USAMRICD-TR-20-11

Subcutaneous Pharmacokinetics of Carfentanil in the African Green Monkey (Chlorocebus aethiops sabeus)

Jeffrey L. Langston
Mark C. Moffett
Jennifer R. Makar
Katherine N. Bullock
Bradley M. Burgan
M. Ross Pennington
Todd M. Myers

August 2020

US Army Medical Research Institute of Chemical Defense
8350 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

an element of the

US Army Medical Research and Development Command

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The views expressed in this technical report are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
31-07-2020	Technical	01 January 2018 – 31 December 2018

4. TITLE AND SUBTITLE

Subcutaneous Pharmacokinetics of Carfentanil in the African Green Monkey (Chlorocebus aethiops sabeus)

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Langston, JL, Moffett, MC, Makar, JR, Bullock, KN, Burgan, BM, Pennington, MR, Myers, TM

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
8350 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-TR-20-11

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Threat Reduction Agency
8725 John J. Kingman Road STOP 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

14. ABSTRACT
Carfentanil is an ultra-potent opioid with a clinical potency up to 100 times greater than that of fentanyl. Little scientific attention has been devoted to the in vivo kinetics of carfentanil in non-human primates. This study characterized the pharmacokinetics of carfentanil administered subcutaneously (1.15 µg/kg) to adult male African green monkeys. To attenuate carfentanil toxicity, an intravenous (71.02 µg/kg) bolus of naloxone was also administered. Blood samples were collected from 2.5 minutes to 24 hours following carfentanil injection, and plasma samples analyzed for carfentanil concentration. The resulting pharmacokinetic data were best described by a one-compartment model with first-order absorption and elimination. The time to peak concentration was 31 minutes and the absorption half-life was 11 minutes. The elimination half-life was 112 minutes. Apparent clearance was 5.5 L/hr/kg and the apparent volume of distribution was 7.9 L/kg. These pharmacokinetic data advance our understanding of carfentanil in a common non-human primate laboratory model, and complement our growing body of research results relating the behavioral and physiological toxicity of carfentanil and its treatment with competitive µ-opioid antagonists in this same primate model, with potential to advance our understanding of human treatment.

15. SUBJECT TERMS
Carfentanil, pharmacokinetics, opioids, non-human primates, toxicity, countermeasure development

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Todd M. Myers
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	14	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-8380

EXECUTIVE SUMMARY

- We evaluated the pharmacokinetic profile of subcutaneous carfentanil citrate in adult male African green monkeys at a dose of 1.15 µg/kg.
- Absorption was relatively fast, with time to maximum concentration (T_{max}) approximating 20 to 30 minutes.
- The half-life of elimination of carfentanil was estimated to be between 0.9 and 1.8 hours.

ABSTRACT

Carfentanil is an ultra-potent opioid with a clinical potency up to 100 times greater than that of fentanyl. Little scientific attention has been devoted to the *in vivo* kinetics of carfentanil in non-human primates. This study characterized the pharmacokinetics of carfentanil administered subcutaneously (1.15 μg/kg) to adult male African green monkeys. To attenuate carfentanil toxicity, an intravenous (71.02 μg/kg) bolus of naloxone was also administered. Blood samples were collected from 2.5 minutes to 24 hours following carfentanil injection, and plasma samples analyzed for carfentanil concentration. The resulting pharmacokinetic data were best described by a one-compartment model with first-order absorption and elimination. The time to peak concentration was 31 minutes and the absorption half-life was 11 minutes. The elimination half-life was 112 minutes. Apparent clearance was 5.5 L/hr/kg and the apparent volume of distribution was 7.9 L/kg. These pharmacokinetic data advance our understanding of carfentanil in a common non-human primate laboratory model, and complement our growing body of research results relating the behavioral and physiological toxicity of carfentanil and its treatment with competitive μ-opioid antagonists in this same primate model, with potential to advance our understanding of human treatment.

