The Effects of Intravenous Lipid Emulsion Therapy in the Prevention of Depressive Effects of Propofol on Cardiovascular and Respiratory Systems: An Experimental Animal Study

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Abstract: Background and objective: Propofol is an anesthetic agent that is frequently used in anesthesia induction, maintenance and sedation. Propofol has severe side effects such as hypotension, bradycardia and respiratory depression. Although propofol is commonly used, there is no known antidote for its toxic effects. An approach to prevent toxic effects of propofol would be beneficial. The aim of this study was to assess the effects of intravenous lipid emulsion (ILE) therapy in the prevention of depressive effects of propofol on cardiovascular and respiratory systems.

Materials and methods: Twenty-eight Sprague-Dawley adult rats were randomly divided into 4 groups. The saline-administered group was determined as the Control group. The second group was administered propofol (PP group); the third group was administered ILE (ILE group), and the fourth was administered propofol with ILE therapy (ILE+PP group). Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Mean arterial blood pressure (MAP), Respiratory rate (RR), Heart rate (HR) and mortality were recorded at 10 points during 60 minutes. A repeated measures linear mixed-effect model with unstructured covariance was used to compare the groups. Results: In the PP group, SBP, DBP, MAP, RR and HR levels were declining steadily; all rats in this group died after 60 minutes. In the ILE+PP group, after a while, the decreased SBP, DBP, MAP, RR and HR levels increased SBP, DBP, MAP, RR and HR levels of the Propofol group were found to be significantly lower than those of the other groups (p<0.01). The mortality rate was 100% (surviving period, 60 min) for the PP group, whereas 0% for the ILE, ILE+PP and Control groups. Conclusion: Our results suggest that undesirable side effects that can be seen after propofol application such as hypotension, bradycardia and respiratory depression might be prevented by using ILE therapy.

Keywords: Acute Toxicity; Cardiovascular Depression; Intravenous Lipid Emulsion; Propofol; Rat Model; Respiratory Depression
1. Introduction

Propofol is the general anesthetic used for anesthesia induction and its continuation. It is also commonly used for sedation [1]. Propofol is generally safe but it does not have an antidote. It has severe side effects such as cardiovascular depression and hypotension. The most common cardiovascular effect of propofol is causing hypotension by lowering systemic vascular resistance, cardiac contractility and cardiac preload.

There are many studies examining the cardiac depressant effects of propofol in the literature. In one of those studies, the incidence of hypotension (SBP <90 mmHg) was found to be at 15.7% in patients receiving induction dose propofol, and 77% of the hypotension attacks were recorded within the first 10 minutes. In the same study, bradycardia was observed in 4.8% of the patients (HR<50 pulse/min), and 42% of the bradycardia attacks were recorded in the first 10 minutes [2]. Abdul Zahoor et al. reported decrease in blood pressure and pulse values in patients who had minor surgery after propofol application [3]. In the study by Ebert et al. on healthy volunteers, propofol and placebo were compared and a significant decrease was reported in respiratory rate, blood pressure and pulse rate in the propofol group [4].

Intravenous lipid emulsion (ILE) therapy was first used to prevent the toxic effects of local anesthetics [5]. Weinberg et al. reported ILE to be an efficient therapy method for hemodynamic recovery in resuscitated rats with bupivacaine overdose and again in dogs with bupivacaine overdose [5, 6]. In later studies, it was shown that it could be used as an effective antidote in poisonings caused by lipophilic drugs [7]. ILE therapy has been used successfully in toxicities due to many lipophilic drugs such as beta blocker [8], calcium channel blocker [9], and tricyclic antidepressants [10], apart from local anesthetics [11]. In the case report of Tsui et al., it was stated that vasopressor and fluid treatment resistant hypotension were recovered with ILE therapy after post-epidural anaesthesia [12].

In addition to these characteristics, it is considered that ILE can be effective on reversing the respiratory depression, vasodilatation and cardiac depressant effects of propofol due to its high lipophilicity.

The aim of this study was to assess the effects of ILE therapy in the prevention of depressive effects of propofol on cardiovascular and respiratory systems.

2. Materials and Methods

The ethical approval for this study was provided by the Ethical Committee of Yeditepe University, Medical Faculty, Experimental Animals Research Laboratory (Atasehir, Istanbul, Turkey 34755) on 05 April 2016 with the protocol number 529. Authors declare here that all applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures and practices performed in the study involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

Consent for this study was taken from the ethical board of experimental animals, and the ethics committee allowed an upper limit of 28 rats.

