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Effects of Buprenorphine, Methylnaltrexone, and Their Combination on Gastrointestinal Transit in Healthy New Zealand White Rabbits

Manuel Martin-Flores,[1*] Bhupinder Singh,[2] Courtney A Walsh,[3] Elizabeth P Brooks,[3] Laci C Taylor,[3] and Lisa M Mitchell[1]

Among the many analgesic agents available, buprenorphine appears to be the analgesic used most often in rabbits. Unfortunately, deleterious side effects of opioids, such as gastrointestinal stasis and anorexia, may discourage the use of these agents. Methylnaltrexone is a peripheral opioid antagonist that ameliorates opioid-induced gastrointestinal stasis in others species yet preserves the analgesic effects of buprenorphine. We evaluated whether methylnaltrexone reversed buprenorphine-induced gastrointestinal stasis in 8 healthy male New Zealand White rabbits. To measure gastrointestinal transit time, each rabbit received 20 barium-filled spheres through an orogastric tube. Rabbits then received 4 treatments in random order: buprenorphine (0.05 mg/kg SC), methylnaltrexone (1 mg/kg SC), both agents combined (B+M), or normal saline (control) every 12 h for 2 d. Fecal production was measured every 6 h, and water and food consumption, and body weight, were measured daily, for 5 d after each treatment. The time to appearance of the first sphere was significantly longer for buprenorphine group than for control and methylnaltrexone groups. Daily fecal output was lowest for buprenorphine and B+M, intermediate for control, and highest for methylnaltrexone. Water and food consumption were lower for groups buprenorphine and B+M than for control and methylnaltrexone. Body weight was not affected. In conclusion, treatment with buprenorphine 0.05 mg/kg BID for 2 d in healthy rabbits decreased food and water consumption, prolonged gastrointestinal transit time and decreased the fecal output. Coadministration of methylnaltrexone at 1 mg/kg did not alleviate these negative side effects.

Abbreviations: B+M: buprenorphine plus methylnaltrexone

During 2014, approximately 150,000 rabbits were used for research in the United States, many of which were used in projects that involved painful procedures requiring analgesia.[11] Among the many analgesic agents available, the opioid buprenorphine appears to be the analgesic used most often in rabbits.[6] Unfortunately, deleterious side effects of opioids, such as gastrointestinal stasis and anorexia, may discourage the use of these agents.[5,6,13]

The effects of opioid analgesics can be antagonized with opioid antagonists, such as naloxone or naltrexone. However, these antagonist agents may also reverse the desirable effects of opioids, namely analgesia or sedation. Methylnaltrexone is a quaternary N-methyl derivative of naloxone that does not cross the blood–brain barrier. This peripheral opioid antagonist can therefore reverse opioid-induced gastrointestinal stasis without antagonizing the analgesic or sedative effects that depend on opioid-receptor activation in the CNS. Methylnaltrexone has been successfully used to treat opioid-induced gastrointestinal stasis in people[7] and in horses.[2]

In the current investigation, we compared the effects of buprenorphine, methylnaltrexone and their combination on gastrointestinal motility in healthy New Zealand White rabbits. We hypothesized that buprenorphine would decrease gastrointestinal motility as evidenced by a decrease in fecal output and a delay in gastrointestinal transit time compared with those in an untreated control group. We further hypothesized that coadministration of methylnaltrexone with buprenorphine would prevent these deleterious side effects and that administration of methylnaltrexone alone would have no effect on gastrointestinal motility.

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[1]Department of Clinical Sciences, College of Veterinary Medicine, [2]Center for Animal Resources and Education, and [3]College of Agriculture and Life Sciences, Cornell University. Ithaca, New York
**Corresponding author. Email: martinflores@cornell.edu*

Materials and Methods

This study was approved by the IACUC of Cornell University (Ithaca, NY) and was conducted in an AAALAC-accredited facility in compliance with the Animal Welfare Act [1]and *Guide for the Care and Use of Laboratory Animals*.[8]

