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Pharmacokinetics of ammonium sulfate gradient loaded liposome-encapsulated oxymorphone and hydromorphone in healthy dogs

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Abstract

Objective—To evaluate the pharmacokinetics, in dogs, of liposome-encapsulated oxymorphone and hydromorphone made by the ammonium sulfate gradient loading technique (ASG).

Animals—Four healthy purpose-bred Beagles aged 9.5 ± 3.2 months and weighing 13.4 ± 2.3 kg.

Study Design—Randomized cross-over design.

Methods—Each dog was given either 4.0 mg kg^{-1} of ASG-oxymorphone or 8.0 mg kg^{-1} of ASG-hydromorphone SC on separate occasions with a 3-month washout period. Blood was collected at baseline and at serial time points up to 1032 hours (43 days) after injection for determination of serum opioid concentrations. Serum opioid concentrations were measured with HPLC-MS and pharmacokinetic parameters were calculated using commercial software and non-compartmental methods.

Results—Serum concentrations of oxymorphone remained above the limit of quantification for 21 days, while those for hydromorphone remained above the limit of quantification for 29 days. C_{max} for ASG-oxymorphone was 7.5 ng mL^{-1}; C_{max} for ASG-hydromorphone was 5.7 ng mL^{-1}.

Conclusions and clinical relevance—Oxymorphone and hydromorphone, when encapsulated into liposomes using the ammonium sulfate gradient loading technique, result in measureable serum concentrations for between 3 to 4 weeks. This formulation may have promise in the convenient use of opioids for clinical treatment of chronically painful conditions in dogs.

Introduction

The mu agonist opioids, oxymorphone and hydromorphone, are commonly recommended for acute pain control in dogs (Hellyer, 2002). Presently available formulations of extended release opioids prescribed for oral administration to humans undergo significant first pass effect in dogs and, thus, are not suitable for oral administration in this species. (Kukanich et al. 2005a; Aragon et al. 2009).

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Oxymorphone is a mu opioid agonist that is approximately 10 times more potent than morphine (Prommer, 2006; Papich 2011a). A pharmacokinetic study from our laboratory of the conventional formulation of oxymorphone in dogs has been published (KuKanich, et al. 2008a). When administered either IV or SC, the conventional formulation of oxymorphone, 0.1 mg kg^{-1}, has a short terminal half-life (0.8 – 1.0 hour) and short mean residence time (1.3 hours), and has a rapid clearance, exceeding hepatic blood flow, after IV administration (KuKanich, et al. 2008a). The short terminal half-life and rapid clearance suggest the duration of effect is short, 2-4 hours, in dogs administered 0.1 mg kg^{-1} (KuKanich, et al. 2008a; Papich 2011a).

Hydromorphone is a mu opioid agonist that is approximately 7 times more potent than morphine (Hennies et al. 1988; Papich, 2011b). Pharmacokinetic studies using conventional formulations of hydromorphone in dogs have also been previously reported (KuKanich et al. 2008b; Guedes, et al. 2008). Despite different study conditions, analytical methods, and pharmacokinetic analyses, the pharmacokinetic results from these two different studies of hydromorphone were similar. The results show that hydromorphone had a short terminal half-life (<1 hour), rapid clearance exceeding hepatic blood flow, and short mean residence time (~ 1.3 hours) after IV administration of 0.1 mg kg^{-1} (Kukanich, et al 2008b; Guedes, et al. 2008). These studies indicate a short duration of approximately 2 hours following 0.1 mg kg^{-1} due to the rapid clearance of hydromorphone (KuKanich et al. 2008b; Guedes, et al. 2008; Papich, 2011b).

The short terminal half-life and rapid clearance of oxymorphone and hydromorphone necessitate frequent administration, up to every 2 hours, to maintain targeted blood concentrations. The frequent administration is labor intensive and costly, produces large fluctuations in blood concentrations, and necessitates frequent and perhaps painful injections for the patient. Peaks in drug concentrations are associated with more adverse effects, such as sedation, nausea, or dysphoria. A strategy to overcome frequent administration and minimize fluctuations in plasma drug concentrations is to administer the drugs by constant rate IV infusion (CRI). However, a CRI requires hospitalization, maintenance of an IV catheter, is labor intensive in the form of constant supervision of the patient, and may result in phlebitis, catheter associated infections, and air embolisms as well as other adverse effects (Mathews et al. 1996; Walsh et al. 2005).

