

Protective Effect of Mito-TEMPO on Sodium Valproate-Induced Hepatotoxicity in Mice

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Abstract- Valproic acid is a broad-spectrum anticonvulsant drug that is also useful for other diseases such as bipolar disorder and migraines. The most important side effect of this drug is hepatotoxicity. Oxidative stress and mitochondrial dysfunction play a role in the pathogenesis of liver toxicity of valproic acid. Mito-TEMPO is an antioxidant-based compound, which is selectively accumulated in mitochondria. The effects of its mitochondrial protection against oxidative damage in various pathologies, such as liver damage, have been observed. The aim of this study was to evaluate the protective effect of Mito-TEMPO on liver toxicity induced by sodium valproate in mice. Animals were divided into five groups and treated intraperitoneally over a 4-week period. Group 1 received normal saline, served as vehicle control, group 2 treated with 12.5 mg/kg of sodium valproate, group 3 was treated with 1 mg/kg of Mito-TEMPO, group 4 received both sodium valproate (12.5 mg/kg) and Mito-TEMPO (1 mg/kg), and group 5 received sodium valproate (12.5 mg/kg), and vitamin E (5 mg/kg) served as a positive control. At the end of the experiment, blood samples were collected by cardiac puncture, and all the animals were killed under ether anesthesia. Biochemical parameters including AST, ALT, ALP, and GGT in serum samples and glutathione (GSH) and malondialdehyde (MDA) contents in liver homogenates were determined. The findings of this study showed that the activity of AST and ALT were significantly lower in the sodium valproate+Mito-TEMPO treated animals as compared to the sodium valproate group (group 2). Furthermore, Mito-TEMPO was able to recover glutathione content (GSH) of liver tissue. The effect of Mito-TEMPO on the activity of ALP and GGT and serum level of MDA was not significant. Taken collectively, Mito-TEMPO has a protective role in sodium valproate hepatotoxicity. Considering the present results, further studies, in view of the potential therapeutic properties of Mito-TEMPO in improving liver damage caused by the use of valproic acid, may lead to the clinical applications of Mito-TEMPO in the treatment of liver disease.

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Keywords: Mito-TEMPO; Valproic acid; Hepatotoxicity

Introduction

Valproic acid is a drug widely used in the treatment of seizures and is also used for other diseases such as bipolar disorder and migraines (1,2). It is well tolerated in consumers but may increase the liver enzyme levels in 20% of consumers (3). Valproic acid causes a mild increase in blood levels of amino acids, alkaline phosphatase, and a minimal increase in bilirubin in 44% of cases in the first month after drug administration

(4,5). Increased liver enzymes correlate with the dose of valproic acid consumed (6). Valproic acid, known as dipropyl acetic acid, 2-propyl valeric acid, 2-propyl pantothenic acid, is an eight-branched fatty acid. Valproic acid increases the amount of gamma-aminobutyric acid in the brain. It is also an inhibitor of neurotransmitters to the brain. It is said that the drug may enhance GABAergic function and mimic its function in postsynaptic receptors. As noted, hepatotoxicity is a well-known side effect of valproic

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acid (7). Liver toxicity of valproic acid occurs due to its effect on the mitochondria of hepatocytes (8). Beta-oxidation inhibition of fatty acids by mitochondria has been reported due to valproic acid consumption, impaired gluconeogenesis, interference with urea synthesis, and inhibition of oxidative phosphorylation in the liver (9).

