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Pharmacokinetics of a controlled-release liposome-encapsulated hydromorphone administered to healthy dogs

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Abstract

The purpose of the study was to assess the pharmacokinetics of liposome-encapsulated (DPPC-C) hydromorphone administered intravenously (IV) or subcutaneously (SC) to dogs. A total of eight healthy Beagles aged 12.13 ± 1.2 months and weighing 11.72 ± 1.10 kg were used. Dogs randomly received liposome encapsulated hydromorphone, 0.5 mg/kg IV ($n = 6$), 1.0 mg/kg ($n = 6$), 2.0 mg/kg ($n = 6$), or 3.0 mg/kg ($n = 7$) SC with a 14–28 day washout between trials. Blood was sampled at serial intervals after drug administration. Serum hydromorphone concentrations were measured using liquid chromatography with mass spectrometry. Serum concentrations of hydromorphone decreased rapidly after IV administration of the DPPC-C formulation (half-life = 0.52 h, volume of distribution = 12.47 L/kg, serum clearance = 128.97 mL/min/kg). The half-life of hydromorphone after SC administration of DPPC-C formulation at 1.0, 2.0, and 3.0 mg/kg was 5.22, 31.48, and 24.05 h, respectively. The maximum serum concentration normalized for dose (C_{MAX}/D) ranged between 19.41–24.96 ng/mL occurring at 0.18–0.27 h. Serum hydromorphone concentrations fluctuated around 4.0 ng/mL from 6–72 h after 2.0 mg/kg and mean concentrations remained above 4 ng/mL for 96 h after 3.0 mg/kg DPPC-C hydromorphone. Liposome-encapsulated hydromorphone (DPPC-C) administered SC to healthy dogs provided a sustained duration of serum hydromorphone concentrations.

INTRODUCTION

Hydromorphone is an opioid that primarily exerts its effects at the μ opiate receptor (Hennies *et al.*, 1988). Previous studies in humans have demonstrated hydromorphone is about seven times more potent than morphine. In comparison to morphine, hydromorphone produces a shorter duration of analgesia and similar adverse effects in humans (Mahler & Forrest, 1975; Dunbar *et al.*, 1996; Coda *et al.*, 1997; Lawlor *et al.*, 1997).

The pharmacokinetics of hydromorphone after administration of the conventional formulation to dogs have been previously studied (Kukanich *et al.*, 2008). Hydromorphone was rapidly eliminated in a dose dependant manner following IV and SC administration with the terminal half-life ranging from ~0.6 (0.1 mg/kg) to ~1.1 h (0.5 mg/kg). The volume of distribution was large (~ 5 L/kg), as expected, and the systemic clearance was rapid, ranging from 65 mL/min/kg (0.5 mg/kg IV) to 110 mL/kg/min (0.1 mg/kg SC). Hydromorphone was rapidly absorbed following SC administration with the T_{MAX} occurring at 0.22 and 0.33 h following 0.1 and 0.5 mg/kg.

Due to the rapid elimination and short terminal half-life of hydromorphone after administration of the conventional formulation, the recommended dosing regimen, 0.1 mg/kg IV or SC q 2 h, requires frequent administration. Based on the human literature, a serum concentration of hydromorphone greater than or equal to 4 ng/mL provides analgesia (Coda *et al.*, 1997), however the concentration-effect profile of hydromorphone has not been described in dogs. In order to maintain serum concentrations greater than 4 ng/mL in dogs, a constant rate infusion (CRI) could be administered, 0.03 mg/kg/h, but would require hospitalization, maintenance of an IV catheter, and use of an infusion pump to accurately deliver the correct dosage. Regardless of the dosage regimen, the administration of the conventional formulation of hydromorphone is labor intensive in order to maintain targeted serum concentrations.

