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Evaluation of Analgesic Efficacy and Associated Plasma Concentration of Tramadol and O-desmethyltramadol Following Oral Administration Post Ovariohysterectomy

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ABSTRACT

Tramadol is used in both human and veterinary medicine to treat postoperative pain. In human subjects, variation in metabolism of tramadol has led to inconsistent analgesia in some individuals. The purpose of the current study was to evaluate tramadol as a sole postoperative analgesic and to compare drug plasma concentration to clinical pain score. A high or low dose of tramadol was randomly assigned and administered to 14 mixed breed female dogs after ovariohysterectomy. The Short-Form of the Glasgow Composite Measure Pain Scale was used for pain evaluation post-operatively. Plasma was collected for evaluation of tramadol and

O-desmethyltramadol concentrations. The effect of weight and dose on pain scores as well as how pain score correlated with plasma concentration of tramadol and O-desmethyltramadol was evaluated. A significant difference in pain score was noted between doses when weight classes were pooled. The plasma concentrations did not correlate with pain score. Based on our results, the use of tramadol as a sole analgesic agent provides inadequate postoperative pain control but further evaluation of tramadol as a postoperative analgesic agent is needed.

INTRODUCTION

Pain management in the veterinary patient can be complicated and frustrating for both practitioners and owners. With patients that do not speak and hide their pain as an evo-

lutionary trait it is difficult to determine the effective analgesic dose in any individual. Finding a medication that is easy to use, effective and safe can be challenging; especially when medicating dogs in the home environment. Failure to control postoperative pain can result in the development of chronic pain and complex pain syndromes which often manifest as behavioral changes. Aggression, fear, and self-mutilation are not uncommon sequelae that can damage the human animal bond and have a substantial influence on patient wellbeing and client satisfaction. (Coderre, 1986; Ward, 1994; Weisman-Orr, 2004)

Due to the strictly enforced legal requirement to appropriately store and document the use of schedule 2 opioids and the questionable efficacy of oral opioid administration in dogs, veterinarians have turned to tramadol for the treatment of postoperative pain. It is often used as an adjunct to other analgesics, but in some cases may be used alone. (KuKanich, et al 2013; Pascoe, et al 2011) In the United States, tramadol is typically administered to dogs orally in the form of 50 mg sustained-release tablets every 6-12 hours.

Compared with other oral opioid preparations tramadol has fewer legislative requirements being classified as schedule IV by the DEA. It is inexpensive, easy for owners to administer and has minimal effects on the kidneys or liver. (Kongara, et al 2008; Tolman, 1998) Current dose recommendations in Plumb's Veterinary Drug Handbook are wide ranging being anywhere from 2-4 mg/kg orally every 12 hours. While veterinary studies to date have found doses in the range of to 5-10 mg/kg orally every 4-6 hours are necessary to observe analgesic effects. (KuKanich, et al 2004; KuKanich, et al 2011) Therefore choosing a clinically effective dose can be challenging and a dose range based on both plasma concentration and corresponding clinical signs of analgesia has yet to be determined.

Tramadol is a synthetic codeine analog composed of a racemic mixture of two en-

antiomers which have effects at the μ -opioid receptor, the δ -opioid receptor, and weakly at the κ -opioid receptor. Tramadol also inhibits endogenous serotonin and norepinephrine uptake. (Valle et al., 2000; Oliva et al., 2002) The majority of tramadol's analgesic effect is derived from the major metabolite O-desmethyltramadol (M1). (Grond and Sablotzki, 2004; KuKanich, et al 2004; KuKanich, et al 2011) Therefore any alterations or variability in metabolism of tramadol could affect the drug's efficacy. (KuKanich, et al 2011) In human subjects mutations of the CYP2D6 gene, a P450 enzyme, are well recognized. These polymorphisms lead to individual variability in metabolism and clearance. (Grond and Sablotzki, 2004) In dogs, there have been reports of low plasma concentrations of the M1 metabolite after tramadol was administered via the oral and rectal routes. (Giorigi, et al 2009) Variability in plasma concentration in dogs could be related to polymorphisms in expression of CYP450 genes as seen in humans and may explain some of the clinical variability observed with tramadol administration for analgesia in dogs.

