Treatment with lipid emulsion decreases high levels of phenytoin in rats.

Kayipmaz AE1*, Kavalci C1, Bayraktar N2, Bacanli D3, Aydos TR4, Giray TA1, Kocalar UG1, Benli S1, Ozel BA1

1Department of Emergency, Faculty of Medicine, Baskent University, Turkey
2Department of Biochemistry, Faculty of Medicine, Baskent University, Turkey
3Experimental Animals Breeding and Research Center, Baskent University, Turkey
4Department of Medical Pharmacology, Faculty of Medicine, Baskent University, Turkey

Abstract

Objective: Phenytoin is a widely used, lipophilic antiepileptic agent. As a result of its narrow therapeutic index, toxicity is not rare. Currently, there is no specific antidote for phenytoin. Therefore, we need a low-cost supportive therapeutic agent that can easily be applied in emergency conditions. The rationale for conducting our study was to evaluate the efficacy of the lipid emulsion to the high levels of phenytoin in an animal model.

Methods: We randomly divided 28 rats into 4 groups: Control group (received no treatment); phenytoin group (received 75 mg/kg phenytoin intraperitoneally); lipid emulsion group (received 4 ml/kg 20% lipid emulsion intravenously); phenytoin+lipid emulsion group (received 75 mg/kg phenytoin intraperitoneally and 4 ml/kg 20% lipid emulsion intravenously). We performed blood analysis twice in each group.

Results: Lipid emulsion significantly decreased the phenytoin level in the treatment group in comparison with the control group (p=0.035 and p=0.026, respectively).

Conclusion: We demonstrated the efficacy of lipid emulsion in reducing serum phenytoin levels in our animal model. Lipid emulsion is a promising method for treatment of phenytoin intoxication.

Keywords: Antiepileptic, Lipid rescue therapy, Poisoning.
Materials and Methods

This study is an experimental animal trial. We conducted the experiments in November 2014. We have received the approval of The Medical and Health Sciences Research Ethics Committee of Baskent University and Baskent University Institutional Animal Care and Use Committee (Project Number: DA 14/20). This study conformed to the Helsinki Declaration of 1975, as revised in 2000 and 2008, concerning Human and Animal Rights. All data underlying the findings described in our manuscript are fully available without restriction.

We used 28 male albino Sprague–Dawley rats. The mean body weight was 372.08 ± 26.73 g. We obtained the rats from Baskent University Experimental Animal Production and Research Center. The rats did not have any food or water restrictions. We conducted all the experiments in a laboratory at the same center. We randomly divided the rats into 4 groups of 7. We designated the rats in Group 1 as the control group and they did not receive any treatment. Blood was withdrawn at 0 (1st measurement) and 15 (2nd measurement) min for measurement of AST and Alanine Aminotransferase : ALT levels. The rats in Group 2 received a single dose of 75 mg/kg phenytoin sodium intraperitoneally, as described previously [10]. We chose single-dose application because we created an acute toxicity model. We did not cause any deaths, and reached the toxic level of phenytoin with the administered dose. We performed additional blood withdrawal at 30 (1st measurement) and 50 (2nd measurement) min to measure phenytoin, albumin, ALT and AST levels. We used the formula recommended by Adole et al. to calculate free phenytoin levels: free phenytoin=total phenytoin/(0.2 × albumin)+0.1 [11]. Group 3 received a single dose of 4 ml/kg 20% lipid emulsion via the tail vein at a dose of 4 ml/kg. Chen et al. obtained maximum plasma phenytoin concentrations at 30 min [13]. Therefore, we administered lipid emulsion at that time. We performed additional blood withdrawal at 35 (1st measurement) and 50 (2nd measurement) min to measure phenytoin, albumin, ALT and AST levels. We withdrew the second blood samples via closed cardiac puncture and sacrificed the rats by cervical dislocation.

Baskent University Hospital Pharmacy provided the drugs used in the study. Phenytoin sodium (Epitoin; 250 mg/5 ml) was from VEM Pharmaceuticals (Istanbul, Turkey) and lipid emulsion (Clinoleic; 20%) was from Eczacibasi-Baxter (Istanbul, Turkey). For anaesthesia, all rats received intraperitoneal 6 mg/kg xylazine and 60 mg/kg ketamine. Blood samples were centrifuged at 4000 × g for 10 min. Serum ALT and AST levels were assayed by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD (without pyridoxal-5’-phosphate), using an Abbott Architect C8000 analyzer (Abbott Laboratories, Chicago, IL, USA). Albumin was estimated by colorimetric assay with the bromocresol green method using the Abbott Architect C8000 analyzer. Phenytoin level was measured with a liquid ready-to-use, homogeneous enzyme immunoassay method, using an Abbott Architect C4000 analyzer. All statistical analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, IL). Shapiro-Wilk test was used to assess whether data had a normal distribution. We used independent samples t test to compare the phenytoin levels between Groups 2 and 4. We used Kruskal-Wallis test to compare AST and ALT levels among the four groups. P values <0.05 were considered to be statistically significant.

| Table 1. The first corrected phenytoin levels of Group 2 and 4. |
|---------------------------------|-------------------|-------------------|-------------------|--------------------|
| Rat number | First phenytoin levels of Group 2 (µg/ml) | First phenytoin levels of Group 4 (µg/ml) | p value |
|-----------|-------------------|-------------------|-------------------|--------------------|
| 1 | 13.33 | 21.78 | 0.035 |
| 2 | 30.48 | 22.41 | |
| 3 | 39 | 15.71 | |
| 4 | 33.22 | 14.85 | |
| 5 | 41.83 | 14.37 | |
| 6 | 10.32 | 9 | |
| 7 | 53.62 | 12.09 | |

<p>| Table 2. The second corrected phenytoin levels of Group 2 and 4. |
|---------------------------------|-------------------|-------------------|-------------------|--------------------|</p>
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Results

The main outcome variable of our study was the levels of phenytoin. The first and second phenytoin levels of Group 2 and 4 are presented in Table 1 and 2. There was a significant difference between the first and second corrected phenytoin levels in Groups 2 and 4 (p=0.035 and p=0.026, respectively). The secondary outcome variable was the levels of liver function tests (AST and ALT). AST and ALT levels in the 4 groups are summarized in Table 3 and 4. There was a significant difference among the groups for the second measurement of AST (p=0.038). However, there were no significant differences among the groups for the first levels of
**Table 3.** The First AST and ALT levels of 4 groups.