INTRODUCTION

Carfentanil is an opioid with a clinical potency approximately 10,000 times that of morphine and 100 times that of fentanyl. Carfentanil is a high potency μ-opioid agonist with limited activity at κ- and δ-opioid receptors (Maguire et al., 1992; Yeadon & Kitchen, 1988). Carfentanil pharmacology has received relatively little scientific attention. In particular, there are few reports on the pharmacokinetics of carfentanil in relevant laboratory species (Bergh, Bogen, Garibay, & Baumann, 2019; Cole et al., 2006; Minkowski, Epstein, Frost, & Gorelick, 2012; Mutlow, Isaza, Carpenter, Koch, & Hunter, 2004; Uddayasankar, Lee, Oleschuk, Eschun, & Ariano, 2018). In one study with humans (Minkowski et al., 2012), the elimination half-life of intravenous (IV) carfentanil was reported to be 40-50 minutes. A case report of a human overdose stated that the elimination half-life of carfentanil to be 5.7 hours (Uddayasankar et al., 2018). The pharmacokinetics of intramuscular (IM) carfentanil in the common eland (the African antelope *Taurotragus oryx*) indicated that absorption was rapid with a T_{max} of 13.8 minutes, but the elimination half-life, 7.7 hours, was protracted (Cole et al., 2006). Studies of carfentanil kinetics in laboratory primates appear to be completely lacking. This study represents a novel contribution in that regard.

The present study characterized the subcutaneous plasma pharmacokinetics of carfentanil citrate using adult male African green monkeys. Each animal completed the entire pharmacokinetic time course characterization (from 2.5 minutes to 24 hours after drug administration) for a single subcutaneous carfentanil dose (1.15 μg/kg body weight); this dose was previously reported under a separate study evaluating naloxone's behavioral safety and efficacy in different animals of the same species, sex, and age (Langston, Moffett, Makar, Burgan, & Myers, 2020). Thus, by advancing our understanding of carfentanil pharmacokinetics, the present study helps relate our previous and growing body of behavioral toxicity and therapeutic treatment data to the plasma kinetics of carfentanil, allowing us to better contextualize previously observed results regarding carfentanil intoxication and treatment outcomes. The carfentanil pharmacokinetics will also provide a stronger rationale for plasma-kinetic-based treatment strategies with medical countermeasures such as naloxone or nalmefene, which are believed to have very different elimination profiles and durations of action compared to each other and to carfentanil. Lastly, the subcutaneous pharmacokinetic results presented here may allow comparison to forthcoming data from other routes of carfentanil exposure (e.g., inhalation) in this and other species (e.g., ferret or marmoset).

METHOD

Subjects

Three experimentally experienced and trained adult male African green monkeys (*Chlorocebus aethiops sabeus*) (5.8-6.02 kg, mean 5.88 kg) of Caribbean origin were individually housed in stainless steel squeeze-back cages (with an effective area equal to ~ 61 cm W X 71 cm D X 86 cm H). The colony was maintained at 21 ± 2 °C with a relative humidity of 50% ± 15% on

a 12 h light/dark cycle (lights on at 0600). Daily allotted food (Certified Primate Diet 5049, Purina Mills, Inc., St. Louis, MO, and fresh fruit and vegetables) was controlled to maintain healthy body weights, and water was available *ad libitum*. On drug administration and training days, the food ration was provided approximately 20 minutes after the 320-minute blood sample was collected. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The USAMRICD is a research facility fully accredited by the AAALAC, International.

Materials

Naloxone HCl dihydrate ((4R,4aS,7aR,12bS)-4a,9-dihydroxy-3-prop-2-enyl-2,4,5,6,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7-one hydrochloride dihydrate) was obtained from Sigma-Aldrich (St. Louis, MO; ≥98% purity; N7758). The naloxone salt was added to sterile physiological saline (0.9%) and passed through a 0.1 µm filter into a sterile vial. Stock solutions of naloxone (up to 40 mg/mL) were made on a weekly basis and kept at 4 °C. On the day of experimentation, an aliquot of naloxone was obtained from the stock solution and diluted to the desired concentration (1 mg/mL) to keep these intravenous naloxone injection volumes at or below 0.5 mL (the actual range was 0.41 to 0.43 mL). Carfentanil citrate (2-hydroxypropane-1,2,3-tricarboxylic acid; methyl 1-(2-phenylethyl)-4-(N-propanoylanilino)piperidine-4-carboxylate; approximately 98% purity), obtained from the U.S. Army Combat Capabilities Development Command Chemical Biological Center, was dissolved in sterile water. Stock solutions of carfentanil were kept at 4 °C. On the day of experimentation, the stock solution of carfentanil was aliquoted, and additional sterile water was added to achieve a final concentration of 22.5 µg/mL to keep injection volumes at approximately 0.3 mL. Drug doses were calculated as the weight of the salt forms listed above.