Animals. Healthy, adult, male New Zealand White rabbits (*Oryctolagus cuniculus*; *n* = 8) were obtained from Charles River Laboratories (Wilmington, MA). The health surveillance report from the vendor indicated that the source colony of these rabbits was negative for *Pasteurella* spp., *Helicobacter* spp., *Salmonella* spp., *Bordetella bronchiseptica*, *Clostridium piliforme*, *Lawsonia* spp., *Pseudomonas aeruginosa*, *Treponema* spp., cilia-associated respiratory bacillus, *Eimeria* spp., *Passalurus ambiguus*, *Cheyletiella parasitovorax*, *Psoroptes cuniculi*, *Leporacarus gibbus*, reovirus, rabbit hemorrhagic disease virus, rotavirus, and lymphocytic choriomeningitis virus. Rabbits were housed individually in stainless steel cages (Allentown, NJ) with perforated floors that allowed collection of feces in a tray. Rabbits were fed Teklad Global Rabbit diet 2030 (Envigo, Madison, WI), and water was provided without restriction in glass water bottles. Hard plastic balls and 4-in. PVC pipe were provided as enrichment manipulanda to each animal. The housing room was maintained at 66 to 70 °F (18.9 to 21.1 °C), at 30% to 70% humidity, and on a 12:12-h light:dark cycle. All rabbits were acclimated for 2 wk prior to study.

Experimental design. Each rabbit was studied on 4 occasions in a crossover, randomized design, and data were collected for 5

consecutive days during each treatment. A washout interval of at least 1 wk was allowed between the treatments. Husbandry parameters, including access to food and water, were not altered during the washout periods.

On day 1 of each study, the rabbits were weighed and then anesthetized in an induction chamber with isoflurane (5%) in oxygen until loss of righting reflex. The rabbits were then removed from the chamber, and 20 barium-filled spheres (diameter, 3.2 mm diameter; weight, 20.5 g; density, 1.12 g/cm^3; LDPE Barium-Filled Ball; Precision Plastic Ball Company, IL) were administered through an orogastric tube by using 20 mL water to flush. Prior to insertion, the tube length needed to reach the stomach was measured. Each rabbit received one of the 4 treatments and was allowed to recover from anesthesia before returning to the cage. The treatment groups consisted of normal saline (1 mL SC, control group), buprenorphine (0.05 mg/kg SC; Buprenorphine Hydrochloride Injection, Hospira, Lake Forest, IL), methylnaltrexone (1 mg/kg SC; Relistor, Salix Pharmaceuticals, Raleigh, NC), and buprenorphine and methylnaltrexone combined (0.05 and 1 mg/kg SC, respectively; B+M). Normal saline was added as a diluent to each treatment such that the final administered volume was 1 mL for all groups. A total of 4 treatments were administered every 12 h for 2 d by the same personnel.

Data collection. The feces of each rabbit were collected and weighed every 6 h for 5 d; during the first 2 d, the feces were collected prior to treatment injection. The collected feces were radiographed to identify the presence of spheres. At each sampling time, animals also were observed for signs of abdominal pain or abdominal distention, the presence of which would lead to subject removal from the study and treatment.

The rabbit's body weight and the amount of food and water consumed (in grams) during the previous day was obtained daily at noon throughout the study period. Because the last data points pertaining to water and food consumption were recorded on the fifth day of each study period, consumption of food and water over the last day was not captured.

Statistical analysis. The distribution of the residuals was evaluated for all results. The first appearance of barium-filled spheres in feces was compared between the groups by using Kruskal–Wallis one-way ANOVA. The weight of feces collected and water and food consumption were normalized to the daily body weight of each rabbit, and the daily change in body weight (as a percentage) was calculated. The effects of treatment and time (and their interaction) on daily fecal weight (measured in grams), change in body weight, and daily consumption of food and water were compared by using individual mixed-effect models and posthoc Tukey tests, with subject as the random effect and time and treatment (and their interaction) as fixed effects. Differences were considered significant when the P value was less than 0.05. Nonparametric results are summarized as median and range, and parametric data are summarized as the mean ± SE. All statistical analyses were performed by using JMP Pro (version 12.0.1. SAS Institute, Cary, NC).

Results

During this study, none of the rabbits in any treatment group exhibited any abnormal signs, except for one rabbit in the buprenorphine group, which showed signs of poor hydration on physical examination on day 6 (1 d after completing data collection); the signs resolved after the administration of subcutaneous fluid.

