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Intramuscular Pharmacokinetics of Naloxone in the Female African Green Monkey (Chlorocebus aethiops sabeus)

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14. ABSTRACT

- We evaluated the pharmacokinetics of intramuscular naloxone at a 10 mg human equivalent dose (HED) in adult female African green monkeys.
- Absorption was rapid, with time to maximum concentration (Tmax) approximating 8 minutes.
- The elimination half-life was short, approximating 37 minutes.
- Intramuscular bolus administration of naloxone in this laboratory non-human primate model exhibited orderly and predictable plasma kinetics that were comparable in both sexes.

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Naloxone; Pharmacokinetics; African Green Monkey (Chlorocebus aethiops sabeus); Intramuscular Injection; Human Equivalent Dose; Allometric Scaling; Female; Sex differences

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EXECUTIVE SUMMARY

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INTRODUCTION

Naloxone is a competitive opioid antagonist that is non-selective for opioid receptor subtypes. Naloxone is the standard, first-line antidote for the treatment of opioid intoxication and overdose. Despite this, pharmacokinetic data of intramuscular naloxone in animals and humans are limited. We recently reported on the pharmacokinetics of intramuscular naloxone in male African green monkeys (Langston, Makar, Bullock, Pennington, & Myers, 2019) across a range of doses. The present study was designed to extend our understanding and assess potential sex differences in the pharmacokinetics of intramuscular naloxone in a non-human primate model. Therefore, we evaluated the intramuscular bolus injection of naloxone in female African green monkeys and compared the pharmacokinetics to our previously established data from male African green monkeys.

The intramuscular plasma pharmacokinetics of naloxone hydrochloride were characterized through a study using conscious adult female African green monkeys. Each animal completed the pharmacokinetic time course characterization (from 2.5 minutes to 24 hours after drug administration) for one intramuscular naloxone dose (355.12 μg/kg) equivalent to the 10 mg human equivalent dose (HED; cf. FDA, 2005) and overlapping with the doses previously reported under separate studies evaluating naloxone's pharmacokinetics in male African green monkeys (Langston et al., 2019) and naloxone's behavioral safety and efficacy in different animals of the same species, sex, and age (Langston, Moffett, Makar, Burgan, & Myers, 2018). The present pharmacokinetic data have the potential to verify observations regarding the time course of naloxone's therapeutic effect and potential limitations therein, particularly in the context of intoxication/overdose from ultra-potent synthetic opioids such as carfentanil, while also addressing concerns regarding potential sex differences in naloxone pharmacokinetics and, thus, therapeutic response.

METHOD

Subjects

Four experimentally experienced and trained adult female African Green monkeys (*Chlorocebus aethiops sabeus*) (3.88-5.10 kg, mean 4.38 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 ºC with a relative humidity of 50% ± 15% on a 12 h light/dark cycle (lights on at 0600). Daily allotted food (Certified Primate Diet 5048, Purina Mills, Inc., St. Louis, MO, and fresh fruit and vegetables) was controlled to maintain healthy body weights, and water was available *ad libitum*. Animals were administered medroxyprogesterone acetate (150 mg) approximately every twelve weeks to control hormone fluctuations and better model human females under birth control. 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. Solutions of naloxone (up to 5 mg/mL) were made on the day of experimentation. Injection volumes were kept below 0.40 mL (range of 0.28 to 0.36 mL).

Procedure

Only one dose of naloxone (355.12 µg/kg) was evaluated in this study. This dose corresponds to a HED of 10 mg administered to a 70 kg human (~143 µg/kg) based on the Food and Drug Administration's body surface area scaling (FDA, 2005). Each naloxone injection was administered into the lateral thigh muscle using a standard 25 gauge 5/8" needle and a 1 mL syringe. Blood samples were taken from the saphenous vein 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 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.

An LC-MS/MS assay was developed and validated using blank, heparinized African green monkey plasma (BioIVT, Chestertown, MD) to prepare calibration curves and quality control samples. Plasma was spiked at 400 ng/mL with naloxone (Cerilliant, Round Rock, TX) and serially diluted from 100-0.0 ng/mL (calibrators). All calibrators were spiked with 5 ng/mL naloxone-d5 (Cerilliant, Round Rock, TX), which served as the internal standard for the study. 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 calibration curves were used (five inter-day and 1 intra-day). Quality control samples were prepared at 150, 15 and 1.5 ng/mL naloxone and spiked with 5 ng/mL naloxone-d5. QC samples were used to determine intra- and inter-day variability. Quantification of the QC samples was accomplished by running a calibration on each day. A linear least squares analysis with a 1/y weighting scheme was used to calculate the values for the calibration curve and QC samples. 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. For this study, the determined quantitative range was 100 ng/mL to 100 pg/mL. Our lower limit of detection (LLOD) was found

to be 30 pg/mL (3σ), and our lower limit of quantitation (LLOQ) was 98 pg/mL (10σ). Our upper limit of quantitation was 100 ng/mL.

