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Evaluation of buprenorphine hydrochloride Pluronic® gel formulation in male C57BL/6NCrl mice

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Abstract

Providing adequate analgesia while minimizing handling and stress post-surgery can be challenging. Recently, there have been commercial products made available for providing long acting analgesia in rodents. However, we find there are limitations for use in mice due to the viscosity of the product and the small dosing volumes needed. This project evaluated an in-house compounded formulation of buprenorphine easily made in the laboratory using pharmaceutical grade products. The release of buprenorphine was evaluated when compounded with two types of hydrogels (Pluronic® F-127 and F-68). Mice given buprenorphine in hydrogel (BP) demonstrated higher serum levels of buprenorphine for a longer period of time compared to mice given standard buprenorphine (Bup). However, the rate of decline in serum levels between the groups was similar; thus, it is more likely that the higher buprenorphine concentration seen in the BP group is due to the higher dose of buprenorphine given, rather than a slower release of product. Feed consumption was decreased in both groups one day after dosing; however, there was no difference in body weights. Increased activity in the open field was observed with both buprenorphine formulations, and lipemia was observed in mice given BP which persisted to at least 96 h. Based on our results, we conclude that this formulation did not sustain the release of buprenorphine or eliminate the increased activity commonly seen in mice given buprenorphine. In addition, the lipemia may confound research parameters, especially in cardiac studies and lipid metabolism studies. Therefore, we cannot recommend this formulation for use.

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AUTHOR CONTRIBUTIONS

T.L.B.-P. conceived of the project. T.L.B.-P., D.R.G., P.H.M. and J.W.D. designed the experiments. T.L.B.-P., D.R.G., P.H.M. and C.A.M. conducted the experiments. D.R.G. and G.E.K. analyzed the data. T.L.B.-P., D.R.G., P.H.M., and J.W.D. prepared the manuscript. T.L.B.-P. supervised the project.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

An important part of animal research is minimizing pain and distress. To accomplish this in those animals undergoing surgery, it is imperative that adequate analgesia be provided. Rodents are commonly used in research and our laboratory has previously evaluated the effectiveness of some analgesic regimes including buprenorphine in rodent surgical models¹⁻³.

Buprenorphine hydrochloride is a semisynthetic, partial μ opioid agonist, partial κ antagonist and is widely used as a post-operative analgesic in mice and rats⁴⁻⁹. When given by injection, dosing is typically done every 8–12 h^{1,10}, necessitating repeated handling of a rodent post-surgery, which may lead to additional stress. Increased activity has been associated with the use of buprenorphine^{1,11} and may be a concern as it can mask pain response such as decreased activity.

Several reports have been published on methods to prolong the efficacy of an analgesic following a single injection¹²⁻¹⁷ to minimize post-surgery handling. One commercially available product, Buprenorphine SR (Zoopharm, Fort Collins, CO) is advertised for use in rodents and its use and efficacy has been published by several laboratories^{12,14,16,18}. However, in the mouse, the concentration and viscosity of the formulation results in the use of very small volumes (less than 50 microliters per mouse) with larger gauge needles (20 g or larger). All of this makes accurate dosing difficult and time consuming (authors' experience).

Pluronic[®] gels have been used for slow release of compounds¹⁹⁻²¹. Pluronic[®] gels, known as thermoreversible hydrogels, are block copolymers that are derived from propylene and ethylene oxides. These act as surfactants and emulsifiers. Hydrogels are viscous liquids at 4 °C and become a semisolid gel above 23 °C. These properties allow drugs to be combined with the gels and then be released over an extended period of time^{19,21-23}.

The purpose of this project was to examine the properties of buprenorphine hydrochloride after compounding in these hydrogels. Our aim was to obtain a slow release of buprenorphine in a larger and more easily measured dosing volume for subcutaneous injection using pharmaceutical grade compounds. Hydrogels were evaluated for gelation temperatures; then, formulations of buprenorphine in hydrogels were evaluated *in vitro* for the release of buprenorphine. Serum levels of buprenorphine were compared between mice given a single subcutaneous injection of buprenorphine hydrochloride formulated in hydrogel and mice given a single dose of regular (standard) buprenorphine. Additionally, physiologic and behavior parameters in mice were measured and compared including feed consumption, body weight, open field activity and running wheel activity.

