



Research Article

Effects of Sodium Selenite and Vitamin E on the Development of Morphine Dependency in Mice

Bohloul Habibi-Asl^{1*}, Alireza Parvizpur¹, Kiarash Fekri^{1,2}, Hadis Jahanpanah¹, Hadis Rezaei³, Mohammad Charkhpour^{1*}

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

²Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

³Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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Abstract

Background: Antioxidant drugs may be useful in preventing morphine-induced dependency by suppressing oxidative stress. Vitamin E which has many essential roles in the body is a powerful antioxidant. On the other hand, selenium is an essential trace element that plays a strong role in various biochemical pathways. The aim of this study was to investigate the effects of sodium selenite and vitamin E on morphine-induced dependency in mice.

Methods: Ninety male mice, weighing 20 to 30 g, were randomly divided into 10 groups and were treated as follows: a) saline and b) morphine groups were pretreated (for 2 days) with normal saline (10 ml.kg⁻¹.day⁻¹, ip) then daily doses of normal saline (10 ml.kg⁻¹.day⁻¹, ip) and morphine (50 mg.kg⁻¹.day⁻¹) were added to the injections for the following 4 days, respectively. c, d, e) sodium selenite, f, g, h) vitamin E, i) vitamin E solvent (almond oil) and j) co-administration groups were pretreated (for 2 days) with sodium selenite (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip), vitamin E (20, 40, 60 IU.kg⁻¹.day⁻¹, ip), vitamin E solvent (10 ml.kg⁻¹.day⁻¹, ip) and combination of the drugs respectively, then morphine doses (50 mg.kg⁻¹.day⁻¹, ip) were added to the injections for the following 4 days. Withdrawal symptoms were evaluated after injecting naloxone (4 mg/kg/day). Biochemical evaluations were also performed.

Results: The results showed that co-administration of sodium selenite and vitamin E (at low doses) significantly reduced morphine dependency ($p < 0.05$).

Conclusion: The synergistic effect of sodium selenite and vitamin E can be a suitable and efficient approach to reduce dependency.

Introduction

The long-term use of opioids leads to tolerance so that patients have to use larger doses of the opioid for the same effect. In which case we will see more severe complications such as dependence, which is characterized by the withdrawal syndrome if the medication is discontinued or an antagonist is prescribed.¹⁻⁴ Among the mechanisms involved in the development process of dependence, we can point to an increase in the amounts of inflammatory cytokines, nitric oxide⁵ and NMDA⁶ or glucocorticoid⁷ receptors. Previous studies have shown that inhibition of NMDA receptors can postpone morphine tolerance. NMDA antagonists such as ketamine, dextromethorphan significantly reduce the tolerance and dependency to opioids.^{1,3}

Oxidative stress, mitochondrial dysfunction, and apoptosis have been known as some destructive effects of drug abuse.⁸ There are evidences that prove the relationship between taking opioids and production of free radicals and

consequently nerve cell oxidative damages.⁹

Selenium is an essential chemical element. Studies have shown that its deficiency is associated with an increased risk of certain types of cancer. It is also an important part of glutathione peroxidase enzyme that destroys peroxides before they cause damages to the various tissues of the body. Besides, vitamin E is a lipid-soluble vitamin that acts as an antioxidant in the cell membrane and suppresses oxidative stress. Altogether, both drugs can inhibit lipid peroxidation and prevent damages caused by active oxygen metabolites.^{10,11}

Given the antioxidant potentials of vitamin E and sodium selenite, they are likely to be effective in reducing opioid dependency and this study aimed to prove this.

Materials and Methods

Drugs

Sodium selenite injection vials and morphine sulfate

*Corresponding Author: Mohammad Charkhpour, E-mail: charkhpour@tbzmed.ac.ir

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ampoules were purchased from Biosyn (Germany) and Darou Pakhsh Pharmaceutical Manufacturing Co (Tehran, Iran) respectively. Vitamin E ampoules were purchased from Osvah Pharmaceutical Co (Tehran, Iran) and were diluted in almond oil to be prepared for injection.