A different strategy to overcome the need for frequent administration is to incorporate the active drug into a repository or sustained release formulation. One approach is to encapsulate drug into liposomal membranes that release slowly over time. In previous work, our laboratory has developed liposome-encapsulated formulations of oxymorphone and hydromorphone that release drug over 3-4 days after subcutaneous administration (Smith et al. 2008; Krugner-Higby et al. 2011). These formulations were made using a freeze-thaw method and liposome membranes composed of dipalmatoyl-phosphatidylcholine (DPPC) and cholesterol. The release time of these formulations made them suitable for peri-operative pain control, but did not offer an extended duration longer than could be achieved with products such as the fentanyl patch (Kyles et al. 1996).

In the present study, we hypothesized that the use of an ammonium sulfate gradient loading method (ASG) would "trap" the opioid inside of the liposome due to relative ion exchange across the liposome membrane, producing a formulation with a longer release time. We tested our hypothesis by measuring serum concentrations of opioid after subcutaneous injection of either ammonium sulfate gradient loaded oxymorphone (ASG-oxymorphone) or hydromorphone (ASG-hydromorphone) into healthy adult male Beagles. Serum opioid concentrations were used to calculate standard pharmacokinetic parameters using commercial software.

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Materials and methods

Animals

The study was approved by the University of Wisconsin School of Veterinary Medicine Animal Care and Use Committee (Protocol # V1285) committee. Four healthy purpose-bred male neutered Beagle dogs (Ridgelan Laboratories, WI, USA) were used, with a mean ± S.D. age of 9.5 ± 3.2 months and body weight of 13.4 ± 2.3 kg. Normal health status was confirmed prior to entry into the study based upon the results of physical examination, complete blood count, and serum chemistry profile. Dogs were housed individually and fed commercial dog chow (Harlan Labs Dog Chow, Harlan Labs, WI, USA) and water *ad libitum*. Dogs were walked outside and socialized in the laboratory on a regular basis prior to testing. After arrival and acclimation, all dogs were anesthetized on one occasion only, for purposes of castration and insertion of a permanent vascular access port (VAP) (Norfolk Medical Products, IL, USA). Anesthesia consisted of 0.05 mg kg^{-1} acepromazine and 0.04 mg kg^{-1} buprenorphine IM, propofol IV to effect, and maintenance on isoflurane in oxygen. Post-operative pain was managed with 0.04 mg kg^{-1} of buprenorphine IM at extubation and 4.0 mg kg^{-1} of carprofen PO once a day for 2 days after surgery. Surgical procedure for castration was routine. To implant the permanent VAP, 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 level of 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 VAP. The port was sutured to subcutaneous fascia using 2-0 absorbable suture at 4 symmetrical points around the disc-shaped port. The overlying subcutaneous layers and skin of both incisions were closed routinely. The internal volume of the VAP and tubing was approximately 1 mL. The port was flushed once at surgery and daily for 3 days after surgery with 2 mL of 100 IU mL^{-1} heparinized saline. The ports thereafter remained patent without flushing until 30 days after they were placed, at which time they were flushed again prior to the start of the study. There was a 30-day recovery period before any dog participated in the study reported here.

At the end of the study, approximately 8 months after dogs had been purchased, they were anesthetized again as described above for removal of the VAP. All dogs were subsequently adopted out to private homes.

Preparation of ASG oxymorphone and hydromorphone

This method has been previously described by our laboratory (Krugner-Higby et al. 2011). Briefly, a mixture of 80 μmol DPPC and 40 μmol cholesterol (Avanti Polar Lipids, AL, USA) was dried by removing chloroform, dissolved in 1 mL sterile tert-butanol (Sigma Aldrich, MO, USA) by heating to 55°C in a water bath, frozen in a dry ice-isopropanol mixture, and lyophilized for 24 hours. Oxymorphone (U.S. Pharmacopeia, MD, USA) or hydromorphone (Professional Compounding Centers of America, TX, USA) was dissolved in double processed tissue culture water (Sigma Aldrich) and brought to pH 7.0, (42.2 mg mL^{-1}, 1.0 mL) and sterilized using a 0.22 mm filter (Millipore, MA, USA). Ammonium sulfate (240 mM or 31.7 mg mL^{-1}) (AMEND Drug and Chemical Company, NJ, USA) was dissolved in double processed tissue culture water (Sigma Aldrich). Then the solution was filtered using a 250 mL, 0.22 mm filter (Nalgene, NY, USA). The solution was used to swell liposomes in a 55°C water bath for 1 hour. The liposomes were frozen in dry ice-isopropanol for 2 minutes and stored at −20°C overnight. The liposomes were thawed at room temperature, washed in 0.9 % sodium chloride, pH 5.5 (XX) and sedimented at 100,000 ***g*** for 30 minutes at 4°C in a Beckman Model L8-M Ultracentrifuge (Beckman-

