

An Evaluation of the Role of OTC [(-)-2-Oxo-4-thiazolidinecarboxylic acid] in Acquisition and Expression of Morphine Dependence in Male NMRI Mice

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ABSTRACT

BACKGROUND AND OBJECTIVE: In morphine dependence, the concentration of natural antioxidants decreases and glutathione is one of the most important ones. Therefore, antioxidants may reduce morphine withdrawal symptoms. Therefore, the present study was conducted to analyze the effect of various concentrations of OTC [(-)-2-Oxo-4-thiazolidinecarboxylic acid] (glutathione transferase activator) on acquisition and expression of morphine dependence.

METHODS: In this experimental study, small male NMRI mice (20 – 30 g) were divided into 9 groups (n = 8). The mice became morphine-dependent using Marshall Method. The animals were administered with various concentrations of OTC (5, 10, 20 mg/kg) on days of inducing dependence (reception) or days of test (expression). Two main symptoms of withdrawal syndrome (the number of jumps and the weight of stools) were evaluated by administration of naloxone.

FINDINGS: Administration of naloxone in the mice that received morphine on previous days increased the weight of stools (0.62 ± 0.2 and 0.3 ± 0.05 in morphine and saline group, respectively) and the number of jumps (46 ± 15 and 1 ± 0.2 in morphine and saline group, respectively; $p < 0.01$). OTC administration did not cause significant changes in the weight of stools and the number of jumps compared with saline group. OTC administration on days of reception increased the weight of stools and the number of jumps (0.53 ± 0.01 , 1.2 ± 0.5 , 1 ± 0.04 and 0.62 ± 0.01 in 5, 10, 20 mg/kg OTC and saline, respectively; $p < 0.01$) and increased the number of jumps on days of test ($p < 0.01$).

CONCLUSION: Results of the study demonstrated that stimulating glutathione transferase by OTC improves the acquisition of physical dependence to morphine.

KEY WORDS: Morphine, Glutathione transferase, Dependence, Expression, Marshall Method, Diarrhea.

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Introduction

Morphine tolerance is one of the main reasons for its long – term consumption restrictions. Clinically speaking, tolerance is defined as a condition in which the effective dose of morphine during the treatment period is less effective in inducing analgesia and requires higher doses of the drug (1). Extensive research has been conducted to find solutions to reduce morphine tolerance, and now the cell-molecular and structural function of morphine in induction of tolerance is known (2).

In terms of structure, when morphine tolerance occurs, wide-cellular and molecular changes occur in various regions of the nervous system. One of the most important changes is the reduction in the size and number of synaptic dopamine (3), opioid (4) and glutamate (5), as well as changes in the shape of neurons and the reduction of neurofilaments within them (6). From a cellular perspective, non-susceptibility or decrease in the number of opioid receptors in different regions of the nervous system (7), increasing the biosynthesis of endogenous opioids such as prodynorphin, endorphin and proenkephalin (8), non-susceptibility of ionotropic glutamate receptors (NMDA and AMPA / Kinase), increased efficiency of glutamate metabotropic receptors and non-susceptibility of dopamine receptors, decreased dopamine and glutamate biosynthesis, increased activity of adenylate cyclase and consequently increased amount of adenosine monophosphate, and increased membrane permeability to sodium ion are the consequences of morphine tolerance in the brain (9 – 12).

Moreover, increased activity of C-gamma protein kinase, as well as extracellular signal-regulated-Kinase (ERK) enzyme, as well as a decrease in the level of IRS2 (Insulin-Receptor-Substrate2) in the ventral tegmental area of rats was observed, while this phenomenon was inhibited by administration of antisense gene and as a result, tolerance was not induced (13). However, tolerance to all effects of morphine is not induced and therefore, as the dose of morphine increases, there is the risk of respiratory interruption, followed by death for the consumer.