A novel approach that overcomes the rapid elimination and short half-life of conventional formulations of hydromorphone, and which prolongs the dosing interval, is to produce a liposome encapsulated (LE) formulation. This formulation has been designed at the University of Wisconsin for prolonged release after subcutaneous injection. Conceptually, encapsulation of a drug in liposomes provides a reservoir of drug that is slowly released after injection into the animal by virtue of passive efflux across the lipid bilayers of the liposome membrane, disruption of the liposome membrane through interaction with lipoproteins and other serum components (Wasan & Lopez-Berestein, 1996), or phagocytosis and breakdown by tissue macrophages (Ishida *et al.*, 2002). Previous studies utilizing oxymorphone and hydromorphone in a liposome encapsulated formulation produced prolonged antinociceptive effects and serum drug concentrations compared to the conventional formulation (Krugner-Higby *et al.*, 2003; Smith *et al.*, 2003, 2004, 2006; Clark *et al.*, 2004). The prolonged drug absorption appeared to result in a flip-flop phenomenon; that is, the rate of absorption is much slower than the rate of elimination with the result that the slope of the terminal portion of the serum profile is actually the absorption rate (Toutain & Bousquet-Melou, 2004). These previous studies were performed using liposomes made with egg phosphatidylcholine both with and without cholesterol, a relatively leaky liposome. In order to find a liposome preparation that allows for very slow drug release, resulting, at least theoretically, in minimal bolus effect after injection while providing a prolonged therapeutic serum profile, a liposome-encapsulated hydromorphone made with dipalmitoylphosphatidylcholine and cholesterol (DPPC-C) was developed. The choice of dipalmitoylphosphatidylcholine, a high phase transition temperature phospholipid with saturated fatty acyl chains, in conjunction with cholesterol, produces highly stable

membranes that are less permeable to drug and more stable in the biological milieu (Senior & Gregoriadis, 1982).

The purpose of the study was to evaluate the serum profile and pharmacokinetics of hydromorphone incorporated into a DPPCC formulation in healthy dogs. Our hypothesis was that hydromorphone would be slowly absorbed after subcutaneous administration of DPPC-C hydromorphone, producing sustained hydromorphone serum concentrations of 4 ng/mL or greater for at least 72 h.

MATERIALS AND METHODS

Preparation of liposome-encapsulated hydromorphone using modified dehydration-rehydration vesicles

Liposomes containing hydromorphone HCl (Sigma-Aldrich, Saint Louis, MO, USA) were prepared from dipalmitoylphosphatidylcholine (Avanti Polar Lipids, Alabaster, AL, USA) and cholesterol (Avanti Polar Lipids, Alabaster, AL, USA), in a molar ratio of 2:1. A mixture of 80 μ mol DPPC and 40 μ mol cholesterol was dried from chloroform solution in a 20 mm screw capped tube, dissolved in 1 mL of sterile tert-Butanol, 99+% A.C.S. (Sigma-Aldrich, Saint Louis, MO, USA) by heating to 55 °C in a water bath, frozen in dry ice-isopropanol, and lyophilized for 24 h. The dried lipid was swollen in 1 mL of 40 mg/mL hydromorphone HCl^a in 10 mM citrate (Sigma-Aldrich, Saint Louis, MO, USA) buffer, pH 4.0, for 30 min in a 55 °C water bath. The liposomes were frozen in dry ice-isopropanol for 2 min, and stored at -20 °C overnight. The liposomes were thawed at room temperature, diluted to 5 mL in 10 mM sodium acetate buffer, pH 4, placed in 5 mL sterile ultra-clear centrifuge tubes (Beckman Coulter, Fullerton, CA, USA) and sedimented at 100 000 g for 30 min at 4 °C in a Beckman Model L8-M Ultracentrifuge. The supernatant was removed from the tube using a sterile 5 mL disposable pipet (Fisher Scientific, Chicago, IL, USA) and the liposome pellet was re-suspended in 10 mM sodium acetate buffer, pH 4.0. Liposome-encapsulated hydromorphone was quantitated spectrophotometrically. There can be variation between liposome preparations owing to differences in the efficiency of drug capture. However, the dose of hydromorphone administered was always the same for each dog in a given treatment group. When liposome preparations are analyzed for their hydromorphone content, multiple measurements are made and the variation between multiple readings is <1%. Once quantitated, the suspension was stored in a dark cabinet at 4 °C for no more than 7 days prior to use. Long-term stability studies, at 4 °C, on liposomes resulted in a low leakage, with only 16% released in 12 months of storage (unpublished data, TD Heath personal communication). Dilution of stock liposomes prior to administration is not found to accelerate leakage. Therefore, it was not anticipated that materials diluted for injection and stored for 7 days would be significantly different from those diluted and injected immediately. It should be noted that the liposomes are not reconstituted for injection, rather they are diluted from a stock solution.

Immediately prior to injection into an animal, the preparation was gently agitated and then slowly drawn up into a syringe using a 22 gauge needle.

