Biomedical Research 2017; Special Issue: *ISSN 0970-938X*
www.biomedres.info

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

Kayipmaz AE[1*], Kavalci C[1], Bayraktar N[2], Bacanli D[3], Aydos TR[4], Giray TA[1], Kocalar UG[1], Benli S[1], Ozel BA[1]

[1]Department of Emergency, Faculty of Medicine, Baskent University, Turkey

[2]Department of Biochemistry, Faculty of Medicine, Baskent University, Turkey

[3]Experimental Animals Breeding and Research Center, Baskent University, Turkey

[4]Department 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.

Accepted on September 14, 2016

Introduction

Phenytoin is an antiepileptic agent that can cause multi-channel blockade (e.g. potassium, sodium, and calcium) [1]. It binds to plasma proteins with high avidity, especially albumin. Plasma albumin concentration is an important factor for binding of phenytoin to proteins [2]. Phenytoin is primarily metabolized in the liver by para-hydroxylation; hence, liver failure is a predisposing factor for phenytoin toxicity [3]. The therapeutic dose range of phenytoin is 10-20 μg/ml, and above this dose, signs of toxicity may occur [4]. Between 20 and 30 μg/ml, ataxia, lateral gaze nystagmus and vertigo can be seen. At higher doses >30 μg/ml, Central Nervous System (CNS) signs such as dysarthria and vertical nystagmus may appear. Sharma et al. reported encephalopathy and death in a patient who had a phenytoin level of 144 μg/ml [5]. As reported by Hwang et al. the most common cause of acute phenytoin intoxication is excessive intake of the drug [6]. Its narrow therapeutic window and variations in metabolic rates among individuals are implicated in phenytoin toxicity. In the same study, iatrogenic causes constituted 11% of intoxications. Sen et al. reported that toxicity was common in patients who used phenytoin for epilepsy; therefore, the drug level should be followed carefully [7]. For example, The American Association of Poison Control Centers reported 1790 cases of intoxication in its 2013 Annual Report: 487 had moderate toxicity, 40 had severe toxicity, and 1 died [8].

Although treatment modalities such as active charcoal, charcoal hemoperfusion and molecular adsorbent recirculation system have been tried in phenytoin toxicity, currently, there is no specific antidote for phenytoin [4,7,9]. Therefore, we need a low-cost supportive therapeutic agent that can easily be applied in emergency situations. We suggest lipid emulsion as a readily applicable, low-cost add-on treatment for phenytoin toxicity because phenytoin is a lipophilic drug. 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. The secondary outcome was to detect the levels of liver function tests (Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)).

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 for 20 s, as described previously [12]. We withdrew blood at 5 (1st measurement) and 20 (2nd measurement) min for ALT and AST measurements. Rats in Group 4 received 75 mg/kg phenytoin sodium intraperitoneally. At 30 min, we administered a 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	

Table 2. *The second corrected phenytoin levels of Group 2 and 4.*

Rat number	Second phenytoin levels of Group 2 (µg/ml)	Second phenytoin levels of Group 4 (µg/ml)	p value
1	14.66	20.15	0.026
2	39.1	27.83	
3	40.86	11.45	
4	41.03	13.87	
5	43.16	17.24	
6	9.31	9.7	
7	57.5	13.33	

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

AST, ALT and the second level of ALT (0.735, 0.537 and 0.499, respectively).

Table 3. *The First AST and ALT levels of 4 groups.*

Rat number	1st levels AST Group (IU/L) of 1	1st levels AST Group (IU/L) of 2	1st levels AST Group (IU/L) of 3	1st levels AST Group (IU/L) of 4	p value	1st levels ALT Group (IU/L) of 1	1st levels ALT Group (IU/L) of 2	1st levels ALT Group (IU/L) of 3	1st levels ALT Group (IU/L) of 4	p value
1	116	106	203	121	0.735	55	65	129	44	0.537
2	121	118	103	132		74	51	62	65	
3	133	133	141	96		87	57	59	46	
4	120	122	124	99		59	65	54		
5	81	108	121	113		44	48	60	59	
6	132	72	96	100		57	45	69	69	
7	125	124	85	120		63	52	48	54	

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

Rat number	2nd levels AST Group (IU/L) of 1	2nd levels AST Group (IU/L) of 2	2nd levels AST Group (IU/L) of 3	2nd levels AST Group (IU/L) of 4	p value	2nd levels ALT Group (IU/L) of 1	2nd levels ALT Group (IU/L) of 2	2nd levels ALT Group (IU/L) of 3	2nd levels ALT Group (IU/L) of 4	p value
1	114	105	109	144	0.038	47	63	62	46	0.499
2	88	158	106	121		60	52	50	59	
3	119	133	85	114		78	60	50	52	
4	85	127	95	120		55	66	49	42	
5	105	122	94	118		37	52	51	42	
6	95	64	100	113		51	40	54	46	
7	113	119	123	115		31	55	58	61	

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

This study was supported by Baskent University Research Fund and presented in an oral session at The 2nd Intercontinental Emergency Medicine Congress, April 16-19, 2015, Antalya, Turkey.