Currently published studies investigating the efficacy and pharmacokinetics of tramadol have only evaluated the antinociceptive effects when the drug was administered prior to surgery or nociceptive testing. (Coderre, et al 1986; Mastrocinque and Fantoni 2003; KuKanich, et al 2004; KuKanich, et al 2011; Davila, et al 2013; Tiexeira, et al 2013). As most veterinary practitioners prescribe tramadol as an analgesic following surgery or to treat an animal already in pain it is difficult to extrapolate a clinically effective dose for canine patients based on the published literature. To the authors' knowledge there have been no published studies in the dog evaluating the clinical analgesia provided by tramadol when administered post celiotomy as an oral tablet and correlating the clinical analgesia to plasma concentration of tramadol and its metabolites.

The objectives of this study were to evaluate the efficacy of tramadol as a sole

postoperative analgesic agent by assessing the clinical analgesic effects using the Glasgow Composite Measure Pain Scale (GCMP-SF). Further we intended to determine the correlation between these pain scores and the plasma concentrations of tramadol and O-desmethyltramadol. Finally, we analyzed the degree of variability in the plasma concentration of tramadol and O-desmethyltramadol amongst individuals within the same dose groups and at two different doses. Based on previous studies evaluating tramadol pharmacokinetics and clinical analgesia following orthopedic procedures (Benitez, et al 2015a; Benitez, et al 2015b) we hypothesized that individual and dose variability in plasma concentration of both tramadol and o-desmethyltramadol would be apparent in the clinical evaluation as assessed by a validated pain scoring system and that tramadol use as a sole analgesic would be inadequate.

MATERIALS AND METHODS:

Animals

Fourteen healthy client-owned female dogs that were pre-enrolled in the University Of Minnesota College Of Veterinary Medicine junior small animal surgery spay/neuter laboratory were recruited for evaluation. On admission to all dogs underwent a physical examination, blood was drawn and a measurement for packed cell volume and total solids was obtained. Dogs were excluded from the study if they were under 6 months of age, if physical examination or history revealed any ongoing illness, or if they were currently receiving any analgesic medications. Dogs were hospitalized overnight prior to their surgical procedure. All study protocols were reviewed, approved, and conducted in accordance with the University of Minnesota Animal Care and Use Committee (IACUC number: 1311-31087A) Written informed consent was obtained from all dog owners prior to enrollment into the study.

Study Design

A prospective, blinded, randomized complete block design, pilot study involving 14 healthy female dogs (ASA status I or

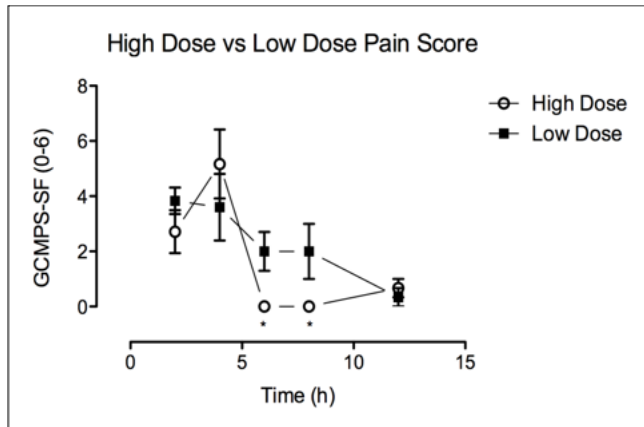
II) undergoing ovariohysterectomy under general anesthesia. Dogs were separated into two groups based on weight (<15 kg and >15 kg). There were 4 dogs less than 15 kilograms enrolled and 10 dogs greater than 15 kilograms enrolled. These groups were further subdivided to receive either a high dose of tramadol (10mg/kg PO) or a low dose (4 mg/kg PO) upon anesthetic recovery. Tramadol was dosed within 0.88 mg of the calculated dose based on a minimum tablet size containing 12.5 mg obtained from quartering tablets. Students were then provided with the tramadol in an envelope to be administered postoperatively. The evaluator (EGD) was unaware of the dose each dog received.

