Received: 24 June 2022 | Accepted: 1 October 2022

DOI: 10.1002/prp2.1031

ORIGINAL ARTICLE

Alfaxalone population pharmacokinetics in the rat: Model application for pharmacokinetic and pharmacodynamic design in inbred and outbred strains and sexes

Kate White[1] | Mohammed Aldurdunji[1,2] | John Harris[3] | Catherine Ortori[4] | Stuart Paine[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK

[2]Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia

[3]School of Biosciences, University of Nottingham, Sutton Bonington, UK

[4]Centre for Analytical Bioscience, Advanced Materials and Healthcare Technologies Division, School of Pharmacy, University of Nottingham, Nottingham, UK

Correspondence
Kate White, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, LE12 5RD, UK.
Email: kate.white@nottingham.ac.uk

Abstract

The translation of new injectable anesthetic drugs from rodent to humans remains slow, despite the realization that reliance on the volatile agents is unsustainable from an environmental perspective. The aim of this study was to investigate the influence of rat sex and strain on the PK and PD of the anesthetic neurosteroid alfaxalone. Forty rats had cannulas inserted under isoflurane anesthesia for drug administration and sampling. Carotid artery blood samples were collected for blood gas analysis, hematology, biochemistry, and plasma concentrations of alfaxalone. Plasma samples were assayed using liquid chromatography-mass spectrometry. Compartmental non-linear mixed effects methods (NLME) models were applied to two rat populations to determine whether body weight, sex, and strain influenced PK parameters. There were significant differences between the sexes for plasma clearance, half-life and mean residence time in Lewis rats, and mean arterial blood pressure was significantly lower in the female rats at 120 min. An initial NLME PK population model was used to design an adjusted alfaxalone infusion for SD females matching plasma concentrations in males and minimizing cardiopulmonary depression but maintaining an appropriate hypnotic effect. A final NLME population model showed that alfaxalone clearance was dependent on both bodyweight and sex, whereas volume of distribution was influenced by strain. NLME PK models offer the advantage of having a single model that describes a population and therefore shares data interpretation between animals unlike the standard deterministic PK approach. This approach can be used to propose bespoke dosing regimens for optimal use of alfaxalone

KEYWORDS
alfaxalone, anesthesia, neurosteroid, pharmacodynamics, population pharmacokinetics, rodent

Abbreviations: CL, clearance; CV, cardiovascular; DNIC, diffuse noxious inhibitory controls; LCBW, log centralized bodyweight; LC–MS, liquid chromatography-mass spectometry; LLOQ, lower limit of quantification; MAP, mean arterial pressure; MRT, mean residence time; NLME, non-linear mixed effects methods; PD, pharmacodynamic; PK, pharmacokinetic; QC, quality controls; SD, Sprague–Dawley; STD, standard quantification; Vdss, steady-state volume of distribution.

1 | INTRODUCTION

Historically, it was recommended that animals of the same strain, sex, age, and weight should be used for biomedical investigations in order to reduce experimental variability.[1] Over 60 years later male animals still predominate in pre-clinical research; however, there is now extensive evidence of sex-specific pharmacology. Indeed, substantial evidence shows that the stage of estrous, and gonadal steroids can affect the response to drugs.[2–4] Translation of preclinical findings with novel molecular entities risks failure if the evidence is based on single-sex research. Sex differences in pharmacology can no longer be ignored and a new paradigm is percolating through biomedical research with major funding agencies now requiring consideration of sex in all applications, and sex to be considered as a categorical variable in studies submitted for publication.[5]

Alfaxalone is a neuroactive steroid that modulates neurotransmission through interaction with a steroid recognition site on the $GABA_A$ receptor complex resulting in inhibition of neuronal excitability. This agent and similar molecules, therefore, have roles in anesthesia, epilepsy, anxiety, insomnia, migraine, postpartum depression, and drug dependence.[6] Alfaxalone is used to induce and maintain anesthesia in a range of animal species and is used as an anesthetic induction agent in humans but anaphylactoid reactions attributed to the polyethoxylated castor oil (Cremophor EL) vehicle made its use redundant. Subsequent formulations of alfaxalone incorporating a cyclodextrin have been devoid of the side effects and Alfaxan® (alfaxalone dissolved in 2-hydroxypropyl-β-cyclodextrin) is now registered for induction and maintenance of anesthesia in dogs, cats, and rabbits. In biomedical research alfaxalone may offer some selective advantages over other anesthetic combinations in terms of a wide safety margin, reflex suppression, cardiopulmonary depression, interaction with pain pathways/modulation, and may also offer additional advantages in influencing CNS development and myelination.[7–9] To date, human trials of alfaxalone (formulated with 13% 7-sulfobutyl ether β cyclodextrin) have been undertaken in healthy male volunteers.[10]

Sex-related differences in anesthetic effects have been reported with old and new formulations of alfaxalone.[11,12] The cause of the sex difference was attributed to the presence of estrogenic hormones potentiating the anesthetic effects, while others have suggested that differences in allopregnanolone concentration may increase the clinical sensitivity of alfaxalone in female rats. However, recent findings suggest that the sex differences observed for alfaxalone are pharmacokinetic (PK) dependent with female Sprague–Dawley (SD) rats having a lower clearance than male SD rats.[13]

The aim of this study was to investigate the influence of sex and strain (SD vs. Lewis rats) on alfaxalone PK, anesthetic, and cardiovascular (CV) effects using standard deterministic and non-linear mixed effects methods (NLME) PK modeling. Furthermore, the resulting model was used to determine sex- and strain-specific dosing regimens for optimal anesthesia.

2 | METHODS

This study was performed in accordance with Project License PPL30/3156 issued under the Animal (Scientific) Procedures Act 2013 (EU Directive 2010/63/EU) and local ethics committee as part of a larger study investigating nociceptive withdrawal reflexes and diffuse noxious inhibitory controls. This study is reported in accordance with the ARRIVE 2.0 guidelines.[14]

2.1 | Study design

The NC3Rs experimental design assistant was used to design the study. The population PK model experiment was conducted in tandem with a larger study investigating nociceptive withdrawal reflexes (NWR) and the influence of anesthesia on diffuse noxious inhibitory controls (DNIC) in the rat. This in vivo experimental system in rats replicates aspects of the human pain pathway.[15–17] This study design facilitated the population PK model generating matched groups of male and female SD and Lewis rats undergoing nociceptive testing and DNIC studies. The motivation for undertaking the study was to interrogate the influence of the anesthesia per se, which is an essential prerequisite although oftentimes overlooked or ignored. This is particularly important in electrophysiology experiments and nociception paradigms conducted under anesthesia, where the anesthesia delivered can have a major influence on the reflexes being studied.

