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Methadone and valproate combination effect on acquisition and expression of morphine dependence and tolerance in male mice

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A B S T R A C T

Background and Objective: According to the issue of dependence and tolerance in addicted patients, the inefficiency of existing treatments and the new recommendation for drug combination therapy for diseases in modern pharmacology, the present study examined the effect of methadone and valproate combination on morphine dependence and tolerance.

Materials and Methods: Ninety-eight male mice were divided into the following groups: saline, morphine, methadone, valproate, and three groups of valproate+methadone, i.e. 1 to 1, 2 to 1 and 1 to 2 ratios. Except for the saline group, the others received escalating dose of morphine for 8 consecutive days. In acquisition group, drugs were injected for 30 minutes before morphine administration. In expression group, the drugs were used only at 8th day (test day). Morphine tolerance was measured by tail immersion test and for dependence assessment, naloxone-induced withdrawal signs was evaluated.

Results: Jumping behavior as a main sign of dependence in both acquisition and expression significantly reduced in methadone1+valproate2 treatment group. Also, acquisition and expression of tolerance significantly decreased in valproate and methadone1+valproate2 more than other groups.

Conclusion: Our results show that methadone and valproate combination treatment could probably reduce tolerance and dependence more than single valproate or methadone treatments.

1. Introduction

Although opioids such as morphine are valuable clinical analgesics, the downsides to their prolonged use are tolerance and addiction (1). Opioid receptors which are present in the ventral side of the tegmentum and the accumbence, act as an inhibitory factor on the GABA-ergic interneurons and promote the "reward" pathway and induce good feeling (2). Insensitivity of such receptors or their reduced reactivity due to adaptation which is followed by the use of opioids are known to cause physiological tolerance to such substances (3). Also, addiction to opioid drugs that are seen in individuals with prolonged usages of these drugs are linked to physiological alterations which occur in the brain in such way that the presence of the foreign substance that opioid is, is needed by the cells for normal functions. The physiological addiction is apparent at best when the drug's administration is stopped or its antagonist is administered to the body (4). In the treatment

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of substance abuse and addiction, methadone is the popular drug of choice; having a structure similar to heroine, and it is a complete agonist for the μ receptors (5), while mimicking the pharmacological traits of morphine like analgesia, tolerance, dependency and other CNS-weakening characteristics (6).

Anti-convulsant drugs which act via activating GABA-ergic systems like valproate that are commonly used to treat convulsions and mood swings in adults can increase the neurotransmitter GABA in the synapses by activating the enzyme called glutamic decarboxylase (the exclusive enzyme which transforms glutamic acid to GABA) and therefore resulting in a decline in the neurons' excitation (7). Lately, valproate is being used to treat physiological addiction to opioids, as a GABA-system excitatory drug (8-10). The mechanism of effect of the latter drug in reducing opioids dependency is through raising the levels of GABA at the synapse level and naturally, an induced decline in the levels of dopamine secreted by the ventral tegmentum to the accumbence nucleus. It has been mentioned before that the in dependency phenomenon, morphine acts as an inhibitor to the GABA-ergic system, resulting in higher levels of dopamine from ventral tegmentum to the accumbence nucleus (2, 11). In this regard, it has been documented that intra-peritoneal administration of GABA-system excitatory drugs such as valproate in rats addicted to morphine reduces withdrawal signs due to naloxane (10). In another study, administration of valproate in rats impeded the sensitivity to morphine, but did not withhold expression of sensitivity to morphine (7). Furthermore, other studies have shown that the anti-convulsant drug divalporax has effects on cocaine addicts in a way that it reduces the intensity and the frequency of compulsion to seek yet another cocaine administration experience and its period of usage (12).

With the issue of substance abuse being so crippling in today's world and the fact that the rehabilitation treatments with commonly used pharmaceuticals is not ideally effective, a newer approach is being taken in which several different pharmaceuticals are used to synergize the treatment (13, 14). Also, with regard to recent studies on GABA-ergic drugs and their effects on addicted lab animals (8-10), this research aimed to look into the complimentary pharmacotherapy of addiction using methadone and valproate combination as a GABA-promoting agents in the CNS.