2.1. Subject Selection

This research was carried out in May 2016. 28 Sprague-Dawley adult rats of 200-300 g weights were used for the study. All experimental animals were kept in a day and night cycle for 12 hours each at 24±4°C for seven days before the start of the experiment, and their adaptation to the environment was provided. Standard diet and water were given to the rats.

2.2. Experiment Protocol and Groups

The hairs in the lower parts of the bodies of the rats were shaved to make the applications easier. They were kept in supine position with their extremities stabilized during the study.

Inhaler isoflurane (Isoflurane, Isofludem 100 ml) was used in all rats, and anesthesia was provided with an animal anesthesia machine. After providing satisfactory anesthetic depth, femoral
artery cannulation was made with 26-G catheter in all groups (i.v. NEO ALPHA, La-med Healthcare, Haryana, India). Using a disposable pressure transducer set (OKUMAN Medikal Sistemler Ltd. Şti., Ankara, Turkey), momentary blood pressure follow-up was provided with a standard cardiac monitor (NIHON KOHDEN Cardiolife Monitor TEC-7721 K, NihonKohden Corporation, Tokyo, JAPAN). Four rats died due to intervention complications during the application of femoral artery cannulation. The study was continued with six rats in each group.

Electrocardiography electrodes were placed on both the forefeet and right hind legs. Respiratory and pulse rates were followed with the same monitor (NIHON KOHDEN Cardiolife Monitor TEC-7721 K, NihonKohden Corporation, Tokyo, JAPAN). Tail vein cannulation was made with 26 G branule (i.v. NEO ALPHA, La-medHealthcare, Hayrana, India) in all groups to apply the drugs used in the study. The propofol used in the study was given with ILE and 0.9% NaCl infusion pump (SwissMade, ArcomedAgVolumed VP7000, Switzerland). ILE (ClinOleic %20 lipid 500 ml, ECZACIBAŞI Baxter/Belgium), Propofol (PROPOFOL-LİPURO %1, 10 mg/ml, I.V. 20 Ml Ampul for Infusion, İlaçsan®/Turkey) and 0.9% NaCl were used.

Dosages, drug application methods and experimental protocols were determined according to the previous studies and the mentioned sources [8, 13, 14]. Rats were randomly separated into four equal groups.

Group 1 (n=6): In the Control group, 0.9% NaCl was applied in the form of infusion in four minutes with a dose of 16 mL/kg as a pro-drug.

Group 2 (n=6): In the PP group, 0.9% NaCl was applied in the form of infusion in four minutes with a dose of 16 mL/kg as a pro-drug. After pretreatment with 0.9% NaCl, propofol was applied i.v. bolus with a dose of 42 mg/kg at the fifth minute.

Group 3 (n=6): In the ILE group, ILE was applied in the form of infusion in four minutes with a dose of 16 mL/kg as a pro-drug.

Group 4 (n=6): In the ILE+PP group, ILE was applied in the form of infusion in four minutes with a dose of 16 mL/kg as a pro-drug. After pretreatment with ILE, propofol was applied i.v. bolus with a dose of 42 mg/kg at the fifth minute.

At 10 different points in the first 60 minutes (0, 1, 3, 5, 6, 8, 10, 20, 30 and 60th minutes), 5 different measurements of each rat (Systolic blood pressure (SBP), Diastolic blood pressure (DBP), mean arterial blood pressure (MAP), Respiratory rate (RR) and Heart rate (HR)) were taken. Additionally, cardiac or respiratory arrest occurrences up to the 60th minute was observed. The mortality times were registered for the rats which died after the drug application. Decapitation was provided in all rats with guillotine under anesthesia at the 60th minute.

2.3. Statistical Examinations

Trend analysis approach examines the relation between the treatment order and response size. Trend analyses in clinical studies focus on causal treatment effects rather than simple comparisons [19]. Of the 24 rats, only 18 of them had completed data. A total of 240 data points were available for statistical analyses (24 rats × 10 time points). In our study, there were missing values due to the death of the rats in the PP group. All rats in the PP group died at different points of the first 60 minutes. We had 15 time points out of 240 (~%6) instead of 6 out of 24 rats (%25). The main aim of the statistical analyses was to demonstrate the significant differences between the repeated measures in the four experimental groups. Thus, due to the unbalanced data structure, we aimed to use the linear mixed-effect model (LMM) to compare the groups. Changes in values in the groups were compared using an unstructured covariance linear mixed model. P values <0.05 were considered statistically significant. All mixed model statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).
3. Results

In the PP group, cardiac arrest developed in 1 rat at the 15th minute, 1 rat at the 20th minute, 1 rat at the 25th minute, 1 rat at the 40th minute, 1 rat at the 44th minute and 1 rat at the 55th minute, and no rats remained in the PP group after this period (Figure 1). Unlike the PP group, mortality was not observed in the other three groups of rats within 60 minutes.

