Int J Physiol Pathophysiol Pharmacol 2022;14(6):324-331
www.ijppp.org /ISSN:1944-8171/IJPPP0135819

*Original Article*

# The effect of sodium benzoate, L-carnitine, and phenylacetate on valproate-induced hyperammonemia in Male Wistar rats

Leila Etemad[1], Ali Roohbakhsh[1,2], Abolfazl Abbaspour[2], Anahita Alizadeh Ghamsari[3], Fatemeh Amin[4], Mohammad Moshiri[3,5]

*[1]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran; [2]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Medical Toxicology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [4]Department of Physiology and Pharmacology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran; [5]Department of Clinical Toxicology, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

Received May 30, 2021; Accepted June 28, 2022; Epub December 15, 2022; Published December 30, 2022

**Abstract:** Introduction: L-carnitine (LC) is commonly used in the treatment of valproate-induced hyperammonemia (VIHA). LC prevents the production of ammonia with no significant effect on renal ammonia excretion. This study was conducted to evaluate the effect of sodium benzoate (SB) and phenyl acetate (PA) on reducing VIHA. Materials and methods: Eight groups treated with Sodium Valproate (SV) at 300 mg/kg and 15 minutes later with normal saline, SB (144 mg/kg), PA (0.3 g/kg), LC (2.5 g/kg), SB (144 mg/kg) plus PA (0.3 g/kg), or SB (144 mg/kg) plus PA (0.3 g/kg) plus LC (2.5 g/kg), intraperitoneally. Other groups were exposed to normal saline, SB, LC or PA alone. Animal's motor function and serum ammonia, lactate, and sodium levels were assessed at 0.5, 1, and 1.5 hours after the SV injection. Results: The results showed that LC reduced SV-induced hyperammonemia just at one and half-hour after treatment ($P<0.001$). PA, alone or in combination with other antidotes, reduced serum ammonia at all evaluated times ($P<0.001$). LC improved the impaired motor function of animals only at 1.5 hours, while PA, alone or in combination decreased the motor function scores at different times. However, SB administration alone did not change SV-induced hyperammonemia or motor function impairment. There was no significant difference in the level of serum aminotransferases, blood urea nitrogen, and creatinine between groups. Conclusion: These findings define that PA had a better therapeutic effect on valproate-induced hyperammonemia in comparison with SB. Co-administration of LC with PA ameliorated the elevated levels of ammonia and may relieve potential therapeutic application against acute SV intoxication.

**Keywords:** Carnitine, hyperammonemia, phenylacetates, sodium benzoate, toxicity, valproic acid

## Introduction

Sodium valproate (SV) is a monocarboxylate compound with anticonvulsant and mood-stabilizing properties. It is widely used in the treatment of epilepsy, bipolar disorder, neuropathic pain, and migraine. SV acts as a histone deacetylase inhibitor and increases the gamma-aminobutyric acid (GABA) level in the brain. It also alters voltage-dependent sodium, potassium and calcium channels activity [1]. SV may cause serious side effects, including hepatotoxicity, coagulopathies, pancreatitis, bone marrow suppression, hyperammonemia and increase the risk of fetal abnormalities [2-4]. SV-induced encephalopathy is frequently correlated with hyperammonemia without any sign of hepatic failure [5]. This serious condition can be controlled if the diagnosis is made at an early stage [6]. Hyperammonemia refers to a clinical condition characterized by elevated blood ammonia level (>40 μmol/l) and manifested by neurological abnormalities such as lethargy, irritability, poor feeding, vomiting, hyperventilation, grunting respiration, and seizure [7, 8]. Serum ammonia level (SAL) more than

200 mmol/l has also been associated with increased intracranial pressure and brain herniation [7].

Currently, few treatment strategies are available for hyperammonemic state. L-carnitine (LC) is commonly used in the treatment of valproate-induced hyperammonemia (VIHA) that prevents the production of ammonia with no significant effect on renal excretion [9]. Sodium benzoate (SB) and phenylacetate (PA) are the main part of hyperammonemia therapy in pediatrics and adults and exert their effect through increasing the renal excretion of nitrogenous compounds (primarily ammonia) [10, 11]. Hippuric acid is formed by the interaction of SB and glycine, excreted from urine and eliminates the extra amount of blood ammonia [12]. PA also maintains normal plasma ammonia level through phenyl acetyl glutamine urinary excretion [13].

Due to LC limitation in decreasing the VIHA, this study was conducted to evaluate the potentials effect of SB and PA, alone or in combination with LC, to reduce the ammonia level as a serious side effect of acute valproate toxicity.