2.2 | Animals

Sixteen adult (8–12 weeks) Lewis rats, nine males (308 ± 49 g) and seven females (222 ± 9 g) and 24 adult (9–12 weeks) SD rats, eight males (422 ± 41 g) and 16 female (304 ± 15 g) (Charles River Laboratories, Margate, UK) were used.

Animals were housed in single-sex groups of 4, in double-layer ventilated cages, given access to food (Teklad 2018, Harlan) and tap water ad libitum and maintained on a 12-hour light/dark cycle. All cages had play tubes, bedding material, and chew blocks for enrichment. All experiments started at 10:00 h each day.

2.3 | General anesthesia

The methods for instrumentation of animals used were identical to those previously described by White et al.[13] Rats were anesthetized with isoflurane (3% for induction of anesthesia, 1–1.5% during surgery) in nitrous oxide/oxygen (2:1) mixture. Lidocaine 2% (Lignol, Dechra, Shrewsbury, UK) 3 mg/kg was infiltrated subcutaneously prior to skin incision. Using aseptic techniques, the left jugular vein was surgically cannulated using 0.63 mm O.D. polyethylene tubing (Fisher Scientific, Loughborough, UK) for the administration of drugs and isotonic fluids. The left carotid artery was surgically cannulated using 1 mm O.D.

polyethylene tubing (Fisher Scientific, Loughborough, UK) to monitor arterial blood pressure and for sampling.

2.4 | Monitoring anesthesia

The hypnotic characteristics of the anesthetic were evaluated by monitoring the paw withdrawal reflex in response to pinch, corneal reflex in response to light brushing, spontaneous blinking and gross purposeful movement, and cardiopulmonary parameters. Arterial blood pressure was monitored by an arterial pressure transducer (SensoNor 840; SensoNor, Horten, Norway) and recorded using a PC running Spike2 software (CED Ltd, Cambridge, UK). Heart rate was recorded via two 25 g needles inserted subcutaneously on the lateral sides of the thoracic wall. The ECG signal was amplified and used to trigger an instant rate meter (Neurolog NL253, Digitimer, Welwyn Garden City, UK) and again recorded using Spike2 software. Respiratory rate and effort were assessed by observing chest excursion and measuring end-tidal carbon dioxide (CapStar 100, Linton, Diss, UK). Intermittent-positive pressure ventilation (IPPV) was instigated (SAV04, Vetronic, Abbotskerwell, UK) in the face of hypoventilation to maintain normocapnia.

2.5 | Infusion of alfaxalone

Infusion regimens of alfaxalone (Alfaxan®, Jurox, Malvern, UK) were administered to rats using a calibrated syringe driver (SP100iz, WPI, Hitchin, UK). All animals received a loading dose (1.67 mg/kg for 2.5 min) followed by a constant rate infusion (CRI). For all animals, isoflurane and nitrous oxide were stopped 2.5 min after starting the alfaxalone infusion, but oxygen was supplied throughout the experiment. The male and female Lewis rats ($n = 16$) were administered a 60-min CRI (0.75 mg/kg/min) followed by a reduced CRI (0.52 mg/kg/min) for the remainder of the experiment. The Sprague–Dawley males ($n = 8$) received a 0.75 mg/kg min CRI throughout the experiment. The Sprague–Dawley females received a 60-min CRI (0.52 mg/kg/min) followed by a reduced dose (0.42 mg/kg/min) for the remainder of the experiment.

2.6 | Sampling

Arterial blood was withdrawn from the carotid cannula into lithium heparin tubes and placed on ice. Blood samples (200 µl) were collected at baseline (prior to alfaxalone administration) and at standardized time points across the alfaxalone infusion period. Arterial blood gases, biochemistry, and hematology parameters (pH, pCO_2, pO_2, bicarbonate, sodium, potassium, chloride, calcium, glucose, lactate, and creatinine concentrations) were also measured (EPOC, Woodley Instrumentation, Bolton, Lancashire, UK). All rats received an equal volume of balanced electrolyte solution after blood sampling (Vetivex 11 [Hartmann's], Dechra, Shrewsbury, UK). Samples were centrifuged (4000*g* for 10 min) within 30 min of collection. Plasma was harvested and stored at −20°C until determination of plasma alfaxalone concentration.

At the end of the experiments, animals were euthanized by intravenous injection of pentobarbitone (pentobarbital, Ayrton Saunders Ltd, Runcorn, UK) followed by cervical dislocation (by a trained individual as required by UK Home Office regulations).

2.7 | Sample analyses

Standard quantification (STD) curves for in vivo plasma samples were generated using authentic alfaxalone standard samples giving concentrations from 200 ng/ml to 40 µg/ml in addition to the use of quality controls (QC). Spiking solutions for standards and QCs were made from a separate accurate weighing of drug compounds. The methanol standard curve and QCs were prepared by spiking 10 µl of a known concentration spike solution into a solution of 40 µl methanol +100 µl methanol containing 3 µM of lansoprazole as internal standard +50 µl of either male or female blank plasma (Charles River, Margate, UK). Alfaxalone in vivo plasma samples were prepared by adding 50 µl of the plasma samples +50 µl methanol +100 µl methanol containing 3 µM of lansoprazole as internal standard.

Samples, standards, and QCs were then vortexed, stored in a freezer at −20°C overnight prior to centrifugation at 4000 g for 20 min at 4°C. The supernatant was then transferred into LC–MS vials for analysis and concentration determination. Finally, the STD curves were analyzed at the beginning and end of the run to determine any variation or deterioration of LC/MS performance. The analytical methods were validated to ensure suitable precision and accuracy, lower limit of quantification (LLOQ), linearity, calibration range, and selectivity. Moreover, all calibration curves had an r2 greater than 0.9 and coefficient of variation (CV %) was less than 20% for QCs.

The samples were analyzed using a Micromass Quattro Premier mass spectrometer incorporating an Agilent 1100 HPLC. An Ascentis® C18 column (2.1 × 50 mm, 3 µm) (Sigma, UK) protected by a Phenomenex C18 guard cartridge (Phenomenex, UK) was used with the following LC conditions: Solvent A = 10% methanol, 90% water, and 0.02% formic acid, Solvent B = 100% methanol and 0.02% formic acid, flow rate = 0.4 ml/min, column temperature = 60°C. LC gradient went from 70% solvent A:30% solvent B to 1% solvent A:99% solvent B over a 3-min interval. The MS/MS method used electrospray-positive mode with a 333.2 >315.2 and 297.2 transitions for the detection of alfaxalone. The lower limit of quantification (LLOQ) was 200 ng/ml. Two separate LC/MS/MS runs were performed for the male and female samples, respectively.

