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Pharmacokinetics of liposomal encapsulated buprenorphine suspension following subcutaneous administration to cats

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We investigated the effects of liposome encapsulation at prolonging the systemic exposure of buprenorphine following subcutaneous administration in cats. Seven healthy male cats were dosed intravenously with 0.02 mg/kg buprenorphine solution (STD-BUP), followed 14 days later by a subcutaneous injection of 0.2 mg/kg buprenorphine as a liposomal suspension (SUS-BUP) containing drug molecules both in liposomes and the suspending vehicle. Buprenorphine time plasma concentration data for both dosing routes were analyzed simultaneously with four compartmental models. Goodness of fit was assessed both graphically and with the Akaike information criterion. The time-course of intravenous STD-BUP was biphasic, with a 4.39 h average terminal half-life. The subcutaneous SUS-BUP produced plasma buprenorphine concentrations above 0.5 μg/L for more than 96 h, with three distinct peaks in the first 15 h. The model with best fit comprised a central and a peripheral compartment, plus three subcutaneous absorption compartments: one of dissolved drug molecules that were absorbed through a first-order process, and two of liposome-encapsulated drug molecules that were transferred to the solution compartment through separate zero-order processes. Liposomes effectively prolonged the systemic exposure of buprenorphine in cats.

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INTRODUCTION

Domestic cats are among the most popular pets in the USA, the UK, and Canada (Turner & Bateson, 2000; Perrin, 2009). As the vast majority of cats are neutered (castrated or spayed) and may be declawed, they will undergo at least one major surgery in their lifetime requiring treatment for pain and discomfort. Opioids are considered an important class of analgesic for the successful management of perioperative and other acute pain in veterinary medicine. Buprenorphine, which has an intermediate plasma half-life of approximately 6–7 h in the cat (Taylor *et al.*, 2001; Robertson *et al.*, 2005), is the most widely used opioid in companion animals in the UK. Further, buprenorphine lacks the vomiting, dysphoria, and hyperthermia side effects of other mu-opioid agonists in cats (Robertson *et al.*, 2003; Robertson & Taylor, 2004). A comprehensive review of the studies using buprenorphine in cats has been published recently (Steagall *et al.*, 2014). Veterinary-approved formulations for use in cats are available in the USA (Simbadol®, Zoetis, Kalamazoo, MI, USA) and Canada (Vetergesic®, Champion Alstoe, Whitby, ON, Canada). At variance with the immediate-release aqueous solution product in Canada, which is formulated at 0.3 mg/mL and administered at 0.01–0.02 mg/kg intramuscular with additional dosages to be given as needed, the USA immediate-release product is formulated at a higher concentration of 1.8 mg/mL and administered at 0.24 mg/kg subcutaneous (s.c.) daily for up to 72 h.

The use of sustained-release human commercial opioid patches including fentanyl and buprenorphine in dogs and cats are subject to highly variable pharmacokinetics as well as accidental human exposure and potential abuse liability risks (Epstein *et al.*, 2015). Alternatively, the encapsulation of buprenorphine into nanoparticles such as liposomes is a promising alternative method for providing sustained drug release in companion animals. For instance, opioids such as oxymorphone, butorphanol, and hydromorphone have been formulated with liposomes for subcutaneous injection and provided encouraging results in dogs and other species (Smith *et al.*, 2003, 2004, 2008; Sladky *et al.*, 2006). Buprenorphine has also been compounded into a sustained-release proprietary

biodegradable matrix that has demonstrated comparable efficacy to injectable buprenorphine provided by the oral transmucosal route in cats undergoing ovariohysterectomy (Catbagan *et al.*, 2011).

Based on concerns with the perceived need for multiple buprenorphine dosing of the immediate-release injection solutions, or the potential for longer lag times for onset of action with sustained-release formulations, we sought to evaluate a prototypical suspension formulation of buprenorphine (SUS-BUP) that included a fraction of unencapsulated buprenorphine in vehicle solution and liposomal encapsulated buprenorphine. Prior to evaluating the efficacy of SUS-BUP for perioperative pain relief in the cat, it is prudent to first understand how this formulation will affect the absorption pharmacokinetics of buprenorphine. Our study objective was to compare the plasma concentration–time profiles of SUS-BUP following s.c. administration with an immediate-release injectable buprenorphine HCl solution (STD-BUP) given intravenous (i.v.) to healthy cats. General health observations and adverse effects accompanying administration of buprenorphine were also observed and reported.

MATERIALS AND METHODS

Animals

Seven healthy intact male research cats (Liberty Research, Inc, Waverly, NY) approximately 6–18 months of age with a weight range of 4.2–5.0 kg were used for this study. Cats were housed in the Central Animal Facility (CAF) at the University of Guelph and maintained according to standards set forth in the Canadian Council on Animal Care guidelines and on recommendations by the CAF. All aspects of this study were approved by the University of Guelph Animal Care Committee. Upon arrival to the facility and prior to study, all cats were group housed and given at least 14 days acclimation accompanied by daily handling. All cats were housed individually from the time of study until completion. All cats were considered healthy based on veterinary physical examination and prescreening clinical pathology (CBC, biochemical profile, FIV/FeLuk). All animals had access to a commercial cat chow and water *ad libitum*, except for dosing days when food was withheld overnight, then returned 8 h postdosing. Animals were maintained on a 12-h daylight cycle for the duration of the study.

Liposomal encapsulation of buprenorphine

Buprenorphine encapsulated liposomes were prepared under aseptic conditions by a modified microencapsulation vesicle method (Nii & Ishii, 2005). To avoid interbatch variability, a single lot of SUS-BUP was prepared, quantified, and used within one week of preparation. Briefly, 100 mg of lyophilized phosphatidylcholine (American Lecithin Company, Oxford, CT, USA) was added to 2 mL of 90% ethanol and the mixture stirred over gentle heating until the phospholipids went into solution. Approximately 13 mg of analytical grade buprenorphine HCl (Sigma, St. Louis, MO, USA) was then added and dissolved in the ethanol phospholipid solution creating a drug–phospholipid–ethanol solution. The final solution was filter-sterilized (0.22 μm pore diameter filter, Fisher Scientific, Ottawa ON, Canada). In a separate beaker, 8 mL of filter-sterilized distilled water was heated to near boiling at high stirring speed. The drug–phospholipid–ethanol solution was added to the distilled water in a drop-wise fashion while maintaining heating and continuous stirring to generate the buprenorphine encapsulated liposomes uniformly. The organic solvent, ethanol, was evaporated with continual heating and stirring, leaving the final buprenorphine suspension containing liposomal encapsulated buprenorphine as well as buprenorphine in vehicle (distilled water) solution. The final SUS-BUP yielded a uniform cloudy suspension with no visible precipitates. The SUS-BUP was cooled to room temperature protected from light. Total buprenorphine concentration (mg/mL) in the SUS-BUP formulation (encapsulated and unencapsulated buprenorphine) was 1.74 ± 0.58 (S.D.), being determined by high-pressure liquid chromatography (HPLC) on multiple aliquots of the final suspension. An encapsulation efficiency of buprenorphine HCl in liposomes of $61.9\% \pm 0.95$ (SD) was determined by spinning 200-μL aliquots of the final suspension (50 000 ***g*** for 10 min), collecting the supernatant containing unencapsulated buprenorphine, then re-suspending the pellet in fresh distilled water and repeating the above procedure. The final pellet was solubilized in 90% ethanol and the concentration of buprenorphine in the re-suspended pellet, and the collected supernatants were determined using HPLC.