METHODS

Analgesic preparations

For the *in vitro* studies, powders for two Pluronic[®] gels (F-127 and F-68) were purchased from Sigma (St. Louis, MO). Solutions were made using the “cold technique,” as described previously²⁴. Each compound was aseptically weighed and dissolved in sterile water. The gel solutions were allowed to equilibrate overnight at 4 °C before addition of the

buprenorphine hydrochloride solution (Buprenex[®] 0.3mg/ml buprenorphine, Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA). For *in vivo* studies, Pluronic[®] F-127 was commercially compounded by a pharmacy accredited by the Pharmacy Compounding Accreditation Board (Triangle Compounding Pharmacy, Cary, NC) as a 20% aqueous solution for injection using pharmaceutical grade hydrogel. Pharmaceutical grade buprenorphine (Buprenex[®]) was then added aseptically to obtain either a solution of 0.1 mg/ml buprenorphine hydrochloride in 17% Pluronic[®] F-127 gel or 0.05mg/ml buprenorphine hydrochloride in 17% Pluronic[®] F-127 gel. The latter concentration was only used for the PK study. All other experiments using BP used the 1 mg/kg in a concentration of 0.1 mg/ml solution which equaled a dose volume of 0.1 ml/10 g body weight.

Gelation temperature experiment

Seven gel formulations (Table 1) containing 0.1 mg/ml of buprenorphine were assessed for gelation temperatures using a water bath. Approximately 1 ml of each solution was measured into a 10 × 75 mm borosilicate tube and allowed to equilibrate to the temperature of the water bath. The beginning temperature was approximately 10 °C, and the water bath was slowly heated and time recorded for each 1 °C rise. The solutions were periodically checked for transition to the gel state. Each formulation was run in triplicate. Three solutions (I, V, and VI) were selected for the *in vitro* dissolution experiment based on gelation temperatures.

In vitro dissolution experiment

The *in vitro* dissolution experiment was similar to that previously described^{21,25}. Briefly, approximately 2 ml of each of the 3 solutions (I, V, VI) containing 0.1 mg/ml of buprenorphine was measured into a 12 × 75 mm borosilicate tube, weighed and kept in an incubator at 37 °C to maintain them in a gel state. The gels were then covered with 2.0 ml of 37 °C phosphate buffered saline (PBS). A small aliquot of PBS was collected at 20, 40 and 60 min for analysis and the gel weighed. Buprenorphine concentration at each time point was measured in triplicate using a commercial ELISA kit (Neogen, Lexington, KY). The gel dissolution rate and the rate of buprenorphine released were calculated. The cumulative amount of buprenorphine released was examined compared to gel dissolution²⁵.

Buprenorphine concentration measurements

A commercially available forensic enzyme linked immunosorbent assay kit (Neogen, Lexington, KY) was used to measure buprenorphine concentrations in the dissolution experiments and in the sera of mice in the pharmacokinetic study similar to others^{18,26}. Standard curves were created using concentrations of 30 ng/ml, 15 ng/ml, 7.5 ng/ml, 3.75 ng/ml, 0 ng/ml of buprenorphine hydrochloride (Buprenex[®]). Samples were diluted in supplied diluent buffer before use. Absorbance was read at 450 nm using an iMark Microplate Reader (Biorad, Hercules, CA).

Animals and housing

All mice used were six to eight week old male C57BL/6NCrl mice (Charles River Laboratories, Raleigh, NC) and acclimated for one week before any experiment. Cages were

