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Research Paper

A Novel, Lipid-Free Nanodispersion Formulation of Propofol and Its Characterization

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Purpose. Propofol is a widely used anesthetic agent with highly desirable fast "on" and "off" effects. It is currently formulated as lipid emulsions, which are known to support microbial growth. In this study, a novel, lipid-free nanodispersion formulation of propofol was characterized.
Methods. The formulation was evaluated for its physical and chemical stability, *in vitro* compatibility with red blood cells, and its antimicrobial effectiveness. *In vivo* pharmacokinetic and pharmacodynamic properties of the formulation were evaluated in rats.
Results. Our data suggest that this lipid-free formulation is physically and chemically stable. Compared to the commercial emulsion formulation Diprivan, it causes less hemolysis with red blood cells and has improved antimicrobial activity. In addition, the lipid-free formulation demonstrates similar pharmacological effects to Diprivan in rats.
Conclusions. This novel, lipid-free formulation exhibits improved *in vitro* properties without compromising *in vivo* effects, therefore representing a promising new alternative for propofol.

KEY WORDS: antimicrobial effectiveness; hemolysis; lipid-free; nanodispersion; propofol.

INTRODUCTION

Propofol (2,6-diisopropyl-phenol) is a lipophilic anesthetic agent currently formulated in two soybean oil–based lipid emulsions for human use (Diprivan from AstraZeneca, Wilmington, DE, USA, and the generic Propofol from Gensia-Sicor, Irvine, CA, USA).

Following intravenous injection of formulations, propofol rapidly crosses the blood-brain barrier and induces fast onset of anesthesia (1–3). Subsequent rapid distribution and high metabolic clearance of propofol allow for fast, clean emergence from anesthesia and short duration of unconsciousness (4).

Despite the highly desirable fast "on" and "off" pharmacological effects, the current commercial formulations have several drawbacks associated with their lipid-emulsion nature. The key component, soybean oil, can cause lipid overload in those patients who are infused for a long duration (>3 days) of the formulation (5–9). The lipid emulsions support microbial growth, and they have to be handled under strict aseptic conditions to avoid bacterial contamination (10–12). Also, the emulsions need to be stored under controlled temperatures (4–22°C) (9). Finally, the emulsions are not thermodynamically stable (13). The oil droplets are stabilized with phospholipids in the form of purified egg lecithin and are sensitive to conditions such as ionic strength, pH, and temperature (14).

All of these drawbacks can be mitigated through a modification to the formulation to remove the lipid emulsion. In this article, a novel, stable, lipid-free nanodispersion formulation of propofol and its *in vitro* and *in vivo* characterization are described.

MATERIALS AND METHODS

Materials

All chemicals were obtained from Spectrum Chemicals (Gardena, CA, USA) except for polysorbate 80, which was obtained from Croda International (Edison, NJ, USA), and propofol, which was purchased from Zambon Group (Vicenza, Italy). All components are USP grade.

Formulation Identification

The formulation, TPI-213F, was identified using TransForm's high-throughput formulation screening technology (15). Briefly, 38 i.v.-acceptable excipients were selected for the formulation screen and prepared as full factorial combinations in glass tubes, with each combination containing 3 of the 38 excipients. These excipient combinations were first inspected using a custom optical inspection station (16) for their physical compatibility. Only those giving minimum background turbidity were selected to be screened with propofol. In total, more than 8000 propofol formulations were prepared. They were agitated and incubated before they were

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inspected again. Formulations giving low turbidities were considered as hits, and their compositions were used as a basis for formulation optimization. TPI-213F has a final optimized composition of 1% w/w propofol, 5% w/w poloxamer 188, 4% w/w PEG 400, 1.5% w/w polysorbate 80, 1% w/w propylene glycol, and 0.2% w/w citric acid.

Formulation Preparation

TPI-213F was prepared by first weighing out all excipients and dissolving them in de-ionized water at room temperature. Propofol was then added to the predissolved excipients, and more water was added if necessary to reach 100% of the target weight. The mixture was stirred for 2 h before it was filtered through an 0.20-μm filter (Sartorius, Goettingen, Germany).

