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The Effect of Propofol on a Forced Swim Test in Mice at 24 Hours

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ABSTRACT

Background: There are few rapidly acting treatments for acute suicidality or treatment-resistant depression. Propofol (2,6-diisopropylphenol) is an intravenous anesthetic agent used in outpatient settings. It is a gamma-aminobutyric acid type A agonist and has affinity at the N-methyl-D-aspartate receptor. Elevation in mood and sociality in humans has been observed following propofol-induced anesthesia. Other authors reported an open-label study of repeated dosing of propofol in treatment-resistant depression in which several patients experienced sustained improvement. Recently, we reported that in a rodent model of despair, a forced swim test, 45 minutes after administration of 50 mg/kg propofol, immobility time was significantly reduced.

Objective: The objective of the experiment was to determine whether the antidepressant-like effects of a single dose of propofol in mice are sustained for 24 hours.

Methods: The time spent immobile during a forced swim test 24 hours after intraperitoneal administration of a single dose of propofol 50 mg/kg or 0.9% saline was evaluated in 24 adult male mice (C57/BL6). Immobility time was quantified and evaluated with a custom video analysis software program.

Results: Propofol-treated mice were immobile for a mean (SEM) time of 115 (13) seconds, whereas saline-treated mice were immobile for a mean (SEM) time of 94 (14) seconds. A 2-tailed unpaired t test found no significant difference between the treatment groups ($t = 1.07$, $df = 22$; $P = 0.30$).

Conclusions: Twenty-four hours after intraperitoneal administration, the effect of propofol on immobility time was not statistically significantly different from vehicle. However, given our previous report of at least a short-term benefit of propofol on struggling time in the forced swim time and an encouraging pilot study in humans with treatment-resistant depression, further evaluation of propofol's antidepressant potential may be warranted.

Introduction

Several recent preclinical and clinical investigations of potential new treatments for depressive illness have addressed central neural systems modulated by N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid (GABA).[1] Ketamine, esketamine, rapastinel, and other investigational agents with varying properties of NMDA receptor activity have produced rapid-onset antidepressant-like effects in rodent models of depression and in human patients with depressive illness.[2–7] A nasally inhaled version of ketamine, esketamine (Spravato; Janssen Pharmaceuticals, Inc, Titusville, New Jersey), was recently approved by the Food and Drug Administration in combination with an orally administered antidepressant for treatment-resistant depression. Intravenously administered brexanolone, which modulates GABA type A (GABA-A) and has a rapid onset of action, was recently approved by the Food and Drug Administration for treatment of postpartum depression.

Propofol (2,6-diisopropylphenol) is a GABA-A agonist intravenous anesthetic agent that also inhibits NMDA receptors.[8–11] These pharmacologic actions overlap with those of esketamine and brexanolone. Propofol is commonly administered in ambulatory

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settings because of its rapid onset, dose-related hypnotic effect, rapid recovery, and favorable safety profile.[10,12] If there was sufficient preclinical evidence of antidepressant potential to further investigate propofol clinically, substantial human pharmacokinetic and safety information would be available as a background for clinical testing.

Recently, we reported that propofol 50 mg/kg ($P < 0.05$) but not 35 mg/kg (P = not significant) administered intraperitoneally 45 minutes before administration of a forced swim test (FST) reduced immobility time compared with in the saline–saline control group (difference between means of 38.42 and 16.46 seconds, respectively).[13] The FST is a commonly applied animal model that assesses behaviors related to depression and resilience to stress. Multiple putative antidepressants that modulate GABAergic and glutaminergic neurotransmission have demonstrated antidepressant-like effects in variations of the FST paradigm.[14–19] Ketamine, R-ketamine, and S-ketamine (esketamine), have been reported to reduce immobility compared to vehicle in the FST 24 hours after intraperitoneal administration in mice preconditioned with stress.[17–19] The GABA-A positive allosteric modulator, MRK-016, has also been reported to demonstrate an antidepressant-like effect on the FST in mice 24 hours postinjection.[20] Moreover, ketamine, R-ketamine, and esketamine, which like propofol modulate the NMDA receptor, have exhibited sustained antidepressant effects in humans in clinical trials.[7,21,22] In some clinical trials with these agents, antidepressant effects have been observed after a single intravenous dose.[7,23,24]