Experimental groups

Ninety male mice weighing 20 g to 30 g were divided into 10 groups as following: Saline and morphine groups were pretreated (for 2 days) with normal saline (10 ml.kg⁻¹.day⁻¹, ip) then daily doses of normal saline (10 ml.kg⁻¹.day⁻¹, ip) and morphine (50 mg.kg⁻¹.day⁻¹) were added to the injections for the following 4 days, respectively. Sodium selenite, vitamin E, vitamin E solvent and co-administration groups were pretreated (for 2 days) with sodium selenite (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip), vitamin E (20, 40, 60 IU.kg⁻¹.day⁻¹, ip), almond oil as vitamin E solvent (10 ml.kg⁻¹.day⁻¹, ip) and combination of the drugs respectively, then morphine doses (50 mg.kg⁻¹.day⁻¹, ip) were added to the injections for the following 4 days.

The animals were housed in standard cages in a room maintained at the ambient temperature (21°C-23°C) with an alternating 12-h light–dark cycle. Food and water were available ad libitum. Each animal was used only once in all experiments. The study protocol was designed and approved by the Ethics Committee for the Use of Animals in Research at Tabriz University of Medical Sciences (Code: IR.TBZMED.REC.1396.571).

Assessment of the withdrawal syndrome

On the sixth day, 2 hours after injecting the last dose of morphine (or normal saline in Saline group), naloxone (4 mg.kg⁻¹, ip) was administered and the withdrawal syndrome (jumping and standing on the feet) was assessed for 30 minutes.

Blood sample preparation

In order to perform the biochemical tests, blood samples were collected through a head cut for each animal following deep anesthesia and according to the ethical guidelines. After collecting the samples, they were centrifuged for 10 minutes at 3500 rpm and were kept in the refrigerator for performing the tests.

Biochemical analyses

Total antioxidant capacity (TAC) was measured by spectrophotometry (Alcyon 300 Biochemistry Analyzer) at wavelength of 600 nm. Malondialdehyde (MDA) measurement was based on reaction with thiobarbituric acid (TBA), extraction with normal butanol, spectrophotometric absorbance measurement, and comparison with the standard curve.

Statistical analysis

The data were expressed as mean ± S.E.M for each group and were analyzed with the independent t-test and analysis of variance (ANOVA) followed by the multiple comparison test of Tukey. Differences between the means were considered statistically significant if P<0.05.

Results

Comparing the withdrawal syndrome between saline and morphine groups

As shown in Figure 1, withdrawal syndrome (jumping and standing on the feet) following naloxone administration in the morphine group was significantly more severe than what was seen in the saline group (P<0.01 and P<0.05).

Effects of sodium selenite administration on the withdrawal syndrome

According to Figure 2, the administration of sodium selenite at various doses (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip), did not make any significant changes to the withdrawal syndrome.

Effects of vitamin E administration on the withdrawal syndrome

As can be seen in Figure 3, the administration of vitamin E, at various doses (20, 40, 60 IU.kg⁻¹.day⁻¹, ip), did not affect the withdrawal syndrome significantly.

Effects of vitamin E solvent (Almond oil) on the withdrawal syndrome

As shown in Figure 4, vitamin E solvent (almond oil) did not make any significant changes to the withdrawal syndrome.

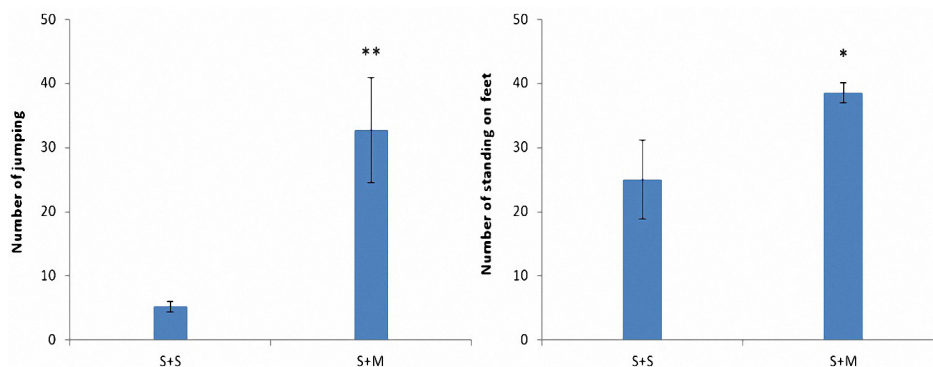


Figure 1. Comparing the withdrawal syndrome (jumping and standing on feet) between saline and morphine groups ($n = 9$ in each group). The left figure: Number of jumping, The right figure: Number of standing on feet, M: Morphine, S: Saline * P<0.05, ** P<0.01

