RESEARCH PAPER

Pharmacokinetics of ropivacaine after intraperitoneal instillation in anaesthetized dogs: a randomized clinical trial

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Abstract

Objective To evaluate the pharmacokinetics of two doses of intraperitoneal ropivacaine in anaesthetized dogs.

Study design Randomized clinical trial.

Animals A total of 20 dogs undergoing ovariectomy/ovariohysterectomy.

Methods Dogs were anaesthetized and randomized to be given 1 (group R1) or 3 (group R3) mg kg^{-1} of ropivacaine intraperitoneally, diluted with 0.9% NaCl to a total volume of 0.8 mL kg^{-1}. Before abdominal wall closure, solution aliquots were instilled over the ovarian pedicles and, if applicable, the uterine stump. Venous blood was sampled 2 minutes before and 5, 10, 15, 30, 45, 60, 120 and 240 minutes after instillation. Cardiovascular signs of toxicity were noted. Plasma concentrations of free and total ropivacaine were measured using ultrahigh-performance liquid chromatography—mass spectrometry. Plasma protein binding (PPB) was determined by rapid equilibrium dialysis. Pharmacokinetic parameters were derived by a noncompartmental analysis, and the maximum total and free ropivacaine plasma concentrations (Cmax) were compared between groups using an independent samples t-test. Data are presented as mean or median (95% confidence intervals). Significance was set at $p < 0.05$.

Results One dog in R3 was excluded. Total ropivacaine Cmax values were 0.27 (0.21—0.33) and 0.67 (0.43—0.92) mg L^{-1} at 15 (8.64—30.35) and 5 (1.77—8.23) minutes (Tmax) for R1 and R3, respectively, and different between groups ($p = 0.005$). Free ropivacaine Cmax values were 0.02 (0.02—0.03) and 0.06 (0.03—0.09) mg L^{-1} at 10 (4.67—15.32) and 5 (0—19.13) minutes (Tmax) for R1 and R3, respectively, and Cmax was different between groups ($p = 0.015$). Median ropivacaine PPB for all dogs was 92.5% (89.7—95.2%). No signs of cardiovascular toxicity were noted.

Conclusions and clinical relevance Although R3 had a greater total and free ropivacaine Cmax than R1, Cmax remained below toxic levels in all dogs.

Keywords canine, intraperitoneal, local anaesthesia, pharmacokinetics, ropivacaine, toxicity.

Introduction

Ovariectomy and ovariohysterectomy are commonly performed surgical procedures in dogs and are associated with a moderate level of pain (Monteiro et al. 2023). The intraperitoneal instillation of local anaesthetics is a simple, low-cost and safe analgesic technique currently recommended for these procedures by the American Animal Hospital

Association and World Small Animal Veterinary Association (Grubb et al. 2020; Monteiro et al. 2023). However, using recommended doses of local anaesthetics, the clinical postoperative benefit provided by this technique in dogs is inconsistent (Guerrero et al. 2016; Lambertini et al. 2018; Brioschi et al. 2023; Kazmir-Lysak et al. 2023).

Ropivacaine is an amide-type local anaesthetic that is longer acting than lidocaine and is less cardiotoxic and neurotoxic than bupivacaine (Feldman et al. 1989; McClure 1996). In dogs, ropivacaine is 99% bound to alpha-1 acid glycoprotein (Feldman et al. 1996). It has been demonstrated in dogs that intravenous injection of 4.88 ± 0.47 mg kg^{-1} resulted in a plasma concentration of 11.4 ± 0.9 mg L^{-1} and signs of local anaesthetic systemic toxicity (LAST)-like seizures, ventricular arrhythmias, hypotension and cardiovascular collapse (Feldman et al. 1989). Therefore, the maximum recommended dose of ropivacaine by all routes is 3 mg kg^{-1} (Barletta & Reed 2019; Grubb & Lobprise 2020). However, the absorption of a local anaesthetic and the associated risk of systemic toxicity depends on several factors related to the drug and to the animal, including the site of application and the vascularity of the injection site (Rosenberg et al. 2004; Christie et al. 2015; Barletta & Reed 2019). Evaluating absorption of a local anaesthetic in a specific tissue remains crucial to provide a recommended dose that is meaningful for a specific analgesic technique.

The pharmacokinetic profile of ropivacaine after intraperitoneal instillation has been studied in humans (Labaille et al. 2002) and pigs (Betton et al. 2010). However, no pharmacokinetic studies on intraperitoneal instillation of ropivacaine have been reported in dogs. In order to determine whether an increase in dose would be safe and potentially result in better analgesic efficacy for the intraperitoneal technique, the pharmacokinetic profile of free and total ropivacaine in dogs should be characterized.

It was hypothesized that intraperitoneal instillation of 1 and 3 mg kg^{-1} of ropivacaine in anaesthetized dogs would result in significantly different maximum plasma concentrations but would remain below reported toxic levels in all cases. An additional hypothesis was that administration of ropivacaine would not result in cardiovascular signs of LAST measured by routine cardiovascular monitoring. Therefore, the aims of this study were twofold: 1) to determine the difference in the maximum plasma concentration of total and free ropivacaine after intraperitoneal instillation of 1 and 3 mg kg^{-1} in anaesthetized dogs undergoing elective ovariectomy or ovariohysterectomy; and 2) to determine whether intraperitoneal instillation of ropivacaine results in cardiovascular signs of LAST in these dogs.