2.8 | Data and statistical analysis

2.8.1 | Pharmacokinetic analyses

Pharmacokinetic analyses were conducted using an IV infusion compartmental model for (a) individual Lewis rat data using dose per kg

and (b) Lewis and Sprague–Dawley population data using total dose. Both analyses used Phoenix® WinNonlin® version 8.3 software (Certara USA, Inc., Princeton, NJ). A two-stage approach was applied to (a) which firstly involved the estimation of clearance (CL), half-life ($T_{1/2}$), mean residence time (MRT), and steady-state volume of distribution (Vdss) for alfaxalone in each rat. Second, statistical tests were performed on pharmacokinetic parameters to determine any differences between male and female rats.

Compartmental non-linear mixed effects methods (NLME) models were applied to (b) using total dose given to each rat to determine whether body weight influenced PK parameters. Two populations were analyzed: population 1 (28 rats) comprised the Lewis rat data with Sprague–Dawley rat data described by White et al.[13] Population 2 (52 rats) comprised population 1 plus the additional Sprague–Dawley rat data (Figure 1). Model residual error was based on a mixed ratio error model. An exponential random effect model was chosen to describe inter-individual variability, that is, parameter = typical parameter x exp(eta). Categorical covariates were implemented for sex (male = 0, female = 1) and strain (Lewis = 0, SD = 1) on the model parameters in a multiplicative exponential way. A continuous covariate for log of centralized body weight (LCBW) was applied in a multiplicative way. The model analysis started from the basic compartmental models without the covariates. Next, the contribution of the covariates on fixed effects and correlation on random effects to the PK parameters were assessed by a reduction in the objective function using stepwise forward inclusion. Selection of the best model was based on the lowest value of the Akaike and Bayesian Information Criteria (AIC and BIC), chi-square p-value based on the likelihood ratio test, visual inspection of the population predicted concentration versus the observed concentrations, and the resulting conditional weighted residual errors. Finally, the best model was checked for robustness using a bootstrap resampling method. Monte Carlo simulations were used to determine a 95% confidence tolerance interval for the 5th and 95th percentile of the population.

2.9 | Statistical analyses

Statistical tests were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA) version 9. The male and female log-transformed Lewis rat pharmacokinetic parameters were compared using an unpaired, two-tailed Student's *t*-test ($\alpha = 0.05$), and a *p*-value of <.05 was considered significant. Data are reported as mean ± standard deviation (*SD*) unless stated otherwise. A one-way ANOVA was used to compare the biochemical, hematological, and blood gas data between groups. The male and female arterial blood pressure or plasma concentration data were compared at different time points using two-way ANOVA with post hoc Sidak multiple comparison test.

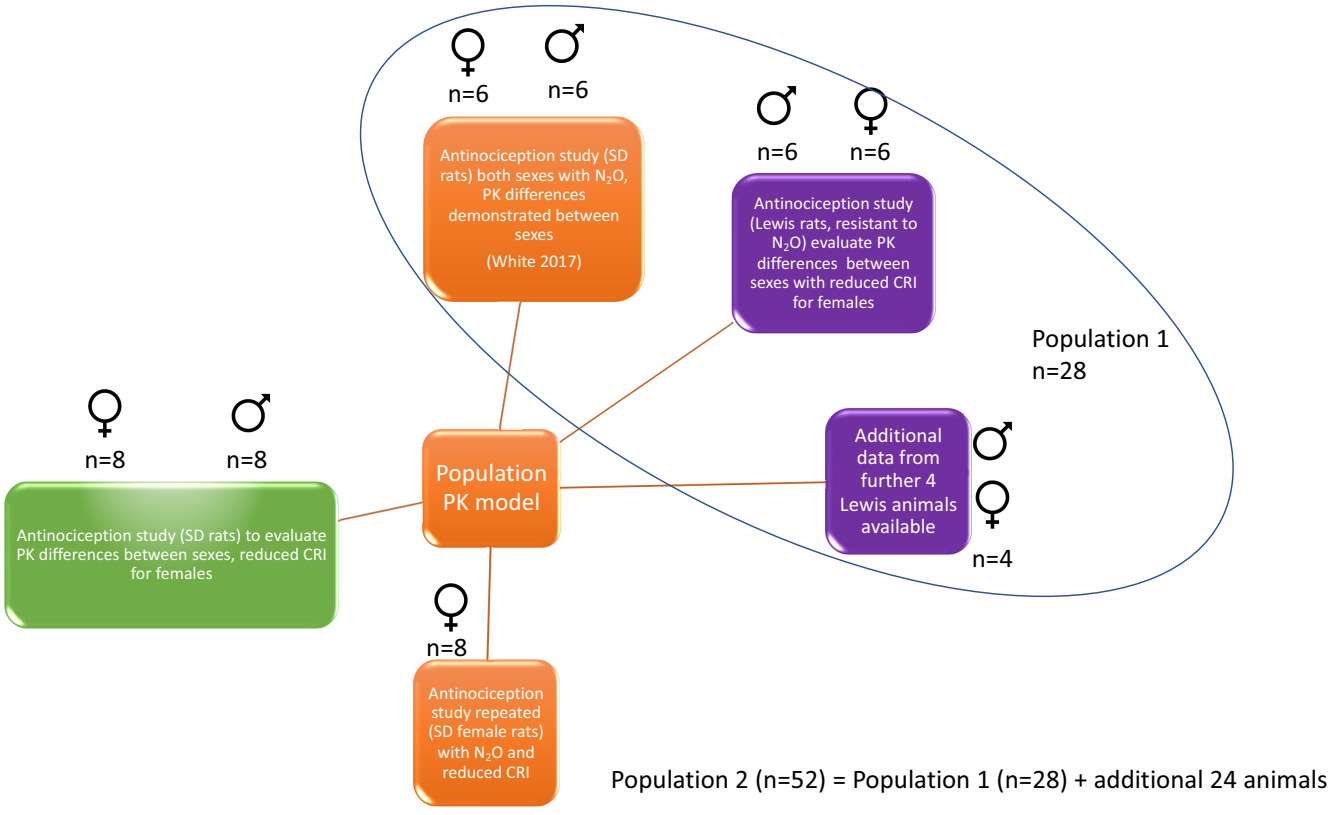

FIGURE 1 Infographic illustrating how the experimental cohorts contributed to the different populations.

3 | RESULTS

3.1 | Alfaxalone pharmacodynamics for Lewis rats

Arterial blood pressure measurements for the Lewis rats during anesthesia are presented in Figure 2. All rats showed an initial short-lived decrease in mean arterial blood pressure (MAP), heart rate, and respiratory rate because of concomitant administration of isoflurane and alfaxalone. Within 5 min following discontinuation of isoflurane, all rats demonstrated an increase in blood pressure from the baseline readings obtained during anesthesia with the gaseous volatile agent. Blood pressure (mean, systolic, diastolic), heart rate, and respiratory rate at baseline were not significantly different between male and female rats under isoflurane anesthesia. Heart rates remained stable during alfaxalone anesthesia and there was no significant difference between the sexes at any time points. Systolic, mean, and diastolic arterial pressures all increased from baseline under isoflurane anesthesia. The MAP remained elevated until 220 min for males before declining, while females showed early signs of a MAP decrease from between 40 and 75 min. Significant differences in MAP were detected between the sexes at 120 min ($p < .05$).