2. Materials and Methods

2.1. Animals

In this study, 98 male albino NMRI rats (with an average weight of 20-25 g) were used. The rats were kept in cages of 7 with normal light/dark cycle and temperature set at 22-24 degrees Celsius and with free access to water and food.

2.2. Chemicals

In this study, morphine sulfate and methadone were procured from Tamad (Iran) and naloxane was obtained from TolidDarou (Iran). Sodium valproate was purchased from Sigma-Aldrich (USA). All drugs were dissolved in normal saline.

2.3. Experimental groups

Of the total 98 rats, they were randomly divided into 7 groups of 14 each as mentioned below:

1. the normal saline group, 2. morphine sulfate group, 3. methadone group (10 mg/kg), 4. valproate (150 mg/kg), 5. valproate + methadone (1:1, 75+5 mg/kg, respectively), 6. valproate + methadone (1:2, 50+7 mg/kg, respectively), and 7. valproate + methadone (2:1, 100+3.5 mg/kg, respectively).

2.4. Method of morphine addiction induction

For this goal, the rats from groups 2 through 7 were intraperitoneally injected with increasing doses of morphine according to body weight for 7 consecutive days, in 2 sets; once in the morning and once in the afternoon as mentioned below:

1st day, 10 mg; 2nd day, 20 mg, 3rd and 4th days; 40 mg, 5th day; 60 mg, 6th day; 80 mg and on the last day (7th day); 100 mg/kg (15). On the 8th day (test day), only 1 dosage of 100 mg/kg was administered to all groups in the morning.

Evaluation of acquisition and expression in morphine sulfate-dependent group In order to isolate the fact that whether the rats were in fact addicted to morphine, naloxane injection was

chosen (5 mg/kg). For this purpose, 4 sets of behavioral manifestations of addiction to morphine was chosen: jumping, diarrhea, standing on both hind legs and the licking of the front legs. To establish the addiction development, naloxane was injected on the 8th day, 2 hours after the administration of the 100 mg/kg dosage of morphine, then the rats were put in Plexiglass cages and monitored for the next 30 minutes (16). In the acquisition group, drugs were administered 30 min before the injection of morphine for a period of 8 days. But in the expression group, the drug was administered 30 min before the injection of morphine only on the 8th day.

Determination of tolerance to analgesic effect of morphine sulfate For this purpose, on the 8th day, 30 minutes before and after the administration of the 100 mg/kg dosage, a hot tail-immersion test was conducted in such a way that the rats would have been kept in normal conditions in restrainer cages for a duration of 15 minutes prior to the test, and then the last 1 cm of their tail would have been inserted into water with the temperature at 56±0.5 degrees Celsius and utilizing a chronometer to measure the time it would take the rat to react to the pain, and when the rat would retract its tail from the water, the chronometer would be pushed to stop. In this test, in order to prevent any tissue damage, a cut-off time of 10 seconds were taken into account. In each group, the delayed reaction time was evaluated 3 times with 3 minutes between each teat, and the averaged result was recorded, both for before and after the administration of morphine sulfate. For the purpose of comparison of the delayed response time in different groups, the "Maximal Possible Effect" formula was applied (17).

2.5. Statistical analysis

In this study, SigmaStat software (version 3.5) was used for statistical analysis. All of the results were reported as Mean± SEM. Statistical comparison between the groups was made by ANOVA and Tukey post-test, and $p<0.05$ was considered significant. Non-parametric data analysis was done by Kruskal-Wallis test and the related post-test.