## Materials and methods

Male Wistar rats weighing 250-300 g (n=60) were obtained from Central Animal Facility, Mashhad University of Medical Science, Mashhad, Iran. They were kept under a 12 h/12 h light/dark cycle and standard temperature. Animal experiments were approved by the Animal Care Committee of Mashhad University of Medical Sciences (IR.mums.sp.1394.42). SB, PA and LC were purchased from Sigma, Germany and SV was purchased from Sanofi, France.

Animals were randomly divided into ten groups, six per each. Six group of animal were treated with SV. Fifteen minutes after the single injection of SV (300 mg/kg), the animal received normal saline (SV group), SB (144 mg/kg), PA (0.3 g/kg), LC (2.5 g/kg), SB+PA, or SB+PA and LC. The other four groups only received normal saline (control), SB, LC or PA without SV treatment. All injections were done intraperitoneally. SV was administrated at a dose that produced a significant increase in blood ammonia levels. The other doses were selected based on the previous studies [14, 15].

*Motor function*

The motor function of animals were evaluated using modified De Bleecker scoring: Grade 0: normal mobility; Grade 1: ataxic gait or slight weakness; Grade 2: stretch movements, decrease in motor activity or weakness in the control of the condition after tail stimulation; Grade 3: severe imbalances and only standing after tail stimulation; Grade 4: no voluntary movements after tail stimulation, no writhing reflexes, and four limb paralysis [16, 17].

*Biochemical parameters*

SAL, serum lactate level (SLL), and sodium concentration were assessed at 0.5, 1, and 1.5 hours after SV treatment. The serum liver aminotransferases (ALT and AST) and creatinine, as well as blood urea nitrogen, were also evaluated at 1.5 hours after SV treatment. Blood samples were taken through rat orbital sinus at 0.5 and 1 hours and via cardiac puncture at 1.5 hours post treatment.

The serum ammonia level was determined by a kit from Sigma (#AA0100). Briefly, in this method, ammonia reacts with α-ketoglutaric acid, and reduced NADPH in the presence of L-glutamate dehydrogenase which results in formation of L-glutamate and NAD+. The oxidation of NAD+ was measured spectrophotometrically at 340 nm. Lactate production assay was also performed using the Lactate Assay kit (#MAK064) from Sigma according to the manufacturer's instructions. The principle of this kit is based on the generation of pyruvate and H2O2 through the reaction between lactate and lactate oxidase. The produced H2O2 interacts with a probe to produce color that was measured at 570 nm. The serum level of AST, ALT, sodium, BUN, and creatinine were assayed using a biochemical auto analyzer instrument (Type 7170, Hitachi, Japan) of Imam Reza Hospital, Mashhad Iran.

*Statistical analysis*

The data were expressed as mean ± SEM. All data, except for motor function, were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's as post-test. The results of motor function were compared by using non-parametric Kruskal-Wallis test. $P<0.05$ was considered statistically significant. All data analyses were conducted using SPSS 11.0 for Windows.

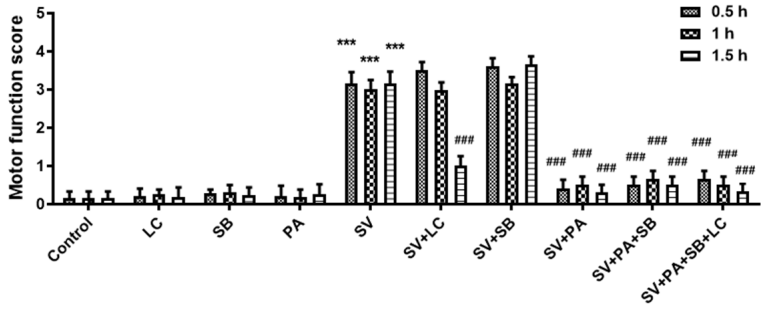


**Figure 1.** Phenylacetate improved motor function scores of sodium valproate intoxicated rats sooner than other antidotes. Data shown as median ± IQR. Each group included six rats. Non-parametric Kruskal-Wallis test was used for statistical analysis. The SV group was compared with control group and other groups were compared with SV group. ***: PV<0.001 compared with the control group at the same time, ###: PV<0.001 compared with the group receiving valproate at the same time. SV: sodium valproate, SB: sodium benzoate, PA: phenylacetate, LC: L-carnitine.