Our report of reduced immobility time in the FST 45 minutes after mice were administered propofol is consistent with multiple anecdotal and other reports of improved mood in humans following propofol induced anesthesia and with a recent small, open-label study in which repeated dosing of propofol reduced Hamilton Depression Rating Scale scores by a mean of 20 points in patients with treatment-resistant depression.[25–27,28–33]

Our rationale for testing an animal model of the persistence of behaviors associated with depression and resilience to stress after administration of propofol was based on recent evidence consistent with a role for disordered GABAergic and glutaminergic neurotransmission in the pathophysiology and treatment of depression[1,14–20,33,34]; propofol's central GABA-A agonist and NMDA antagonist activity,[8–11] which overlaps with the mechanisms of compounds that have exhibited antidepressant-like activity in animal models for up to 24 hours and in clinical trials; our earlier finding of propofol's reduction of immobility in an FST model of resiliency and depressive-like behavior 45 minutes after administration[13]; anecdotal reports of mood elevation after propofol anesthesia[25–27,28–33]; the pilot study by Mickey et al[27] observations of sustained antidepressant effects from propofol in patients with treatment-resistant depression.

Based on the above observations, we hypothesized that 24 hours after administration, mice that received propofol would demonstrate statistically significantly decreased immobility time during an FST compared with mice that received an inactive control. The dose and sample size in the current study were informed by our earlier study of propofol 45 minutes after administration.[13] The current study was conducted at a subsequent time point on a separate sample of mice under a different protocol than our first study and is therefore reported separately.

Method

The present study was designed to test the effects of 50 mg/kg propofol on the behavior of mice in an FST performed 24 hours postadministration. The time spent immobile during the FST following intraperitoneal dosing was compared with a control group administered 0.9% saline. Saline was selected as the control for consistency with our earlier investigation of the effects of propofol on an FST in mice 45 minutes after administration.[13] The biotechnical experiments described in the report were performed at Charles River Laboratories (South San Francisco, California). All raw data are located in the archive of Charles River Laboratories by study number Key 1745. The internal study report was authored by Popescu and Janssens.[35] Data will be stored for a period of 10 years after completion of the final report.

Table 1
Compounds

Group	Substance	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Route
1	Saline	0.9% saline	5	IP	
2	Propofol	50	10		

IP = intraperitoneal.

Animals

Twenty-four adult male mice (C57/BL6) provided by Charles River Laboratories and aged 8 to 9 weeks and weighing 20 to 25 g were used in the experiment. Before the experiment, the animals were group housed in plastic cages (2–4 animals/cage) and had access to food and water ad libitum. Animals were kept on a 12/12 hour light/dark cycle and acclimated to the housing environment for at least 5 days. Experiments were approved by the Institutional Animal Care and Use Committee of Charles River Laboratories. The sample size of 12 mice per arm of the study and male sex were selected for consistency with our earlier investigation of the effects of propofol on a FST 45 minutes after administration.[13]

Compounds formulation

A mixture of 0.9% saline and a commercially available formulation of propofol injectable emulsion (10 mg propofol and 100 mg soybean oil/mL) (Zoetis, Parsippany, New Jersey) were used as is, preformulated by the vendors. The dose, concentration, volume, and route of administration of saline and propofol are shown in Table 1.

Experimental procedures

Twenty-four hours before testing, mice were randomly assigned (ie, A, B, A, B, and so on) to the treatment groups, and dosed intraperitoneally as described in Table 1. On the day of the FST, animals were brought to the experimental room and allowed to acclimate for at least 60 minutes before the beginning of the experiment. The FST was performed during the lights on phase during daytime hours.