3. Results

3.1. Valproate, methadone and valproate+ methadone and their effects on the acquired dependency to morphine sulfate

As it is shown in Fig. 1, all groups subjected to valproate, valproate+methadone (both 1:2 and 2:1 ratio groups) significantly showed less physiological manifestations of jumping, diarrhea, licking the front legs and standing on both hind legs as a result of administering naloxane as compared to the morphine group. Jumping was chosen as the key index of dependence in the treatment groups and treatment groups showed 66%, 84% and 67% decline, respectively. The results for declines in diarrhea, licking and standing were (78%, 68%, 81%), (56%, 56%, 88%) and (51%, 13%, 56%), respectively. Also, regarding diarrhea, licking and standing, the case groups treated with valproate and methadone was significantly different than the methadone group.

3.2. Valproate, methadone and valproate+methadone and their effect on the expression of dependency to morphine sulfate

As it is shown in Fig. 2, the effect of high/acute dosage of valproate, methadone and their combination is seen for the signs of morphine withdrawal. In the valproate treatment group and the valproate+methadone groups of 1:2 and 2:1 ratio, jumping, diarrhea, licking of the front legs and standing on the hind legs were significantly lower as compared to the morphine group. Jumping in the said groups was lowered by 55%, 66% and 63%, respectively. Regarding diarrhea, the valproate treatment group showed 84% decline and for licking, the valproate+methadone group with the ratio of 2:1 showed a 72% decline, which were significant as compared to the morphine group. With respect to standing on hind legs, the same mentioned groups showed 51% and 52% decline, respectively. Another noteworthy fact was that regarding the "jumping" manifestation, the treatment group which was treated with both valproate and methadone showed a more promising result than the group being treated with methadone alone.