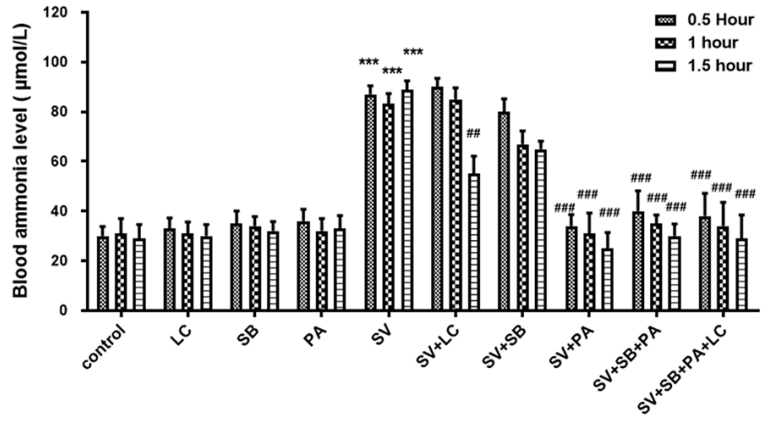


**Figure 2.** Phenylacetate decreased the serum ammonia level of sodium valproate intoxicated rats sooner than other antidotes. The data are presented as mean ± SEM. Each group included six rats. The SV group was compared with control group and other groups were compared with SV group. ***: PV<0.001 compared with the control group at the same time. ##: P<0.01 and ###: PV<0.001 compared with the SV group at the same time. SV: sodium valproate, SB: sodium benzoate, PA: phenylacetate, LC: L-carnitine.

## Results

*The effect of sodium benzoate, L-carnitine, and phenyl acetate on motor function of valproate intoxicated rat*

The results showed that the motor function of the SV group was significantly lower than control group at 0.5, 1, and 1.5 hours (P<0.001). There was no significant difference between SV and SV+SB group at any mentioned time. LC improved the impaired motor function of animals only after 1.5 hours in comparison with the SV group (P<0.001). PA-treated animals showed lower impaired motor function scores than the SV group at all evaluated times (P<0.001). Co-administration of SB+PA or SB+PA+LC and SV, also developed the motor function impairment (PV<0.001) (**Figure 1**). There was no significant difference between the results of three sham groups and control group.

*The effect of sodium benzoate, L-carnitine, and phenyl acetate on biochemical parameters of valproate intoxicated rat*

SV-administration induced elevated SAL in comparison with control group (P<0.001). LC reduced the raised SAL just at the half and one hour after treatment (P<0.01). PA alone or in combination with other compounds reduced SAL at all times in SV-intoxicated rats (P<0.001). However, SB was not effective at any time (**Figure 2**).

SV administration also significantly increased the SLL compared to the control group (P< 0.001). PA, SB, and LC, alone or in combination therapy significantly decreased the SLL at 1.5 hours. But only LC, alone or in combination with others, could reduce the SV-induced hyperlactatemia at just around 1 hour (P<0.05 and P<0.01, respectively) (**Figure 3**).

In comparison with the control group, SV also significantly raised the serum sodium concentration at 0.5 and 1 hours (P<0.001). However, neither LC nor SB or PA could significantly change the SV-induced hypernatremia (**Figure 4**).

There was no significant difference in the level of serum aminotransferases, blood urea nitrogen, and creatinine between any groups (**Table 1**).

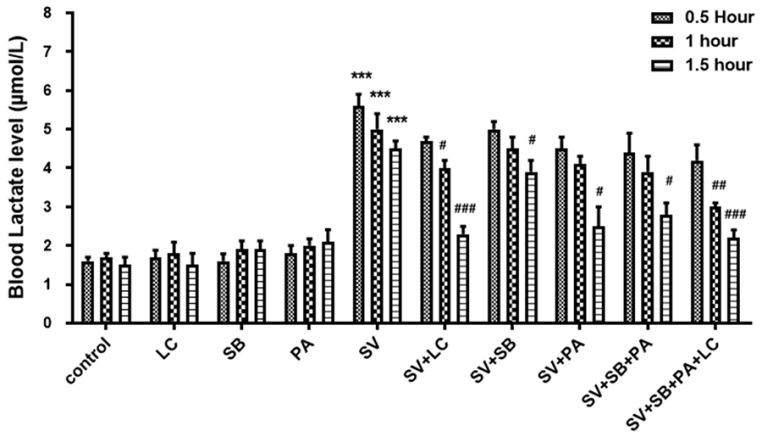


Figure 3. L-carnitine decreased the serum Lactate level of sodium valproate intoxicated rats sooner than other antidotes. The data were presented as mean ± SEM from the experiment on six rats. To test the statistical difference, repeated measures ANOVA and Tukey's follow up tests were used. The SV group was compared with control group and other groups were compared with SV group. ***: P<0.001 compared with the control group at the same time. #: P<0.05, ##: P<0.01, and ###: P=0, compared with the SV group at the same time. SV: Sodium Valproate, SB: Sodium benzoate, PA: Phenylacetate, LC: L-carnitine.

