Perioperative analgesia associated with oral administration of a novel methadone-fluconazole-naltrexone formulation in dogs undergoing routine ovariohysterectomy

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OBJECTIVE
To determine perioperative analgesia associated with oral administration of a novel methadone-fluconazole-naltrexone formulation in dogs undergoing routine ovariohysterectomy.

ANIMALS
43 healthy female dogs.

PROCEDURES
Dogs were randomly assigned to receive the methadone-fluconazole-naltrexone formulation at 1 of 2 dosages (0.5 mg/kg, 2.5 mg/kg, and 0.125 mg/kg, respectively, or 1.0 mg/kg, 5.0 mg/kg, and 0.25 mg/kg, respectively, PO, q 12 h, starting the evening before surgery; n = 15 each) or methadone alone (0.5 mg/kg, SC, q 4 h starting the morning of surgery; 13). Dogs were sedated with acepromazine, and anesthesia was induced with propofol and maintained with isoflurane. A standard ovariohysterectomy was performed by experienced surgeons. Sedation and pain severity (determined with the Glasgow Composite Pain Scale—short form [GCPS-SF]) were scored for 48 hours after surgery. Rescue analgesia was to be provided if the GCPS-SF score was > 6. Dogs also received carprofen starting the day after surgery.

RESULTS
None of the dogs required rescue analgesia. The highest recorded GCPS-SF score was 4. A significant difference in GCPS-SF score among groups was identified at 6:30 AM the day after surgery, but not at any other time. The most common adverse effect was perioperative vomiting, which occurred in 11 of the 43 dogs.

CONCLUSIONS AND CLINICAL RELEVANCE
Oral administration of a methadone-fluconazole-naltrexone formulation at either of 2 dosages every 12 hours (3 total doses) was as effective as SC administration of methadone alone every 4 hours (4 total doses) in dogs undergoing routine ovariohysterectomy. Incorporation of naltrexone in the novel formulation may provide a deterrent to human opioid abuse or misuse. (*Am J Vet Res* 2020;81:699–707)

Methadone is a μ-opioid receptor agonist that has been approved in countries other than the United States for the control of postoperative pain in dogs. It is labeled for IV, IM, and SC administration with an approximate duration of action of 4 hours.[1] This short duration of action is typical of opioids in dogs and is a result of rapid hepatic metabolism and clearance, resulting in a short half-life.[2,3] Methadone is not effective when administered orally to dogs at relevant doses because of low oral bioavailability presumably due to a substantial first-pass effect by which most of the drug is metabolized in the intestines or liver prior to reaching the systemic circulation.[3–5]

ABBREVIATIONS

CRI	Constant rate infusion
GCPS-SF	Glasgow Composite Pain Scale—short form
OHE	Ovariohysterectomy

A drug that increases another drug's oral bioavailability or prolongs its duration of action is termed a pharmacokinetic enhancer. Pharmacokinetic enhancers are included with several drugs approved for use in human patients. For example, quinidine is a pharmacokinetic enhancer that has been used with dextromethorphan[a] to increase plasma dextromethorphan concentrations and extend the drug's duration of action.[6] Similarly, a treatment for HIV infection combines the antiviral darunavir with the pharmacokinetic enhancer cobicistat.[7,b]

Recent studies of dogs have documented several pharmacokinetic enhancers for orally administered methadone. Chloramphenicol is an effective pharmacokinetic enhancer of methadone in dogs, but con-

cerns with the potential for aplastic anemia following human exposure to chloramphenicol could limit its widespread use.[4,8] Additional studies have documented that fluconazole is a pharmacokinetic enhancer of methadone in dogs.[5] In contrast to chloramphenicol, fluconazole is not associated with severe adverse effects following unintentional human exposure. Additionally, fluconazole is well tolerated in dogs. The dosage at which no observable hepatic adverse effects are observed in dogs is 7.5 mg/kg/d for 6 months, and doses as high as 20 mg/kg/d are routinely used to treat systemic fungal diseases in dogs.[9,10] Although other adverse effects are not reported in the literature, the authors (BK and KK) have observed anorexia with fluconazole administration to dogs.

A recently developed novel oral formulation containing methadone, fluconazole, and naltrexone has been shown to provide long-lasting (approx 12 hours) opioid effects in dogs.[5] Fluconazole is included in the formulation as a pharmacokinetic enhancer of methadone; naltrexone is included as a deterrent to human opioid abuse and misuse.[11,12] Additionally, the authors hypothesize that naltrexone might mitigate the opioid effects of methadone if inadvertent human exposure to the formulation occurs (eg, ingestion by a child). Opioid effects are maintained in dogs despite the inclusion of naltrexone owing to naltrexone's low bioavailability and the poor formation of its active metabolite 6β-naltrexol in this species.[13] In contrast, orally administered naltrexone antagonizes opioid effects in humans and, in opioid-dependent humans, elicits symptoms of withdrawal owing to the absorption of naltrexone and the formation of 6β-naltrexol.[11,12]

We believe that this novel methadone-fluconazole-naltrexone formulation may be useful for providing perioperative analgesia in dogs. However, its clinical efficacy has not been studied. Therefore, the purpose of the study reported here was to compare, in dogs undergoing standard OHE, perioperative analgesia obtained with a methadone-fluconazole-naltrexone formulation administered PO every 12 hours at 1 of 2 dosages with the perioperative analgesia obtained with methadone alone administered SC every 4 hours. The primary outcome measure was defined as the need for rescue analgesia; the secondary outcome measure was GCPS-SF scores at various time points. We hypothesized that both treatments would be well tolerated and that there would be no significant differences in the need for rescue analgesia or GCPS-SF scores among groups throughout the 48-hour postoperative period.