Materials and methods

Animals and study design

The study was approved by the National (Bundesministerium für Bildung Wissenschaft und Forschung) and Institutional (University of Veterinary Medicine Vienna; GZ 2023-0.623.479) Ethical Committees. Informed, written owner consent was obtained for all dogs enrolled. The study was designed as a prospective, clinical trial and reported following the Consolidated Standards of Reporting Trials statement (Fig. 1; Moher et al. 2010) and Clinical Pharmacokinetic Study Guidelines (Kanji et al. 2015).

An *a priori* sample size estimation was performed for the primary outcome (i.e. maximum plasma ropivacaine concentration). Due to the lack of pharmacokinetic studies on intraperitoneal ropivacaine in dogs, available literature on intraperitoneal technique in any mammals using any local anaesthetic was checked to find the highest and lowest peak total ropivacaine plasma concentration (Cmax). The lowest Cmax was 0.92 mg L^{-1}, obtained from a study in pigs using 3 mg kg^{-1} ropivacaine (Betton et al. 2010), and the highest Cmax was 1.45 mg L^{-1}, obtained from a study in dogs using 10 mg kg^{-1} lidocaine (Wilson et al. 2004). Because Betton et al. (2010) did not present a standard deviation (SD), a common SD of 0.36 was used to estimate the sample size. For an 80% statistical power and level of significance of $p < 0.05$, an estimated eight animals per group would be necessary to detect an arbitrarily selected (guided by Cmax data presented above) difference in total maximum ropivacaine plasma concentration of 0.53 mg L^{-1} between two doses. To account for possible attrition, 10 dogs were enrolled per group. Using a balanced design and a random sequence for treatments generated in variable size blocks, dogs were randomized to receive either 1 or 3 mg kg^{-1} of ropivacaine intraperitoneally by one author (PAD; Stata 13, StataCorp, TX, USA). Allocation was implemented using sequentially numbered opaque envelopes.

Recruitment occurred between April 2024 and April 2025. Dogs included were adults, female, with an actual body weight over 15 kg and a body condition score (BCS) of 4–6/9, not pregnant, assigned an American Society of Anesthesiologists physical status classification of I or II/V and scheduled to undergo ovariectomy or ovariohysterectomy. Health was assessed based on medical history, physical examination, haematology and serum biochemistry. A gynaecological examination, which included vaginal inspection, cytology and ultrasound scanning was performed to ensure anoestrus and a non-gravid uterus. Dogs with cardiac dysrhythmias, severe systemic disease or pregnancy were excluded.