Subcutaneous Pharmacokinetic Studies

Naloxone (71.02 µg/kg; 2 mg human equivalent dose) was administered intravenously through a catheter placed into the saphenous vein. Following a bolus injection of naloxone, the catheter was flushed with approximately 1 mL sterile saline. Approximately 2 minutes following the naloxone injection, carfentanil (1.15 µg/kg) was administered into the subcutaneous space in the upper back or abdominal region using a standard 25-27 gauge 5/8" needle and a 1 mL syringe. Blood samples were obtained from the saphenous vein prior to naloxone administration and at prescribed post-administration time points of 2.5, 5, 10, 20, 40, 80, 160, 320, and 1440 minutes using a 22-25 gauge needle or catheter. Each sample was collected into a heparinized syringe, dispensed into a heparinized (15 IU/mL) micro-centrifuge tube, and centrifuged at 10K RPM for 5 minutes at 4 °C. The supernatant (plasma) was carefully removed via transfer pipette and dispensed into a separate blank micro-centrifuge tube and flash frozen in a bath of dry ice and ethanol.

Analytical Procedures

A liquid chromatography-tandem mass spectrometry (LC-MS-MS) assay was developed for the analysis of carfentanil-exposed African green monkey plasma samples. The developed assay was validated using blank, heparinized African green monkey plasma (BioIVT, Chestertown, MD, USA) to prepare calibration curves and quality control samples. Plasma was spiked at 100 ng/mL with standardized carfentanil (100 µg/mL carfentanil oxalate, Cerilliant, Round Rock, TX, USA) and serially diluted with plasma to produce the following concentrations, which served as calibrators: 25, 6.25, 1.56, 0.391, 0.098, 0.024 ng/mL. Isotopically labeled carfentanil (100 µg/mL carfentanil-D_5 oxalate, Cerilliant, Round Rock, TX, USA) was spiked into each sample to produce a final concentration of 2 ng/mL in each sample. The assay was validated according to the FDA guidelines regarding bioanalytical method development (FDA, 2018). Calibration curves were generated in duplicate and analyzed in triplicate, and a total of 6 sets of calibration curves were prepared over non-consecutive days (five inter-day and 1 intra-day). Quality control (QC) samples were prepared at 20, 2 and 0.2 ng/mL. QC samples were used to determine intra- and inter-day variability. Quantification of the QC samples was accomplished by running a calibration curve on each day. A linear least squares analysis with a 1/y weighting scheme was used to calculate the calibration parameters. The precision (%CV) was calculated using the formula %CV = (SD/mean) x 100%, and the accuracy (%error) was calculated using the formula % error = ((calculated concentration – actual concentration)/actual concentration) x 100%. Precision and accuracy were below 15% for all validation samples and QCs.

Prior to processing, animal samples were stored at -80 °C. Samples were thawed and 200 µL was transferred to clean microcentrifuge tubes. Isotopically labeled carfentanil (100 µg/mL carfentanil-D_5 oxalate, Cerilliant, Round Rock, TX, USA) was spiked into each sample to produce a final concentration of 2 ng/mL in each sample. A calibration curve was prepared each day that samples were processed. All calibrators, QCs and samples were extracted by solid-phase extraction (SPE) using Oasis 1 cc MCX cartridges with 30 mg sorbent (Waters Corporation, Milford, MA). The SPE procedure was as follows: 1) wash with 2 mL methanol containing 1% formic acid; 2) wash with 2 mL water containing 1% formic acid; 3) load 100 µL sample; 4) wash with 2 mL water containing 1% formic acid; 5) wash with 2 mL methanol containing 1% formic acid; and 6) elute with 2 mL 20%/78% isopropanol/methylene chloride with 2% ammonium hydroxide. The eluent for all calibrators, QCs, and samples was evaporated under a dry nitrogen stream at 40 °C. Samples were reconstituted in 90 µL of 30% methanol in 0.1% formic acid in water. Extraction was performed in duplicate, and the replicates were analyzed via LC-MS/MS in triplicate.