Effects of vitamin E (20 IU.kg⁻¹.day⁻¹, ip) and sodium selenite (0.25 mg.kg⁻¹.day⁻¹, ip) co-administration on the withdrawal syndrome

According to Figure 5, in this experimental group (unlike

the previous groups), changes in withdrawal syndrome were statistically significant ($P < 0.01$ and $P < 0.05$). This demonstrated that co-administration of vitamin E and sodium selenite at low doses had a synergistic protective

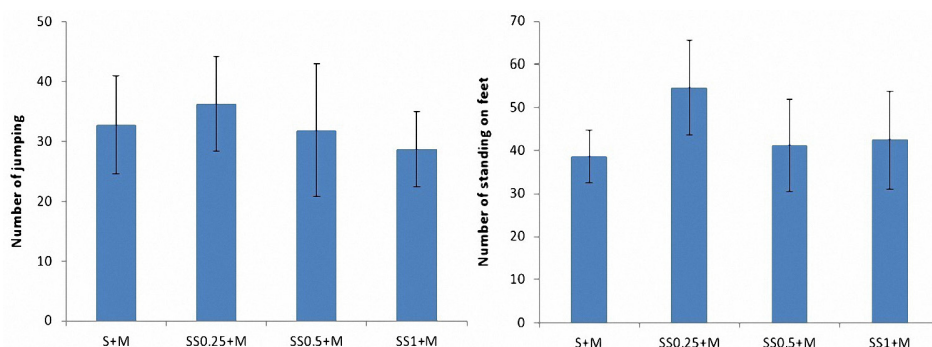


Figure 2. Effects of sodium selenite (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip) administration on the withdrawal syndrome. The left figure: Number of jumping, the right figure: Number of standing on feet ($n = 9$ in each group). M: Morphine, S: Saline, SS: Sodium selenite.

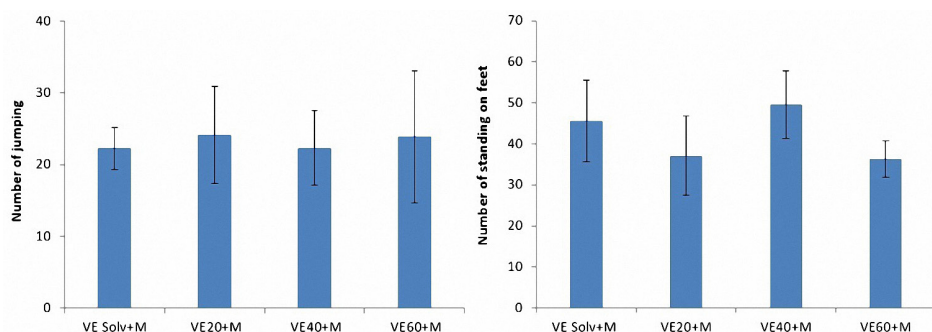


Figure 3. Effects of vitamin E (20, 40, 60 IU.kg⁻¹.day⁻¹, ip) administration on the withdrawal syndrome. The left figure: Number of jumping, The right figure: Number of standing on feet ($n = 9$ in each group). VE Solv: Vitamin E solvent, VE: vitamin E, M: Morphine.

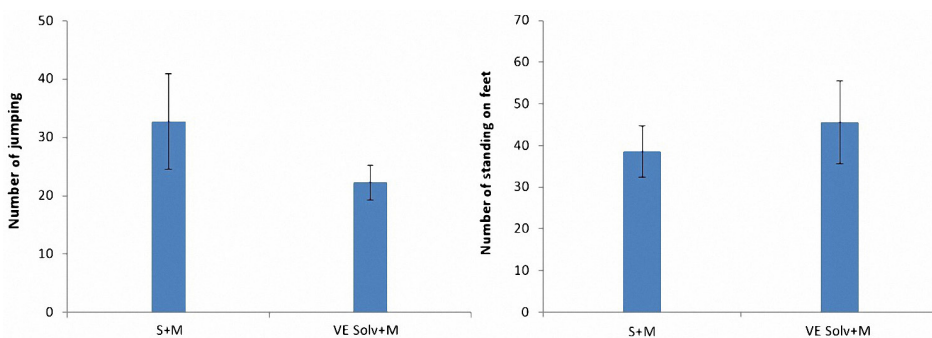


Figure 4. Effects of vitamin E solvent on the withdrawal syndrome. The left figure: Number of jumping, The right figure: Number of standing on feet ($n = 9$ in each group). VE Solv: Vitamin E solvent, M: Morphine, S: Saline.