Formulation Characterization

Propofol and impurities in TPI-213F were separated and quantified using a high performance liquid chromatography (HPLC) method. The sample was diluted in 60% acetonitrile-water mixture. Chromatographic separation of propofol from other components in the formulation was achieved with the conditions listed in Table I. A Waters C18 column (Waters, Milford, MA, USA; 3 × 150 mm, 5-μm particle size, heated to 45°C) was used with an injection volume of 10 μl and a flow rate of 0.8 ml/min. Propofol was detected at a wavelength of 272 nm.

The particle size distribution (as characterized by mean particle size and polydispersity index) of the formulation was measured using photon correlation spectroscopy (Zetasizer $3000HS_A$, Malvern Instrument, Southborough, MA), which covers a particle size range of 5 nm to 3 μm.

Physical stability of TPI-213F was assessed using gravitational stress. The formulation was centrifuged at 2000 × *g* for 2 h. Particle size distribution was measured before and after the centrifugation.

Formulation Stability

Chemical and physical stability of TPI-213F were assessed under different storage conditions. The formulation was subdivided into 2-ml type I borosilicate glass vials with Teflon-coated butyl stoppers and aluminum crimped tops. The vials were stored upright at long-term storage (25 ± 2°C/60 ± 5% RH) and accelerated (40 ± 2°C/75 ± 5% RH) conditions. At various time points, chemical degradation of propofol was detected and quantified using the HPLC method described above. Physical changes (in terms of mean particle size and polydispersity index) were monitored with photon correlation spectroscopy.

Table I. Chromatographic Separation Method for Detection of Propofol and Impurities in TPI-213F

Time (min)	A: Deionized water with 0.05% trifluoroacetic acid B: Acetonitrile with 0.05% trifluoroacetic acid
0	60% A, 40% B
25	60% A, 40% B
45	0% A, 100% B
47	60% A, 40% B
55	60% A, 40% B

Antimicrobial Effectiveness Test

The ability of TPI-213F to resist microbial growth was tested at Lancaster Labs (Lancaster, PA, USA) and compared to that of Diprivan. Suspensions of four standard USP preservative efficacy test organisms, *Staphylococcus aureus, Escherichia coli, Candida albicans*, and *Pseudomonas aeruginosa*, were added to TPI-213F or Diprivan at approximately 100 and 200 colony-forming units (CFUs) per ml. The inoculated formulations were incubated at 20–25°C and 30–35°C and tested for viable counts after 24 and 48 h.

In Vitro Hemolysis

In vitro hemolysis of TPI-213F was assessed using fresh human whole blood. This study was performed at MDS Pharma Services (Montreal, Canada). Blood was obtained from two human volunteers of different gender and compatible blood type. Both volunteers signed written consent forms. Blood samples were pooled and subdivided into two portions before each of the portions was spiked with Diprivan or TPI-213F. Final concentrations of propofol in both samples were 10 μg/ml. Both samples were incubated at 37°C. At 0.25, 0.75, 1, 1.5, and 2 h post-onset of incubation, aliquots (in triplicate) of the whole blood were removed from each sample and centrifuged at 3200 × *g* for 10 min to obtain plasma. The plasma samples were analyzed for hemoglobin content by measuring the absorbance at 415 nm.

Pharmacokinetic Study

The pharmacokinetic study was carried out at MDS Pharma Services in male Sprague-Dawley rats (7 weeks old, average weight 280 g, from Charles River Canada, St-Constant, QC, Canada). All animals were handled according to established guidelines and principles. Following an overnight fast, 2 groups of 4 rats each were dosed 10 mg/kg TPI-213F over the period of about 1 min (slow push) via jugular venipuncture.

Following the dose administration, blood samples were collected by jugular venipuncture at various time points into tubes containing heparin. Blood samples from group 1 were centrifuged at 3200 × *g* and 4°C for 10 min. The resulting plasma samples were harvested and stored at –20°C for analysis of propofol. Blood samples from group 2 were stored directly at –20°C for analysis. All animals were observed constantly during dose administration and blood sampling period. At the end of the sampling, all rats were humanely sacrificed.

The samples were analyzed for propofol using a liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. A Zorbax Extend C18 column (4.6 × 12.5 mm, 3.5 μm) and an isocratic mobile phase consisting of 0.1% ammonia in 30/70 (v/v) water/acetone were used. Run time was 5 min at a flow rate of 1.0 ml/min. Detection of propofol was performed on a triple quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization source operated in the negative ion mode.