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Coulter Inc., CA, USA). The supernatant was removed and the liposome pellet was re-suspended in 1 mL of oxymorphone or hydromorphone and placed in a 55° C water bath for 1 hour. After this, the excess opioid was removed by washing the liposomes with more 0.9% sodium chloride, pH 5.5, (XX) and sedimented at 100,000 ***g*** for 30 minutes at 4°C in a Beckman Model L8-M Ultracentrifuge. The supernatant was removed and the liposome pellet was re-suspended in 1 mL of 10 mM sodium acetate buffer (pH 4.0). Encapsulation efficiency of the opioid was quantified via spectrophotometric analysis at 281 nm (oxymorphone) or 282 nm (hydromorphone) against a known concentration of a standard opioid mixture. There can be variation between liposome preparations owing to differences in the efficiency of drug capture. However, the dose of either oxymorphone or hydromorphone administered was always the same for each dog in a given treatment group. When liposome preparations are analyzed for their opioid content, multiple measurements are made and the variation between multiple readings was < 1%. Hydromorphone concentrations of the preparation were 40.7 mg mL^{-1} ± 3.2 (mean ± S.D.) and oxymorphone concentrations were 30.2 mg mL^{-1} ± 2.3 (mean ± S.D.). Thus, at the doses tested, all dogs were injected with a drug volume of less than 3 mL.

Drug dosing and blood collection

All drug treatments were administered via a 3 mL syringe attached to a 22-gauge 2.5 cm needle. Dogs were weighed on the morning prior to each drug treatment and doses were calculated on a mg kg^{-1} basis for the dog's weight on that day. All subcutaneous (SC) drug injections were given in the loose skin caudal to the scapulae at least 5 cm distal to the permanent vascular access port. All injections were administered over a period of 60-90 seconds. Treatments were administered in random order, determined by research randomizer software for the first treatment and the dogs received the other treatment on the second occasion. Each dog received either ASG-oxymorphone (4.0 mg kg^{-1}) or ASG-hydromorphone (8.0 mg kg^{-1}) on 1 of 2 experimental days, separated by a 3-month washout. In this study, we did not inject a liposome mixture containing just the buffer, as a previous study reported that no dogs injected with the vehicle control had measurable serum concentrations of opioid, nor did they demonstrate any detectable opioid-related side-effects (Smith et al. 2008).

The VAPs were used for all blood sampling. The ports were flushed with 1 mL of 10 IU mL^{-1} 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-hour period of the day of drug administration did not exceed 25 IU kg^{-1}. Whole blood samples (3-4 mL each) were obtained prior to dosing (baseline), and at 3, 6, 12, 24 hours, and at 2, 3, 4, 5, 7, 10, 14, 18, 21, 25, 29, 32, 36, 40, and 43 days after drug dosing. For the daily blood collections, all samples were taken between 0700 and 1000 hours. For purposes of the LC/MS results, days were converted to hours by multiplying the day by 24 (e.g. day 2 = 48 hours). Blood was immediately transferred into serum separator tubes and held on ice for no more than 2 hours, then centrifuged for 15 minutes 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.

Side effects

At the same time points in which blood was collected, dogs were assessed for sedation using a visual analog scale (0 – 10), where 0 was no detectable sedation and 10 was non-responsive to stimulus. Trained observers who recorded sedation were blinded to the

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treatment. Nausea and vomiting, temperature, heart rate, and respiratory rate were recorded at the same time points.

HPLC / MS method

Serum concentrations of hydromorphone and oxymorphone were determined by high-pressure liquid chromatography (Shimadzu Prominence, Shimadzu Scientific Instruments, MD, USA) with triple quadruple mass spectrometry (API 4000, Applied Biosystems, CA, USA). Plasma standards were made by adding hydromorphone (Cerilliant, TX, USA) or oxymorphone (Cerilliant) to untreated canine serum. Analytes from the serum samples and serum standards were extracted using solid phase extraction. The serum sample or serum standard, 0.2 mL, was added to a 1.5 mL microcentrifuge tube. The internal standards for hydromorphone and oxymorphone were hydromorphone D3 and oxymorphone D3 (Cerilliant, TX, USA), respectively at 1000 ng mL^{-1}. The internal standard, 0.02 mL, was added to the microcentrifuge tube followed by 0.2 mL 0.1 M borate buffer and then vortexed. The microcentrifuge tubes were centrifuged for 5 minutes at 14 000 × ***g*** to sediment any particulates. Solid phase extraction cartridges were conditioned with 1 mL methanol followed by 1 mL deionized water. The serum mixture was then loaded, followed by rinsing with 1 mL deionized water which was repeated for a total of 3 washes. The analytes were eluted with 1 mL methanol, evaporated to dryness in a water bath maintained at 40 °C under a stream of air. The eluates were reconstituted with 0.2 mL 0.1% formic acid and transferred to injection vials.