Study design and sampling

All cats underwent two test periods; first receiving STD-BUP (Temgesic®, Schering-Plough, Kenilworth, NJ, USA, 0.3 mg/mL) at 0.02 mg/kg i.v. followed by at least a 14-day washout period, then crossed over receiving s.c. SUS-BUP at 0.2 mg/kg. The anticipated equipotent dose of SUS-BUP chosen for study was designed at 10 × the STD-BUP dose based on previous work with other liposomal encapsulated opioid formulations undertaking similar formulation comparisons (Smith *et al.*, 2003, 2004).

STD-BUP was administered i.v. into a preplaced cephalic catheter followed by flushing with physiologic saline to ensure complete delivery of the dose. Whole blood samples were collected from catheterized jugular veins predose and at 1, 3, 5, 7, 10, 15, 20, 30, 45, and 60 min, and 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h postdosing. SUS-BUP was given s.c. in the dorsum of each animal being placed between the scapula and following shaving of the injection site. Whole blood samples were also collected from jugular vein catheters following s.c. administration of SUS-BUP at predose and 5, 15, 30, and 60 min and 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h postdosing. Jugular vein catheters were placed and secured under isoflurane anesthesia 24 h prior to each test period. The volume of blood collected over a single test period was adjusted to be less than 10% of each cat's total blood volume (1.0–1.5 mL per sample) and was replaced s.c. with an equal volume of

physiologic saline at the completion of each test period. All whole blood samples were collected into heparinized tubes, then centrifuged (400 ***g*** for 10 min) with plasma from samples stored frozen at −80 °C until assayed for buprenorphine concentrations.

Health observations and s.c. injection site were carried out by the examining veterinarian(s) at each blood sample time point for the evidence of adverse reactions following dosing. Clinical pathology (CBC, biochemistry profile) was evaluated on each cat at the end of each test period following the last time point.

At the completion of the second test period of the study, and following a 14-day washout period, one cat chosen at random received a single s.c. injection of unloaded liposomes in vehicle; that is, no buprenorphine at a volume equivalent to the average volume, on a mL/kg basis, received by cats in the SUS-BUP phase of the study. Health observations and plasma were collected predose and 10, 30 and 60 min and 2 and 8 h after dosing of unloaded liposomes. Plasma was stored at −80 °C until assayed for buprenorphine concentrations along with samples collected from test periods one and two of the study. All samples were assayed within 4 months of collection.

Buprenorphine assay

Analysis of plasma buprenorphine concentrations in collected samples was performed using a commercial buprenorphine ELISA kit (Neogen, Lexington, KY) that was validated for feline plasma, and only interacts with buprenorphine molecules outside the liposomes. All assay plates used were from the same lot # to reduce interplate variability. Reference standards (0.25, 0.5, 1, 2, 4, 8, and 16 μg/L) were prepared by spiking blank feline plasma with analytical grade buprenorphine HCl. If HPLC peaks were above 16 μg/L, then samples were diluted and back calculated. Quality control (QC) samples (5 and 10 μg/L) were prepared separately. Nine calibration runs were performed for assay validation with calibration standards run in triplicate. The coefficient of determination (R^2) was greater than 0.99 for each curve. The intraday precision showed assay coefficients of variation of less than 15% for all reference standards and QC samples. The true value for each calibration standard was within 15% of the actual value. Norbuprenorphine, a metabolite of buprenorphine identified in several animal species, has been shown to possess only weak analgesic activity in animal models of pain when compared to buprenorphine (Ohtani *et al.*, 1995) and was not quantified in this study. Cross-reactivity with norbuprenorphine, up to 60 μg/L, was <1% according to the manufacturer of the ELISA kit. Cross-reactivity with other metabolites of buprenorphine in the cat is unknown and may have contributed to buprenorphine determinations in this study.

Pharmacokinetic analysis

An exploratory data analysis was performed to assist with the creation of compartmental Pharmacokinetic (PK) models suitable for the analysis of the time–concentration data following s.c. dosing of SUS-BUP suspension. This consisted in (i) visually identifying the number of peak plasma concentrations after s.c. administration and the number of decay slopes for each type of dosing, (ii) performing statistical moment PK analysis using the area under the plasma concentration curves calculated with linear trapezoidal methods, and (iii) performing deconvolution analysis with Kinetica™ software (ThermoFisher Scientific, Waltham, MA, USA) to identify the absorption kinetic processes and obtain initial values of their associated lag times.

Next, the time–concentration data of i.v. dosing of STD-BUP were analyzed with multicompartmental PK models; the model with the highest goodness of fit to the observed data was selected on the basis of the Akaike information criterion (AIC) and visual inspection of the scattering of regression residuals. Afterward, the following compartmental PK models were coded to test the following hypotheses regarding the absorption of buprenorphine (Fig. 1): (i) whether drug release from the liposomes followed zero-order or first-order kinetics, (ii) whether the liposomes are homogeneous with respect to their drug-releasing kinetics, (iii) whether the drug molecules can transfer from one type of liposome to the other after s.c. administration, and (iv) whether the liposomes act as a reservoir of drug molecules or as a distinct drug absorption site. Because the actual distribution of the dose across the liposomes and solution compartments at the time of dosing may vary among animals, all models included the parameters f_{sol} (fraction drug in solution) and f_{lipo_X} (fraction drug within the X^{th} pool of liposomes). In addition, the PK models included separate measurement error parameters for each type of drug administration.

In the final stage of compartmental analysis, the i.v. and s.c. data of all cats were analyzed simultaneously (i.e., nonlinear mixed-effects modeling) with each compartmental model, using the estimates of total clearance (CL_t), steady-state volume of distribution (V_{ss}), terminal half-life ($t_{½}$-λ Z), and drug bioavailability (%F) after s.c. administration derived from statistical moment PK analysis, the central volume of distribution (V_c) estimated from back extrapolation of the first slope of the PK profile, and the 62% encapsulation efficiency as starting values for iteration. All analyses were performed using Adapt v.5 using maximum-likelihood expectation maximization (MLEM) methods (D'Argenio *et al.*, 2009). The estimates of population PK parameters, their associated variances and covariances (i.e., full covariance matrix), and the combined (i.e., additive and proportional) residual error parameters were estimated iteratively with importance sampling methods: 3000 samples of data per iteration were collected until asymptotic solutions of all parameters were reached. All PK parameters were assumed to be log-normally distributed. At the end of this analysis, the conditional individual estimates of PK parameters were calculated using the population PK estimates as prior information.

Asymptotic convergence of the models was assessed by visual inspection of the plots of estimated PK parameters as a function of iteration number, by comparing the accuracy of estimated PK parameters, and by assessing goodness of fit to the data (AIC).