Liquid chromatography was performed using an Agilent 1290 Infinity liquid chromatograph (Agilent Technologies, Santa Clara, CA). Separation was performed on a Halo C18 column (2.7 µm, 2.1 mm x 50 mm) (Advanced Materials Technology, Wilmington, DE) with a chromatographic ramp with mobile phase B = 0.2% formic acid in methanol and mobile phase A = 0.2% formic acid, consisting of the following schedule: 0 min → 3min (10% mobile phase A →

95% mobile phase A), 3 min → 4 min (95% mobile phase A), 4.0 min → 4.1 min (95% mobile phase A → 10% mobile phase A), 4.1 →7 minutes (10% mobile phase A). The flow rate was 500 µL/min, and an injection volume of 5 µL was used. A retention time of 2.2 min was observed.

Tandem mass spectrometry was accomplished using a Sciex 6500 QTrap triple quadrupole mass spectrometer (Sciex, Ottawa, CA). It was operated in electrospray mode using multiple reaction monitoring (MRM). The ion source temperature was 700 °C. Capillary voltage was +5500V, curtain gas was 30 and the collision assisted dissociation gas was medium. Ion source gas 1 and 2 were 50 and 70. Declustering potential was 50V and entrance potential was 10V. For carfentanil, the quantifier ion transition was 395.2 Da to 246.1 Da with collision energy of 27eV and collision exit potential of 11V, while the qualifier ion transition was 395.2 Da to 335.1 Da with collision energy of 15eV and collision exit potential of 8V. For carfentanil-D_5, the quantifier ion transition was 400.2 Da to 284.0 Da with collision energy of 26eV and collision exit potential of 12V, while the qualifier ion transition was 400.2 Da to 340.2 Da with collision energy of 15eV and collision exit potential of 9V. Peak areas were integrated using Analyst software (Sciex, Ottawa, Ontario).

Data Analysis

Plasma concentration-time data for subcutaneous carfentanil were fit using nonlinear least squares regression and adequately described by a one-compartment model with first-order absorption and elimination (Gibaldi & Perier, 1982). The differential equations governing the PK model are

$$\frac{dX_a}{dt} = -k_a X_a \quad (1)$$

$$\frac{dX_c}{dt} = k_a X_a - K X_c \quad (2)$$

where k_a is the absorption rate and K is the elimination rate. Additional pharmacokinetic parameters (e.g., $t_{½}$ k_a, $t_{½}$ ke, C_{max}, t_{max}, etc.) were estimated according to the methods of Gibaldi and Perrier (1982). Individual plasma concentration-time data were fit using the PKfit (v.1.3.8) package for R (v.3.2.5; R Core Team, 2013; Vienna, Austria). Non-compartmental analyses were conducted using the PKNCA (v.0.8.1) package for R (Denney, Duvvuri, & Buckeridge, 2015).

RESULTS

The pharmacokinetic data were best described by a one-compartment model with first-order absorption and elimination after incorporating a lag time component for two animals that showed no measurable carfentanil plasma concentration until 5 minutes following administration (see Appendix A). The mean plasma concentrations across the first 5 hours and 20 minutes of sampling for carfentanil (1.15 µg/kg) are presented in Figure 1.

Pharmacokinetic estimates were determined for each animal, and the group means are shown in Table 1. Carfentanil absorption was relatively rapid, with a maximal plasma concentration (C_{max}) of 0.11 ng/mL occurring at approximately 30 minutes (T_{max}). Absorption half-life was approximately 11 minutes, and the half-life of elimination was 112 minutes. Apparent clearance was 5.5 L/h/kg and apparent volume of distribution was 7.9 L/kg.

In addition to the one-compartmental analysis of the pharmacokinetic data, a secondary *non-compartmental* analysis was conducted. The non-compartmental analysis estimates are also shown in Table 1. The observed T_{max} averaged approximately 20 minutes and the C_{max} was 0.14 ng/mL. According to the non-compartmental analysis, the half-life of elimination ($T_{1/2}$) was 55 minutes.