The mobile phase consisted of A, 0.1% formic acid and B, 90% methanol in 0.1% formic acid at a flow rate of 0.3 mL $minute^{-1}$. A mobile phase gradient was used starting at 100% A with a linear gradient to 7.5% B at 5 minutes, to 100% B at 5.5 minutes, and returning to 100% A at 6 minutes with a total run time of 6.5 minutes. A 2.1×50 mm, 5 μM phenyl column was used (Waters Xbridge, Waters Corporation, MA, USA). The qualifying ion for hydromorphone was 286.3 with 185.0 used as the quantifying ion. The qualifying ion for hydromorphone d3 was 289.3 with 185.0 used as the quantifying ion. The qualifying ion for oxymorphone was 302.2 with 227.2 used as the quantifying ion. The qualifying ion for oxymorphone D3 was 305.2 with 230.2 used as the quantifying ion.

The standard curves were accepted if the measured values were within 15% of the actual values. The range of the standard curve was 0.4 – 51.2 ng mL^{-1} for hydromorphone and oxymorphone. The limit of quantification (LoQ) was 0.4 ng mL^{-1} for hydromorphone and oxymorphone, as determined by the lowest standard on a linear standard curve with measured concentrations within 15% of actual concentrations. The accuracy of the hydromorphone assay was 98 ± 10% of the actual concentration and the coefficient of variation was 5% determined on replicates of three each at 0.4, 1.6, 6.4, and 25.6 ng mL^{-1}. The accuracy of the oxymorphone assay was 99 ± 8% of the actual concentration and the coefficient of variation was 7% determined on replicates of three each at 0.4, 1.6, 6.4, and 25.6 ng mL^{-1}.

Pharmacokinetics

Pharmacokinetic analysis used computer software (WinNonlin 5.2, Pharsight Corporation, CA, USA) and noncompartmental methods. The area under the curve extrapolated to infinity (AUC) was estimated using the linear trapezoidal rule. The percent of the AUC extrapolated ($AUC_{extrapolated}$), area under the moment curve extrapolated to infinity (AUMC), maximum plasma concentration (C_{max}), plasma clearance per dose administered (Cl/D), plasma terminal half-life (T 1/2), terminal slope (λz), mean residence time extrapolated to infinity (MRT), time to C_{max} (T_{max}), and volume of distribution (area method) per dose administered (Vz/D) were also determined. Cl/D and Vz/D were calculated based on the

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administered dose, not necessarily the absorbed dose. Therefore, these parameters may not truly represent the actual Cl and Vz and are not intended to define the true Cl and Vz, but can be useful in extrapolating dose simulations for an extravascular route of administration.

Statistics

Differences in side effects between time points within a treatment group, and differences between treatment groups at each given time point were analyzed with ANOVA using standard software (Graphpad Software, Prism 4, CA, USA).

Results

Side effects

Dogs in both groups exhibited signs of mild sedation after drug administration between 3 and 6 or 12 hours. Median (range) VAS scores in the ASG-oxymorphone group at 3, 6, and 12 hours were 1.5 (1.5 – 2); 0.8 (0 – 1.5); and 0 (0 – 1), respectively. Median (range) VAS scores in the ASG-hydromorphone group at 3 and 6 hours were 2 (1.5 – 2) and 1 (1 – 1.5), respectively. All VAS scores in the ASG-hydromorphone group were 0 by 12 hours. There was no significant difference in VAS scores between groups at any time point. Rectal temperatures decreased modestly during the first 12 hours, with a nadir (mean) of 36.1°C at 3 hours in both groups. There was no difference in temperature between groups at any time point. Similarly, heart rate decreased between 3 and 12 hours in both groups, with a nadir of 60 beats minute^{-1} at 3 hours in the ASG-oxymorphone group and 54 beats minute^{-1} at 6 hours in the ASG-hydromorphone group. Median (range) heart rates in the ASG-oxymorphone group at 3, 6, and 12 hours were 84 (60 – 90); 84 (72 – 96); and 84 (84 – 108) beats minute^{-1}, respectively. Median (range) heart rates in the ASG-hydromorphone group at 3, 6, and 12 hours were 81 (60 – 84); 69 (54 – 102) and 93 (84 – 120) beats minute^{-1}, respectively. Panting was observed in 1-2 dogs in each group at variable time points after baseline up to 12 hours, and other dogs had a mild decrease in respiratory rate. There was no significant difference between the groups with respect to respiratory rate at any time point. Two dogs in the ASG-oxymorphone group vomited or had signs of nausea (salivation, lip smacking, non-productive retching) immediately after the drug was injected; no dogs in the ASG-hydromorphone group vomited at any observed time point.