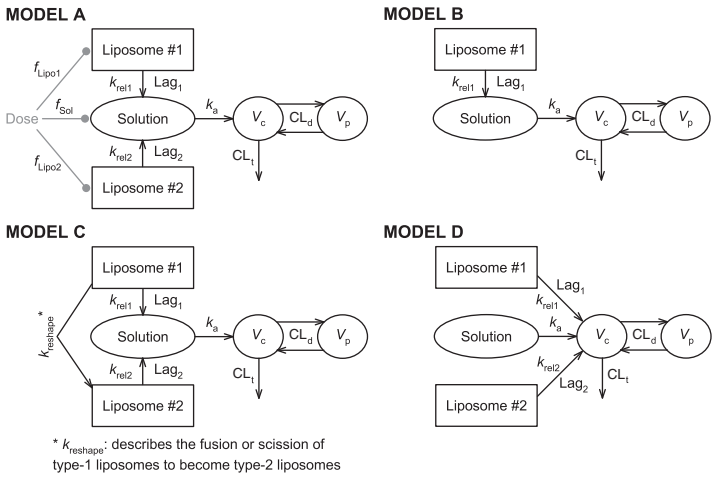

Fig. 1. Graphical representations of the compartmental models created for the pharmacokinetic analysis of buprenorphine in cats (Note #1: the parameters estimating dose fractions of the s.c. administered liposomal drug suspension SUS-BUP distributed between solution and liposomal compartments is depicted only in Model A, but are present also in Models B, C, and D, with f_{Lipo2} being non-existent in Model B. Note #2: to avoid overdrawing the graphs, the symbol expressing drug input following i.v. administration of the aqueous buprenorphine solution STD-BUP was omitted). Legend: CL_t, total clearance; CL_d, distribution clearance; f_{Sol}, buprenorphine subcutaneous dose fraction associated with the vehicle of liposomal suspension; f_{Lipo1}, buprenorphine subcutaneous dose fraction associated with the first (i.e. fast releasing) pool of liposomes; f_{Lipo2}, buprenorphine subcutaneous dose fraction associated with the second (i.e. slow releasing) pool of liposomes; k_a, first-order absorption rate constant of buprenorphine molecules in solution at the site of subcutaneous injection; k_{rel1}, zero-order buprenorphine release rate from the first (i.e. fast releasing) pool of liposomes; k_{rel2}, zero-order buprenorphine release rate from the second (i.e. slow releasing) pool of liposomes; Lag_1, buprenorphine release lag time of the first (i.e. fast releasing) pool of liposomes; Lag_2, buprenorphine release lag time of the second (i.e. slow releasing) pool of liposomes; Liposome #1, compartment of drug molecules associated with the first (i.e. fast releasing) liposomes; Liposome #2, compartment of drug molecules associated with the second (i.e. slow releasing) liposomes; Solution, compartment of drug molecules in solution within the dosing vehicle; V_c, volume of the central disposition compartment; V_p, volume of the peripheral disposition compartment.

RESULTS

No significant adverse effects were noted in any of the cats throughout the study including no evidence of injection site reactions in any cats receiving s.c. SUS-BUP, or the unloaded liposomes in vehicle. No significant findings on clinical pathology were noted in any of the cats for either test period. Temperatures were recorded at baseline, 1, 4, and 24 h postdosing in all cats in both test periods. All cats, in both the STD-BUP and SUS-BUP groups, recorded mild temperature elevations, with values just over 39.0 °C occurring between 1 and 4 h postdosing. Two cats in the STD-BUP group achieved temperature elevations just over 40.0 °C, also for transient periods of time between 1 and 4 h postdosing (data not shown). In consequence, the PK data analysis included the buprenorphine concentration–time data from all seven cats receiving STD-BUP i.v. and SUS-BUP s.c.

The exploratory statistical moment PK analysis of STD-BUP data yielded average values of CL_t of 1.05 L/h/kg (range: 0.53–1.7 L/h/kg), V_{ss} values of 5.29 L/kg (range: 2.1 and 10.2 L/kg), V_c values of 1.14 L/kg (range: 0.9–1.5 L/kg), and the %F values of the s.c.-administered buprenorphine liposomal suspension (SUS-BUP) at a dose of 200 μg/kg averaged 139% (range: 63–258%). The average mean residence time (MRT) of STD-BUP was 6.84 h (range: 1.2–15.7 h).

The time-course of i.v.-administered buprenorphine (STD-BUP) was found to be multiphasic upon visual inspection, with at least two distinct decay slopes. In some cats, measurable plasma buprenorphine concentrations were recorded at sampling times exceeding 12 h postdosing. Therefore, the i.v. datasets were analyzed with open, two-compartment, and three-compartment PK models with drug output proceeding exclusively from the central compartment (i.e., mammillary PK models). The visual inspection (Fig. 2) and calculated goodness of fit of each model to the i.v. data revealed that the two-compartment model (AIC = 254.4) more accurately predicted the i.v. data as compared to the three-compartment model (AIC = 263.4).

The time–concentration data for SUS-BUP revealed three distinct peaks in most subjects. The deconvolution analysis of individual sets of s.c. data suggested the presence of three absorption processes in all seven cats: a first fraction of drug was absorbed upon dosing, a second fraction started being absorbed between 0.5 and 2 h after dosing, and a third fraction started being absorbed between 6 and 36 h after dosing. All three processes apparently were linear in a % dose remaining to be absorbed versus time plot: Therefore, we refined our analysis by discarding the PK models with first-order absorption kinetics, and use instead a variant set of PK models with zero-order drug release from the compartments of liposome-encapsulated drug molecules.

The best model structures for this compartmental PK analysis included a linear two-compartment disposition PK system, connected to an absorption PK system comprising a

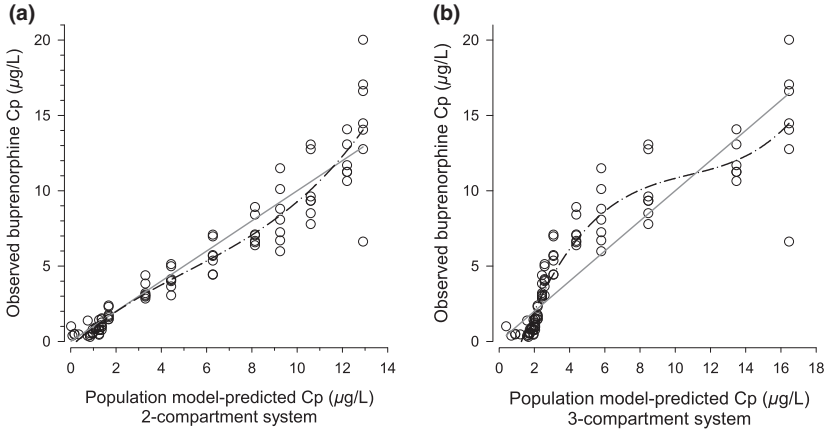

Fig. 2. Observed plasma buprenorphine concentrations following a single i.v. administration of 20 μg/kg of STD-BUP, as a function of the population model-predicted concentrations generated by open, mammilary, two-compartment (panel a), and three-compartment (panel b) disposition systems. An identitiy line (i.e. Observed Cp = Predicted Cp; solid gray line) and a 3rd-degree polynomial trend (hatched black line) are added to the scatter plots to allow the goodness of fit assessment.

compartment representing the drug molecules dissolved in the aqueous vehicle of suspension, plus one (default) or two compartments representing the drug molecules entrapped within liposomes (Fig. 1). Of these, only Model A with zero-order liposomal drug release provided accurate prediction of the measured plasma buprenorphine concentrations: Its estimated population PK parameters are presented in Table 1, its individual conditional estimates of dose fractions in each absorption compartment are listed in Table 2, and its goodness of fit to the observed concentration data is illustrated in Fig. 3. Model B did not fit well to the data (data not shown), and models C and D could not reach asymptotic solution of the PK parameters.

The time-courses of the observed and the mean population model-predicted concentrations of Model A are depicted in Fig. 4. The predicted i.v. kinetic profile of STD-BUP fits closely the data, excepting for some concentrations that were recorded between 24 and 48 h after dosing (Fig. 4a). For the s.c. kinetic profile of SUS-BUP, the mean predicted concentration well represents the observed concentrations, which are highly scattered in the first 12 h following the injection of liposomal buprenorphine but become much narrower afterward (Fig. 4b).

