Alfaxalone population pharmacokinetics in the rat: model application for PK/PD design in inbred and outbred strains and sexes

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

[1]University of Nottingham School of Veterinary Medicine and Science
[2]Umm Al-Qura University College of Pharmacy
[3]University of Nottingham School of Biosciences
[4]University of Nottingham

May 24, 2022

Abstract

Background and purpose The translation of new injectable anaesthetic drugs from rodent to humans remains slow, despite the realisation 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 anaesthetic neurosteroid alfaxalone. Experimental approach Forty rats had cannulas inserted under isoflurane anaesthesia for drug administration and sampling. Carotid artery blood samples were collected for blood gas analysis, haematology, 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 Key Results 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 minutes. An initial NLME PK population model was used to design an adjusted alfaxalone infusion for SD females matching plasma concentrations in males and minimising 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. Conclusion and implications 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 alphaxalone.

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 (Lewis, 1960). Over sixty 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 oestrous, and gonadal steroids can affect the response to drugs (Pham et al., 2001; Nakagawa et al., 2006; Salama and Bett, 2014). 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 (Docherty et al., 2019).

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 anaesthesia, epilepsy, anxiety, insomnia, migraine, postpartum depression and drug dependence (Belelli et al., 2020). Alfaxalone is used to induce and maintain anaesthesia in a range of animal species and was used as an anaesthetic 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 anaesthesia in dogs, cats, and rabbits. In biomedical research alfaxalone may offer some selective advantages over other anaesthetic 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 (Rupprecht and Holsboer, 1999; Yawno et al., 2011; Shaw et al., 2021). To date human trials of alfaxalone (formulated with 13% 7-sulfobutyl ether β cyclodextrin) have been undertaken in healthy male volunteers (Goodchild et al., 2020).

Sex related differences in anaesthetic effects have been reported with old and new formulations of alfaxalone (Fink et al., 1982; Arenillas and Gomez de Segura, 2018). The cause of the sex difference was attributed to the presence of oestrogenic hormones potentiating the anaesthetic effects while others have suggested that differences of 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 (White et al., 2017).

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

Methods

This study was performed in accordance with Project Licence 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 (du Sert et al., 2020).

Study design

The NC3Rs experimental design assistant (EDA) 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 anaesthesia on diffuse noxious inhibitory controls (DNIC) in the rat. This *in vivo* experimental system in rats replicates aspects of the human pain pathway (Le Bars et al., 1992, 2001; White et al., 2018). 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 anaesthesia *per se* , which is essential prerequisite although oftentimes overlooked or ignored. This is particularly important in electrophysiology experiments and nociception paradigms conducted under anaesthesia, where the anaesthesia delivered can have a major influence on the reflexes being studied.

Animals

Sixteen adult (8-12 weeks) Lewis rats, 9 males (308 ± 49 g) and 7 females (222 ± 9 g) and 24 adult (9-12 weeks) SD rats, 8 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.

General anaesthesia

The methods for instrumentation of animals used were identical to those previously described by (White et al., 2017). Rats were anaesthetised with isoflurane (3% for induction of anaesthesia, 1-1.5% during surgery) in nitrous oxide/oxygen (2:1) mixture. Lidocaine 2% (Lignol, Dechra, Shrewsbury, UK) 3 mg kg^{-1} 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 administration of drugs and isotonic fluids. The left carotid artery was surgically cannulated using 1mm O.D. polyethylene tubing (Fisher Scientific, Loughborough, UK) to monitor arterial blood pressure and for sampling.

Monitoring anaesthesia

The hypnotic characteristics of the anaesthetic 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 25g 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.

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^{-1} for 2.5 minutes) followed by a constant rate infusion (CRI). For all animals isoflurane and nitrous oxide were stopped 2.5 minutes after starting the alfaxalone infusion, but oxygen was supplied throughout the experiment. The male and female Lewis rats (n=16) were administered a 60-minute CRI (0.75 mg $kg^{-1}min^{-1}$) followed by a reduced CRI (0.57 mg kg^{-1} min^{-1}) for the remainder of the experiment. The Sprague Dawley males (n=8) received a 0.75 mg kg^{-1} min^{-1} CRI throughout the experiment. The Sprague Dawley females received a 60-minute CRI (0.57 mg kg^{-1} min^{-1}) followed by a reduced dose (0.42 mg kg^{-1} min^{-1}) for the remainder of the experiment.