Pharmacokinetics

Figures 1 and 2 depict the serum concentration over time of oxymorphone after SC injection of ASG-oxymorphone at 4.0 mg kg^{-1}. For purposes of clarity, Figure 1 depicts serum concentrations of oxymorphone at time points from 0 to 96 hours and. and figure 2 from 96 to 700 hours. In this group, no oxymorphone was detected in any sample after 700 hours, so time points from 700 to 1032 hours are not shown.. Figures 3 and 4 depict the serum concentrations of hydromorphone after SC injection of ASG-hydromorphone at 8.0 mg kg^{-1}. Figure 3 displays time points from 0 to 96 hours and Figure 4 displays time points from 96 to 1032 hours.

The pharmacokinetics of liposome encapsulated oxymorphone are presented in Table 1. Subcutaneous ASG-oxymorphone resulted in a mean C_{max} of 7.5 ng mL^{-1} at a mean T_{max} of 11.4 hours. The mean terminal half-life was 160.6 hours. The mean residence time was 256.1 hours. Serum concentrations of oxymorphone were detected above the LoQ, for at least 504 hours (21 days) in all four dogs after 4 mg kg^{-1} SC.

The pharmacokinetics of liposome encapsulated hydromorphone are presented in Table 2. Subcutaneous ASG-hydromorphone resulted in a mean C_{max} of 5.7 ng mL^{-1} at a mean T_{max} of 11.4 hours. The mean terminal half-life was 161.1 hours. The mean residence time was

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345.7 hours. Serum concentrations of hydromorphone were detected above the LoQ for at least 696 hours (29 days) in all four dogs after 8 mg kg^{-1} SC.

Discussion

Liposome-encapsulated oxymorphone and hydromorphone prepared by ammonium sulfate gradient loading provided extended release characteristics *in vivo* that are of longer duration than any extended-release opioid formulation that is currently available and useful in dogs. This method resulted in significantly longer release kinetics than those for liposome-encapsulated opioid formulations that have previously been reported by our laboratory for freeze-thaw DPPC liposome preparations of the same drugs (Smith et al. 2008). Future studies including a direct comparison between the two formulations in the same animals would be needed to confirm longer release kinetics with the ASG-loaded preparation. Earlier studies in our laboratory have produced liposome-encapsulated formulations of oxymorphone and hydromorphone that offer serum concentrations for 3-4 days, which may make them suitable for perioperative use in domestic and laboratory animals (Krugner-Higby et al. 2003; Krugner-Higby et al. 2011; Smith et al. 2003; Smith et al. 2006). These preparations were made using either dehydration rehydration vesicles (Gregoriadis, 1984) or freeze-thaw vesicles (Krugner-Higby et al. 2003). Data from the current study suggest that ASG-oxymorphone or ASG-hydromorphone provide release kinetics that allow for a much longer dosing interval. In humans, a serum hydromorphone concentration equal to 4 ng mL^{-1} has been associated with excellent clinical analgesia (Coda et al. 1997). Our data suggested that hydromorphone serum concentrations were maintained at or above this value for approximately 10 days, but it is not known if the effective analgesic serum concentration of hydromorphone in dogs is similar to that of humans. Oxymorphone is effective for the control of moderate to severe chronic osteoarthritis pain in humans with steady state plasma concentrations of approximately 2.5 ± 1.4 ng mL^{-1} (Adams & Ahdieh 2004). Our data suggest that oxymorphone serum concentrations were maintained within this range for approximately 20 days in at least two of the four dogs tested. Again, the effective serum concentration of oxymorphone in dogs is not known, but the effective morphine serum concentration is similar between dogs and humans (Dahlstrom et al. 1982; Skarke et al. 2003, Kukanich et al. 2005a; Kukanich et al. 2005b).