The individual conditional fits to plasma buprenorphine concentrations are depicted in Fig. 5. Examination of this set of graphs, displayed both in semi-logarithmic and log-log scales, reveals that their measured plasma buprenorphine concentrations scatter closely around the curves of their respective model-predicted concentrations throughout the whole sampling period. In addition, the i.v. PK profiles of five cats reveal transient rises in plasma buprenorphine concentration starting at 6 h postdosing or after (Fig 5b, d, f, l, n).

Table 1. Pharmacokinetic parameters of buprenorphine in cats, following a single i.v. bolus injection of 20 μg/kg drug in a solution (STD-BUP), and following a single s.c. injection of 200 μg/kg drug in a liposomal suspension (SUS-BUP). Parameters have been estimated by means of nonlinear mixed-effect modeling, where the structural model comprises two compartments of liposome-encapsulated drug molecules releasing their content at distinct zero-order rates to a compartment of dissolved drug molecules, which in turn are subject to first-order absorption into an open, two-compartment disposition system (i.e., zero-order variant of Model A; see Fig. 1 and Appendix)

Parameter	Unit	Mean	SD	SD as CV%
CL_t	L/h/kg	1.04	0.473	45.7
CL_d	L/h/kg	1.93	0.471	24.4
V_{ss}	L/kg	4.99	2.63	52.7
V_c	L/kg	1.32	0.120	9.06
V_p	L/kg	3.67	2.55	69.7
f_{Sol}	Dimensionless	0.111	0.082	73.7
f_{Lipo1}	Dimensionless	0.556	0.364	65.5
f_{Lipo2}	Dimensionless	0.745	0.436	58.5
k_a	per h	4.84	4.38	90.6
k_{rel1}	μg/h	0.139	0.097	69.8
Lag_1	h	0.762	0.482	63.2
k_{rel2}	μg/h	0.024	0.013	52.6
Lag_2	h	13.7	2.03	14.9
$t_{1/2}$ (λ_1)	h	0.265	0.106	40.0
$t_{1/2}$ (λ_Z)	h	4.39	4.25	96.8

CL_t, total clearance; CL_d, distribution clearance; f_{Sol}, buprenorphine subcutaneous dose fraction associated with the vehicle of liposomal suspension; f_{Lipo1}, buprenorphine subcutaneous dose fraction associated with the first pool of liposomes; f_{Lipo2}, buprenorphine subcutaneous dose fraction associated with the second pool of liposomes; k_a, absorption rate constant; k_{rel1}, zero-order buprenorphine release rate from the first pool of liposomes; k_{rel2}, zero-order buprenorphine release rate from the second pool of liposomes; Lag_1, buprenorphine release lag time of the first pool of liposomes; Lag_2, buprenorphine release lag time of the second pool of liposomes; $t_{1/2}$ (λ_1), half-life of the first (distribution) slope of i.v.-administered buprenorphine; $t_{1/2}$ (λ_Z), half-life of the last (terminal) slope of i.v.-administered buprenorphine; V_c, volume of the central disposition compartment; V_p, volume of the peripheral disposition compartment; V_{ss}, steady-state distribution volume.

DISCUSSION

There is concern that the current management of feline pain in clinical practice is inadequate. The opioids are an important class of analgesic; however, current formulations require frequent dosing due to short half-lives and duration of action. A relatively new formulation of buprenorphine, Simbadol®, is approved in the USA for once every 24 h dosing, but the requirement is for administration by injection, limiting its usefulness as a method of providing analgesia during the critical first 72 h following surgery when most animals have returned

Table 2. Individual conditional estimates (SE as CV%) of the buprenorphine dose fractions present in each absorption compartment at the time of s.c. dosing of 200 μg/kg of buprenorphine as a liposomal suspension (SUS-BUP), sum of the estimated dose fractions, and absolute bioavailability estimated by means of statistical moment pharmacokinetic analysis

Subject	f_{Sol}	f_{Lipo1}	f_{Lipo2}	Sum of f (%)	%F
07pdc3	0.08 (14.29)	0.34 (15.55)	1.11 (19.98)	153	73
07fco2	0.09 (10.62)	0.25 (7.56)	0.32 (17.05)	66	63
07fco1	0.07 (7.84)	0.65 (10.31)	0.99 (12.76)	171	171
07fbp2	0.28 (7.12)	1.62 (11.01)	0.98 (12.24)	288	258
07fck1	0.04 (10.92)	1.18 (11.57)	1.14 (14.54)	236	140
07fby1	0.38 (9.03)	0.50 (10.82)	1.17 (11.86)	204	201
06jrc1	0.09 (11.22)	0.33 (8.63)	0.30 (18.10)	73	64

f_{Sol}, buprenorphine subcutaneous dose fraction associated with the vehicle of liposomal suspension; f_{Lipo1}, buprenorphine subcutaneous dose fraction associated with the first pool of liposomes; f_{Lipo2}, buprenorphine subcutaneous dose fraction associated with the second pool of liposomes; Sum of f: sum of the subcutaneous dose fractions as a percentage; %F: absolute bioavailability, as estimated from statistical moment PK analysis.

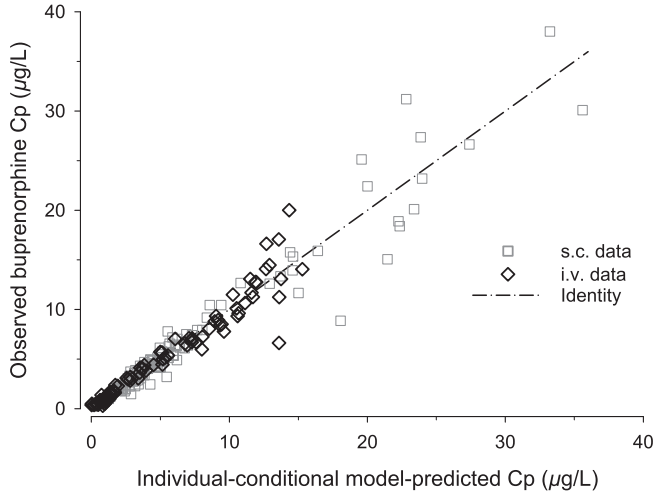

Fig. 3. Observed vs. individual-conditional model predicted plasma buprenorphine concentrations (Cp) in cats dosed intravenously an aqueous drug solution STD-BUP (open diamonds), and dosed the liposomeencapsulated drug suspended in an aqueous drug solution SUS-BUP by the subcutaneous route (open squares). An identity line (i.e. Observed Cp = Predicted Cp) is added to the scatter plot to allow the goodness-of-fit assessment.

home. There is a formulation of buprenorphine compounded in a proprietary biodegradable matrix for use in cats and dogs (Catbagan *et al.*, 2011), which is available in the USA and Canada through Zoopharm compounding pharmacy (WY, USA). However, this compounded product is not approved by regulatory agencies in either country. Hence, acquisition of the compounded product is subject to the relevant regulations governing the use of compounded drugs in veterinary patients in each country, and there is a paucity of data available on both potential efficacy and adverse effects. Liposomal encapsulation of drugs is a delivery system with proven efficacy at providing sustained drug release for periods that can last even days (Plumb, 2005). Veterinary approval of buprenorphine for use in cats in North America has contributed substantially to acceptance of buprenorphine as a safe and effective opioid in cats (Robertson *et al.*, 2003, 2005; Robertson & Taylor, 2004; Nii & Ishii, 2005; Steagall *et al.*, 2014). As such, we believe that this opioid will be an ideal candidate for the development of a liposome-encapsulated formulation with improved systemic drug exposure, safety, and therapeutic effect duration when compared to immediate-release buprenorphine formulations administered by the same route.