Effects of buprenorphine and methylnaltrexone on gastrointestinal motility. Barium-containing spheres were recovered from the feces of all 8 rabbits in the control group and from 7 of the 8 rabbits in each of the remaining 3 groups (Figure 1 A). The time to the appearance of spheres was 18 (18 to 18) h after control treatment, 30 (24 to 48) h in the buprenorphine group, 24 (18 to 48) h in the B+M group, and 18 (18 to 24) h in the methylnaltrexone group. The time to the appearance of the first sphere was longer for the buprenorphine group than for the control and methylnaltrexone groups ($P = 0.0005$); this parameter did not differ significantly between any other groups. In 2 rabbits receiving buprenorphine and in one animal in the B+M group, spheres first appeared 48 h after drug administration. In the control and methylnaltrexone groups, spheres first appeared in 24 h or less in all rabbits. The cumulative recovery of spheres (absolute and percentage) is shown in Figure 1 B.

Effect of buprenorphine and methylnaltrexone on fecal output. The daily fecal weight was affected by both time ($P < 0.0001$) and treatment ($P < 0.0001$); it was lowest on day 1 and higher thereafter (Figure 2). When compared between treatments, daily fecal weight was highest ($P < 0.0001$) for methylnaltrexone, intermediate for control, and lowest for buprenorphine and B+M (did not differ between these 2 treatments). There was no significant interaction between time and treatment ($P = 0.09$).

Effect of buprenorphine and methylnaltrexone on body weight and water and food consumption. Neither treatment nor time affected either the absolute body weight or the change in body weight during the 5 d of observation (all $P > 0.1$; Figure 3). However, both time and treatment altered water and food intake (all $P < 0.009$). Water and food consumption were lower for groups buprenorphine and B+M than for either control or methylnaltrexone, and food and water consumption were greater in the methylnaltrexone group than in control rabbits ($P < 0.0001$ for both comparisons; Figure 4).

Discussion

The main findings of this investigations suggest that: 1) the administration of buprenorphine 0.05 mg/kg SC twice daily for 2 d prolonged gastrointestinal transit time, reduced the daily fecal output, and reduced the daily intake of water and food in healthy male New Zealand White rabbits; 2) coadministration of methylnaltrexone (1 mg/kg SC) with buprenorphine did not effectively counter the undesirable gastrointestinal effects of buprenorphine; and 3), the administration of methylnaltrexone alone increased daily food intake and fecal production in our animals.

Buprenorphine is likely the most commonly used opioid analgesic agent in rabbits.[6] However, like most opioids, the administration of buprenorphine can delay gastrointestinal transit time, constipation, and anorexia. In this investigation, we had hypothesized that buprenorphine, administered twice daily for 2 d, would delay gastrointestinal transit and reduce the amount of feces excreted. To quantify gastrointestinal transit, we administered 20 barium-filled spheres to isoflurane-anesthetized rabbits by using an orogastric tube. We had previously used this technique in horses, which each received 200 spheres.[9,10] In horses, these spheres were recovered over several days, and a curve of recovery of spheres over time was plotted.[2,10] In many of our rabbits, most of the spheres were excreted during the first day of data collection; we therefore focused the analysis on the time to first appearance of spheres and used that variable to reflect gastrointestinal transit time. Those results showed that gastrointestinal transit was fastest for the control group and significantly slower after buprenorphine; they also showed that coadministration of 1 mg/kg methylnaltrxone did not mitigate the hypomotility associated with buprenorphine in rabbits.