Anesthesia

A standardized anesthetic plan was used in all patients undergoing ovariohysterectomy in the laboratory. Premedication with acepromazine at 0.02-0.04mg/kg and Morphine at 0.6-1 mg/kg was administered intramuscularly to allow for intravenous catheter placement using aseptic technique. After intravenous catheter placement, anesthesia was induced by administering propofol intravenously to effect (loss of palpebral reflex, loose jaw-tone) with or without ketamine at 2 mg/kg intravenously. The trachea was intubated and anesthesia was maintained with isoflurane to effect in oxygen. A balanced isotonic crystalloid solution (5-10 mL/kg/h, IV) was administered during surgery. Blood pressure was measured using a Doppler and sphygmomanometer, arterial hemoglobin saturation using pulse oximetry, heart rate via an esophageal stethoscope, and respiratory rate by direct observation, and intermittent end-tidal carbon dioxide concentration using capnometry were monitored during anesthesia. Anesthetic time was no less than 2 hours and no more than 6 hours from premedication to extubation for all dogs.

Appropriate level of recovery from general anesthesia was based on the patient's ability to swallow and right herself. Patients were then medicated with either the high or

Figure 1, Pain Score for High and Low Dose: Average pain score on the GCMPs-SF for high (10 mg/kg PO) and low (4mg/kg PO) doses of tramadol for both weight groups. Time in hours is represented on the x-axis and pain score is represented on the y-axis. Patients with a pain score higher than 6 were removed from the study and provided rescue analgesia. (*) represent significant difference from first pain score Student's t-test ($p < 0.05$).



low dose (as previously described) of oral tramadol based on previous random assignment. The time of successful administration of the oral tramadol was the start time for evaluation. Patients were hospitalized overnight in a small animal ward.

Pain Scoring

One blinded observer (EGD) evaluated dogs at 2, 4, 6, 8, and 12 hours post tramadol administration. Evaluation incorporated the Short Form of the Glasgow Composite Measure Pain Scale (GCMPs-SF). (Reid, et al 2007) Any dog that scored greater than 6/24 on GCMPs-SF was rescued with injection of meloxicam (0.1 mg/kg SQ) or carprofen (2.2 mg/kg SQ) and hydromorphone (0.1mg/kg IM). The hydromorphone treatment was repeated at the same dose as deemed necessary (EGD). Once rescued, the dog was removed from further pain scoring and blood collection.

Plasma Collection and Evaluation

Peripheral venipuncture was performed at 2, 4, 8, and 12 hours post tramadol administration in dogs greater than 15 kg. Venipuncture was performed after pain scores were obtained. Blood collection was not

performed in the smaller dogs (<15 kg). Approximately 3 mL of whole blood was collected into EDTA tubes and was immediately centrifuged at 3000 rpm for 12 minutes. Plasma was pipetted into a screw top vial and labelled with time and patient I.D. All samples were stored in -30°C freezer until time of plasma evaluation. Samples were shipped overnight to University of Tennessee Veterinary Pharmacology Laboratory. Plasma samples were evaluated using commercially available reference standards. An LC-MS assay for tramadol and O-desmethytramadol (M1) was used to evaluate levels of drug at each time point. (Ceccato, et al 2000; Patel, et al 2009)

After the 12 hour evaluation, the study was completed and any dogs that had not received rescue analgesia during the study period were treated with meloxicam (0.1 mg/kg SQ) or carprofen (2.2 mg/kg SQ). Hydromorphone (0.05 mg/kg IM) was administered as needed.

Statistical analysis

Explanatory values included, high dose versus low dose, weight class, and time while outcome variables included, plasma drug concentration (tramadol and O-desmethytramadol) and pain scale (GCMPs-SF). All data was input into a spreadsheet program then transferred to a statistical software package.^{c, d}

The Shapiro-Wilks test was used to evaluate goodness of fit. All outcome variable data was entered as a mean. Bivariate analysis was used to compare outcome variables (pain score, drug plasma level) to the explanatory variable data (weight, dose). Student's t-test was used to evaluate difference in pain score between doses and weight classes, and the difference in plasma concentration of tramadol and O-desmethytramadol

between weight classes and doses. Significance was set at $P < 0.05$

RESULTS

14 intact female dogs were enrolled in the study, one dog from the greater than 15 kg group was excluded from further evaluation after a dysphoric recovery. The patient was assumed to be painful and immediate analgesia and sedation was administered without formal pain scoring. Thirteen dogs received tramadol postoperatively and were pain scored. Data for these 13 dogs was analyzed.

Pain Scoring

There was a significant difference in average pain score between the high dose and low dose groups at 6 and 8 hours when weight classes were pooled. (Figure 1) When pain scores were pooled, for both weight classes and drug doses, there was a significant reduction in pain score over time after the 6 hour time point. (Figure 2).