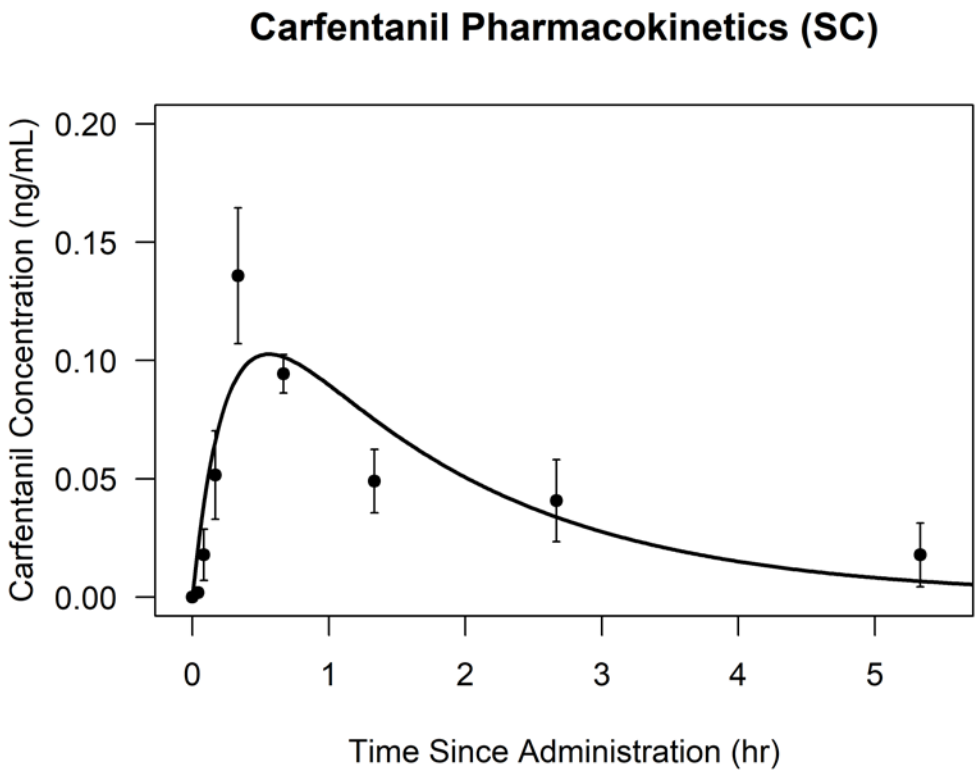

Figure 1. Pharmacokinetics of subcutaneous carfentanil in adult male African green monkeys. Points are mean plasma concentrations of n=3 animals and vertical bars are ± SEM. Solid line is the predicted plasma concentration across time, derived from the average parameter estimates.

Table 1. Mean (SD) of pharmacokinetic parameter estimates for carfentanil following subcutaneous administration to African green monkeys (n = 3).

Pharmacokinetic parameter	One-compartment analysis	Non-compartmental analysis
T_{max} (min)	30.92 (6.26)	19.8 (0.0)
$t_{½}$ ka (min)	10.92 (5.19)	n/a
C_{max} (ng/mL)	0.11 (0.03)	0.14 (0.05)
$t_{½}$ ke (min)	111.53 (127.13)	55.2 (16.92)
$AUC_{0\text{-}inf}$ (µg.h/L)	0.41 (0.29)	0.21 (0.14)
Cl/F (L/h/kg)	5.47 (4.19)	n/a
V/F (L/kg)	7.93 (5.12)	n/a

DISCUSSION

We evaluated the pharmacokinetic profile of subcutaneous carfentanil at a dose of 1.15 µg/kg in adult male African green monkeys. Absorption was relatively quick, with time to maximum concentration (T_{max}) approximating 20 to 30 minutes. Subcutaneous administration of carfentanil in this laboratory non-human primate model exhibited orderly and predictable plasma kinetics. The half-life of elimination of carfentanil in this non-human primate model was estimated to be between 55 and 112 minutes.

