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Pharmacokinetics of fentanyl citrate and norfentanyl in Holstein calves and effect of analytical performances on fentanyl parameter estimation

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Abstract

This study describes the pharmacokinetics of intravenously administered (IV) fentanyl citrate, and its primary metabolite norfentanyl in Holstein calves.

Eight calves (58.6 +/- 2.2 kg), aged 3-4 weeks, were administered fentanyl citrate at a single dose of 5.0 µg/kg IV. Blood samples were collected from 0 to 24 hours. Plasma (nor)fentanyl concentrations were determined using liquid chromatography with mass spectrometry and a lower limit of quantification (LLOQ) of 0.03 ng/mL. The noncompartmental pharmacokinetic analysis was then repeated with a hypothetical LLOQ value of 0.05 ng/mL.

Terminal elimination half-life was estimated at 12.7 and 3.6 hours for fentanyl and norfentanyl, respectively. For fentanyl, systemic clearance was estimated at 2.0 L/hr/kg, volume of distribution at steady state was 24.8 L/kg, and extraction ratio was 0.42. At a hypothetical LLOQ of 0.05 ng/mL fentanyl half-life, volume of distribution at steady state, and clearance were respectively of 3.0 hr, 8.8 L/kg, and 3.4 L/kg/hr.

Fentanyl citrate administered IV at 5.0 µg/kg can reach levels associated with analgesia in other species. Pharmacokinetic parameters should be interpreted with respect to LLOQ, as lower limits can influence estimated parameters, such as elimination half-life or systemic clearance and have significant impact on dosing regimen selection in clinical practice.

Key words.

Fentanyl, Cattle, Calves, Norfentanyl

Introduction

Analgesia for cattle during production, surgical, and medical procedures is an important tool for promoting animal welfare. While cattle are commonly subjected to potentially painful production procedures and non-routine surgical procedures, practitioners have limited options in terms of pain management as in the US there are currently no drugs labelled for analgesia in cattle.

The synthetic mu receptor opioid agonist fentanyl is commonly used to provide analgesia in veterinary species. Morphine and butorphanol are opioid analgesics that currently are currently used as an intravenous (IV) bolus in cattle. Morphine, is a primary mu opioid agonist that is used for the treatment of pain in a wide variety of veterinary species. Butorphanol has also been described for use in many veterinary species and is a partial opioid agonist with activity as an agonist for the kappa receptor and weak mu receptor antagonist activity. Butorphanol is thought to have an analgesic value of approximately four to seven times that of morphine.

With a potency that is approximately 100 times more than morphine, and a rapid onset, fentanyl is an ideal clinical analgesic in veterinary medicine. Fentanyl is primarily metabolized by cytochrome P450 3A enzymes to norfentanyl(Clavijo, Thomas et al., 2011). There are several additional minor pathways it the metabolism of fentanyl, primarily amide hydrolysis to despropionyl fentanyl as well as alkyl hydroxylation to hydroxyfentanyl.

Among large animal species, the pharmacokinetics (PK) of IV fentanyl has been described in sheep(Ahern, Soma et al., 2010), goats(Carroll, Hooper et al., 1999), alpacas(Lovasz, Aarnes et al., 2017), and horses(Maxwell, Thomasy et al., 2003). In small animals, the IV pharmacokinetics of fentanyl has also been described. Adverse reactions to fentanyl include an increase in locomotor activity in horses (Kamerling, DeQuick et al., 1985), and respiratory depression when too high systemic concentrations are reached (30 ng/mL) in dogs (Arndt, Mikat et al., 1984). Pharmacokinetics of fentanyl metabolites, while readily available in human medical studies, are limited in veterinary medicine. Currently limited to studies reporting norfentanyl concentrations in chickens(Delaski, Gehring et al., 2017), and

primates(Koch, Isaza et al., 2004), as well as not detecting measurable quantities of norfentanyl in dogs(Lin, Wang et al., 1981).

While practitioners routinely utilize analgesic drugs in a legal extra-label manner, there are few reports of the pharmacokinetics of fentanyl in ruminant species, and no reports of the use of this analgesic therapy in cattle. Due to the increased analgesic activity of fentanyl compared to morphine and butorphanol it may have clinical uses for bovine analgesia during surgical procedures.

