

Intravenous Lipid Emulsion Increased Muscles Power and Survival Time of Phenobarbital in Intoxicated Rats

Mohammad Moshiri ¹, Fatemeh Aftabi ², Maryam Esmaeili ², Leila Etemad ³ and Hossein Hosseinzadeh ^{3*}

1. Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

2. Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

3. Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Background: Phenobarbital (PHB) is an anticonvulsant drug that poisoning with it leads to stupor or coma and in higher doses may induce severe respiratory depression and cardiovascular collapse. Intravenous Lipid Emulsion (ILE) has been used to treat drug toxicity and for parenteral nutrition.

Methods: Ten male rats that had been intoxicated by 100 mg/kg of PHB were treated intravenously by 18.6 ml/kg of ILE20 or similar dose of normal saline. Subject rats were checked before PHB injection (zero time), 0.5 hr before ILE20% (or normal saline infusion) and 1, 3, and 6 hr after PHB toxic injections. On each check point, we tested the blood pressure, muscular power score and mortality rate.

Results: There were no significant differences between blood pressures of two groups at all. ILE was able to increase rats' muscular power scores at the 3rd and 6th hours check points ($p=0.007$ and 0.0371 , respectively). ILE also increased the survival period of intoxicated rats; however it did not change the mortality rate.

Conclusion: ILE was able to reverse the muscles power reduction and could improve the survival period among intoxicated rats.

Keywords: Phenobarbital, Intravenous lipid emulsion, Acute toxicity, Muscular power

Introduction

Phenobarbital (PHB) is an anticonvulsant, which causes acute poisoning due to easy availability ^{1,2}. Suicidal actions are the major cause of phenobarbital intoxication ¹. PHB poisoning leads to stupor or coma and in high dose induces severe respiratory depression and cardiovascular collapse. These may result fatal outcome up to 7% of intoxicated patients ¹. Authors of literatures suggest no especial antidote; however, they recommended some methods such as alkaline diuresis, activated charcoal and hemodialysis.

*Corresponding author

Hossein Hosseinzadeh, PhD

Pharmaceutical Research Center,
Pharmaceutical Technology Institute,
Mashhad University of Medical Sciences,
Mashhad, Iran

Tel: +98 51 3881 9042

Fax: +98 51 3882 3251

E-mail: Hosseinzadehh@mums.ac.ir

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Administration of Intravenous Lipid Emulsion (ILE) to severe intoxicated patients could reverse poisoning and rescue their lives³. Sirianni and his colleagues reported the use of ILE following 70 min of unsuccessful standard cardiopulmonary resuscitation in a 17-year-old girl who developed seizures and cardiovascular collapse after intentionally ingesting an overdose of bupropion, lamotrigine and amphetamine. Her cardiovascular status has been improved and she recovered with near-normal neurologic function⁴.

In past ten years, there are several animal studies that revealed antidotal effect of ILE against drug intoxication^{3,5-9}. Moshiri *et al* reported that ILE could reversed the hypotension, hypothermia, catalepsy and miosis of haloperidol intoxicated rabbits sooner than normal saline^{5,6}. His colleagues evaluated the ILE effect on tramadol induced seizure and hypotension in rabbit and found similar effects⁷. We evaluated the effect of ILE on PHB poisoning in this issue.

Materials and Methods

Ten male rats weighting 200-250 g were housed in cages with a 12 hr light/dark cycle and easy access to food and water. Animals were divided into two groups consisting of five rats.

Ampoules of PHB (200 mg/ml, Chemidarou, Iran) were diluted and administrated to all animals. They also received ILE 20% [Intralipid Emulsion (Frese-nius Kabi AB) Spain].

We applied 18.6 ml/kg ILE 20% or 18.6 ml/kg Normal Saline (NS), as a control, 30 min after 100 mg/kg PHB injection. ILE or NS were infused over 15 min. Rats received the injections through the vein cannula.