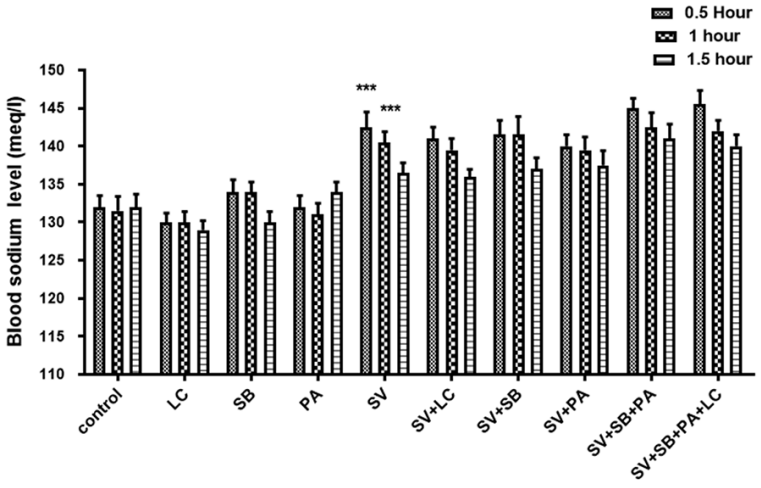


Figure 4. None of antidotes did not change the SV-induced hypernatremia in rats. The data were presented as mean ± SEM from the experiment on six rats. To test the statistical difference, repeated measures ANOVA and Tukey's follow up tests were used. The SV group was compared with control group and other groups were compared with SV group. ***: P<0.001 compared with the control group at the same time. SV: Valproate, SB: Sodium benzoate, PA: Phenylacetate, LC: L-carnitine.

## Discussion

The results of the present study showed that the administration of SV significantly increased the SAL. It is well documented that SV administration at single or repeated doses can induce hyperammonemia in animal models [18, 19]. The intravenous injection of SV at 200 mg/kg leading to 100% increase in the arterial ammonia that persist up to 100 min [20]. Patients receiving treatment with SV also experienced an increased level of ammonia, and there was a relationship between SAL and clinical manifestations [21]. Valproate toxicity decreased the level of consciousness due to hyperammonemia [22]. Our results showed a direct correlation between the motor function and SAL, however, we did not evaluate the brain ammonia level of rats. In this study, PA, alone or in combination with other compounds, completely reversed the impaired motor function of SV-intoxicated rats.

Omega oxidation metabolites of valproate interfere with carbamoyl phosphate synthase, a mitochondrial enzyme that plays a significant role in the first step of the urea cycle and result in hyperammonemia [23]. Currently, LC is the recommended compound for the treatment of VIHA that acts through improving the beta-oxidation of valproate [9, 24, 25]. Case reports as well as clinical trials have also raised possibility in alleviating the ammonia level and encephalopathy with carnitine therapy in subjects with VIHA [9, 26, 27]. Metabolism of SV will not shift toward omega oxidation if there is a present sufficient concentration of LC. Another contributor to SV-induced hyperammonemia is renal ammonia excretion [28]. In renal tubules, ammonia is excreted as a result of the glutamine-to-glutamate conversion by glutaminase [29]. The VIHA of renal origin is related to increase glutaminase activity and glutamine transport across the mitochondrial membrane [30]. LC has no effect on this

**Table 1.** There was no significant difference in biochemical parameters between groups that received sodium valproate alone or in combination with antidotes

| Group | Biochemical parameters | | | |
|---|---|---|---|---|
| | BUN mg/dl | CR mg/dl | AST IU/L | ALT IU/L |
| Control | 17.8±2.0 | 0.58±0.031 | 118.2±22.9 | 60.2±8.8 |
| PA | 17.4±3.1 | 0.64±0.030 | 119.6±25.8 | 54.6±9.5 |
| SB | 16.3±1.3 | 0.60±0.012 | 115.8±28.5 | 57.8±7.6 |
| LC | 18.0±2.7 | 0.56±0.065 | 122.0±18.2 | 59.9±9.2 |
| SV | 16.5±3.0 | 0.59±0.084 | 116.9±19.8 | 55.3±6.5 |
| SV+PA | 17.1±2.2 | 0.63±0.063 | 117.8±20.5 | 56.1±9.3 |
| SV+SB | 16.9±1.8 | 0.61±0.039 | 120.0±23.2 | 58.7±10.2 |
| SV+LC | 17.9±2.6 | 0.59±0.041 | 119.4±19.3 | 55.5±6.8 |
| SV+PA+SB | 16.8±3.3 | 0.62±0.035 | 112.1±21.8 | 59.3±9.5 |
| SV+PA+SB+LC | 17.6+2.3 | 0.58±0.043 | 118.8±25.6 | 57.2±7.6 |