The aim of this study was to describe the pharmacokinetics of fentanyl citrate and its primary metabolite norfentanyl when administered as an IV bolus in calves, as well as to report any adverse reactions. A secondary goal of this study was to examine the impact of the bioanalytical quantification limit of fentanyl with respect to pharmacokinetic parameter estimation.

Materials and Methods

Experimental Animals

This study was completed at the Iowa State University Dairy Farm. Eight female Holstein calves were enrolled in the study. The age of these calves ranged from 23 to 30 days, weighed 58.6 +/- 2.2 kg, and were procured from a single source farm. Approval for the study was secured from the Institution Animal Care and Use Committee (Log # 7-16-8318-B) at Iowa State University. The calves were housed in individual pens since birth, and the study took place in the same individual pens for each calf. The calves were housed in a climate-controlled calf raising facility, and no alterations to feeding or handling schedule was made for this study. During the pre-study time period, all calves were trained to be restrained by a hand placed under the mandible and behind the poll. Criteria for enrollment in this study included a physical assessment by a veterinarian that yielded vital signs within the normal limits for a bovine calf, no previous history of medical illness as well as no history of a previously administered medication. Prior to and during the study all calves were fed a diet that wither met or exceeded the NRC requirements for maintenance and growth of bovine calves.

Twenty hours prior to initiation of the study the calves were restrained and 2 IV jugular catheters were aseptically placed. The skin was aseptically prepared utilizing 4 alternating scrubs of chlorhexidine surgical scrub and 70% isopropyl alcohol. Prior to catheter placement the skin at the catheter site was infiltrated with 2% lidocaine. The calf was restrained by study personnel and a 14-gauge catheter was placed in each jugular vein. An injection port was placed and the catheters were sutured to the skin and wrapped for security.

Experimental Design and Sample Collection

Calves were administered a single 5.0 µg/kg IV bolus of fentanyl citrate (Fentanyl Citrate, Hospira Inc, Lake Forrest, Il) via a catheter inserted in the left jugular vein. Blood collection was achieved through a catheter in the right jugular vein at 2, 5, 10, 30,

45, and 60 minutes, and 1.5, 2, 2.5, 3, 4, 6, 10, 16, and 24 hours after administration. Starting at the 2-hour sampling time point, heart and respiratory rates were measured at each sampling timepoint up to 24 hours.

At each sampling timepoint blood was collected from the catheter using a 12-mL syringe and placed into sodium heparin tubes (BD Vacutainer, Franklin Lakes, NJ). The samples were then centrifuged at 1500 G for 10 minutes. The plasma was pipetted off and transferred to cryovials which were then stored at -80 C until analysis.

Sample Analysis

Plasma concentrations of fentanyl, and its metabolite norfentanyl were determined by liquid chromatography-mass spectrometry (LC-MS) after precipitation of proteins by acetonitrile. Briefly, plasma samples were thawed and vortexed, and 200uL aliquots were transferred into a vial with 800uL of internal standard, fentanyl-D5, in acetonitrile with 0.1% formic acid added. Samples were vortexed and then centrifuged at 7500 rpm for 20 minutes. The supernatant was then transferred and the samples were dried down, then reconstituted in 125uL of 25% acetonitrile in water, vortexed and transferred into an autosampler vial (with glass insert) and then centrifuged for 20 minutes at 2400 rpm and analyzed via LC-MS/MS. The LC-MS system consisted of an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled to a Thermo LTQ ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA). The lower limit of quantification (LLOQ) for fentanyl and its metabolite was 0.03 ng/mL for this assay.

Pharmacokinetic Analysis

Pharmacokinetic analysis of total fentanyl and norfentanyl plasma concentrations was completed using a statistical moment (i.e. non-compartmental) approach in commercial software (Phoenix WinNonlin 7.0, Certara, Princeton, NJ, USA). Time versus concentration figures for fentanyl and norfentanyl were produced via a commercial program (GraphPad Prism 7, GraphPad Software, Inc, La Jolla, CA, USA).