All Lewis rats required IPPV as judged by apnea, or a rise in end-tidal carbon dioxide coupled with a decrease in respiratory rate and effort at some point and for different time periods. Blood gas parameters and biochemistry values are presented in Table 1. There were no significant differences between sexes for all parameters.

3.2 | Hypnotic effect

The plane of anesthesia was continually evaluated by serial cardiopulmonary measurements, blood gas analysis, and reflex responses. Subjective evaluation of this hypnotic effect of the alfaxalone in all Lewis rats was excellent.

3.3 | Deterministic individual alfaxalone PK for Lewis rats

A one-compartment infusion model was shown to have the best fit to the individual Lewis rat PK data according to AIC. Figure 3 compares the male versus female Lewis rat PK data and model fit (curves) for each rat. Plasma concentrations of alfaxalone during maintenance CRI were greater in the females compared to male rats. PK parameters obtained from the model fit are shown in Table 2. Logarithmic transformed pharmacokinetic parameters were shown to be significantly different between the male and female rats. The mean CL of alfaxalone for male rats was more than twice that of female rats and with a higher Vdss for the latter, resulted in an almost 5-fold longer half-life in female rats compared to males.

3.4 | NLME PK model for alfaxalone (population 1)

The most parsimonious NLME model obtained for population 1 was a one-compartment model with random effects included on all parameters and no correlation (diagonal omega matrix). Goodness of model fit can be found in Figures S1–S7. The covariates for sex and rat strain had the most significant influence on clearance (CL) and rat strain having the most significant influence on volume of distribution (Vd). The CL and Vd for individual rats (expressed per total body weight) within the population model are described as follows:

$$CL = CL_{TV} * e^{(-0.841 * \text{sex covariate})} * e^{(0.478 * \text{strain covariate})} * e^{(\text{CL eta})}. \quad (1)$$

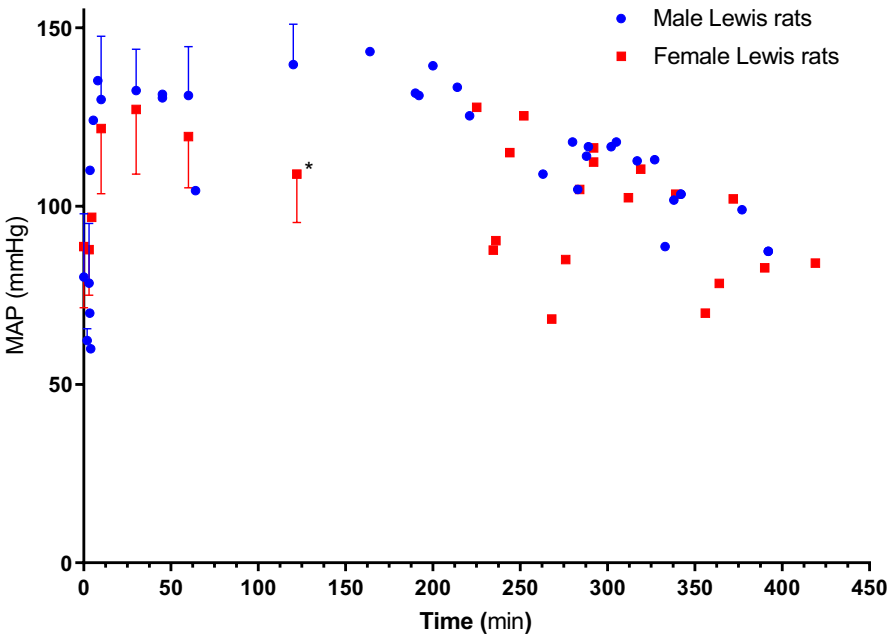

FIGURE 2 Mean arterial pressure (MAP) versus time; comparison between male (blue circles) and female (red squares) in Lewis rats. The difference was significant * at 120 min. Data are presented as mean ± *SD*; later time points are presented as single recorded points as they were measured at different times.

Strain, sex, number	Lewis male ($n = 9$)	Lewis female ($n = 7$)	SD male ($n = 8$)	SD female ($n = 16$)
pH	7.38 ±0.07	7.38 ±0.09	7.34 ±0.03	7.37 ±0.04
P_aCO_2 (mm Hg)	51 ±13	45 ±10	50 ±13	48 ±7
P_aO_2 (mm Hg)	428 ±103	350 ±71	495 ±140	405 ±168
HCO_3 (mmol/L)	29 ±3	26 ±4.4	27 ±7	28 ±3.3
Base Excess (mmol/L)	4.1 ±2.8	1.3 ±5.1	1.2 ±7	2.4 ±3.3
Sodium (mmol/L)	145 ±3	145 ±3.2	146 ±4	142 ±2.3
Potassium (mmol/L)	4 ±0.6	3.8 ±0.5	3.8 ±0.8	3.7 ±0.7
Ionized Calcium (mmol/L)	1.28 ±0.08	1.2 ±0.1	1.22 ±0.2	1.29 ±0.07
Chloride (mmol/L)	107 ±3	110 ±4.2	107 ±4	105 ±5
Anion Gap (mmol/L)	13 ±3	12 ±1.1	12 ±1.6	12 ±1.9
Hematocrit (%)	35 ±5	31 ±6	31 ±6	28 ±7
Hemoglobin (g/dl)	11.8 ±1.8	10.7 ±2	10.6 ±2	10.0 ±2.2
Glucose (mmol/L)	6.2 ±1.2	6.3 ±1.5	6.4 ±2	5.9 ±1.3
Lactate (mmol/L)	0.6 ±0.2	0.6 ±0.4	1.7 ±2.3	1.9 ±2.6
Creatinine (mmol/L)	31 ±13	27 ±11	43 ±14	35 ±11

Note: Data are mean ± *SD*.

Abbreviations: HCO_3, bicarbonate; P_aCO_2, partial arterial carbon dioxide pressure; P_aO_2, partial arterial oxygen pressure; SD, Sprague–Dawley.

