Anaesthetic effects of propofol polymeric micelle: a novel water soluble propofol formulation

F. Ravenelle¹*, P. Vachon², A. E. Rigby-Jones³, J. R. Sneyd³, D. Le Garrec¹, S. Gori¹, D. Lessard¹ and D. C. Smith¹

¹Labopharm Inc., 480 Blvd. Armand-Frappier, Laval, Québec, Canada H7V 4B4. ²Department of Veterinary Biomedicine, Faculty of Veterinary Medicine, University of Montreal, CP 5000, Saint-Hyacinthe, Québec, Canada J2S 7C6. ³Peninsula College of Medicine and Dentistry, The John Bull Building, Research Way, Tamar Science Park, Plymouth PL6 8BU, UK

*Corresponding author. E-mail: fravenelle@labopharm.com

Background. As a result of its very low water solubility, propofol is generally presented as a lipid-based formulation with well-characterized limitations.

Methods. Propofol (99.7%) was added directly to an aqueous solution of poly(N-vinyl-2-pyrrolidone)-block-poly(D,L-lactide) copolymers (PVP-PLA) block copolymers and stirred in order to obtain a clear solution. This formulation was filtered sterile and then lyophilized to form Propofol-PM (propofol polymeric micelle) which reconstitutes to a 1%w/v (10 mg ml⁻¹) clear aqueous solution of 30–60 nm propofol-containing micelles.

Population pharmacokinetic data from whole blood and plasma were obtained by administering reconstituted Propofol-PM formulations and a 1% oil in water formulation, Diprivan to male Sprague–Dawley rats (n = 40) at a dose of 10 mg kg⁻¹. Preliminary recovery data were obtained from a further small study.

Results. The pharmacokinetics were best described using a two-compartment mamillary population model, which incorporated sample matrix (blood or plasma) and propofol formulation (Diprivan or Propofol-PM) as covariates. Sample matrix was applied to all structural model parameters as a dichotomous covariate. An influence of propofol formulation was observed for all parameters (excluding distributional clearance) but only when plasma was used for propofol quantification. In this preliminary pharmacodynamic study, there was no statistically significant difference in the timing of the recovery endpoints between the Propofol-PM formulation and Diprivan groups.

Conclusions. Propofol-PM formulations produce anaesthesia in rats. Whole blood pharmacokinetics of Propofol-PM did not differ from those observed with Diprivan.

Br J Anaesth 2008; 101: 186–93

Keywords: anaesthetics i.v, propofol; copolymer; formulations, polymeric micelles; pharmacodynamics; pharmacokinetics, propofol

Accepted for publication: March 24, 2008

Major problems with commercially available lipid formulations of propofol include pain on injection which may be partially addressed by co-administration with lidocaine¹ and support of bacterial growth² which may be moderated by the addition of benzyl alcohol, EDTA³ or sulphite.⁴ After prolonged infusion of lipid containing propofol formulations a syndrome of metabolic acidosis and progressive organ dysfunction may rarely follow.⁵ Numerous approaches to propofol formulation have recently been reviewed.⁶ Non-lipid formulations have been described but their bioequivalence cannot be assumed.⁷

We have recently used amphiphilic diblock copolymers of poly(N-vinyl-2-pyrrolidone) and poly(D,L-lactide), PVP-PLA, to dissolve propofol inside micelles of ~30–60 nm in diameter and evaluated their in vivo and in vitro characteristics.⁸ This transparent and clear formulation is then lyophilized to form a solid lyophilizate, Propofol-PM (propofol polymeric micelle) that instantaneously reconstitutes to a clear solution.

© The Board of Management and Trustees of the British Journal of Anaesthesia 2008. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/bja/article-abstract/101/2/186/260314 by guest on 29 July 2018
upon addition of an aqueous medium. Figure 1 illustrates how a propofol-loaded PVP-PLA micelle transports and delivers its cargo. In the present study, we have compared the pharmacokinetics in blood and plasma of propofol administered as propofol 1% in soya oil (Diprivan®) and three lipid and preservative-free propofol 1% solutions reconstituted from Propofol-PM of various drug:polymer ratios; 7, 10, and 12%, respectively. We also undertook a preliminary pharmacodynamic (PD) study comparing the same formulations.