Animal groups and instrumentation

The study was approved by the University of Wisconsin School of Veterinary Medicine Research Animal Care and Use committee. A total of eight healthy purpose-bred male neutered Beagle dogs (Ridgelan Laboratories, Mount Horeb, WI, USA) were used, with an age of (mean $\pm$ SD) 12.13 ± 1.2 months and body weight 11.72 ± 1.10 kg. Normal health status was confirmed prior to entry into the study based upon the results of physical examination, CBC, and serum chemistry profile. After arrival and acclimation, all dogs were anesthetized on one occasion only for purposes of castration and insertion of a permanent vascular access port (Companion Port®, Norfolk Medical, Skokie, IL, USA). Anesthesia consisted of 0.05 mg/kg acepromazine and 0.04 mg/kg buprenorphine IM, propofol IV to effect, and maintenance on isoflurane in oxygen. Postoperative pain was managed with 0.04 mg/kg of buprenorphine IM at extubation and 4.0 mg/kg of carprofen PO once a day for 2 days after surgery. Surgical procedure for castration was routine. To implant the permanent vascular access ports, a cut-down incision was made over the right jugular vein with the dog in left lateral recumbency and approximately 7' of the distal end of the silastic catheter was threaded into the jugular vein, using a needle introducer, to approximately the right atrium. A second incision was made between the scapulae on the dorsal surface of the dog and a curved hemostat was used to tunnel subcutaneously to the proximal end of the catheter, which was grasped and threaded over the receiving end of the vascular access port. The port was sutured to subcutaneous fascia using 2-0 absorbable suture at four symmetrical points around the disc-shaped port. The overlying subcutaneous layers and skin of both incisions were closed routinely. The port was flushed once at surgery and daily for 3 days after surgery with 2 mL of 100 IU/mL heparinized saline. At least 30 days were allowed as a recovery period prior to the initiation of the study.

Drug dosing protocols

All drug treatments were administered via a 3 mL syringe attached to a 22 SWG 1' needle. Dogs were each weighed on the morning prior to each drug treatment and doses were calculated on a mg/kg basis for the dog's weight on that day. All subcutaneous (SC) drug administrations were given in the loose skin caudal to the scapulae at least 5 cm distal to the permanent vascular access port. Intravenous (IV) drug administration was performed by one experienced person (LJS) into either the cephalic or saphenous vein. All drug administrations were injected slowly over a period of 60–90 sec. Acetate buffer was added such that all dogs received the same volume of drug for each treatment (regardless of dose) on a mL/kg basis. The following groups were included in the final study: 1. $n = 6$ DPPC-C at 0.5 mg/kg hydromorphone HCl IV; 2. $n = 6$ DPPC-C at 1.0 mg/kg hydromorphone HCl SC; 3. $n = 6$ DPPC-C at 2.0 mg/kg hydromorphone HCl SC; 4. $n = 7$ DPPC-C at 3.0 mg/kg hydromorphone HCl SC; 5. $n = 2$ blank DPPC-C liposomes that contained acetate buffer at 0.2 mL/kg SC. Treatments were administered in random order, determined by research randomizer software, to the dogs that participated in this study. Most dogs were administered multiple treatments, but 2–4 weeks were allowed as a washout between treatments. Although eight dogs were used for this study, the numbers reflected in the data sets are less than $n = 8$. This is because 1–2 data sets were lost for each drug challenge due

to technical difficulties in either sample collection, storage, and/or HPLC analysis in a different laboratory from the one reporting data here.

Blood sampling for serum analysis of hydromorphone

The vascular access ports were used for all blood sampling. The ports were flushed with 1 mL of 10 IU/mL heparinized saline, then 3 mL of blood/heparinized saline was aspirated and set aside. The sample was aspirated and the 3 mL of blood/heparinized saline was re-injected into the port, followed by a 1 mL flush of heparinized saline. In this way, the total amount of blood drawn from each dog was minimized while ensuring a clean sample uncontaminated with heparin. The total amount of heparin injected within the 24-h period of the day of drug administration did not exceed 25 IU of heparin per kg body weight. Whole blood samples (3–4 mL each) were obtained prior to dosing (baseline), and at 5, 10, 15, 20, 30, 45, 60 and 90 min and 2, 4, 6, 8, and 12 h post injection. Additional blood samples (4 mL) were taken daily as described above for 7 days postinjection. Blood was immediately transferred into serum separator tubes (Vacutainer SST 5 mL; Becton-Dickson, Franklin Lakes, NJ, USA) and held on ice for no more than 2 h, then centrifuged for 15 min at 10 °C and approximately 1000 g. Serum was separated and frozen at –70 °C until analysis. In the current study, no samples were stored for more than 9 months.