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**Table 4.** The Second AST and ALT levels of 4 groups.

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**Discussion**

Toxicity is not rare in epilepsy patients who use phenytoin because of the narrow therapeutic index of this drug [6]. CNS signs such as dizziness and encephalopathy, and death may occur in accordance with the exposed dose [3,5]. Although phenytoin is a widely used drug, it has no specific antidote. Treatment such as charcoal hemoperfusion and molecular adsorbent recirculation system have been tested [4,7,9]. However, these methods are difficult to apply and costly in the emergency department. Lipid emulsion treatment is potentially useful in cases of high doses of lipophilic phenytoin as a low-cost and easily applicable add-on treatment. In our study, lipid emulsion significantly decreased the phenytoin level in the treatment group in comparison with the control group (p=0.035 and p=0.026, respectively). There was a significant difference among the 4 groups for the second level of AST (p=0.038).

McNamara et al. reported that intravenous phenytoin was more effective than intraperitoneal application in male Sprague–Dawley rats [14]. However, Loscher et al. failed to demonstrate any difference in plasma phenytoin levels between the two routes of administration [10]. We used different modes (intraperitoneal vs. intravenous) for administration of lipid emulsion to rats. We aimed to prevent any interactions between these two agents prior to their entry into the systemic circulation. Blood samples obtained from rats who had lipid emulsion treatment were more lipidemic. Lipidemia was also reported in a case published by Meaney et al. [15]. They stated that lipidemia complicates laboratory tests and to prevent this, “blood samples should be taken prior to administration of lipid emulsion” [15]. Lipidemia was also evident in 4 out of 9 cases analyzed retrospectively by Levine et al. [16]. Lipidemia was actually seen in all rats who had received lipid emulsion; thus, it is probably the only side effect directly associated with lipid emulsion [16].

There were no significant differences among the blood samples obtained for the second measurements of ALT levels in all groups (p>0.05). Moreover, no significant difference was seen for the first measurements of ALT and AST among the 4 groups (p>0.05). There was a significant difference among the 4 groups for the second levels of AST (p=0.038). We consider that the short observation time caused there to be no difference among the groups. ALT and AST levels in the control group...
(Group 1) were similar to those at 13 weeks in another study using rats of the same species and sex (92.8 ± 25.3 and 53.7 ± 18 IU/L, respectively) [17].

Although it is not a first-line treatment method for intoxication with other lipophilic agents, intravenous lipid emulsion is a promising add-on treatment for emergency cases with hemodynamic imbalance [18]. There are 3 potential mechanisms of action for lipid emulsion as an antidote: the lipid sink theory, basic hemodilution, and fatty acid metabolism [19]. According to the lipid sink theory described by Weinberg, lipid emulsion is found as fat droplets or multi lamellar vesicles in the blood [20]. These in turn bind to lipophilic toxin and eliminate it from target tissue [20]. Considering fatty acid metabolism, myocardial tissue supplies most of its energy needs from mitochondrial oxidation of fatty acids [21]. Recent studies have supported the theory that lipid emulsion increases the intracellular concentration of fatty acids and supplies extra energy to myocardial tissue, which in turn increases calcium levels and causes a positive inotropic effect [22,23]. In our study, the drop in phenytoin level can be explained by basic hemodilution, lipid sink, or increased fatty acid oxidation.

In our literature review, we could not find any in vivo randomized controlled trial of lipid emulsion treatment of phenytoin toxicity. The potential beneficial effects of lipid emulsion against toxicity of phenytoin have been shown in vitro [24]. However, those results should be compared with in vivo studies. There is one case report on the Lipid Rescue website, in which Werstler described a case of phenytoin intoxication successfully treated with lipid emulsion [25].

Conclusion

We demonstrated the efficacy of lipid emulsion treatment in reducing serum phenytoin levels in our animal model. Lipid emulsion significantly decreased the phenytoin level compared with the control group. Our findings show that lipid emulsion treatment, which has a safe adverse effect profile, is a promising method for treatment of phenytoin intoxication. In future studies, the neurological biomarkers and histopathological evaluation can be done to detect the toxic effects of phenytoin on the CNS and liver. Also, these results should be consolidated with clinical trials in real patients. In the light of our results, the use of lipid emulsion treatment should be kept in mind for emergency cases of phenytoin intoxication.

Limitations

Our study investigated the efficacy of lipid emulsion treatment of high-dose phenytoin exposure. Phenytoin reaches a maximum blood level at 30 min, and the duration of observation was limited to 50 min extending the duration of observation might be beneficial to record clinical findings of animals and investigate the long-term effects of lipid emulsion on phenytoin levels. The short observation time may be the reason why there were no differences in the first measurements of AST and ALT among the groups. Although 30% lipid emulsion is more efficacious than 20% lipid emulsion, only the latter is available in Turkey and we used this form in our study.

Acknowledgement

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References

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*Correspondence to*

Afsin Emre Kayipmaz
Department of Emergency
Baskent University Faculty of Medicine
Ankara
Turkey
E-mail: aekayipmaz@baskent.edu.tr