There was no significant difference in pain score between large and small dogs. Two dogs in the less than 15 kg group ($n=4$)

Figure 3, Pain Score Variation between Weight Classes: Average pain score at each time point for dogs less than 15 kg and dogs greater than 15 kg. Time in hours is represented on the x-axis and pain score is represented on the y-axis. Patients with a pain score higher than 6 were removed from the study and provided rescue analgesia.

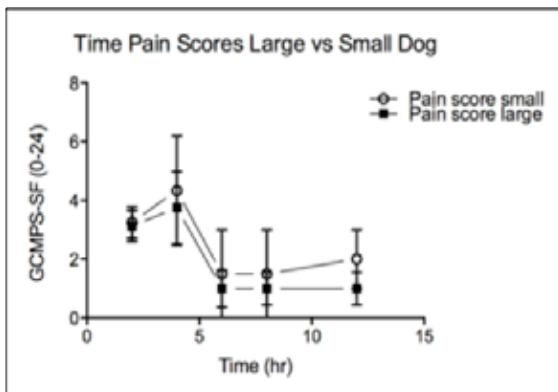
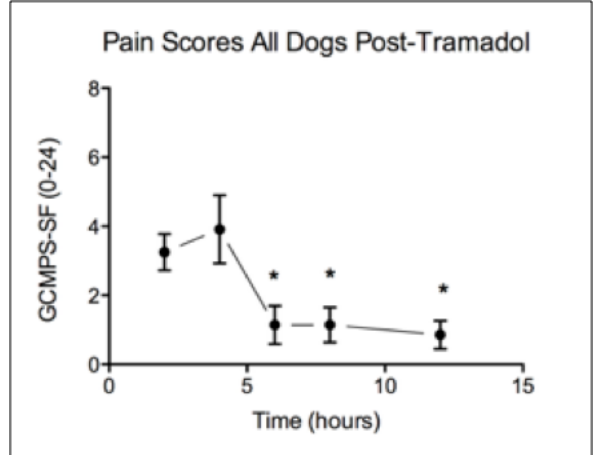


Figure 2, Pain Score for All Doses and All Weights: Pooled average pain score on the GCMPS-SF for all dogs. Time in hours is represented on the x-axis and pain score is represented on the y-axis. Patients with a pain score higher than 6 were removed from the study and provided rescue analgesia. (*) represent significant difference from baseline using the Student's t-test ($p < 0.05$).



required rescue analgesia, one from the high dose group and one from the low dose group. For the dogs in the greater than 15 kg group ($n=9$), 4/9 of dogs in the high dose group required rescue analgesia and 2/4 of the dogs in the low dose group required rescue analgesia. (Figure 3)

Plasma Drug Concentration

Plasma concentration of tramadol and O-desmethyltramadol for each dose group was plotted against time for dogs over 15 kg. Only dogs that were assessed to the 12 hour point are represented. Of the 8 dogs in which plasma concentrations were measured, all but one dog exhibited a peak level of tramadol at approximately 8 hours after oral dosing. Three of the 8 dogs required rescue analgesia prior to the 12 hour evaluation. All three of these dogs were rescued after the 4 hour assessment. Two of the rescued dogs had a tramadol plasma concentration that decreased from the 2 hour measurement to the 4 hour measurement. One dog had an increase in the tramadol

plasma concentration at the 4 hour measurement compared with the 2 hour measurement. Further plasma concentration measurements or pain evaluations were not performed in the three dogs requiring rescue analgesia. (Figure 4A and Figure 4B)

Plasma Drug Concentration and Pain Score

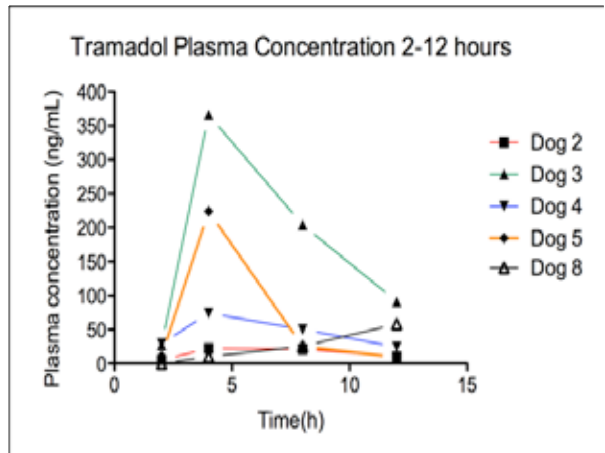
Average pain score for dogs over 15 kg was plotted against tramadol and O-desmethyltramadol concentration at 2, 4, 8, and 12 hours. A correlation between peak tramadol plasma concentration and increased pain score was noted, however, this was not significant. When pain score was compared with time, pain score consistently decreased over time from 4 hours after drug administration. There was also a positive association between decreasing plasma concentrations (for both tramadol and M1) and increasing time. (Figure 5A and Figure 5B)