Other investigators have studied the pharmacokinetics of nonliposomal sustained-release buprenorphine formulations injected subcutaneously to cats (Taylor *et al.*, 2016), humans (Nasser *et al.*, 2014; Laffont *et al.*, 2016), laboratory mice (Clark *et al.*, 2014), minipigs (Thiede *et al.*, 2014), and juvenile northern elephant seals (Molter *et al.*, 2015). Interspecies, drug formulation, and methodological differences preclude any comparison between the tested products and our liposomal formulation prototype. But noteworthy, Taylor *et al.* (2016) correlated the plasma buprenorphine concentrations of their cats with increases of thermal threshold, a measure of analgesic efficacy: the median of reported individual plasma drug concentrations at which the thermal threshold returned to baseline values was 1.6 μg/L (interquartile range = [0.98, 2.62] μg/L). As shown in Fig. 5, we recorded plasma buprenorphine concentrations above this median value for periods between 48 and 72 h postdosing SUS-BUP.

The pharmacokinetics of i.v.-administered buprenorphine in cats has been documented so far with statistical moment approaches (Taylor *et al.*, 2001; Robertson *et al.*, 2005): the first reported mean systemic clearances (CL_t) and steady-state distribution volumes (V_{ss}) of 1.0 ± 0.4 L/h/kg and 7.1 ± 3.2 L/kg, respectively, and the last reported median CL_t and V_{ss} values of 0.6 L/h/kg and 4.8 L/kg, respectively. These reported values are in the range of our estimated PK parameters, both the ones of our exploratory moment PK analysis and those of our final compartmental PK analysis reported in Table 1. This supports the assumption that our simultaneous PK analysis of the time–concentration data of i.v.-administered STD-BUP and of s.c.-administered SUS-BUP correctly estimated the disposition kinetics of buprenorphine in cats.

The time-course of i.v.-administered buprenorphine in our cats was better described by a model with two disposition compartments, as based both on the lower AIC value and the random scattering of its residuals. A three-compartment mammillary model had been used in some PK studies of i.v.-administered buprenorphine in dogs, but may overestimate its apparent distribution volume, as suggested by the disparity of V_{area} average estimates across published studies, for example a reported 29 L/kg by Garrett and Chandran (1990) versus a calculated 6.1 L/kg by Andaluz *et al.* (2009). In our study, the addition of a third disposition compartment considerably deteriorated the goodness of fit of the resulting model because fitting

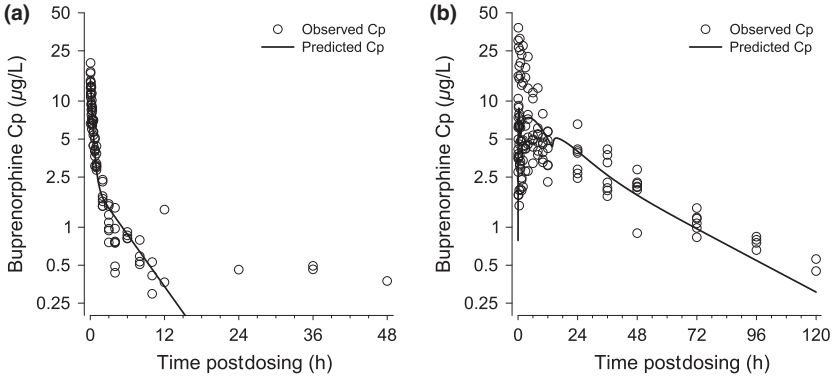

Fig. 4. Time-course of observed (circles) and population typical model-predicted (solid black lines) plasma buprenorphine concentrations in cats, following a single i.v. administration of 20 μg/kg of the aqueous drug solution STD-BUP (panel a), and a single s.c. administration of 200 μg/kg of the liposomal drug suspension SUS-BUP (panel b).

to the plasma drug concentrations found in some subjects at 12 h postdosing and later was made at the expense of all earlier concentrations (Fig. 2).

Alternatively, the recorded late concentrations of the i.v. STD-BUP data may result from entero-hepatic circulation, which already has been reported in rhesus monkeys and dogs (Brewster *et al.*, 1981), humans (Cone *et al.*, 1984), and rats (Ohtani *et al.*, 1994). Although no studies evaluating the metabolism and biliary excretion of buprenorphine and metabolites in cats have been published to our knowledge, it is conceivable that our cats excreted bile containing sizable amounts of unchanged buprenorphine at times corresponding to their *ad libitum* feeding. In this regard, cats have a very low capacity to glucuronate the phenolic moieties of morphine and other opioids (van Beusekom *et al.*, 2014), and buprenorphine glucuronide is the major metabolite found in the bile of dogs (Garrett & Chandran, 1990) and rats (Ohtani *et al.*, 1994). The plasma buprenorphine concentrations recorded by beyond 4 h following the i.v. dosing did not follow a distinct, homogeneous decay slope but appeared as gusts of short duration. This is especially visible in the graphics where the time-course of plasma buprenorphine concentration is delineated on log–log coordinates (Fig. 5b, d, f, l, n). This repeated feature of the individual i.v. pharmacokinetic profiles supports the alternative interpretation of the presence of a drug entero-hepatic cycle over the presence of a third disposition compartment.

The presence of multiple peaks after s.c. administration of SUS-BUP (Figs 3b & 5) is consistent with the administration of a liposomal suspension whose drug molecules are distributed between several vehicle phases with distinct release or absorption kinetics. Because a dose fraction of buprenorphine is also present in the aqueous phase of SUS-BUP, our liposomal suspension should be considered as a heterogeneous drug formulation. The liposomes might act either as passive reservoirs of drug molecules that burst to release their content to the fluids present at injection site, or as drug vectors that deliver their drug content directly to the central compartment. In addition, the liposomes can behave as a single, kinetically homogeneous set or as several, kinetically distinct subsets. Moreover, the lymphatics can uptake small-sized liposomes (Oussoren & Storm, 2001): Factors such as size (≤70 nm), liposome bilayers containing phosphatidylserine, surface modification with biotin or other substances, a high lymph flow, or a small interstitial space size at injection site may increase the extent of liposome absorption and their evasion from regional lymph nodes (Oussoren & Storm, 2001). Because our liposomes were free of substances that increase their lymphatic delivery to the bloodstream, and because their injection site has large interstitial space and was not massaged, it is likely that their lymphatic carriage was low and halted by the lymph nodes.

The compartmental PK analysis was essential for studying SUS-BUP first because the drug absorption kinetics from its liposomes may infringe the equivalent source constraint that the moment PK analysis assumes (DiStefano & Landaw, 1984). This was anticipated because the drug release process of liposomal drug formulations reportedly is heterogeneous (Arndt *et al.*, 1999) and requires compartmental analysis methods to decipher their atypical absorption kinetics. Second, the elucidation of how the buprenorphine molecules are absorbed from this liposomal suspension will assist us in the further refinement of SUS-BUP formulations prior to its clinical efficacy testing.

Our PK models were purposefully built to test specific hypotheses about the partition of buprenorphine molecules in the SUS-BUP formulation, about its liposomal drug release kinetics, and about the drug absorption kinetics (Fig. 1). Models A, B, and C hypothesized that only drug molecules in solution may be absorbed, implying that the liposomes are passive drug reservoirs that start bursting after their specific lag times have passed. In contrast, model D hypothesize that the liposomes are vectors that directly deliver the drug to the fluids at instant equilibrium with the systemic bloodstream. Two variants of each PK model were used: the first where liposomal burst rate declines exponentially (i.e., first-order release kinetics), and the other where the liposome burst rate is constant over time (i.e., zero-order drug release kinetics).