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 standardised time points across the alfaxalone infusion period. Arterial blood gases, biochemistry and haematology 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 (4000g for 10 minutes) within 30 minutes of collection. Plasma was harvested and stored at -20°C until determination of plasma alfaxalone concentration.

At the end of the experiments animals were euthanised 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).

Sample analyses

Standard quantification (STD) curves for in vivo plasma samples were generated using authentic alfaxalone standard samples giving concentrations from 200 ng ml^{-1} to 40 µg ml^{-1} in addition to the use of quality controls (QC). Spiking solutions for standards and QCs were made from 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 4000g for 20 minutes at 4 °C. The supernatant was then transferred into LC-MS vials for analysis and concentration determination. Finally, the STD curves were analysed 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.

The samples were analysed 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 minute 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^{-1}. Two separate LC/MS/MS runs were performed for the male and female samples, respectively.

Data and Statistical analysis

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 2-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. Secondly, 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 analysed: population 1 (28 rats) comprised the Lewis rat data with Sprague Dawley rat data described by White et al. (2017); 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 i.e. 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 centralised 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 5^{th} and 95^{th}percentile of the population.

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 < 0.05 was considered significant. Data are

reported as mean ± standard deviation (SD) unless stated otherwise. The male and female arterial blood pressure or plasma concentration data were compared at different time points using 2-way ANOVA with post hoc Sidak multiple comparison test.

Results

Alfaxalone pharmacodynamics for Lewis rats

Arterial blood pressure measurements for the Lewis rats during anaesthesia 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 minutes following discontinuation of isoflurane, all rats demonstrated an increase in blood pressure from the baseline readings obtained during anaesthesia 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 anaesthesia. Heart rates remained stable during alfaxalone anaesthesia 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 anaesthesia. The MAP remained elevated until 220 minutes for males before declining, while females showed early signs of a MAP decrease from between 40-75 minutes. Significant differences in MAP were detected between the sexes at 120 minutes ($p < 0.05$).

All Lewis rats required IPPV as judged by apnoea, 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.

Hypnotic effect

The plane of anaesthesia 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.

Deterministic individual alfaxalone PK for Lewis rats

A 1-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. 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.

NLME PK model for alfaxalone (population 1)

The most parsimonious NLME model obtained for population 1 was 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 the supplementary data file. 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:

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

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

Where CL_{TV} and Vd_{TV} are the typical values (fixed effect) for Clearance (25.2 ml min^{-1}) 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 alphaxalone concentration for male Lewis and female Lewis rats is depicted in Figure 4 (A and B). However, MAP decreases for female Lewis rats (B) were evident when alfaxalone concentration exceeded approximately 20 μg ml^{-1}.

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 minimising cardiopulmonary depression but maintaining an appropriate hypnotic effect.

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 9/16 female SD, and 5/8 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.

Hypnotic effect

The plane of anaesthesia 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 anaesthesia however surgical anaesthesia was not an outcome measure of the model.

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.

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 the supplementary data file. 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:

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

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

where CL_{TV} and Vd_{TV} are the typical values for Clearance (35.2 ml min^{-1}) 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 (normalised 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.

Discussion

This study demonstrated a sex difference in alfaxalone PK parameters using a 2 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 by Visser et al. (2002) and Lau et al. (2013) have shown alfaxalone clearance to be 158 ± 29 ml min^{-1} kg^{-1} and 54.3 ± 6.8 ml min^{-1} kg^{-1} for male and female Wistar rats respectively, and White et al. (2017) 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 towards 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 were 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 (White, et al. 2017). 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 co-variate 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 body-weight 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 normalised 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 real reason for the difference, as was the case for model 1, and 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 i.e. a decrease in blood pressure (Khan et al., 2014; Muir et al., 2009; Sear, 1996) but to a lesser extent compared to other anaesthetics such as thiopental and propofol (Goodchild et al., 2015; Visser et al., 2002). 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 as a result of the 30% reduction phase. One explanation of 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 anaesthesia. 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 whilst simultaneously minimising cardiopulmonary depression enhancing the profile particularly for prolonged anaesthesia.