Therefore, finding ways to reduce morphine tolerance and increase analgesia is one of the most important goals of research programs in this area. Studies have shown that morphine-induced tolerance is inhibited by co-administration of NMDA glutamate receptors (14). It has also been shown that administration of LY235959, which is an NMDA

receptor antagonist, causes analgesia in morphine-tolerated mice using tail flick method (15). On the other hand, intrathecal MK801 administration, which is an NMDA receptor antagonist, strengthens analgesia to morphine in the tail flick method (16). Since the NMDA glutamate receptors do at least a part of their work through nitric oxide, various researchers have shown that administration of nitric oxide enzyme inhibitors can reduce morphine tolerance in different pain assessment models (17-19).

On the other hand, it has been shown that glutathione and one of its metabolites, called S-Nitrosoglutathione, can act as an agonist for NMDA receptors and induce their responses (20). OTC [(-)-2-Oxo-4-thiazolidinecarboxylic acid] acts as a stimulant for intracellular glutathione production (21). Due to the rapid evacuation of glutathione after the administration of morphine, some studies have reported that the concentration of normal antioxidants in the body decreases when morphine dependence occurs (22). Moreover, the administration of various antioxidants may prevent the occurrence of withdrawal syndrome in animal models as well as in addicted humans. Morphine seems to increase metabolism in the brain after injection, and antioxidant drugs can inhibit morphine activity by inhibiting these effects (23).

Therefore, considering that glutathione is one of the strongest antioxidants and numerous studies have shown that administration of morphine can lead to the evacuation of the body's internal antioxidant system, and that glutathione system is most effective one in this condition, the stimulating effect of this system on morphine-related complications was investigated in this study. Therefore, two main symptoms of withdrawal syndrome in animals, jumping and feces (diarrhea), were investigated in this study. In other words, the present study was conducted to analyze the effect of various concentrations of OTC [(-)-2-Oxo-4-thiazolidinecarboxylic acid] (glutathione transferase activator) on acquisition and expression of morphine dependence.

Methods

Animals: This experimental study was conducted on male NMRI mice with a mean weight of 25–30 g. Animals were purchased from the Pasteur Institute. After being transferred to the animal room, they were kept in transparent plastic cages with dimensions (15×30×45) in standard laboratory conditions with fresh food and water, in natural circadian condition and at 22–

24 °C. Experiments were conducted in accordance with international ethical guidelines and they were approved by the Ethics Committee.

Medications: Morphine sulfate (Temad-Iran), Naloxone hydrochloride (Toliddaru – Iran) and OTC [(–)-2-Oxo-4-thiazolidinecarboxylic acid] were used in this study. Medications were dissolved in saline and injected subcutaneously at 10 mg / kg.

Method of inducing physical dependence to morphine: The animals became morphine-dependent using Marshall Technique (24). Morphine sulfate was injected three times a day with doses of 50, 75, 100, 125 mg / kg at the first hour, three hours later and eight hours later (Table 1). This continued for three days. On day 4 (day of test), a single dose of morphine (50 mg / kg) was injected to animals at 10:00 am.

Table 1. Marshall Technique to induce morphine dependency (24)

Hour of injection Days	first hour	three hours later	eight hours later
Day 1	50 mg/kg Morphine	50 mg/kg Morphine	75 mg/kg Morphine
Day 2	75 mg/kg Morphine	75 mg/kg Morphine	100 mg/kg Morphine
Day 3	100 mg/kg Morphine	100 mg/kg Morphine	125 mg/kg Morphine

Test design and test groups: In the present study, 9 experimental groups were used, each group consisting of 8 animals. All drugs were administered subcutaneously. One group was subcutaneously administered with different concentrations of Morphine sulfate (50, 75, 100, 125 mg / kg) three times a day for three days. On day 4 (day of test), morphine sulfate (50 mg / kg) was injected to animals at 10 a.m. and two hours later, naloxone (2 mg / kg) was administered to animals. Immediately, in order to investigate the symptoms of the withdrawal syndrome, the animals were placed under a cylindrical chamber for 30 minutes and the number of jumps and the amount of feces were measured.