Materials and Methods

The study protocol was approved by the Kansas State University Institutional Animal Care and Use Committee. The enrollment target was 45 healthy sexually intact female dogs from local animal shelters or foster homes undergoing elective OHE. All clients signed an informed consent form describing the drug treatments as well as the surgery and study. Dogs were brought to the hospital the morning of the day prior to surgery. Preoperatively, dogs were confirmed to be healthy on the basis of physical examination results, measurement of PCV and total solids concentration, and a negative heartworm test result.[14] All dogs were classified as American Society of Anesthesiologists class I (normal healthy patients).

After blocking by weight, dogs were randomly assigned to 1 of 3 treatment groups by drawing treatment assignments from a bowl. The 3 treatment groups consisted of methadone alone (0.5 mg/kg, SC, q 4 h; positive control group), methadone-fluconazole-naltrexone (0.5 mg/kg, 2.5 mg/kg, and 0.125 mg/kg, respectively, PO, q 12 h; 0.5-mg/kg PO group), and methadone-fluconazole-naltrexone (1.0 mg/kg, 5.0 mg/kg, and 0.25 mg/kg, respectively, PO q 12 h; 1-mg/kg PO group). An injectable methadone formulation[c] approved by the US FDA for use in humans was used, because no comparable veterinary formulation was available for use in the United States.

Methadone-fluconazole-naltrexone capsules for oral administration were formulated in-house by a single individual (BK) using FDA-approved products for human use: methadone hydrochloride (10-mg tablets),[d] fluconazole (200-mg tablets),[e] and naltrexone hydrochloride (50-mg tablets).[f] Briefly, tablets were combined at a methadone-to-fluconazole-to-naltrexone ratio of 1:5:0.25 on the basis of stated tablet content. Tablets were weighed with a balance calibrated daily, combined, and then pulverized and homogenized with a mortar and pestle. The resultant powder was weighed and transferred to size-0 gelatin capsules[g] to achieve the desired dose for each dog.

Oral treatments were initiated between 5:00 and 6:00 PM the day prior to surgery (day 0), with additional doses given approximately 12 and 24 hours later, for a total of 3 total doses. At the same times, dogs in the control group were given a capsule containing lactose PO. Dogs in the control group were given methadone (0.5 mg/kg, SC) starting between 7:15 and 7:45 AM on the day of surgery, with additional doses given at 12:00 PM, 4:00 PM, and 8:00 PM (within a 1-hour range) that day. At the same times, dogs in the 2 treatment groups were given injections of saline (0.9% NaCl) solution[h] at volumes equivalent to the volume of methadone injections based on body weight.

Acepromazine (0.05 mg/kg, SC)[i] was administered to all dogs between 7:15 and 7:45 AM on the day of surgery, with the second oral dose of methadone administered at the same time. Dogs underwent surgery between 8:30 AM and 12:00 PM. All dogs were administered carprofen[j] (3.3 to 4.4 mg/kg, PO, q 24 h) beginning at 6:30 AM the day after surgery and continuing for a total of 5 days, consistent with then-current clinical practice.

Intravenous catheter placement started at 8:00 AM on the day of surgery. Anesthesia was induced with propofol[k] (target dose, 4 mg/kg, IV), administered in quarter-dose increments until intubation was ac-

complished. Anesthesia was maintained with isoflurane[l] in oxygen administered by veterinary technicians (RO and GJ). Lactated Ringer solution[m] was administered at a rate of 5 mL/kg/h, IV, throughout surgery. Anesthetic monitoring included continuous pulse oximetry; every 5 minutes, systolic blood pressure measured by means of Doppler ultrasonography was recorded, along with heart rate and respiratory rate.

In all dogs, a standard OHE was performed by an experienced surgeon (DAU, AC, BC, KB, or EEK). Two surgeons performed surgeries on each surgical day, with a total of 8 to 10 surgeries performed each day. A standard ventral midline approach to the abdomen was performed beginning at the umbilicus and extending a third of the distance from the umbilicus to the pubis. A modified 3-clamp technique was used with polydioxanone suture[n] to double ligate the ovarian pedicles. The uterine body was then identified, and a 3-clamp technique was used with polydioxanone suture to double ligate the uterine body. The incision was closed in 3 layers with polydioxanone suture in a continuous pattern for each layer. Tattoo ink was placed along a 2- to 3-cm-long incision parallel to the abdominal incision.