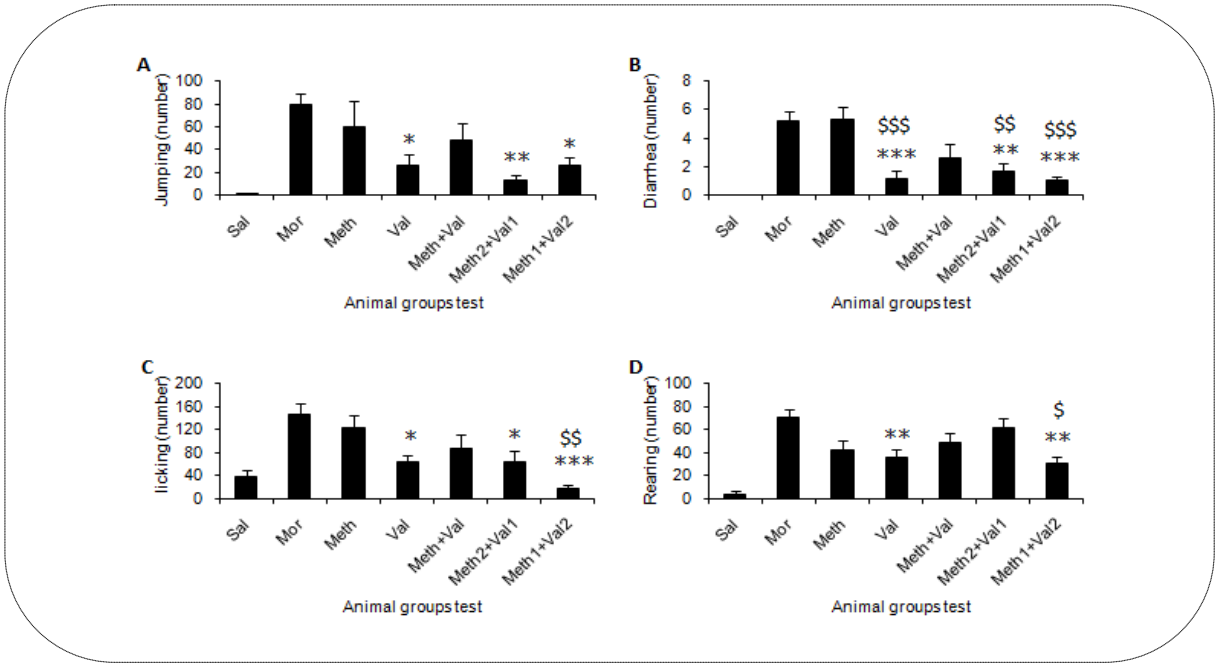

Figure 1. The effect of valproate and methadone both individually and combined on the dependence acquisition phenomenon. Signs of jumping (A), diarrhea (B), licking (C), and standing on the hind legs (D) in control group and morphine and the treatment groups. The columns show average±S.E.M. (n = 7 in each group).
The symbols * and $ are the significant differences regarding the morphine group and the methadone group. * p<0.05, ** p<0.01, *** p<0.001; $ p<0.05, $$ p<0.01, $$$ p<0.001.

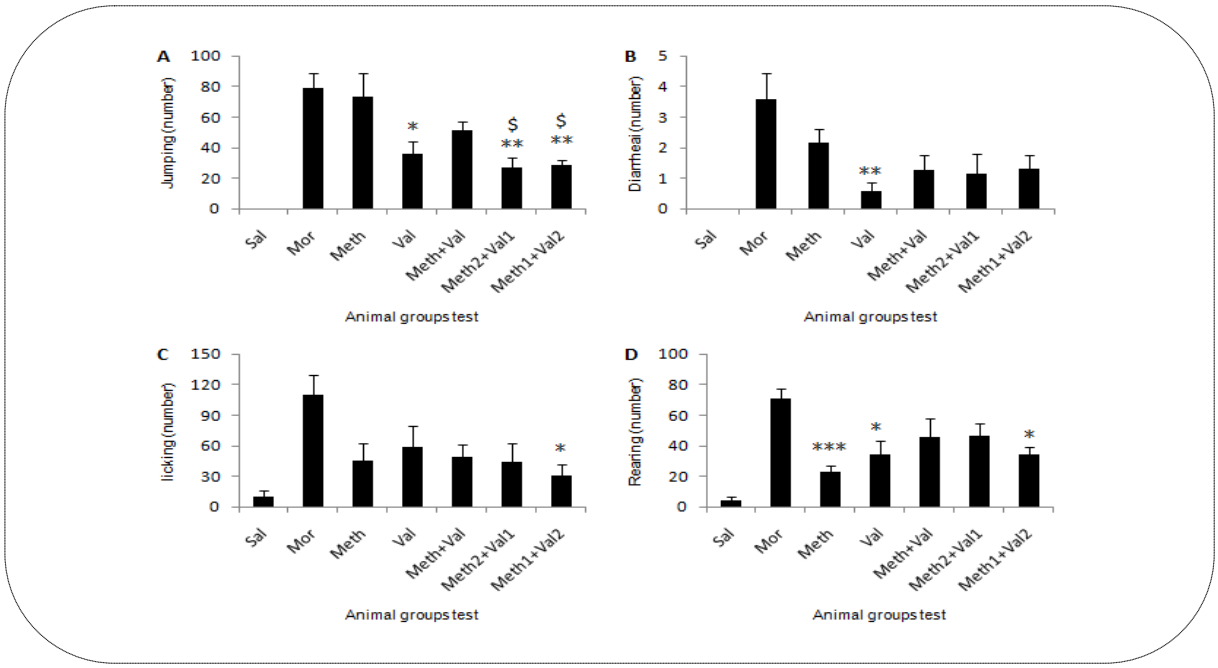

Figure 2. The effect of valproate and methadone both individually and combined on the expression of dependency phenomenon. Signs of jumping (A), diarrhea (B), licking (C), and standing on the hind legs (D) in control, morphine and the treatment groups. The columns show average±S.E.M. (n = 7 in each group).
The symbols * and $ are the significant differences regarding the morphine group and the methadone group. * p<0.05, ** p<0.01, *** p<0.001; $ p<0.05.