Hydromorphone and pharmacokinetic analyses

Serum samples were analyzed by liquid chromatography with mass spectrometry for hydromorphone according to previously published methods (Kukanich *et al.*, 2008). Solid phase extraction of the serum samples was used to extract drug from serum. The mobile phase consisted of 88% of 0.1% citric acid and 12% acetonitrile. Hydromorphone d3 was used as an internal standard. The lower limit of quantification was 2 ng/mL. The accuracy (deviation from true value) was within $5 \pm 1\%$ (mean $\pm$ SEM), and the precision (coefficient of variation) was $7 \pm 1\%$ performed on replicates of 5 with a known amount of hydromorphone reference standard added to serum at concentrations of 2, 20, and 500 ng/mL handled in an identical manner as the incurred samples. For the calculation of mean serum concentrations (Table 1), values below the LOQ of the assay were entered as 0, therefore the mean values for some of the time points are less than 2 ng/mL (LOQ).

Pharmacokinetic parameters were estimated with computer software (WinNonlin 5.0, Pharsight Academic License, Pharsight Corporation, Mountain View, CA, USA) using noncompartmental analysis. The estimated variables included the area under the curve from time 0 to infinity ($AUC_{0-\infty}$) and the area under the curve from 0 to the last time point above the LOQ of the assay (AUC_{0-LAST}) using the linear trapezoidal rule. The area under the first moment curve from time 0 to infinity ($AUMC_{0-\infty}$), area under the first moment curve from time 0 to the last time point above the LOQ of the assay ($AUMC_{0-LAST}$) serum clearance per fraction of the dose absorbed (Cl/F), apparent volume of distribution at steady state per fraction of the dose absorbed (Vd_{ss}/F), apparent volume of distribution of the area during the elimination phase per fraction of the dose absorbed (Vd_{area}/F), first-order rate constant (λ_z), terminal half-life ($t_{1/2} \lambda_z$), biologic half-life ($t_{1/2} BIOL = 0.693 * MRT_{0-LAST}$), mean residence time extrapolated to infinity ($MRT_{0-\infty}$), mean residence time from 0 to the last measured time point (MRT_{0-LAST}), maximum serum concentration (C_{MAX}), and time to maximum

serum concentration (T_{MAX}) were also estimated. The C_{MAX} per dose (C_{MAX}/D) was calculated by dividing the C_{MAX} by the actual dose administered as hydromorphone base. The concentration at time 0 (C_0) was calculated by log-linear regression utilizing the first two time points. Serum drug concentrations below the LOQ of the assay were not used in the determination of the pharmacokinetic variables.

RESULTS

Minor adverse effects were noted in the dogs after IV or SC administration of the DPPC-C hydromorphone formulation. Moderate sedation was noted in most dogs for the first 3–4 h after drug administration and was associated with transient mild bradycardia, nausea and panting. The most profound sedation was noted in the IV DPPC-C treated groups, while the sedation between the three doses of DPPC-C hydromorphone given subcutaneously was subjectively assessed as similar. There was no swelling detected at the injection sites at any time during the study.

Table 1 displays mean serum concentrations vs. time for the 0.5 mg/kg IV DPPC-C hydromorphone group and for 1.0, 2.0, and 3.0 mg/kg SC DPPC-C hydromorphone groups. Hydromorphone was rapidly eliminated after IV administration of the DPPC-C formulation with a 1.12 h (geometric mean) terminal half-life (Table 2, Fig. 1) and a 0.52 h biologic half-life. The volume of distribution per fraction of the dose absorbed was larger than expected ($V_{d_{area}}/F = 12.47$ L/kg, $V_{d_{ss}}/F = 8.64$ L/kg) and the serum clearance per fraction of the dose absorbed was rapid, 128.97 mL/min/kg.

Hydromorphone was not detected in any time 0 serum sample or from 0–24 h in the two animals treated with the unloaded DPPC-C liposomes, in which hydromorphone was not included. The mean T_{MAX} of hydromorphone after SC administration of DPPC-C formulation was rapid (0.18–0.27 h), varying slightly with the dose (Table 3, Figs 2–4). The mean C_{MAX} increased proportionally with dose. The mean C_{MAX}/D was similar between each of the SC doses (19.41–24.96 ng/mL). A secondary peak was noted following each SC dose at 24 h post administration, but was of a lesser magnitude than the C_{MAX} . As expected, the AUC_{0-LAST} increased with increasing doses from 1.0 mg/kg to 3.0 mg/kg. Due to the prolonged terminal portion of the serum profile the $AUC_{0-\infty}$ may not be accurate due to the large portion extrapolated for the 1 and 2 mg/kg DPPC-C hydromorphone groups. The large amount of extrapolation is due to the serum concentrations decreasing below the LOQ of the assay. However the mean AUC% extrapolated (16.31%) for the 3 mg/kg group is within the targeted value of less than 20% which suggest it is a valid extrapolation.