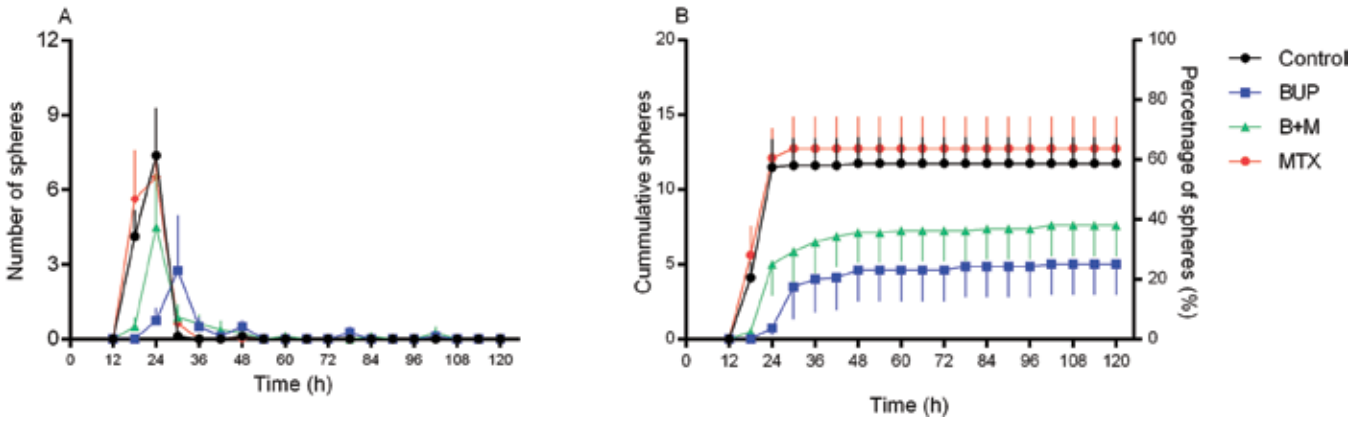

Figure 1. (A) Number (mean ± SE) number of spheres in feces and (B) cumulative spheres (absolute number and percentage) in 8 New Zealand White rabbits receiving normal saline, buprenorphine (BUP, 0.05 mg/kg), methylnaltrexone (MTX, 1 mg/kg), or both agents combined (B+M). Treatments were administered subcutaneously at 0, 12, 24 and 36 h. Rabbits received 20 spheres at 0 h. The time to appearance of the first sphere was significantly ($P = 0.0005$) longer for buprenorphine group than for control and methylnaltrexone groups.

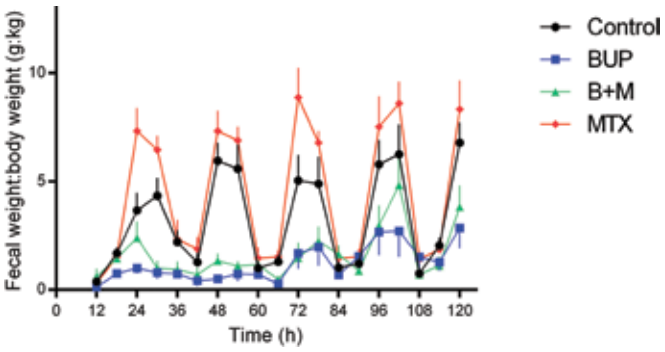

Figure 2. Fecal weight to body weight (mean ± SE) in in 8 New Zealand White rabbits receiving normal saline, buprenorphine (BUP, 0.05 mg/kg), methylnaltrexone (MTX, 1 mg/kg), or both agents combined (B+M). Treatments were administered subcutaneously at 0, 12, 24, and 36 h. Fecal weight was greatest ($P < 0.0001$) for methylnaltrexone, intermediate for the control treatment, and lowest for buprenorphine and B+M.

Approximately 60% of the total spheres administered were recovered in the control and methylnaltrexone groups, compared with 25% to 38% of the spheres in the groups that received buprenorphine. We expected that buprenorphine would delay transit time but not that fewer spheres would be recovered during the observation period. Moreover, in one animal in each treatment group (except for the control group), no spheres were recovered in the feces; dorsoventral abdominal radiography of these rabbits failed to identify any spheres once data collection was complete. However, we observed opacities that appeared to be fragments of spheres. Perhaps several spheres were eliminated with cecotropes and reingested. Those spheres could then be masticated and account for the absence of intact spheres in feces and the presence of small radiopaque fragments. Because the feces were collected from a stainless steel tray situated under the rabbits' cages, we could confirm that no feces or spheres were missed during sample collection.