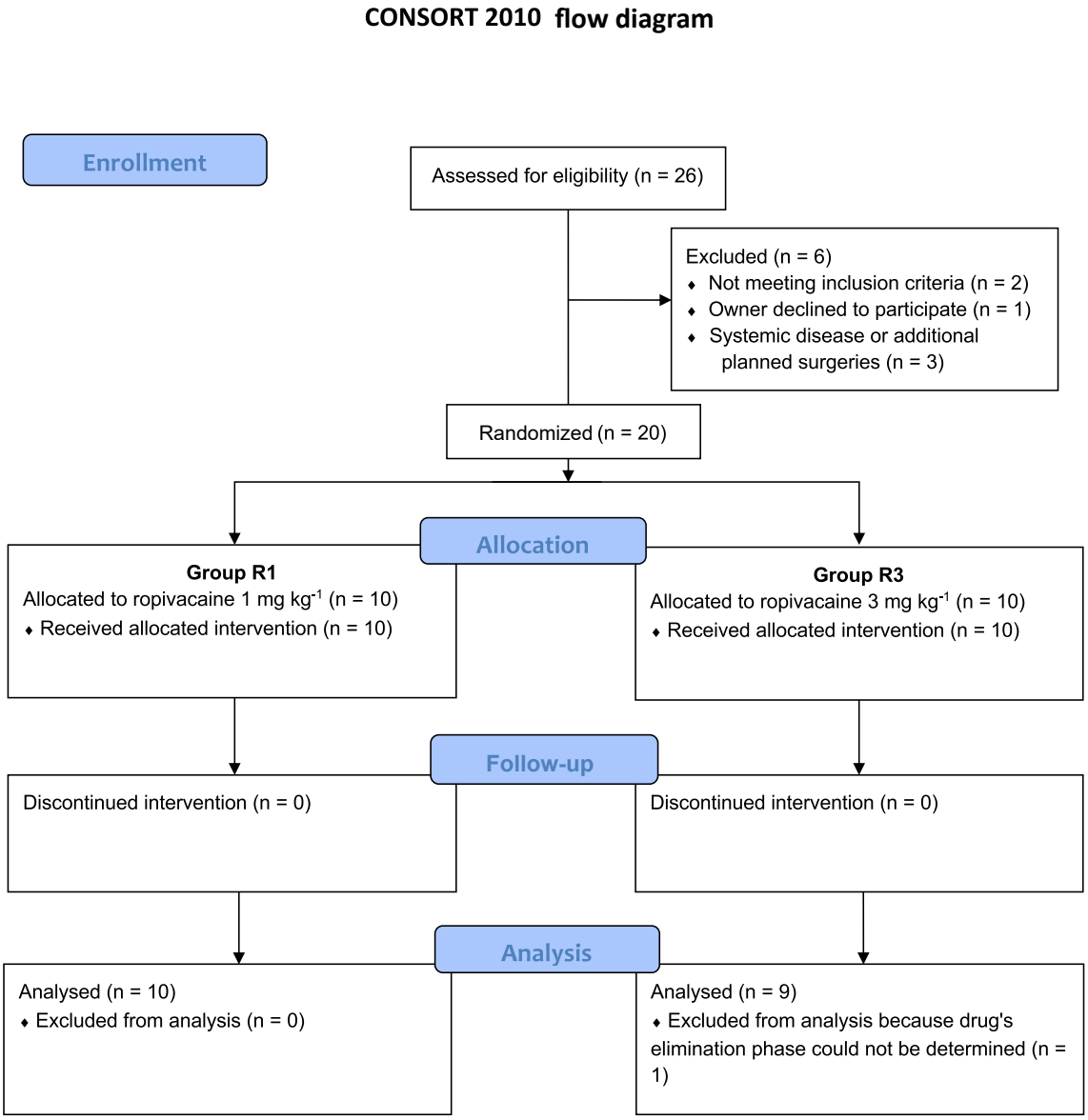

Figure 1 Consolidated Standards of Reporting Trials (CONSORT) 2010 flow diagram outlining the progression of all animals in this randomized clinical trial to evaluate the pharmacokinetics of two doses of intraperitoneal ropivacaine in anaesthetized dogs.

Anaesthesia, instrumentation and intraperitoneal drug instillation

Dogs were fasted overnight (8 hours) with water available *ad libitum*. All dogs were given acepromazine (0.02 mg kg^{-1}; Tranquisol KH 0.5 mg mL^{-1}, CP Pharma, Germany) and methadone (0.2 mg kg^{-1}; Insistor 10 mg mL^{-1}, VetViva Richter, Austria) intramuscularly as anaesthetic premedication. After 30 minutes, an intravenous (IV) catheter was placed in the right cephalic vein. A balanced crystalloid solution (Elo-Mel Isoton, Fresenius Kabi, Austria) at a rate of 5 mL kg^{-1} $hour^{-1}$ was infused IV throughout the procedure. Following preoxygenation by mask, general anaesthesia was induced with IV propofol (Propofol Fresenius 1%, Fresenius Kabi, Austria), titrated to effect, and the trachea was intubated with an appropriately sized cuffed orotracheal tube. Anaesthesia was maintained with isoflurane (Vetflurane 1000 mg g^{-1}, Virbac, Austria) in 70% oxygen using a circle system. Volume controlled mechanical ventilation was initially set to a tidal volume of 10 mL kg^{-1}, positive end-expiratory pressure of 5 cmH_2O, and respiratory rate adjusted to maintain an end-tidal carbon dioxide partial

pressure ($PÉCO_2$) between 4.66 and 6.66 kPa (35 and 50 mmHg). Pre- and intraoperative monitoring consisted of electrocardiogram (ECG) (at 50 mm $second^{-1}$), pulse oximetry, oesophageal temperature, invasive and noninvasive arterial blood pressure, $PÉCO_2$ and end-tidal isoflurane concentration. A central venous catheter (Certofix Monopaed S 110, B. Braun, Germany) was placed in a jugular vein for subsequent blood sampling. An arterial catheter (Vasofix Safety, B. Braun, Germany) was placed in a dorsal pedal artery to measure invasive blood pressure. Once in the operating room, the arterial catheter was connected to a new blood pressure transducer (Combitrans Monitoring Set Arteriell, B. Braun, Germany), which was levelled to the right atrium (i.e. scapulohumeral joint) and zeroed to atmospheric pressure, following the manufacturer's instructions. A fentanyl (Fentanyl-Janssen 0.05 mg mL^{-1}, Janssen-Cilag Pharma, Austria) constant-rate IV infusion was given at 5 μg kg^{-1} $hour^{-1}$ throughout surgery and adjusted when appropriate to maintain a heart rate above 40 beats $minute^{-1}$ and below 180 beats $minute^{-1}$. A camera (GoPro Hero 7 White, GoPro Inc., CA, USA) was used to record the screen of the multiparametric monitor throughout the intraoperative period, for additional *post hoc* evaluation of cardiovascular signs of LAST.

Dogs were randomized to one of two groups: intraperitoneal instillation of 1 (R1) or 3 (R3) mg kg^{-1} of ropivacaine (Ropinaest 5 mg mL^{-1}, Gebro Pharma, Austria), diluted with 0.9% NaCl (NaCl 0.9% 10 mL, B. Braun, Germany) to a total volume of 0.8 mL kg^{-1}. The dose of ropivacaine was calculated based on actual body weight. If the dog underwent ovariectomy, the injectate solution was divided into two equal aliquots and applied over the ovarian pedicles. If the dog underwent ovariohysterectomy, the solution was divided into three equal aliquots and applied on both ovarian pedicles and uterine stump. In all cases, the solution was applied after removal of the mentioned structures and immediately before abdominal wall closure.

Intraoperative cardiovascular signs of LAST were considered as follows: a 25% decrease in mean arterial pressure from values 2 minutes before ropivacaine instillation (baseline), QRS complex widening, ventricular dysrhythmias from visual assessment of ECG, and cardiac arrest (Feldman et al. 1989).

Postoperative monitoring continued until the last blood sampling point and consisted of heart rate, respiratory rate and rectal temperature. During recovery, all dogs were given meloxicam (0.2 mg kg^{-1}; Metacam, Boehringer Ingelheim Vetmedica, Germany) and buprenorphine (10 μg kg^{-1}; Bupaq, VetViva Richter, Austria) IV. Anaesthetic management, instrumentation, application of ropivacaine solution and monitoring for cardiovascular signs of LAST (in real time and *post hoc* by video evaluation) were performed in all dogs by the same investigator (NV), who was unaware of group allocation.