**Methods**

Poly(N-vinyl-2-pyrrolidone)-block-poly(D,L-lactide) (PVP-PLA) copolymers (Fig. 1) were prepared as previously described. Briefly, N-vinyl-2-pyrrolidone is polymerized using a radical initiator in isopropyl alcohol using mercaptoethanol as a chain transfer agent. This procedure yields poly(N-vinyl-2-pyrrolidone), PVP, bearing one hydroxyl end-group (PVP-OH) of low number average molecular weight ($M_n = 1900$ Da) and of relatively narrow molecular weight distribution ($M_w/M_n 1.5$). Using sodium hydride, a PVP-OH macroinitiator is activated to polymerize D,L-lactide by ring opening anionic polymerization in tetrahydrofuran, THF. Block copolymers used in the presented studies met the predetermined specifications with 36.7% weight of PLA and number average molecular weight ($M_n$) of 4000 Da ($M_w/M_n ≤ 1.15$). Weight percentage of PLA in PVP-PLA was determined by elementary analysis.

Propofol (99.7%) was provided by Albemarle Corporation as lot PL-PROP-62.

**Propofol-PM formulation**

Propofol-PM formulations were prepared at different drug loading levels according to the following process. The ratio of polymer to propofol was varied in order to evaluate drug (propofol) loading levels of 7, 10 and 12%. These correspond to the following equation:

$$\text{Loading\%} = \frac{\text{Weight}_{\text{propofol}}}{(\text{Weight}_{\text{propofol}} + \text{Weight}_{\text{polymer}})} \times 100\%$$

As a first step, PVP-PLA block copolymer was dissolved in sodium phosphate buffer (pH 7.4). Propofol was then added to the polymer solution and stirred until a homogenous clear solution, free of precipitate or floating droplets, was obtained. The solution was then filtered using 0.2 μm filters before being frozen and lyophilized to a solid freeze-dried cake and stored at room temperature. The formulations were reconstituted with water for injection, which spontaneously dissolved the freeze-dried cake to form a clear, isotonic and neutral, aqueous solution of propofol 1% w/v (10 mg ml$^{-1}$). We studied four propofol formulations: propofol 1% in soya oil (Diprivan®) and three lipid-free propofol 1% solutions reconstituted from Propofol-PM 7, 10 and 12% loading, respectively.

![Fig 1](https://academic.oup.com/bja/article-abstract/101/2/186/260314/187)

Fig 1 (a) Chemical structure and schematic representation of a poly(N-vinyl-2-pyrrolidone)-block-poly(D,L-lactide) (PVP-PLA) copolymer. Number of repeating units are distributions with number average values where $n ≈ 22$ and $m ≈ 20$. The dark grey segment of copolymer represents the water-soluble PVP moiety and the light grey segment represents the lipophilic PLA moiety. (b) Schematic representation of a propofol-loaded micelle of PVP-PLA (Propofol-PM) in solution above its critical micelle concentration (CMC ~ 6 ppm) (left) and after injection and dilution below its CMC (right).
High-performance liquid chromatography (HPLC) method

Propofol assay in solid (dry) and reconstituted Propofol-PM formulations were performed using a validated HPLC method. The system comprised a Hewlett Packard HPLC, model 1100 equipped with a variable wavelength detector set at 275 nm, a normal phase column (Inertsil SIL-150A, 250 × 4.6 mm, 5 μm) and a hexane:acetonitrile:ethanol (990:7.5:1) mobile phase at 25°C. System suitability tests performed for each analytical run showed a percentage recovery of 99–101%. Relative standard deviation (SD) of standards and quality control samples assay were <2%. All assays were performed in triplicate.

Animal welfare

All experiments were conducted in accordance with guidelines from the Canadian Council on Animal Care and use of laboratory animals.