Unfortunately, two of our models could not converge to an asymptotic solution, a situation that hampers our ability to verify some of our hypotheses. However, the poorer fit of Model B with respect to Model A rejects the hypothesis stating that the liposomes acted as a kinetically homogeneous drug release compartment. In addition, the results of deconvolution analysis directed us to the use of model variants with zero-order drug release kinetics. Model C was designed to test an interaction

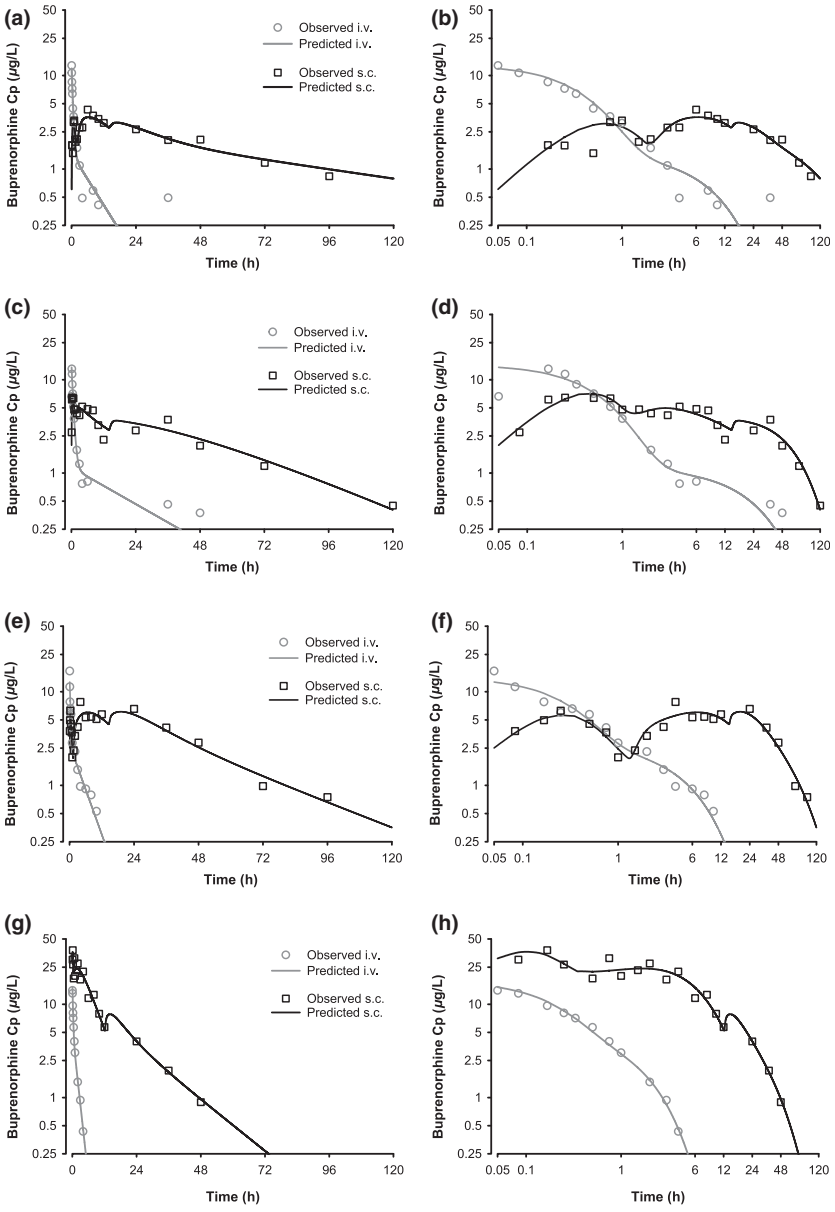

Fig. 5. Individual time-courses of observed and individual-conditional model-predicted plasma buprenorphine concentrations (Cp) in seven cats, following the administration of a single i.v. dosing of 20 µg/kg of the aqueous drug solution STD-BUP (respectively open circles and gray solid lines), and a single s.c. dosing of 200 µg/kg of the liposomal drug suspension SUS-BUP (respectively open squares and black solid lines). The time-concentration data are depicted both in semi-logarithmic coordinates (left panels), and in log-log coordinates (right panels), the latter allowing a better visualization of the curve fit to the early time-course of plasma drug concentrations. Legend: Panels a and b: subject #07pdc3. Panels c and d: subject # 07fco2. Panels e and f: subject # 07fco1. Panels g and h: subject # 07fbp2. Panels i and j: subject 07fck1. Panels k and l: subject # 07fby1. Panels m and n: subject # 06jrc1.

between liposomes such as fusion, scission, or aggregation (Oussoren & Storm, 2001), but failed to converge to a solution, and therefore, we are unable to confirm this possibility. This may be either because its structure was incorrect (e.g., a wrongly directed k_{reshape}), or because the available data were insufficient to globally identify Model C. Model D was designed to test the direct delivery of liposome-encapsulated drug to the central compartment, for example by lymphatic uptake and transport to the regional lymph nodes (Oussoren & Storm, 2001). Again, the failure of reaching an asymptotic solution may be associated with errors in model structure (e.g., only one liposomal compartment was needed for *in situ* release and the other for lymphatic transport) or because our data were unsuited to verify this hypothesis. But also, the numerical instability that precluded reaching a solution for Models C and D could be associated to our choice to estimate the full covariance matrix of PK parameters, which summed up to 13 variances and 65 covariances. We conclude that Model A with zero-order drug release from the liposomes offered a reasonable compromise of model parsimony and accuracy at predicting the time-course of plasma buprenorphine concentrations in cats.

High variability was recorded for some parameter estimates describing the absorption of buprenorphine from its s.c. injection site. In addition to the low number of cats and the complexity of the PK models used in this study, this finding may also be associated with the use of a liposomal suspension prototype, which manufacturing had not been optimized to obtain a pharmacokinetic profile with minimal interindividual variation.

The sum of estimated dose fractions directed to the solution and liposomal compartments of SUS-BUP largely exceeded 100% in some cats. This was a consistent finding both for the

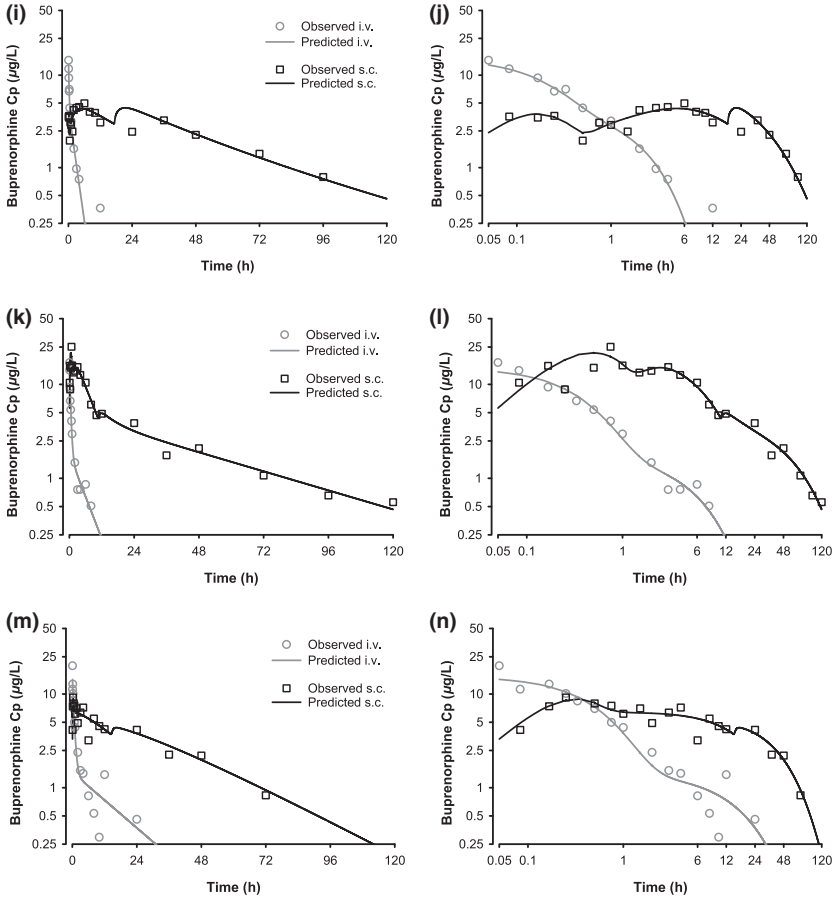

Fig. 5. Continued.