Data shown as mean ± SE. ANOVA test was used for statistical analysis. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CR: creatinine, LC: L-carnitine, PA: phenylacetate, SB: sodium benzoate, SV: sodium valproate.

pathway and is mainly effective in inhibition of ammonia production rather than increasing the renal excretion. It was demonstrated that pretreatment with LC failed to reduce non-valproate hyperammonemia in rat model [15]. In the present study, LC was administrated when serum ammonia concentration was above normal level and reduced the raised ammonia concentration up to one and a half hours after treatment. As the half-life of SV is 1 to 5 hours in rats [31], it seems that ammonia reduction at 1.5 hour is related to the synergism of valproate elimination kinetic and the effect of LC on ammonia production. Previous studies have shown that SB was able to reduce SAL, alleviate the symptoms, and even reduce the rate of mortality following hepatic encephalopathy [32, 33]. Moreover, there are a few case reports regarding the successful treatment of VIHA with SB [34]. However, in the present study, SB treatment did not significantly decrease the SAL.

It has been reported that one mole of glycine conjugated to benzoate removes one mole of ammonia [32]. This process is energy consuming and is interfered by coenzyme A ester. SV reduces the levels of necessary cofactors of the urea cycle; free coenzyme A, acetyl-CoA, and carnitine in the cytoplasm [28] and results in raising the blood and hepatocyte concentrations of glycine [32, 35]. On the other hand, 2-propyl-4-pentenoic acid, a metabolite of omega oxidation of SV, inhibits benzoyl-CoA synthesis [36]. For the above-mentioned reasons, it may be suggested that the total capacity of SB in conjugation with glycine and decreasing the ammonia level was decreased. In this study, SB administration alone could not improve the motor function, the increased blood ammonia level, and lactate level. Owing to the above reason, the failure of SB on ammonia detoxification and reduction of VIHA can be justified. It was also reported that treatment of ammonium acetate-induced hyperammonemia with a non-toxic dose of SB in rats enhanced mortality due to the strong inhibition of ornithine trans carbamylase, as a major mitochondrial enzyme of the urea cycle [37]. Furthermore, experimental and clinical studies showed the increased mortality rate following administration of SB in hyperammonemia [38, 39]. Palekar and colleagues treated rats with L-norvaline, a strong inhibitor of ornithine trans carbamylase, and SB. They reported that SB increased serum and liver ammonia instead of returning SAL to the normal level [40]. SB was also ineffective in the prevention of ammonia accumulation in isolated hepatocytes [40, 41].

It was indicated that micromolar levels of monocarboxylate compounds, such as SB and SV, initiated the mitochondrial permeability transition in isolated liver mitochondria [42]. Mitochondrial permeability transition is a process that leads to failure of oxidative phosphorylation, the formation of reactive oxygen species, and cell death by apoptosis or necrosis [43]. It may also have a role in the pathogenesis of Reye's syndrome [44], which is characterized by hyperammonemia, hypoglycemia, micrvesicular steatosis, and encephalopathy [42]. Therefore, the administration of SB during SV intoxication could increase the risk of liver dysfunction problems such as Reye's syndrome and hyperammonemia. Although there are, many documents regarding the use of SB for hyperammonemia, in this study, SB could not reduce VIHA, although the levels of liver transaminases were normal [33, 34].

Sodium phenylbutyrate, a pro-drug of PA that is known as a monocarboxylate compound theoretically induces mitochondrial permeability transition [44]. However, we did not find any evidence for mitochondrial toxicity of the PA metabolite in the literature. In the present study, the administration of PA, alone or in combination with SB and LC, return the blood ammonia level to the normal range. A small cohort study in in the United States and Canada showed that the combination of intravenous sodium phenyl acetate and sodium benzoate was more effective in decreasing the plasma ammonium levels and improving survival in patients with historically lethal urea-cycle enzyme disorder [45]. The combined therapy with sodium phenyl acetate and sodium benzoate has been approval by the US Food and Drug Administration (FDA) to treat hyperammonemia in patients with urea cycle enzyme disorders since 1980s [45].