The DPPC-C formulation exhibited prolonged hydromorphone concentrations after SC administration. The biologic half-lives were 5.22 h (1.0 mg/kg), 31.48 h (2.0 mg/kg), and 24.05 h (3.0 mg/kg) after SC administration. Mean serum concentrations decreased below 4 ng/mL at the 4 h time point after IV administration, 4 h time point after 1.0 mg/kg SC, and 96 h time point following 3.0 mg/kg SC. The mean serum concentrations fluctuated around 4 ng/mL between 6 and 72 h at which point it decreased below 4 ng/mL and continued to decrease for the 2.0 mg/kg dose group.

DISCUSSION

Commercially available, conventional formulations of hydromorphone are rapidly eliminated after SC administration of a clinically relevant dose (0.1 mg/kg) with a short terminal half-life, 0.7 h (Kukanich *et al.*, 2008). The short half-life of conventional formulations of hydromorphone results in the need for frequent drug administration (every 2 h) in order to maintain targeted serum concentrations.

Previous studies have indicated that controlled release of opioids can be achieved after SC administration by using a liposome-encapsulated formulation (Krugner-Higby *et al.*, 2003; Smith *et al.*, 2003, 2004, 2006; Clark *et al.*, 2004). These previous studies employed liposomes made with egg phosphatidylcholine, and showed that there was some early bolus release of drug, based upon behavioral effects of the opioids on the animals and on serum drug concentrations obtained with the ELISA assay. The results of the current study indicate that hydromorphone can be effectively incorporated into a more stable liposome formulation made with dipalmitoylphosphatidylcholine and cholesterol to achieve a sustained drug release with near steady state levels through 72 h.

The volume of distribution of hydromorphone after IV administration of the DPPC-C formula ($V_{d_{ss}}/F = 8.64$ L/kg, and $V_{d_{area}}/F = 12.47$ L/kg) was larger than reported for the values of IV conventional formulations of hydromorphone ($V_{d_{ss}} = 4.24$ L/kg, and $V_{d_{area}} = 5.25$ L/kg.) The difference is most likely caused by the rapid sequestration of the liposomes in the liver, which should occur within the first hour following administration (Senior & Gregoriadis, 1982; Ishida *et al.*, 2002). Such sequestration will limit bioavailability, since hydromorphone will be released slowly from the liposomes and metabolized in the liver without release back into the systemic circulation resulting in a situation analogous to first pass metabolism. The resultant AUC of the IV DPPC-C formulation is lower than that estimated for the conventional formulation of hydromorphone with a calculated fraction of the dose absorbed being 0.47. The less than complete bioavailability may increase the safety of the DPPC-C formulation if it is inadvertently administered IV.

The longer and steeper distribution phase of the IV DPPC-C formulation, compared to IV conventional hydromorphone (Fig. 5), is suggestive the DPPC-C formulation of hydromorphone is being sequestered in some area of the body and removed prior to reentering systemic circulation. The inflexion point of the plasma profile of the conventional hydromorphone HCl formulation occurs at approximately 10 min (~100 ng/mL) whereas the inflexion point for the DPCC-C formulation occurs at approximately 45 min (~25 ng/mL). The similar terminal half-lives of IV conventional hydromorphone HCl (1.00 h) and IV DPCC-C hydromorphone (1.12 h) are also similar suggesting the actual elimination is occurring at a similar rate.

From the IV DPPC-C-H data we could not determine whether the amount of hydromorphone measured in serum was free drug released from the liposomes as well as encapsulated drug. However, the C0 for the conventional formulation of hydromorphone (157.21 ng/mL) and the C0 for the DPCC-C formulation of hydromorphone (148.40 ng/mL)

are similar suggesting that both free hydromorphone and liposome encapsulated hydromorphone are measured by the mass spectrometry assay.