exploratory moment PK analysis (with F%) and in the final PK analysis using compartmental models (Table 2). Noteworthy, the cats that had measurable plasma drug concentrations between 12 and 48 h after i.v. drug administration of STD-BUP had F values in the range of 70%, but the cats that lacked this feature in their i.v. STD-BUP kinetic profiles had *F* values in the range of 200%. We suspect that in the latter cats, the entero-hepatic circulation of buprenorphine was lower, either because the intestinal uptake of the biliary excreted drug was not as efficient, or because the intestinal and/or hepatic first-pass metabolism was more efficient in these cats. Either mechanism may cause an underestimation of the extent of systemic exposure (i.e., AUC) to buprenorphine after i.v. administration of STD-BUP because the drug was given in bolus administration at a low dose to prevent adverse opiate effects. None of our compartmental PK models was designed to account for this entero-hepatic circulation of buprenorphine, but enhancing our PK models to consider this drug recycling may end up with parameter identifiability problems (Williams, 1990) unless a more sophisticated animal model (e.g., cats instrumented to enable bile and/or portal vein blood collection) or study designs (e.g., simultaneous measurement of parent drug and metabolite, higher i.v. dose amounts of STD-BUP, other dosing routes and/or rates) are used.

In conclusion, the pharmacokinetic behavior of buprenorphine administered subcutaneously as a liposomal suspension in cats was successfully modeled with a nonlinear mixed-effect pharmacokinetic modeling. The model that most accurately predicted the data suggests that there are two kinetically distinct pools of liposomes that entrap variable fractions of the administered dose and act as slow-release drug vehicles. In addition, there is a small fraction of the dose that is solubilized in the vehicle of liposomal suspension and is absorbed readily after s.c. dosing, a fact that may accelerate the production of therapeutic drug concentrations. The results of this study may form the basis for further drug formulation refinements that may be assisted by *in vitro* dissolution testing, until obtaining a product suitable for a multicenter efficacy study that evaluates the efficacy and safety of liposomal encapsulated buprenorphine for perioperative and chronic pain management in cats.

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APPENDIX

The following are the sets of differential equations of the models used in this study. Two variants of each model were created, one with zero-order drug release from the liposomal compartments, and the other with first-order drug release from the liposomal compartments. Each model comprises two disposition subsystems, the first receiving the absorbed s.c. dose of SUS-BUP (Fig. 1), and the second the i.v. dose of STD-BUP. Both subsystems share the same disposition PK parameters for enabling the simultaneous analysis of i.v. and extravascular data of separate experiments (D'Argenio *et al.*, 2009). All differential equations were coded with clearance parameterization.

Model A, zero-order variant

+++ Subsystem #1 +++.

$$\frac{dX_{\text{sol}}}{dt} = -k_a \cdot X_{\text{sol}} + \text{Input}_1 + \text{Input}_2;\ X_{\text{sol}}(0) = f_{\text{Sol}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo1}}}{dt} = -\text{Input}_1 = \begin{bmatrix} 0 \text{ if } t < \text{Lag}_1 \text{ or } X_{\text{LIpo1}} = 0 \\ k_{\text{rel1}} \text{ if } t \geq \text{Lag}_1 \text{ and } X_{\text{Lipo1}} > 0 \end{bmatrix};\ X_{\text{Lipo1}}(0) = f_{\text{Lipo1}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo2}}}{dt} = -\text{Input}_2 = \begin{bmatrix} 0 \text{ if } t < \text{Lag}_2 \text{ or } X_{\text{LIpo2}} = 0 \\ k_{\text{rel2}} \text{ if } t \geq \text{Lag}_2 \text{ and } X_{\text{Lipo2}} > 0 \end{bmatrix};\ X_{\text{Lipo2}}(0) = f_{\text{Lipo2}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\text{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\text{Sol}};\ X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p1} + \frac{\text{CL}_d}{V_c} \cdot X_{c1};\ X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\text{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\text{Sol}};\ X_{c2}(0) = \text{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p2} + \frac{\text{CL}_d}{V_c} \cdot X_{c2};\ X_{p2}(0) = 0$$

Model A, first-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\text{sol}}}{dt} = -k_a \cdot X_{\text{sol}} + \text{Input}_1 + \text{Input}_2;\ X_{\text{sol}}(0) = f_{\text{Sol}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo1}}}{dt} = -\text{Input}_1 = \begin{bmatrix} 0 \text{ if } t < \text{Lag}_1 \\ k_{\text{rel1}} \cdot X_{\text{Lipo1}} \text{ if } t \geq \text{Lag}_1 \end{bmatrix};\ X_{\text{Lipo1}}(0) = f_{\text{Lipo1}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo2}}}{dt} = -\text{Input}_2 = \begin{bmatrix} 0 \text{ if } t < \text{Lag}_2 \\ k_{\text{rel2}} \cdot X_{\text{Lipo2}} \text{ if } t \geq \text{Lag}_2 \end{bmatrix};\ X_{\text{Lipo2}}(0) = f_{\text{Lipo2}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\mathrm{CL}_t + \mathrm{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\mathrm{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\mathrm{Sol}}; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\mathrm{CL}_d}{V_p} \cdot X_{p1} + \frac{\mathrm{CL}_d}{V_c} \cdot X_{c1}; X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\mathrm{CL}_t + \mathrm{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\mathrm{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\mathrm{Sol}}; X_{c2}(0) = \mathrm{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\mathrm{CL}_d}{V_p} \cdot X_{p2} + \frac{\mathrm{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$

Model B, zero-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\mathrm{sol}}}{dt} = -k_a \cdot X_{\mathrm{sol}} + \mathrm{Input}_1;\ X_{\mathrm{sol}}(0) = f_{\mathrm{Sol}} \cdot \mathrm{Dose}_{s.c.}$$

$$\frac{dX_{\mathrm{Lipo1}}}{dt} = -\mathrm{Input}_1 = \begin{bmatrix} 0 \text{ if } t < \mathrm{Lag}_1 \text{ or } X_{\mathrm{LIpo1}} = 0 \\ k_{\mathrm{rel1}} \text{ if } t \geq \mathrm{Lag}_1 \text{ and } X_{\mathrm{Lipo1}} > 0 \end{bmatrix}; X_{\mathrm{Lipo1}}(0) = f_{\mathrm{Lipo1}} \cdot \mathrm{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\mathrm{CL}_t + \mathrm{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\mathrm{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\mathrm{Sol}}; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\mathrm{CL}_d}{V_p} \cdot X_{p1} + \frac{\mathrm{CL}_d}{V_c} \cdot X_{c1}; X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\mathrm{CL}_t + \mathrm{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\mathrm{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\mathrm{Sol}}; X_{c2}(0) = \mathrm{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\mathrm{CL}_d}{V_p} \cdot X_{p2} + \frac{\mathrm{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$