A previous report from this laboratory evaluated the behavioral safety and efficacy of naloxone against carfentanil intoxication in male African green monkeys (Langston et al., 2020). In that report, the effects of a 0.575 µg/kg dose of carfentanil in the absence of naloxone treatment were evaluated using an automated behavioral assay. Behavioral suppression was observed in under 13 minutes, and maximal behavioral effects of that dose (0.575 µg/kg) of carfentanil were evident approximately 28 minutes following subcutaneous administration, then sustained for the remainder of the 2-hour behavioral assessment. Furthermore, in the same report, the investigators conducted a separate experiment (Experiment 2) to estimate the ED_{50} dose of naloxone required for reversal of subcutaneously administered carfentanil intoxication using the same carfentanil dose (1.15 µg/kg) as in the current study. In that study, the average time to naloxone administration, which occurred at the onset of severe signs (bradypnea and/or loss of posture), was approximately 28 minutes following carfentanil. The high degree of concordance between these previously reported data describing the time course of behavioral intoxication produced by carfentanil and the plasma pharmacokinetics observed here lends credibility to the current estimates of the pharmacokinetics of carfentanil.

To our knowledge, this is the first report of carfentanil pharmacokinetics in a common non-human primate species. The elimination half-life of subcutaneous carfentanil in the present study was estimated to be between approximately 1 to 2 hours. There is one report examining the binding of [^{11}C]-carfentanil in human cocaine users and healthy controls that estimated the elimination half-life of intravenous carfentanil to be 40-50 minutes (Minkowski et al., 2012). In

contrast, a case report of a human overdose of carfentanil reported an elimination half-life of 5.7 hours (Uddayasankar et al., 2018). These apparent discrepancies may be partially explained by differences in the administered dose of carfentanil between these two reports as well as differences in the number of individuals the pharmacokinetic parameters were based on. In the case report, the carfentanil dose was unknown; in contrast, in the imaging study, the carfentanil dose (19-37 ng/kg) was substantially lower than the dose used in the present study, and the pharmacokinetics of carfentanil were determined using twenty-six individuals. The differences in the elimination half-life of carfentanil between these two reports in humans may also be partially explained by a recent report of carfentanil pharmacokinetics in rodents (Bergh et al., 2019). In that study, rats were subcutaneously administered carfentanil at doses of 1, 3, and 10 μg/kg, and the pharmacokinetics were determined for both carfentanil and its metabolite nor-carfentanil. The authors reported significant, dose-related differences in pharmacokinetic parameters for both analytes and reported evidence for non-linearity in the dose-response pharmacokinetics of carfentanil, such that, at the highest dose, the AUC and elimination half-lives were significantly greater than would be predicted under the assumption of linearity. One potential explanation for the reduced clearance of carfentanil following high doses was kidney dysfunction. Bergh et al. (2019) reported a delayed increase in plasma creatinine concentrations in rats administered 3 or 10 μg/kg carfentanil and speculated that impaired renal function could have contributed to the decreased drug clearance at these higher doses. In the Bergh et al. (2019) study, the elimination half-lives for 1, 3, and 10 μg/kg carfentanil were 35.4 ± 2.5, 55.1 ± 6.3, and 66.4 ± 8.4 minutes, respectively. In the present study, naloxone was administered prophylactically to minimize the toxic effects of carfentanil and, thus, may have attenuated or precluded any renal dysfunction that may have occurred in untreated animals at similar carfentanil doses.

One limitation of the present study was the lack of inclusion of the pharmacokinetics of the major carfentanil metabolite nor-carfentanil. However, to our knowledge, there have been no published reports on the biological activity of this metabolite. Feasel et al. (2016) speculated that nor-carfentanil would have little biological activity based on the structural similarities to fentanyl metabolites with low levels of activity. Furthermore, nor-carfentanil was reported to have low activity in an *in vitro* assay of μ-opioid receptor activation at high concentrations (1 μM/326 ng/mL) (Cannaert, Ambach, Blanckaert, & Stove, 2018). Future studies may endeavor to evaluate the biological activity and pharmacokinetics of nor-carfentanil in non-human primates.

In conclusion, subcutaneously administered carfentanil showed orderly and predictable plasma pharmacokinetics in male African green monkeys. These pharmacokinetic data advance our understanding of carfentanil in a common non-human primate laboratory model, and complement our growing body of research results relating the behavioral and physiological toxicity of carfentanil and its treatment with competitive μ-opioid antagonists in this same primate model, with potential to advance our understanding of human treatment.