All dogs were maintained on a circulating warm water blanket from the time of anesthetic induction until they were returned to their individual runs. Dogs remained in the recovery area until they were extubated, their rectal temperature was at least 37.2°C, and they were able to walk.

In all dogs, sedation and GCPS-SF scores were assigned and rectal temperature was measured at 5 PM on the day before surgery (day 0; baseline); at 12:00, 1:00, 4:00, 6:00, and 8:00 PM the day of surgery (day 1); at 6:30 AM and 5:00 PM the day after surgery (day 2); and at 7:00 AM the second day after surgery (day 3) before dogs were discharged. Sedation was scored on a scale from 0 to 4 **(Appendix 1)**, as described.[15,16] Pain scores were based on the GCPS-SF, as described.[15–20] A GCPS-SF score > 6 (or > 5 if the dog was not mobile) was the cutoff for administration of rescue analgesia (morphine; 0.25 mg/kg, IV, IM, or SC). Adverse effects were recorded when observed. Sedation and GCPS-SF scores were assigned by a single individual (PC) who was unaware of the treatments.

Blood samples were obtained from all dogs at 4:00 and 8:00 PM on the day of surgery (day 1) and at 5:00 PM on the day after surgery (day 2) by venipuncture of a jugular or cephalic vein. Plasma was separated and stored at –70°C for later determination of plasma methadone, naltrexone, 6β-naltrexol, naltrexone glucuronide, and fluconazole concentrations.

Plasma drug concentrations were measured by means of ultrahigh-pressure liquid chromatography[o] with triple quadrupole mass spectrometry.[p] Pass-through plates were used to prepare plasma samples.[q] The mobile phase consisted of deionized water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) with the following gradient: 85% A at time 0; followed by a linear gradient to 5% A at 0.8 minutes, which was held until 1.2 minutes, and then a linear gradient to 85% A with a total run time of 2 minutes. Separation was achieved with a column[r] maintained at 40°C at a flow rate of 0.6 mL/min **(Appendix 2)**.

Statistical analysis

Kruskal-Wallis 1-way ANOVA on ranks was used to compare time from preanesthetic drug administration to catheter placement, induction dose of propofol, anesthesia time, surgery time, GCPS-SF scores, and plasma methadone concentrations among groups. Within each group, rectal temperatures were assessed for differences over time with 1-way ANOVA; however, differences between groups were not assessed because the power was < 0.8. Statistical analyses were performed with standard software.[s] Values of $P < 0.05$ were considered significant.

Results

A total of 43 dogs were enrolled, with 15 dogs assigned to each of the 2 treatment groups and 13 dogs assigned to the positive control group. Specific ages of the dogs were not known, but both juvenile and adult dogs were included, as determined by the presence or absence of deciduous teeth. One dog was a Boxer; the remainder were mixed-breed dogs. Overall mean weight was 17.7 kg (range, 2.7 to 28.0 kg). Mean weight was 17.7 kg (range, 2.7 to 19.1 kg) for the control group, 19.3 kg (range, 4.9 to 28.0 kg) for the 0.5-mg/kg PO group, and 16.2 kg (range, 6.0 to 25.0 kg) for the 1-mg/kg PO group. Results of statistical comparisons of body weights among groups were not reported owing to the low statistical power.

All dogs had IV catheters placed prior to surgery without the need for additional sedation **(Table 1)**. The time from preanesthetic drug administration to IV catheter placement was not significantly (P = 0.934) different among groups. There were no significant (P = 0.679) differences in propofol induction dose among groups, with mean doses of 4.3 mg/kg (range, 2.8 to 8.4 mg/kg) for the control group, 3.9 mg/kg (range, 2.7 to 5.5 mg/kg) for the 0.5-mg/kg PO group, and 3.7 mg/kg (range, 2.5 to 5.9 mg/kg) for the 1-mg/kg PO group. Total anesthesia time was also not significantly (P = 0.694) different among groups, with mean anesthesia times of 55.9 minutes (range, 42 to 84 minutes) for the control group, 52.4 minutes (range, 35 to 70 minutes) for the 0.5-mg/kg PO group, and 51.5 minutes (range, 38 to 78 minutes) for the 1-mg/kg PO group. Finally, surgery times did not differ significantly (P = 0.928) among groups, with mean surgery times of 26.5 minutes (range, 19 to 45 minutes) for the control group, 27.4 minutes (range, 17 to 42 minutes) for the 0.5-mg/kg PO group, and 27.3 minutes (range, 16 to 45 minutes) for the 1-mg/kg PO group.