Study design

Pharmacokinetic study

Forty rats were randomly allocated to one of eight groups (five rats/group). Ten rats were used for each of the four propofol formulations: five rats for the collection of blood samples and five for plasma samples. PD data were not collected. Male Sprague–Dawley rats weighing 325 (SD 10) g (mean ± SD) were anaesthetized with isoflurane and both jugular veins exposed through a single small incision. Isoflurane administration was then discontinued and anaesthesia allowed to lighten until they responded with movement after interdigital hind paw pinch. Animals then received a single dose of propofol 10 mg kg\(^{-1}\) in a volume of 1 ml kg\(^{-1}\) via the jugular vein over 1 min. Blood samples (0.3 ml) were obtained from the jugular vein contralateral to the dosing site at 1, 3, 5, 7.5, 10, 15, 30, 60, and 75 min after the end of the injection. Animals that had recovered from propofol anaesthesia were briefly exposed to isoflurane to abolish movement before sampling.\(^{10}\)

Sample processing and bioanalysis

Blood samples were frozen (−80°C) pending assay, plasma samples were prepared by centrifugation of fresh blood at 3200 g for 10 min (4°C) and the collected plasma was frozen (−80°C). Blood and plasma samples were stored for less than 2 weeks before analysis by a validated Liquid Chromatography Mass Spectrometry assay.\(^{11}\)

Sensitivity and selectivity

The precision (coefficient of variance) and accuracy at the lower limit of quantification were determined in six replicates of plasma samples containing propofol 20 ng ml\(^{-1}\). The precision obtained was 5.7% and the accuracy was 95.0%. Extracted blank plasma did not show any interference from endogenous substances. The calibrated analytical range was set from 0.020 to 20 μg ml\(^{-1}\).

Precision and accuracy

The intra-batch reproducibility of the method was evaluated by analysing six replicates of plasma samples containing propofol at the nominal concentration of 0.020, 1 and 20 μg ml\(^{-1}\) in three individual runs. The coefficient of variation observed was 6.0, 7.7, and 5.7%, respectively. The observed accuracy was 99.0, 97.3, and 99.8%, respectively.

Pharmacokinetic (PK) and PD parameters

Determination of PK parameters

Mixed-effects population models\(^{12}\) were fitted to the log-transformed propofol blood and plasma concentration vs time data. The program NONMEM version 6\(^{13}\) was used.

Determination of PD effects

We undertook a preliminary study to compare the PD effects of the different propofol formulations. Male Sprague–Dawley rats weighing 319 (9) g (mean ± SD) were anaesthetized with isoflurane and a single jugular vein exposed. Isoflurane administration was then discontinued and anaesthesia allowed to lighten until they responded with movement after interdigital hind paw pinch. Animals then received a single dose of propofol 10 mg kg\(^{-1}\) in a volume of 1 ml kg\(^{-1}\) via the jugular vein over 1 min. No blood samples were collected.

Anaesthetic concentration was determined using the withdrawal reflex by pinching interphalangeal skin with fine forceps.\(^{14}\) The withdrawal reflex was tested immediately at the end of the injection and every 30 s until a positive response was obtained. Time of the first spontaneous movement and time to recover (righting reflex) were also recorded. The PD and PK studies were performed on the same day using the same drug batch preparations. The number of animals receiving a single 10 mg kg\(^{-1}\) i.v. dose of propofol as Diprivan\(^{13}\) 1%, and aqueous solutions 1% reconstituted from Propofol-PM 7, 10, and 12% were 5, 4, 5 and 6, respectively. These numbers were determined by the amount of Propofol-PM remaining after completion of the PK study.

Statistical analysis

Statistical analysis was performed using StatXact (Cytel Software, Cambridge, MA, USA), a package that is particularly suited to the analysis of small, unbalanced data sets. A non-parametric ANOVA test (Kruskal–Wallis) was conducted to test the null hypothesis that there was no difference between the underlying (population) medians for each of the recovery endpoints in each of the formulation groups. Where a difference was indicated by the Kruskal–Wallis test, this was further investigated by conducting a series of Mann–Whitney tests with subsequent...
Bonferroni correction of the resulting P-values. Statistical significance was set a priori at P < 0.05.

Results
No animals suffered any ill effects after the administration of Diprivan® or the Propofol-PM micelle formulations. Measured concentrations of propofol were 10.15, 9.91, and 10.29 mg ml\(^{-1}\) for injectates reconstituted from Propofol-PM 7, 10, and 12% DLL respectively, and these were used to calculate the administered doses before injection.