REFERENCES

Bergh, M. S., Bogen, I. L., Garibay, N., & Baumann, M. H. (2019). Evidence for nonlinear accumulation of the ultrapotent fentanyl analog, carfentanil, after systemic administration to male rats. *Neuropharmacology, 158*, 107596. doi:10.1016/j.neuropharm.2019.04.002

Cannaert, A., Ambach, L., Blanckaert, P., & Stove, C. P. (2018). Activity-based detection and bioanalytical confirmation of a fatal carfentanil intoxication. *Front Pharmacol, 9*, 486. doi:10.3389/fphar.2018.00486

Cole, A., Mutlow, A., Isaza, R., Carpenter, J. W., Koch, D. E., Hunter, R. P., & Dresser, B. L. (2006). Pharmacokinetics and pharmacodynamics of carfentanil and naltrexone in female common eland (Taurotragus oryx). *J Zoo Wildl Med, 37*(3), 318-326. doi:10.1638/05-070.1

Denney, W. S., Duvvuri, S., & Buckeridge, C. (2015). Simple, automatic noncompartmental analysis: The PKNCA R package. *Journal of Pharmacokinetics and Pharmacodynamics, 42*(1S), 65.

FDA. (2018). *Guidance for Industry: Bioanalytical Method Validation*. Silver Spring, MD: U.S. Department of Health and Human Services.

Feasel, M. G., Wohlfarth, A., Nilles, J. M., Pang, S., Kristovich, R. L., & Huestis, M. A. (2016). Metabolism of carfentanil, an ultra-potent opioid, in human liver microsomes and human hepatocytes by high-resolution mass spectrometry. *AAPS J, 18*(6), 1489-1499. doi:10.1208/s12248-016-9963-5

Gibaldi, M., & Perier, D. (1982). *Pharmacokinetics* (2nd ed.). New York: Marcel Dekker.

Langston, J. L., Moffett, M. C., Makar, J. R., Burgan, B. M., & Myers, T. M. (2020). Carfentanil toxicity in the African green monkey: Therapeutic efficacy of naloxone. *Toxicol Lett, 325*, 34-42. doi:10.1016/j.toxlet.2020.02.008

Maguire, P., Tsai, N., Kamal, J., Cometta-Morini, C., Upton, C., & Loew, G. (1992). Pharmacological profiles of fentanyl analogs at mu, delta and kappa opiate receptors. *Eur J Pharmacol, 213*(2), 219-225. doi:10.1016/0014-2999(92)90685-w

Minkowski, C. P., Epstein, D., Frost, J. J., & Gorelick, D. A. (2012). Differential response to IV carfentanil in chronic cocaine users and healthy controls. *Addict Biol, 17*(1), 149-155. doi:10.1111/j.1369-1600.2010.00256.x

Mutlow, A., Isaza, R., Carpenter, J. W., Koch, D. E., & Hunter, R. P. (2004). Pharmacokinetics of carfentanil and naltrexone in domestic goats (Capra hircus). *J Zoo Wildl Med, 35*(4), 489-496. doi:10.1638/03-074

Uddayasankar, U., Lee, C., Oleschuk, C., Eschun, G., & Ariano, R. E. (2018). The pharmacokinetics and pharmacodynamics of carfentanil after recreational exposure: A case report. *Pharmacotherapy, 38*(6), e41-e45. doi:10.1002/phar.2117

Yeadon, M., & Kitchen, I. (1988). Comparative binding of mu and delta selective ligands in whole brain and pons/medulla homogenates from rat: affinity profiles of fentanyl derivatives. *Neuropharmacology, 27*(4), 345-348. doi:10.1016/0028-3908(88)90141-4

APPENDIX A

Table A-1. Carfentanil plasma concentrations (ng/mL) following SC administration of 1.15 µg/kg carfentanil citrate.

Time (h)	Monkey 1	Monkey 2	Monkey 3
0	0.0000	0.0000	0.0000
0.04	0.0000	0.0057	0.0000
0.08	0.0059	0.0394	0.0084
0.16	0.0150	0.0762	0.0636
0.33	0.1079	0.1933	0.1062
0.67	0.0843	0.1105	0.0883
1.33	0.0356	0.0758	0.0358
2.67	0.0599	0.0562	0.0062
5.33	0.0443	0.0092	0.0000
24	0.0000	0.0000	0.0000