None of the dogs required rescue analgesia while hospitalized (ie, during the 48 hours after surgery). The highest recorded GCPS-SF score was 4, which

Table 1—Preanesthetic sedation scores, ease of IV catheter placement, and time from preanesthetic drug administration to IV catheter placement in dogs undergoing routine OHE that received methadone alone (0.5 mg/kg, SC, q 4 h; positive control group; n = 13), methadone-fluconazole-naltrexone (0.5 mg/kg, 2.5 mg/kg, and 0.125 mg/kg, respectively, PO, q 12 h; 0.5-mg/kg PO group; 15), or methadone-fluconazole-naltrexone (1.0 mg/kg, 5.0 mg/kg, and 0.25 mg/kg, respectively, PO, q 12 h; 1-mg/kg PO group; 15). All dogs were given acepromazine (0.05 mg/kg, SC) the morning of surgery.

	Group		
Variable	**Control**	**0.5-mg/kg PO**	**1-mg/kg PO**
Preanesthetic sedation score*			
0 (no sedation)	0	1	0
1 (slight sedation)	7	14	13
2 (moderate sedation)	3	0	1
3 (profound sedation)	3	0	1
4 (unresponsive)	0	0	0
Ease of IV catheter placement*			
Unable to restrain the dog	0	0	0
Dog could be restrained with difficulty	2	5	3
Dog was restrained with little effort	8	10	12
Dog was unable to remain in a sternal position	3	0	0
Time from preanesthetic drug administration to catheter placement (min)†	101 (33–176)	97 (32–195)	102 (29–217)

*Data represent number of dogs. †Data represent mean (range).

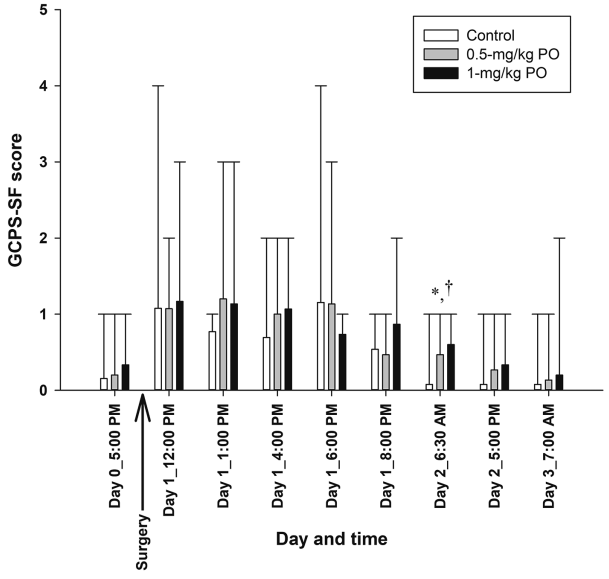

Figure 1—Mean GCPS-SF scores in dogs undergoing routine OHE that received methadone alone (0.5 mg/kg, SC, q 4 h; positive control group; n = 13), methadone-fluconazole-naltrexone (0.5 mg/kg, 2.5 mg/kg, and 0.125 mg/kg, respectively, PO, q 12 h; 0.5-mg/kg PO group; 15), or methadone-fluconazole-naltrexone (1.0 mg/kg, 5.0 mg/kg, and 0.25 mg/kg, respectively, PO, q 12 h; 1-mg/kg PO, group; 15). *Values were significantly (P = 0.027) different between the control group and the 0.5-mg/kg PO group. †Values were significantly (P = 0.005) different between the control group and the 1-mg/kg PO group. Error bars represent maximum score.

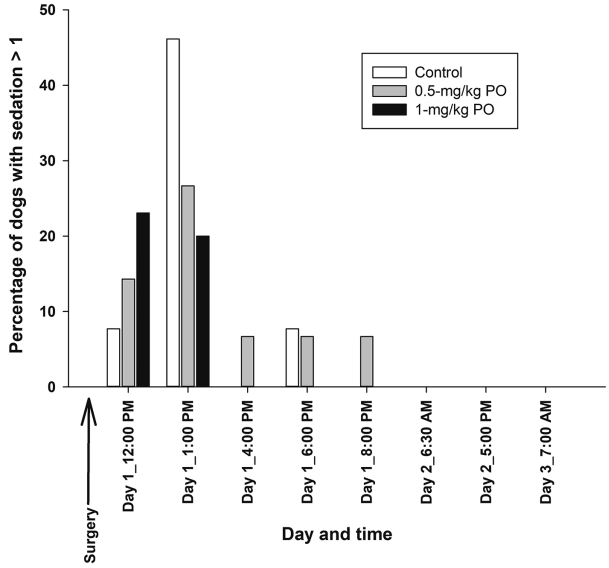

Figure 2—Percentages of dogs in the groups described in Figure 1 with sedation scores > 1 at various times after surgery. Sedation was scored on a scale from 0 to 4, with 0 = no sedation, 1 = slight sedation, 2 = moderate sedation, 3 = profound sedation, and 4 = unresponsive.

was recorded for dogs in the control group. For both treatment groups, the highest GCPS-SF score at any time point was 3 **(Figure 1)**. A significant difference in GCPS-SF score among groups was identified at 6:30 AM on day 2, with mean score for the control group (mean ± SD, 0.077 ± 0.277) significantly lower than that for the 0.5-mg/kg PO group (0.467 ± 0.516; P = 0.027) and that for the 1-mg/kg PO group (0.600 ± 0.507; P = 0.005) even though all individual scores in all 3 groups were 0 or 1. There were no significant differences in GCPS-SF scores among groups at any other time point.

