Received: 25 October 2017 | Accepted: 14 February 2018

DOI: 10.1111/jvp.12501

ORIGINAL ARTICLE

WILEY Journal of Veterinary Pharmacology and Therapeutics

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

J. S. Smith[1] | J. F. Coetzee[2] | I. W. G. Fisher[1] | D. J. Borts[1] | J. P. Mochel[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA

[2]Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA

Correspondence
J. S. Smith, Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA.
Email: jss303@iastate.edu

This study describes the pharmacokinetics of intravenously administered (i.v.) 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 i.v. Blood samples were collected from 0 to 24 hr. Plasma (nor)fentanyl concentrations were determined using liquid chromatography with mass spectrometry and a lower limit of quantification (LLOQ) of 0.03 ng/ml. To explore the effect of analytical performance on fentanyl parameter estimation, 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 hr for fentanyl and norfentanyl, respectively. For fentanyl, systemic clearance was estimated at 2.0 L hr^{-1} kg^{-1}, 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^{-1} hr^{-1}. Fentanyl citrate administered i.v. 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 dosage regimen selection in clinical practice.

1 | 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 nonroutine surgical procedures, practitioners have limited options in terms of pain management as in the United States, 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 (i.v.) 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 4–7 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 et al., 2011). There are several additional minor pathways in 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 i.v. fentanyl has been described in sheep (Ahern, Soma, Rudy, Uboh, & Schaer, 2010), goats (Carroll, Hooper, Boothe, Hartsfield, & Randoll, 1999), alpacas (Lovasz et al., 2017) and horses (Maxwell, Thomasy, Slovis, & Kollias-Baker, 2003). In small animals, the i.v. pharmacokinetics of fentanyl has also been described. Adverse reactions to fentanyl include an increase in locomotor activity in horses (Kamerling,

DeQuick, Weckman, & Tobin, 1985), and respiratory depression when too high systemic concentrations are reached (30 ng/ml) in dogs (Arndt, Mikat, & Parasher, 1984). Pharmacokinetics of fentanyl metabolites, while readily available in human medical studies, is limited in veterinary medicine. Currently limited to studies reporting norfentanyl concentrations in chickens (Delaski, Gehring, Heffron, Negrusz, & Gamble, 2017), and primates (Koch, Isaza, Carpenter, & Hunter, 2004), as well as not detecting measurable quantities of norfentanyl in dogs (Lin, Wang, Caprioli, & Mo, 1981).

While practitioners routinely utilize analgesic drugs in a legal extralabel 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 i.v. 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.

2 | MATERIALS AND METHODS

2.1 | 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 was 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 were made for this study. During the prestudy time period, all calves were trained to be restrained by a hand placed under the mandible and behind the poll. Criteria for enrolment 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 either met or exceeded the NRC requirements for maintenance and growth of bovine calves. Study calves were fed a pasteurized whole milk diet (three quarts) every 8 hr with ad libitum access to a commercial calf starter.

Twenty hours prior to initiation of the study, the calves were restrained and two i.v. jugular catheters were aseptically placed. The skin was aseptically prepared utilizing four 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.

2.2 | Experimental design and sample collection

Calves were administered a single 5.0 μg/kg i.v. 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 min and 1.5, 2, 2.5, 3, 4, 6, 10, 16 and 24 hr after administration. Starting at the 2-hr sampling timepoint, heart and respiratory rates were measured at each sampling timepoint up to 24 hr.

The 5.0 μg/kg dose was determined as a pilot study investigating a 2.5 μg/kg i.v. bolus of fentanyl citrate (Fentanyl Citrate) as reported for sheep (Ahern et al., 2010) failed to achieve a concentration above 1.0 ng/ml and enough data points to accurately analyse pharmacokinetics. Thus, this study was conducted in a new cohort of calves with twice the pilot dosage.

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 1,500 *g* for 10 min. The plasma was pipetted off and transferred to cryovials which were then stored at −80°C until analysis.