Each mole of PA conjugated to glutamine removes two moles of ammonia [35]. Combination of PA and LC can be a potential treatment for VIHA through increasing the ammonia excretion and reducing the ammonia production.

Based on the results, all animals treated with valproate had significantly high serum levels of lactic acid and only LC, alone or in combination with the other two antidotes, returned SLL to the normal level. In liver cells, LC attaches valproate-Co A instead of acetyl coenzyme A and enters into the mitochondria. Decreasing the storage of LC and acetyl coenzyme A during SV toxicity shifts cellular metabolism to fatty acids and induces lactic acidosis [46]. Lactic acidosis has been reported in SV intoxicated patients with serum valproate level greater than 850 mg/l [47]. LC improves the fatty acid metabolism by transferring long-chain free fatty acids to mitochondria and buffering the ratio of free CoA to acyl-CoA [48, 49]. It seems that the role of LC in getting the lactic acid level back to normal is related to improve lipid metabolism and increase the amount of acyl-coenzyme A [50].

Cooke et al. (2009) reported a 43-year-old man who ingested 40 grams of SV with serum concentration of 1470 mg/l (serum levels above 1000 mg/l may cause death) and SAL of 850 μmol/l. He was unresponsive and hypotensive. He became hemodynamically unstable, and his general condition deteriorated rapidly with rising lactic acidosis despite supportive treatment. Treatment by combination of LC, SB and sodium phenyl acetate resulted in rapid normalization of serum valproate, ammonia levels and neurological function without hemodialysis. The authors did not report the serum sodium level and other biochemical parameters [51].

High sodium serum levels up to 1.5 hours after treatment were due to the sodium salt of valproic acid. The sodium levels of SB-treated rats did not return to normal level even after 1.5 hours. As we used sodium-free PA, PA+SV-treated rats showed similar sodium levels. However, SB or PA, alone or in combination therapy for valproic acid-induced toxicity have been associated with hypernatremia.

The result of this study did not show significant differences in the level of serum aminotransferases, blood urea nitrogen, and creatinine in SV-intoxicated group. Also, Farooq and colleagues reported a 44-year-old male with valproate-induced hyperammonemic encephalopathy with normal liver function tests [52].

## Conclusion

The results of this study showed that during acute SV poisoning, administration of PA resulted in better therapeutic effects in comparison with SB. Moreover, co-administration of LC with PA may show a better therapeutic response.

## Acknowledgements

This article was extracted from a doctoral thesis, which is conducted based on the research funding (grant no. 941313) in Mashhad University of Medical Sciences.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Mohammad Moshiri, Medical Toxicology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; Department of Clinical Toxicology, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +985138022718; E-mail: Moshirim@mums.ac.ir

## References


[1] Rahim F, Azizimalamiri R, Sayyah M and Malayeri A. Experimental therapeutic strategies in epilepsies using anti-seizure medications. J Exp Pharmacol 2021; 13: 265-290.

[2] Etemad L, Moshiri M and Moallem SA. Epilepsy drugs and effects on fetal development: potential mechanisms. J Res Med Sci 2012; 17: 876-881.

[3] Watkins LV and Angus-Leppan H. Valproate, sexual health, and men: a narrative review. Epilepsy Behav 2020; 103: 106835.

[4] Habhab SF, Ulvin LB, Tauboll E, Svalheim S, Olsen KB, Horn MA and Heuser K. Influence of valproate-induced hyperammonemia on treatment decision in an adult status epilepticus cohort. Epilepsy Behav 2020; 111: 107193.

[5] Zhao L, Walline JH, Gao Y, Lu X, Yu S, Ge Z, Zhu H and Li Y. Prognostic role of ammonia in critical care patients without known hepatic disease. Front Med (Lausanne) 2020; 7: 589825.

[6] Segura-Bruna N, Rodriguez-Campello A, Puente V and Roquer J. Valproate-induced hyperammonemic encephalopathy. Acta Neurol Scand 2006; 114: 1-7.

[7] Auron A and Brophy PD. Hyperammonemia in review: pathophysiology, diagnosis, and treatment. Pediatr Nephrol 2012; 27: 207-222.

[8] Abbasi Jennatabad N, Fayyazi Bordbar MR, Etemad L, Eizadi-Mood N, Jomehpour H and Moshiri M. Methamphetamine-associated hyperammonemic encephalopathy: case reports. J Clin Psychopharmacol 2022; 42: 92-94.

[9] Maldonado C, Guevara N, Silveira A, Fagiolino P and Vazquez M. L-Carnitine supplementation to reverse hyperammonemia in a patient undergoing chronic valproic acid treatment: a case report. J Int Med Res 2017; 45: 1268-1272.