Sedation had resolved in all dogs by 6:30 AM on day 2, which was < 24 hours after surgery **(Figure 2)**. The highest percentage of dogs with a sedation score > 1 (ie, dogs with moderate or higher sedation) occurred in the control group

Table 2—Rectal temperatures (°C) of the dogs in the groups described in Table 1.

	Group					
	Control		0.5-mg/kg PO		1-mg/kg PO	
Day and time	Mean (range)	*P* value	Mean (range)	*P* value	Mean (range)	*P* value
Day 0	39.1 (38.6–39.8)	NA	38.8 (38.2–39.9)	NA	38.7 (38.1–39.4)	NA
Day 1; 12:00 PM	37.9 (36.3–39.0)	< 0.001	38.1 (37.4–38.8)	< 0.001	37.9 (36.4–39.0)	0.002
Day 1; 1:00 PM	37.6 (36.5–39.0)	< 0.001	38.3 (37.4–39.2)	0.025	38.1 (36.8–39.3)	0.033
Day 1; 4:00 PM	38.5 (37.4–39.2)	0.038	38.6 (37.1–39.6)	0.441	38.2 (37.4–39.3)	0.095
Day 1; 6:00 PM	37.7 (37.0–39.1)	< 0.001	38.6 (37.2–39.4)	0.287	38.4 (37.3–39.2)	0.187
Day 1; 8:00 PM	38.1 (37.2–38.9)	< 0.001	38.5 (37.9–39.3)	0.275	38.3 (37.6–39.3)	0.190
Day 2; 6:30 AM	38.6 (38.2–39.2)	0.119	37.9 (37.2–38.6)	< 0.001	37.6 (36.9–38.3)	< 0.001
Day 2; 5:00 PM	38.9 (38.4–40.4)	0.489	38.5 (37.6–39.1)	0.136	37.9 (36.6–39.0)	< 0.001
Day 3; 7:00 AM	38.6 (38.3–39.7)	0.093	38.7 (38.0–39.3)	0.472	38.5 (37.3–39.3)	0.433

Day 1 was the day of surgery (all surgeries were performed between 8:00 AM and 12:00 PM). *P* values are for the comparison of temperatures at each time point with the day 0 temperature for the same group.

NA = Not applicable.

Table 3—Plasma methadone concentrations (ng/mL) for the dogs in the groups described in Table 1.

	Group		
Day and time	Control	0.5-mg/kg PO	1-mg/kg PO
Day 1; 4:00 PM	14.4 (5.4–32.9)[a]	20.6 (2.6–30.3)[a]	36.2 (3.2–67.7)
Day 1; 8:00 PM	22.4 (14.8–47.7)	17.6 (2.0–29.8)[a]	27.7 (2.1–54.8)
Day 2; 5:00 PM	2.0 (0–10.3)[a,b]	14.1 (3.6–41.7)[a]	46.7 (1.4–98.5)

Data are given as median (range). Day 1 was the day of surgery (all surgeries were performed between 8:00 AM and 12:00 PM).

[a]Significantly different from the value for the 1-mg/kg PO group. [b]Significantly different from the value for the 0.5-mg/kg PO group.

at 1:00 PM on the day of surgery. Profound sedation (sedation score of 3) was noted in 6 dogs across all treatment groups on day 1 at 1:00 PM and in 1 dog in the 0.5-mg/kg PO group on day 1 at 4:00 PM. All dogs were ambulatory by 6:00 PM the day of surgery. One dog in the 0.5-mg/kg PO group had moderate sedation at 8:00 PM on day 1. At that time, this dog had a plasma methadone concentration of 18.1 ng/mL, which was near the median plasma concentration at that time for the group.

Mean rectal temperature was significantly decreased from baseline temperature (ie, rectal temperature the night prior to surgery) in all 3 groups at various times after surgery **(Table 2)**. Median plasma methadone concentration for the 1-mg/kg PO group was significantly higher than concentrations for both the control group and the 0.5-mg/kg PO group at 4:00 PM on day 1 **(Table 3)**. At 8:00 PM on day 1, median plasma methadone concentration for the 1-mg/kg PO group was significantly higher than median concentration for the 0.5-mg/kg PO group but was not significantly different between the control and 1-mg/kg PO groups or between the control and 0.5-mg/kg PO groups. Median plasma methadone concentration for the 1-mg/kg PO group was significantly higher than median concentrations for the control and 0.5-mg/kg PO groups, and median concentration for the 0.5-mg/kg PO group was significantly higher than median concentration for the control group at 5:00 PM on day 2.

Naltrexone was not detected in any plasma sample from any dog, and naltrexone-glucuronide, 6β-naltrexol, and fluconazole were not detected in plasma samples from dogs in the control group. Naltrexone glucuronide was detected in most plasma samples from dogs in the 2 treatment groups, but 6β-naltrexol was rarely detected (7/120 samples), with 1.7 ng/mL as the highest measured concentration (for this assay, the limit of quantitation was 1 ng/mL). Fluconazole was detected in all plasma samples from dogs in the 2 treatment groups, with concentrations ranging from 1.1 to 7.7 µg/mL.