2.3 | 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 200 μl aliquots was transferred into a vial with 800 μl of internal standard, fentanyl-D5, in acetonitrile with 0.1% formic acid added. Samples were vortexed and then centrifuged at 4402 *g* for 20 min. The supernatant was then transferred and the samples were dried down, then reconstituted in 125 μl of 25% acetonitrile in water, vortexed and transferred into an autosampler vial (with glass insert) and then centrifuged for 20 min at 768 *g* and analysed 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.

2.4 | Pharmacokinetic analysis

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

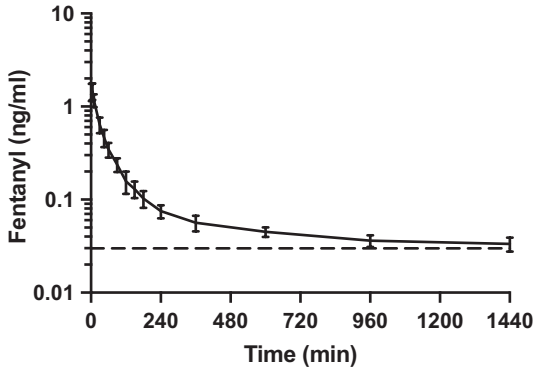

FIGURE 1 Individual fentanyl pharmacokinetic time-course (log10, mean ± 1*SD*) following intravenous bolus dosing at 5.0 μg/kg

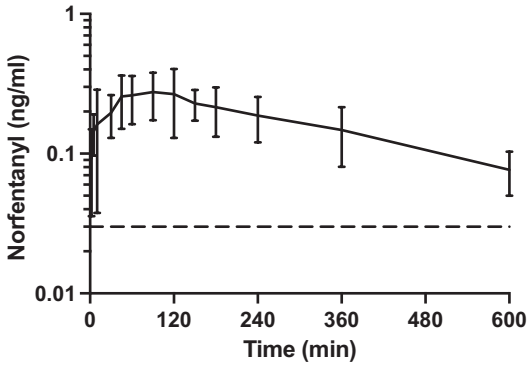

FIGURE 2 Individual norfentanyl pharmacokinetic time-course (log10, mean ± 1*SD*) following intravenous bolus dosing of fentanyl at 5.0 μg/kg

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 $\lambda_{z,}$ computed by linear regression of the logarithmic concentration vs. time curve during the elimination phase;
- (Nor)fentanyl terminal half-life, $T_{1/2}$ (λ_z) = ln (2)/λ_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 × 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 and Bousquet-Melou (2004), with:

$$E_{Body} = \text{Systemic clearance}/\text{Cardiac output} \quad (1)$$

First calculated for each individual calf, and then combined for a mean value. With the calf cardiac output calculated according to Toutain and Bousquet-Melou (2004) as follows:

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

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 et al., 2017), was applied and the pharmacokinetic analysis for fentanyl only was repeated using the same workflow as described above.

3 | RESULTS

3.1 | Animal health

At enrolment, 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 behaviour or physical assessment.

3.2 | 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 Table 1 for fentanyl and norfentanyl. Among individuals, there appears to be limited variation of time vs. concentration data for fentanyl as opposed to norfentanyl. For the LLOQ of 0.03 ng/ml 4.2% (5/120) of the postadministration, data points had values below the LLOQ. For the theoretical LLOQ of 0.05 ng/ml 21.7% (26/120) of the postadministration, data points had values below the LLOQ.

Table 1 summarizes the pharmacokinetic parameters for fentanyl and norfentanyl when administered i.v. For fentanyl, the systemic clearance was almost 2 L kg^{-1} hr^{-1}. The average extraction ratio was

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^{-1} kg^{-1}	1999	2167	1505	2821
	$T_{1/2}$ (λ_z)	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
	C_{max}	ng/ml	0.3	0.3	0.2	0.5
	T_{max}	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
	$T_{1/2}$ (λ_z)	hr	3.6	3.2	2.9	5.4

The following parameters were calculated for i.v. administration: C_0, plasma concentration back extrapolated to time 0 using log-linear regression of the first two timepoints; C_{max}, maximum concentration; T_{max}, time of maximum concentration; AUC_{inf}, area under the curve extrapolated to infinity, using the linear trapezoidal method; $\%AUC_{extrap}$, per cent 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.

