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Effects of Vitamin C and Citicoline on Morphine-Inducing Tolerance In Mice

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Abstract

Introduction: The issue of evolving tolerance to opioid analgesia is a major problem in relation to pain management within the field of medicine, as in cancer patients. This paper seeks to remedy this problem by evaluating *vitamin C* and *citicoline* on tolerance postponement, depending on proven founded inhibiting effects on "glutamatergic system" of which hyper activation will result in tolerance.

Aim: This study set out to assess effects of vitamin C and citicoline on morphine-induced tolerance postponement in male mice.

Methods: Nine male albino mice groups (n=8, 0.02-0.03 g) received certain drug regimens for four days concurrently with daily morphine in order to tolerance development. On the fifth day, through *the Hot-plate test*, pain response to thermal stimulation at 15, 30, 45 and 60 minutes after the test dose of morphine was recorded. MDA and TAC were also assayed through blood serum. Statistical analyses were carried out using one-way analysis of variance (ANOVA) and Tukey post-test, where differences with *p* values less than 0.05 were intended significant.

Results: Vitamin C could significantly attenuate the tolerance, with maximum analgesic effect against thermally induced pain at 15 and 60 min (p-values<.05). However, no significant variation of MDA and TAC levels was remarked.

Conclusion: Vitamin C could postpone morphine-induced tolerance due to possible mechanisms, N-Methyl-D-Aspartate (NMDA) receptor antagonism and glutamate release inhibition, respectively.

Keywords:

Pain;

Morphine;

Tolerance;

Vitamin C;

Citicoline;

Mice.

1. Introduction

Opioids are one of the most widely used groups of antinociceptive agents. Morphine, an important component, plays a key role in severe and chronic pain management especially within cancer patients. Despite its safety and efficacy, prolonged application suffers from several major drawbacks. A major problem with this kind of application is tolerance evolution which has been a controversial and much disputed subject and received considerable critical attention within the field of medicine. By the evolution of tolerance within chronic consumption, opioid administered dose must be increased to achieve the prior analgesic effect[1,2].

The causes of this event have been the subject of intense debate within the scientific community. A number of factors found to be influencing tolerance development have been explored in several studies which show an unambiguous relationship between N-methyl-D-aspartate receptor (NMDAR, which found at most excitatory synapses and belongs to the family of glutamate receptors) and tolerance. Previous studies have indicated that NMDA antagonisms have a positive impact on tolerance postponement[3-6]. Related findings have identified that redox

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phenomena regulate the function of NMDA receptor in brain. Vitamin C (L-ascorbic acid), a well-known example of an antioxidant, has been found to oppose NMDA actions by inhibiting glutamate-NMDAR binding and subsequent events rooted from NMDA-gated currents in neurons[7-9].

As Hurtado et al. (2005) have indicated, citicoline (cytidine-5′-diphosphocholine or CDP-choline), contributing factor in neuronal membrane proteins generation from choline, plays the neuroprotective role in cerebral ischemic injury by decreasing brain glutamate release via glutamate uptake enhancement through astrocyte stimulation and increasing expression of EAAT2 glutamate transporter[10].

The main purpose of this study was to contest the claim whether vitamin C and citicoline can postpone tolerance development or not.

2. Materials and methods

2.1. Materials

The compounds which were utilized, are: Morphine sulfate and Vitamin C (Ascorbic acid) (Darupakhsh pharmaceutical company - Iran), Citicoline (Caspiantamin pharmaceutical company - Iran), Normal saline 0.9% (Shahid Qazi factory - Iran), Ketamine 10% (Alfasan veterinary medicines manufacturer- Holland), Xylazine 2% (Alfasan veterinary medicines manufacturer- Holland).

2.2. Animals

Nine groups of male albino mice (0.02-0.03 kg, n=8) were treated under conditions in which constant temperature (25 ± 0.5°C), air ventilation and available food and water were provided.

Prior to commencing the study, ethical clearance was sought from the Ethic Committee for Animal Experiments of Tabriz University of Medical Sciences (code: IR.TBZMED.REC.1396.670) and the procedures were carried out according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. Treatment

Drugs were administered by intraperitoneal (i.p.) injection over the course of a 4-day period, according to the following protocols designed for examined groups:

1) Normal saline (10 ml/kg) + Normal saline (10 ml/kg)

2) Normal saline (10 ml/kg) + Morphine (50 mg/kg); control group

3,4,5) Citicoline (5, 10, 15 mg/kg) + Morphine (50 mg/kg)

6,7,8) Vitamin C (25, 50, 75 mg/kg) + Morphine (50 mg/kg)

9) Citicoline (10 mg/kg) + Vitamin C (50 mg/kg) + Morphine (50 mg/kg)

The morphine treated group was considered as the "control group".