labeled with an experimental group assignment the day before animals were received. Three of the authors (PM, DG, TB) then arbitrarily selected a mouse from the shipping crate upon receipt and placed it in one of the labeled cages without examining the assignment of that particular cage. Male mice were used to compare with past research done by this laboratory. Also, each mouse was required to be individually housed to assess wheel activity and measure feed intake. Each mouse was housed in a micro-isolator static cage (Techniplast, Exton, PA) with autoclaved nesting material (Nestlet, Ancare Corp., Bellmore, NY) and housed on hardwood bedding (Sani-chips, PJ Murphy, Montville, NJ). Mice were maintained on a 12:12-h light:dark cycle at 22+/- 0.5 °C and relative humidity of 40% to 60%. Mice were provided ad libitum autoclaved rodent diet (NIH31, Harlan Laboratories, Madison, WI) and deionized water treated by reverse osmosis. Mice were negative for mouse hepatitis virus, Sendai virus, pneumonia virus of mice, mouse parvovirus 1 and 2, epizootic diarrhea of infant mice, mouse norovirus, *Mycoplasma pulmonis*, *Helicobacter* spp., and endo- and ectoparasites upon receipt and no pathogens were detected in sentinel mice during this study. All animal procedures were reviewed and approved by National Institute of Environmental Health Sciences Animal Care and Use Committee. All animals were housed, cared for, and used in compliance with the *Guide for the Care and Use of Laboratory Animals* and housed and used in an Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) Program.

Pharmacokinetic (PK) study and blood collection

A single buprenorphine-gel formulation (BP-buprenorphine in 17% Pluronic® F-127) was selected based on the results of the gelation and dissolution experiments for comparison with regular injectable buprenorphine. Mice were then given either 1.0 mg/kg BP or 0.1 mg/kg Bup in a volume 0.1 ml/10 g of body weight subcutaneously. The BP dose was based on a previous long acting analgesia study formulation¹⁴. Six mice were used per group/time point. Mice were observed at each time point for clinical signs such as changes in activity, breathing, and posture. At each time point, six mice were euthanized with carbon dioxide and blood was collected by cardiac puncture. Blood was collected from the Bup group at 1, 4, 8, and 12 h. Previous reports^{18,26–29} have shown that buprenorphine is not detectable after 12 h so mice were not used for the longer time points in this group to minimize animal use. Blood was collected from the 1.0 mg/kg BP injected mice at 1, 4, 8, 12, 24, and 48 h. A separate group of mice was injected with 0.5 mg/kg of BP (using a concentration of 0.05 mg/ml) after the PK data from the first two groups had been compared. Six mice/time point were euthanized and blood collected at 4, 8 and 24 h. Following centrifugation, serum was stored at -20 °C until buprenorphine concentration was measured. As lipemia was observed in all of the BP serum samples, two mice were injected with 0.1 ml/10 g body weight with 17% pluronic gel (no buprenorphine) and two mice were injected with the same volume of saline. One from each group was sacrificed at 72 and 96 h for blood collection. Three additional mice were dosed with 0.01 ml/10 g body weight of 17% pluronic gel and sacrificed at 96 h for blood collection.

Behavior studies

Locomotor activity in the open field—Twelve mice were assigned to one of three groups to measure locomotor activity. Activity was measured using commercially available

open field instrumentation (OptoMax Activity, Columbus Instruments, Columbus, OH). Each mouse was placed in an open acrylic chamber (45.7 cm × 45.7 cm) containing photocells spaced one inch from the chamber bottom and approximately 1.3 cm apart linearly around the chamber. The beam diameter was 0.32 cm and activity was measured by recording the number of photocell disruptions by using Opto-Max version 2.27 software (Columbus Instruments, Columbus, OH). The number of photocell disruptions per 5 min was recorded. Each mouse was allowed to acclimate and freely explore the chamber for 30 min. Time 0 was the beginning of the acclimation period. At 30 min, each mouse was injected subcutaneously with either 0.9% saline, Bup (0.1 mg/kg), or BP (1 mg/kg) and placed back into the activity chamber for an additional 45 min (total time of 75 min). Chambers were cleaned with 70% alcohol between mice and with 4% chlorhexidine diacetate solution and water between sessions.

Running wheel activity—Forty-eight mice were assigned to one of three groups (16/group). Each home cage was equipped with a stainless steel running wheel (Vital View Data Acquisition, Respironics, Bend, OR) that continuously recorded voluntary wheel revolutions of each mouse. Total wheel revolutions were recorded every fifteen minutes and running wheel activity was reported in light and dark period totals for each day. Mice were removed daily for body weight data collection. Data was collected continuously beginning four days before injection until 5 days after injection. Day 0 signifies the injection day. Injections were done at 10 a.m. Mice were given either 0.9% saline, BP (1 mg/kg) or Bup (0.1 mg/kg). The pre-dose running wheel data was the baseline data and was used to remove the novelty effect of the wheels and to compare post-injection activity.