Prior to processing, samples were stored at -80 °C. Samples were thawed, and 200 µL transferred to clean micro-centrifuge tubes. Plasma (20 µL) containing 55 ng/mL naloxone-d5 was added to each sample. Solid-phase extraction was performed using Oasis 1 cc HLB 96-well plates with 30 mg sorbent (Waters Corporation, Milford, MA) which were prepared for the samples by adding 1.0 mL of methanol followed by 1.0 mL water. The cartridges were loaded with 100 µL of animal samples, calibrators and QCs. All calibrators, QCs, and samples were extracted with 800 µL of methanol. The eluent was collected in 96-well plates and evaporated under a dry nitrogen stream at 40 °C. Samples were reconstituted in 90 µL of 10% methanol in 0.1% formic acid in water. Solid-phase 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 (2.7µm, 2.1mm x 50mm) (Advanced Materials Technology, Wilmington, DE) with a chromatographic ramp with mobile phase A = 100% methanol with 0.2% formic acid and mobile phase B = 100% water with 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→4.1 minutes (95% mobile phase A → 10% mobile phase B), 4.1→7 minutes (10% mobile phase A). The flow rate was 500 µL/min, and an injection volume of 5 µL was used.

Tandem mass spectrometry was completed using a Sciex 6500 QTrap triple quadpole mass spectrometer (Sciex, Ottawa, CA). It was operated in electrospray mode using multiple reaction monitoring (MRM). The ion source temperature was 600 °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. The quantifier ion transition was 328Da to 212.2Da with collision energy of 61eV, and collision exit potential of 22V was monitored. The qualifier ion transition was 328Da to 253Da with collision energy of 35eV, and collision exit potential of 30V was used. The deuterated naloxone ion transition was 333Da to 217.1Da with collision energy of 61eV and collision exit potential of 21V. Peak areas were integrated using Analyst software (Sciex, Ottawa, Ontario).

Plasma concentration-time data for intramuscular naloxone 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

All monkeys were monitored for any adverse reactions throughout the experiment and at each blood draw. Consistent with our previous naloxone work using an identical route and dose, at no time were any adverse reactions noted.

The pharmacokinetic data were best described by a one-compartment model with first-order absorption and elimination. The mean plasma concentrations across the first 5 hours and 20 minutes of sampling for all four animals following 355 µg/kg naloxone are presented in Figure 1. Given the rapid elimination observed, plasma concentration values from the 24-hour time sample are not shown.

Pharmacokinetic estimates were determined for each animal, and the group means are shown in Table 1 along with the pharmacokinetic estimates previously obtained from male monkeys using this same dose and administration route (Langston et al., 2019). Naloxone absorption was rapid, with measurable concentrations occurring 2.5 min following IM administration. For female animals, maximal plasma concentration (T_{max}) occurred at approximately 8 minutes for the 10 mg HED. For male animals, maximal plasma concentrations (T_{max}) appeared to be dose dependent and occurred at approximately 3, 4, and 13 minutes for the 2, 10, and 40 mg HEDs, respectively (Langston et al., 2019). As previously reported for males, C_{max} and $AUC_{0\text{-}inf}$ both increased linearly with dose ($R^2 > 0.99$ for both). Elimination half-life appeared to decrease with increasing doses of naloxone, but not greatly. Neither apparent volume of distribution (V/F) nor apparent clearance (Cl/F) demonstrated dose-related trends.

Figure 2 provides a visual comparison of the male and female pharmacokinetic results at the 10 mg HED. The male data are from Langston et al., 2019, and are characterized by slightly greater variability. Nevertheless, the pharmacokinteic curves are clearly similar, and any differences are small and subsumed within the standard error of the mean.

Although one dose of naloxone was evaluated for the females monkeys, results were largely as expected and not appreciably different from the derived parameters for the male monkeys. Thus, no apparent differences were observed in the pharmacokinetics of intramuscular naloxone between male and female African green monkeys.