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 (V_{ss}) was 24.8 L/kg. The elimination half-life $T_{1/2}$ (λ_z) was estimated at approximately 12 hr.

The AUC% extrapolation of 7.2% for norfentanyl was less than that of fentanyl. C_{max} and T_{max} of norfentanyl were 0.3 ng/ml, and 1.1 hr, respectively. The elimination half-life $T_{1/2}$ λ_z was estimated at 12.7 hr.

3.3 | 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).

4 | 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 orthopaedic 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, Sear, & Keuhnel, 2005), dogs (Robinson 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

TABLE 2 Average (±*SD*) 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^{-1} kg^{-1}	2061 ± 491	3371 ± 813
$T_{1/2}$ (λ_z)	hr	14.9 ± 9.9	3.0 ± 0.9
λ_z	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

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 et al. (1999)	Sheep Ahern et al. (2010)	Alpacas Lovasz et al. (2017)
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
$T_{1/2}$ (λ_z)	hr	14.9	3.0	1.2	3.1	1.2
MRT	hr	15.3	2.7	0.80	–	1.3

species, although currently the threshold required for analgesia in calves is unknown.

Other studies have evaluated the pharmacokinetics of intravenously fentanyl in horses (Maxwell et al., 2003), sheep (Ahern et al., 2010; Christou, Oliver, Rawlinson, & Walsh, 2015), goats (Carroll et al., 1999) and alpacas (Lovasz et al., 2017). The mean maximum concentration of 1.5 μg/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 hr), goats (1.2 hr) and alpacas (1.2 hr) (Lovasz 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 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 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 (3 hr) despite a very low quantification limit (0.01 ng/ml); therefore, between-species differences in 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 dosage 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, Greenslade, & Wright, 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^{-1} kg^{-1}). 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):

$$\text{Elimination half- life} = (0.693 \times \text{Volume of Distribution}) / \textit{Clearance} \quad (3)$$

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 (Mochel et al., 2013, 2015). 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 et al., 2017). The estimated systemic clearance (2.1 L kg^{-1} hr^{-1}) was

consistent with other reported clearances in similar large animal species of sheep (3.6 L kg^{-1} hr^{-1}), goats (2.1 L kg^{-1} hr^{-1}) and alpacas (1.1 L kg^{-1} hr^{-1}) (Lovasz 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 et al., 1995). As reported by Toutain et al. (Toutain & Bousquet-Melou, 2004), 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 et al., 2011). Two other minor metabolites, despropionyl fentanyl and hydroxyfentanyl, are accomplished by amide hydrolysis and alkyl hydroxylation, respectively (Clavijo et al., 2011). The pharmacokinetics of norfentanyl is 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 hr vs. 12.7 hr for its parent. As a metabolite cannot be eliminated faster than it is being formed, the elimination half-life of norfentanyl can either be similar or be 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 hr 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, & Kaye, 2016). Further studies of norfentanyl are necessary to determine the clinical significance of this metabolite in cattle.

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 carried out to evaluate the suitability of these routes for bovine practice. Based on comparison to similar ruminant species, it appears that the pharmacokinetic parameters calculated with an LLOQ of 0.05 ng/ml may be more useful for calculating dosage regimens in calves. However, there are species differences in location and distribution of opioid receptors, and as such, differences in responses to opioids have been described (Livingston, 2010). Future studies should also focus on tissue residue depletion, so that withdrawal guidance could be generated for practitioners.

5 | 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.

6 | CONCLUSIONS

In conclusion, fentanyl citrate administered intravenously reaches systemic peak concentrations associated with analgesia in other veterinary species. An i.v. dose of 5.0 μg/kg i.v. appears to be safely tolerated in calves. 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 dosage regimen selection in clinical practice.

CONFLICTS OF INTERESTS

The authors have no conflicts of interests.