Sampling schedule

Venous blood samples of 2 mL were taken from jugular catheter 2 minutes before and 5, 15, 30, 45, 60, 120 and 240 minutes after ropivacaine intraperitoneal instillation. Before collection of each sample, 2 mL of blood were taken from jugular catheter and immediately injected through the catheter in the cephalic vein. Blood was collected in sterile syringes and immediately transferred to heparin-containing tubes, which were kept refrigerated for up to 5 hours. Plasma was separated by centrifugation for 10 minutes at 1200 ***g*** and then stored at −80 °C until analysed.

Analytical method

Free and total ropivacaine plasma concentrations were analysed by a highly sensitive, precise and accurate ultrahigh-performance liquid chromatography (UHPLC)—mass spectrometry (MS) method (Verdier et al. 2025). The method was developed for dog plasma, according to a previously validated method for human plasma (Lamy et al. 2020) and followed the European Medicines Agency ICH M10 guidelines on bioanalytical method validation and study sample analysis (European Medicines Agency 2022). Free and total ropivacaine were separated using rapid equilibrium dialysis devices, with plates containing single-use inserts made of two side-by-side chambers, separated by a vertical cylinder of an 8 kDa molecular weight cut-off dialysis membrane (Fisher Scientific, Austria). After UHPLC separation (Agilent 1290 Infinity II; Agilent Technologies, CA, USA), detection and quantification of ropivacaine was performed using a triple quadrupole tandem mass spectrometer with electrospray ionization (QTRAP 6500+; AB Sciex, MA, USA). The lower limit of quantification was as low as 0.05 ng mL^{-1} and 0.30 ng mL^{-1} for free and protein-bound ropivacaine, respectively. Precision and accuracy of the method including rapid equilibrium dialysis was determined at low (100 ng mL^{-1}) and high (2000 ng mL^{-1}) concentration levels and ranged from 3.2% to 10.8% and 91.4% to 115.7%, respectively. Using this method, ropivacaine plasma protein binding (PPB, %) was determined for each dog.

Pharmacokinetic analysis

Noncompartmental analysis was conducted using freely available software (R version 4.3.0; R Core Team 2025) with the PKNCA package (version 0.12.0). The following pharmacokinetic parameters (using actual body weight) were calculated for each dog: Cmax (mg L^{-1}), time to reach Cmax (Tmax; minutes), area under the curve from zero to last timepoint measured (AUClast; mg*minute L^{-1}), area under the curve from zero to infinity (mg*minute L^{-1}), last observed

Table 1 Age, weight, body condition score (BCS), duration of anaesthesia and duration of surgery of dogs undergoing ovariectomy or ovariohysterectomy after intraperitoneal instillation of 1 (R1) or 3 (R3) mg kg^{-1} of ropivacaine. Data are expressed as median ± standard deviation or median (interquartile range), with the corresponding 95% confidence intervals (CIs).

Variable	R1 ($n = 10$)	R3 ($n = 9$)
Age (months)	45.6 ± 21.3 (95% CI, 30.3–60.9)	67.1 ± 54.5 (95% CI, 25.2–109.0)
Weight (kg)	26.1 ± 5.6 (95% CI, 22.1–30.1)	24.3 ± 6.3 (95% CI, 19.5–29.1)
BCS (1–9)	5 (5–5)	5 (5–6)
	(95% CI, 4.9–5.1)	(95% CI, 4.3–5.7)
Duration of anaesthesia (minutes)	150 (146–163)	170 (158–175)
	(95% CI, 138–162)	(95% CI, 155–184)
Duration of surgery (minutes)	59 (52–65)	55 (42–65)
	(95% CI, 51–67)	(95% CI, 37–72)

concentration (mg L^{-1}), elimination half-life (minutes), apparent clearance (Cl/F; L $minute^{-1}$), and apparent volume of distribution (Vz/F; L). The terminal elimination rate constant (λz) was estimated by log-linear regression of the terminal portion of the concentration–time curve, using at least three points. For profiles in which at least three datapoints were not well aligned on the log-linear terminal portion of the concentration curve, data were excluded from the pharmacokinetic analysis.

Statistical analysis

Data distribution was assessed using histograms and the Shapiro–Wilk test. Quantitative variables are presented as mean ± SD or as median and interquartile range (IQR), for normally and non-normally distributed data, respectively. The corresponding 95% confidence intervals (CIs) are also reported. For non-normally distributed variables, the 95% CI of the median was estimated using a nonparametric bootstrap approach with 1000 replications to generate an empirical distribution of the sample median. Group comparisons (R1 *versus* R3) for total and free ropivacaine Cmax were performed using an independent samples t-test. Differences were considered significant with p values of < 0.05. Statistical analyses were performed using commercial software (Stata Version 13.0, StataCorp, TX, USA).

Results

Animals, procedures and toxicity

A total of 20 female dogs were included in this study. For one dog in group R3, λz could not be determined because the predefined criteria for selecting time points in the terminal log-linear phase were not met. Consequently, this dog was excluded from the pharmacokinetic analysis. Therefore, data from 19 dogs were analysed (R1, $n = 10$; R3, $n = 9$). In one dog in R1, the last two blood samples were arterial because the jugular vein catheter was no longer patent.

Age, actual body weight and BCS, the duration of anaesthesia and the duration of surgery are presented in Table 1. Out of the 10 dogs in R1, four underwent ovariectomy and six ovariohysterectomy. Out of the nine dogs in R3, eight dogs underwent ovariectomy and one ovariohysterectomy. No intraoperative cardiovascular signs of LAST were observed in any dog. All dogs recovered uneventfully from anaesthesia.