Ammonium sulfate gradient loading is a method that has been used to produce several types of liposomal chemotherapeutic drug preparations including doxorubicin and vincristine. These formulations are used for extended release after intravenous administration, giving up to 2 weeks of release for doxorubicin and vincristine (Krishna et al. 2001; Webb et al. 2007). Following gradient loading, doxorubicin and vincristine are precipitated within the liposomes as insoluble sulfate salts, which considerably slow their release from liposomes. In contrast, oxymorphone and hydromorphone do not precipitate. Therefore, the drug is retained by a different mechanism.

One possible explanation for the reduced leakage rate from these liposomes involves the potential effect of residual ammonium in the liposomes following loading. Both protonated opioid and ammonium will be retained in liposomes, and their leakage will occur indirectly through deprotonation and loss of their respective free bases. Therefore, leakage will generate protons in the liposomes that will reduce the intraliposomal pH and thereby reduce the rate of leakage. Consequently, leakage will be a self-limiting process, and the leakage of the one species, if inherently faster, will effectively reduce the rate of leakage of the other species. Owing to its higher diffusion coefficient, we would expect ammonia to leak more rapidly than an opioid from the liposomes. Therefore, loss of residual ammonium from the liposomes through leakage as ammonia may effectively slow the leakage of the corresponding opioid.

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The pharmacokinetics of subcutaneously injected ASG-oxymorphone and ASG-hydromorphone were well described in this study. In contrast to the conventional formulations of oxymorphone and hydromorphone which had an MRT of 1.3 hours, the MRT of ASG-oxymorphone was 256.1 hours and the MRT of ASG-hydromorphone was 345.7 hours in dogs. The mean residence time is the average amount of time that the drug molecules remain in the body. We hypothesize that the primary reason the MRT was prolonged in the ASG formulations was due to the drug molecules remaining trapped within the liposomes, which circulate from the subcutaneous injection site, through the lymphatic system, while slowly releasing the opioid drug. The slow absorption provided a relatively consistent serum profile over 480 hours (20 days) although some fluctuation did occur.

In the present study, side effects associated with the injection of ASG-oxymorphone or ASG-hydromorphone were mild and well within clinically acceptable limits for healthy dogs. We did not observe any adverse reactions at the injection sites, and by 12 hours no dog showed appreciable sedation, bradycardia, hypothermia, or nausea, despite the fact that measurable concentrations of drug were present for up to 29 days after injection. The magnitude of side effects were actually less than those observed after injection of standard formulations of oxymorphone and hydromorphone (Kukanich et al. 2008a; Kukanich et al. 2008b). This is consistent with the relatively low C_{max} values that we report in this study, despite the fact that oxymorphone and hydromorphone were dosed at 40 times the standard conventional dose (Pettifer and Dyson, 2000; Hellyer, 2002) in mg kg^{-1}. This observation is again consistent with the hypothesized release rate mechanism when lipid soluble opioids are encapsulated into liposomes using an ammonium sulfate gradient. Our pharmacokinetic analyses have revealed that there are essentially two peaks in serum concentration of opioid, one initially and likely due to early bolus release of the drug, and a second larger peak at Tmax. The greater incidence of adverse effects observed initially compared with at later time points might be attributable to some degree of opioid tolerance or to a slower rate of rise in serum drug concentration during the onset to Tmax as compared to the initial peak.

In the current study we did not attempt to assess analgesia or threshold changes to a noxious stimulus. Results from the pharmacokinetic analysis do suggest that analgesia may persist for 3 – 4 weeks after a single subcutaneously administered dose of ASG-oxymorphone or ASG-hydromorphone. Further studies are warranted to test analgesia in both a research and clinical environment, and to assess the safety and efficacy of these formulations in a wider population of dogs.

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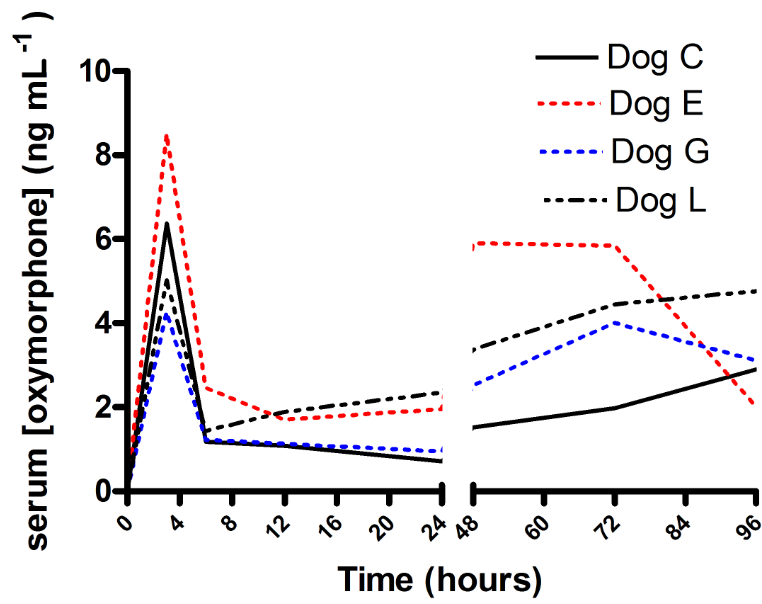