Model B, first-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\mathrm{sol}}}{dt} = -k_a \cdot X_{\mathrm{sol}} + \mathrm{Input}_1;\ X_{\mathrm{sol}}(0) = f_{\mathrm{Sol}} \cdot \mathrm{Dose}_{s.c.}$$

$$\frac{dX_{\mathrm{Lipo1}}}{dt} = -\mathrm{Input}_1 = \begin{bmatrix} 0 \text{ if } t < \mathrm{Lag}_1 \\ k_{\mathrm{rel1}} \cdot X_{\mathrm{Lipo1}} \text{ if } t \geq \mathrm{Lag}_1 \end{bmatrix}; X_{\mathrm{Lipo1}}(0) = f_{\mathrm{Lipo1}} \cdot \mathrm{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\mathrm{CL}_t + \mathrm{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\mathrm{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\mathrm{Sol}}; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\mathrm{CL}_d}{V_p} \cdot X_{p1} + \frac{\mathrm{CL}_d}{V_c} \cdot X_{c1}; X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\mathrm{CL}_t + \mathrm{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\mathrm{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\mathrm{Sol}}; X_{c2}(0) = \mathrm{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\mathrm{CL}_d}{V_p} \cdot X_{p2} + \frac{\mathrm{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$

Model C, zero-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\text{sol}}}{dt} = -k_a \cdot X_{\text{sol}} + \text{Input}_1 + \text{Input}_2;\ X_{\text{sol}}(0) = f_{\text{Sol}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo1}}}{dt} = -k_{\text{reshape}} \cdot X_{\text{Lipo1}} - \text{Input}_1; \text{Input} = \begin{bmatrix} 0 \textit{ if } t < \text{Lag}_1 \text{ or } X_{\text{Lipo1}} = 0 \\ k_{\text{rel1}} \textit{ if } t \geq \text{Lag}_1 \text{ and } X_{\text{Lipo1}} > 0 \end{bmatrix}; X_{\text{Lipo1}}(0) = f_{\text{Lipo1}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo2}}}{dt} = k_{\text{reshape}} \cdot X_{\text{Lipo1}} - \text{Input}_2; \text{Input} = \begin{bmatrix} 0 \textit{ if } t < \text{Lag}_2 \text{ or } X_{\text{Lipo2}} = 0 \\ k_{\text{rel2}} \textit{ if } t \geq \text{Lag}_2 \text{ and } X_{\text{Lipo2}} > 0 \end{bmatrix}; X_{\text{Lipo2}}(0) = f_{\text{Lipo2}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\text{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\text{Sol}}; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p1} + \frac{\text{CL}_d}{V_c} \cdot X_{c1}; X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\text{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\text{Sol}}; X_{c2}(0) = \text{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p2} + \frac{\text{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$

Model C, first-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\text{sol}}}{dt} = -k_a \cdot X_{\text{sol}} + \text{Input}_1 + \text{Input}_2;\ X_{\text{sol}}(0) = f_{\text{Sol}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo1}}}{dt} = -k_{\text{reshape}} \cdot X_{\text{Lipo1}} - \text{Input}_1; \text{Input}_1 = \begin{bmatrix} 0 \textit{ if } t < \text{Lag}_1 \\ k_{\text{rel1}} \cdot X_{\text{Lipo1}} \textit{ if } t \geq \text{Lag}_1 \end{bmatrix}; X_{\text{Lipo1}}(0) = f_{\text{Lipo1}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo2}}}{dt} = -k_{\text{reshape}} \cdot X_{\text{Lipo1}} - \text{Input}_2; \text{Input}_2 = \begin{bmatrix} 0 \textit{ if } t < \text{Lag}_2 \\ k_{\text{rel2}} \cdot X_{\text{Lipo2}} \textit{ if } t \geq \text{Lag}_2 \end{bmatrix}; X_{\text{Lipo2}}(0) = f_{\text{Lipo2}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\text{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\text{Sol}}; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p1} + \frac{\text{CL}_d}{V_c} \cdot X_{c1}; X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\text{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\text{Sol}}; X_{c2}(0) = \text{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p2} + \frac{\text{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$

Model D, zero-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\text{sol}}}{dt} = -k_a \cdot X_{\text{sol}};\ X_{\text{sol}}(0) = f_{\text{Sol}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo1}}}{dt} = -\text{Input}_1 = \begin{bmatrix} 0 \; if \; t < \text{Lag}_1 \text{ or } X_{\text{LIpo1}} = 0 \\ k_{\text{rel1}} \; if \; t \geq \text{Lag}_1 \text{ and } X_{\text{Lipo1}} > 0 \end{bmatrix}; X_{\text{Lipo1}}(0) = f_{\text{Lipo1}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo2}}}{dt} = -\text{Input}_2 = \begin{bmatrix} 0 \; if \; t < \text{Lag}_2 \text{ or } X_{\text{LIpo2}} = 0 \\ k_{\text{rel2}} \; if \; t \geq \text{Lag}_2 \text{ and } X_{\text{Lipo2}} > 0 \end{bmatrix}; X_{\text{Lipo2}}(0) = f_{\text{Lipo2}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\text{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\text{Sol}} + \text{Input}_1 + \text{Input}_2; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p1} + \frac{\text{CL}_d}{V_c} \cdot X_{c1}; X_{p1}(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\text{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\text{Sol}}; X_{c2}(0) = \text{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p2} + \frac{\text{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$

Model D, first-order variant

+++ Sub-system #1 +++

$$\frac{dX_{\text{sol}}}{dt} = -k_a \cdot X_{\text{sol}}; \; X_{\text{sol}}(0) = f_{\text{Sol}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo1}}}{dt} = -\text{Input}_1 = \begin{bmatrix} 0 \; if \; t < \text{Lag}_1 \\ k_{\text{rel1}} \cdot X_{\text{Lipo1}} \; if \; t \geq \text{Lag}_1 \end{bmatrix}; X_{\text{Lipo1}}(0) = f_{\text{Lipo1}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{\text{Lipo2}}}{dt} = -\text{Input}_2 = \begin{bmatrix} 0 \; if \; t < \text{Lag}_2 \\ k_{\text{rel2}} \cdot X_{\text{Lipo2}} \; if \; t \geq \text{Lag}_2 \end{bmatrix}; X_{\text{Lipo2}}(0) = f_{\text{Lipo2}} \cdot \text{Dose}_{s.c.}$$

$$\frac{dX_{c1}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c1} + \frac{\text{CL}_d}{V_p} \cdot X_{p1} + k_a \cdot X_{\text{Sol}} + \text{Input}_1 + \text{Input}_2; X_{c1}(0) = 0$$

$$\frac{dX_{p1}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p1} + \frac{\text{CL}_d}{V_c} \cdot X_{c1}; X_p(0) = 0$$

+++ Sub-system #2 +++

$$\frac{dX_{c2}}{dt} = -\left(\frac{\text{CL}_t + \text{CL}_d}{V_c}\right) \cdot X_{c2} + \frac{\text{CL}_d}{V_p} \cdot X_{p2} + k_a \cdot X_{\text{Sol}}; X_{c2}(0) = \text{Dose}_{i.v.}$$

$$\frac{dX_{p2}}{dt} = -\frac{\text{CL}_d}{V_p} \cdot X_{p2} + \frac{\text{CL}_d}{V_c} \cdot X_{c2}; X_{p2}(0) = 0$$