Pharmacokinetic analysis

A noncompartmental analysis was used to analyse the time–plasma ropivacaine concentration data. The log base of 10 of the mean plasma concentrations and their SD of free and total ropivacaine at all sample times for both groups are presented in Figs 2 and 3, respectively. Pharmacokinetic parameters for total and free ropivacaine for both groups are presented in Tables 2 and 3, respectively. Median ropivacaine PPB for all dogs was 92.5% (IQR 86.1–94.0%; 95% CI 89.7–95.2%).

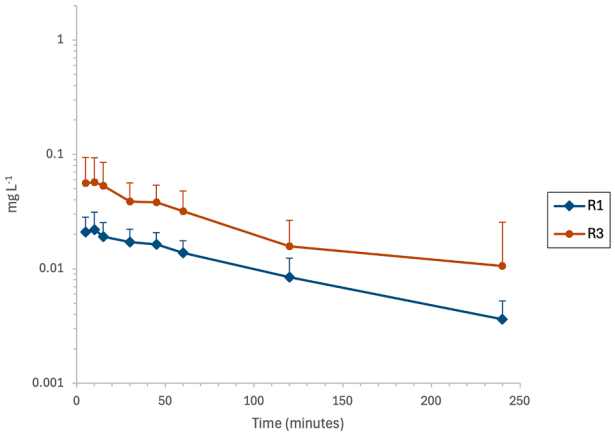

Figure 2 Semilogarithmic plot of the mean plasma free concentrations of ropivacaine and their positive standard deviation over time, after intraperitoneal instillation of 1 (R1, $n = 10$) and 3 (R3, $n = 9$) mg kg^{-1} to anaesthetized dogs. Only the positive standard deviation is shown to facilitate visualization.

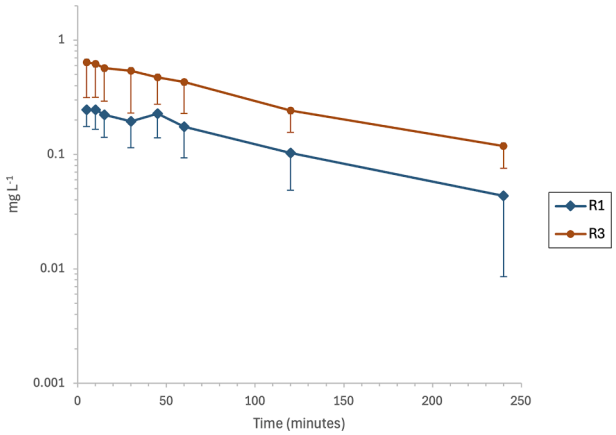

Figure 3 Semilogarithmic plot of the mean plasma total concentrations of ropivacaine and their negative standard deviation over time, after intraperitoneal instillation of 1 (R1, $n = 10$) and 3 (R3, $n = 9$) mg kg^{-1} to anaesthetized dogs. Only the negative standard deviation is shown to facilitate visualization.

Discussion

This study provides objective information on the pharmacokinetic profile of both total and free ropivacaine instilled intraperitoneally to anaesthetized dogs undergoing ovariectomy or ovariohysterectomy. The hypothesis that Cmax of both total and free ropivacaine differed between the groups was confirmed, showing that dose given plays a major role in the disposition of ropivacaine. Importantly, despite the observed differences, Cmax remained well below the reported toxic levels in all dogs (Feldman et al. 1989). No clinical cardiovascular signs of LAST were observed in any dog.

This study was powered to detect significant differences in the Cmax of total ropivacaine between R1 and R3. Consequently, inferential statistical testing was not performed on other outcomes as they are unsupported by an *a priori* sample estimation, with a resultant risk of type II error and potential for inaccurate estimates of the true effect. The sample size estimation was speculative and based on studies in other species using other local anaesthetics. The 95% CI for the mean or median difference is reported as an indication of the precision of the estimated differences.

The Cmax of both total and free ropivacaine was significantly higher in R3 than in R1. It appears that the dose given played a major role in the resultant maximum plasma concentration, because the groups were otherwise comparable. There are no previous studies on the pharmacokinetics of ropivacaine after intraperitoneal instillation in dogs. However, similar pharmacokinetic studies in humans and pigs comparing the instillation of two doses showed that a higher dose of ropivacaine results in a higher plasma concentration, which is in agreement with our findings (Labaille et al. 2002; Betton et al. 2010). In our study, although the dose used in R3 was threefold greater than in R1, the Cmax and the AUClast in R3 were 2.5- and 2.6-times greater than in R1, respectively. This, together with the results obtained for the half-life, Vz/F and Cl/F, suggests that ropivacaine, as in humans (Arthur & Covino 1991), followed linear kinetics in dogs. This finding should be considered when planning future studies on the analgesic efficacy of the intraperitoneal technique using ropivacaine.

The Tmax of total ropivacaine was achieved in 15 (10–30) minutes in R1 and only 5 (5–10) minutes in R3, showing that dose plays a role in time to achieve peak concentration.

Table 2 Mean ± standard deviation or median (interquartile range), and 95% confidence interval for TOTAL ropivacaine after intraperitoneal instillation of 1 (R1) and 3 (R3) mg kg^{-1} of ropivacaine in 19 anaesthetized dogs undergoing ovariectomy or ovariohysterectomy. AUCinf, area under the curve from zero to infinity; AUClast, area under the curve from zero to last point measured; Cl/F, apparent clearance; Clast, last observed concentration; Cmax, maximum total ropivacaine plasma concentration; T1/2, elimination half-life; Tmax, time to reach Cmax; Vz/F, apparent volume of distribution.