The animals were monitored before PHB injection (zero time), 0.5 hr (before ILE 20% or NS infusion) and 1, 3, and 6 hr after PHB injection. The muscular powers of animals were evaluated by scoring scale as Grade 4: normal mobility; Grade 3: ataxic gait; Grade 2: stretch movements after tail stimulation; Grade 1: standing after tail stimulation; Grade 0: no voluntary movements after tail stimulation, four limb paralysis⁹. All animals were evaluated by an individual unaware of the groups. Blood pressures of all animals were measured using tail-cuff method (Power lab, Data Acquisition Systems, US). Mortality rates and survival

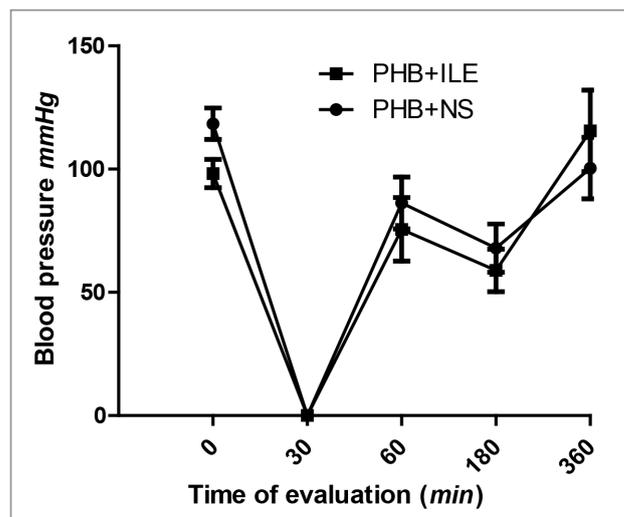


Figure 1. Evaluation of blood pressure in Phenobarbital (PHB) intoxicated rats that received Intravenous Lipid Emulsion (ILE) or Normal Saline (NS). Starting of PHB injection: zero time, ILE or NS were infused at 0.5 hr. At 0.5 hr the blood pressure of animals could not be detectable by our instrument. There was no significant difference between blood pressure of NS and ILE treated groups at all evaluating times.

times were also recorded.

The data were expressed as the mean \pm SEM. Unpaired T test was used to determine the significance of a difference in blood pressure and survival times between groups. Non parametric tests were used if necessary. Fisher's exact test was used to evaluate differences in mortality rate of different groups. The alpha level was set at 0.05. All statistical analyses were performed in SPSS 11.5.

Results

Half an hour after PHB toxic dose administration, the blood pressure of animals could not be detectable and animals were hypotensive. We found no significant difference between blood pressure of NS and ILE treated groups at all evaluating times, and there was no difference in returning to normal blood pressure between two groups (Figure 1).

There was no significant difference in animal muscular power scores before PHB administration. The scores significantly increased 3 and 6 hr after ILE infusion compare with control group ($p=0.007$ and 0.0371 , respectively), (Figure 2).

There was no statistically significant difference between mortality rates of ILE treated and control

Table 1. Comparing mortality rate and means of survival times of groups

	Intravenous lipid emulsion	Normal saline	p value
Mortality rate	0%	60%	0.167
Means of survival time (min)± standard error	360 ± 1.58	171 ± 77.18	0.0400

Mortality rates were compared by Fisher's exact test. Means of survival times were compared by T test. Numbers of animals in each group at beginning=5

groups (0% and 60% respectively; $p=0.167$), (Table 1). However, the mean survival time of ILE group was higher than NS group (Table 1).

Discussion

PHB is a relative lipid-insoluble barbiturate that at high doses induces cardiovascular depression with hypotension, nystagmus, ataxia, severe respiratory depression, coma and death¹⁰. Blocking the sympathetic ganglion and inhibition of medullar vasomotor centers by PHB at high doses induce vasodilatation, hypotension, bradycardia and low cardiac output¹¹. ILE could reverse hypotension induced by lipophilic-drug overdose such as haloperidol, methamphetamine and propranolol^{6,8,12}. However, it is not able to reverse non lipophylic drug induced hypotension, such as atenolol¹³. In our study, the administration of ILE could not reverse PHB induced hypotension sooner than normal saline, as volume expander.

The most important hypothesized mechanism of ILE therapy in lipophilic drug induced toxicity is lipid sink. Based on this theory, ILE relieves the toxicity of lipophilic xenobiotics via redistribution from action site and receptors in tissue to plasma or inert lipid induced compartment¹⁴⁻²¹. It has been shown that the lipid solubility of drug has a direct relationship with ILE detoxification capacity^{16,17}. PHB is relatively lipid-insoluble barbiturate with volume of distribution of 0.5-1 mg/L²². It seems that the ILE effect on survival and muscles power of PHB intoxicated rats are not explained by lipid sink theory and some other mechanisms might be involved^{5,7,18-21}.