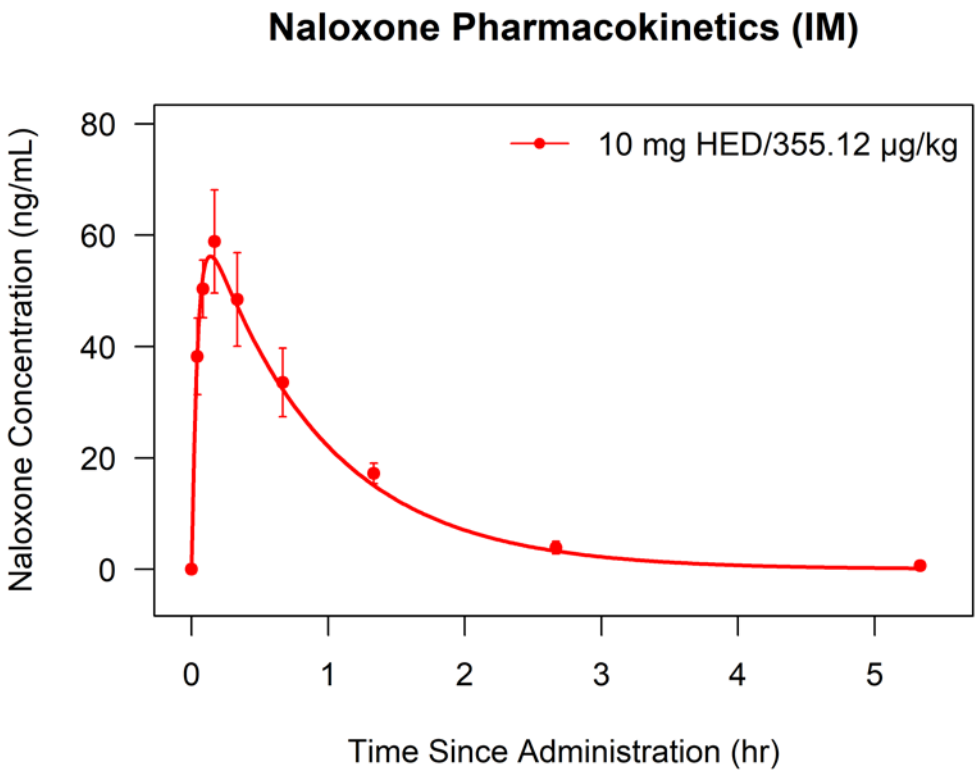

Figure 1. Pharmacokinetics of intramuscular naloxone in adult female African green monkeys. Points are mean plasma concentrations of n=4 animals. Solid line is the predicted plasma concentration derived from the average parameter estimates.

Table 1. Mean (SD) of pharmacokinetic parameter estimates for naloxone HCl following intramuscular administration to African green monkeys (n=4).

Parameter	355.12 µg/kg (10 mg HED) Male	355.12 µg/kg (10 mg HED) Female
T_{max} (min)	4.31 (5.50)	8.54 (3.07)
$t_{½}$ ka (min)	0.69 (1.45)	2.02 (1.11)
C_{max} (ng/mL)	60.94 (23.99)	57.50 (16.03)
$t_{½}$ ke (min)	50.26 (50.88)	37.06 (8.33)
$AUC_{0\text{-}inf}$ (µg.h/L)	71.77 (21.98)	73.14 (21.99)
Cl/F (L/h/kg)	4.54 (1.95)	6.63 (3.24)
V/F (L/kg)	5.49 (2.02)	5.71 (2.36)