3.3. Valproate, methadone and their combination and their tolerance-promoting effect to the analgesic effect of morphine sulfate

In the "A" section of Fig. 3, the effect of valproate and methadone to the obtained tolerance is shown. In the valproate and the combination group of valproate+methadone 2:1, the average analgesic response to morphine was 18.77±2.24 and 20.93±2.03, respectively as compared to the morphine group with an average increase of 7.91±1.52. In the mentioned groups, there was a decline in the occurrence of morphine tolerance. In the "B" section of the Fig. 3, the effect of valproate and methadone on the promotion of morphine tolerance occurrence was observed. The same aforementioned groups were able to express a heightened state of analgesia to morphine with averages of 20.32±1.94 and 28.75±5.86, respectively as compared to the morphine group with an average of 7.05±1.49 and a lowered occurrence of tolerance to morphine.

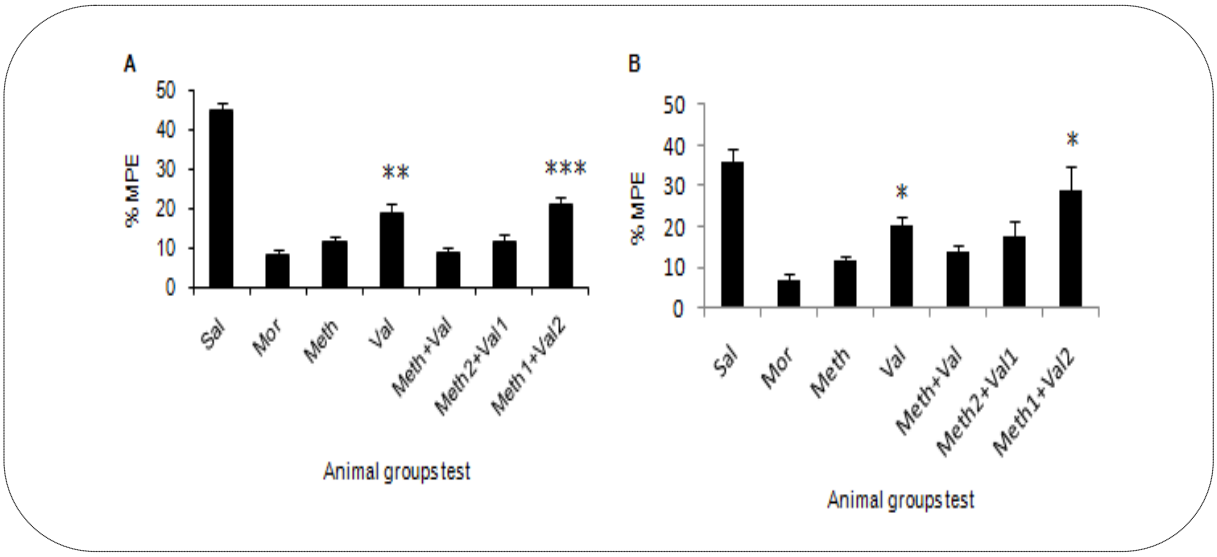

Figure 3. The effect of valproate and methadone both individually and combined on the acquired tolerance to morphine. A: acquisition treatment group, B: expression treatment group. The columns show average±S.E.M. (n = 7 in each group).
*P<0.05, **P<0.01, ***P<0.001 as compared to the morphine group.

4. Discussion

In this study, to induce morphine addiction and tolerance in experimental rats, a method of intraperitoneal and increasing injection of morphine was taken upon with the administered dosages starting from 10 mg/kg and ending with 100 mg/kg over 7 days, twice daily, in the morning and in the afternoon. It has been documented that continued administration of morphine would result in a physiological state of tolerance to the drug in which the analgesic effects would be lowered over the period of usage, in a way that in the addicted group, even the 100 mg/kg dosage could not induce a state of analgesia as was hypothesized before; but the treatment group of valproate, either a single dosage on the test day or its combined administration with morphine over the 1 week period of addiction induction was shown to hinder the mentioned physiological tolerance to the analgesic effect of morphine. The same effect on reducing the occurrence of tolerance was seen even more prominently in the group treated with valproate+methadone with 2:1 ratio. Also, previous reports exist showing a lowered occurrence of morphine tolerance where the treatment groups were administered with a combination of valproate and morphine (18). It has been reported that valproate acts as an inhibitor of the enzyme GABA transaminase and succinic semialdehyde dehydrogenase (19). Therefore, valproate which acts upon promoting GABA, is hypothesized to be an agent acting