Feed consumption and body weights—Feed and body weights were recorded on mice used in the running wheel experiment to correlate feed intake, body weight, and activity. Feed consumption was averaged over 3 days before dosing for the baseline data. The feed was then weighed and consumption calculated daily for the remainder of the study. Feed consumption is reported as average grams of feed/day/animal. Body weights were recorded on day -3, day 0 and daily for the remainder of the study.

Statistics

Prior to the study start, the number of mice per dose group required to achieve 80% power to detect pre-specified differences at the 0.05 level of significance was calculated for each experiment. Locomotor activity over time in the open field was analyzed by two-way repeated measures ANOVA (RM-ANOVA) and Tukey's multiple comparisons test. The running wheel data were evaluated using a two-way RM-ANOVA. Tukey's multiple comparisons test was used to compare group performance each day. Body weight and feed consumption on the running wheel were evaluated using two-way RM-ANOVAs and Tukey's multiple comparisons tests were used to compare groups each day as well as to compare day-to-day fluctuations in body weight and feed consumption within each group. For the PK study, the decline in serum buprenorphine concentrations after the peak at 8 h was compared between each BP formulation and Bup using linear regression and F-tests for equality of slopes³⁰. All statistical analyses were conducted using Prism, version 6.02 (GraphPad, La Jolla, CA).

RESULTS

Gelation temperature

The gelation temperatures of the seven formulations varied from 27 to over 38 °C (Table 1 and Fig. 1). The ideal temperature range for gelation was 30–33 °C. At higher temperatures, the gel does not solidify sufficiently; and at lower gelation temperatures, the product is too viscous to inject³¹. Of the seven solutions evaluated, three solutions (I, V and VI) matched these criteria (Table 1, Fig. 1). These three solutions were then used in the *in vitro* dissolution experiment to assess buprenorphine rate of release and in the pharmacokinetic study.

In vitro dissolution

The *in vitro* gel dissolution results are presented in Figure 2. All three solutions released similar amounts of buprenorphine per milligram of gel dissolved (Fig. 2). Solution I had the least amount of dissolution of gel over time and the slowest rate of buprenorphine release. Solution V and VI had 2 and 3 fold higher rate of dissolution respectively (Fig. 2) and a corresponding increased rate of buprenorphine released.

PK study

Both the Bup and the 1 mg/kg BP solutions peaked within the first eight hours (Table 2). It is suspected that the 0.5 mg/kg BP peaked as well at 8 h with 9 ng/ml, but the 12 h time point was not evaluated. The 1 mg/kg BP solution peaked at 20 ng/ml compared to 8 ng/ml for standard buprenorphine. The BP solution maintained high blood concentrations for as long as 48 h, while the levels achieved by standard buprenorphine had fallen considerably by 12 h. The slopes of all the curves were calculated and found to be the same between and among all the groups. Lipemia was noted in all the mice dosed with BP at each time point. This was not present in the mice dosed with Bup. Lipemia was present at 72 and 96 h in the serum of the mice dosed with 0.1 ml/10 g body weight of 17% pluronic gel but not present in the saline mice. All three mice dosed with 0.01 ml/10 g body weight had visible lipemia at 96 h.

Locomotor activity in the open field

Figure 3 shows the results of the locomotor activity in the open field. Increased activity was seen in both the Bup and the BP groups compared to saline. There was a significant difference in activity between saline and BP groups at 60, 65, 70, and 75 min ($P < 0.05$) and between saline and Bup groups at 70 min ($P < 0.05$). There were no differences between the Bup and BP groups at any time points.

Running wheel

The running wheel activity is shown in Figure 4. A two-way RM-ANOVA of running wheel activity indicated no significant main effect of treatment. The Bup group showed an increase in the 24 h revolution count at day 4 and day 5 compared to the BP group ($P < 0.05$); however, there was no significant difference between the saline and BP groups at any of the time points.

Feed consumption and body weights

Feed consumption data is presented in Figure 5. Feed consumption was decreased in the Bup and BP groups compared to saline treatment groups the day after dosing. Body weight differences among the groups were not significantly different before or after dosing (Fig. 6). Tukey's multiple comparisons test showed no significant differences between groups each day.