DISCUSSION

Previously published data on the use of tramadol in dogs has shown that a dose of less than 9-10 mg/kg PO does not consistently provide adequate analgesia. (KuKanich, et al 2004, KuKanich, et al 2011) Based on our results it is possible that even the 10 mg/kg recommended dose will not guarantee efficacy. We suspect that variability in metabolism between individuals is the cause for this general trend toward insufficient analgesia observed in our study. Human subjects exhibit polymorphisms of the CYP450 enzymes, these polymorphisms lead to individual variability in metabolism and clearance. (Grond, et al 2004; Meyer, et al 2015) We suspect that similar polymorphism is seen in canine

Figure 4, Plasma Concentration for Tramadol and O-desmethyltramadol Over Time: (A) Tramadol concentrations for dogs over 15 kg. both high and low dose individuals are represented but only dogs that were not rescue and were evaluated for 12 hours are illustrated. Dog 1, dog 6, and dog 7 all required rescue analgesia prior to the 12 hour evaluation. Hours are represented on the x-axis and concentration in ng/mL as well as pain score is represented on the y-axis. (B) O-desmethyltramadol (M1) concentrations for dogs over 15 kg. both high and low dose individuals are represented but only dogs that were not rescue and were evaluated for 12 hours are illustrated. Dog 1, dog 6, and dog 7 all required rescue analgesia prior to the 12 hour evaluation. Hours are represented on the x-axis and concentration in ng/mL as well as pain score is represented on the y-axis.

(A)



(B)

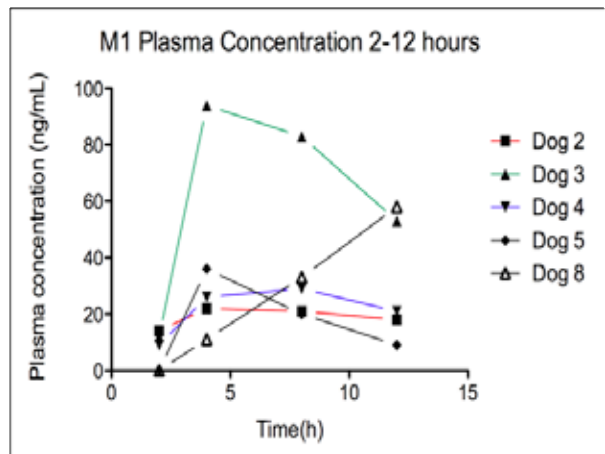
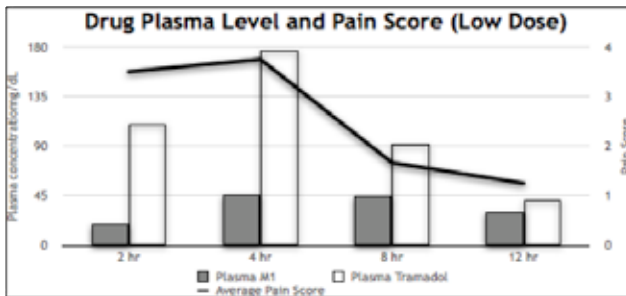
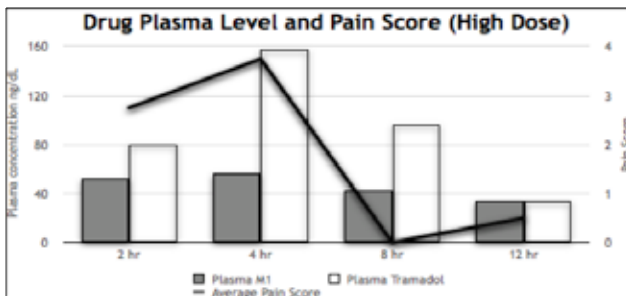