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 anaesthesia for instrumentation. Isoflurane can cause significant cardiovascular depression in a dose dependent manner (Yang, et al., 2014), as such, no arterial pressure baselines in conscious rats were available. Furthermore,

after discontinuing the gases, 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 anaesthesia and compatibility with other adjuncts and analgesics is advised to move away from reliance on the volatile agents, thereby minimising their detrimental environmental impact.

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Tables

Table 1. Blood gas and biochemistry values 90-120 minutes after commencing the alfaxalone infusion. P_aCO_2: partial arterial carbon dioxide pressure, P_aO_2: partial arterial oxygen pressure, HCO_3: bicarbonate, SD: Sprague Dawley. Data are mean ± SD.

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^{-1})	29 ± 3	26 ± 4.4	27 ± 7	28 ± 3.3
Base Excess (mmol L^{-1})	4.1 ± 2.8	1.3 ± 5.1	1.2 ± 7	2.4 ± 3.3
Sodium (mmol L^{-1})	145 ± 3	145 ± 3.2	146 ± 4	142 ± 2.3
Potassium (mmol L^{-1})	4 ± 0.6	3.8 ± 0.5	3.8 ± 0.8	3.7 ± 0.7
Ionized Calcium (mmol L^{-1})	1.28 ± 0.08	1.2 ± 0.1	1.22 ± 0.2	1.29 ± 0.07
Chloride (mmol L^{-1})	107 ± 3	110 ± 4.2	107 ± 4	105 ± 5
Anion Gap (mmol L^{-1})	13 ± 3	12 ± 1.1	12 ± 1.6	12 ± 1.9
Haematocrit (%)	35 ± 5	31 ± 6	31 ± 6	28 ± 7
Haemoglobin (g dl^{-1})	11.8 ± 1.8	10.7 ± 2	10.6 ± 2	10.0 ± 2.2
Glucose (mmol L^{-1})	6.2 ± 1.2	6.3 ± 1.5	6.4 ± 2	5.9 ± 1.3
Lactate (mmol L^{-1})	0.6 ± 0.2	0.6 ± 0.4	1.7 ± 2.3	1.9 ± 2.6
Creatinine (mmol L^{-1})	13 ± 13	27 ± 11	43 ± 14	35 ± 11

Table 2: Pharmacokinetic parameters for 16 (9 male & 7 female) Lewis rats after intravenous administration of alfaxalone at a rate of 1.67 mg $kg^{-1}minute^{-1}$ for 2.5 minutes followed by 0.75 $mg.kg^{-1}minute^{-1}$ for the maintenance stage, then by 0.52 mg $kg^{-1}minute^{-1}$ for the end of the experiment.

Lewis rats	**CL (ml min kg^{-1})**	**$T_{\frac{1}{2}}$ (min)**	**MRT (min)**	**Vdss (L kg^{-1})**
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	0.0003	0.0001	0.0001	0.0286

Table 3: Outputted adjusted typical values and post hoc primary PK parameters (normalised per kg body-weight) for the most parsimonious model of population 1

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Rat groups	**Adjusted typical values**	**Adjusted typical values**	**Post hoc (mean ± SD)[a]**	**Post hoc (me**
	Vd (L)	CL (mL min^{-1})	Vd (L Kg^{-1})	CL (mL min^{-1}
Male Lewis rat	0.57	25.2	1.90 ± 0.34	101 ± 28.1

Rat groups	Adjusted typical values	Adjusted typical values	Post hoc (mean ± SD)[a]	Post hoc (me
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

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

Rat groups	Adjusted Typical values[a]	Adjusted Typical values[a]	Post hoc (mean ± SD)[b]	Post hoc (mean ± SD)[b]
	Vd (L)	CL (mL min^{-1})	Vd (L kg^{-1})	CL (mL $min^{-1}kg^{-1}$)
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

Figures

Hosted file

`image1.emf` available at https://authorea.com/users/484755/articles/570387-alfaxalone-population-pharmacokinetics-in-the-rat-model-application-for-pk-pd-design-in-inbred-and-outbred-strains-and-sexes

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

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 minutes. Data presented as mean ± SD; later time points are presented as single recorded points as they were measured at different times.