Variable	Units	Group R1 ($n = 10$)	Group R3 ($n = 9$)	*p* value	Times difference R3 to R1
AUClast	mg*minute L^{-1}	2.99 ± 0.90 (2.35–3.64)	7.69 ± 3.77 (4.79–10.59)		2.57
AUCinf	mg*minute L^{-1}	28.66 (23.40–42.91) (19.04–38.27)	81.22 (65.59–95.08) (62.07–100.37)		2.83
Cmax	mg L^{-1}	0.27 ± 0.08 (0.21–0.33)	0.67 ± 0.32 (0.43–0.92)	0.005	2.48
Tmax	minutes	15 (10–30) (8.64–30.35)	5 (5–10) (1.77–8.23)		
Clast	mg L^{-1}	0.031 (0.027–0.047) (0.019–0.044)	0.11 (0.09–0.12) (0.08–0.13)		
T1/2	minutes	75.85 (74.01–88.21) (61.91–89.79)	93.86 (84.11–124.00) (81.99–123.58)		
Cl/F	L minute^{-1}	0.90 ± 0.42 (0.60–1.20)	0.89 ± 0.34 (0.63–1.16)		
Vz/F	L	106.36 ± 44.42 (74.58–138.14)	139.72 ± 78.55 (79.35–200.09)		

Table 3 Mean ± standard deviation or median (interquartile range), and 95% confidence interval for FREE ropivacaine after intraperitoneal instillation of 1 (R1) and 3 (R3) mg kg^{-1} of ropivacaine in 19 anaesthetized dogs undergoing ovariectomy or ovariohysterectomy. AUCinf, area under the curve from zero to infinity; AUClast, area under the curve from zero to last point measured; Cl/F, mean clearance; Clast, last observed concentration; Cmax, maximum total ropivacaine plasma concentration; T1/2, elimination half-life; Tmax, time to reach Cmax; Vz/F, apparent volume of distribution.

Variable	Units	Group R1 ($n = 10$)	Group R3 ($n = 9$)	*p* value	Times difference R3 to R1
AUClast	mg*minute L^{-1}	0.26 ± 0.09 (0.19–0.33)	0.66 ± 0.43 (0.35–0.97)		2.53
AUCinf	mg*minute L^{-1}	2.88 (2.40–3.45) (2.30–3.45)	5.32 (3.39–7.81) (1.46–9.17)		1.84
Cmax	mg L^{-1}	0.02 ± 0.008 (0.017–0.029)	0.06 ± 0.037 (0.033–0.091)	0.015	3.00
Tmax	minutes	10.00 (5–10) (4.67–15.32)	5.00 (5–15) (0–19.13)		
Clast	mg L^{-1}	0.003 (0.002–0.005) (0.002–0.005)	0.005 (0.003–0.010) (0–0.011)		
T1/2	minutes	84.10 (72.17–110.11) (58.89–109.31)	87.02 (60.29–101.25) (53.53–119.51)		
Cl/F	L $minute^{-1}$	7.65 (7.22–9.32) (5.91–9.38)	15.30 (8.98–19.35) (8.53–22.06)		
Vz/F	L	1093.45 (871.42–1716.09) (687.85–1499.05)	1328.13 (1127.07–1580.25) (669.25–1987.01)		

Other intraperitoneal studies observed a Tmax at 59 minutes after instillation of 3 mg kg^{-1} of ropivacaine to pigs (Betton et al. 2010), a Tmax at 22 minutes after a high dose of lidocaine (8.8 mg kg^{-1}) to dogs (Wilson et al. 2004) and a Tmax at 30 minutes after 2 mg kg^{-1} of bupivacaine to cats (Benito et al. 2016). These differences in Tmax could be further explained by several factors: species and drug differences, the use of vasodilator agents like acepromazine or azaperone and the type of surgery (laparotomy *versus* laparoscopy with pneumoperitoneum and potential blood vessel collapse). Contrary to bupivacaine and lidocaine, ropivacaine can exert dose-dependent local vasoconstriction (Åkerman et al. 1988; Kopacz et al. 1989; Nakamura et al. 1993) that may also affect its systemic uptake. Further studies are necessary to explain the rapid absorption of ropivacaine and subsequent short Tmax observed in the dogs included in the current study.

Uptake of local anaesthetics depends not only on the tissue vascularity and structure of surrounding tissues, but also on the drug properties, presence of vasoactive substances, and patient-related factors (Rosenberg et al. 2004). It has been reported that general anaesthesia and the consequential physiological changes on the central nervous and cardiovascular systems may also affect the disposition of a drug (Copeland et al. 2008). Interestingly in our study, the plasma concentration of total ropivacaine in R1 showed a tendency to increase again at 45 minutes, which coincided with the time when most of the dogs were allowed to recover from anaesthesia. Whether the transition into recovery or positional changes played a role in the disposition of ropivacaine, and why this was not observed for the free fraction or in R3, remains to be determined.