In addition, rabbits treated with buprenorphine had decreased fecal excretion and water and food consumption, when compared with the control and methylnaltrexone groups. The reduction in fecal excretion during the days of treatment was not compensated by an increase in fecal output during subsequent days (Figure 2). This finding suggests that treatment with buprenorphine not only delayed transit of feces through the gastrointestinal tract but also reduced the amount of feces produced, likely secondary to a reduction in food intake. Although fecal production increased over time in the rabbits that received buprenorphine, daily fecal output remained substantially decreased compared with that of the control group, even on day 5. Given that buprenorphine is commonly administered as an analgesic, its negative effects on gastrointestinal motility and appetite may exceed the duration of its perceived analgesic effects.

Although buprenorphine delayed gastrointestinal transit and reduced fecal output, no severe consequences requiring medical intervention occurred during the experimental period, with the exception of one rabbit that required supplemental subcutaneous fluids after the experimental period. This pattern was true even though buprenorphine was administered at a high dose and to rabbits that did not receive supplementary fiber in their diet or did not exercise routinely. Therefore, even though buprenorphine has an undeniably negative effect gastrointestinal motility, the clinical consequences of buprenorphine administration are not always grave necessarily and may not require treatment in many subjects. Moreover, the use of buprenorphine in rabbits that underwent ovariohysterectomy was no more detrimental to gastrointestinal motility than was a NSAID.[5] These combined data suggest that buprenorphine can be used in the postoperative period as long as food intake and fecal output are monitored.

The coadministration of methylnaltrexone with buprenorphine failed to normalize fecal output and consumption of water and food. Methylnaltrexone is a competitive antagonist of opioid receptors, and its ability to antagonize the effects of an opioid agonist may potentially be affected, at least in part, on the dose, and the affinity with which each agent might interact with the receptor.[3] Buprenorphine is considered a potent opioid with high affinity for the receptors and a long dissociation constant.[4] The dissociation constant of methylnaltrexone is shorter than that of buprenorphine, and these characteristics may reduce the ability of methylnaltrexone to antagonize the effects of buprenorphine.[4] Nevertheless, the opioid antagonist naloxone can reverse buprenorphine-induced respiratory depression in man, albeit only when high doses are administered;[12] naloxone, like methylnaltrexone, has a shorter dissociation constant than that of buprenorphine.[4] Therefore perhaps the dose of methylnaltrexone used in this study was simply insufficient to effectively antagonize buprenorphine and that more effective antagonism might be achieved with higher doses, as occurs when naloxone

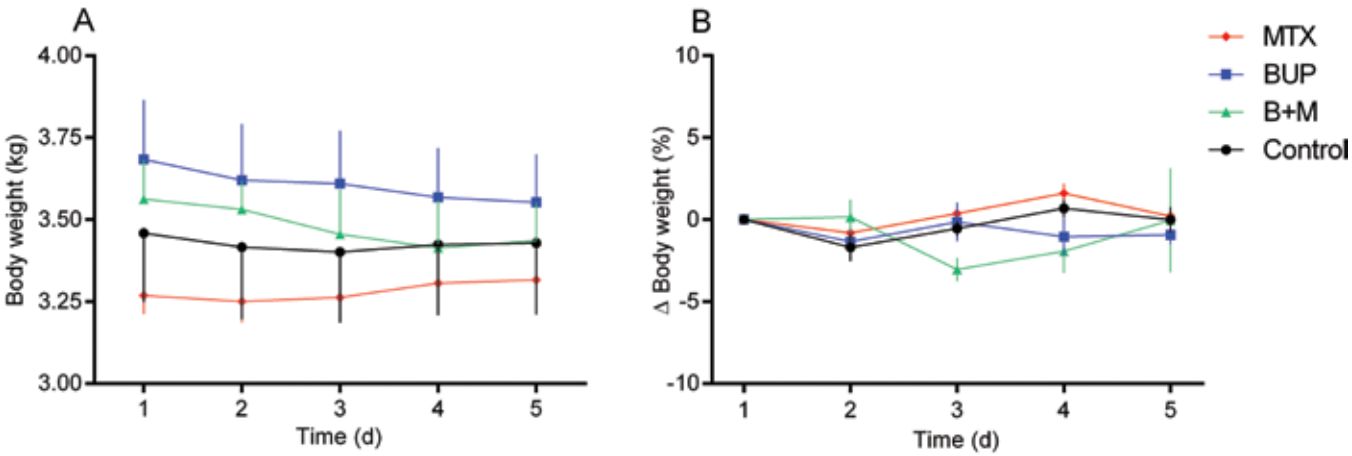

Figure 3. (A) Body weight (mean ± SE) and (B) change in body weight (mean ± SE) in 8 New Zealand White rabbits receiving normal saline, buprenorphine (BUP, 0.05 mg/kg), methylnaltrexone (MTX, 1 mg/kg), or both agents combined (B+M).

