Anesthetic Profile of a Non-lipid Propofol Nanoemulsion

Roberto Takashi Sudo, TSA [1], Laura Bonfá [2], Margarete Manhães Trachez, TSA [3], Roberto Debom [4], Marisa D. R. Rizzi [5], Gisele Zapata-Sudo [6]

Summary: Sudo RT, Bonfá L, Trachez MM, Debom R, Rizzi MDR, Zapata-Sudo G – Anesthetic Profile of a Non-lipid Propofol Nanoemulsion.

Background and objectives: The clinical use of a lipid propofol formulation causes pain during injection, allergic reactions, and bacterial growth. Propofol has been reformulated in different non-lipid presentations to reduce the incidence of adverse effects, but those changes can modify its pharmacokinetics and pharmacodynamics. In the present study, we investigate the pharmacology and toxicology of lipid propofol (CLP) and the non-lipid nanoemulsion (NLP).

Methods: Conventional lipid formulation of propofol and NLP were infused in the jugular veins of rats and blood pressure (BP), heart rate (HR), and respiratory rate (RR) were measured. Both formulations (1%) were infused (40 μL.min^{-1}) over 1 hour. Hypnotic and anesthetic doses as well as recoveries were determined. The pain induced by the CLP and NLP vehicles was compared by counting the number of abdominal contortions ("writhing test") after the intraperitoneal (i.p.) injection in mice. Acetic acid (0.6%) was used as positive control.

Results: Hypnotic and anesthetic doses of 1% CLP (6.0 ± 1.3 and 17.8 ± 2.6 mg.kg^{-1}, respectively) and 1% NLP (5.4 ± 1.0 and 16.0 ± 1.4 mg.kg^{-1}, respectively) were not significantly different. Recovery from hypnosis and anesthesia was faster with NLP than with CLP. Changes in HR, BP, and RR caused by NLP were not significantly different from those caused by CLP. Acetic acid and the vehicle of CLP caused 46.0 ± 2.0 and 12.5 ± 0.6 abdominal contortions 20 min after i.p. injection, respectively. The absence of abdominal contractions was observed with the vehicle of NLP. Abdominal inflammatory response was not observed after the i.p. injection of both propofol vehicles.

Conclusions: Non-lipid formulation of propofol can be a better alternative to CPL for intravenous anesthesia with fewer adverse effects.

Keywords: ANESTHESICS, Intravenous: propofol; ANIMALS: rats.

Financial Support: CNPq, FAPERJ, FUJB/UFRJ, INCT/INOFAR, Instituto do Milênio.

[Rev Bras Anestesiol 2010;60(5): 475-483] ©Elsevier Editora Ltda.

INTRODUCTION

Propofol 2,6 diisopropylphenol is the most commonly used intravenous anesthetic agent and is formulated in a lipid solution. High lipophilicity permits its wide distribution and fast penetration in the central nervous system (CNS) which confers efficient control of the onset and recovery from anesthesia [1]. For clinical use propofol was initially formulated in cremophor 16% [1,2] which was excluded of the clinical research because of the high incidence of anaphylactic reactions [3].

Despite being widely used in anesthesia and intensive care, propofol promotes undesirable effects such as pain on injection and allergic reactions, which are characteristics related to its macroemulsion formulation [4]. Although intravenous emulsions are made as a sterile product, the presence of fatty acids can increase the likelihood of bacterial contamination or fungal growth which has led manufacturers to add antimicrobial agents to some commercial formulations of the lipid emulsion [5]. Additionally, the lipid emulsion was reported to induce hyperlipidemia [6], causing a propofol infusion syndrome that is characterized by severe metabolic acidosis, rabdomyolisis, and renal and acute cardiac failure [7]. Hyperlipidemia can occur in children and adults who received infusion of over 4mg.kg^{-1}.h^{-1} of propofol for a period longer than 24 hours. There have been many attempts to alter the formulation and propose alternatives for clinical use in order to reduce the adverse effects of propofol [8,9].

The aim of the present work was to compare the anesthetic properties, including the induction and recovery from anesthesia and the incidence of pain induced by a conventional lipid macroemulsion of 1% propofol (CLP) with a non-lipid nanoemulsion of 1% propofol (NLP).