The free or unbound fraction of a drug in plasma is free to diffuse through membranes and interact with receptors, and it is therefore the pharmacologically active fraction. Local anaesthetics are basic drugs that are in plasma mainly bound to alpha-1 acid glycoprotein (Feldman et al. 1996). The extent of ropivacaine being bound to plasma protein has been reported as 94% in humans and 99% in dogs (Feldman et al. 1996; McClure 1996; Thomas & Schug 1999). In our study, we observed a lower PPB of 92.5%. This difference could be partly due to the different quantification methods used: (Feldman et al. 1996) used a method originally developed for bupivacaine, while we developed a novel and highly ropivacaine-specific method, which included rapid equilibrium dialysis. Rapid equilibrium dialysis is considered the gold-standard approach to determine the fraction of free and total protein-bound drugs (Stumpe et al. 2000; Banker & Clark 2008). The PPB could also be influenced by the level of alpha-1 acid glycoprotein. This is an acute-phase protein that increases serum concentrations during inflammation and has been described to result in a higher lidocaine PPB (Belpaire et al. 1987; Rosenberg et al. 2004). Although we did not measure alpha-1 acid glycoprotein concentration, it could be inferred that the stress associated with surgery in the current study caused alpha-1 acid glycoprotein to increase, leading to an increase in ropivacaine PPB and consequently a lower free fraction. As the unbound fraction of local anaesthetics is

responsible for toxic effects, further studies are warranted to determine the variation of ropivacaine PPB in stressed *versus* unstressed dogs.

In anaesthetized dogs, signs of LAST mainly include cardiac dysrhythmias, hypotension and eventually cardiovascular collapse (Feldman et al. 1989). In the current study, signs of LAST were defined as a widening of the QRS complex and a decrease greater than 25% in mean arterial blood pressure (from baseline). Based on these criteria, none of the dogs developed signs associated with LAST. Furthermore, the highest Cmax of all the studied dogs was 1.20 mg L^{-1}, which is well below the 11.40 ± 0.90 mg L^{-1} concentration associated with systemic toxicity (Feldman et al. 1989) and below a reported concentration of 2.41 ± 0.52 mg L^{-1} that resulted in neurological signs in one out of six dogs (Arthur et al. 1988). This is in agreement with several other studies in humans, dogs, cats and pigs, in which different doses of local anaesthetics, given intraperitoneally, resulted in plasma concentrations lower than those associated with toxicity for each species and did not result in signs of LAST (Labaille et al. 2002; Wilson et al. 2004; Betton et al. 2010; Benito et al. 2016).

This study has several limitations. First, the study was limited to healthy, adult female dogs undergoing a specific surgical procedure, which may limit generalizability. It has been reported in humans that sex may affect drug kinetics (Soldin & Mattison 2009); therefore, the disposition of ropivacaine in male dogs remains to be determined. Second, due to the clinical nature of this study, the last three or four samples were collected after recovery from anaesthesia, which could have altered the disposition of ropivacaine. However, this reflects a common clinical scenario, where drugs are given by the intraperitoneal route near the end of surgery, so the pharmacokinetic data are relevant to current clinical practice. Third, ECG changes were observed using a multiparametric physiological monitor, and no objective measurements of the QRS complex were performed. This, together with dogs being placed in dorsal recumbency, may have hindered a proper ECG analysis. Fourth, because the dogs were under general anaesthesia, central nervous system signs of LAST could not be evaluated. Fifth, LAST is presumably driven by arterial, not venous local anaesthetic concentrations. Therefore, it would have been preferable to sample arterial rather than venous blood.

This study also has important strengths: the total and free fractions of ropivacaine were determined by a using UHPLC–MS and rapid equilibrium dialysis, currently considered the gold-standard technique. This study was performed as a randomized clinical trial to evaluate the pharmacokinetics of a commonly used local anaesthetic in a clinical setting, making the results directly applicable to clinical practice. Furthermore, the protocol was standardized, and a single investigator blinded to treatment performed the intraperitoneal application of ropivacaine and the clinical evaluation of cardiovascular toxicity.

Future studies investigating the effect of sex and the role of alpha-1 acid glycoprotein during surgery are necessary to determine whether an increase in the dose of ropivacaine given intraperitoneally to female dogs undergoing ovariectomy or ovariohysterectomy would improve its analgesic efficacy without causing LAST.

Conclusions

The results of this study show that the intraperitoneal instillation of 1 and 3 mg kg^{-1} of ropivacaine in anaesthetized female dogs resulted in significantly different maximum plasma concentrations, but drug concentrations remained well below reported toxic levels. Signs of LAST, based on cardiovascular monitoring, were not observed.

Conflict of interest statement

The authors have no conflict to declare. A disclosure is noted that NV is an Associate Editor of this journal but had no access to the review process.

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Author contributions

NV: conceptualization, methodology, validation, formal analysis, investigation, resources, writing — original draft. KH: conceptualization, methodology, validation, formal analysis, investigation, resources, visualization, supervision. VAJ: conceptualization, formal analysis, investigation, visualization. SC: methodology, investigation. PAD: formal analysis, visualization. DAP: conceptualization, formal analysis, supervision. ERF: conceptualization, resources, supervision. MM: conceptualization, formal analysis, resources,

supervision, project administration. All authors participated in the review and editing of the manuscript and approved its submission.

References

Åkerman B, Hellberg I-B, Trossvik C (1988) Primary evaluation of the local anaesthetic properties of the amino amide agent ropivacaine (LEA 103). Acta Anaesthesiol Scand 32, 571–578.

Arthur GR, Covino BG (1991) Pharmacokinetics of local anaesthetics. Baillieres Clin Anaesthesiol 5, 635–658.

Arthur RG, Feldman HS, Covino BG (1988) Comparative pharmacokinetics of bupivacaine and ropivacaine, a new amide local anesthetic. Anesth Analg 67, 1053–1058.

Banker M, Clark T (2008) Plasma/serum protein binding determinations. Curr Drug Metab 9, 854–859.

Barletta M, Reed R (2019) Local anesthetics pharmacology and special preparations. Vet Clin North Am Small Animal Pract 49, 1109–1125.