The 24.05–31.48 h half-life of the DPPC-C formulation of hydromorphone administered SC was markedly longer than that previously reported for conventional hydromorphone administered SC (1.11 h). The controlled release of the drug results in a flip-flop phenomenon where drug absorption is the rate-limiting step in drug elimination. The biologic half-life is presented since the terminal half-life of the SC DPPC-C formulation of hydromorphone may not be accurate due to the flattening of the curve and the serum concentrations being close to the lower limit of quantification of the assay. After subcutaneous administration of all three doses of DPPC-C hydromorphone, the release pattern was triphasic, with immediate release of a portion of drug causing an initially high serum concentration, followed by a rapid decline, a small secondary peak, and finally a prolonged terminal phase. The stability of the formulation of liposome-encapsulated hydromorphone used in this study and the amount of drug released from the liposomes into the vehicle is only 3% in the first month of storage. *In vitro* release studies also show an initially fast release rate for the drug. The cause of the multiphasic serum profile is not currently known.

The mean and median values of the half-life varied markedly between the 1.0, 2.0, and 3.0 mg/kg SC DPPC-C hydromorphone formula groups, and the values varied within each dose group as noted by the range of the 25th and 75th percentiles. The half-life in the 3.0 mg/kg dose group appeared to be the least variable as indicated by the smaller range in the 25th and 75th percentiles. The variability within the 1.0 and 2.0 mg/kg groups may be in part due to the serum concentrations being close to the LOQ of the assay. Although the repeatability of the assay at the LOQ was within acceptable range ($\leq \pm 15\%$ of the actual value), serum concentrations below the LOQ could not be utilized in the determination of the terminal slope. As a result, fewer time points were available for the determination of late phases of the serum profile in the 1.0 and 2.0 mg/kg groups, and the estimates may not be as robust as the 3.0 mg/kg dose group resulting in the increased variability. It is also possible that the absorption from the 1.0 and 2.0 mg/kg group was more variable, resulting in a more variable serum profile.

The prolonged half-life of hydromorphone after administration of the DPPC-C formulation indicates accumulation may occur with multiple doses. However the purpose of the current study was to assess the pharmacokinetics of hydromorphone in the DPCC-C formulation after a single dose. More studies are needed to assess the pharmacokinetics, pharmacodynamics, and safety after multiple doses of the DPCC-C formulation of hydromorphone to dogs.

The AUC_{0-LAST} increased with increasing doses of DPPC-C hydromorphone, which is expected because the AUC is an estimate of drug exposure. However the disproportionate increase in AUC_{0-LAST} between the 1.0 mg/kg and 2.0 mg/kg doses is probably due to the serum drug concentrations in the 1 mg/kg dose group being close to the LOQ of the assay resulting in a poor estimation of the pharmacokinetic parameters including the AUC.

Therefore the pharmacokinetic estimates for the 1 mg/kg group may not be accurate and cautious interpretation is needed.

A previous study using different dogs administered hydromorphone as the commercially available solution, 0.5 mg/kg IV yielded a mean $AUC_{0-\infty}$ of 122.55 h·ng/mL (Kukanich *et al.*, 2008). The fraction of the drug absorbed (F) can be estimated by comparing the $AUC_{0-\infty}$ for the conventional formulation and the 3 mg/kg DPPC-C formulation with the following equation:

$$F = \frac{AUC_{0-\infty}(DPPC-C-H_{SC})}{Dose(DPPC-C-H_{SC})} \times \frac{Dose(conventional_{IV})}{AUC_{0-\infty}(conventional_{IV})}$$

where $AUC_{0-\infty}$ (DPPC-C- H_{SC}) is the $AUC_{0-\infty}$ for the group administered DPPC-C formulation subcutaneously, Dose (DPPC-C- H_{SC}) is the dose of DPPC-C formulation administered subcutaneously, Dose (conventional_{IV}) is the dose of the conventional formula administered IV in a previous study (0.5 mg/kg), and $AUC_{0-\infty}$ (conventional) is the $AUC_{0-\infty}$ for the group administered the conventional formulation of hydromorphone IV in a previous study (122.55 h·ng/mL). The estimated fraction of the dose absorbed for the 3 mg/kg DPPC-C formulation group was 1.03. However since the results are not from a crossover design the fraction of the dose absorbed needs to be interpreted cautiously, but is suggestive that hydromorphone is well absorbed from the DPPC-C formulation. Due to the large extrapolation of the $AUC_{0-\infty}$ for the 1 and 2 mg/kg DPCC-C an estimation of the fraction of the dose absorbed for the groups was not made.