METHODS

The protocols used in this study were approved by the Animal Care and Use Committee at the Universidade Federal do Rio

Received from the Program of Drug Development, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro.

1. PhD, Full Professor of the Program of Drug Development – UFRJ
2. Masters Degree, Anesthesiologist - Hospital da Lagoa - RJ
3. Physician, Professor– UFF
4. Pharmacy Graduation, RD&I Director – Cristália
5. Pharmacy Graduation, RD&I Supervisor – Cristália
6. Post-Doctorate, Professor of the Program of Drug Development – UFRJ

Submitted on March 2, 2010.
Approved on May 3, 2010.

Correspondence to:
Dr. Roberto Takashi Sudo
Rua Republica do Peru, 114 apt 802
Copacabana
22021-040 – Rio de Janeiro, RJ, Brazil
E-mail: rtsudo@farmaco.ufrj.br

de Janeiro. The NLP, CLP and their vehicles were kindly donated by Cristália Produtos Químicos e Farmacêuticos Ltda (São Paulo, SP, Brazil).

Preparation of NLP

NLP (1%) was prepared by combining propofol, macrogol hydroxy stearate (Soluthol HS15™, BASF Corporation, Florham Park, NJ) and glycerol under continuous agitation until completely homogenized. Then, the pH was adjusted to 6-8.5 using NaOH to form a clear nanoemulsion containing droplets of 5-140 nm.

Determination of HD_{50} and LD_{50}

One hundred and sixty,male Swiss mice (20-25 g) were randomly divided into two sets of four groups (10 mice per group). Ten mice were used for each dose of CLP or NLP. Sample size for mice populations was estimated to reach valid conclusions for $p < 0.05$ with a power of 90% to estimate difference to 40% between groups. The mice received a single intravenous injection of increasing dose of 1% CLP or 1% NLP. The effect dose to promote hypnosis in 50% of the animals (HD_{50}) and the lethal dose for 50% of the mice (LD_{50}) were obtained through intravenous bolus injections of both formulations. The therapeutic index was then calculated as LD_{50}/HD_{50}. The induction of hypnosis was considered effective when the mice lost their forepaw righting reflex.

Blood pressure and electrocardiogram recordings

Male Wistar rats (220-250 g) were anesthetized with sevoflurane (Sevocris™, Cristália Produtos Químicos e Farmacêuticos Ltda, São Paulo, SP, Brazil) and a catheter was placed into the right carotid artery to measure the arterial blood pressure (BP) using a calibrated pressure transducer (Statham, P022). A pair of external electrodes was placed on the animal's chest to record the electrocardiogram (EKG). Additionally, a catheter was placed in a jugular vein of each animal for intravenous infusions of the propofol formulations. Two hours after the surgical procedure, the 1% CLP or NLP was infused at a rate of 40 $\mu L.min^{-1}$ for 1 h. Both the BP and EKG were continuously recorded on a polygraph (AstroMed Grass Physiological Recorder, Model 7400) before and during administration of the CLP or NLP. The hypnotic and anesthetic doses were determined by measuring the time required to lose the forepaw righting reflex and inhibit the pinprick reflex, respectively. The time required for recovery from hypnosis and anesthesia was also evaluated.

Writhing test

The writhing test was used to evaluate the pain caused by the injection of the two types of propofol formulations. Thirty male Swiss mice (20-25 g) were randomly divided into three groups, and each group was treated with either 5 $mL.kg^{-1}$ of a 0.6% acetic acid solution (positive reference) or the vehicles of CLP or NLP. The frequency of writhing was measured for 20 min after the intraperitoneal injection (i.p.) of either acetic acid or the vehicles.

Histological evaluation

The presence of local tissue lesion or inflammation induced by the both propofol formulations was assessed following intraperitoneal administration of acetic acid, CLP or NLP. For the histological analysis, peritoneal membranes were excised, fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin. The hematoxylin- and eosin-stained sections were observed under 400x (16 µm) magnifications.

Statistical analysis

All data were expressed as the mean ± SD. Experimental differences between different doses were considered statistically significant when $p < 0.05$ using one-way analysis of variance (ANOVA) followed by a Dunnett's post hoc test. To compare multiple groups, ANOVA was used followed by the Newman-Keuls post hoc test.

