International Journal of Pharmaceutics 486 (2015) 69–76

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Evaluation of pharmacokinetic properties and anaesthetic effects of propofol in a new perfluorohexyloctane (F6H8) emulsion in rats – A comparative study

Charalambos Tsagogiorgas [a,1,*], Sonja Theisinger [b,1], Elisabeth Heesch [a], Jörg Krebs [a], René Holm [c], Grietje Beck [a], Benito Yard [d]

[a] Department of Anaesthesiology and Intensive Care Medicine, University Medical Centre Mannheim, Medical Faculty Mannheim of the University of Heidelberg, Mannheim, Germany
[b] Novaliq GmbH, Heidelberg, Germany
[c] Biologics and Pharmaceutical Science, H.Lundbeck A/S, DK-2500 Valby, Denmark
[d] Department of Medicine V, University Medical Centre Mannheim, Medical Faculty Mannheim of the University of Heidelberg, Mannheim, Germany

ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form 13 March 2015
Accepted 16 March 2015
Available online 19 March 2015

Keywords:
Drug delivery
Propofol
Semifluorinated alkane
F6H8
Perfluorocarbon
Emulsion

ABSTRACT

Propofol (2,6-diisopropylphenol) is a safe and widely used anaesthetic, but due to low water solubility and high lipophilicity a difficult compound to formulate. The solubility of propofol in the semifluorinated alkane perfluorohexyloctane (F6H8) is very high (>300 mg/ml). In the present work we investigate if a F6H8-based emulsion could be used as a new intravenous drug delivery system for propofol from a pharmacokinetic, pharmacodynamic and safety point of view. The pharmacokinetic parameters were evaluated after an intravenous bolus injection of either Disoprivan® or a F6H8-based propofol emulsion in Wistar rats. The onset and end of sedation after multiple dosings (5, 10 and 15 mg/kg bw) were examined. Clinical chemistry and histology were assessed. No significant difference was found for any of the pharmacokinetic parameters. No differences in the onset nor the end of sedation in the tested dosages could be detected. Histology scores revealed no differences. A slightly increased alanine aminotransferase (ALT) was measured after multiple application of the F6H8-propofol emulsion. In conclusion, the F6H8-propofol emulsion showed no significant different pharmacokinetics and sedation properties, compared to a commercial soy-based propofol emulsion. Further, no toxic effects could be detected on the F6H8 emulsion indicating it was a safe excipient in rats.

1. Introduction

Propofol (2,6-diisopropylphenol) is a safe and widely used intravenous anaesthetic agent, initially developed for induction and maintenance of anaesthesia. As a highly lipophilic compound (log $P = 4.16$), it is a challenging compound to formulate (Thompson and Goodale, 2000) and all commercial available propofol formulations are supplied as oil-in-water emulsions. Extensive efforts have been devoted to alter propofol carrier and reduce the side effects due to the lipid emulsion of soy oil (Rodrigues et al., 2012), *e.g.* rapid growth of micro-organisms (Bennett et al., 1995; Jansson et al., 2006; Langevin et al., 1999), hyperlipidemia (Wolf et al., 2001) and pain upon injection (Auerswald et al., 2005; Jalota et al., 2011). The advances in modern drug discovery have generated an increasing number of new chemical entities with extremely poor aqueous solubility. Therefore, new solvents or lipid excipients to solubilise highly lipophilic drugs are constantly sought, because a low aqueous solubility not necessarily lead to high solubility in excipients used in lipid based formulations (Hauss, 2007).