[10] Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW and Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med 2007; 356: 2282-2292.

[11] Savy N, Brossier D, Brunel-Guitton C, Ducharme-Crevier L, Du Pont-Thibodeau G and Jouvet P. Acute pediatric hyperammonemia: current diagnosis and management strategies. Hepat Med 2018; 10: 105-115.

[12] Irwin C, van Reenen M, Mason S, Mienie LJ, Westerhuis JA and Reinecke CJ. Contribution towards a metabolite profile of the detoxification of benzoic acid through glycine conjugation: an intervention study. PLoS One 2016; 11: e0167309.

[13] Haberle J, Chakrapani A, Ah Mew N and Longo N. Hyperammonaemia in classic organic acidaemias: a review of the literature and two case histories. Orphanet J Rare Dis 2018; 13: 219.

[14] Davies NA, Wright G, Ytrebo LM, Stadlbauer V, Fuskevag OM, Zwingmann C, Davies DC, Habtesion A, Hodges SJ and Jalan R. L-ornithine and phenylacetate synergistically produce sustained reduction in ammonia and brain water in cirrhotic rats. Hepatology 2009; 50: 155-164.

[15] Therrien G, Rose C, Butterworth J and Butterworth RF. Protective effect of L-carnitine in ammonia-precipitated encephalopathy in the portacaval shunted rat. Hepatology 1997; 25: 551-556.

[16] Moshiri M, Vahabzadeh M, Etemad L and Hosseinzadeh H. Failure of intravenous lipid emulsion to reduce diazinon-induced acute toxicity: a pilot study in rats. Iran J Pharm Res 2013; 12: 897-902.

[17] De Bleecker J, Willems J, De Reuck J, Santens P and Lison D. Histological and histochemical study of paraoxon myopathy in the rat. Acta Neurol Belg 1991; 91: 255-270.

[18] Ardianto C, Wardani HA, Nurrahmi N, Rahmadi M and Khotib J. Alpha-lipoic acid ameliorates sodium valproate-induced liver injury in mice. Vet World 2020; 13: 963-966.

[19] Shakya R, Hoque MK, Sapkota AS and Gupta PK. Differential hepatotoxic effects of sodium valproate at different doses in albino rats. Kathmandu Univ Med J (KUMJ) 2018; 16: 78-82.

[20] Warter JM, Imler M, Marescaux C, Chabrier G, Rumbach L, Micheletti G and Krieger J. Sodium valproate-induced hyperammonemia in the rat: role of the kidney. Eur J Pharmacol 1983; 87: 177-182.

[21] Baddour E, Tewksbury A and Stauner N. Valproic acid-induced hyperammonemia: Incidence, clinical significance, and treatment management. Ment Health Clin 2018; 8: 73-77.

[22] Ge Y, Xu B, Zhu S, Li C, He Q, Zhu T, Fan R and Gong D. Severe acute valproic acid intoxication successfully treated with liver support therapy. Basic Clin Pharmacol 2017; 121: 368-370.

[23] Brown LM, Cupples N and Moore TA. Levocarnitine for valproate-induced hyperammonemia in the psychiatric setting: a case series and literature review. Ment Health Clin 2018; 8: 148-154.

[24] Lheureux PE and Hantson P. Carnitine in the treatment of valproic acid-induced toxicity. Clin Toxicol 2009; 47: 101-111.

[25] Mishra MK, Kukal S, Paul PR, Bora S, Singh A, Kukreti S, Saso L, Muthusamy K, Hasija Y and Kukreti R. Insights into structural modifications of valproic acid and their pharmacological profile. Molecules 2021; 27: 104.

[26] Nakamura M and Nagamine T. The effect of carnitine supplementation on hyperammonemia and carnitine deficiency treated with val-