RESULTS

To determine the HD_{50} and LD_{50}, the percentages of hypnosis and death were plotted against the formulation doses, respectively. Linear regression was used to calculate the HD_{50} and LD_{50}, which were 15.4 and 44.4 $mg.kg^{-1}$ for 1% CLP, respectively. Similar results were observed with 1% NLP, where the HD_{50} and LD_{50} were 11.5 and 49.0 $mg.kg^{-1}$, respectively. Thus, no significant difference in the therapeutic index was observed between CLP (2.88) and NLP (4.26).

Table I – Hypnotic and Anesthetic Doses of CLP and NLP Induced by Intravenous Infusion in Rats

	Hypnosis			Anestesia		
Formulation	Latency (min)	Dose ($mg.kg^{-1}$)	Recovery (min)	Latency (min)	Dose ($mg.kg^{-1}$)	Recovery (min)
1% CLP	4.0 ± 0.9	6.0 ± 1.3	40.6 ± 3.1	11.9 ± 1.8	17.8 ± 2.6	19.8 ± 1.5
1% NLP	5.4 ± 1.0	7.5 ± 1.1	19.3 ± 3.4*	11.2 ± 1.2	16.0 ± 1.4	7.9 ± 0.7*

The Data represent mean ± SD (n = 8). *p < 0.05 NLP vs CLP.

The latencies to lose the forepaw righting reflex and inhibit the pinprick reflex upon intravenous infusion of propofol were used to calculate the doses required to induce hypnosis and anesthesia. There was no significant difference in the hypnotic and anesthetic doses of CLP and NLP (Table I). The doses of 1% NLP that produced hypnosis and anesthesia were 7.5 ± 1.1 and 16.0 ± 1.4 mg.kg^{-1}, respectively and for 1% CLP were 6.0 ± 1.3 and 17.8 ± 2.6 mg.kg^{-1}, respectively. In contrast, the recovery from hypnosis and anesthesia were faster ($p < 0.05$) following 1 hour of NLP infusion than CLP infusion. The times required for complete recovery from hypnosis and from anesthesia were 19.3 ± 3.4 and 7.9 ± 0.7 min, respectively for NLP. The recovery times following CLP exposure were twice as long (Table I).

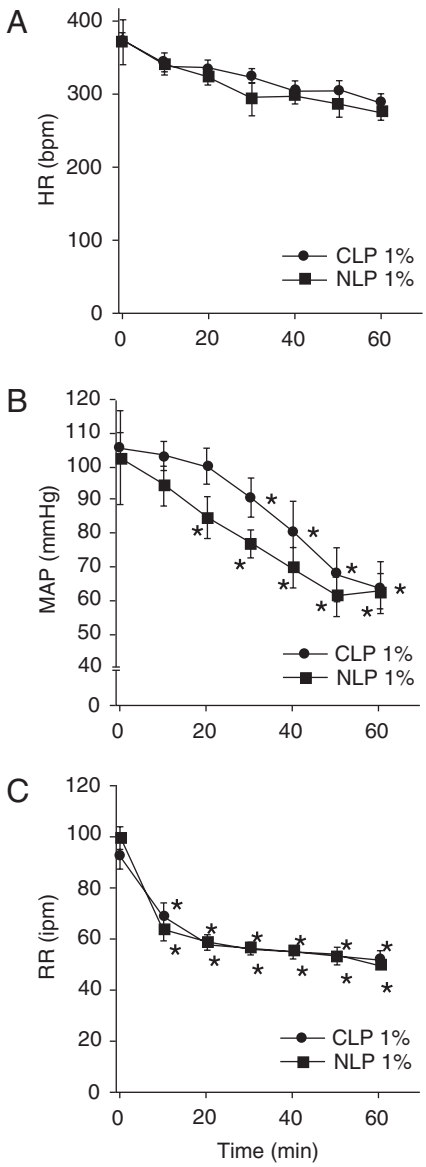

Figure 1. Effect of 60 min Infusion of CLP and NLP on Heart Rate (HR) (A), mean arterial blood pressure (MAP) (B) and respiratory rate (RR) (C) of rats. The data represent mean ± SEM (n = 6). *$p < 0.05$ vs control (time 0).