Potential new excipients for formulations of lipophilic, poorly soluble drug substances, include semifluorinated alkanes (SFAs), which are colourless non-aqueous amphiphilic liquids consisting of diblock molecules with a hydrophobic/lipophobic perfluorocarbon (R_F) and a hydrophobic/lipophilic hydrocarbon (R_H) segment. They are considered to be chemically and biologically inert (Krafft and Riess, 1998, 2009; Meinert and Knoblich, 1993). As near chemical relatives of perfluorocarbons (PFCs), Krafft and Riess demonstrated that SFAs stabilize PFC emulsions as co-surfactants in blood substitutes (Krafft et al., 2003; Krafft and Riess, 2009; Riess, 2001). SFAs alone can form a stable emulsion and could also

* Correspondence to: Klinik für Anästhesiologie und Operative Intensivmedizin, UMM- Universitätsmedizin Mannheim, Theodor-Kutzer-Ufer 1-3, D-68167 Mannheim, Germany. Tel.: +49 621 383 2415; fax: +49 621 383 2164.
E-mail address: charalambos.tsagogiorgas@umm.de (C. Tsagogiorgas).
[1] Equal contributors.

http://dx.doi.org/10.1016/j.ijpharm.2015.03.037
0378-5173/

be used as oxygen-carriers instead of PFCs (Meinert and Knoblich, 1993). These emulsions show no toxic side effects and are nearly lipid-free. Moreover, in contrast to PFCs, SFAs have a solubilizing capacity for selected lipophilic drugs, which qualifies them as a potential new and interesting excipient class (Meinert and Roy, 2000). There is evidence in the literature that SFAs can be used as excipients in aerosol formulations for inhalative drug delivery of lipophilic drugs (Tsagogiorgas et al., 2010, 2012a) and for buccal drug delivery of propofol (Tsagogiorgas et al., 2013). In the buccal study, the solubility of propofol in SFAs was shown to be very high (>300 mg/ml) (Tsagogiorgas et al., 2013), why SFAs potentially could be used as an excipient in submicron emulsions as the disperse phase.

The purpose of the present study was, therefore, to investigate if SFA-based emulsions could be used as a new drug delivery system for intravenous administration of propofol. This was done by comparing a propofol emulsion consisting of lecithin (S75), aqueous glucose solution and 5% SFA perfluorohexyloctane (F6H8) as the oil phase with a commercial available lipid-based propofol emulsion (Disoprivan® 1%) with respect to pharmacokinetic profile, sedation potential, clinical chemistry and histology in rats.

2. Material and methods

2.1. Materials

The (1-perfluorohexyloctane) F6H8-propofol emulsion was manufactured by Novaliq GmbH (Heidelberg, Germany) as described below. Soy lecithin (S75) was purchased from Lipoid AG (Ludwigshafen, Germany) and Disoprivan® (1%) from Astra Zeneca (Wedel, Germany). Propofol was purchased from Biotrend Chemikalien (Wangen, Switzerland) and the internal standard Thymol from Sigma–Aldrich (St. Louis, MO, USA). Formic acid, methanol, acetonitrile and ammoniac solution were all of HPLC grade and purchased from Merck (Darmstadt, Germany). All other reagents were of analytical or HPLC grade. Purified water was obtained from a Millipore Milli-Q Ultrapure water purification system (Billerica, MA, USA). All other test substances were from Sigma–Aldrich (St. Louis, MO, USA) if not differently indicated.

2.2. F6H8-propofol formulation

Lecithin (8 wt%; related to organic phase) was added to a glucose solution (5%, BBraun AG, Melsungen, Germany) stirred for 10 min to achieve a clear solution. Propofol (equal to 10 mg/ml in the final emulsion) and F6H8 (equal to 5 wt% in the final emulsion) were mixed and added under vigorous stirring to the surfactant solution. This mixture was stirred for 1 h at 2000 rpm. The emulsion was then prepared by high pressure homogenization process using an Avestin EmulsiFlex® apparatus (Avestin Inc., Ottawa, Canada) at a pressure of 1100 bar in continuous process mode for 1 h. The pH was adjusted to Ph 7.3–7.5 by adding sodium hydroxide. The final emulsion was filled into vials, closed and sealed after blanketing with nitrogen. Subsequently the vials were sterilized at 121 °C for 10 min. The propofol concentration in the final emulsion was confirmed by HPLC (Prolytic GmbH, Frankfurt, Germany).