2.4. Tolerance evaluation method

For the purpose of tolerance evaluating, on the fifth day, pain response (hand licking) to thermal stimulation was examined at intervals of 15 minutes (till one hour) after the 9 mg/kg test dose of morphine, test latency time; while the procedure is called Hot-plate test, although base latency time was recorded before test dose administration. The temperature of the Hot-plate instrument was stabilized at 54 °C (varied from 53.7 to 54.8°C). With the provision of outlasting threshold of stimulation more than 40 seconds, the test was discontinued. Maximum Possible Effect (MPE %) was calculated in accordance with the equation below:

MPE% = [(TL-BL) / (cut off time- BL)] x 100; where TL indicates time latency and BL, base latency time.

2.5. Evaluation of preventing effects on oxidative stress

On completion of analyzing vitamin C and citicoline effects on tolerance, the process of evaluating oxidative stress was carried out. Traditionally, tissue damage caused by oxidative stress have been assessed by measuring Malondialdehyde (MDA) and Total antioxidant capacity (TAC)[11,12], although there are certain drawbacks associated with the current MDA measuring method resulting in unreliability, which have been amended by recent studies[13-15]. Thus, it can be considered as a source for error affecting the result. However, for our purpose, the animal was deeply anesthetized by ketamine and xylazine (80 and 8 mg/kg, i.p., respectively). Blood sample was then obtained through cutting off the head[16] and poured in non-anticoagulant tube. Subsequently, the plasma serum was isolated by centrifuge

device (rpm= 3500, temperature=4°C, centrifuging time: 20 minutes). Finally, the samples were frozen with liquid nitrogen, recovered and stored at -70 °C until TAC and MDA assays.

2.6. Statistical analysis

The data were managed and analyzed using SPSS 16.0 (2010). To compare the average scores of treated groups with the control group, one-way ANOVA (analysis of variance) Tukey post hoc test were used; where significant levels were set at less than 5%.

3. Results

3.1. Development of tolerance to analgesia

As can be seen from the Figure 1, feeling of pain in daily *morphine-treated* mice occurred significantly more quickly (p<.05) at 15 minutes after the morphine test dose administration than *normal saline-treated* ones; accordingly, incidence of tolerance turns out to be definite.

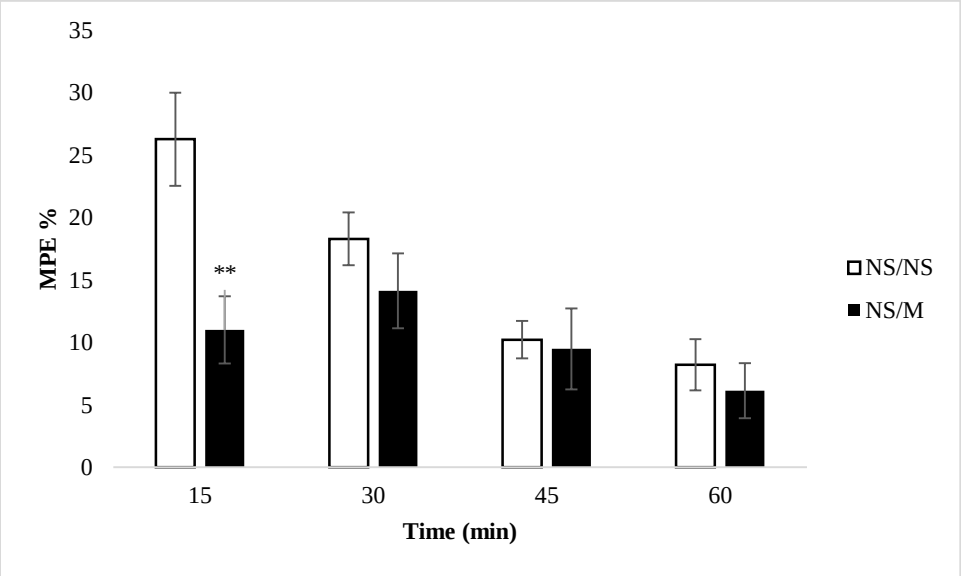

Figure 1. Mean MPE (%) (± SEM), pertaining to tolerant and non-tolerant mice (respectively treated with daily morphine 50 mg/kg, i.p. and normal saline 10 mL/kg, i.p.); $^{**}p < .01$

3.2. Effects of Vitamin C on morphine-inducing tolerance

Figure 2 presents the experimental data on vitamin C. Results showed that vitamin C (50, 75 mg/kg) attenuated the degree of tolerance to morphine.