AUTHOR CONTRIBUTIONS

JSS was involved in study design and execution, sample collection and analysis, manuscript preparation and submission. JFC was involved in study design and manuscript preparation. IWGF was involved in study design, execution and manuscript preparation. DJB was involved in study design, method development, sample analysis and manuscript preparation. JPM was involved in study design, sample analysis and manuscript preparation. All authors have read and approved the final manuscript.

ORCID

J. S. Smith http://orcid.org/0000-0002-4288-2262
J. P. Mochel http://orcid.org/0000-0002-0997-3111

REFERENCES

Ahern, B. J., Soma, L. R., Rudy, J. A., Uboh, C. E., & Schaer, T. P. (2010). Pharmacokinetics of fentanyl administered transdermally and intravenously in sheep. *American Journal of Veterinary Research*, *71*(10), 1127–1132. https://doi.org/10.2460/ajvr.71.10.1127

Arndt, J. O., Mikat, M., & Parasher, C. (1984). Fentanyl's analgesic, respiratory, and cardiovascular actions in relation to dose and plasma concentration in unanesthetized dogs. *Anesthesiology*, *61*(4), 355–361. https://doi.org/10.1097/00000542-198410000-00001

Carroll, G. L., Hooper, R. N., Boothe, D. M., Hartsfield, S. M., & Randoll, L. A. (1999). Pharmacokinetics of fentanyl after intravenous and transdermal administration in goats. *American Journal of Veterinary Research*, *60*(8), 986–991.

Christou, C., Oliver, R. A., Rawlinson, J., & Walsh, W. R. (2015). Transdermal fentanyl and its use in ovine surgery. *Research in Veterinary Science*, *100*, 252–256. https://doi.org/10.1016/j.rvsc.2015.04.006

Clavijo, C. F., Thomas, J. J., Cromie, M., Schniedewind, B., Hoffman, K. L., Christians, U., & Galinkin, J. L. (2011). A low blood volume LC-MS/MS assay for the quantification of fentanyl and its major metabolites norfentanyl and despropionyl fentanyl in children. *Journal of Separation Science*, *34*(24), 3568–3577. https://doi.org/10.1002/jssc.201100422

Delaski, K. M., Gehring, R., Heffron, B. T., Negrusz, A., & Gamble, K. C. (2017). Plasma concentrations of fentanyl achieved with transdermal application in chickens. *Journal of Avian Medicine and Surgery*, *31*(1), 6–15. https://doi.org/10.1647/2015-153

Gauntlett, I. S., Fisher, D. M., Hertzka, R. E., Kuhls, E., Spellman, M. J., & Rudolph, C. (1988). Pharmacokinetics of fentanyl in neonatal humans and lambs: Effects of age. *Anesthesiology*, *69*(5), 683–687. https://doi.org/10.1097/00000542-198811000-00008

Greenblatt, D. J. (1985). Elimination half-life of drugs: Value and limitations. *Annual Review of Medicine*, *36*(1), 421–427. https://doi.org/10.1146/annurev.me.36.020185.002225

Hing, J. P., Woolfrey, S. G., Greenslade, D., & Wright, P. M. (2001). Analysis of toxicokinetic data using NONMEM: Impact of quantification limit and replacement strategies for censored data. *Journal of Pharmacokinetics and Pharmacodynamics*, *28*(5), 465–479. https://doi.org/10.1023/A:1012247131190

Kamerling, S. G., DeQuick, D. J., Weckman, T. J., & Tobin, T. (1985). Dose-related effects of fentanyl on autonomic and behavioral responses in performance horses. *General Pharmacology*, *16*(3), 253–258. https://doi.org/10.1016/0306-3623(85)90078-3

Koch, D. E., Isaza, R., Carpenter, J. W., & Hunter, R. P. (2004). Simultaneous extraction and quantitation of fentanyl and norfentanyl from primate plasma with LC/MS detection. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(3), 577–584. https://doi.org/10.1016/S0731-7085(03)00652-6

Kuhls, E., Gauntlett, I. S., Lau, M., Brown, R., Rudolph, C. D., Teitel, D. F., & Fisher, D. M. (1995). Effect of increased intra-abdominal pressure on hepatic extraction and clearance of fentanyl in neonatal lambs. *Journal of Pharmacology and Experimental Therapeutics*, *274*(1), 115–119.