PK study
The observed blood propofol concentrations for all formulations were similar. Plasma concentrations were lower than blood concentrations and plasma concentrations after administration of the Propofol-PM formulations were generally lower than those after Diprivan®.

The optimal PK model for this data set was one that incorporated both sample matrix (blood vs plasma) and propofol formulation as covariates. Sample matrix, as a dichotomous variable, was applied to all PK structural model parameters.

An influence of propofol formulation was observed on the values for all the structural parameters (excluding Q, distributional clearance) but only when plasma was used as the sample matrix. The effect of formulation was not apparent when whole blood concentrations of propofol were measured. The influence of propofol formulation in the plasma group animals was coded as a dichotomous covariate: Diprivan® or Propofol-PM formulation (all types). Models with separate estimates of theta (i.e. structural model parameters) for each formulation (Diprivan®, Propofol-PM 7%, Propofol-PM 10%, Propofol-PM 12%) were either (1) not statistically superior to the dichotomous model or (2) were better statistically but the confidence intervals (CI) for the estimates of theta for each of the PM formulations overlapped entirely and there was no improvement in the diagnostic plots relative to the dichotomous model.

Average population PK model predicted profiles after a single dose of 1% propofol are presented in Figure 2. The typical population PK parameter values and their 95% CI are given in Table 1. (For a full explanation of the model build, please see the supplementary data at British Journal of Anaesthesia online.) Figure 3 shows the basic goodness-of-fit plots for the final model. The median performance error (MDPE) and median absolute performance error (MDAPE) were calculated for the final population model as described by Varvel et al.\(^{15}\) The MDPE, a measure of the extent of bias in the population PK model, was −0.7% indicating that the model is essentially unbiased. The MDAPE, a measure of the inaccuracy of the model, was 19.5%.

PD study
Figure 4 shows the median, range, and interquartile range of each recovery endpoint for each of the propofol formulations. The Kruskal–Wallis analysis suggested that statistically significant differences (P < 0.01) existed between the formulation groups for time of withdrawal reflex and time of first movement. However, when this was investigated further using a series of Mann–Whitney tests, none of the formulations produce significantly different times for any of the PD endpoints after the P-values were adjusted (Bonferroni correction) to account for the multiple comparisons.

Discussion
We studied PK and PD separately. This constraint was imposed by the cannula-free technique used for central venous access, which has been demonstrated to minimize the stress response and leaves the animals in a suitable state for subsequent full recovery.\(^{10}\) Intermittent isoflurane administration achieves the necessary immobility for sampling with less stress than that imposed using
Table 1 Parameter estimates for the final pharmacokinetic model. The typical values are the geometric means of the individualized parameter estimates. The 95% confidence intervals (CI) are symmetric approximations and were calculated as the parameter estimate ± 1.96 × the standard error of the estimate. The coefficient of variation was calculated, where possible, as the typical magnitude of the ETA variable (reflecting inter-individual variability) associated with that parameter. NA, not applicable (no ETA parameter associated with the parameter).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical value</th>
<th>95% CI</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (CL), ml min⁻¹</td>
<td>28.5</td>
<td>24.7–32.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Plasma Diprivan®</td>
<td>42.8</td>
<td>38.3–47.3</td>
<td></td>
</tr>
<tr>
<td>Plasma Propofol-PM</td>
<td>12.9</td>
<td>12.0–13.8</td>
<td></td>
</tr>
<tr>
<td>Blood (all formulations)</td>
<td>17.5</td>
<td>12.2–22.8</td>
<td></td>
</tr>
<tr>
<td>Distributional clearance (Q), ml min⁻¹</td>
<td>50.7</td>
<td>34.0–67.4</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of central compartment (V1), ml</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Plasma Diprivan®</td>
<td>235</td>
<td>158–312</td>
<td></td>
</tr>
<tr>
<td>Plasma Propofol-PM</td>
<td>519</td>
<td>438–600</td>
<td></td>
</tr>
<tr>
<td>Blood (all formulations)</td>
<td>169</td>
<td>153–185</td>
<td></td>
</tr>
<tr>
<td>Volume of peripheral compartment (V2), ml</td>
<td></td>
<td>34.9</td>
<td></td>
</tr>
<tr>
<td>Plasma Diprivan®</td>
<td>345</td>
<td>250–440</td>
<td></td>
</tr>
<tr>
<td>Plasma Propofol-PM</td>
<td>658</td>
<td>484–832</td>
<td></td>
</tr>
<tr>
<td>Blood (all formulations)</td>
<td>185</td>
<td>149–221</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacokinetics

We observed lower propofol concentrations in plasma than whole blood samples. Propofol is unevenly distributed between red blood cells and plasma with literature figures for whole blood:plasma partition coefficient for rats of 2:3 receiving the commercial oil in water formulation.\(^1\)\(^7\)\(^–\)\(^1\)\(^9\) Figure 2 suggests that the preferential distribution to red cells (and hence, lower propofol concentrations in plasma) is even more marked in animals receiving the Propofol-PM formulations possibly because of the greater total surface area of the micelle formulations compared with the emulsion. Propofol 1% (10 mg ml⁻¹) solution reconstituted from Propofol-PM 7% contains the largest amount of the PVP-PLA block copolymer and that reconstituted from Propofol-PM 12% contains the least. The quantity of the copolymer in plasma does not seem to influence the PK of propofol in this study.

With a drug which is preferentially distributed to cellular elements in the blood it is to be expected that plasma concentrations will be lower than whole blood concentrations and this is consistent with our finding that preferential distribution to red cells occurs to an even greater extent with the Propofol-PM formulations than with Diprivan® (Fig. 1).

Other researchers have demonstrated differences in propofol concentrations when measured in plasma and in whole blood.\(^2\)\(^0\)\(^–\)\(^2\)\(^1\) Fan et al. found biphasic differences in whole blood vs plasma propofol concentrations in humans after a bolus injection of propofol (2 mg kg⁻¹).\(^2\)\(^1\) Plasma concentrations were 5–10% higher than whole blood concentrations during the first 10 min and subsequently ~10% lower during the terminal phase (at 60, 120 and 240 min post-dose). The initially higher plasma concentrations were attributed to a lag in propofol distribution into erythrocytes and the lower plasma concentrations during the terminal phase to slow redistribution of propofol from blood cells to plasma against rapid plasma clearance. Fan et al. also compared propofol concentrations in plasma from blood which had been immediately centrifuged, and plasma from blood which had been kept at 4°C for 1 h prior to centrifugation. Storage increased the plasma propofol concentration by 5–10%. In the present study, blood samples were stored for up to half an hour before centrifugation.

Pharmacodynamics

We were unable to show any statistically significant differences in any of the recovery endpoints, neither between any of the test micelle formulations and the control formulation (Diprivan®) nor between the three different micelle formulations relative to each other. However, the number of animals in the PD study was small (n = 4–6 per formulation) and there was a high degree of variability in the recovery endpoints. Given this, it is likely that our study was underpowered to detect small differences. Figure 4 shows a trend towards longer recovery times for each endpoint as drug loading increases and the slowest recovery was seen in those animals receiving injectate reformulated from Propofol-PM 12%. As this formulation has the highest propofol loading level there would have been proportionately less of the PVP-PLA block copolymers than in the injectate reformulated from Propofol-PM 7%. If propofol continues to partition between red blood cells, the polymer, and the aqueous phase after injection, then this might alter the amount of ‘free’ propofol available to diffuse towards the effect site. In man, free propofol comprises only 1.2–1.7% of the whole blood concentration.\(^1\)\(^9\) Any change in the free fraction of propofol could result in an increase in intrinsic potency if this results in a higher concentration being achieved in the brain. This would be equivalent to giving a larger dose and a slightly longer effect would be anticipated. Such an interpretation must be considered speculative and could be investigated using appropriate in vitro techniques.