Figure 1. Survival rate of rats in PP group versus time.

Linear mixed model was conducted to compare Systolic Blood Pressure (SBP), Diastolic blood pressure (DBP), Mean Arterial Blood Pressure (MAP), Respiratory Rate (RR) and Heart Rate (HR) values between groups. SBP, DBP, MAP, RR and HR values in the PP group were found to be lower than those of the Control, ILE and ILE+PP groups (p<0.05). (Table 1, Figure 2B, 3B, 4B, 5B, 6B).

Table 1: Differences of least squares means (MD) and adjusted p (Adj p) value of all pairwise comparisons among groups (1: Control, 2: PP, 3: ILE, 4: ILE+PP).

<table>
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<td>-3.78 1.000</td>
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<td>-28.41 &lt;.0001</td>
<td>-4.95 0.937</td>
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<td>MAP</td>
<td>28.68 &lt;.0001</td>
<td>0.91 1.000</td>
<td>0.08 1.000</td>
<td>-27.77 &lt;.0001</td>
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<td>RR</td>
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<td>-27.25 &lt;.0001</td>
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<td>HR</td>
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<td>-24.08 0.183</td>
<td>22.85 0.234</td>
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<td>- &lt;.0001</td>
<td>46.93 0.0012</td>
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Bonferroni’s correction was used for multiple comparisons.
Figure 2. **A)** Least squares means of Systolic blood pressure (SBP) values of Control, PP, ILE and ILE+PP groups of rats versus time. **B)** Least squares means of SBP values (Error bars: 95% confidence interval).

Figure 3. **A)** Least squares means of Diastolic blood pressure (DBP) values of Control, PP, ILE and ILE+PP groups of rats versus time. **B)** Least squares means of DBP values (Error bars: 95% confidence interval).
Figure 4. A) Least squares means of Mean arterial blood pressure (MAP) values of Control, PP, ILE and ILE+PP groups of rats versus time. B) Least squares means of MAP values (Error bars: 95% confidence interval).

Figure 5. A) Least squares means of Respiratory rate (RR) values of Control, PP, ILE and ILE+PP groups of rats versus time. B) Least squares means of RR values (Error bars: 95% confidence interval).
Figure 6. A) Least squares means of Heart rate (HR) values of Control, PP, ILE and ILE+PP groups of rats versus time. B) Least squares means of HR values (Error bars: 95% confidence interval).

As a result of the pairwise comparisons, we found that RR values of the ILE group were higher than those of the Control group (mean difference=13.13 Count/Min., p<0.0001). The difference between SBP, DBP, MAP and HR values in the Control and ILE groups was not significant (p>0.05) (Table 1, Figure 2B, 3B, 4B, 5B, 6B).

SBP values of the ILE+PP group were found to be significantly lower than the Control group (mean difference=7.80 Count/Min., p=0.009). The difference between DBP, MAP, RR and HR values in the ILE+PP and Control groups was not significant (p>0.05) (Table 1, Figure 2B, 3B, 4B, 5B, 6B).

SBP, RR and HR values of the ILE group was found to be higher than those of the ILE+PP group. Mean differences were 7.42, 14.20, 46.93, respectively, and p values were p=0.013, p<0.0001, p=0.0012, respectively. The difference between other values (DBP and MAP) in the ILE and ILE+PP groups was not significant (p>0.05) (Table 1, Figure 2B, 3B, 4B, 5B, 6B).

4. Discussion

Cardiac side effects of propofol are related to the channel blockage of molecular level voltage-gated sodium (Na) and potassium (K) channel [15, 16]. Propofol stimulates the release of nitric oxide, blocks the calcium channel and activates protein kinase C. [17, 18]. Therefore, administration of propofol results in a decrease in cardiac output and arterial blood pressure. [2, 19]. Propofol suppresses baroreceptor reflex and decreases sympathetic nerve activity. In previous studies, it was reported that propofol formed baroreceptor inhibition-related bradycardia depending on the dose [20]. The decrease in blood pressure can be explained by supression of sympathetic nerve activity, vasodilatation and depression of myocardial contractility. Propofol is also a strong dose-related respiratory depressant [21]. The mechanisms that explain the respiratory depressant effect of propofol have not been fully clarified. Kashigawi et al. found that propofol reduced the activity of inspiratory neurons located in the medulla and caused an inhibitory effect by activation of GABA\textsubscript{A} receptors [22].