Lin, S. N., Wang, T. P., Caprioli, R. M., & Mo, B. P. (1981). Determination of plasma fentanyl by GC-mass spectrometry and pharmacokinetic analysis. *Journal of Pharmaceutical Sciences*, *70*(11), 1276–1279. https://doi.org/10.1002/jps.2600701124

Livingston, A. (2010). *Pain and analgesia in domestic animals*. In F. Cunningham, J. Elliott, & P. Lees (Eds.), *Pain and analgesia in domestic animals* (pp. 159–189). Heidelberg, Berlin: Springer Berlin Heidelberg.

Lovasz, M., Aarnes, T. K., Hubbell, J. A., Bednarski, R. M., Lerche, P., & Lakritz, J. (2017). Pharmacokinetics of intravenous and transdermal fentanyl in alpacas. *Journal of Veterinary Pharmacology and Therapeutics*, *40*, 663–669. https://doi.org/10.1111/jvp.12403

Maxwell, L. K., Thomasy, S. M., Slovis, N., & Kollias-Baker, C. (2003). Pharmacokinetics of fentanyl following intravenous and transdermal administration in horses. *Equine Veterinary Journal*, *35*(5), 484–490.

Mochel, J. P., Fink, M., Peyrou, M., Soubret, A., Giraudel, J. M., & Danhof, M. (2015). Pharmacokinetic/Pharmacodynamic modeling of renin angiotensin aldosterone biomarkers following angiotensin converting enzyme (ACE) inhibition therapy with benazepril in dogs. *Pharmaceutical Research*, *32*, 1931–1946. https://doi.org/10.1007/s11095-014-1587-9

Mochel, J. P., Gabrielsson, J., Collard, W., Fink, M., Gehring, R., Laffont, C., … Riviere, J. (2013). Animal Health Modeling & Simulation Society: a new society promoting model-based approaches in veterinary pharmacology. *Journal of Veterinary Pharmacology and Therapeutics*. [Epub ahead of print]. https://doi.org/10.1111/jvp.12060.

Peng, P. W., & Sandler, A. N. (1999). A review of the use of fentanyl analgesia in the management of acute pain in adults. *Anesthesiology*, *90*(2), 576–599. https://doi.org/10.1097/00000542-199902000-00034

Robertson, S., Taylor, P., Sear, J., & Keuhnel, G. (2005). Relationship between plasma concentrations and analgesia after intravenous fentanyl and disposition after other routes of administration in cats. *Journal of veterinary pharmacology and therapeutics*, *28*(1), 87–93. https://doi.org/10.1111/j.1365-2885.2004.00628.x

Robinson, T. M., Kruse-Elliott, K. T., Markel, M. D., Pluhar, G. E., Massa, K., & Bjorling, D. E. (1999). A comparison of transdermal fentanyl versus epidural morphine for analgesia in dogs undergoing major orthopedic surgery. *Journal of the American Animal Hospital Association*, *35*(2), 95–100. https://doi.org/10.5326/15473317-35-2-95

Ruan, X., Chiravuri, S., & Kaye, A. D. (2016). Using postmortem blood and urine norfentanyl/fentanyl ratios in the investigation of fentanyl-related deaths. *Clinical Toxicology (Philadelphia, PA)*, *54*(9), 893. https://doi.org/10.1080/15563650.2016.1199030

Toutain, P. L., & Bousquet-Melou, A. (2004). Plasma clearance. *Journal of Veterinary Pharmacology and Therapeutics*, *27*(6), 415–425. https://doi.org/10.1111/j.1365-2885.2004.00605.x

How to cite this article: Smith JS, Coetzee JF, Fisher IWG, Borts DJ, Mochel JP. Pharmacokinetics of fentanyl citrate and norfentanyl in Holstein calves and effect of analytical performances on fentanyl parameter estimation. *J vet Pharmacol Therap*. 2018;00:1–7. https://doi.org/10.1111/jvp.12501