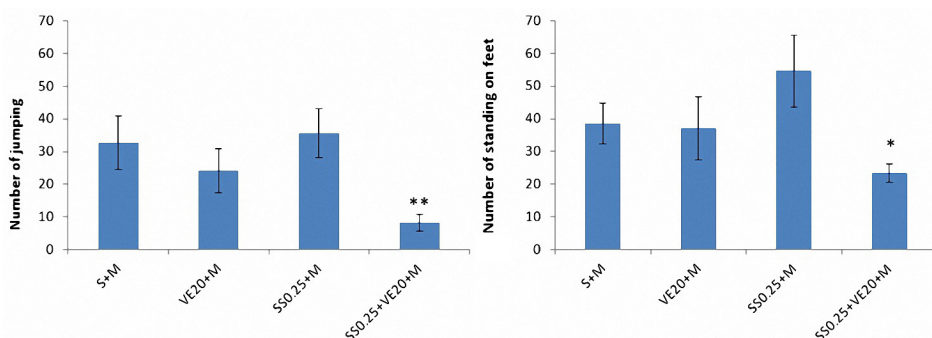


Figure 5. Effects of vitamin E (20 IU.kg⁻¹.day⁻¹, ip) and sodium selenite (0.25 mg.kg⁻¹.day⁻¹, ip) co-administration on the withdrawal syndrome. The left figure: Number of jumping, The right figure: Number of standing on feet ($n = 9$ in each group), * $p < 0.05$, ** $p < 0.01$ compared to S+M group. M: Morphine, S: Saline, VE: Vitamin E, SS: sodium selenite.

effect against the development of morphine-induced dependency.

Biochemical analyzes: MDA and TAC

Effects of vitamin E (20, 40, 60 IU.kg⁻¹.day⁻¹, ip), sodium selenite (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip) and co-administration of the two on TAC and MDA levels were evaluated and the results have been presented in Table 1. As shown in the table, changes in sodium selenite groups (compared to Morphine group) and VitE 60 group (compared to Vit E solvent group) were statistically significant.

Discussion

In recent years, many studies have been conducted to prove the efficacy of compounds with the potential ability to attenuate morphine tolerance and dependency.^{1,12} Throughout these studies a significant number of underlying mechanisms and pathways have been investigated. As a result some receptors (especially G protein-coupled receptors), hormones (for example cortisol) and neurotransmitters (such as orexins, noradrenaline, serotonin and etc.) have been introduced to be somehow involved in the process of morphine tolerance and dependency. So that pharmacological manipulation of any of the above can be an approach to deal with this challenge.¹²⁻¹⁷ NMDA receptors, which are part of the glutamatergic system, have been found to play a key role in these processes.^{18,19} The use of NMDA receptor antagonists has been shown to prevent morphine tolerance and dependency.²⁰ On the other hand, stimulation of NMDA receptors results in increased nitric oxide (NO) production, and many studies have found this increase to be an important factor in the development of morphine tolerance and dependency.²¹⁻²⁴ Selenium has been found involved in regulating inflammation²⁵ and has the ability to reach the brain.²⁶ It is able to inhibit the NOS enzyme and also to decrease the sensitivity of NMDA receptors to excitatory amino acids such as aspartate or glutamate. Therefore, sodium selenite may decrease the sensitivity of NMDA receptors to this type of amino acids (aspartate/

glutamate) and on the other hand, may inhibit morphine tolerance and dependence through NOS inhibition. Furthermore, due to the presence of selenium in the glutathione peroxidase construct, the neuroprotective and antioxidant role of selenium would be justifiable.²⁷

Oxidative stress is another mechanism involved in the process of morphine dependency. Studies have shown that heroin withdrawal would increase lipid peroxidation and decrease the amounts of endogenous antioxidants. It also reduces the antioxidant capacity of serum enzymes and the amounts of antioxidants such as superoxide dismutase, catalase, and glutathione peroxide in the brain, thereby increasing the production of reactive oxygen species and causing damages to carbohydrates, amino acids, phospholipids and nucleic acids.²⁸ As one of the microelements necessary for body function selenium is considered as the main component of glutathione peroxidase.²⁹ Selenium has an important role in maintaining GPx enzyme levels and proper regulation of glutathione which results in reducing the harmful effects of oxidative stress consequently.³⁰ Selenium is widely distributed throughout the body and as the main component of cerebral selenoproteins, has a crucial role in maintaining brain function. Furthermore, like glutathione peroxidase cofactor, it accelerates hydrogen peroxide reduction and converts organochlorine hydroxide to non-toxic compounds.²⁸ In the present study, sodium selenite (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip) administration did not attenuate morphine withdrawal syndrome. But changes in the serum levels of TAC and MDA were significant.