The feces weight was measured in the bottom of the cylinder chamber of weighted paper. After the test, the paper was reweighed and the weight difference of the paper at the beginning and end of the test showed the amount of feces in the animal. Normal saline was injected into the sham group instead of morphine

sulfate. In order to investigate the effect of OTC on the physical dependence of Morphine, three groups of animals were injected with different doses of OTC (5, 10 and 20 mg/kg) 30 minutes prior to morphine injection.

This was done three times a day for three days. On the fourth day, animals only received 50 mg/kg morphine. Two hours later, naloxone was injected, and for 30 minutes, the symptoms of withdrawal syndrome (number of jumps and amount of feces) in animals were measured. To investigate the effect of OTC on the expression of physical dependence on morphine, three groups of animals received different doses of morphine three times a day. This continued for three days. On day 4 at 10 a.m. only 50 mg/kg morphine was injected. 30 minutes before injection of naloxone, different doses of OTC (5, 10, 20 mg/kg) were injected to animals. One hour later, test was performed with naloxone and the amount of feces and number of jumps were measured for 30 minutes.

Data analysis: Data were analyzed using SPSS 22 software, one-way ANOVA and Tukey test. P-value <0.05 was considered significant.

Results

The effect of recurrent administration of morphine on dependence in small mice: In this part, mice were divided into two groups. One group received different doses of morphine (50, 75, 100 and 125 mg/kg), and another group received saline (as described in the procedures section). Naloxone was administered on day four. Experiments showed that administration of naloxone increased the number of jumps (46 ± 15 and 1 ± 0.2 in the morphine and saline groups, respectively) ($p < 0.001$) and the amount of feces (0.62 ± 0.2 and 0.3 ± 0.05 g in the morphine and saline groups, respectively) ($p < 0.01$) in the morphine group compared to the saline group, indicating that administration of increasing doses of morphine induces dependence in these animals.

Investigating of the effect of OTC administration on physical dependence in small laboratory mice: In this experiment, animals were divided into four groups. Three groups of animals received different doses of OTC (5, 10, 20 mg/kg) and the fourth group received saline for three consecutive days (like morphine). On the fourth day, all groups received naloxone. The results showed that administration of different doses of OTC alone did not have a significant effect ($p < 0.05$) on the

number of jumps and the amount of feces (The number of jumps: 2 ± 0.5 , 4 ± 2 , 3 ± 1.5 and 5 ± 2 , the amount feces: 0.2 ± 0.01 , 0.4 ± 0.04 , 0.2 ± 0.02 and 0.3 ± 0.01 , respectively at doses of 5, 10, 20 mg / kg OTC and saline) ($p<0.05$) (Table 3).

Table 3. Effect of naloxone injection in mice treated with OTC at different doses

Variable Group	Amount of feces (g) Mean $\pm$ SEM	Number of jumps in 30 minutes Mean $\pm$ SEM
Saline	0.2 ± 0.01	2 ± 0.05
OTC mg/kg 5	0.3 ± 0.01	5 ± 2
OTC g/kg 10	0.2 ± 0.02	3 ± 1.5
OT mg/kg 20	0.4 ± 0.04	4 ± 2

Investigating the effect of OTC on the acquisition of morphine dependence in small laboratory mice: In this series of experiments, four animal groups were used. Three groups of animals received one of the three doses of 5, 10, 20 mg / kg OTC on the days of induction before receiving morphine and the other group received saline.

The results showed that OTC administration increases the effect of morphine on the induction of jumps (40 ± 18.5 , 97 ± 14.2 , 90.8 ± 13.26 , and 68.2 ± 17.3 at 5, 10, 20 mg / kg OTC and saline, respectively, $p<0.01$) and the amount of feces (0.62 ± 0.01 , 1 ± 0.04 , 1.2 ± 0.5 and 0.53 ± 0.01 at 5, 10, 20 mg/kg OTC and saline, respectively, $p<0.01$), and these increases are dose-dependent in both cases (jumping and feces) (Figure 1).

