to be effective. Gabapentin has been reported to have a strong effect on reducing mechanical hypersensitivity, yet variable effects on treating thermal hypersensitivity^{8,18} which is consistent with the findings of this current study. Our study of carprofen tablets also attenuated mechanical, but not thermal, hypersensitivity for 3 d which is consistent with previous data, in which carprofen in gel preparation attenuated mechanical hypersensitivity for at least 2 d in the same model in rats, but did not attenuate thermal hypersensitivity during the postopera-

tive period.³³ The mechanisms of thermal hypersensitivity may differ from those of mechanical hypersensitivity, and therefore doses required for each hypersensitivity differ. Carprofen (at 5 mg/kg SC) is a promising NSAID because it effectively provides postoperative analgesia in the laparotomy model in rats.^{13,23,44} Although plasma concentration was not measured in our study, the plasma concentration in rats was reported to be at least 16 μ g/mL and was maintained for 24 h after discontinuation of oral carprofen (approximately 4.5 mg/kg).³³ In this current study, an effective dose of carprofen tablet was 5.5 to 5.8 mg/kg from Day -1 to Day +2, which is consistent with oral doses of carprofen (5mg/kg)^{13,23,44} that were previously found to be effective. Other NSAID (flunixin, celecoxib, etoricoxib and indomethacin) have previously shown to reduce mechanical hypersensitivity in the rat incisional pain model^{35,40,41} and future studies of these drugs in tablet formulations could prove valuable.

To study multimodal analgesia, we investigated the combination of gabapentin and carprofen. In an earlier study that combined gabapentin with tramadol, the combination did not attenuate thermal hypersensitivity.²⁵ This is again consistent with our current data, which show that when gabapentin was combined with carprofen, although not in the same drug class as tramadol, the combination did not attenuate thermal hypersensitivity. By combining these medications, the authors expected to see profound attenuation of mechanical hypersensitivities.¹⁸ However, the gaba/car combination did not attenuate mechanical hypersensitivity on Day +1, only on Days +2 and Day +3. This may be explained by studies which show that anesthesia and surgery reduce food and water consumption.¹³ This was supported in our current study, which observed an overall slight decrease in tablet consumption the day after surgery. However, a

Table 2. Drug ingestion presented as mg/kg.

	Day -3	Day -2	Day -1	Surgery day	Day +1	Day +2	Average
Gabapentin (<i>n</i> = 10)	137.1	132.7	160.8	101.4	124.7	133.1	131.6
Carprofen (<i>n</i> = 10)	5.9	5.8	5.6	5.7	5.8	5.5	5.7
Gaba/Car (<i>n</i> = 8)	119.1/2.7	138.1/3.1	119.3/2.7	86.0/1.9	104.4/2.3	136.7/3.0	117.3/2.6

Data are shown as mg/kg of ingested medication, taking into account individual percentage of tablet ingested, against individual rats' daily weight.

substantially lower amount of the gaba/car tablet was ingested the day after surgery, falling from 43% to 32% (Table 1) and lowering drug intake (Table 2). Given the dose ingested the day after surgery was on average lower than historically effective dosages (gabapentin 100 mg/kg^{8,18,31,38} and carprofen 5 mg/kg^{13,23,44}), the ingested dosages may have been insufficient to attenuate mechanical hypersensitivity immediately after surgery on Day +1 when subjects were expected to be most sensitive.² Similarly, the combination of 2 medications may have decreased the overall palatability of the tablet.³¹ To our knowledge, this is the first study involving gaba/car in a flavored tablet. Our attempts to provide an adequately flavored tablet appear successful for the individual compounds, even though the combination of medications was not palatable enough to overcome the appetite suppressing effects of anesthesia and surgery. In the case of gaba/car, we plan to study different drug per tablet ratios, along with alternative tablet flavors, in the future.