The most common adverse effect was perioperative vomiting, which occurred in 2 of the 13 dogs in the control group, 2 of the 15 dogs in the 0.5-mg/kg PO group, and 7 of the 15 dogs in the 1-mg/kg PO group. Post hoc power analysis yielded a sample size of 40 dogs needed to achieve an α of 0.05 with a power of 0.8 to detect a statistical difference among these proportions. Two of the dogs in the 1-mg/kg PO group were administered ondansetron after vomiting ≥ 3 times. Both of these dogs were considered puppies because they had all of their deciduous teeth and tested negative for parvovirus with an ELISA.[t]

Discussion

Results of the present study suggested that PO administration of a novel methadone-fluconazole-naltrexone formulation at either of 2 dosages every

12 hours (for 3 total doses) was as effective as SC administration of methadone alone every 4 hours (for 4 total doses) in dogs undergoing routine OHE. Injectable formulations of methadone have been approved as being safe and efficacious for use as an analgesic in dogs in some countries other than the United States. Therefore, we used methadone as our positive control in the present study. Although compliance was not examined in our study, we expect that compliance in a typical veterinary clinic would be higher with twice-daily PO administration of a medication than with SC injection every 4 hours.

Our 2 outcome measures for analgesic efficacy in the present study were the need for rescue analgesia and GCPS-SF score, and we hypothesized that neither outcome would be significantly different among groups. In alignment with this hypothesis, none of the 43 dogs in the study required rescue analgesia. In contrast, there was 1 time point (6:30 AM on day 2) when mean GCPS-SF score for the control group was significantly lower than mean scores for the 2 treatment groups. However, GCPS-SF scores were 1 or 0 for all dogs at this time. Dogs assigned a GCPS-SF score of 1 were either vocalizing (crying or whimpering) or had a quieter than normal demeanor, which we suspected was due to persistent opioid effects. A previous study[21] documented increased whining in pain-free dogs after methadone administration and cautioned that it could be misinterpreted as pain or discomfort.

Median plasma methadone concentrations were significantly higher at 5:00 PM on day 2 (approx 21 hours after the last dose of methadone was administered to all dogs) in the 2 treatment groups than in the control group. No differences were detected among groups in GCPS-SF scores at that time, but all dogs had also been administered carprofen earlier that day, which might have minimized the potential to evaluate the benefits of prolonged methadone concentrations.

Opioids can decrease rectal temperature in dogs by stimulating opioid receptors in the hypothalamus.[22] However, low rectal temperature recorded in the dogs of our study did not require any intervention in excess of placing blankets on the concrete floor of the runs where the dogs were housed. Opioid-mediated decreases in rectal temperature have been demonstrated to be dose dependent, similar to antinociceptive effects in healthy, pain-free dogs.[5,23–25] In the present study, dogs received a number of drugs other than methadone that might also affect body temperature, including acepromazine, propofol, and isoflurane. Additionally, baseline temperatures might have been artificially elevated owing to recent transport, excitement, or agitation. Therefore, although multiple confounders could have contributed to the significant decreases in rectal temperature, compared with baseline temperature, the observed temperature decrease could have been an indicator of central opioid effects. It is interesting to note that rectal temperature was significantly decreased from the baseline temperature in both treatment groups at 6:30 AM on day 2 and additionally in the 1-mg/kg PO group at 5:00 PM. These decreased temperatures might have been an indication of prolonged opioid effects in the dogs in these 2 groups, compared with dogs in the control group, because these times corresponded to times when significantly higher plasma methadone concentrations were detected in the treatment groups.

Ovariohysterectomy has been used in previous studies[26–36] of the efficacy of analgesics in dogs, with the GCPS-SF used to assess postoperative pain severity. All of those studies used a GCPS-SF score ≥ 5 as an indication of the need for rescue analgesia, and only 3 of the treatments (fentanyl CRI, buprenorphine-carprofen, and lidocaine-ketamine-dexmedetomidine CRI) were sufficiently effective that none of the dogs required rescue analgesia.[27,34] Two of those treatments were CRIs that are not commonly used for dogs undergoing OHE, can be technically challenging to administer, and require appropriate infusion equipment and monitoring to attain accurate and precise dosing. The remaining studies had treatment failure rates ranging from 5% to 90% and included studies evaluating the efficacy of the following analgesics: buprenorphine, buprenorphine-carprofen, buprenorphine-cimicoxib, butorphanol, carprofen, cimicoxib, dexketoprofen, dexmedetomidine CRI, ketamine CRI, lidocaine CRI, morphine, morphine-tramadol, and tramadol. These data suggest not only that is OHE a feasible model for assessing postoperative analgesia in dogs undergoing soft tissue surgery, but also that dogs undergoing OHE require effective postoperative analgesia. Importantly, comparisons between these studies and the present study to assess relative analgesic efficacy may be inaccurate because of differences in study design, surgeon skill, concurrent anesthetics, enrollment criteria (eg, age, weight, and breed), and surgical technique. However, these studies demonstrate that analgesia is a necessary component of OHE in dogs despite the routine nature of this procedure in companion animal practice. The present study demonstrated that SC administration of methadone (0.5 mg/kg) every 4 hours and PO administration every 12 hours of methadone-fluconazole-naltrexone at dosages of 0.5 mg/kg, 2.5 mg/kg, and 0.125 mg/kg, respectively, or 1.0 mg/kg, 5.0 mg/kg, and 0.25 mg/kg, respectively, were effective at providing postoperative analgesia in dogs undergoing OHE.