OTC on the day of test increased the number of jumps (41 ± 15.23 , 94.5 ± 15.68 , 48.84 ± 16.11 and 75.67 ± 19.41 at 5, 10, 20 mg / kg OTC and saline, respectively, $p<0.01$), but decreased the amount of feces significantly (0.54 ± 0.013 , 0.47 ± 0.010 , 0.62 ± 0.014 and 0.62 ± 0.012 at 5, 10, 20 mg / kg OTC and saline, respectively, $p<0.05$).

Investigating the effect of OTC on the expression of morphine dependence in small laboratory mice: Experiments showed that OTC administration has contradictory effects on the expression of morphine dependence; significantly increases the number of jumps in 5 and 20 mg / kg doses and significantly decreases diarrhea ($p<0.05$) at 10 mg / kg (number of jumps: 41 ± 15.23 , 94.5 ± 15.68 , 48.84 ± 16.11 and 75.67 ± 19.41 at 5, 10, 20 mg/kg OTC and saline, respectively; amount of feces: 0.54 ± 0.013 , 0.47 ± 0.010 , 0.62 ± 0.014 and 0.62 ± 0.012 at 5, 10, 20 mg / kg OTC and saline, respectively, $p<0.05$) (Figure 2).

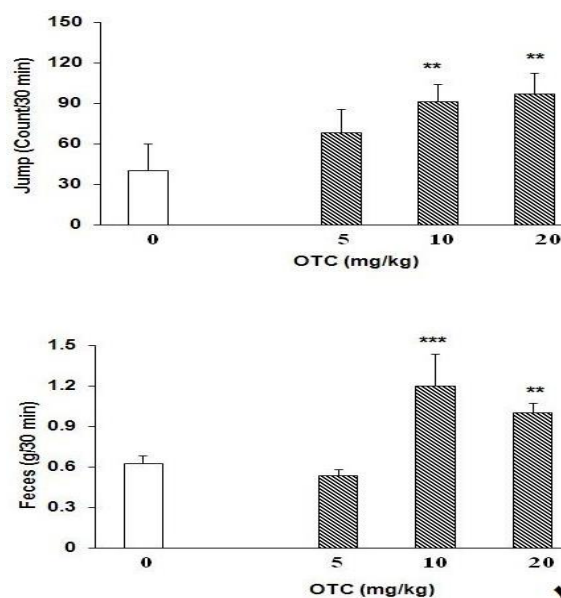


Figure 1. The effect of OTC on the acquisition of morphine dependence

Before receiving morphine on induction days, animals received one of three doses of 5, 10 and 20 mg / kg OTC and the other group received saline. The results are shown as (Mean $\pm$ SEM) for 8 animals. ** $p<0.01$, *** $p<0.001$

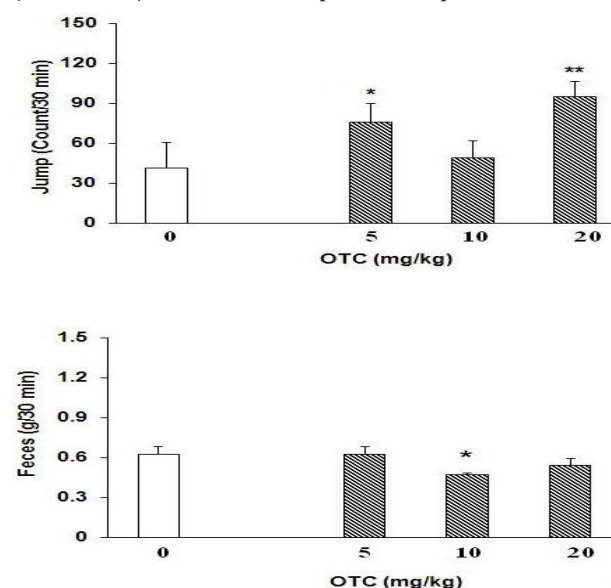


Figure 2. The effect of OTC on the acquisition of morphine dependence

The animals were injected with morphine sulfate as described in the procedures. On the day of the test, different doses of OTC (5, 10, 20 mg / kg) were injected 30 minutes before naloxone injection, and jumps and diarrhea were tested for 30 minutes after administration of naloxone. * $p<0.05$, ** $p<0.01$

Discussion

Results of this study demonstrated that OTC administration increases the effect of morphine in the

induction of jumping and feces, and these increases are dose – dependent in both cases (in terms of morphine dependence). On the other hand, OTC increased the number of jumps, but decreased the amount of feces significantly on the day of test (in the expression of dependence).