As for limitations within our project, while we recognize that previous studies have shown no effect to measured baseline while preloaded with carprofen or gabapentin,^{4,15,33} we want to acknowledge that the carprofen, gabapentin, and gaba/car groups' baseline measure was taken after 2 d of oral preloading. The baselines for each of the various cohorts were not statistically different on Day -1. Also, 2 rats were excluded from the study; the first due to inappetence of both tablet and commercial chow, resulting in weight loss prior to surgery, the second due to lack of any sensitivity response during the 3 d acclimation period, which is abnormal for this model.³⁴ These 2 rats were from different treatment group assignments, thus we were not concerned that these exclusions would have any correlation to any particular drug group. Lastly, because our focus was on clinical responses, pharmacokinetics datum was not analyzed in the current study and is a potential area of future study.

When providing self-selective or "ad libitum" dosing of medications, numerous factors must be considered. These factors include the need to singly house animals while dosing to assure adequate tablet ingestion, and to prevent over or under ingestion by target animal and cage mates. In addition, providing a daily tablet sets the maximum dosage allowable, assuming no adjunct medication by another route is being provided. As ingestion of the medicated tablet is voluntary, under medicating is a possibility if an animal does not ingest the entire tablet. Standard chow was never removed or restricted during the study, as we wanted to test the tablets without the added factor of food restriction. In our study, the rats showed some variation in the amount of tablet ingested. We anticipated the addition of medications might alter the flavor of the tablet and thus collected and weighed any remaining tablets when a new daily tablet was added. The combination of gabapentin with carprofen (gaba/car) decreased the average amount of tablet ingested, particularly on Day +1; however on average the ingestion rates were comparable to standard dosing of our target medications.^{8,13,18,23,44} Furthermore, rats received medicated flavored tablets for 3 d prior to surgery both to preload and to decrease neophobia. Neophobia was not a major concern in this current study as, in congruence with our inhouse testing

and Bio-Serv's recommendations, it appeared the rats liked the bacon flavor. In case they had rejected the tablet, we tried chocolate flavor on a separate cohort of rats, and they appear to have no adverse reaction to either. Recently peanut butter has successfully been used with training to overcome concerns with palatability, providing yet another possible oral dosing method and flavor.¹⁹ Others have had success with buprenorphine delivery via gelatin.²² Tablets should be monitored daily to assure rats ingest them, an obvious important requirement to their dosing. Bioavailability from human literature indicates an inversely dependent dose curve for oral gabapentin,²⁶ with approximately 60% bioavailability for a 300 mg/kg dose³⁸ that declines to 40% for a 600 mg/kg oral dose.³⁶ Monitoring ingestion before and after surgery is essential to assure that adequate dosing has been achieved. If appropriate dosing has not been reached, alternative dosing routes should be used to achieve the necessary therapeutic levels.

The approximate cost of using these flavored tablets is similar to BupSR injection based on a 300g rat. Administering BupSR (1.2 mg/kg once) was \$7.45 per rat, with gabapentin tablets (1 tab/day for 6 d) at \$16.20, carprofen tablets (1 tab/day for 6 d) at \$3.50, and gaba/car tablets (1 tab/day for 6 d) at \$16.20 per rat. Given the gabapentin and gaba/car tablets were specially formulated for this project, we expect the price to drop if general application and demand is present. Although using the oral route reduces the amount of handling and personal skill required, it must be monitored closely at all times and used consistently to maintain appropriate pain management and improved animal welfare. Although not done in this study, multimodal analgesia with a combination of injectables (during anesthesia) can also be used.

The primary goal of our project was to expand available options for postoperative analgesia in rats, with the goal of attenuating pain in a way that would reduce animal stress by increasing ease of administration. This study successfully demonstrates that the use of nonopioid analgesics in the form of flavored gabapentin or carprofen tablets, voluntarily consumed, effectively attenuated mechanical hypersensitivity for 3 d in a rat incisional pain model, providing a refinement by reducing animal stress from frequent handling and thereby improving animal welfare.

Acknowledgments

We thank Mike Alvarez for helping us secure a behavioral testing room, Mikhail Klukinov for his assistance in the experimental set-up, Marlon Pailano for his support and extra care of our rats, and Rhondra Zude for her assistance in formatting and editing the manuscript. This study was supported by the Department of Comparative Medicine, Stanford School of Medicine.