Standard PK parameters were generated for individual calves, as follows:

- Maximum (nor)fentanyl concentration, C_0 (fentanyl) or C_{max} (norfentanyl);
- Time of maximum norfentanyl concentration, T_{max};
- Area under (nor)fentanyl concentration-time curve, AUC_{last} and AUC_{inf};
- Area under the moment curve, $AUMC_{inf}$;
- (Nor)fentanyl mean residence time, $MRT = AUMC_{inf} / AUC_{inf}$;
- Slope of the elimination phase λ_z, computed by linear regression of the logarithmic concentration vs. time curve during the elimination phase;
- (Nor)fentanyl terminal half-life, $T_{1/2}(\lambda z) = \ln(2) / \lambda z$;
- Fentanyl systemic clearance, $CL = Dose / AUC_{inf}$;
- Volume of distribution of fentanyl during the elimination phase, $V_{area} = Dose / (AUC_{inf} \times \lambda z)$;
- Volume of distribution of fentanyl at steady-state, $V_{ss} = CL \times MRT$

For data analysis, the first value below the LLOQ was inferred to be LLOQ/2, and subsequent data points were excluded from the analysis. A linear/log trapezoidal rule was used to estimate the area under the (nor)fentanyl time-curves. Summary statistics on the individual PK parameters were performed thereafter to derive the geometric mean, median and (min-max) range.

For fentanyl, the extraction ratio (E_{body}) was calculated as reported by Toutain et al(Toutain & Bousquet-Melou, 2004), with:

$$E_{Body} = \text{Systemic clearance} / \text{Cardiac output} \quad \text{[Equation 1]}$$

First calculated for each individual calf, and then combined for a mean value. With the calf cardiac output calculated as follows:

$$\text{Cardiac output} = 180 \times BW(kg)^{-0.19} \quad \text{[Equation 2]}$$

In a second step and using the same raw source data, an hypothetical analytical LLOQ of 0.05 ng/mL, as reported in the literature in other species(Lovasz, Aarnes et al., 2017), was applied and the pharmacokinetic analysis for fentanyl only was repeated using the same workflow as described above.

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Results

Animal Health

At enrollment, all study subjects were assessed to be healthy and to have parameters within the normal limits for calves of their respective ages. The injections were well tolerated by all calves, with no adverse effects noted throughout the entire study period. For heart rate, respiratory rate, and temperature no significant elevation or depression from baseline was reported, with the exception of excitement at the timepoints that coincided with the feeding of the calves. Follow up examination 2 weeks and 2 months after the study revealed no abnormalities in behavior or physical assessment.

Pharmacokinetics of fentanyl and its metabolites using a LLOQ of 0.03 ng/mL

No calf had detectable fentanyl or metabolites in plasma at time zero. The individual time-course of fentanyl and norfentanyl total concentrations in plasma can be found in Figures 1 and 2, respectively. Geometric mean and standard deviations disposition profiles are presented in Figures 3 and 4 for fentanyl and norfentanyl, respectively. Among individuals there appears to be limited variation of time versus concentration data for fentanyl as opposed to norfentanyl. For the LLOQ of 0.03 ng/mL 4.2% (5/120) of the post administration data points had values below the LLOQ. For the theoretical LLOQ of 0.05 ng/mL 21.7% (26/120) of the post administration data points had values below the LLOQ.

Table 1 summarizes the pharmacokinetic parameters for fentanyl and norfentanyl when administered IV. For fentanyl, the systemic clearance was almost 2 L/kg/hr. The average extraction ratio was calculated to be 0.41 ± 0.10. The AUC% extrapolation was estimated to be inferior to 20% (15.4%), while the steady-state volume of distribution (Vss) was 24.8 L/kg. The elimination half-life $T_{1/2}$ (λz) was estimated at approximately 12 hours.

For norfentanyl the AUC% extrapolation was estimated to be inferior to 20% (7.2%). C_{MAX} and T_{MAX} of norfentanyl were 0.3 ng/mL, and 1.1 hr respectively. The elimination half-life $T_{1/2}$ λz was estimated at 12.7 hours.

Pharmacokinetics of fentanyl and its metabolites using a LLOQ of 0.05 ng/mL

A comparison of the fentanyl estimated PK parameters with a LLOQ of 0.03 vs. 0.05 ng/mL is provided in Table 2. Despite this relatively small difference in analytical sensitivity (0.02 ng/mL), a noted lack of agreement among parameters was observed. Compared to the quantification limit of 0.03 ng/mL, the clearance of fentanyl was markedly increased (164 % increased) when a hypothetical quantification limit of 0.05 ng/mL was utilized on the study data. In contrast, the estimated volume of distribution markedly decreased (by 68%), and the elimination half-life was 12 hr shorter as compared with the 0.03 ng/mL LLOQ threshold. Interestingly, with the higher quantification limit, the estimated elimination half-life was closer in value to what is reported in the literature for other ruminant species, with a LLOQ ranging from 0.01 (sheep) to 0.1 (goat) ng/mL (Table 3).