Animals were placed in cylindrical containers filled with water warmed to 26°C (±2°C). Each session lasted 5 minutes, after which animals were dried, allowed 5 minutes to recover on a heating pad and returned to their home cage. The entire procedure was video recorded for off-line analysis. The individuals who conducted the behavioral assay and data analyses were blind to treatment group and were different individuals than those who administered the compound.

Data analysis

The duration of animal immobility during FST was evaluated: a mouse was considered immobile when it ceased struggling and remained floating in the water making only those movements necessary to keep its head above water. Immobile behavior was quantified with custom video analysis software.

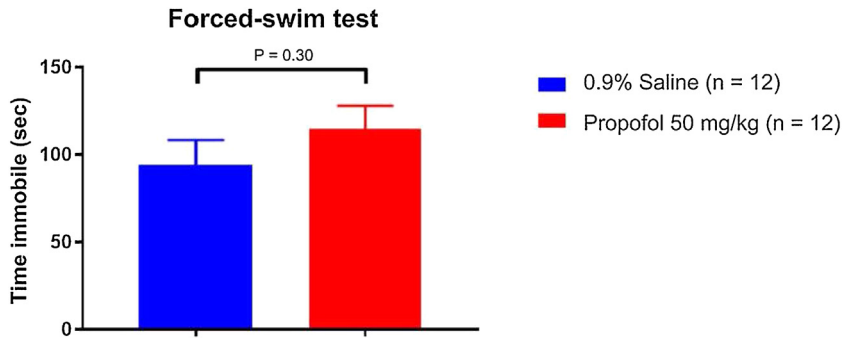

Fig. 1. Time spent immobile during the forced swim test, 24 hours after intraperitoneal dosing, expressed as mean (SEM).

Table 2
Summary statistics (time immobile in seconds).

	Saline 0.9% (n = 12)	Propofol 50 mg/kg (n = 12)
Minimum	1	10
25th percentile	68	89
Median	88	116
75th percentile	121	155
Maximum	176	170
Mean	94	115
Standard deviation	49	45
Standard error of the mean	14	13
	Saline 0.9% vs propofol 50 mg/kg (n = 24)	
Difference between means*	21 (19)	
95% confidence interval	−19 to 61	
η^2	0.05	

* Value is presented as mean (SD).

Behavioral data were analyzed, and results plotted by Charles River Laboratories using Prism Graphpad software (San Diego, California). A 2-tailed unpaired *t* test was used to compare the results from the 2 treatment groups. The Grubb test was used to identify statistically significant outliers. No statistically significant outliers were identified by Grubb test and no mice were excluded from analysis. Significance was set at $P < 0.05$.

Results

Mice received intraperitoneal administration of compounds, and 24 hours later underwent 5 minutes of FST. Sessions were video recorded and scored offline, to identify the percentage of time spent immobile. The Fig. 1 shows the average results for each group of treatment. The average time spent immobile during the 5 minute FST test is presented for the groups of mice injected 24 hours prior with either saline or propofol, as described in Table 1.

As shown in Table 2, propofol-treated mice were immobile for a mean (SEM) 115 (13) seconds, whereas saline treated mice were immobile for mean (SEM) 94 (14) seconds. A 2-tailed unpaired *t* test found no significant difference between the treatment groups ($t = 1.07$, df = 22; $P = 0.30$).

Discussion

Previously, we reported that compared with saline, propofol 50 mg/kg IP significantly decreased immobility time during an FST administered 45 minutes after the injection. To assess whether this effect was enduring, we conducted a second study in which wild-type mice were administered either vehicle, or 50 mg/kg propofol, and 24 hours later tested via an FST. The time spent immobile (floating behavior) was quantified. Animals treated with 50 mg/kg propofol 24 hours prior did not show a statistically significant difference time spent immobile during the FST compared with vehicle.