TABLE 1 Blood gas and biochemistry values 90–120 min after commencing the alfaxalone infusion

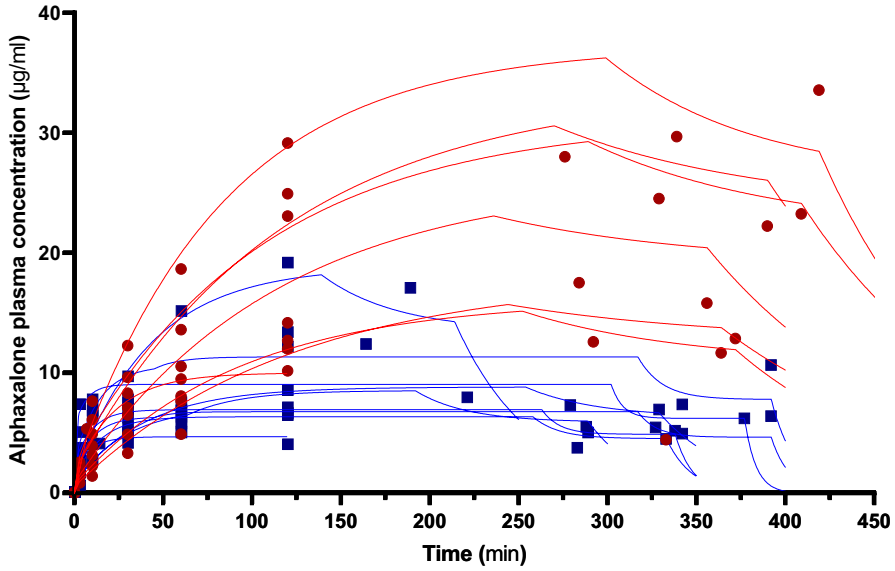

FIGURE 3 Alfaxalone plasma concentration–time curves; comparison between observed/simulated male (blue squares/lines) and female (red circles/lines) in Lewis rats for the same dosing regimen: 1.67 mg/kg/min for 2.5 min followed by 0.75 mg mg/kg/min until the end of the electrophysiological stage, then by 0.52 mg/kg/min.

TABLE 2 Pharmacokinetic parameters for 16 (9 male & 7 female) Lewis rats after intravenous administration of alfaxalone at a rate of 1.67 mg/kg/min for 2.5 min followed by 0.75 mg/kg/min for the maintenance stage, then by 0.52 mg/kg/min for the end of the experiment

Lewis rats	CL (ml/min/kg)	T½ (min)	MRT (min)	Vdss (L/kg)
Male	98.3 ±32.2	13.5 ±9.6	19.4 ±13.8	1.7 ±1.0
Female	36.8 ±19.7	64.7 ±23.3	93.3 ±33.6	3.0 ±1.3
p-value	.0003	.0001	.0001	.0286

$$Vd = Vd_{TV} * e^{(-0.0237 * \text{strain covariate})} * e^{(Vd\ eta)} \quad (2)$$

Where CL_{TV} and Vd_{TV} are the typical values (fixed effect) for Clearance (25.2 ml/min) and volume of distribution (0.57 L) within population 1. These fixed effects are adjusted by the covariates (0 for male and Lewis, 1 for female, and Sprague–Dawley) to give an adjusted typical value for each group. CL eta and Vd eta represent the random effects, such as inter-individual variability, in the population for clearance and volume of distribution. Sex and strain outputted adjusted typical values (per total bodyweight) and post hoc PK parameters (per kg bodyweight) for the most parsimonious NMLE model are shown in Table 3. These PK parameters were encompassed by the 2.5 and 97.5% confidence intervals of the bootstrap resampling analysis indicating a robust model. Mean arterial pressure plotted against plasma alfaxalone concentration for male Lewis and female Lewis rats is depicted in Figure 4A,B. However, MAP decreases for female Lewis rats (B) were evident when alfaxalone concentration exceeded approximately 20 µg/ml.

TABLE 3 Outputted adjusted typical values and post hoc primary PK parameters (normalized per kg bodyweight) for the most parsimonious model of population 1

	Adjusted typical values		Post hoc (mean ± SD)[a]	
Rat groups	Vd (L)	CL (ml/min)	Vd (L/Kg)	CL (ml/min/kg)
Male Lewis rat	0.57	25.2	1.90 ± 0.34	101 ± 28.1
Female Lewis rat	0.57	10.9	2.57 ± 0.10	40.8 ± 14.4
Male SD	0.56	40.6	1.49 ± 0.23	138 ± 114
Female SD	0.56	17.5	2.01 ± 0.11	63.5 ± 19.4

[a]Individual body weights used.

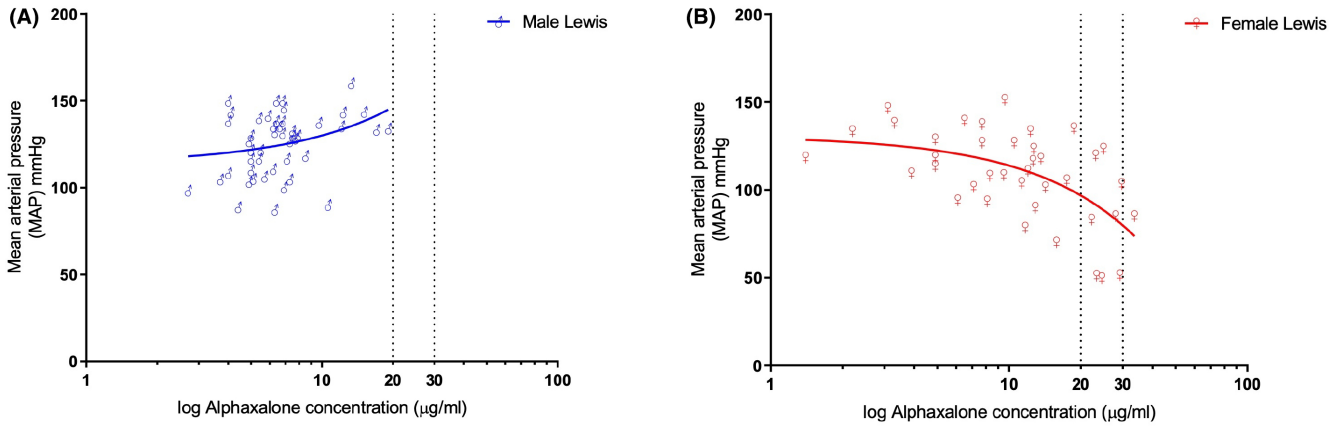

FIGURE 4 Mean arterial pressure (MAP) versus alfaxalone concentration; comparison between (A) male Lewis, (B) female Lewis rats.

3.5 | Adjusted alfaxalone infusion regimen for female SD rats

The NLME PK model for alfaxalone using population 1 was used to design an adjusted alfaxalone infusion regimen for female SD rats that matches male SD plasma concentrations minimizing cardiopulmonary depression but maintaining an appropriate hypnotic effect.

3.6 | Alfaxalone pharmacodynamics for SD male and females

There was no statistical difference between dose-adjusted female and male SD rats' mean arterial blood pressure (Figure 5).

IPPV was required for nine of 16 female SD and five of eight male SD rats. Blood gas parameters and biochemistry values are presented in Table 1. There were no significant differences between sexes or strains for all parameters.