Discussion

To the best of our knowledge, this is the first report of the pharmacokinetics of fentanyl in calves. Although the cohort sampling could potentially be a source of bias for this study, it was thought to be minimal as calves had acclimated to the individual pens prior to the study, and the group of individual pens used for the study was from the same block of eight stalls in the temperature, humidity, and ventilation controlled barn. The age and size of the calves utilized for this study was designed to mimic the age of calves presented to the author's hospital for surgical procedures that could potentially benefit from fentanyl analgesia.

In the United States, there is currently no approved formulation of fentanyl citrate for cattle. However, in practice calves routinely undergo orthopedic and other surgical procedures that warrant post-operative analgesia. Several concentrations of fentanyl have been associated with analgesia in various veterinary species. Plasma fentanyl values of 1.07, 0.95, and 0.6 ng/mL or greater have been associated with analgesia in cats(Robertson, Taylor et al., 2005), dogs(Robinson, Kruse-Elliott et al., 1999) and people(Peng & Sandler, 1999), respectively. In humans, few reports suggest that values as low as 0.2 ng/mL may provide analgesia for individuals that are "opioid naïve" and have not been previously treated with any drugs in the class(Peng & Sandler, 1999). The maximum concentration reported in this study (1.5 ng/mL), would be above what is reported to be an analgesic concentration is other veterinary species, although currently the threshold required for analgesia in calves is unknown.

Other studies have evaluated the pharmacokinetics of intravenously fentanyl in horses(Maxwell, Thomasy et al., 2003), sheep(Ahern, Soma et al., 2010; Christou, Oliver et al., 2015), goats(Carroll, Hooper et al., 1999), and alpacas(Lovasz, Aarnes et al., 2017). The mean maximum concentration of 1.5 ug/L reported in our study was less than described by earlier reports in other large animal species when normalized with the input dose (Table 3). The estimated elimination half-life of fentanyl in calves was apparently longer compared with other large animal species, such as sheep (3.1 hours), goats (1.2 hours) and alpacas (1.2 hours)(Lovasz, Aarnes et al., 2017). This must be interpreted with caution however, as these values are compared to mature animals in

these previous studies, and drug metabolism can be different between young and older animals of the same species. In lambs aged between 3 and 37 days it has been noted that clearance and volume of distribution increase with age(Gauntlett, Fisher et al., 1988). Fentanyl is extracted by the liver via the cytochrome P450 system, and initial activity of this system is low at birth and increases with age(Gauntlett, Fisher et al., 1988). It is uncertain how adult cattle would metabolize this drug, as there would be potential for variation from calves.

It is noteworthy that when the estimated elimination half-life is considered (with a LLOQ of 0.05 ng/mL is applied), the value is much lower (3.0 hr vs 14.9 hr), and this lower value appears to reconcile with other species when a higher quantification limit is applied in calves. However, the HL in sheep was fairly short (3h) despite a very low quantification limit (0.01 ng/mL), therefore, between species differences for fentanyl metabolism are also expected independent of the analytical method.

While the different quantification limits create different pharmacokinetic parameter values, these differences are not trivial.

For calculating dosing regimens, clearance is the most important pharmacokinetic parameter(Toutain & Bousquet-Melou, 2004). A lower LLOQ can have multiple effects of the pharmacokinetic parameters reported, including clearance. By reducing the number of samples that are below the limit of quantification (BQL), clearance can be overestimated(Hing, Woolfrey et al., 2001). A higher LLOQ would theoretically result in more sample values BQL, and therefore result in a higher clearance. This finding is supported by the higher average clearance reported for the theoretical 0.05 ng/mL LLOQ for these calves than the average clearance reported for the 0.03 ng/mL LLOQ (3371 vs 2061 mL/hr/kg). Similarly, elimination half-life, important in predicting time to steady-state, as well as drug accumulation, would also be affected by a lower LLOQ. The relationship between elimination half-life and clearance is as follows(Greenblatt, 1985) :

Elimination half-life = (0.693 x Volume of Distribution) / Clearance. [Equation 3]

Therefore, increasing clearance would serve to underestimate the elimination half-life. This is also supported by the theoretical exercise as the elimination half-life was much shorter for the theoretical LLOQ of 0.05 ng/mL vs the theoretical calculation with a LLOQ at 0.03 ng/mL. These differences in calculated parameters could have effects on patients when treated with fentanyl, depending on the pharmacodynamics of the drug. While there is a relative paucity of the effects of fentanyl in cattle, adverse effects from overdosing have been reported in multiple species.