References

1. Balcombe JP, Barnard ND, Sandusky C. 2004. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci* 43:42–51.
2. Brennan TJ, Vandermeulen EP, Gebhart GF. 1996. Characterization of a rat model of incisional pain. *Pain* 64:493–502. [https://doi.org/10.1016/0304-3959\(95\)01441-1](https://doi.org/10.1016/0304-3959(95)01441-1).

3. **Buvanendran A, Kroin JS.** 2009. Multimodal analgesia for controlling acute postoperative pain. *Curr Opin Anaesthesiol* **22**:588–593. <https://doi.org/10.1097/ACO.0b013e328330373a>.
4. **Castel A, Vachon P.** 2013. Gabapentin reverses central pain sensitization following a collagenase-induced intrathalamic hemorrhage in rats. *J Pain Res* **7**:5–12.
5. **Chandran P, Pai M, Blomme EA, Hsieh GC, Decker MW, Honore P.** 2009. Pharmacological modulation of movement-evoked pain in a rat model of osteoarthritis. *Eur J Pharmacol* **613**:39–45. <https://doi.org/10.1016/j.ejphar.2009.04.009>.
6. **Chen YL, Law PY, Loh HH.** 2006. Nuclear factor κ B signaling in opioid functions and receptor gene expression. *J Neuroimmune Pharmacol* **1**:270–279. <https://doi.org/10.1007/s11481-006-9028-0>.
7. **Cheppudira BP.** 2006. Characterization of hind paw licking and lifting to noxious radiant heat in the rat with and without chronic inflammation. *J Neurosci Methods* **155**:122–125. <https://doi.org/10.1016/j.jneumeth.2006.01.001>.
8. **Chogtu B, Bairy KL, Smitha D, Dhar S, Himabindu P.** 2011. Comparison of the efficacy of carbamazepine, gabapentin and lamotrigine for neuropathic pain in rats. *Indian J Pharmacol* **43**:596–598. <https://doi.org/10.4103/0253-7613.84980>.
9. **Chum HH, Jampachaisri K, McKeon GP, Yeomans DC, Pacharinsak C, Felt SA.** 2014. Antinociceptive effects of sustained-release buprenorphine in a model of incisional pain in rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci* **53**:193–197.
10. **Cloutier S, Wahl KL, Panksepp J, Newberry RC.** 2015. Playful handling of laboratory rats is more beneficial when applied before than after routine injections. *Appl Anim Behav Sci* **164**:81–90. <https://doi.org/10.1016/j.applanim.2014.12.012>.
11. **Curtin LJ, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LBE, Kristal MB.** 2009. Evaluation of buprenorphine in a postoperative pain model in rats. *Comp Med* **59**:60–71.
12. **Dobkin AB.** 1977. Buprenorphine hydrochloride: determination of analgesic potency. *Can Anaesth Soc J* **24**:186–193. <https://doi.org/10.1007/BF03006231>.
13. **Flecknell PA, Orr HE, Roughan JV, Stewart R.** 1999. Comparison of the effects of oral or subcutaneous carprofen or ketoprofen in rats undergoing laparotomy. *Vet Rec* **144**:65–67. <https://doi.org/10.1136/vr.144.3.65>.
14. **Foley PL, Liang H, Crichlow AR.** 2011. Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. *J Am Assoc Lab Anim Sci* **50**:198–204.
15. **Folkesson A, Honoré PH, Bjerrum OJ.** 2010. Co-administered gabapentin and venlafaxine in nerve injured rats: Effect on mechanical hypersensitivity, motor function and pharmacokinetics. *Scand J Pain* **1**:91–97. <https://doi.org/10.1016/j.sjpain.2009.12.001>.
16. **Gärtner K, Büttner D, Döhler K, Friedel R, Lindena J, Trautschold I.** 1980. Stress response of rats to handling and experimental procedures. *Lab Anim* **14**:267–274. <https://doi.org/10.1258/002367780780937454>.
17. **Gerhold KJ, Drdla-Schutting R, Honsek SD, Forsthuber L, Sandkühler J.** 2015. Pronociceptive and antinociceptive effects of buprenorphine in the spinal cord dorsal horn cover a dose range of four orders of magnitude. *J Neurosci* **35**:9580–9594. <https://doi.org/10.1523/JNEUROSCI.0731-14.2015>.
18. **Hayashida K, Eisenach JC.** 2008. Multiplicative interactions to enhance gabapentin to treat neuropathic pain. *Eur J Pharmacol* **598**:21–26. <https://doi.org/10.1016/j.ejphar.2008.09.004>.
19. **Hocking AJ, Elliot D, Hua J, Klebe S.** 2018. Administering fixed oral doses of curcumin to rats through voluntary consumption. *J Am Assoc Lab Anim Sci* **57**:508–512. <https://doi.org/10.30802/AALAS-JAALAS-17-000143>.
20. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
21. **Kehlet H, Holte K.** 2001. Effect of postoperative analgesia on surgical outcome. *Br J Anaesth* **87**:62–72. <https://doi.org/10.1093/bja/87.1.62>.