DISCUSSION

This study sought to create a more easily administered, pharmaceutical grade, injectable, long acting buprenorphine for mice by compounding buprenorphine with a thermoreversible, biodegradable hydrogel (Pluronic[®] gel). Several buprenorphine/gel formulations were evaluated for gelation temperatures *in vitro*. Formulations that were in the desirable range of gelation temperature were then evaluated for dissolution *in vitro*. And one of those was selected for the *in vivo* pharmacokinetic study. Solution I had the slowest rate of buprenorphine release (buprenorphine concentration of 0.1 mg/ml in 17% gel). When injected at 1 mg/kg in mice, the BP maintained blood levels significantly higher than standard buprenorphine. However, when the slopes of the curves were analyzed, the rate of decline of buprenorphine between the groups was not different. This suggests that the higher serum concentrations at the time points were due to the higher dose given and not due to sustained release, though this would require a group of mice receiving Bup at 1 mg/kg (the same dose used in the BP group) to confirm. In addition, lipemia was noted during the PK study. This study used physiologic and behavioral parameters to evaluate buprenorphine formulated in hydrogel: body weight, feed consumption, voluntary running wheel activity, and open field activity. Both the BP and the Bup groups had decreased feed consumption on day 1, but body weight was not affected. There were no differences in body weight among the groups before or after dosing. Increased activity was observed in the BP group similar to the Bup group. This product is readily compounded with pharmaceutical grade products and could be compounded in house or by a reputable compounding pharmacy. It is easily measured and injected. The physiologic and behavior parameters were similar between BP and Bup. However, the PK data suggests that sustained release was not achieved. And the lipemia may interfere with research, especially any involving lipid metabolism, atherosclerosis, and cardiovascular mechanisms.

Pain and stress relief is an important animal welfare issue in laboratory animal research. Adequate analgesia post-surgery is important in minimizing adverse effects on experimental results; but it is also important to minimize other stressors such as repeated handling and dosing. Many studies have evaluated methods to prolong analgesia in a way that minimizes handling^{9,13,16,32-42}. A commercially available, injectable, sustained-release formulation of buprenorphine (buprenorphine HCl SR[®], Zoopharm, Fort Collins, CO) has been evaluated by several laboratories in rodents^{12,14,18,43}. Although this commercial formulation is advertised as being for laboratory animals, in our hands, the viscosity and concentration of the product (1 mg/ml) makes it time consuming and challenging to adequately and accurately use in mice. Chum, *et al.*¹⁵ formulated their own product; and although viscosity was not reported, volumes were still small. It was of interest to evaluate other vehicles to

prolong buprenorphine release in a subcutaneous dosing volume routinely used by this laboratory (0.1 ml/10 g of body weight).

Biodegradable synthetic hydrogels are continuously being evaluated *in vitro* and *in vivo* for use in drug and cell delivery^{19–23,35,44–48}. Specific for buprenorphine, Graves, Freeman, and Mandal²⁵ created an *in situ* buprenorphine gel formulation and developed an *in vitro* method to evaluate its dissolution. Barichello, *et al.*²¹ specifically used F-127 to formulate a longer releasing insulin in a rat model. We used a similar dissolution method to evaluate our gel formulations *in vitro* and found Solution I (17% Pluronic® F-127) had the slowest gel dissolution rate which directly correlated with the buprenorphine release rate. Two types of commercially available biodegradable gels were used in the *in vitro* portion of this study, Pluronic® F-127 and Pluronic® F-68, based on biochemical properties. The basic structure consists of a central hydrophobic propylene oxide chain flanked by two hydrophilic ethylene oxide chains, the only difference between the two being the length of these chains^{19,22,23}. These products can be obtained commercially in pharmaceutical grade forms and are readily available for use in compounding water soluble products such as buprenorphine hydrochloride. They can also be compounded with buprenorphine using pharmaceutical grade products reliably by reputable compounding pharmacies.