Volume of distribution at steady state (27.5 L/kg) was also greater than reported values of other ruminant species such as 8.9 L/kg (sheep), 1.5 L/kg (goats), and 1.5 L/kg (alpacas)(Lovasz, Aarnes et al., 2017). The estimated systemic clearance (2.1 L/kg/hr) was consistent with other reported clearances in similar large animal species of sheep (3.6 L/kg/hr), goats (2.1 L/kg/hr), and alpacas (1.1 L/kg/hr)(Lovasz, Aarnes et al., 2017).

Extraction data does not appear to be well described for fentanyl in large animal species. The total extraction of the body, reported in this study as E_{body}, can be described as a percentage or ratio of the drug eliminated through one pass of the different organs contributing to clearance(Toutain & Bousquet-Melou, 2004). The extraction ratio reported for the calves in this study (0.41 ± 0.10) would be consistent with an extraction percentage of 41.0 ± 10%. This appears to be greater than what has been described in neonatal lambs, as a fentanyl extraction percentage of 16.5 ± 3.0% has been reported(Kuhls, Gauntlett et al., 1995). As reported by Toutain et al[11], an extraction value of 0.3 (30%) or higher is indicative of high a clearance of fentanyl in calves.

In adult humans fentanyl is mainly metabolized by cytochrome P450 3A enzymes to norfentanyl(Clavijo, Thomas et al., 2011). Two other minor metabolites, desproprionyl fentanyl, and hydroxyfentanyl are accomplished by amide hydrolysis and alkyl hydroxylation respectively(Clavijo, Thomas et al., 2011). The pharmacokinetics of norfentanyl are not widely described in veterinary species, with one recent report identifying parameters in chickens administered fentanyl via a transdermal patch system.

Norfentanyl pharmacokinetics in this study significantly varied from that of the parent compound fentanyl. Notably, the elimination half life of norfentanyl was estimated at only 3.6 hours vs. 12.7 hours for its parent. Since a metabolite cannot be eliminated faster than it is being formed, the elimination half-life of norfentanyl can either be similar or longer than that of fentanyl, but not shorter. Therefore, the apparent '*shorter*' half-life of norfentanyl is most likely a consequence of the bioanalytical cut-off, such that the reported half-life of 3.6 hours relate to the distribution, rather than the elimination of norfentanyl. This is supported by the similarities in the estimated half-life between fentanyl and norfentanyl as the theoretical LLOQ for the parent increased from 0.03 to 0.05 ng/mL. As no norfentanyl concentrations were measured below 0.05 ng/mL, the PK parameters remain unchanged if re-evaluated with the theoretical LLOQ of 0.05 ng/mL.

At this time the significance of the norfentanyl pharmacokinetic parameters is unknown as a relative paucity of comparative data for this metabolite exists in the veterinary literature. Among human toxicologists it is speculated that the smaller the ratios of blood and urine norfentanyl/fentanyl, the larger the probability of acute fentanyl intake with coexistent fentanyl abstinence, which then predisposes to fentanyl toxicity(Ruan, Chiravuri et al., 2016). Further studies of norfentanyl are necessary to determine the clinical significance of this metabolite in cattle.

Limitations

A limitation of this study was the relatively small number of calves used. While eight animals are commonly used in PK studies, it might not account for population variability. Similarly, all of the animals were calves of the approximate same age which may not be reflective of adult cattle. Norfentanyl calculations were limited, as a metabolite, clearance and volumes of distribution cannot be calculated without a priori knowledge on the fractional conversion of fentanyl into norfentanyl. Additional pharmacokinetic studies with norfentanyl per se should consider intravenous injection of the metabolite to derive such parameters.

Conclusions

In conclusion, fentanyl citrate administered intravenously reaches systemic peak concentrations associated with analgesia in other veterinary species. Further work needs to be completed to investigate the analgesic properties of fentanyl in calves. In addition, more work into alternative dosing formulations, such as continuous rate infusion and transdermal patches needs to be done to evaluate the suitability of these routes for bovine practice. Finally, interpretation of pharmacokinetics warrants close investigation of the quantification limits used, as increased or decreased limits of quantification can significantly alter the estimation of pharmacokinetic parameters, which could have important implications for dosing regimen selection in clinical practice.

