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RESEARCH PAPER

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

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Abstract

Objective To characterise, as a clinical study, the pharmacokinetics and pharmacodynamics and describe the hypnotic effect of the neurosteroid alfaxalone (3α-hydroxy-5 α-pregnane-11, 20-dione) formulated with 2-hydroxypropyl-β-cyclodextrin in male and female rats.

Study design Prospective, experimental laboratory study.

Animals A total of 12 (six male and six female) adult, aged-matched Sprague Dawley rats.

Methods Surgery and instrumentation was performed under isoflurane anaesthesia in an oxygen/nitrous oxide mixture (1:2) and local anaesthetic infiltration. All animals received a loading dose (1.67 mg kg^{-1} $minute^{-1}$) for 2.5 minutes followed by a constant rate infusion (0.75 mg kg^{-1} $minute^{-1}$) for 120 minutes of alfaxalone. Isoflurane and nitrous oxide was discontinued 2.5 minutes after the alfaxalone infusion started. Cardiorespiratory variables (heart rate, respiratory rate, arterial blood pressure and end tidal carbon dioxide tension) and clinical signs of anaesthetic depth were evaluated throughout anaesthesia. Carotid artery blood samples were collected at strategic time points for blood gas analysis, haematology, biochemistry, and plasma concentrations of alfaxalone. Plasma samples were assayed using liquid chromatography-mass spectrometry.

Results There were significant differences between the sexes for plasma clearance ($p = 0.0008$), half-life ($p = 0.0268$) and mean residence time ($p = 0.027$). Mean arterial blood pressure was significantly higher in the male rats ($p = 0.0255$).

Conclusions and clinical relevance This study confirms that alfaxalone solubilised in 2-hydroxypropyl-β-cyclodextrin provides excellent total intravenous anaesthesia in rats. Sex-based differences in pharmacokinetics and pharmacodynamics were demonstrated and must be considered when designing biomedical research models using alfaxalone.

Keywords alfaxalone, anaesthetics, intravenous, rat, steroid.

Introduction

Alfaxalone is a neuroactive steroid that modulates neurotransmission through interaction with a steroid recognition site on the $GABA_A$ receptor complex causing a positive allosteric modulation of the ligand-gated chloride channel, resulting in the inhibition of neuronal excitability (Harrison & Simmonds 1984; Turner et al. 1989). Therefore, such agents have roles in anaesthesia, epilepsy, anxiety, insomnia, migraine and drug dependence (Rupprecht & Holsboer 1999). Alfaxalone had been used as an anaesthetic induction agent in humans and veterinary species for almost half a century, but anaphylactoid reactions attributed to the polyethoxylated

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castor oil (Cremophor EL) vehicle (Tammisto et al. 1973) made its use redundant. Subsequent formulations of alfaxalone incorporating a cyclodextrin have hitherto been devoid of the previous side effects and Alfaxan (Jurox, UK; alfaxalone dissolved in 2-hydroxypropyl-β-cyclodextrin) is now registered for the induction and maintenance of anaesthesia in dogs and cats and has been used in horses (Goodwin et al. 2011), sheep (Andaluz et al. 2012; del Mar Granados et al. 2012), rabbits (Navarrete-Calvo et al. 2014) and other more exotic species (Bouts & Karunaratna 2011; McMillan & Leece 2011; Bauquier et al. 2013; Kischinovsky et al. 2013; Knotek et al. 2013; Villaverde-Morcillo et al. 2014).

The use of alfaxalone in biomedical research and clinical veterinary medicine is gaining popularity as it may offer some selective advantages over other anaesthetic combinations in terms of safety, reflex suppression, cardiopulmonary depression, interaction with receptors involved in pain pathways/modulation and pain on injection (Child et al. 1972; Michou et al. 2012; Santos González et al. 2013) but may also offer additional advantages in influencing central nervous system development and myelination (Yawno et al. 2014). Alfaxalone is popular for neuroendocrine studies for its sparing of various forebrain functions (Sarkar et al. 1976; Sherwood et al. 1980). Human trials of alphaxalone in cyclodextrin are currently underway (Monagle et al. 2015).

The majority of animals used in basic science pain research, however, are young healthy male laboratory rodents, and indeed it has been suggested that a more heterogeneous and diverse population must be used to improve the translational relevance to a human population (Mogil 2009). The inclusion of female rodents must be encouraged despite the additional complexities that the variability of the oestrous cycle and sexual dimorphism poses; well-designed studies can include both sexes without needless increase in animal numbers (Clayton & Collins 2014). With respect to alfaxalone usage, the pharmacokinetics of a single intravenous (IV) dose have been defined in dogs (Ferré et al. 2006), cats (Whittem et al. 2008b; Muir et al. 2009), female rats (Lau et al. 2013) and male rats after a 5-minute infusion (Visser et al. 2002). Therefore, the novelty and primary aim of this study was to characterise the pharmacokinetics, pharmacodynamics and hypnotic characteristics of a constant rate infusion of alfaxalone in male *versus* female rats.

Materials and 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 control (DNIC). This study is reported in accordance with the ARRIVE guidelines (Kilkenny et al. 2014).

Animals

In total, 12 (age, 9–12 weeks), six male (397 ± 16 g) and six female (286 ± 20 g), Sprague Dawley rats (Charles River Laboratories, UK) were used. Animals were housed in single sex groups of four, given access to food (Teklad 2018, Harlan, UK) and tap water *ad libitum* and maintained on a 12-hour light/dark cycle. All experiments started at 10:00 hours each day.

General anaesthesia

Anaesthesia was induced using 3% isoflurane (Isoflo; Abbott, UK) in oxygen and nitrous oxide mixture (1:2). Once the rat had lost its righting reflex, it was transferred to a heating blanket (Harvard Apparatus Ltd., UK) coupled to a rectal probe for the maintenance of body temperature (37.5 ± 0.5 °C). Anaesthesia was maintained using 2.00–2.25% (vaporiser setting) isoflurane in oxygen/nitrous oxide delivered via a nosecone. Lidocaine 2% (Lignol; Dechra, UK) 3 mg kg^{-1} was infiltrated subcutaneously prior to skin and sternohyoid incision. The trachea was surgically cannulated using 2.42 mm O.D. polyethylene tubing (Fisher Scientific, UK). Respiratory rate and effort was assessed by observing chest excursion and measuring end tidal carbon dioxide (CapStar 100, Linton Instrumentation, UK). In animals exhibiting respiratory depression as judged by a low respiratory rate and rising end tidal carbon dioxide values, intermittent positive pressure ventilation was initiated (Harvard 683 ventilator, Harvard Apparatus) at 60–80 breaths minute^{-1} to maintain end tidal carbon dioxide at 35–45 mmHg (4.67–6.00 kPa). The left jugular vein was surgically cannulated using 0.63 mm O.D. polyethylene tubing (Fisher Scientific) for the administration of alfaxalone. The left carotid artery was surgically cannulated using 1 mm O.D. polyethylene tubing (Fisher Scientific) to monitor arterial blood pressure and for sampling. Arterial blood pressure was monitored by an arterial pressure transducer (SensoNor 840; SensoNor, Norway) and

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