We also investigated whether NLP infusion interfered with the heart rate and blood pressure in Wistar rats. No significant differences were observed between NLP- and CLP-infused animals when comparing changes in the hemodynamic parameters. The control heart rate (HR) of 372.0 ± 30.0 beats. min^{-1} was not significantly altered by 1% NLP (Figure 1A). However, there was a reduction in the mean arterial pressure (MAP) following infusion of NLP for 1 h. NLP reduced the MAP from 102.4 ± 13.8 to 62.4 ± 5.3 mmHg ($p < 0.01$) and CLP reduced the MAP from 105.3 ± 4.3 to 63.5 ± 7.5 mm Hg ($p < 0.01$) (Figure 1B). Also, as shown in Figure 1C, both NLP and CLP significantly reduced the respiratory rate and there was no significant difference between the effects.

To investigate whether NLP induced pain like CLP, we compared the number of abdominal contortions induced by i.p. injection of acetic acid (0.6%) and the CLP and NLP vehicles in mice. Acetic acid and the CLP lipid vehicle produced 46.0 ± 2.0 (n = 10) and 12.5 ± 0.6 (n = 10) contortions during the 20 min following the i.p. injections, respectively. No abdominal contortions were observed after treatment of mice with the NLP vehicle.

Analysis of photomicrography shows no significant peritoneal inflammatory response observed after i.p. injection of either propofol formulations (Figure 2).

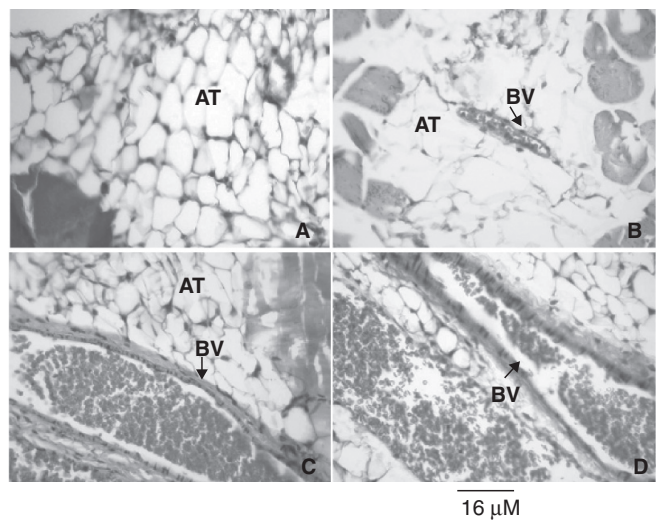

Figure 2. Representative Photomicrograph of Peritoneal Tissues from Mice Stained with Hematoxilin and Eosin after i.p. Injection of Saline (A), acetic acid (B), NLP (C) and CLP (D). Arrows in the panels indicate AT = adipose tissue and BV = blood vessel.

DISCUSSION

The lipid emulsion of propofol which contains soy oil (10%) and long-chain triglycerides, is the most commonly used intravenous anesthetic and causes adverse side-effects, including arterial hypotension, reduced heart rate and pain upon injection in 80 to 90% of the patients [10,11]. Propofol increases the risk of embolism and hypertriglyceridemia after

prolonged intravenous infusion. These adverse reactions have motivated attempts to develop new, safer propofol formulations. There are several reformulations of propofol, including: 1. the basic presentation with the addition of ethylene diamine tetraacetic acid or sulfite to minimize the bacterial growth [12]; 2. emulsion containing different long- and medium-chain triglycerides to reduce the amount of serum lipids [13,14,15]; 3. propofol prodrug [16]; 4. water-soluble analogues of propofol [17,18] and 5. non-lipid cyclodextrin-based formulation of propofol [6,19,20].