Figure 5, Plasma Concentration for Tramadol and O-desmethyltramadol Over Time Compared with Pain Score Over Time: (A) Average plasma concentrations for dogs over 15 kg administered the low dose (4 mg/kg) at 4 time points post tramadol administration. Hours are represented on the x-axis and concentration in ng/dL as well as pain score is represented on the y-axis. dark bars represent tramadol plasma concentration and light bars represented O-desmethyltramadol (M1) plasma concentration. The line is the average pain score at each time point. (B) Average plasma concentrations for dogs over 15 kg administered the high dose (10 mg/kg) at 4 time points post tramadol administration. Hours are represented on the x-axis and concentration in ng/dL as well as pain score is represented on the y-axis. dark bars represent O-desmethyltramadol (M1) plasma concentration and light bars represented tramadol plasma concentration. The line is the average pain score at each time point.

(A)



(B)



subjects and accounts for the variability in plasma concentration observed in our study. Further investigation into the metabolism of tramadol, genetic mutations in metabolic enzymes, and how this variation within different breeds of dog affects tramadol efficacy is warranted.

Based on the data presented here, the currently recommended dosing interval of every 6-8 hours is inadequate. These

findings are in agreement with previous studies which recommend a shorter dosing interval of 4-6 hours. (KuKanich, et al 2004, KuKanich, et al 2011) In our study, a peak in plasma concentration of tramadol and O-desmethyltramadol was observed at approximately 4 hours for most of the studied individuals. It is worth noting that the peak plasma concentration for tramadol and M1 noted at 4 hours did not correspond with decreasing pain score. Pain score was not significantly lower until the 6 and 8 hours assessments in patients who received the high dose of tramadol. It is possible that alternative active metabolites, that were not measured, are contributing to the analgesic effects or that the majority of the acute pain caused by the surgical procedure has subsided by 6-8 hours postoperatively.

In addition to possible polymorphisms or alternative active metabolites this study also introduces the concept of non-responders. In human subjects a small number of those treated with tramadol show no analgesic effect even at high dose ranges. These patients have a mutation in the CYP2D6 gene can greatly affect the plasma concentration of O-desmethyl tramadol. (Stamer, et al 2003; Grond, et al 2004) We believe that further investigation into

breed variation in clinical analgesic response to tramadol would allow us to explain why some of the dogs in this study had markedly higher pain scores than other dogs despite similar procedures and identical dosing regimens. The authors believe that repeating this study with a larger sample size would yield more significant results and may reveal more consistent trends in breed or size variation in

plasma concentration and response.

While many patients may have a higher pain score in the initial hours following surgery and individual observation may vary, the Short Form of the Glasgow Composite Measure Pain Scale has been well validated for evaluation of postoperative pain in the canine patient and a rescue level of 6/24 is recognized as ethical and standard.(Reid, et al 2007) We feel confident that the pain scoring in this study was accurate and representative of clinical analgesia.

Limitations to this study include the small sample size and potentially the marked differences in individual students' surgical technique and tissue handling. It is expected that seasoned veterinary practitioners would have both better surgical skills and much shorter procedure duration. A non-treatment control group was not included in this study due to ethics. While the tramadol dose administered to each dog was within 0.88 mg of the calculated dose, this variation may have led to some variability in plasma concentration and clinical analgesia.

While collection of data on more animal to evaluate the analgesia and pharmacokinetics of tramadol after ovariohysterectomy was considered, the very high rescue analgesia requirements in this pilot raised concerns about continuing.

Although large statistical variation was not observed, due to small sample size, is important to recognize that this study has revealed clinically significant information. Thirty to fifty percent of dogs in both the high and low dose groups across weight classes required rescue analgesia. In as many as half of the dogs treated, oral tramadol was not adequate as a sole analgesic agent when administered post operatively.

In conclusion, tramadol was unable to consistently provide adequate analgesia to dogs following an abdominal surgery. The authors feel confident in recommending that tramadol should not be used as the sole postoperative analgesic in dogs following invasive surgical procedures.

FOOTNOTES

- a. Tramadol, Amneal Pharmaceuticals, Bridgewater, NJ
- b. LRS, Abbot Laboratories Chicago, IL
- c. Excel 2015. Microsoft. Redmond, WA
- d. GraphPad Prism. 2015. San Diego, CA

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