Morphine dependence is one of its most important long-term complications, which is associated with several symptoms when discontinued, and it stimulates an addict to re-use morphine. Morphine dependence is associated with several symptoms, the most important of which are diarrhea, nausea, palpitations, pain, stiffness of the hair and snarl, which are clearly observed in addicts. Signs of sagging stomach and jumps are observed in small laboratory mice (25-29). The present study showed that despite the expected stimulation of the glutathione system, morphine dependence increased and morphine-dependent animals treated with different OTC dosages showed more jumps and the amount of feces increased in them. This was a dose-dependent increase in both cases. In addition to the role of morphine in reducing glutathione, it seems that other factors should be considered in this regard. It should be considered that stimulation of the glutathione system reduces the storage of glutamate by conversion to glutathione. Previous studies have shown that stimulation of the glutamate system has no effect on morphine induction, but inhibition of glutamate system inhibits morphine dependence and tolerance in small laboratory mice. This effect is more pronounced in glutamate NMDA receptors, and it seems that these receptors play a significant role in this regard (30). Therefore, in the present study, it was expected that the stimulation of the glutathione system by depleting the intracellular glutamate reserve would reduce morphine tolerance, but the results were the opposite of this prediction. Previous studies have shown that glutathione can act as an agonist for the NMDA receptor in the neurons cultured in the hippocampus of a laboratory rat (20). Similarly, glutathione may also act as an agonist on NMDA receptors in other parts of the nervous system. Therefore, OTC seems to produce more glutathione by stimulating the glutathione system, and as this substance is released, effects of glutathione similar to glutamate are induced on the cells of the nervous system, and its outer manifestation was an increase in the number of jumps and the amount of feces in morphine-dependent animals receiving morphine before each OTC injection. Although in previous studies direct stimulation of the glutamate system did

not show a significant effect on morphine dependence, this does not mean that the glutamate system is not effective in this regard, because inhibition of NMDA receptors inhibits morphine dependence. Therefore, probably if the morphine dose is reduced, the effectiveness of agonists can be used to induce dependence from the initial dose of 25 mg to increase the effectiveness of OTC. Moreover, OTC administration in the present study showed a dual effect on the expression of morphine dependence; the number of jumps first increased, then decreased and increased again. Although the increase in the number of jumps was not different from the control group, the increases were quite significant. On the other hand, OTC administration decreased the amount of feces in animals, which was only significant at 10 mg / kg. This suggests the existence of various systems for the incidence of diarrhea and jumps in morphine-dependent mice, because stimulation of the glutathione system increased jumps and reduced diarrhea when expressing morphine dependence. It seems that the role of glutathione system in the jumps and diarrhea is not the same in morphine-dependent mice. The role of antioxidant role glutathione in this matter cannot be very important, because previous studies using ascorbic acid showed results that were not consistent with the results of the present study (31, 32). However, it should be kept in mind that each of the antioxidant medications also affects the neurotransmitter systems of the brain, thus activating several factors simultaneously and inducing complicated responses.

In addition, it should be noted that in our study, the effect of OTC was a dose-dependent effect, indicating the existence of an effective regulatory system for its function. Of course, it should be kept in mind that there might be an unknown pharmacokinetic interference between morphine and OTC, leading to the obtained results. The results of this study showed that stimulation of glutathione transferase by OTC promotes physical dependence on morphine (increased jumps and feces increase, and dose-dependent in both cases) and inconsistent effects in expressing morphine dependency (increased jumps and decreased diarrhea) in the small laboratory mice.

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