PHB induces Central Nervous System (CNS) depression by enhancing GABAergic transmission²². There are some evidence on cross talking of N-methyl-D-aspartate (NMDA) and GABAA receptors on neurons, two important excitatory and inhibitory receptors²³. NMDA receptor activation cross-inhibits

GABAA receptor in a concentration dependent manner²⁴. Cong *et al* reported that the inhibitory effect of NMDA receptor activation on GABAA receptor mediated by calcium influx through NMDA receptors²⁴. Stelzer and coworkers evaluated the effect of intralipid on cell membrane of mice cultured cortical neurons, by patch clamp technique. They revealed that intralipid activates NMDA receptor channels in a concentration-dependent manner in cortical neurons²³. So it seems that part of effect of ILE on PHB toxicity is related to reversing GABAA receptor inhibition through cross talking of NMDA activation by lipid. However, it has been stated by Weigt *et al* that ILE has a dual effect on NMDA receptor channels. According to their research projects ILE can decrease^{25,26} and increase²⁷ NMDA receptor activity.

PHB is excreted unchanged through kidney and due to its low pKa (7.24), urine alkalinization to pH of 7.5-8.0 can increase PHB excretion up to 5-10 folds^{11,22,28}. Intralipid has alkaline pH (pH=8)²⁹. The blood volume of a male rat is 68.6 ml/kg³⁰ and we administrated ILE in 27% of total rat blood volume (18.6/68.6). It is suspected that ILE by raising the blood pH can increase the PHB renal excretion. However, we have not recorded rat's blood pH or urine pH. On the other hand, our control group received normal saline without bicarbonate, and we could not able to evaluate this hypothesis. Fox *et al* evaluated pH of premature infants after continues administration of 5 ml/kg (1 g/kg) intralipid and they found no difference on pH of their patients at 4 hours later³¹. We used much higher dose and infusion rate.

Nutritional theory may be another suggested mechanism to explain rising muscles activity score of ILE group. However, this theory is expressed for cardiac muscles³, there are some evidences that ILE has similar effects on skeletal muscles. Free fatty acid oxidation is an energy source of skeletal muscles cells. This

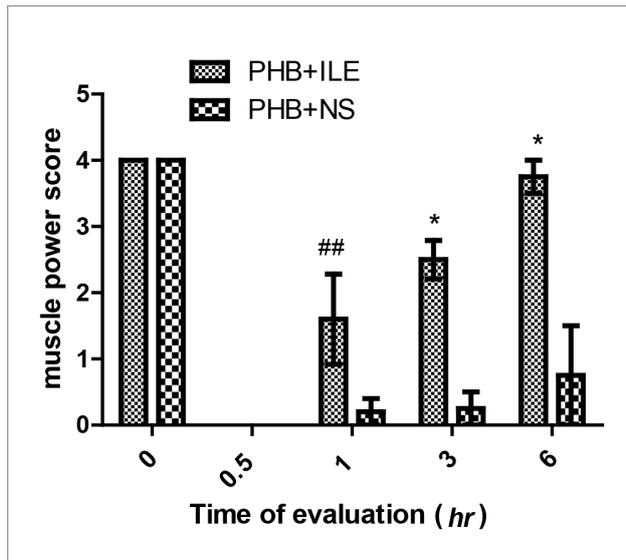


Figure 2. Evaluation of muscles power score in Phenobarbital (PHB) intoxicated rats that received Intravenous Lipid Emulsion (ILE) or Normal Saline (NS). Starting of Phenobarbital injection at zero time, ILE or NS were infused at 0.5 hr. * = $p < 0.05$, ##: not significant, $N=5$.

process is limited by two steps, the availability of free fatty acid and the abundance of fatty acid transports

into mitochondria³²⁻³⁴. The infusion of high dose ILE raises the serum level of free fatty acid secondary to activation of lipoprotein lipase³⁵, so it seems that improving rat muscles power after ILE infusion is partly due to free fatty acid availability to skeletal muscles.

Conclusion

In conclusion, ILE could reverse the reduction of muscles power and improve survival time of PHB intoxicated rats. However, further detailed research needed to confirm this finding and to reveal the exact mechanisms.

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Conflict of Interest

The authors report no declarations of interest.

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