Figure 1.

Oxymorphone serum profiles after SC administration of ASG-oxymorphone 4.0 mg kg^{-1} to healthy dogs from baseline to 96 hours.

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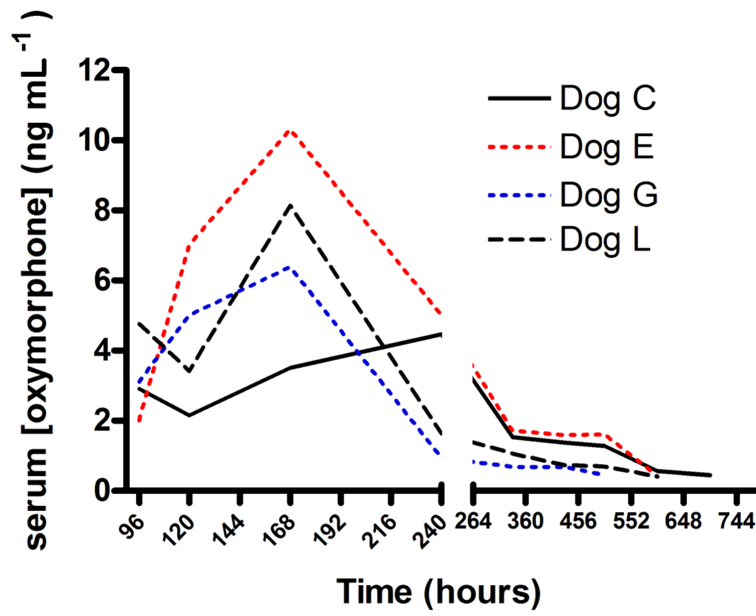

Figure 2.
Oxymorphone serum profiles after SC administration of ASG-oxymorphone 4.0 mg kg^{-1} to healthy dogs from 96 to 700 hours.

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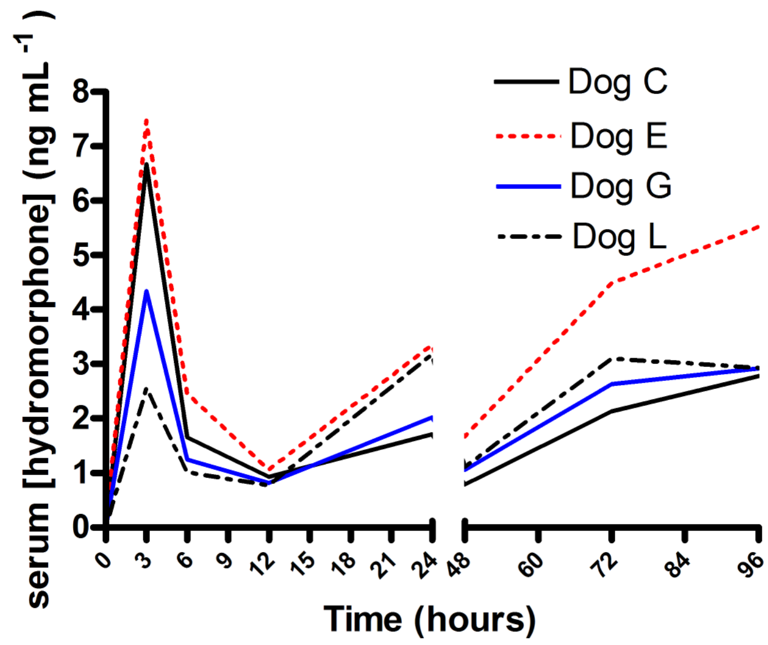

Figure 3.
Hydromorphone serum profiles after SC administration of ASG-hydromorphone 8.0 mg kg^{-1} to healthy dogs from baseline to 96 hours.