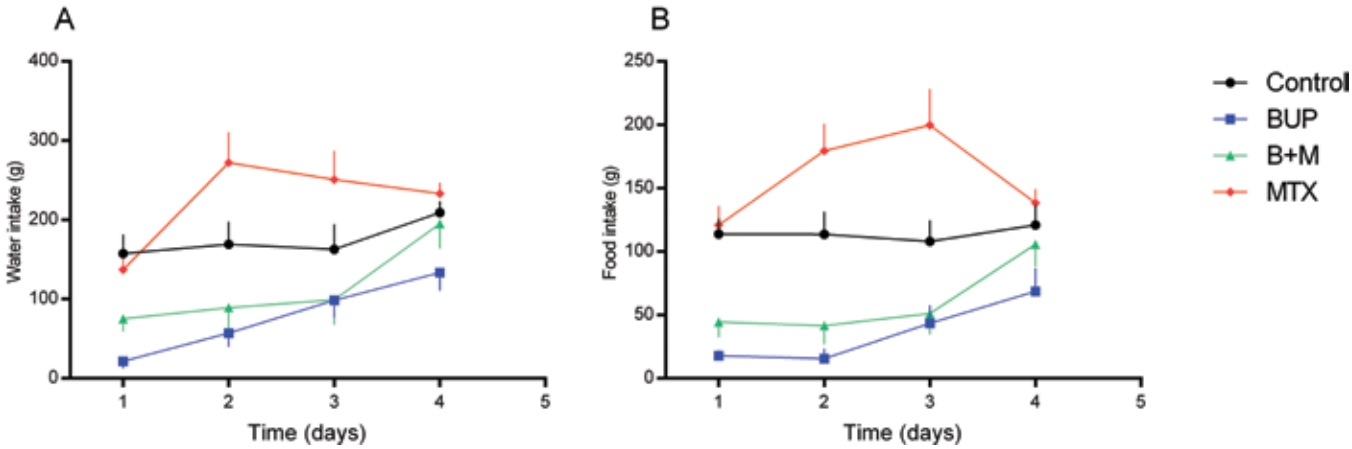

Figure 4. (A) Water and (B) food consumption (mean ± SE) in 8 New Zealand White rabbits receiving normal saline, buprenorphine (BUP, 0.05 mg/kg), methylnaltrexone (MTX, 1 mg/kg), or both agents combined (B+M). Water and food consumption were significantly ($P < 0.0001$) lower for rabbits treated with buprenorphine or B+M than for control and methylnaltrexone groups; in addition, food and water consumption were greater in the methylnaltrexone group than in control animals ($P < 0.0001$ for both comparisons).

interacts with buprenorphine. Methylnaltrexone doses of 1 mg/kg or less have been successfully used in humans and horses to antagonize morphine-induced gastrointestinal ileus.[2,14] The discrepancies between those previous results and our current findings may reflect interspecies differences or differences in the efficacy of methylnaltrexone when other opioid agonists are used.

Unexpectedly, the administration of methylnaltrexone alone increased water and food consumption and fecal production. The increase in fecal production might simply reflect the increased food consumption in these rabbits. Our data do not allow us to determine whether this increase was due to a direct effect of methylnaltrexone on appetite or to an antagonistic effect on endogenous endorphins, which might have been upregulated subsequent to the stress associated with general anesthesia and orogastric intubation. In horses, the administration of methylnaltrexone increased the quantity of feces excreted, however, whether food consumption increased as well is not reported.[2]

Several study limitations should be considered: buprenorphine typically is administered to relieve pain, whereas our rabbits had not been exposed to any noxious stimulus, and the effects of buprenorphine on gastrointestinal transit might differ, at least in their magnitude, when animals experience painful stimuli. Reduced fecal output occurs in rabbits during the postoperative period, regardless of the method of analgesia used;[5] therefore, buprenorphine will not necessarily worsen postoperative gastrointestinal hypomotility in rabbits. The method for measuring gastrointestinal transit time, the administration of radiopaque spheres, likely affected gastrointestinal motility, because it required general anesthesia. Moreover, gastrointestinal transit can differ with various diets. Therefore, our results regarding gastrointestinal transit time do not represent normal transit time in rabbits but rather may only apply to these particles. However, because this technique was used in the same way in all treatment groups, assessment of gastrointestinal time is still a useful parameter when comparing the effects of drugs on gastrointestinal motility.