Oxidative stress imbalances the production of reactive oxygen species and the ability of the biological system to detoxify reactive mediators, repairing their damage. Normal disruption of cell swelling can produce toxic effects through the production of peroxides and free radicals that cause damage to all cellular components, including proteins, lipids, and DNA (10,11). Therefore, oxidative stress can impair cell function. Oxidative stress plays a role in the development of many diseases or exacerbation of many of their symptoms (12). However, reactive oxygen species can be useful; for example, they are used by the immune system as a way to attack and kill pathogens (11). An antioxidant is a molecule that prevents the oxidation of other molecules. Oxidation is a chemical reaction in which electrons or hydrogen are transferred from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. These radicals can initiate chain reactions. Antioxidants end the chain reaction by displacing free radicals and prevent other oxidation reactions. Antioxidants do this by oxidizing themselves. So antioxidants are often regenerative agents such as thiol, ascorbic acid, or polyphenols. Oxidation reactions are vital to life and can also be destructive. Plants and animals have a variety of antioxidants such as glutathione, vitamin C, vitamin E, catalase, and superoxide dismutase, but Mito-TEMPO is a combination of a piperidine nitroxide antioxidant (TEMPO) and a lipophilic cation of triphenylphosphonium TPP (9). In the present study, the effect of hepatotoxicity of Mito-TEMPO on the prevention of sodium valproate-induced toxicity in liver tissue was investigated.

Materials and Methods

The animals used in this study, 30 mice (weighing 25-30 g), were kept at $22\pm 2^\circ$ C and sufficient light (12 hours of light and 12 hours of darkness) in order to adapt to the new environment of mice ten days in the animal house of the Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, and were kept sterilized in polyethylene glycol in large cages.

Sodium valproate, Vitamin E and Mito-TEMPO

were obtained from Sigma Aldrich (USA). Kits for assessment of ALT, AST ALP, and GGT were purchased from Pars Azmoon, an Iranian company. Other assessments were conducted by MDA and GSH kits, which were purchased from Zell- Bio Company in Germany. Thirty male mice were divided into five groups of 6 each. The first group of mice was treated with normal saline. The second group received sodium valproate (12.5 mg/kg). The third group of mice treated with Mito-TEMPO 1 mg/kg. The fourth group of mice treated with sodium valproate (12.5 mg/kg) and Mito-TEMPO (1 mg/kg). The fifth group received sodium valproate (12.5 mg/kg) and Vitamin-E (5 mg/kg). All injections were intraperitoneal. After these processes, the animals were anesthetized by ketamine (80 mg/kg) and xylazine (5 mg/kg). For biochemical studies, about 2 mL of the whole blood was collected from the retro-orbital plexus of each mouse, and the serums of samples were extracted by centrifuge.

Samples were used to measure markers of liver injury and oxidative stress. After blood sampling, the whole liver was removed and transferred to -80° C for subsequent tests.

Statistical analysis

Data were analyzed in Excel using one-way ANOVA and Turkey-Kramer post-test. Results were reported as Mean \pm SEM. Significance level $P<0.05$ was considered.

Results

Effect of Mito-TEMPO on ALT activity

Valproic acid increased the activity of ALT, but the used of Mito-TEMPO accompany by Valproic acid caused a reduction of this activity. On the other hand, using of vitamin-E as a positive control with valproic acid had the same effect of using Mito-TEMPO with valproic acid on the activity of ALT (** $P<0.01$).

Effect of Mito-TEMPO on AST activity

Valproic acid increased the activity of AST, but the used of Mito-TEMPO accompany by Vaporic acid caused a reduction of this activity. Using of vitamin-E as a positive control with valproic acid had the same effect of using Mito-TEMPO with valproic acid on the activity of AST (* $P<0.05$).

Effect of Mito-TEMPO on ALP activity

Valproic acid had no effect on the activity of ALP compared to the control group. Otherwise, like vitamin

Protective effect of Mito-TEMPO

E, Mito-TEMPO accompany valproic acid had no effect on the activity of ALP also.