Belpaire FM, Rick AD, Dello C et al. (1987) A1-acid glycoprotein and serum binding of drugs in healthy and diseased dogs. J Vet Pharmacol Ther 10, 43–48.

Benito J, Monteiro B, Lavoie A-M et al. (2016) Analgesic efficacy of intraperitoneal administration of bupivacaine in cats. J Feline Med Surg 18, 906–912.

Betton D, Greib N, Schlotterbeck H et al. (2010) The pharmacokinetics of ropivacaine after intraperitoneal administration: instillation versus nebulization. Anesth Analg 111, 1140–1145.

Brioschi FA, Ravasio G, Ferrari F et al. (2023) Comparison of intraperitoneal and incisional lidocaine or ropivacaine irrigation for postoperative analgesia in dogs undergoing major abdominal surgeries. PLoS One 18, e0284379.

Christie LE, Picard J, Weinberg GL (2015) Local anaesthetic systemic toxicity. Continuing Educ Anaesth Critical Care Pain 15, 136–142.

Copeland SE, Ladd LA, Gu X-Q, Mather LE (2008) The effects of general anesthesia on whole body and regional pharmacokinetics of local anesthetics at toxic doses. Anesth Analg 106, 1440–1449.

European Medicines Agency (2022) ICH guideline M10 on bioanalytical method validation and study sample analysis, step 5. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf. (Accessed 31 October 2025).

Feldman HS, Arthur GR, Covino BG (1989) Comparative systemic toxicity of convulsant and supraconvulsant doses of intravenous ropivacaine, bupivacaine, and lidocaine in the conscious dog. Anesth Analg 69, 794.

Feldman HS, Dvoskin S, Arthur GR, Doucette AM (1996) Antinociceptive and motor-blocking efficacy of ropivacaine and bupivacaine after epidural administration in the dog. Reg Anesth 21, 318–326.

Grubb T, Lobprise H (2020) Local and regional anaesthesia in dogs and cats: descriptions of specific local and regional techniques (part 2). Vet Med Sci 6, 218–234.

Grubb T, Sager J, Gaynor JS et al. (2020) 2020 AAHA anesthesia and monitoring guidelines for dogs and cats. J Am Anim Hosp Assoc 56, 59–82.

Guerrero KSK, Campagna I, Bruhl-Day R et al. (2016) Intraperitoneal bupivacaine with or without incisional bupivacaine for postoperative analgesia in dogs undergoing ovariohysterectomy. Vet Anaesth Analg 43, 571–578.

Kanji S, Hayes M, Ling A et al. (2015) Reporting guidelines for clinical pharmacokinetic studies: the ClinPK statement. Clin Pharmacokinet 54, 783–795.

Kazmir-Lysak K, Steblaj B, Torgerson PR et al. (2023) Intraperitoneal and incisional ropivacaine did not improve postoperative analgesia after multimodal anaesthesia compared with saline in dogs undergoing ovariohysterectomy. Schweiz Arch Tierheilkd 165, 634–643.

Kopacz DJ, Carpenter RL, Mackey DC (1989) Effect of ropivacaine on cutaneous capillary blood flow in pigs. Anesthesiology 71, 69–74.

Labaille T, Mazoit JX, Paqueron X et al. (2002) The clinical efficacy and pharmacokinetics of intraperitoneal ropivacaine for laparoscopic cholecystectomy. Anesth Analg 94, 100–105.

Lambertini C, Kluge K, Lanza-Perea M et al. (2018) Comparison of intraperitoneal ropivacaine and bupivacaine for postoperative analgesia in dogs undergoing ovariohysterectomy. Vet Anaesth Analg 45, 865–870.

Lamy E, Fall F, Boigne L et al. (2020) Validation according to European and American regulatory agencies guidelines of an LC-MS/MS method for the quantification of free and total ropivacaine in human plasma. Clin Chem Lab Med 58, 701–708.

McClure JH (1996) Ropivacaine. Br J Anaesth 76, 300–307.

Moher D, Hopewell S, Schulz KF et al. (2010) CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 340, c869.

Monteiro BP, Lascelles BDX, Murrell J et al. (2023) 2022 WSAVA guidelines for the recognition, assessment and treatment of pain. J Small Animal Pract 64, 177–254.

Nakamura MD, Toda H, Kakuyama M et al. (1993) Direct vascular effect of ropivacaine in femoral artery and vein of the dog. Acta Anaesth Scand 37, 269–273.

R Core Team (2025) R: a language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/. Last accessed 1 August 2025.

Rosenberg PH, Veering BTh, Urmey WF (2004) Maximum recommended doses of local anesthetics: a multifactorial concept. Reg Anesth Pain Med 29, 564–575.

Soldin OP, Mattison DR (2009) Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 48, 143–157.

Stumpe M, Morton NS, Watson DG (2000) Determination of free concentrations of ropivacaine and bupivacaine in plasma from neonates using small-scale equilibrium-dialysis followed by

liquid chromatography—mass spectrometry. J Chromatogr B Biomed Sci Appl 748, 321—330.

Thomas JM, Schug SA (1999) Recent advances in the pharmacokinetics of local anaesthetics. Clin Pharmacokinet 36, 67—83.

Verdier N, Hummel K, Razzazi-Fazeli E (2025) A method for analysis of free and total ropivacaine in dog plasma using UHPLC—MS/MS. Biomed Chromatogr 39, e70214.

Wilson DV, Barnes KS, Hauptman JG (2004) Pharmacokinetics of combined intraperitoneal and incisional lidocaine in the dog following ovariohysterectomy. J Vet Pharmacol Ther 27, 105—109.

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