A negative control group of dogs that did not receive postoperative analgesics was not included in the present study because of ethical concerns. Previous studies[19,20,36] assessing the efficacy of analgesics in dogs undergoing soft tissue surgery, including OHE, in which the GCPS-SF was used to assess pain severity had placebo failure rates between 36% and 76%. Given the results of those studies, the authors could not justify inclusion of a negative control group.

Assessing postoperative pain in dogs is difficult. A variety of methods have been proposed including, but not limited to, the GCPS-SF, visual analog scales, and numeric rating scales.[37] Limitations of the GCPS-SF are known and include confounding effects of sedation, dysphoria, concurrent orthopedic or neurologic conditions, and individual animal behavior, including anxiety. Visual analog and numeric rating scales, however, have limitations similar to those described for the GCPS-SF and might be biased by expected pain results. We decided to use the GCPS-SF in the present study because it has been used as an assessment tool for analgesic drug approval by the US FDA.[15,17,19,20]

For dogs in the present study, carprofen was administered beginning the day after surgery, consistent with our current clinical practice. Nonsteroidal antiinflammatory drugs have proven efficacy for postoperative pain, are convenient for dispensing to clients, and lack the potential for illicit abuse. A limitation of NSAIDs used alone in the immediate perioperative period is reported treatment failure rates ranging from 6% to 28% for dogs undergoing soft tissue surgery, when pain severity was measured with the GCPS-SF with the same cutoff used in the present study.[17,19,20] None of the dogs in the present study required rescue analgesia, but a randomized controlled trial would be needed to determine whether greater analgesic effects can be documented in the immediate postoperative period with an opioid versus an NSAID.

In the novel drug formulation used in the present study, fluconazole was included as a pharmacokinetic enhancer of methadone because of its ability to inhibit the cytochrome P450 enzyme. However, fluconazole might also affect the clearance and, subsequently, the duration of effects of other drugs administered in the postoperative period. On the basis of clinical experience of 2 authors (BK and KK), fluconazole appears to have limited interactions with other commonly administered perioperative drugs, including acepromazine, butorphanol, propofol, and isoflurane. A recent study[38] evaluating the effects of fluconazole on the pharmacokinetics and clinical effects of ketamine-midazolam following IV administration described increases in the half-life (approx 50% for ketamine and midazolam) and time to standing (73 vs 36 minutes), but no difference in time to attaining a sternal position (32 vs 25 minutes). Although significant effects were demonstrated, no contraindications to the use of fluconazole were identified.

Vomiting was the most common adverse effect in the present study. The 1-mg/kg PO treatment group had the highest proportion of dogs vomiting; however, there was not sufficient statistical power to assess differences in the proportion of dogs vomiting among groups. Interestingly, the 2 dogs treated with ondansetron because of excessive vomiting were both puppies, which might indicate that puppies are more prone to vomiting than older dogs. However, additional studies including larger numbers of dogs are needed to assess the emetic potential of the methadone-fluconazole-naltrexone formulation and determine whether any of its adverse effects are age dependent.

Excessive sedation can be an adverse effect of postoperative opioid administration. Sedation scores varied within all 3 groups in the present study, which was expected. Nevertheless, all dogs were ambulatory by 6:00 PM the day of the surgery, and only 1 dog was moderately sedate at 8:00 PM on that day. The latter dog might possibly have been more sensitive to the opioid effects of the methadone-fluconazole-naltrexone formulation or to the effects of anesthesia, because plasma methadone concentration in this dog was similar to the median plasma concentration for that group at the same time. Regardless, our findings suggested that excessive sedation in the postoperative period was not common.

All drugs used in the present study had been approved by the US FDA for use in humans or animals. Use of human-approved drugs in animals is allowed under the extralabel drug use provisions of the AMDUCA. Currently, there are no opioid analgesics approved for use in dogs in the United States, regardless of the route of administration. Some veterinarians are hesitant to prescribe opioids because of the risk of misuse or diversion. For example, 37% of veterinarians surveyed in South Dakota altered their prescribing of opioids on the basis of public perception of opioid misuse and not on the basis of perceived need.[39] However, inclusion of naltrexone in the methadone-fluconazole-naltrexone formulation mitigates the risk of illicit human use.