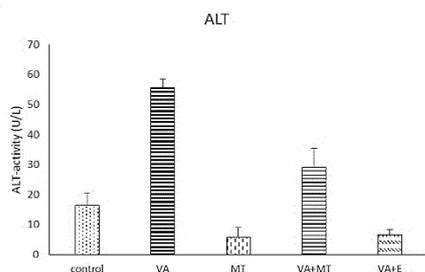


Figure 1. Effect of drugs (mg/kg) on serum ALT activity of animal groups [Control, VA= Valproic acid (12.5 mg/kg), MT= Mito-TEMPO (1 mg/kg), E=Vitamin E (5 mg/kg)] after 4 weeks treatment
** $P<0.01$, *** $P<0.001$

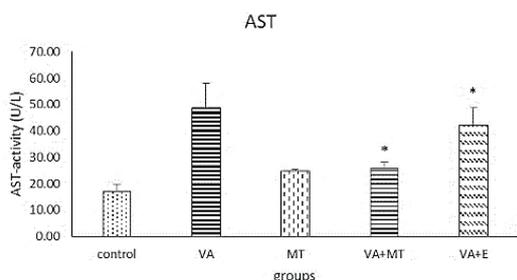


Figure 2. Effect of drugs on serum AST activity of animal groups [Control, VA= Valproic acid (12.5 mg/kg), MT= Mito-TEMPO (1 mg/kg), E=Vitamin E (5 mg/kg)] after 4 weeks treatment
* $P<0.05$, ** $P<0.01$

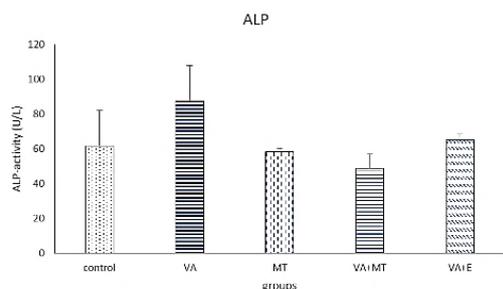


Figure 3. Effect of drugs on serum ALP activity of animal groups [Control, VA= Valproic acid (12.5 mg/kg), MT= Mito-TEMPO (1 mg/kg), E=Vitamin E (5 mg/kg)] after 4 weeks treatment

Effect of Mito-TEMPO on ALP activity

Valproic acid had no effect on the activity of GGT compared to the control group. Otherwise, like vitamin E, Mito-TEMPO accompany valproic acid had no effect on the activity of GGT also.

Effect of Mito-TEMPO on GSH content

Valproic acid decreased GSH, but the use of Mito-

TEMPO alone and accompany valproic acid increased GSH content, but vitamin E had no effect on GSH content when used with valproic acid (** $P<0.01$).

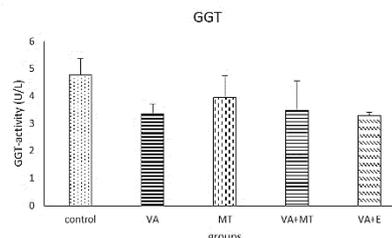


Figure 4. Effect of drugs on serum GGT activity of animal groups [Control, VA= Valproic acid (12.5mg/kg), MT= Mito-TEMPO (1 mg/kg), E=Vitamin E (5mg/kg)] after 4 weeks treatment

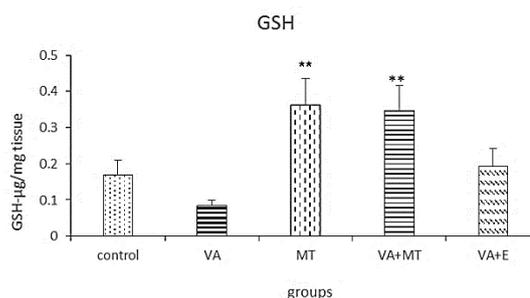


Figure 5. Effect of drugs on liver tissue GSH activity of animal groups [Control, VA= Valproic acid (12.5mg/kg), MT= Mito-TEMPO (1mg/kg), E=Vitamin E (5mg/kg)] after 4 weeks treatment
** $P<0.01$

Effect of Mito-TEMPO on MDA activity

Valproic acid had no effect on the activity of MDA compared to the control group. Otherwise, like vitamin E, Mito-TEMPO accompany valproic acid had no effect on the activity of MDA also.