3.7 | Hypnotic effect

The plane of anesthesia was deemed inadequate for injection of capsaicin as part of the antinociception study for two female SD rats during the final reduced phase of the CRI in view of a very faint sluggish paw withdrawal and spontaneous blinking. No rats demonstrated gross purposeful movement or required a change in the infusion rate to improve the plane of anesthesia however surgical anesthesia was not an outcome measure of the model.

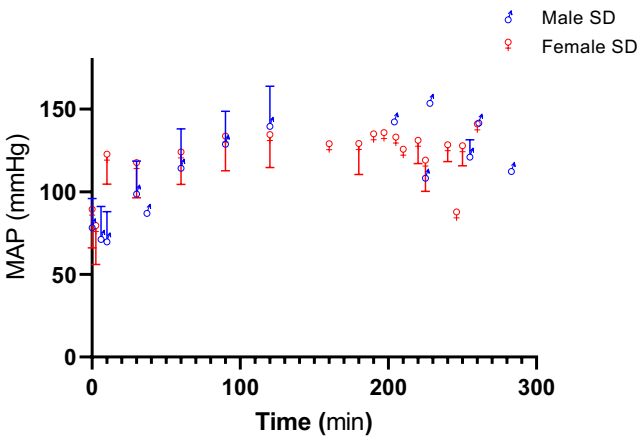

FIGURE 5 Mean arterial pressure (MAP) versus time; comparison between male (blue) and dose-adjusted female (red) SD rats

3.8 | Alfaxalone pharmacokinetics for SD males and females

Figure 6 shows the measured alfaxalone plasma concentrations versus time for male and female SD rats using the adjusted regimen for the latter along with the simulated median, upper and lower 95%

confidence intervals using the NMLE PK model. Plasma concentrations were similar with no statistical difference between male and female SD rats during the plateau phase.

Figure 7 shows no relationship between MAP and alfaxalone concentration for male SD (A) and dose-adjusted female SD (B) rats.

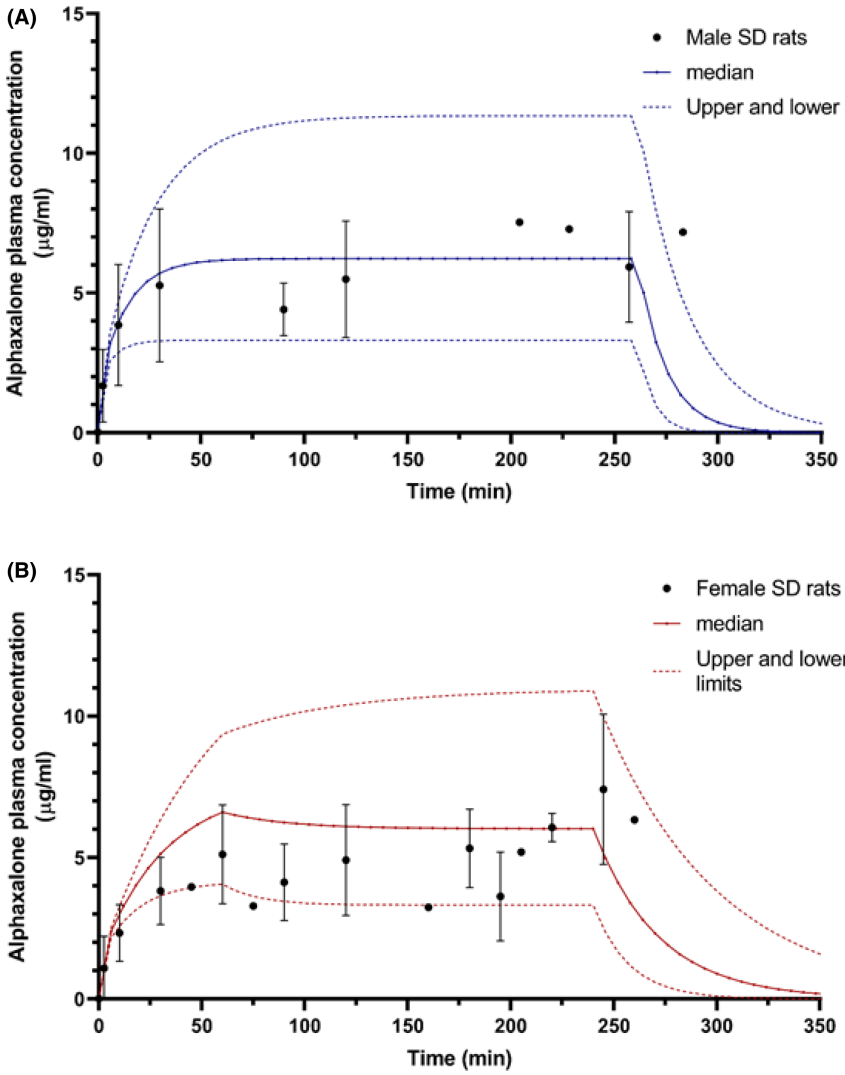

FIGURE 6 Measured (•) and simulated (lines) alfaxalone plasma concentrations with 5th and 95th percentile prediction (dashed lines) for male (A) and female (B) SD rats.

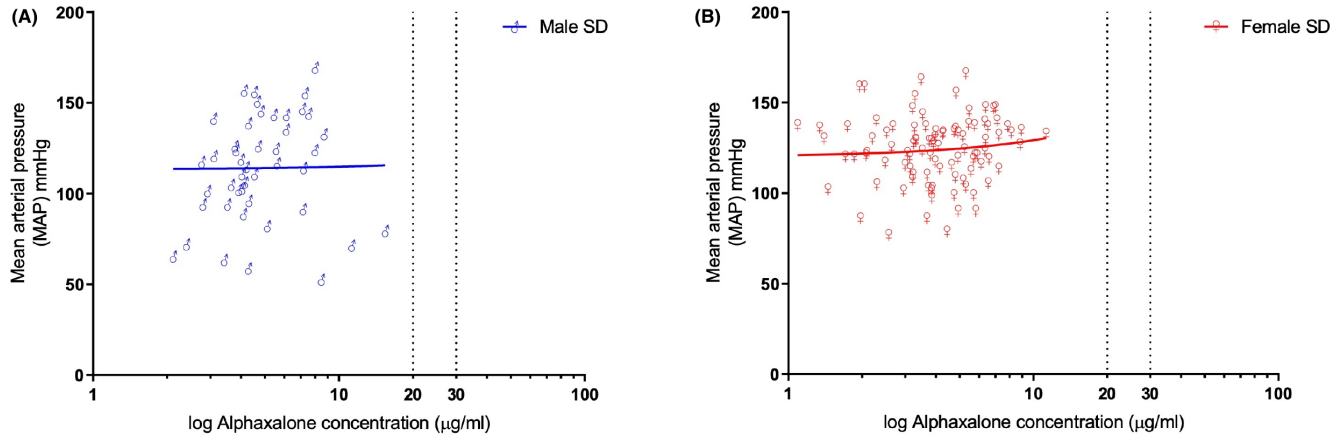

FIGURE 7 Mean arterial pressure (MAP) versus alfaxalone concentration; comparison between (A) male Lewis, (B) female Lewis, (C) male SD, and (D) dose-adjusted female SD rats.