22. **Leach MC, Forrester AR, Flecknell PA.** 2010. Influence of preferred foodstuffs on the antinociceptive effects of orally administered buprenorphine in laboratory rats. *Lab Anim* **44**:54–58. <https://doi.org/10.1258/la.2009.009029>.
23. **Liles JH, Flecknell PA.** 1994. A comparison of the effects of buprenorphine, carprofen and flunixin following laparotomy in rats. *J Vet Pharmacol Ther* **17**:284–290. <https://doi.org/10.1111/j.1365-2885.1994.tb00247.x>.
24. **Mao J, Chen LL.** 2000. Gabapentin in pain management. *Anesth Analg* **91**:680–687. <https://doi.org/10.1213/00005539-200009000-00034>.
25. **McKeon GP, Pacharinsak C, Long CT, Howard AM, Jampachaisri K, Yeomans DC, Felt SA.** 2011. Analgesic effects of tramadol, tramadol-gabapentin, and buprenorphine in an incisional model of pain in rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci* **50**:192–197.
26. **McLean MJ.** 1994. Clinical pharmacokinetics of gabapentin. *Neurology* **44**:S17–S22.
27. **Molina-Cimadevila MJ, Segura S, Merino C, Ruiz-Reig N, Andrés B, de Madaria E.** 2014. Oral self-administration of buprenorphine in the diet for analgesia in mice. *Lab Anim* **48**:216–224. <https://doi.org/10.1177/0023677214532454>.
28. **Morgan D, Carter CS, DuPree JP, Yezierski RP, Vierck CJ Jr.** 2008. Evaluation of prescription opioids using operant-based pain measures in rats. *Exp Clin Psychopharmacol* **16**:367–375. <https://doi.org/10.1037/a0013520>.
29. **Oifa S, Sydoruk T, White I, Ekstein MP, Marouani N, Chazan S, Skornick Y, Weinbroum AA.** 2009. Effects of intravenous patient-controlled analgesia with buprenorphine and morphine alone and in combination during the first 12 postoperative hours: A randomized, double-blind, four-arm trial in adults undergoing abdominal surgery. *Clin Ther* **31**:527–541. <https://doi.org/10.1016/j.clinthera.2009.03.018>.
30. **Rivat C, Ballantyne J.** 2016. The dark side of opioids in pain management: basic science explains clinical observation. *Pain Rep* **1**:1–9. <https://doi.org/10.1097/PR9.0000000000000570>.
31. **Rose MA, Kam PCA.** 2002. Gabapentin: pharmacology and its use in pain management. *Anaesthesia* **57**:451–462. <https://doi.org/10.1046/j.0003-2409.2001.02399.x>.
32. **Rubio F, Seawall S, Pocolinko R, DeBarbieri B, Benz W, Berger L, Morgan L, Pao J, Williams TH, Koehlin B.** 1980. Metabolism of carprofen, a nonsteroid anti-inflammatory agent, in rats, dogs, and humans. *J Pharm Sci* **69**:1245–1253. <https://doi.org/10.1002/jps.2600691104>.
33. **Seymour TL, Adams SC, Felt SA, Jampachaisri K, Yeomans DC, Pacharinsak C.** 2016. Postoperative analgesia due to sustained-release buprenorphine, sustained-release meloxicam, and carprofen gel in a model of incisional pain in rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci* **55**:300–305.
34. **Sluka KA.** 1997. Activation of the cAMP transduction cascade contributes to the mechanical hyperalgesia and allodynia induced by intradermal injection of capsaicin. *Br J Pharmacol* **122**:1165–1173. <https://doi.org/10.1038/sj.bjp.0701486>.
35. **St A Stewart L, Martin WJ.** 2003. Evaluation of postoperative analgesia in a rat model of incisional pain. *Contemp Top Lab Anim Sci* **42**:28–34.
36. **Turck D, Vollmer KO, Brockbrader H, Sedman A.** 1989. Dose—linearity of the new anticonvulsant gabapentin after multiple oral doses. *Eur J Clin Pharmacol* **36** (Suppl):8–11.
37. **Vallejo R, de Leon-Casasola O, Benyamin R.** 2004. Opioid therapy and immunosuppression: A review. *Am J Ther* **11**:354–365. <https://doi.org/10.1097/01.mjt.0000132250.95650.85>.
38. **Vollmer KO, Anhut H, Thomann P, Wagner F, Jaehnchen D.** 1989. Pharmacokinetic model and absolute bioavailability of the new anticonvulsant gabapentin, p 209–211. *Advances in Epileptology Series, vol 17; Xviiith Epilepsy International Symposium, Jerusalem, Israel, 8–11 September 1987.* New York (NY): Raven Press.
39. **Wala EP, Holtman JR Jr.** 2011. Buprenorphine-induced hyperalgesia in the rat. *Eur J Pharmacol* **651**:89–95. <https://doi.org/10.1016/j.ejphar.2010.10.083>.
40. **Whiteside GT, Harrison J, Boulet J, Mark L, Pearson M, Gottshall S, Walker K.** 2004. Pharmacological characterisation of a rat model of incisional pain. *Br J Pharmacol* **141**:85–91. <https://doi.org/10.1038/sj.bjp.0705568>.
41. **Yamamoto T, Sakashita Y, Nozaki-Taguchi N.** 2000. Anti-allodynic effects of oral COX2 selective inhibitor on postoperative pain