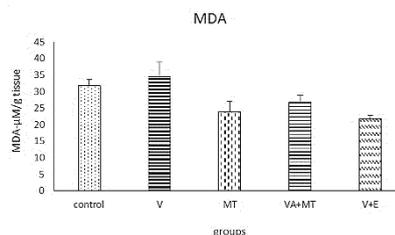


Figure 6. Effect of drugs on liver tissue MDA activity of animal groups [Control, VA= Valproic acid (12.5mg/kg), MT= Mito-TEMPO (1 mg/kg), E=Vitamin E (5mg/kg)] after 4 weeks treatment

Discussion

The liver is the body's primary organ for metabolism and the breakdown of many anticonvulsants, so it is also at risk for drug damage. Damage to the liver encompasses a range of hepatotoxic reactions, ranging

from mild to a temporary damage to fatal liver failure (13). Liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are used as markers to detect liver cell injury. Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are also used as diagnostic markers of bile duct obstruction. These enzymes can increase secondary to a liver disease and pathology, or even in the absence of an underlying disease and in response to enzymatically inducing drugs. Most anticonvulsants, except the two drugs gabapentin and vigabatrin, have hepatic metabolism. Lipophilic drugs for urinary excretion and renal excretion must be in the form of hydrophilic and water-soluble. The process of converting lipophilic drugs into hydrophilic consists of two phases. Phase 1 reactions include oxidation, redox, and hydroxylation. Phase two also involves conjugation reactions, *e.g.*, glucuronidation, which results in the formation of active and inactive metabolites. Just as anticonvulsants can damage and increase liver enzymes, liver diseases can impair the metabolism of these drugs in various ways. The metabolism of these drugs depends on the level of hepatic blood flow, the amount of albumin bonded, the hepatic drug uptake by the liver, and the cell metabolism potency (14,15). Anticonvulsants, such as carbamazepine and valproic acid, can cause severe lethal reactions. The prevalence of these lethal drug reactions is low, but they are more dangerous than other drugs that damage the liver and can lead to death and liver failure and require liver transplantation. Generally, a variety of medications can cause liver damage through one or more of the following mechanisms: Valproic acid is a drug widely used to treat seizures and is also used in other diseases such as bipolar disorder and migraines (2). It is well tolerated in consumers but may increase the liver enzyme levels in 20% of consumers (3). Valproic acid causes a mild increase in blood levels of amino acids, alkaline phosphatase, and a minimal increase in bilirubin in 44% of cases within the first month after starting the drug (6). The mechanism of injury to valproic acid is by interfering with beta-oxidation of endogenous lipids. Valproic acid causes carnitine deficiency and mitochondrial damage through the formation of conjugated esters with carnitine. The occurrence of oxidative stress in liver tissue as well as in other tissues exposed to toxic compounds has been well documented (15). Lipid peroxidation in biological membranes is one of the most common events that occur after the toxicity and oxidative stress in cells. Increased levels of lipid peroxidation can damage the cell wall. Measurement of MDA caused by oxygen free radicals

indicates damage to biological membranes. Glutathione is one of the most important molecules in the cellular defense system against chemical reactions of toxic compounds and stress. A form of glutathione resuscitation is needed to eliminate toxicity. Decreased GSH levels, which indicate glutathione depletion, make cells more susceptible to chemical damage (16).

Therefore, the use of cell preservatives can prevent this damage to the liver and other organs. Vitamin E is a fat-soluble vitamin that has mainly reductive properties of oxidation reactions. Vitamin E has various biological functions, and its antioxidant properties are the most important and prominent feature. It has a great ability to neutralize free radicals, and as a potent antioxidant, prevent the damage caused by free radicals, and can play a key role in delaying the pathogenesis of various degenerative diseases of the liver and kidney (17). Mito-TEMPO is a combination of antioxidant properties. This compound consists of a piperidine nitroxide antioxidant (TEMPO) and a lipophilic triphenylphosphonium compound (TPP) (9). TEMPO is a compound that is produced by superoxide dismutase in the catalytic cycle, while TPP is a highly permeable cation that has high membrane potential for mitochondrial accumulation (9). Mito-TEMPO is a compound that can easily cross two lipid layers and selectively accumulate in mitochondria. Mito-TEMPO is actually a compound that is targeting mitochondria (18). This compound inhibits oxidative stress and reduces cardiopulmonary alterations in type 1 and type 2 diabetic mice by inhibiting the production of reactive oxygen species (ROS) in the mitochondria. In vitro and in vivo studies have shown the antioxidant role of this drug in mitochondria (18-20).