Conflicts of interest

The authors have no conflicts of interest.

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Table 1. Pharmacokinetic parameters for fentanyl and norfentanyl in study calves.

Compound	Parameter	Unit	Geomean	Median	Min	Max
	C_0	ng/mL	1.5	1.6	1.0	2.0
	AUC_{last}	ng/mL*hr	2.0	2.1	1.6	2.3
Fentanyl	AUC_{inf}	ng/mL*hr	2.5	2.3	1.8	3.3
	$\%AUC_{extr}$	%	15.4	11.0	7.0	48.1
	$AUMC_{inf}$	$ng/mL*hr^2$	31.1	17.1	16.2	131.1
	MRT	hr	12.4	8.8	7.3	39.3
	CL	mL/hr/kg	1999	2167	1505	2821
	$T_{1/2}$ (λz)	hr	12.7	9.1	7.5	35.1
	V_{ss}	L/kg	24.8	23.3	15.8	58.8
	V_{area}	L/kg	36.7	34.0	23.4	76.1
	Cmax	ng/mL	0.3	0.3	0.2	0.5
	Tmax	hr	1.1	1.5	0.08	2.5
Norfentanyl	AUC_{inf}	ng/mL*hr	1.8	2.2	0.9	2.9
	$\%AUC_{extr}$	%	7.2	7.2	3.6	13.1
	$AUMC_{inf}$	$ng/mL*hr^2$	10.6	13.4	4.6	16.5
	MRT	hr	5.9	6.0	4.8	7.9
	$T_{1/2}$ (λz)	hr	3.6	3.2	2.9	5.4

The following parameters were calculated for IV administration: C0, plasma concentration back extrapolated to time 0 using log-linear regression of the first two time points; Cmax, maximum concentration; Tmax, time of maximum concentration; AUC_{inf}, area under the curve extrapolated to infinity, using the linear trapezoidal method; $\%AUC_{extrap}$, percent of the AUC extrapolated to infinity; CL, plasma clearance; $T_{½}$ λ_z, terminal half-life; λ_z, terminal rate constant; MRT, mean residence time; V_{ss}, Volume of distribution at steady state; V_{area}, volume of distribution during the elimination phase.

Table 2. Average (± S.D) fentanyl pharmacokinetic parameters with the study lower limit of quantification (LLOQ) of 0.03 ng/mL compared to a theoretical LLOQ of 0.05 ng/mL. See **Table 1** for definition of abbreviated terms.

Parameter	Unit	Calves (Current)	Calves (Hypothetical)
LLOQ	**ng/mL**	**0.03**	**0.05**
AUC_{inf}	ng/mL*hr	2.6 ± 0.6	1.5 ± 0.3
CL	mL/hr/kg	2061 ± 491	3371 ± 813
$T_{1/2}$ (λz)	hr	14.9 ± 9.9	3.0 ± 0.9
λz	1/hr	0.06 ± 0.03	0.30 ± 0.1
MRT	hr	15.3 ± 11.6	2.7 ± 0.6
V_{ss}	L/kg	27.5 ± 14.7	8.8 ± 1.2
V_{area}	L/kg	39.6 ± 17.1	13.9 ± 3.0

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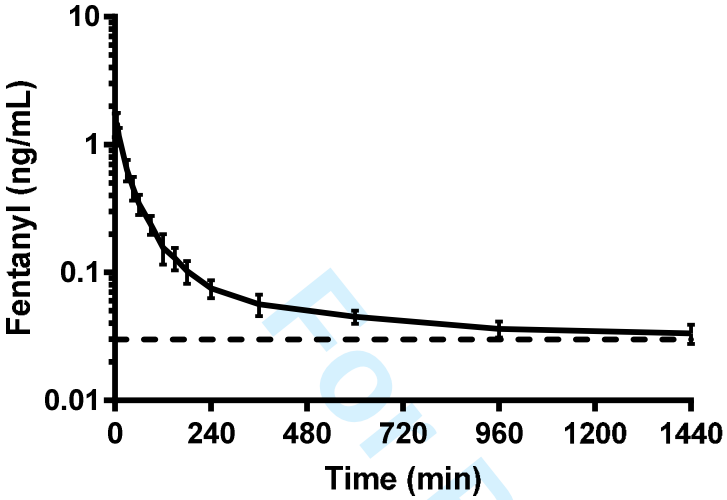
Table 3. Pharmacokinetic parameters of fentanyl in other large animal species. See Table 1 for definition of abbreviated terms.