Buprenorphine and its metabolites can be detected and measured by various techniques^{13,14,25–27,49–51}. We modified a commercially available enzyme linked immunoassay (ELISA) developed to detect buprenorphine in human blood and urine similar to what others have done^{18,26,27}. This technique was used for *in vitro* studies to determine the rate of release of buprenorphine as well as for the *in vivo* pharmacokinetic study. In our experience, the assay has many variables when used in this manner. Samples are required to be diluted to ensure results are readable within the standards used which can add a variable each time the test is run. We used one animal for one time point (versus using an animal for several time points) which also may have added to the variability we saw. The type of diluent used can also affect both the standards and the samples. Future studies will examine various methods of standard and serum dilution in this assay. This will be compared with mass spectrometry which is the “gold standard” for buprenorphine and its metabolites in order to better quantify and qualify the results.

Our aim using the assay was to compare buprenorphine serum levels between BP and Bup groups. Although our data showed that BP had higher serum levels of buprenorphine when compared to Bup; there was no difference in the slopes of the curves. It was expected that our BP group would have a less steep slope for it to be sustained release. The PK results indicate that the buprenorphine levels in the BP group declined at the same rate as the Bup group. As our goal was to create a sustained release of the buprenorphine, the PK data suggests this was not accomplished.

Buprenorphine hydrochloride serum concentration has been shown to increase rapidly after subcutaneous administration; however, it also declines rapidly and is undetectable 8–12 h after administration at standard anesthetic doses^{26,49}. Buprenorphine levels of 1 ng/ml has been correlated with prolonged tail flick and paw withdrawal latency in rats^{40,52} and this blood level has been associated with pain relief in human clinical trials; however different

serum levels may be needed relative to the type of painful procedure induced⁴⁹. Mice given transdermal formulation of buprenorphine demonstrated prolonged latency on tail flick that correlated with 1 ng/ml buprenorphine in the plasma, however, 8 ng/ml was the plasma therapeutic level to achieve adequate analgesia on the writhing test⁴⁹. This is supported by a recent publication that found different levels of buprenorphine are required relative to the type of painful procedure to which the mouse is subjected⁵³. Although our data suggests we still had buprenorphine levels higher than 1 ng/ml at 48 h with the 1 mg/kg BP, this was associated with the total amount of buprenorphine injected and not achieved by sustained-release.

We incorporated several parameters: feed consumption, body weight, clinical signs, open field activity, and running wheel to evaluate physiologic and behavior changes as previously done by this laboratory^{1,3}. Buprenorphine has been shown to decrease feed intake and reduce body weight or weight gain in rodents^{4,32,54–60}. The purpose of monitoring feed intake and body weight was to evaluate if the BP would eliminate decreased feed intake and weight loss that can be seen with standard buprenorphine. We specifically measured these parameters in the running wheel study to determine whether feed or weight changes were correlated with changes in activity. Decreased feed consumption was observed in the 24 h after dosing with BP but weights were not significantly different.

Buprenorphine is known to cause increased activity in non-manipulated mice. Another aim of this project was to determine whether a BP formulation would attenuate or eliminate this increased activity in dosed mice. The open field locomotor activity was used to assess acute changes in activity immediately following injection and we found increased activity in open field in both the Bup and the BP groups. No visible signs of sedation were seen in mice given this higher dose of buprenorphine. Running wheel activity was used to assess long term changes in activity and thus data was collected every 15 min and presented in 24 h increments for 5 days post injection. There were no changes in running wheel activity when examined this way. However, when we examined the running wheel data immediately following injection we observed a similar increase in activity as in the open field for several hours that was masked when viewing the data in 24 h increments (data not shown). The lower dose of 0.5 mg/kg BP was not evaluated in the activity experiment as the PK data indicated that sustained release had not been achieved. In addition, the lipemia observed was a concern.