In conclusion, PO administration of a novel methadone-fluconazole-naltrexone formulation at either of 2 dosages was effective in controlling postoperative pain in dogs undergoing routine OHE, as was SC administration of methadone alone. The incorporation of naltrexone in the novel formulation might provide a deterrent to human opioid abuse and misuse and may mitigate the effects of accidental human exposure. Further studies are needed to assess the effectiveness of the methadone-fluconazole-naltrexone formulation in dogs undergoing various other soft tissue surgery procedures, in dogs undergoing orthopedic surgery procedures, and in dogs with chronic pain conditions (eg, osteoarthritis, cancer-associated pain, and intervertebral disk disease).

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Kansas State University holds a provisional patent and a pending patent on the methadone-fluconazole-naltrexone formulation.

Footnotes

a. Nuedexta, Avanir Pharmaceuticals Inc, Aliso Viejo, Calif.
b. Prezcobix, Janssen Therapeutics, Division of Janssen Products, Titusville, NJ.

c. Methadone hydrochloride (10 mg/mL), Akorn Inc, Lake Forest, Ill.
d. Elite Pharmaceuticals Inc, Northvale, NJ.
e. BluePoint Laboratories, manufactured by Glenmark Pharmaceuticals Ltd, Calvale-Bardaz, Goa, India.
f. Mallinckrodt Inc, Hazelwood, Mo.
g. Capsuline, Pompano Beach, Fla.
h. Sterile saline, VetOne, Boise, Idaho.
i. VetOne, Boise, Idaho.
j. Dechra Veterinary Products, Overland Park, Kan.
k. 10 mg/mL, Hospira Inc, Lake Forest, Ill.
l. Primal Critical Care Inc, Bethlehem, Pa.
m. Vetivex, Dechra Veterinary Products, Overland Park, Kan.
n. Oasis, Mettawa, Ill.
o. Acquity Prominence UPLC, Waters Corp, Milford, Mass.
p. TQD, Waters Corp, Milford, Mass.
q. Ostro Pass-through Sample Preparation Plate, Waters Corp, Milford, Mass.
r. Acquity UPLC HSS T3 column (1.8 µm; 2.1 X 50 mm), Waters Corp, Milford, Mass.
s. Sigma Plot, version 12.5, Systat Software Inc, San Jose, Calif.
t. Snap Parvo Test, Idexx Laboratories Inc, Westbrook, Me.

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Appendix 1

Scoring system used to assess sedation in dogs.

Score	Brief description	Detailed description
0	No sedation	Normal
1	Slight sedation	Almost normal; able to stand easily but appears somewhat fatigued, subdued, or somnolent
2	Moderate sedation	Able to stand but prefers to be recumbent; sluggish; ataxic or uncoordinated
3	Profound sedation	Unable to rise but can have some awareness of environment; responds to stimuli through body movement; may be in lateral or sternal recumbency
4	Unresponsive	In a state of coma or semicoma from which little or no response can be elicited; remains in lateral recumbency

Appendix 2

Settings, internal standards, and validation of an ultrahigh-pressure liquid chromatography with triple quadrupole mass spectrometry assay for measuring methadone, naltrexone, 6β-naltrexol, naltrexone glucuronide, and fluconazole concentrations in canine plasma samples.

Variable	Methadone	Fluconazole	Naltrexone	6β-naltrexol	Naltrexone glucuronide
m/z*	310 → 265	307 → 220	342 → 324	344 → 326	516 → 113
Internal standard	Methadone d9	Voriconazole	Naltrexone-d3	Naltrexone-d3	Naltrexone glucuronide-d3
m/z* of internal standard	319 → 268	350 → 281	345 → 270	345 → 270	519 → 113
Accuracy	1 ng/mL = 114%	0.5 μg/mL = 93%	0.5 ng/mL = 96%	1 ng/mL = 95%	10 ng/mL = 106%
	10 ng/mL = 105%	10 μg/mL = 98%	25 ng/mL = 93%	25 ng/mL = 91%	50 ng/mL = 94%
	100 ng/mL = 113%	50 μg/mL = 90%	50 ng/mL = 103%	50 ng/mL = 99%	100 ng/mL = 99%
Precision	1 ng/mL = 15%	0.5 μg/mL = 9%	0.5 ng/mL = 14%	1 ng/mL = 29%	10 ng/mL = 3%
	10 ng/mL = 6%	10 μg/mL = 8%	25 ng/mL = 5%	25 ng/mL = 6%	50 ng/mL = 4%
	100 ng/mL = 9%	50 μg/mL = 6%	50 ng/mL = 2%	50 ng/mL = 3%	100 ng/mL = 4%
Lower limit of quantification	0.5 ng/mL	0.2 μg/mL	0.5 ng/mL	1 ng/mL	10 ng/mL

*Given as precursor ion → quantifying ion.

Note that for 6β-naltrexol, the value for 1 of the quality control samples was –30.3%; however, values for the other 2 quality control samples were –0.5% and 8.2%, meaning that values were within the acceptable range for 2 of the 3 samples.