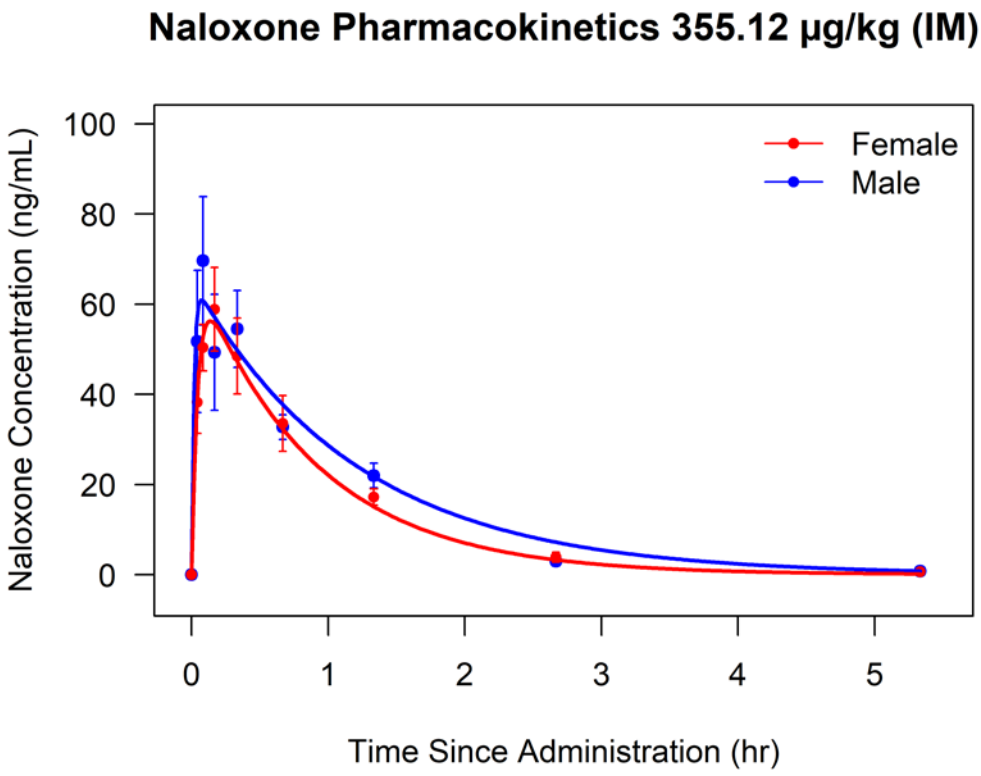

Figure 2. Pharmacokinetics of intramuscular naloxone (355.12 µg/kg) in male and female African green monkeys. Points are mean plasma concentrations of n=4 animals; vertical bars are ± SEM. Solid lines are the predicted plasma concentrations derived from the average parameter estimates. Male data from Langston et al., 2019.

DISCUSSION

We evaluated the pharmacokinetic profile of intramuscular naloxone at the 10 mg human equivalent dose (HED) in adult female African green monkeys. Absorption was rapid, with time to maximum concentration (T_{max}) approximating 8 minutes for 10 mg HED. Intramuscular administration of naloxone in this laboratory non-human primate model exhibited orderly and predictable plasma kinetics that appear largely comparable to available human data at a similar scaled dose (c.f. EVZIO package insert; Full Prescribing Information; Reference ID: 4001455). Compared with previously published data from this laboratory (Langston et al., 2019), there were no significant differences in the pharmacokinetic profile of 10 mg HED intramuscular naloxone in male and female monkeys.

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APPENDIX A

Table A-1. Naloxone plasma concentrations (ng/mL) following IM administration of 355.12 µg/kg naloxone HCl dihydrate.

Time (h)	Subject 1	Subject 2	Subject 3	Subject 4
0	0	0	0	0
0.04	26.67548	26.563	53.55002	46.13163
0.08	37.66607	61.64393	54.98001	47.10854
0.16	33.0644	72.29325	57.59004	72.48769
0.33	23.73784	61.52765	53.81487	54.76659
0.67	15.13088	41.0025	39.63211	38.41014
1.33	11.97056	17.53917	19.99734	19.35924
2.67	0.875096	6.100192	4.857	3.741342
5.33	0.541994	0.885768	0.489091	0.76458
24	0	0	0	0

APPENDIX B

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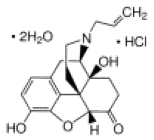
Product Specification

Product Name:
Naloxone hydrochloride dihydrate – ≥98% (TLC and titration), powder

Product Number:	**N7758**
CAS Number:	51481-60-8
MDL:	MFCD00150901
Formula:	C19H21NO4 · HCl · 2H2O
Formula Weight:	399.87 g/mol
Storage Temperature:	2 - 8 °C



TEST	Specification
Appearance (Color)	White to Off-White
Appearance (Form)	Powder
Solubility (Color)	Colorless to Yellow
Solubility (Turbidity) 50 mg/ml, H2O	Clear
Infrared spectrum	Conforms to Structure
Water (by Karl Fischer)	≤ 11 %
HCLO4 Titration (dry basis)	≥ 98 %
Purity (TLC)	≥ 98 %

Specification: PRD.0.ZQ5.10000061949