3.9 | NLME pharmacokinetic model for alfaxalone (population 2)

The most parsimonious NLME model obtained for population 2 was again a 1-compartment model with random effects included on all parameters and no correlation (diagonal omega matrix). Goodness of model fit can be found in Figures S1–S7. The covariates for LCBW and sex had the most significant influence on alfaxalone clearance. Similar to the model for population 1, rat strain had the most significant influence on Vd. The CL and Vd for individual rats (expressed for total body weight) within the population model are described as follows:

$$CL = CL_{TV}{}^{*}\,(1 + LCBW * 3.64)^{*}\, e^{(-0.43 * \text{sex covariate})} * e^{(\text{CL eta})} \quad (3)$$

$$Vd = Vd_{TV}{}^{*}\, e^{(-0.692 * \text{strain covariate})} * e^{(\text{Vd eta})} \quad (4)$$

where CL_{TV} and Vd_{TV} are the typical values for Clearance (35.2 ml/min) and volume of distribution (0.51 L) within population 2. These fixed effects are adjusted by the covariates (0 for male and Lewis, 1 for female, and Sprague–Dawley and LCBW) to give an adjusted typical value for each group. Sex and strain outputted adjusted typical values and post hoc PK parameters (normalized per kg bodyweight) for the most parsimonious NMLE model are shown in Table 4. These PK parameters were encompassed by the 2.5 and 97.5% confidence intervals of the bootstrap resampling analysis indicating a robust model.

4 | DISCUSSION

This study demonstrated a sex difference in alfaxalone PK parameters using a two-stage deterministic approach for male and female Lewis rats. Sex differences were observed for both CL and Vd, where the former influences steady-state concentrations and the latter loading dose. As steady-state conditions are of the most interest only differences in CL will be discussed. Alfaxalone CL for female Lewis rats was significantly lower than for male animals. Separate studies[18,19] have shown alfaxalone clearance to be 158 ± 29 ml/min/kg and 54.3 ± 6.8 ml/min/kg for male and female Wistar rats, respectively, and[13] also identified a sex difference for alfaxalone CL in SD rats. The stepping down of the infusion rates in the Lewis rats was for purposes of creating dynamic change toward the end of the experiment, however, no significant change in concentration was apparent. For this reason, the stepping down was omitted for the SD animals and the reduced CRI is in fact the maintenance CRI designed to achieve maintenance levels faster to replicate the male model.

NLME PK models offer the advantage of having a single model that describes a population and therefore shares data interpretation between animals unlike the standard deterministic PK approach. Moreover, sub-populations such as the sex and strain can be described by co-variates that adjust the fixed effects (typical values). NLME models for populations 1 and 2 exemplify the sex difference in alfaxalone clearance where in both cases sex is a highly significant covariate. However, the deterministic estimated clearance parameter for Lewis rats for both sexes in the current study was in the lower range compared to the reported values by the Visser and Lau groups for Wistar rats as well as the reported values by White et al. for SD rats.[13] This is consistent with strain being a significant covariate on clearance in the population 1 NLME PK model.

However, the conclusion of the larger population 2 model suggests that LCBW is the more significant covariate source for clearance. This may be partly due to body weight having a wider range in population 2 compared to the narrower range in population 1 as the models were based on total dose. However, LCBW contains an allometric transformation for bodyweight and therefore Lewis rats, which have a lower bodyweight compared to age-matched SD animals, have an enhanced lower clearance compared to SD animals. This is reflected in Table 4 where the estimated post hoc clearance in SD female rats is significantly higher than female Lewis rats when normalized per kg bodyweight. However, the allometric relationship between clearance and bodyweight is an inverse one, which is unusual. Alternatively, as there is a correlation between weight and strain, it may be the case that strain is the underlying reason for the difference in population 1. It is also possible that population 2 is biased by the majority SD data, leading to LCBW being the most statistically significant co-variate.

The present study used arterial pressure measurements as a clinical biomarker for the PD investigation. Cardiovascular effects were chosen as a biomarker because alfaxalone exerts a dose-dependent depression of the cardiorespiratory system, that is, a decrease in blood pressure[20–22] but to a lesser extent compared to other anesthetics such as thiopental and propofol.[18,23] As such, a dose reduction (33%) of alfaxalone near the end of infusion for Lewis rats was designed to observe a dynamic change in the cardiovascular effect to determine an IC_{50}. However, there was no noticeable change in the cardiovascular response because of the

TABLE 4 Outputted adjusted typical values and post hoc primary PK parameters (normalized per kg bodyweight) for the most parsimonious model of population 2

	Adjusted Typical values[a]		Post hoc (mean ± *SD*)[b]	
Rat groups	**Vd (L)**	**CL (ml/min)**	**Vd (L/kg)**	**CL (ml/min/kg)**
Male Lewis rat	0.51	33.5	1.58 ± 0.78	103 ± 30.6
Female Lewis rat	0.51	10.1	3.03 ± 1.3	40.4 ± 17.4
Male Sprague–Dawley	0.26	48.6	2.18 ± 1.1	111 ± 34.9
Female Sprague–Dawley	0.26	20.6	3.84 ± 1.9	74.6 ± 20.4

[a]LCBW covariate applied to each group using average body weight for each group.

[b]Individual body weights used.

30% reduction phase. One explanation for the blood pressure discrepancies might be the female Lewis were most responsive, being impacted by the higher alfaxalone plasma concentrations and this was manifest as a relative hypotension compared to the male animals where a stable blood pressure was maintained during anesthesia. The population 1 NLME PK model was used to simulate a dosing regimen for female SD rats that would give a similar alfaxalone plasma profile to that of male SD, while simultaneously minimizing cardiopulmonary depression enhancing the profile particularly for prolonged anesthesia.

There are some limitations in the present study for the PD measurements. All the rats had a sampling cannula secured in the left carotid artery which required surgery and the use of gaseous anesthesia for instrumentation. Isoflurane can cause significant cardiovascular depression in a dose-dependent manner[24] as such, no arterial pressure baselines in conscious rats were available. Furthermore, after discontinuing the gases and transitioning to alfaxalone infusions, most rats' arterial pressure rebounded rapidly to a higher blood pressure. However, in view of the concurrent cessation of the volatile agent and a loading dose of alfaxalone infusion, it is not clear what the value of the real baseline is. Subsequent work evaluating the suitability of the alfaxalone model for surgical anesthesia and compatibility with other adjuncts and analgesics is advised to move away from reliance on the volatile agents, thereby minimizing their detrimental environmental impact.