Pharmacokinetic analysis of mean propofol concentrations in plasma and blood was performed using MDS's PhAST Software Program (Version 2.3, Phoenix International Life Sciences Inc., Montreal, Canada). The area under the concentration vs. time curve to the last measurable con-

centration (AUC_{0-t}) was calculated using the linear trapezoidal method (17). The observed terminal phase rate constant (k_{el}) was calculated as the slope of the terminal portion of the log concentration vs. linear time curve by linear regression. The area under the concentration vs. time curve from zero to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_{0-t} and the ratio of the last measurable concentration by k_{el}. The terminal phase half-life ($t_{1/2}$) was calculated by dividing 0.693 by k_{el}. In addition, the plasma or blood clearance (CL) and the apparent volume of distribution (V_{dss}) were also calculated.

The red blood cell (RBC)/plasma partition coefficient K_P was determined for TPI-123F using the following equation:

$$Kp = AUC_{RBC}/AUC_{plasma} = [(AUC_{blood}/AUC_{plasma}) - (1 - H)]/H$$

where AUC is $AUC_{0-\infty}$ and H is the hematocrit. A nominal value of H = 0.45 was used for rats.

Pharmacological Study

Male Sprague-Dawley rats from Charles River Canada were used for the pharmacological study at MDS Pharma Services. The animals were purchased precannulated in the femoral vein. Shortly after their arrival, they were fitted in infusion jackets and tether systems designed to allow injection of the formulations in the implanted catheter while allowing for free movement of the animals. Eighteen animals were divided into three study groups (Table II). On day 1 (period 1), rats were dosed their respective formulations (Diprivan, TPI-213F, or saline control) over a period of 1 min (slow push) via the surgically implanted catheter. Following dose administration, 0.5 ml of saline was used to flush the catheter to ensure complete administration of the dose. The end of the saline flush was taken as time zero (t = 0). After each administration, the time to loss of locomotor activity was recorded for each animal. This was defined as the time at which the animal lost the capability to regain ventral recumbency once manually placed on its side. Rats were maintained in dorsal or lateral recumbency during evaluation, and the time to regain locomotor activity was recorded. Following recovery from anesthesia after period 1, animals were returned to their respective cages and offered food. Period 2 took place after a 72-h washout period. The animals were crossed over and dosed their respective formulations in a similar manner as period 1 and were monitored in the same fashion. After period 2, the rats were humanely sacrificed.

RESULTS AND DISCUSSION

Formulation Characterization and Stability

TPI-213F is a lipid-free nanodispersion of propofol dispersed in mixed surfactants polysorbate 80 and poloxamer 188 and cosolvents polyethylene glycol 400 and propylene glycol. It is a very slightly opalescent liquid. Based on the photon correlation spectroscopy measurements, the formulation has an average diameter of 90 nm and a polydispersity index of 0.6, and the maximum particle size in the formulation is 200 nm.

After the formulation was centrifuged at 2000 × *g* for 2 h, no phase separation or changes in particle size distribution was observed.

Table II. Dosing Schedule for the Pharmacodynamics Study in Rats

Period	Group ID	Test article	Dose (mg/kg)	Dose volume (ml/kg)	No. of rats
1	1	Saline	0	1	6
	2	Diprivan	10		6
	3	TPI-213F	10		6
2	1	Saline	0		6
	2	TPI-213F	10		6
	3	Diprivan	10		6

With the HPLC method used, propofol eluted at around 21 min. The two major degradants of propofol, the quinone and the propofol dimer, eluted at around 19 min and 36 min, respectively. Both degradants are known impurities and oxidation by-products for propofol (18). Figure 1 indicates that the formulation maintained its chemical stability over 9 months. Minimal degradation of propofol (<0.5%) was detected at both 25°C/60% RH and 40°C/75% RH. In addition, no detectable changes in mean particle size, polydispersity index, or maximum particle size were observed at either storage condition during the course of the stability study.