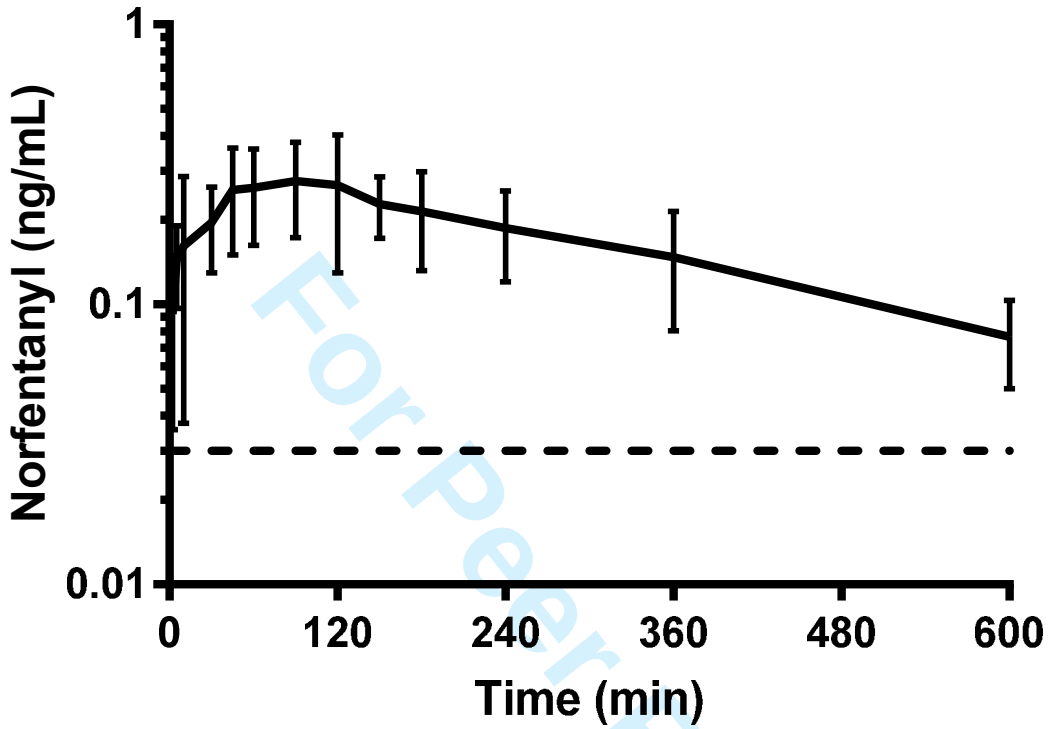
Parameter	Unit	Calves (Actual)	Calves (Hypothetical)	Goats (Carroll, 1999)	Sheep (Ahern, 2010)	Alpacas (Lovasz, 2016)
LLOQ	ng/mL	0.03	0.05	0.10	0.01	0.05
Dose	µg/kg	5.0	5.0	2.5	2.5	2
$T_{1/2}$ (λz)	hr	14.9	3.0	1.2	3.1	1.2
MRT	hr	15.3	2.7	0.80	-	1.3

Figure 1: Individual fentanyl pharmacokinetic time-course (log10, mean ± 1 S.D) following intravenous bolus dosing at 5.0 µg/kg.



An initial fentanyl concentration of 1.5 ng/mL was observed. As evident by the standard deviation bars very little individual to individual variation was noted. The dashed line represents the lower limit of quantification for the assay (0.03 ng/mL).

Figure 2: Individual norfentanyl pharmacokinetic time-course (log10, mean ± 1 S.D) following intravenous bolus dosing of fentanyl at 5.0 µg/kg.



A maximum norfentanyl concentration of 0.3 ng/mL was observed, with a time to maximum concentration of 1.1 hours. As evidenced by the standard deviation bars more individual to individual variation was noted as opposed to fentanyl. After 600 minutes all values were below the LLOQ of 0.03 ng/ml (LLOQ represented as dashed line).