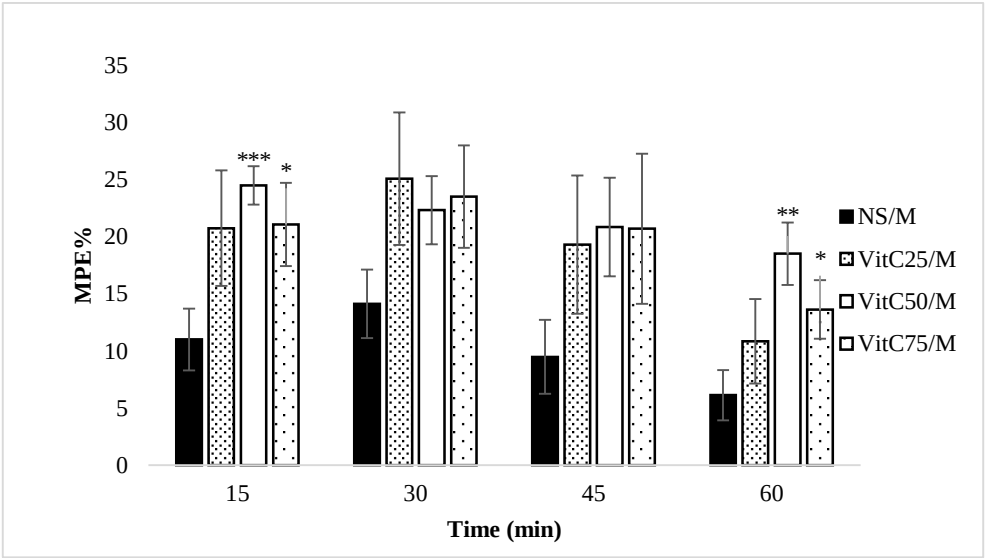

Figure 2. Mean MPE (%) (± SEM), pertaining to vitamin C treated groups (25, 50, 75 mg/kg, i.p.) and the control group through the Hot-plate test; *$p < .05$, **$p < .01$, ***$p < .001$

3.3. Effects of Citicoline on morphine-inducing tolerance

It can be seen from the Figure 3 that citicoline failed to significantly reduce tolerance to morphine analgesia.

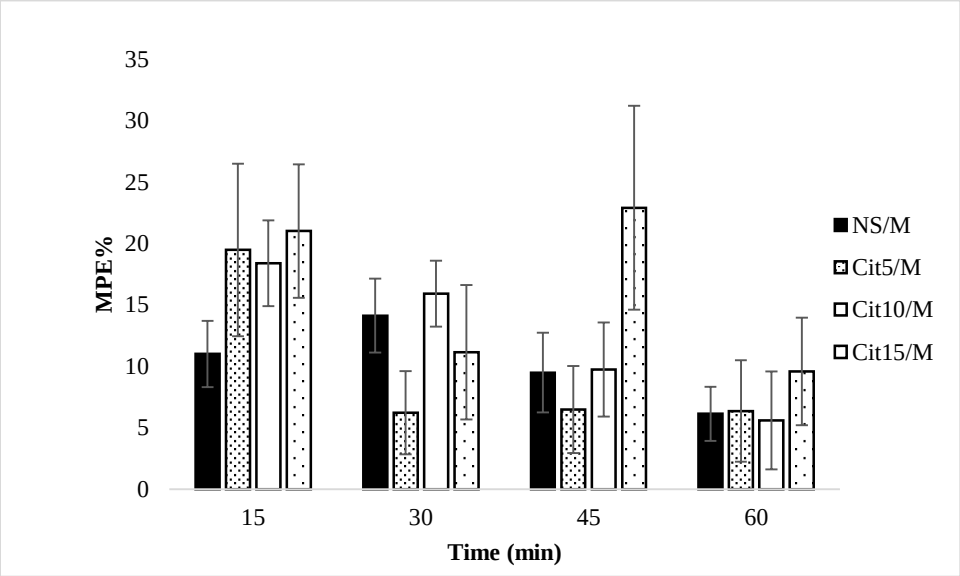

Figure 3. Mean MPE (%) (± SEM), pertaining to citicoline treated groups (5, 10, 15 mg/kg, i.p.) and the control group through the Hot-plate test.