AUTHOR CONTRIBUTIONS

KW, JH, SP, and MA participated in research design. KW and MA conducted experiments and KW, MA, and SP performed data analysis. All authors contributed to drafting the manuscript.

ACKNOWLEDGMENTS

M.A. received stipend and laboratory costs from the Saudi Arabian Government. The funding agency had no influence on the design, execution, or publishing of this work.

CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Kate White https://orcid.org/0000-0002-1439-0228
Stuart Paine https://orcid.org/0000-0001-9443-2311

REFERENCES

1. Lewis JJ. *An Introduction to Pharmacology*. Livingstone; 1960:49.
2. Pham TV, Sosunov EA, Gainullin RZ, Danilo P, Rosen MR. Impact of sex and gonadal steroids on prolongation of ventricular repolarization and arrhythmias induced by Ik-blocking drugs. *Circulation*. 2001;103(17):2207-2212. doi:10.1161/01.CIR.103.17.2207
3. Salama G, Bett GCL. Sex differences in the mechanisms underlying long QT syndrome. *Am J Physiol Hear Circ Physiol*. 2014;307(5):640-648. doi:10.1152/ajpheart.00864.2013
4. Nakagawa M, Ooie T, Takahashi N, et al. Influence of menstrual cycle on QT interval dynamics. *Pacing Clin Electrophysiol*. 2006;29:607-613. doi:10.1111/j.1540-8159.2006.00407.x
5. Docherty JR, Stanford SC, Panettieri RA, et al. Sex: a change in our guidelines to authors to ensure that this is no longer an ignored experimental variable. *Br J Pharmacol*. 2019;176(21):4081-4086. doi:10.1111/bph.14761
6. Belelli D, Hogenkamp D, Gee KW, Lambert JJ. Realising the therapeutic potential of neuroactive steroid modulators of the GABAA receptor. *Neurobiol Stress*. 2020;12:1-11. doi:10.1016/j.ynstr.2019.100207
7. Rupprecht R, Holsboer F. Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci*. 1999;22(9):410-416. doi:10.1016/S0166-2236(99)01399-5
8. Yawno T, Yan EB, Hirst JJ, Walker DW. Neuroactive steroids induce changes in fetal sheep behavior during normoxic and asphyxic states. *Stress*. 2011;14:13-22. doi:10.3109/10253890.2010.504789
9. Shaw JC, Crombie GK, Palliser HK, Hirst JJ. Impaired oligodendrocyte development following preterm birth: promoting GABAergic action to improve outcomes. *Front Pediatr*. 2021;9:618052. doi:10.3389/fped.2021.618052
10. Goodchild CS, Serrao JM, Sear JW, Anderson BJ. Pharmacokinetic and pharmacodynamic analysis of Alfaxalone administered as a bolus intravenous injection of Phaxan in a phase 1 randomized trial. *Anesth Analg*. 2020;130(3):704-714.
11. Fink G, Sarkar DK, Dow RC, et al. Sex difference in response to alphaxalone anaesthesia may be oestrogen dependent. *Nature*. 1982;298:270-272.
12. Arenillas M, de Segura IAG. Anaesthetic effects of alfaxalone administered intraperitoneally alone or combined with dexmedetomidine and fentanyl in the rat. *Lab Anim*. 2018;52(6):588-598.
13. White KL, Paine S, Harris J. A clinical evaluation of the pharmacokinetics and pharmacodynamics of intravenous alfaxalone in cyclodextrin in male and female rats following a loading dose and constant rate infusion. *Vet Anaesth Analg*. 2017;44:865-875. doi:10.1016/j.vaa.2017.01.001
14. du Sert NP, Hurst V, Ahluwalia A, et al. The arrive guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7):1-12. doi:10.1371/journal.pbio.3000410
15. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev*. 2001;53(4):597-652.
16. Le Bars D, Villanueva L, Bouhassira D, Willer JC. Diffuse noxious inhibitory controls (DNIC) in animals and in man. *Patol Fiziol i Eksp Ter*. 1992;4(4):55-65. doi:10.1016/0304-3959(90)92659-E
17. White K, Targett M, Harris J. Gainfully employing descending controls in acute and chronic pain management. *Vet J*. 2018;237:16-25. doi:10.1016/j.tvjl.2018.05.005
18. Visser SAG, Smulders CJGM, Reijers BPR, Van der Graaf PH, Peletier LA, Danhof M. Mechanism-based pharmacokinetic-pharmacodynamic modeling of concentration-dependent hysteresis and biphasic electroencephalogram effects of alphaxalone in rats. *J Pharmacol Exp Ther*. 2002;302(3):1158-1167. doi:10.1124/jpet.302.3.1158
19. Lau C, Ranasinghe MG, Shiels I, Keates H, Pasloske K, Bellingham M. Plasma pharmacokinetics of alfaxalone after a single intraperitoneal or intravenous injection of Alfaxan in rats. *J Vet Pharmacol Ther*. 2013;36:516-520. doi:10.1111/jvp.12055.SHORT

20. Khan KS, Hayes I, Buggy DJ. Pharmacology of anaesthetic agents II: inhalation anaesthetic agents. *Contin Educ Anaesthesia Crit Care Pain*. 2014;14(3):106-111.
21. Muir W, Lerche P, Wiese A, Nelson L, Pasloske K, Whittem T. The cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in cats. *Vet Anaesth Analg*. 2009;36(1):42-54. doi:10.1111/j.1467-2995.2008.00428.x
22. Sear JW. Steroid anesthetics: old compounds, new drugs. *J Clin Anesth*. 1996;8(3 Suppl):91S-98S. doi:10.1016/S0952-8180(96)90021-5
23. Goodchild CS, Serrao JM, Kolosov A, Boyd BJ. Alphaxalone reformulated: a water-soluble intravenous anesthetic preparation in Sulfobutyl-ether-β-cyclodextrin. *Anesth Analg*. 2015;120(5):1025-1031. doi:10.1213/ANE.0000000000000559
24. Hanley PJ, Loiselle DS. Mechanisms of force inhibition by halothane and isoflurane in intact rat cardiac muscle. *J Physiol*. 1998;506(1):231-244. doi:10.1111/j.1469-7793.1998.231bx.x

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: White K, Aldurdunji M, Harris J, Ortori C, Paine S. Alfaxalone population pharmacokinetics in the rat: Model application for pharmacokinetic and pharmacodynamic design in inbred and outbred strains and sexes. *Pharmacol Res Perspect*. 2022;10:e01031. doi:10.1002/prp2.1031