When the recovery endpoint times are plotted on the population PK model predicted plasma concentrations (using the model developed from the different animals
the PK study group), the predicted plasma concentrations were consistently higher in the Diprivan® animals (data not shown). However, because of the small number of animals in each group for the PD study, and the large within-group variability, this finding should be interpreted cautiously. Additionally, it must be borne in mind that these data refer to predicted plasma concentrations and not to predicted concentrations at the effect site. Furthermore, others have suggested that, in rats, drug bound to the formed elements of blood participates in the uptake and transfer of propofol to its effect site. Hence, it may be more appropriate to consider whole blood rather than plasma concentrations.

Other lipid-free propofol formulations
Changing the formulation of an i.v. anaesthetic might alter its PD – possible by delaying or altering redistribution or access to the effect site. A lipid-free propofol formulation in hydroxypropyl-P-cyclodextrin 20% caused bradycardia and hypotension which did not occur with control administrations of either the cyclodextrin vehicle or Diprivan®. However, a similar formulation of propofol in hydroxypropyl-beta-cyclodextrin was comparable with Diprivan® when evaluated in rabbits. Propofol in sulphobutyl ether-β-cyclodextrin had nearidentical pharmacokinetics and PD to Diprivan® when evaluated in a porcine model.

Dutta and Ebling prepared a propofol/ethanol/water formulation and found that this produced an arterial plasma concentration–time profile markedly different from that of Diprivan®. Post-infusion propofol concentrations declined in a biexponential manner for the emulsion formulation whereas the lipid-free formulation exhibited a monoexponential decline. The differences were attributed to extensive lung sequestration of the ethanol-based formulation and manifest as a delay in time to maximal effect and delayed offset of sedation. However, this study used an unusual method of drug administration with the formulation created in situ by mixing the flow from two syringe drivers. It is unclear whether the formulation could be considered stable and it is possible that large droplet formation could have contributed to the substantial depot effect the authors observed in the lung.

Certainly, other non-lipid propofol formulations appear bioequivalent with no significant difference in the efficacy...
of the Cremophor® EL (another micellar solution) and lipid emulsion formulations other than a small increase in the minimum sleep dose with the emulsion formulation in rats.25 However, Cremophor® EL should be regarded as a special case of a micellar formulation in that it possesses a terminal half-life of ~80 h.26 It is possible that this slow elimination combined with a small volume of distribution may counteract the increased surface area for Cremophor® EL micelles, that is it is retaining propofol preferentially in the plasma similar to oil droplets in Diprivan®. Results presented here seem to indicate that PVP-PLA is rapidly eliminated and thus allowing the combination of both high surface area and rapid elimination.

Reconstituted Propofol-PM formulations are anaesthetic in rats with whole blood PK equivalent to those of the standard emulsion formulation. The possibility of small differences in PD and their possible relationship to the propofol loading dose of the dried formulation requires further investigation. Studies using a continuous measure of drug effect such as an EEG derivative would permit clearer understanding of any differences. Caution is appropriate before extrapolating from a rat model to the likely effects of Propofol-PM were it to be administered to man.

**Conclusion**

Propofol-PM formulations cause similar anaesthesia in rats to Diprivan®. Results have shown equivalent blood pharmacokinetic profiles while identifying differences in plasma profiles. Plasma Propofol-PM formulations present slightly lower concentrations which may reflect a faster distribution to erythrocytes and potential higher potency.

**Supplementary data**

For a full explanation of the model build, please see the supplementary data at *British Journal of Anaesthesia* online.

**Acknowledgement**

We are grateful to Dr Francis Beaudry for his expertise in performing the bioanalytical LC/MS/MS work.
Funding
This study was funded by Labopharm Inc., 480 Blvd. Armand-Frappier, Laval, Québec, Canada, H7V 4B4.

References
12. Wright PMC. Population based pharmacokinetic analysis: why do we need it; what is it; and what has it told us about anaesthetics? Br J Anaesth 1998; 80: 488–501
23. Egan TD, Kern SE, Johnson KB, Pace NL. The pharmacokinetics and pharmacodynamics of propofol in a modified cyclodextrin formulation (Captisol) versus propofol in a lipid formulation (Diprivan): an electroencephalographic and hemodynamic study in a porcine model. Anesth Analg 2003; 97: 72–9