References

1. Nilsson MF, Ritchie H, Webster WS. The effect on rat embryonic heart rate of Na+, K+, and Ca2+ channel blockers, and the human teratogen phenytoin, changes with gestational age. Birth Defects Res Part B Dev Reprod Toxicol 2013; 98: 416-427.
2. Kilpatrick CJ, Wanwimolruk S, Wing LM. Plasma concentrations of unbound phenytoin in the management of epilepsy. Br J Clin Pharmacol 1984; 17: 539-546.
3. Chua HC, Venketasubramanian N, Tjia H, Chan SP. Elimination of phenytoin in toxic overdose. Clin Neurol Neurosurg 2000; 102: 6-8.
4. Doyon S. Anticonvulsants. In: Goldfrank's Toxicologic Emergencies. 8th ed McGraw-Hill, New York 2006; 733-735.
5. Sharma B, Handa R, Prakash S, Nagpal K, Gupta P. Phenytoin toxicity presenting as encephalopathy with fatal outcome: a case report. J Neurol Res 2013; 3: 184-186.
6. Hwang WJ, Tsai JJ. Acute phenytoin intoxication: causes, symptoms, misdiagnoses, and outcomes. Kaohsiung J Med Sci 2004; 20: 580-585.
7. Sen S, Ratnaraj N, Davies NA, Mookerjee RP, Cooper CE, Patsalos PN, Williams R, Jalan R. Treatment of phenytoin toxicity by the Molecular Adsorbents Recirculating System (MARS). Epilepsia 2003; 44: 265-267.
8. Mowry JB, Spyker DA, Cantilena LR, McMillan N, Ford M. 2013 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 31st Annual Report. Clin Toxicol (Phila) 2014; 52: 1032-1283.
9. Kumar PP, Lingappa L, Shah M, Shaikh FA. Charcoal hemoperfusion for phenytoin intoxication. Indian Pediatr 2012; 49: 152-153.
10. Loscher W, Cramer S, Ebert U. Limbic epileptogenesis alters the anticonvulsant efficacy of phenytoin in Sprague-Dawley rats. Epilepsy Res 1998; 31: 175-186.
11. Adole PS, Singh A, Kharbanda PS, Sharma S. Phenotypic interaction of simultaneously administered isoniazid and phenytoin in patients with tuberculous meningitis or tuberculoma having seizures. Eur J Pharmacol 2013; 714: 157-162.
12. Fettiplace MR, Akpa BS, Ripper R, Zider B, Lang J, Rubinstein I, Weinberg G. Resuscitation with lipid emulsion: dose-dependent recovery from cardiac

pharmacotoxicity requires a cardiotonic effect. Anesthesiology 2014; 120: 915-925.

13. Chen Y, Wang C, Xiao X, Wei L, Xu G. Multidrug resistance-associated protein 1 decreases the concentrations of antiepileptic drugs in cortical extracellular fluid in amygdale kindling rats. Acta Pharmacol Sin 2013; 34: 473-479.
14. McNamara JO, Rigsbee LC, Butler LS, Shin C. Intravenous phenytoin is an effective anticonvulsant in the kindling model. Ann Neurol 1989; 26: 675-678.
15. Meaney CJ, Sareh H, Hayes BD, Gonzales JP. Intravenous lipid emulsion in the management of amlodipine overdose. Hosp Pharm 2013; 48: 848-854.
16. Levine M, Skolnik AB, Ruha AM, Bosak A, Menke N, Pizon AF. Complications following antidotal use of intravenous lipid emulsion therapy. J Med Toxicol 2014; 10: 10-14.
17. Petterino C, Argentino-Storino A. Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol 2006; 57: 213-219.
18. Jamaty C, Bailey B, Larocque A, Notebaert E, Sanogo K, Chauny JM. Lipid emulsions in the treatment of acute poisoning: a systematic review of human and animal studies. Clin Toxicol (Phila) 2010; 48: 1-27.
19. Cave G, Harrop-Griffiths W, Harvey M, Meek T, Picard J, Short T, Weinberg G. AAGBI Safety Guideline: Management of Severe Local Anaesthetic Toxicity 2010; 37.
20. Weinberg GL. Lipid emulsion infusion: resuscitation for local anesthetic and other drug overdose. Anesthesiology 2012; 117: 180-187.
21. Van der Vusse GJ, van Bilsen M, Glatz JF. Cardiac fatty acid uptake and transport in health and disease. Cardiovasc Res 2000; 45: 279-293.
22. Stehr SN, Ziegeler JC, Pexa A, Oertel R, Deussen A, Koch T, Hubler M. The effects of lipid infusion on myocardial function and bioenergetics in L-bupivacaine toxicity in the isolated rat heart. Anesth Analg 2007; 104: 186-192.
23. Partownavid P, Umar S, Li J, Rahman S, Eghbali M. Fatty-acid oxidation and calcium homeostasis are involved in the rescue of bupivacaine-induced cardiotoxicity by lipid emulsion in rats. Crit Care Med 2012; 40: 2431-2437.
24. Willers JW. A comparison of the relative sequestration of free and albumin-bound phenytoin by Intralipid: further insights to determine the utility of the Lipid Sink in clinical practice. UK: AAGBI 2012.
25. Werstler EK. Intralipid in phenytoin toxicity. Lipid Rescue Lett 2010.

* Correspondence to

Afsin Emre Kayipmaz

Department of Emergency

Baskent University Faculty of Medicine

Ankara

Turkey

E-mail: aekayipmaz@baskent.edu.tr