Antimicrobial Effectiveness

As expected, Diprivan, which contains 0.005% disodium edetate as the bacteriostatic agent, did not support further microbial growth from the initial inoculations. Compared to Diprivan, TPI-213F killed all of the organisms at both microbial challenge levels (Fig. 2 A and B). These data suggest that the lipid-free TPI-213F formulation not only inhibits further bacterial growth, it also has an intrinsic preservative effect in the absence of any added antimicrobial agents. The exact mechanism behind this observed intrinsic preservative effect is not clear and is beyond the scope of this study. However, literature does suggest that citric acid (19–20) and poloxamers (21–22) have some antimicrobial activities. So they may have both contributed to the intrinsic resistance of TPI-213F to microbial growth.

In Vitro Hemolysis

Visual appraisal of hemolysis prior to hemoglobin content determination indicated that there was evidence of he-

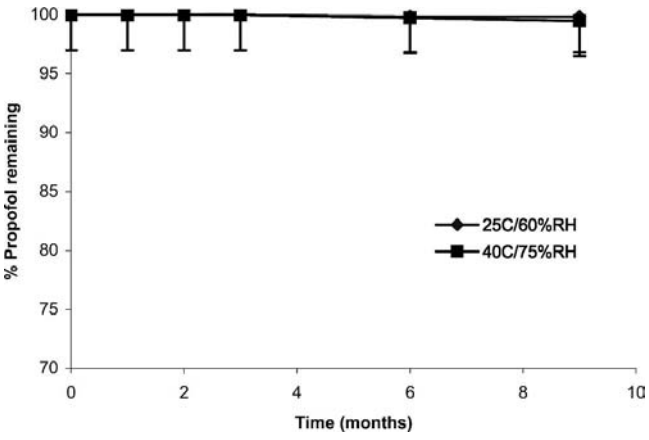

Fig. 1. Chemical stability of TPI-213F. The HPLC method used has a precision of 3%, as established by three injections of a propofol standard solution.

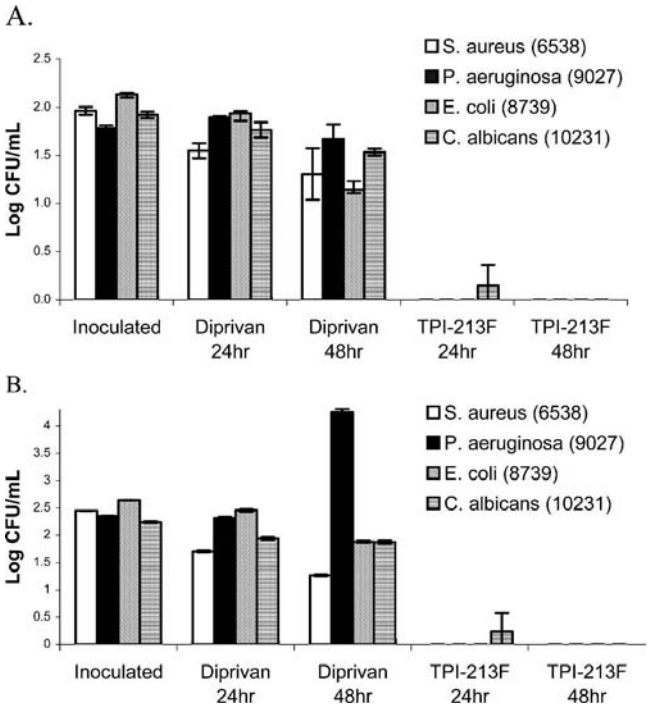

Fig. 2. Comparison of microbial resistance of TPI-213F and Diprivan after (A) 100 CFU/ml inoculation and incubation at 20–25°C and (B) 200 CFU/ml inoculation and incubation at 30–35°C.

molysis in the Diprivan sample at 2 h following onset of incubation. In contrast, no visual evidence of hemolysis was observed for the TPI-213F sample at any of the time points.

Mean concentrations of hemoglobin in plasma following incubation with Diprivan and TPI-213F are plotted in Fig. 3. Consistent with visual observations, TPI-213F showed lower hemoglobin ($p < 0.05$, Student's *t* test) concentrations at all time points compared to Diprivan (Fig. 3). This indicates that TPI-213F is milder on red blood cells than Diprivan.