The C_{MAX} increased with increasing doses, as expected. However the C_{MAX}/D was similar regardless of the dose administered indicating peak serum drug concentrations are proportional to the dose administered. Adverse effects of opioids are often proportional to the drug concentration achieved or dose administered. For example, the respiratory depressant effects of methadone are well correlated with plasma drug concentration in dogs (Schlitt *et al.*, 1978). Therefore the dose proportional C_{MAX} achieved following SC hydromorphone in the DPPC-C formula indicate consistency between the different doses administered and adverse effects are expected to increase proportionally with increasing doses.

The C_{MAX} after 2.0 mg/kg DPPC-C hydromorphone (35.50 ng/mL) is similar to the C_{MAX} (34.8 ng/mL) after 0.1 mg/kg of the conventional formulation of hydromorphone (Kukanich *et al.*, 2008). The adverse effects may not be more frequent in the larger dose DPPC-C hydromorphone group (2 mg/kg SC) as compared to the 0.1 mg/kg conventional hydromorphone if the adverse effects of hydromorphone are proportional to its serum concentration.

The hydromorphone T_{MAX} was rapid and similar regardless of the dose of the DPPC-C formulation administered subcutaneously, ranging from 0.18–0.27 h. The rapid rise in serum hydromorphone concentrations is expected to produce a rapid effect. The T_{MAX} for hydromorphone for the DPPC-C formulation was similar to that achieved by the conventional hydromorphone formulation, 0.22–0.33 h, after SC dosing (Kukanich *et al.*,

2008). The rapid increase in serum concentrations is expected to result in a rapid onset of drug effect, regardless of the formulation administered, but a prolonged effect is also expected with DPPC-C formulation due to persistent serum hydromorphone concentrations.

The 3.0 mg/kg SC DPPC-C formulation maintained mean serum hydromorphone concentrations above the targeted 4 ng/mL for at least 72 h. Based upon the previous pharmacokinetic study with conventional hydromorphone, the total CRI dose (0.03 mg/kg/h) needed to maintain a serum concentration of 4 ng/mL for 72 h (total dose = 2.2 mg/kg) is similar to the single SC dose of the DPPC-C formulation needed to maintain mean serum concentration above 4 ng/mL for at least 72 h. In humans, a serum concentration of approximately 4 ng/mL is correlated with excellent clinical analgesia (Coda *et al.*, 1997). The data reported in the current study suggest that one SC dose of the DPPC-C formulation of hydromorphone administered to healthy dogs will provide mean serum concentrations greater than or equal to 4 ng/mL for up to 72 h. However it is unknown if a serum concentration of 4 ng/mL provides analgesia in dogs.

In conclusion, the DPPC-C formulation rapidly achieved maximum serum hydromorphone concentrations, and also produced prolonged drug concentrations through sustained release. Although the ideal serum concentrations of hydromorphone needed to achieve antinociceptive effects in dogs are unknown, 3.0 mg/kg of the DPPC-C formulation of hydromorphone achieves a targeted mean serum concentration of 4 ng/mL, within 5 min, and maintains it for at least 72 h in dogs. A 3.0 mg/kg dose of DPPC-C hydromorphone is suggested for future studies assessing the efficacy and adverse effects of this novel formulation of hydromorphone in dogs.

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Fig. 1. Individual hydromorphone serum concentrations following 0.5 mg/kg IV of the DPPC-C formulation of hydromorphone; $n = 6$.

Fig. 2. Individual hydromorphone serum concentrations following 1 mg/kg SC of the DPPC-C formulation of hydromorphone; $n = 6$.

Fig. 3. Individual hydromorphone serum concentrations following 2 mg/kg SC of the DPPC-C formulation of hydromorphone; $n = 6$.

Fig. 4. Individual hydromorphone serum concentrations following 3 mg/kg SC of the DPPC-C formulation of hydromorphone; $n = 7$.

Fig. 5. Comparative plasma profiles (mean $\pm$ SEM) of the conventional hydromorphone formulation and of the DPCC-C formulation of hydromorphone both administered 0.5 mg/kg IV.