Similarly in our study, a significant decrease was observed in SBP, DBP, MAP, HR and RR values in the group that was only administered propofol, and all the rats in this group died at the end of the 60th minute. (Figure 1).
The mechanism of action of ILE therapy is not completely explained and different hypotheses are used to try to clarify this. The first hypothesis asserted on the mechanism of action of ILE is that it provides the separation of lipophilic drugs from the target tissue by forming lipid-rich compartments in the plasma (lipid-sink) [23-24]. Scavenging Effect is not only a static sink effect; it is considered a dynamic shuttle effect. The lipid compartment in the blood creates the shuttle effect by transferring the lipophilic drugs from the organs. In this way, organs with high blood flow are detoxified from the drug [25]. In one study, it has been shown that ILE therapy acutely decreased drug concentrations in the heart and brain and increased concentrations in the liver. [23].

Other hypotheses assert ILE’s cardiotonic and inotropic effects to explain the mechanism of action. In one study, it has been indicated that ILE administration increased the left ventricular contractility [26]. A study performed by Umpierrez et al. showed that ILE administration increased blood pressure in African-American patients with type 2 diabetes [27]. Nonetheless, both the inotropic and lusitropic effects of ILE have been shown in both in-vivo cardiac studies and isolated heart studies, which were conducted in the absence of drug toxicity [28, 29].

Huang et al. demonstrated that, similar to calcium (Ca) channel opening agents, long-chain fatty acids activated voltage-gated Ca channels in isolated cardiomyocytes [30]. In another study, it was also observed that ILE showed an improvement of myocyte functions by increasing intracellular Ca level [31]. Also, there are studies, the findings of which are opposite to those in the literature. XIAO et al. conducted a study on Neonatal Rat Ventricular Myocytes to examine the role of polyunsaturated fatty acids (PUFAs) on L-type Ca channel currents and sarcoplasmic reticulum Ca release. They found that PUFAs reduced intracellular Ca level by both blocking the L-type Ca channel currents and decreasing sarcoplasmic reticulum Calcium release [32]. In another study, ILE and verapamil were administered both together and separately. Both L-type Ca channel activity and intracellular Ca level on ventricular cardiomyocytes were evaluated. They found that ILE had no effect on intracellular Ca level and myocardial contractility in the absence of verapamil administration [33]. The validity of the calcium hypothesis has not been clarified yet and needs further studies.

In our study, it was observed that SBP, DBP, MAP and HR values in the group that was administered Propofol with ILE (ILE+PP group) had a decreasing course from the 5th minute, when propofol was started, until the 8th minute. After the 8th minute, an increase was observed in SBP, DBP, MAP and HR values, and these values got closer to the Control group. A similar course was detected in SBP, DBP, MAP and HR values in the Control group after the 30th minute. At this point, it is considered that ILE treatment is advantageous for preventing the hypotension and bradycardia side effects of propofol (Figure 2, 3, 4, 6).

According to findings of this study, decreased values of SBP, DBP, MAP and HR due to propofol, increased with ILE administration. The difference between SBP, DBP, MAP and HR values in the ILE and Control groups was not significant (Table 1). Therefore, We did not observe any inotropic effects in the group in which only ILE was administered. Our findings suggest that ILE has no direct inotropic effect if it is administered alone. Again, parallel to other studies, it was observed that RR values in the ILE+PP group had a decreasing course from the 5th minute, when propofol was started, until the 8th minute. After the 8th minute, an increase was observed in RR values, and these values got closer to the Control group. A similar course was detected in RR values in the Control group after the 30th minute. We observed that ILE treatment is also advantageous in preventing the respiratory depressant side effect of propofol (Figure 5).
The mortality rate of the PP group was detected as 100% (Figure 1); the survival rate was found to be 100% in the ILE+PP group. Our study is important as it shows that ILE therapy decreased propofol-related mortality. However, it is not possible to compare the results of our study with the literature, because ILE therapy was not tried to prevent propofol-induced mortality.

According to our findings, the RR level of the ILE group was found to be significantly higher than those of the other groups (Table 1). More detailed studies should be designed to explain the tachypneic effect of ILE therapy.

5. Conclusion

Due to the findings obtained during the research, it was concluded that side effects such as hypotension, bradycardia and respiratory depression, which can be seen after propofol application in rats, could be recovered with ILE therapy, and mortality due to these side effects were prevented. More extensive studies are required.

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Conflict of Interest: The authors declare that they have no conflict of interest.

References