proic acid in a psychiatric setting. Innov Clin Neurosci 2015; 12: 18-24.
[27] Nojiri S, Fujiwara K, Matsuura M, Iio E, Matsunami K, Nomura T, Sobue S, Takahashi Y and Joh T. L-carnitine reduces ammonia levels and alleviates covert encephalopathy: a randomized trial. J Transl Sci 2018; 4: 1-6.
[28] Lheureux PE, Penaloza A, Zahir S and Gris M. Science review: carnitine in the treatment of valproic acid-induced toxicity-what is the evidence? Critical Care 2005; 9: 431.
[29] Limon ID, Angulo-Cruz I, Sanchez-Abdon L and Patricio-Martinez A. Disturbance of the glutamate-glutamine cycle, secondary to hepatic damage, compromises memory function. Front Neurosci 2021; 15: 578922.
[30] Yue YGW, Wai CK, Peter WYM, Tung WH and Yau CK. A fatal case of valproate-induced hyperammonemic encephalopathy: an update on proposed pathogenic mechanisms and treatment options. Int J Epilepsy 2017; 4: 181-183.
[31] Löscher W. Basic pharmacology of valproate. CNS Drugs 2002; 16: 669-694.
[32] Husson MC, Schiff M, Fouilhoux A, Cano A, Dobbelaere D, Brassier A, Mention K, Arnoux JB, Feillet F, Chabrol B, Guffon N, Elie C and de Lonlay P. Efficacy and safety of i.v. sodium benzoate in urea cycle disorders: a multicentre retrospective study. Orphanet J Rare Dis 2016; 11: 127.
[33] Misel ML, Gish RG, Patton H and Mendler M. Sodium benzoate for treatment of hepatic encephalopathy. Gastroenterol Hepatol 2013; 9: 219.
[34] Amanat S, Shahbaz N and Hassan Y. Valproic acid induced hyperammonaemic encephalopathy. J Pak Med Ass 2013; 63: 72-75.
[35] De Las Heras J, Aldamiz-Echevarria L, Martinez-Chantar ML and Delgado TC. An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease. Expert Opin Drug Metab Toxicol 2017; 13: 439-448.
[36] Beyoglu D and Idle JR. The glycine deportation system and its pharmacological consequences. Pharmacol Ther 2012; 135: 151-167.
[37] O'Connor JE, Ribelles M and Grisolia S. Potentiation of hyperammonemia by sodium benzoate in animals. A note of caution. Eur J Pediatr 1982; 138: 186-187.
[38] O'Connor JE, Costell M and Grisolia S. The potentiation of ammonia toxicity by sodium benzoate is prevented by L-carnitine. Biochem Biophys Res Commun 1987; 145: 817-824.
[39] Maswoswe SM, Cyr DM, Griffith AD and Tremblay GC. The effect of sodium benzoate on ammonia toxicity in rats. Biochem Biophys Res Commun 1986; 138: 369-373.
[40] Palekar AG, Canas JA, Kalbag SS, Kim SJ, Castro-Magana M and Angulo MA. Failure of sodium benzoate to alleviate plasma and liver ammonia in rats. Biochem Med Metab Biol 1989; 41: 64-69.
[41] Tremblay CC and Wendler PA. On benzoate therapy for ammonia toxicity. Pediatrics 1982; 70: 503-504.
[42] Prabhakar Kamath P. Valproic acid administration in management of status epilepticus causing Reye's syndrome. Int J Recent Surg Med Sci 2021; 44-46.
[43] Bonora M, Patergnani S, Ramaccini D, Morciano G, Pedriali G, Kahsay AE, Bouhamida E, Giorgi C, Wieckowski MR and Pinton P. Physiopathology of the permeability transition pore: molecular mechanisms in human pathology. Biomolecules 2020; 10: 998.
[44] Piper JD and Piper PW. Benzoate and sorbate salts: a systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. Compr Rev Food Sci Food Saf 2017; 16: 868-880.
[45] Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW and Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med 2007; 356: 2282-2292.
[46] Doyon S. Antiepileptics. In: Hoffman RS, Howland MA, Lewin NA, Nelson LS, Goldfrank lR, editors. Goldfrank's Toxicologic Emergencies 10. McGrow Hill; 2019. pp. 770-783.
[47] Nguyen P, Chevillard L, Gouda AS, Gourlain H, Labat L, Malissin I, Deye N, Voicu S and Megarbane B. L-carnitine does not improve valproic acid poisoning management: a cohort study with toxicokinetics and concentration/effect relationships. Ann Intensive Care 2022; 12: 7.
[48] Harmeyer J. The physiological role of L-carnitine. Lohman Information 2002; 27: 15-21.
[49] Hoppel C. The role of carnitine in normal and altered fatty acid metabolism. Am J Kidney Dis 2003; 41: S4-S12.
[50] Gnoni A, Longo S, Gnoni GV and Giudetti AM. Carnitine in human muscle bioenergetics: can carnitine supplementation improve physical exercise? Molecules 2020; 25: 182.
[51] Cooke A, Mac Sweeney R, Bell C, Loughrey C and Allen G. Sodium valproate toxicity, a novel approach. Intensive Care Med 2009; 35: 154-154.
[52] Farooq F, Sahib Din J, Khan AM, Naqvi S, Shagufta S and Mohit A. Valproate-induced hyperammonemic encephalopathy. Cureus 2017; 9: e1593.