2.2.1. Droplet size measurement

F6H8-propofol emulsion average droplet size was measured by dynamic light scattering (DLS) using a PSS Nicomp 380 (Santa Barbara, CA, USA) after sterilization on the day of production ($t = 0$ day) and on day 84 ($t = 84$ day) after storage at room temperature (21 °C) in a dark and dry place. The F6H8-propofol formulation was used within a 84 day time period.

2.3. Animal study

The study was approved by the Institutional Review Board for the care of animal subjects (University of Heidelberg, Medical faculty Mannheim, Germany). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, USA.

2.3.1. Pharmacokinetic study – animal preparation and experimental protocol

For the pharmacokinetic study a total of 10 pathogen free male Wistar rats (472 ± 54 g) housed in standard condition with food and water *ad libitum* were anesthetized by intraperitoneal (i.p.) injection of ketamine hydrochloride (50 mg/kg; Ketanest 10%®, Pfizer, Karlsruhe, Germany) and xylazine (2 mg/kg; Rompun®, BayerVital, Leverkusen, Germany). Anaesthesia was maintained as needed with intravenous ketamine throughout the experiment. The femoral artery and the femoral vein were cannulated with polyethylene catheter tubing (PE-50, neoLab Heidelberg, Germany).

The arterial line was used for continuous monitoring of heart rate (HR), mean arterial pressure (MAP) and to collect intermittent blood samples (200 μl) for the analysis of propofol.

The animals were then tracheotomised, intubated with a 14G polyethylene tube (Klinject, KLINIKA Medical GmbH, Usingen, Germany) and mechanically ventilated with a neonatal respirator (Babylog 8000, Draeger, Luebeck, Germany) using a pressure-controlled mode with a PEEP of 2 cm H_2O, inspiratory/expiratory ratio (I:E) of 1:1 and fraction of inspired oxygen (FiO_2) of 0.5, a tidal volume of 6 ml/kg, a positive end expiratory pressure of 3 cm H_2O and a variable respiratory rate of 80–100 min^{-1} to maintain a $PaCO_2$ value within the physiological range. Body temperature was maintained between 37 °C and 38.5 °C with a heating pad.

Baseline blood samples (200 μl) were collected prior to administration of propofol. F6H8-propofol 1% or Disoprivan® propofol 1% (Astra Zeneca, Wedel, Germany) were injected as bolus (10 mg/kg bw) into the femoral vein within 30 s. The end of injection was taken as time zero ($t = 0$ min). Blood samples (200 μl) were collected into lithium heparin coated tubes after 1, 5, 10, 15, 30, 60 min after administration. Plasma was harvested immediately by 10 min of centrifugation at 4 °C, 3000 × *g* (Multifuge 1 S-R, Heraeus, Hanau, Germany) and stored at −20 °C until analyzed.

2.3.2. Anaesthetic activity – animal preparation and experimental sedation protocol

For the sedation animal study a total of 12 pathogen free male Wistar rats (450–500 g) housed under standard condition with food and water *ad libitum* were anesthetized by intraperitoneal (i.p.) injection of ketamine hydrochloride (50 mg/kg; Ketanest 10%®, Pfizer, Karlsruhe, Germany) and xylazine (2 mg/kg; Rompun®, BayerVital, Leverkusen, Germany).

An intravenous catheter was applied in all animals through the femoral vein, fixed and diverted subcutaneously to the crest of the neck. The intraluminal volume of all catheters was measured before application, blocked with heparin (1 U/ml) and tightly sealed. All wounds were sutured. Rats were placed on a heating pad to maintain body temperature at 38 ± 1 °C during the period of anaesthesia and recovery.

After regaining consciousness the animals were allowed to recover from operational procedures for 7 days, where the animals were housed individually under standard condition with food and water *ad libitum*. All animals were examined daily for potential side effects of the procedure. After 7 days all animals were randomly assigned to one of two sedation groups receiving either