- in the rat. *Can J Anaesth* **47**:354–360. <https://doi.org/10.1007/BF03020953>.
42. **Yanarates O, Dogrul A, Yildirim V, Sahin A, Sizlan A, Seyrek M, Akgül O, Kozak O, Kurt E, Aypar U.** 2010. Spinal 5-HT₇ receptors play an important role in the antinociceptive and antihyperalgesic effects of tramadol and its metabolite, O-desmethyltramadol, via activation of descending serotonergic pathways. *Anesthesiology* **112**:696–710. <https://doi.org/10.1097/ALN.0b013e3181cd7920>.
43. **Yang JY, Lee WI, Shin WK, Kim CH, Baik SW, Kim KH.** 2013. Administration of four different doses of gabapentin reduces awakening from breakthrough pain and adverse effects in outpatients with neuropathic pain during the initial titration. *Korean J Anesthesiol* **65**:48–54. <https://doi.org/10.4097/kjae.2013.65.1.48>.
44. **Zegre Cannon C, Kissling GE, Goulding DR, King-Herbert AP, Blankenship-Paris T.** 2011. Analgesic effects of tramadol, carprofen or multimodal analgesia in rats undergoing ventral laparotomy. *Lab Anim (NY)* **40**:85–93. <https://doi.org/10.1038/labani0311-85>.
45. **Zhu CZ, Nikkel AL, Martino B, Bitner RS, Decker MW, Honore P.** 2006. Dissociation between post-surgical pain behaviors and spinal fos-like immunoreactivity in the rat. *Eur J Pharmacol* **531**:108–117. <https://doi.org/10.1016/j.ejphar.2005.12.019>.