Table 1

Mean $\pm$ SEM serum hydromorphone concentrations (ng/mL) following administration of the DPPC-C formulation either IV or SC. Serum concentrations below the LOQ of the assay were entered as 0 ng/mL for the determination of the mean serum drug concentrations

Time (h)	Serum concentration (ng/mL)											
	0.5 mg/kg IV		1 mg/kg SC		2 mg/kg SC		3 mg/kg SC					
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0.083	95.63	14.98	13.60	6.14	50.11	26.56	43.16	6.24				
0.167	55.62	5.52	26.95	13.89	59.52	29.30	60.00	7.71				
0.25	40.52	5.08	31.74	18.34	62.93	31.25	65.69	9.93				
0.33	29.22	3.73	31.51	21.08	58.20	27.82	53.46	5.41				
0.5	21.09	2.59	22.57	14.06	54.97	28.24	44.47	5.09				
0.75	15.81	2.62	15.62	8.82	41.41	21.10	33.02	4.27				
1	12.62	1.51	10.90	5.35	32.06	15.56	27.82	5.07				
1.5	8.59	0.93	7.44	3.32	20.38	9.17	18.84	3.76				
2	5.83	0.81	5.90	2.43	17.72	7.49	15.39	3.33				
4	1.31	0.42	2.05	1.01	7.56	2.43	7.31	1.48				
6			1.39	0.65	3.38	0.96	6.19	1.32				
8			1.07	0.74	4.43	1.32	6.28	1.36				
12			0.37	0.37	2.67	0.25	4.62	0.85				
24			1.55	0.50	4.16	0.41	8.18	3.54				
48			1.19	0.53	4.11	0.61	6.05	2.06				
72			0.34	0.34	3.67	0.49	4.40	0.94				
96					2.50	0.55	2.93	0.78				
120					2.28	0.48	1.38	0.69				
144					0.76	0.48	0.32	0.32				

Table 2

Geometric mean, median, 25th, and 75th percentiles following 0.5 mg/kg IV DPCC-C formulation of hydromorphone; $n = 6$

Parameter	Units	Mean	Percentiles		
			25th	75th	
AUC _{0-LAST}	h-ng/mL	52.23	48.17	59.00	
AUC _{0-∞}	h-ng/mL	57.29	51.27	67.42	
AUC _{Extrapolated}	%	8.29	6.04	10.18	
AUMC _{0-∞}	h-h-ng/mL	63.95	54.49	65.85	
AUMC _{0-LAST}	h-h-ng/mL	39.23	31.80	48.66	
C ₀	ng/mL	148.20	88.94	245.68	
T _{1/2BIOL}	h	0.52	0.40	0.70	
T _{1/2λ_z}	h	1.12	0.73	1.38	
λ _z	1/h	0.621	0.50	0.95	
MRT _{0-LAST}	h	0.75	0.57	1.01	
MRT _{0-∞}	h	1.12	0.86	1.40	
Cl/F	mL/min/kg	128.97	109.59	144.12	
Vd _{disev} /F	L/kg	12.47	11.79	22.24	
Vd _{ss} /F	L/kg	8.64	8.45	14.67	

Table 3

Geometric mean, median, 25th, and 75th percentiles of the pharmacokinetics parameters of hydromorphone SC DPCC-C formulation

Parameter	Units	1 mg/kg DPCC SC; n = 6				2 mg/kg DPCC SC; n = 6				3 mg/kg DPCC SC; n = 7			
		Percentiles				Percentiles				Percentiles			
		Mean	Median	25th	75th	Mean	Median	25th	75th	Mean	Median	25th	75th
AUC _{0-∞}	h-ng/mL	191.58	339.46	147.06	519.00	760.97	731.63	660.63	793.89	756.81	756.81	611.81	1013.12
AUC _{Extrapolated}	%	40.27	67.72	30.32	69.76	29.41	29.90	21.14	37.73	16.31	16.60	9.83	23.54
AUC _{0-LAST}	h-ng/mL	78.97	130.93	71.02	150.54	511.96	518.73	456.08	554.74	618.47	572.12	408.62	917.48
AUMC _{0-LAST}	h-h-ng/mL	594.95	1719.26	204.85	2873.76	23 253.22	26 884.07	18 602.50	32 045.46	24 740.19	27 017.78	15 689.50	41 541.42
C _{MAX}	ng/mL	17.21	10.56	7.38	43.84	35.50	16.89	16.30	163.96	66.39	63.28	48.21	87.28
C _{MAX/D}	ng/mL	19.41	11.90	8.32	49.44	20.02	9.52	9.19	92.46	24.96	23.79	18.12	32.81
T _{MAX}	h	0.27	0.29	0.25	0.33	0.18	0.25	0.083	0.25	0.22	0.25	0.17	0.25
T _{1/2BIOL}	h	5.22	9.688	1.02	16.15	31.48	36.93	22.16	48.71	24.05	26.91	25.29	34.70
MRT _{0-LAST}	h	7.53	13.98	1.48	23.31	45.42	53.29	31.97	70.28	40.00	38.84	36.50	50.07