In conclusion, twice-daily treatment of healthy rabbits with buprenorphine (0.05 mg/kg) for 2 d decreased food and water consumption, prolonged gastrointestinal transit time, and decreased fecal output. However, no serious complications requiring intervention occurred during the experimental period. The coadministration of methylnaltrexone (1 mg/kg SC) did not alleviate these negative side effects.

Acknowledgment

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References

1. **Animal Welfare Act as Amended**. 2013. 7 USC §2131–2159.
2. **Boscan P, Van Hoogmoed LM, Pypendop BH, Farver TB, Snyder JR.** 2006. Pharmacokinetics of the opioid antagonist N-methylnaltrexone and evaluation of its effects on gastrointestinal tract function in horses treated or not treated with morphine. Am J Vet Res **67:**998–1004.
3. **Boas RA, Villiger JW**. 1985. Clinical actions of fentanyl and buprenorphine. The significance of receptor binding. Br J Anaesth **57:**192–196.
4. **Cassel JA, Daubert JD, DeHaven RN.** 2005. [^{3}H]Alvimopan binding to the μ opioid receptor: comparative binding kinetics of opioid antagonists. Eur J Pharmacol **520:**29–36.
5. **Cooper CS, Metcalf-Pate KA, Barat CE, Cook JA, Scorpio DG.** 2009. Comparison of side effects between buprenorphine and meloxicam used postoperatively in Dutch Belted rabbits (*Oryctolagus cuniculus*). J Am Assoc Lab Anim Sci **48:**279–285.
6. **Coulter CA, Flecknell PA, Leach MC, Richardson CA.** 2011. Reported analgesic administration to rabbits undergoing experimental surgical procedures. BMC Vet Res **7:**1–6.
7. **Foss JF.** 2001. A review of the potential role of methylnaltrexone in opioid bowel dysfunction. Am J Surg **182 Suppl 1:**S19–S26.
8. **Institute for Laboratory Animal Research**. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
9. **Martin-Flores M, Campoy L, Kinsley MA, Mohammed HO, Gleed RD, Cheetham J.** 2014. Analgesic and gastrointestinal effects of epidural morphine in horses after laparoscopic cryptorchidectomy under general anesthesia. Vet Anaesth Analg **41:**430–437.
10. **Sano H, Martin-Flores M, Santos LC, Cheetham J, Araos JD, Gleed RD.** 2011. Effects of epidural morphine on gastrointestinal transit in unmedicated horses. Vet Anaesth Analg **38:**121–126.
11. **US Department of Agriculture**. [Internet]. 2015. Annual report animal usage by fiscal year. [Cited 31 Jan 2015]. Available at: https://www.aphis.usda.gov/animal_welfare/downloads/7023/Animals%20Used%20In%20Research%202014.pdf
12. **van Dorp E, Yassen A, Sarton E, Romberg R, Olofsen E, Teppema L, Danhof M, Dahan A.** 2006. Naloxone reversal of buprenorphine-induced respiratory depression. Anesthesiology **105:**51–57.
13. **Weaver LA, Blaze CA, Linder DE, Andrutis KA, Karas AZ.** 2010. A model for clinical evaluation of perioperative analgesia in rabbits (*Oryctolagus cuniculus*). J Am Assoc Lab Anim Sci **49:**845–851.
14. **Yuan CS, Foss JF, O'Connor M, Toledano A, Roizen MF, Moss J.** 1996. Methylnaltrexone prevents morphine-induced delay in oral–cecal transit time without affecting analgesia: a double-blind randomized placebo-controlled trial. Clin Pharmacol Ther **59:**469–475.