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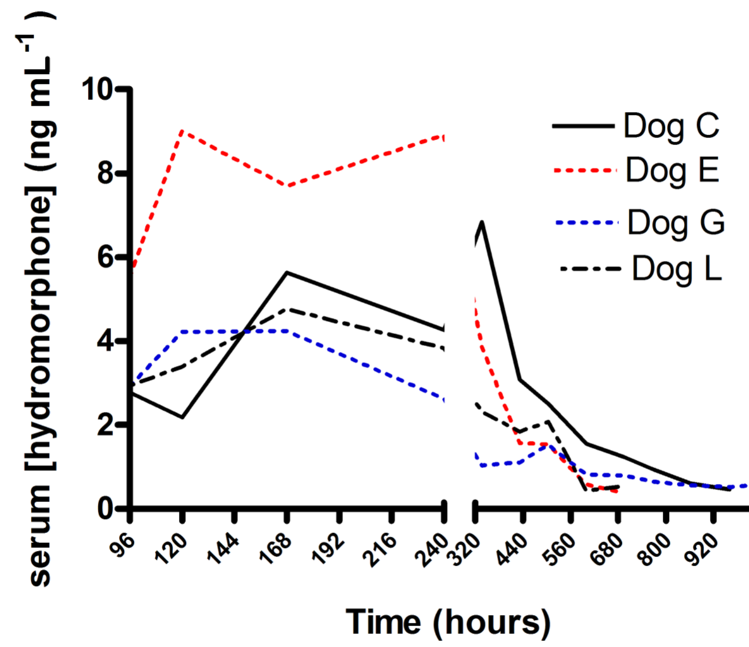

Figure 4.
Hydromorphone serum profiles after SC administration of ASG-hydromorphone 8.0 mg kg^{-1} to healthy dogs from 96 to 1032 hours.

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Table 1

ASG-oxymorphone pharmacokinetic parameters after 4.0 mg kg^{-1} SC to healthy Beagles.

Parameter	Units	Dog C	Dog E	Dog G	Dog L	Harmonic Mean
$AUC_{extrapolated}$	**%**	6.9	2.9	13.8	7.6	5.8
AUC	**hour ng mL^{-1}**	1403	2224	1223	1484	1507
AUMC	**hour hour ng mL^{-1}**	434363	499181	326853	353728	392464
Cl/D	**mL minute kg^{-1}**	47.5	30.0	54.5	44.9	42.1
C_{max}	**ng mL^{-1}**	6.4	10.3	6.4	8.1	7.5
T ½ λz	**hours**	148.4	108.2	271.4	190.8	160.6
λz	**1/hours**	0.00470	0.00640	0.00260	0.00360	0.00388
MRT	**hours**	309.6	224.5	267.3	238.4	256.1
T_{max}	**hours**	3.0	168.0	168.0	168.0	11.4
Vz/D	**L kg^{-1}**	613.7	278.8	1264.1	739.9	543.6

Definition of Terms: AUC = area under the curve extrapolated to infinity, $AUC_{extrapoiated}$ = the percent of the AUC extrapolated, AUMC = area under the moment curve extrapolated to infinity, C_{max} = maximum plasma concentration, Cl/D = plasma clearance per the dose administered, T ½ = plasma terminal half-life, λz = terminal slope, MRT = mean residence time extrapolated to infinity, T_{max} = time to C_{max} , Vz/D = volume of distribution (area method) per dose administered.

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Table 2

ASG-hydromorphone pharmacokinetic parameters after 8.0 mg kg^{-1} SC to healthy Beagles (see table 1 for definitions of terms)

Parameter	Units	Dog C	Dog E	Dog G	Dog L	Harmonic Mean
$AUC_{extrapolated}$	**%**	4.8	2.1	18.2	6.4	4.4
AUC	**hour ng mL^{-1}**	2641	2678	1811	1704	2115
AUMC	**hour hour ng mL^{-1}**	1050901	658719	1039021	524553	749275
Cl/D	**mL minute kg^{-1}**	50.5	49.8	73.6	78.3	60.4
C_{max}	**ng mL^{-1}**	6.9	9.0	4.3	4.8	5.7
T ½ λz	**hours**	189.3	100.7	401.3	140.2	161.1
λz	**$hour^{-1}$**	0.0037	0.0069	0.0017	0.0049	0.00331
MRT	**hours**	397.9	246.0	573.8	307.9	345.7
T_{max}	**hours**	336.0	120.0	3.0	168.0	11.4
Vz/D	**L kg^{-1}**	827.2	434.1	2557.5	949.8	807.0