3.4. Effects of parallel Vitamin C, Citicoline treatment on morphine-inducing tolerance

As Figure 4 shows, the concurrent treatment of vitamin C and citicoline did not significantly eventuate in tolerance postponement.

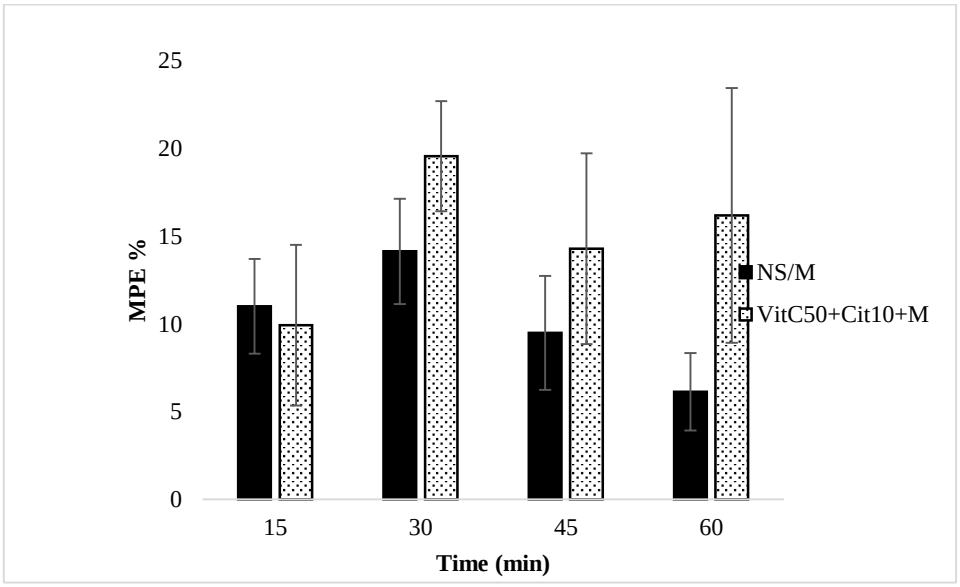

Figure 4. Mean MPE (%) (± SEM), pertaining to citicoline concurrently with vitamin C treated group (10 and 50 mg/kg, i.p.) and the control group through the Hot-plate test.

3.5. Effects of parallel Vitamin C, Citicoline treatment on MDA and TAC serum levels

Table 5 presents the results of malondialdehyde (MDA) and total antioxidant capacity (TAC) assay. No significant differences were found compared with the control group.

Table 5: Malondialdehyde (MDA) and total antioxidant capacity (TAC) assay

Groups of mice	MDA (μM)	TAC (Mm)
Ns/Ns	2.04 ± 0.22	1.05 ± 0.31
Ns/M*	2.34 ± 0.2	1.05 ± 0.23
Cit5+M	2.7 ± 0.6	1.23 ± 0.16
Cit10+M	1.62 ± 0.99	1.13 ± 0.06
Cit15+M	1.56 ± 0.1	1.19 ± 0.78
VitC25+M	1.74 ± 0.22	1.14 ± 0.11
VitC50+M	1.7 ± 0.24	1.09 ± 0.08
VitC75+M	1.68 ± 0.14	1.00 ± 0.07
VitC50+ Cit10+M	1.89 ± 0.24	0.99 ± 0.09

4. Discussion

The present study was designed with the aim of assessing vitamin C, citioline and the co-administration effects on tolerance postponement during morphine consumption based on prior studies noting their importance for modifying the glutamatergic system in neurons, of which hyper activation results in *tolerance*[17-19]. Contrary to expectations, only vitamin could significantly put off tolerance.

In correlation with this study, a number of important limitations need to be considered. First, the numbers of subjects were relatively small which could have prominently affected the evaluations of which especially oxidation biomarkers. However, approaches of behavior studies carry with them noticeable limitations including influencing environmental conditions such as unavoidable noise, researcher's mood, etc.

Thirdly, another weakness of the investigation is the use of current measuring method for MDA as well as small sample size.

In addition, there are, however, other possible explanations for the unanticipated results. For instance, inappropriate doses, inefficaciousness of intraperitoneal injection and drug kinetics or dynamics being affected by the other drug or in the body. It is recommended that further studies which take these variables into account be undertaken.

5. Conclusion

Although the study has successfully demonstrated postponing ability of tolerance to morphine analgesia for vitamin C, the precise mechanisms interfering in different findings of citicoline and concurrent utilization of vitamin c and citicoline remain to be elucidated.

6. Acknowledgments

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