Vitamin E is a lipophilic antioxidant in the cell membrane which acts as a free radical scavenger.³¹ Vitamin E protects cells from free radical-induced lipid peroxidation in cell membranes and as an efficient antioxidant, eliminates radical peroxides in the chain of radical fatty acids.³²

In the present study, vitamin E (20, 40, 60 IU.kg⁻¹.day⁻¹, ip) administration did not attenuate morphine withdrawal syndrome. But coadministration of vitamin E and sodium selenite at low doses had synergic protective effects against the development of morphine-induced dependency. On the other hand, changes in the serum levels of TAC and MDA in the co-administered group were not significant. So that, attenuation of the withdrawal syndrome cannot be due to the antioxidant effects and a variety of other underlying mechanisms may be involved. For example, pharmacological manipulations on the GABAergic and serotonergic systems, Orexin type-1 receptors and inflammatory cytokines have shown to play roles in attenuating morphine tolerance and dependency.³³⁻³⁶ Each of the above can be a candidate for being focused in future studies.

Regarding the improvement of anti-oxidative factors in the groups treated with monotherapies and the reversal effects in the co-treated group, there may be some underlying pharmacodynamic or pharmacokinetic interactions.

In general, anti-inflammatory activity of sodium selenite (by inhibiting phospholipase A2), its inhibitory effect on

Table 1. Effects of vitamin E (20, 40, 60 IU.kg⁻¹.day⁻¹, ip), sodium selenite (0.25, 0.5, 1 mg.kg⁻¹.day⁻¹, ip) and co-administration of the two on the Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) levels.

Group	TAC	MDA
Saline	1.15±0.11	0.95 ±0.12
Morphine	0.99±0.02	1.1±0.05
Sodium Selenite 0.25	1.33±0.15*	1.44±0.5
Sodium Selenite 0.5	1.34±0.1**	0.92±0.03*
Sodium Selenite 1	1.12±0.04*	1.1±0.08
Vit E solvent	0.97±0.046	1.1±0.06
Vit E 20	1.07±0.06	1.12±0.08
Vit E 40	1.03±0.045	0.98±0.07
Vit E 60	1.187±0.07#	0.96±0.07
Vit E 20 + Sodium Selenite 0.25	0.99±0.02	1.08±0.07

*P<0.05, **P<0.01 compared to Morphine group; # P<0.05 compared to Vit E solvent group.

NOS enzyme and the potential to reduce the sensitivity of NMDA receptors to excitatory amino acids may also be involved in its effects on the withdrawal syndrome. Vitamin E can reduce interleukin-8 (the inflammatory cytokine) levels and also inhibit protein kinase C, 5-lipoxygenase, and cyclooxygenase 2 and may thereby attenuate the morphine dependency. So the synergistic effect of the drugs on attenuating the morphine withdrawal syndrome would be justifiable. In sum, co-administration of vitamin E and sodium selenite can be considered as an approach to attenuate the morphine withdrawal syndrome. For clarifying the exact mechanisms of the two drugs in attenuating the morphine withdrawal syndrome, further studies are needed.

Conclusion

Although the antioxidant pathways did not seem to be involved (the underlying mechanisms need to be investigated in future studies), the results showed that co-administration of sodium selenite and vitamin E would effectively attenuate morphine dependency. Therefore, the obvious effects of this combination therapy on the withdrawal syndrome can be considered as a basis for further preclinical and clinical studies.

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Ethical Issues

This project has met the principles of the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.571).

Author Contributions

BH was the adviser who designed and oversaw the whole stages of the research. MC and AP were the supervisors and led the project. KF summarized the contents and prepared the manuscript for publication. HJ performed the practical procedures of the research and HR helped her in some cases. All authors read and gave approval of the final manuscript.

Conflict of Interest

The authors declared no conflict of interest for this study.

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