Figure 3: Alphaxalone 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 $kg^{-1}minute^{-1}$ for 2.5 minutes followed by 0.75 mg $kg^{-1}minute^{-1}$until the end of the electrophysiological stage, then by 0.52 mg $kg^{-1}minute^{-1}$

A B

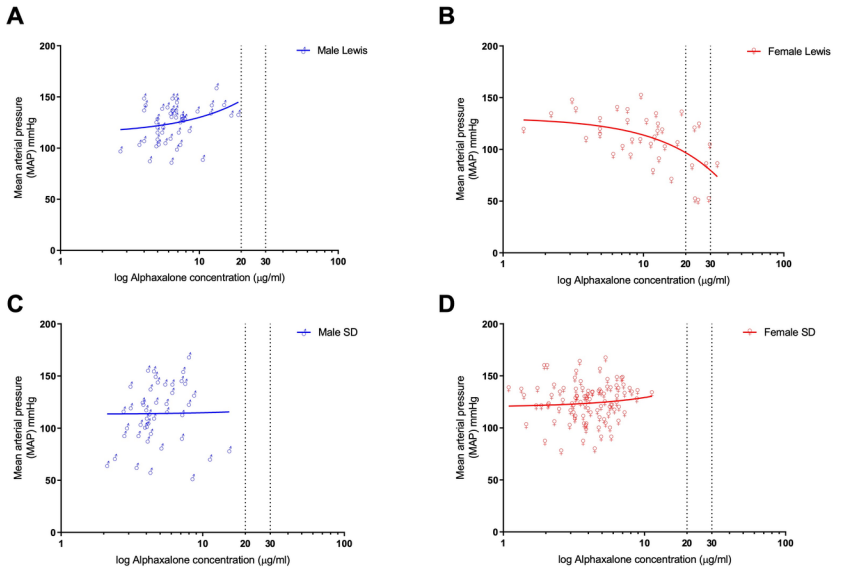
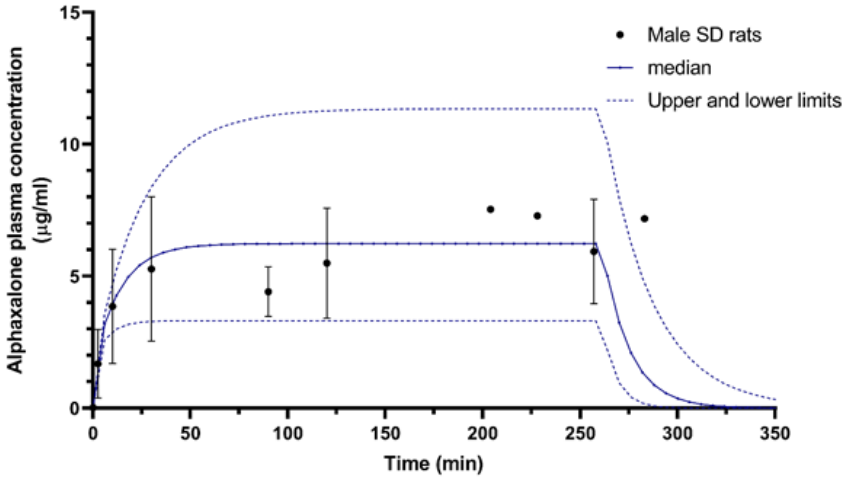


Figure 4: Mean arterial pressure (MAP) versus alphaxalone concentration; comparison between (A) male Lewis, (B) female Lewis rats