In spite of the diversity of propofol preparations, the incidence of pain upon injection was not significantly altered. This pain can be minimized by previous injection of lidocaine. The pharmacological mechanism of pain-induced by propofol is still unknown [21]. It has been hypothesized involvement of kallikrein-kinin-bradykinin cascade [22,23]. Another hypothesis is related to directly chemical activation of nociceptors induced by propofol on the vascular endothelium [24]. Propofol belongs to a group of phenols which have chemical stability and low toxicity, but can cause irritation of the skin and mucosal tissues. Thus, a bolus injection of propofol would be expected to cause pain [25]. We tested whether NLP produced visceral pain in a model in which number of abdominal contortions was measured after i.p. injection of compounds. Contortions induced by acetic acid were used as a positive control [26]. The NLP vehicle produced fewer abdominal contortions than the CLP vehicle, indicating that the animals experienced less pain upon injection of NLP.

Injection of 1% NLP produced hypnotic and analgesic effects similar to CLP and had the advantage of faster recovery. The therapeutic index for NLP was similar to that of other intravenous anesthetics and ranged from 2 to 4, indicating its safety. The changes in hemodynamic parameters induced by NLP were not significantly different from the changes induced by CLP. Therefore, the present work provides an incentive to further develop the non-lipid propofol nanoemulsion. Our results demonstrate that NLP may be used clinically in anesthesiology as an intravenous anesthetic that lacks adverse reactions.

ACKNOWLEDMENTS

This work was supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação Universitária Jose Bonifácio (FUJB), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). In addition, this work was supported, in part, by fellowships from CNPq (to GZS, RTS). NLP was kindly donated by Cristália Produtos Químicos e Farmacêuticos (Itapira, SP, Brazil).

REFERENCIAS / REFERENCES

01. Glen JB – Animal studies of the anesthetic activity of ICI 35-868. Br J Anaesth, 1980;52: 731-742.
02. Rolly G, Versichelen L, Zubair NA – Use of ICI 35868 as an anesthetic induction agent. Acta Anaesth Belg, 1980;31:241-247.
03. Shao X, Li H, White PF et al – Bisulfite-containing propofol: is it a cost-effective alternative to Diprivan for induction of anesthesia? Anesth Analg, 2000;91:871-875.
04. Kay B, Rolly G – ICI35868 – The effect of a change of formulation on the incidence of pain after intravenous injection. Acta Anesthesiol Belg, 1997;28:317-322.
05. Jones C – Fundamentals of emulsions. Am J Anesth, 2000;27(Suppl): 12-15.
06. Egan TD – Target-controlled drug delivery. progress toward an intravenous "vaporizer" and automated anesthetic administration. Anesthesiology, 2003;99:1214-1219.
07. Sabsovich I, Rehman Z, Yunen J et al – Propofol infusion syndrome: a case of increasing morbidity with traumatic brain injury. Am J Crit Care, 2007;16:82-85.
08. Dubey PK, Kumar A – Pain on injection of lipid-free Propofol and Propofol emulsion containing medium-chain triglyceride: a comparative study. Anesth Analg, 2005;101:1060-1062.
09. Baker MT, Naguib M – Propofol: the challenges of formulation. Anesthesiology, 2005;103:860-876.
10. Abad-Santos F, Galvez-Mugica MA, Santos MA et al – Pharmacokinetics and pharmacodynamics of a single *bolus* of propofol 2% in healthy volunteers. J Clin Pharmacol, 2003;43:397-405.
11. Ando R, Watanabe C – Characteristics of propofol-evoked vascular pain in anaesthetized rats. Br J Anaesth, 2005;95:384-392.
12. Hart B – Diprivan: a change of formulation. Eur J Anesthesiol, 2000;17:71-73.
13. Rau J, Roizen MF, Michael F, Doenicke AW, O'Connor M, Strohschneider U – Propofol in a emulsion of long- and medium-chain triglycerides: the effect on pain. Anesth Analg, 2001;93:382-384.
14. Doenicke AW, Roizen MF, Rau J et al – Pharmacokinetics and pharmacodynamics of propofol in a new solvent. Anesth Analg, 1997;85:1399-1403.
15. Paul M, Dueck M, Kampe S, et al – Pharmacological characteristics and side effects of a new galenic formulation of propofol witout soyabean oil. Anaesthesia, 2003;58:1056-1062.
16. Banaszczyk MG, Carlo AT, Milan V et al. – Propofol phosphate, a waterdrug soluble propofol prodrug: in vivo evaluation. Anesth Analg, 2002;95:1285-1292.
17. Cooke A, Anderson A, Buchanan K et al. – Water-soluble propofol analogues with intravenous anaesthetic activity. Bioorg Med Chem Lett, 2001;11:927-930.
18. Trapani A, Laquintana V, Lopedota A et al. – Evaluation of new propofol aqueous solution for intravenous anesthesia. Int J Pharm, 2004;278:91-98.
19. Bielen SJ, Lysco GS, Cough WB – The effect of a cyclodextrin vehicle on the cardiovascular profile of propofol in rats. Anesth Analg, 1996;82:920-924.
20. Trapani G, Latrofa A, Franco M – Inclusion complexation of propofol with 2-hydroxypropyl-beta-cyclodextrin: physicochemical, nuclear magnetic resonance spectroscopic studies, and anesthetic properties in rat. J Pharm Sci, 1998;87:514-518.
21. Huang YW, Buerkle H, Lee TH et al – Effect of pretreatment with ketorolac on propofol injection pain. Acta Anaesthesiol Scand, 2002;46:1021-1024.