Inhibition of mitochondrial ROS treatment using Mito-TEMPO reduced cardiac changes and cardiac dysfunction. Therefore, Mito-TEMPO, as a mitochondrial antioxidant, can be an effective treatment for diabetes-related heart problems (21). Du and colleagues also administered Mito-TEMPO to mice for an hour and a half after administering 300 mg/kg fasting mice to assess the protective effects of Mito-TEMPO on acetaminophen-induced hepatotoxicity. Mito-TEMPO dose-dependently reduced acetaminophen-induced liver injury, which was manifested by increased serum ALT activity and lobular centric necrosis. They concluded that Mito-TEMPO could protect acetaminophen overdose by reducing mitochondrial oxidative stress and preventing peroxynitrite build-up and mitochondrial dysfunction and could be a promising treatment for acetaminophen poisoned patients (22). In another study, Dikalova and colleagues showed that mitochondrial

Protective effect of Mito-TEMPO

superoxide radicals play an important role in the development and spread of blood pressure and that mitochondrial antioxidants such as Mito-TEMPO have good therapeutic effects on this disease and possibly other diseases (18). Xilan Lu *et al.*, in a study, investigated the efficacy of Mito-TEMPO in improving sperm quality during the semen cryopreservation process. The results showed that the addition of Mito-TEMPO significantly improved sperm motility after thawing (23). In the present study, the protective effect of Mito-TEMPO on sodium valproate-induced hepatotoxicity in mice was investigated. The results of this study showed that the administration of sodium valproate to mice increased the activity of ALT and AST enzymes in comparison with control. Administration of sodium valproate and Mito-TEMPO to mice did not have a significant effect on GGT, ALP, and malondialdehyde levels. These results are largely consistent with other studies. According to the above, elevated levels of ALT and AST enzymes as a result of sodium valproate administration may be a sign of hepatocellular vulnerability to sodium valproate. However, no significant difference in the activity of GGT and ALP enzymes indicates that sodium valproate had no toxic effects on bile ducts. Administration of Mito-TEMPO with sodium valproate decreased the activity of ALT and AST enzymes. However, this decrease was more severe for ALT. However, it can be argued that administration of Mito-TEMPO along with sodium valproate can prevent liver cell damage and have protective effects, which is in line with the findings of Du *et al.*, Although all intracellular targets of sodium valproate and Mito-TEMPO are not fully understood, these findings confirm that mitochondrial is one of the target organelles of these compounds. Given that the level of hepatic glutathione was not significantly different between the sodium valproate and control groups, it seems that sodium valproate did not significantly decrease hepatic glutathione consumption and a significant and similar increase in glutathione in the Mito-TEMPO group alone or in combination with sodium valproate also confirms this. Although the levels of malondialdehyde were higher in the groups receiving sodium valproate (alone or with Mito-TEMPO) than in the other groups, this difference was not statistically significant, especially in the group receiving vitamin E with sodium valproate. MDA levels are almost similar to other groups, so it is likely that sodium valproate did not cause significant lipid peroxidation in liver cells, at least at the dose we used (1).

Conclusion As mentioned, valproic acid is widely

used in the treatment of seizures and other diseases such as bipolar disorder and migraines, but may also cause liver damage. Based on this study, we may conclude that sodium valproate may cause hepatic impairment and that Mito-TEMPO as an antioxidant may reduce these damages. Confirmation of these findings requires further studies.

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