Because the main difference between the lipid-free TPI-213F and Diprivan is that Diprivan is formulated as an oil-in-water emulsion, where propofol is dissolved in soybean oil and emulsified by egg lecithin and the emulsion tonicity is adjusted by glycerol, our data suggest that one or more of these emulsion components are facilitating the disruption of the red blood cell membranes. For instance, previous literature observations suggest that glycerol is hemolytic (23).

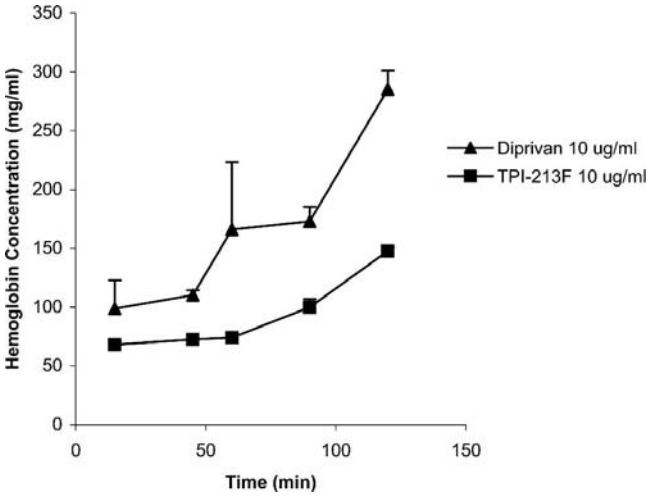

Fig. 3. Comparison of *in vitro* red blood cell hemolysis of TPI-213F and Diprivan. Values plotted are mean ± standard deviation, n = 3.

Pharmacokinetic Study

The plasma and blood concentration-time profiles of TPI-213F are shown in Fig. 4. The pharmacokinetic parameters calculated are shown in Table III. The formulation showed higher propofol concentrations and AUC values in blood than in plasma, suggesting significant partition of propofol into rat red blood cells. This is consistent with published literature (24). The RBC/plasma partition coefficient (K_p) was estimated to be 5.5 for TPI-213F, suggesting a high degree of preferential distribution of propofol into the rat red blood cells when it is formulated in the emulsion-free formulation TPI-213F. In comparison, Diprivan shows slightly lower preferential partitioning into rat red blood cells with a K_p of 3.8 (24).

This difference in preferential red blood cell partitioning observed for TPI-213F in rats may be explained by the following hypothesis based on the physical and chemical differences of the two formulations. Propofol is a highly lipophilic drug, with a log octanol:water partition coefficient of 4.33 (4,25). In Diprivan, propofol is dissolved in the soybean oil phase of the oil-in-water emulsion droplets. When Diprivan is injected into the systemic circulation, propofol exists in the blood as an equilibrium among free propofol, protein-bound propofol, soybean oil–dissolved propofol, and propofol in red blood cell membranes. TPI-213F, on the other hand, is a nanodispersion of propofol stabilized with mixed surfactants and cosolvents. In the blood, the mixed surfactant micelles are likely diluted below their critical micelle concentrations and de-aggregate into single surfactant molecules, hence giving the "naked" propofol a greater thermodynamic driving force to partition into the red blood cell membranes. Although not examined in this study, we can hypothesize that distribution of propofol into other lipid-rich components of blood (e.g., chylomicrons) likely also differs between the two formulations, with propofol from TPI-213F partitioning more into these other lipid-rich phases than that from Diprivan.

Other calculated pharmacokinetic parameters are shown in Table III. V_{dss} values for TPI-213F were calculated to be 5912 ± 1511 for plasma and 1282 ± 275 ml/kg for blood. Clear-

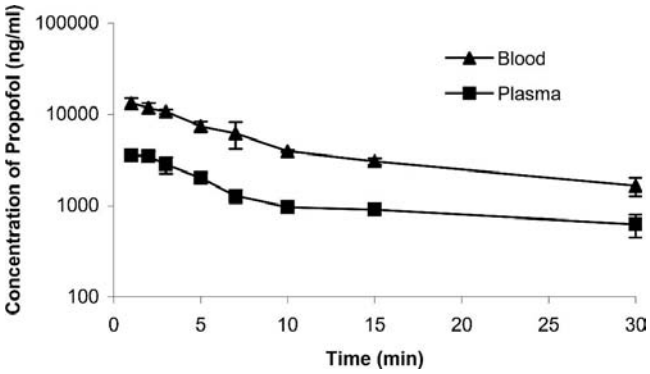

Fig. 4. Propofol plasma and blood concentration *vs.* time curves for TPI-213F in rats. Values plotted are mean ± standard deviation, n = 4.