22. Iwama H, Nakane M, Ohmori S et al – Nafamostat mesilate, a kalicrein inhibitor, prevents pain on injection with propofol. Br J Anesth, 1998;81:963-964.
23. Ohmizo H, Obara S, Iwana H – Mechanism of injection pain with long and long-medium chain triglyceride emulsive propofol. Can J Anaesth, 2005;52:595-599.
24. Fujii Y, Nakayama M – A lidocaine/metoclopramide combination decreases pain on injection of propofol. Can J Anesth, 2005;52:474-477.
25. Doenick AW, Roizen MF, Rotzen MF et al – Reducing pain during propofol injection: the role of solvent. Anesth Analg, 1996;82:472-474.
26. Martinez V, Coutinho SV, Thakur S et al – Differential effects of chemical colonic irritation on behavioral pain response to intraperitoneal acetic acid in mice. Pain, 1999;81:179-186.

Resumen: Sudo RT, Bonfá L, Trachez MM, Debom R, Rizzi, MDR, Sudo, GZ – Caracterización Anestésica de la Nanoemulsión no Lipídica de Propofol.

Justificativa y objetivos: El uso clínico de la formulación lipídica del propofol, causa dolor durante la inyección, reacción alérgica y crecimiento microbiano. El propofol ha sido reformulado en diferentes presentaciones no lipídicas para reducir los efectos adversos, pero esos cambios pueden modificar su farmacocinética y farmacodinámica. En este trabajo, investigamos la farmacología y la toxicología del propofol lipídico (CLP) y de la nanoemulsión no lipídica (NLP).

Método: El CLP y el NLP fueron infundidos en la vena yugular de ratones midiendo la presión arterial (PA), frecuencia cardíaca (FC) y frecuencia respiratoria (FR). Las dos formulaciones (1%) fueron infundidas (40 μL.min-1) durante 1 hora. Dosis hipnóticas y anestésicas y recuperaciones, fueron determinadas. El dolor inducido por el vehículo del CLP y NLP se comparó por medio del conteo del número de contorciones abdominales ("writhing test") después de la inyección intraperitoneal en ratones. El ácido acético (0,6%) fue usado como control positivo.

Resultados: Las dosis hipnóticas y anestésicas con 1% CLP (6,0 ± 1,3 y 17,8 ± 2,6 $mg.kg^{-1}$, respectivamente) y 1% NLP (5,4 ± 1,0 y 16,0 ± 1,4 $mg.kg^{-1}$, respectivamente), no fueron significativamente diferentes. La recuperación de la hipnosis y de la anestesia fue más rápida con NLP que con CLP. Las alteraciones de FC, PA y FR causadas por el NLP no fueron significativamente diferentes de las del CLP. El ácido acético y el vehículo del CLP provocaron 46,0 ± 2,0 y 12,5 ± 0,6 contorciones en 20 minutos después de la inyección *i.p.*, respectivamente. No se observaron contorciones abdominales con vehículo de NLP. Ninguna respuesta inflamatoria abdominal fue notada con la inyección *i.p.* de los dos vehículos de propofol.

Conclusiones: El NLP puede representar una mejor alternativa que el CLP para la anestesia venosa, con menores efectos adversos.