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

A



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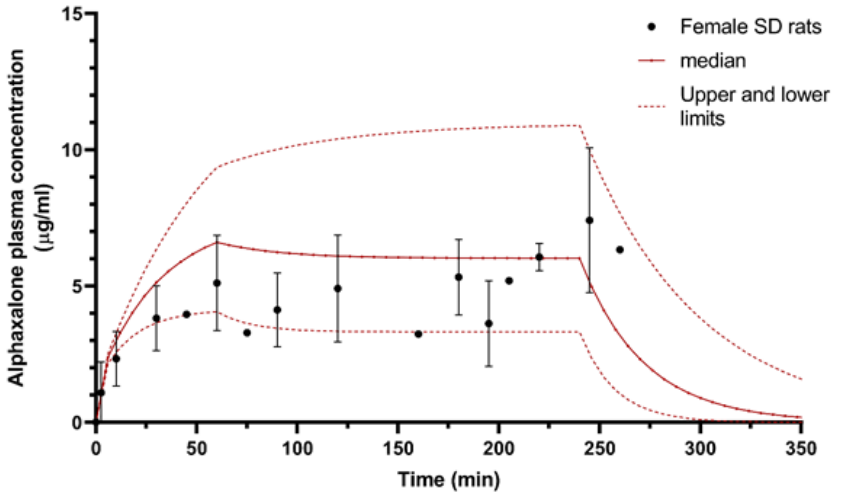

Figure 6: Measured (*) and simulated (lines) alphaxalone plasma concentrations with 5^{th} and 95^{th}percentile prediction (dashed lines) for male (a) and female (b) SD rats.

A B

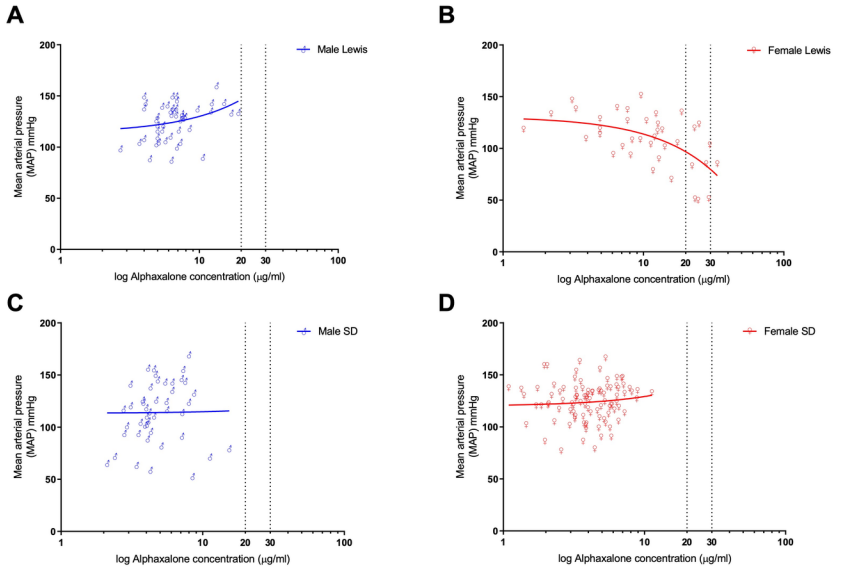

Figure 7: Mean arterial pressure (MAP) versus alphaxalone concentration; comparison between (A) male Lewis, (B) female Lewis, (C) male SD and (D) dose adjusted female SD rats

What is already known

The ideal anaesthesic drug does not yet exist

Designing new and repurposing old anaesthesic drugs is essential for progress in basic science and for translation

What this study adds

Compartmental non-linear mixed method effects (NLME) offers advantages over the standard deterministic pharmacokinetic approach

Alfaxalone CL is dependent on bodyweight and sex, whereas Vd is influence by rat strain

Clinical significance

This NLME model can be used for predicting and designing future alfaxalone experiments

Sex and strain differences must be addressed, incorporated and studied in anaesthetised rodent models