The ineffectiveness of a single injection of propofol in decreasing FST immobility at 24 hours might have been influenced by relatively rapid elimination of propofol from the mouse brain. Guan, Wu and Jiang[36] reported that the half-life of propofol in the brain of mice was 9.6 (0.5) minutes. The current study did not address whether propofol would have significantly influenced immobility time after a less ambitious interval (eg, 4–12 hours) or with a higher dose or with repeated dosing. Rapid clearance and awakening after a bolus of propofol occurs in humans as well as mice.[10,37] However, the behavioral, cognitive, neurophysiological and synaptic plasticity effects of propofol, ketamine, and brexanolone and other anesthetic agents have been reported to persist in rodents and humans substantially longer than would be anticipated by their pharmacokinetic and pharmacodynamic profiles alone.[2,4,11,17,26,27,33,34,38–41] For example, in their review, Browne and Lucki[41] observed that "the majority of studies indicate that the FST remains sensitive to the protracted effects of ketamine up to 1 week after a single injection." Thus, the rapid clearance of propofol is unlikely to explain the lack of influence on an FST at 24 hours.

Feng et al[38] reported that a single dose of propofol 75 mg/kg IP statistically significantly shortened struggling time in the FST and tail suspension test, reduced expression of CD11b and increased expression of p-STAT-3 in the brain tissues of C57BL/6 mice. They speculated that the decreased struggling time might have been mediated by interference in microglial function stemming from these brain tissue changes. In contrast, Wu et al[11] found potentially beneficial effects of propofol in reducing microglial inflammation through NMDA receptor inhibition. Nevertheless, taken together, the study by Feng et al[38] and ours are consistent with the notion that a single dose of propofol in C57BL/6 mice does not have enduring antidepressant-like effects.

Yang et al[17] reported that in C57BL/6 mice conditioned by the social defeat stress model, using the tail suspension and forced swimming tests, R-ketamine (10 mg/kg IP) and rapastinel (10 mg/kg IP) (both of which, in common with propofol, have an affinity for the NMDA receptor) significantly attenuated immobility time compared with the vehicle-treated group. In contrast, our mice and Feng's mice were not subjected to stress or depressive model preconditioning.[38] This may have been a factor in the lack of a salutary effect of propofol on immobility time in our study as well as the study by Feng et al.[38]

The results of our experiment could also have been influenced by testing during the lights-on phase because mice are normally inactive during daylight. For example, the work by Rantamaki et al[42] work is consistent with notion that the timing of the experiment with relation to the dark-light cycle has the potential to influence sleep or the slow-wave activity necessary to consolidate neuroplastic effects related to antidepressant-like activity.

Conclusions

In contrast to our previous report in which testing was performed 45 minutes after injection, propofol 50 mg/kg IP did not impact FST immobility time, a mouse model used to screen antidepressants, statistically significantly differently than vehicle 24h after injection in 24 C57/BL6 mice. Our study design had a number of significant limitations that caveat interpretation of our findings, including, for example, use of a single mouse strain, the fixed dose design, single administration, one behavioral assay, lack of stress preconditioning, and the single test point at 24 hours. A lipid emulsion might be advantageous as a control in future studies because of its similarity to the propofol emulsion vehicle. Mickey et al's encouraging open-label findings in treatment resistant depression as well as our earlier FST findings 45 minutes after injection suggest that additional exploratory preclinical and clinical work to investigate the potential antidepressant effects of propofol may be warranted.

Declaration of Competing Interset

D. G. Daniel is president of Bioniche Global Development, LLC, which provided funding for this study. All authors are inventors or co-inventors of a pending patent that includes propofol for the rapid treatment of depression and suicidality. N. Daniel, D. T. Daniel, L. Copeland-Flynn, and M. Allen are consultants to Bioniche Global Development, LLC.

No author has a financial interest in the video system used to record and analyze the results of the experiment.

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