Table III. Mean Pharmacokinetic Parameters for Propofol in Plasma and Blood of Rat Following a Single Intravenous Dose of TPI-213F[a]

Group	Test article	Matrix	Dose (mg/kg)	$AUC_{0-\infty}$ (ng · min/ml)	$t_{1/2}$ (min)	V_{dss} (ml/kg)	CL (ml/min · kg)
1	TPI-213F	Plasma	10	69,826 (22,629)	34.1 (16.5)	5912 (1511)	156 (54)
2	**TPI-213F**	**Blood**	**10**	**178,518 (39,373)**	**19.8 (9.6)**	**1282 (275)**	**58 (12)**
	Diprivan	Plasma	12.5	Not reported	27.0 (23.2)	3200 (2432)	160 (43.2)

$AUC_{0-\infty}$, the area under the concentration *vs.* time curve from time zero to infinity; $t_{1/2}$, terminal phase half-life; V_{dss}, apparent volume of distribution; CL, plasma or blood clearance.

[a] The numbers in parentheses are the standard deviations. Plasma pharmacokinetic parameters for Diprivan obtained from Dutta *et al.* (26).

ance for TPI-213F for plasma and for blood was 156 ± 54 and 58 ± 12 ml min^{-1} kg^{-1}, respectively (Table III).

The plasma pharmacokinetic parameters for TPI-213F can be compared to those for Diprivan obtained from the literature (26) (Table III). Based on the Student's *t* test, there was no statistically significant difference in any of the parameters from the two formulations.

Pharmacological Study

The mean time values to loss and re-appearance of locomotor activity are plotted in Fig. 5 for TPI-213F and Diprivan. None of the animals dosed with the saline control exhibited loss of locomotor activity during either dose period. Following administration of Diprivan and TPI-213F in both periods, animals rapidly lost locomotor activities (within 1 min of dose administration). On average, both groups of animals given propofol formulations regained their locomotive activities after 8 min. The time for the animals to lose and regain locomotor activity was essentially the same for both formulations of propofol ($p < 0.005$).

Results from this preliminary pharmacodynamic study in rats suggest that TPI-213F has very similar pharmacological effects as Diprivan. Based on these data, it may be inferred that the difference in propofol RBC partitioning does not significantly affect the partition of the drug to the site of action (e.g., the central nervous system) in rats, hence explaining the similarity in the pharmacological effects observed.

It remains to be seen whether these pharmacokinetic and pharmacodynamic observations we made in the rats would translate to humans; and if they do, what impact they would have on the clinical end points.

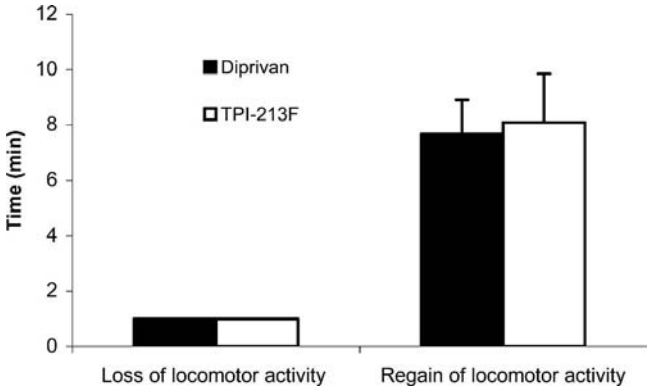

Fig. 5. Pharmacological effects of TPI-213F and Diprivan in rats. Values plotted are mean ± standard deviation, n = 6.

CONCLUSIONS

In this article, we characterized a novel, lipid-free propofol formulation, TPI-213F. This formulation, which is a clear aqueous nanodispersion of propofol, was shown to have chemical and physical stability, and it had a better red blood cell compatibility and improved microbial resistance compared to Diprivan. The formulation also had a similar pharmacological effect in rats as Diprivan. The combined data suggest that this novel nanodispersion formulation of propofol can be an excellent alternative to the current emulsion formulations.

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