Side effects of any compound must be evaluated when considering its use. Compound osmolality, pH, and volume are just a few parameters that must be considered⁶¹. The development of skin lesions in mice given the 2.0 mg/kg Bup SR Lab product was reported¹⁸. Cannon *et al.*³ reported skin lesions in rats given tramadol which was later determined to be due to hyperosmolality of the product (unpublished data). Buprenorphine has also been reported to have respiratory depression when given at 10 mg/kg doses⁶². The lipemia observed was not expected. The lipemia was further evaluated by injecting mice with saline or hydrogel without buprenorphine—lipemia was still significant at 96 h. In discussions with our compounding pharmacists, we found that Pluronic[®] gel 127 is also known as poloxamer 407 which has been reported to cause lipemia in mice, rats, and rabbits^{63–66}. A recent publication further examined the role of block copolymers interaction

with lipoproteins. A dose of 0.25 g/kg induces lipemia in C57BL/6 mice⁶³. Our study mice were dosed with 0.1 ml/10 g body weight of 17% Pluronic[®] gel which calculates to 1.7 g/kg body weight. Three mice were dosed with one tenth of this amount in volume and dose (0.01 ml/10 g body weight; 0.17 g/kg) and lipemia was still observed (results not shown) at 96 h. This volume also was extremely difficult to measure and administer which contradicted the purpose of the study.

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FIGURE 1.

Transition temperatures from solid to gel. Red and green lines define the ideal temperature range for *in vivo* use. Above the red line, the solution will not gel sufficiently at physiological temperatures; whereas below the green line, it will be too viscous for easy handling and injection or may gel at room temperature.

FIGURE 2.

Data on buprenorphine release from gel solutions. **(a)** Mass of buprenorphine released versus mass of gel dissolved. **(b)** Buprenorphine release rate showing cumulative mass of drug released over time. **(c)** Gel dissolution rate showing cumulative mass of gel dissolved over time.

FIGURE 3.

Comparison of locomotor activity using Optomax Activity monitor program. Vertical lines denote time period that animals were removed from open field and dosed with either saline, Bup, or BP. Data represent the mean $\pm$ s.e.m. ($n = 4$). Asterisk indicates significant difference between saline and BP groups at 60 min (*, $P = 0.05$), saline and BP groups at 65 min (*, $P = 0.05$), saline and Bup groups at 70 min (*, $P = 0.05$), saline and BP groups at 70 min (***, $P = 0.0001$), and saline and BP groups at 75 min (*, $P = 0.05$).

FIGURE 4.

Daily wheel revolutions. Data was collected daily 4 days before dosing through day 5. Data represent the mean $\pm$ s.e.m. ($n = 16$). Asterisks at day 4 and day 5 indicate significant differences between Bup and BP groups (*, $P < 0.05$).

FIGURE 5.

Average daily feed consumption baseline (BL) was average over 3 days before dosing. The feed was weighed and consumption calculated daily for the remainder of the study. Feed consumption is reported as average grams of feed/day/animal. Data represent the mean $\pm$ s.e.m. ($n = 5-9$). Asterisks indicate significant difference between Saline and Bup groups (**, $P < 0.01$) at day 1 and Saline and BP groups (***, $P < 0.001$) at day 1.

FIGURE 6.

Average daily body weights were measured 3 days before dosing for the baseline (BL) pretreatment data. Additional body weight measurements were taken when animals were dosed (day 0), and then daily through day 7. Data represent the mean $\pm$ s.e.m. ($n = 5$).

Table 1

Formulations of Pluronic® gel solutions prepared with sterile water using the cold technique referenced.

Solution	F-127 (%)	F-68 (%)	NaCl (%)	Buprenorphine (mg/mL)	Gelation temperature (°C)
I	17	0	0	0.1	31
II	19	0	0	0.1	27
III	15	17	0	0.1	38
IV	15	17	0.61	0.1	>38
V	16	0	0.67	0.1	30
VI	20	5	0	0.1	32
VII	22	5	0	0.1	27

Table 2

Pharmacokinetic data showing mean serum levels (ng/ml) of buprenorphine in mice following a single subcutaneous injection of either buprenorphine HCl or buprenorphine in hydrogel.

Column 1	Column 2	Column 3	Column 4
Time	Buprenorphine HCl (0.1 mg/kg)	Buprenorphine HCl in Pluronic™ gel (1.0 mg/kg)	Buprenorphine HCl in Pluronic™ gel (0.5 mg/kg)
1 h	2.89	10.83	*
4 h	1.63	6.74	10.55
8 h	8.6	20.93	9.87
12 h	1.31	8.89	*
24 h	*	16.66	0.71
48 h	*	2.01	*

* denotes blood collection not done.