towards an analgesic state. Also, it has been reported that the analgesic state promoted by valproate is linked to the activation of pro-enkephalin system in the rat brain (20). In the present study, valproate was able to reduce the morphine tolerance due to prolonged usage of morphine by the subject. It has been reported that a change on the distribution level of serotonin in the CNS affects analgesic properties of morphine, although it is known that morphine administration leads to serotonin release in the CNS through inhibiting GABA discharge. So, it has been hypothesized that exogenic GABA-ergic substances act as an inhibitor to the increased serotonin and dopamine flow caused by morphine. Also it has been reported that the administration of GABA agonists (mucimol) will affect the posterior raphe nucleus and counteract the serotonin-releasing effects of morphine (21). Also, valproate reduces the levels of serotonin in hypothalamus and the cerebellum (22). This matter might explain one possible mechanism of valproate effect. Furthermore, the effect of naloxan and the withdrawal syndrome manifestations were observed and documented in all groups. Regarding jumping, diarrhea, standing, and licking, the treatment group receiving valproate+methadone at a ratio of 2:1 showed the best results. In previous studies, it was documented that the intraperitoneal administration of GABA-promoting drugs like valproate can reduce the withdrawal syndrome signs of naloxane (10). The mechanism of action of valproate, as mentioned before, is through the promotion of GABA-ergic system and the suppression of glutamatergic system (7). It has been known that opioid substances are vital for the dopaminergic neurons of the ventral tegmentum and the causality of the induction of state of "reward" in the subject. Also, the dopaminergic system of the mesolimbic system causes a focused function towards behavior alterations which occur after being exposed to opioid substances. It has been reported that morphine activates the opioid μ receptors in the ventral tegmentum and increases the dopaminergic transmission in the mesolimbic system (2). Methadone as the foremost prescribed drug to treat drug addiction, is a full agonist for the opioid receptors with higher affinity for μ receptors and less affinity for δ and κ receptors. But the downside is that methadone blocks the potassium receptors, NMDA glutamic receptors and serotonin receptors which eventually cause its side effects (5). On the contrary, it has been defined that central GABA-ergic interneurons balance out the activity of dopaminergic neurons of the ventral tegmentum nucleus through tonic inhibition. Glutamic neurons which originate from the amygdala also activate the dopaminergic neurons. Valproate can promote the central GABA-ergic system through the inhibition of GABA-transaminase and therefore, blockage of destruction of active GABA neurotransmitters and through increasing the synthesis of GABA by activating glutamic acid decarboxylase enzyme (9-11).

Regarding our obtained results, this hypothesis that valproate acts as an antagonist to morphine seems highly factual. One possibility is that the inhibition of GABA-transaminase plays the key role for valproate's modulation and that the inhibition of GABA's deactivation by GABA-transaminase, the increased level of extracellular GABA and its synthesis and eventually the promotion caused in the GABA-ergic system. Hence, the morphine-promoted pathways of dopaminergic activity is then halted and the induced tolerance to morphine is then repressed. From another viewpoint, it has been reported that chronic exposure to opioid substances like morphine results in certain intracellular signal conduction alterations. One example is the incremental regulation of cAMP as an adaptation mechanism to prolonged exposure to opioid substances and the same mechanism is linked to both tolerance and dependency to morphine (23). Sensitivity-lowering mechanism of opioid receptors which is induced by the opioid substances happen through phosphorylation of the said receptors. For instance, a decline in a receptor's sensitivity to the substance is equal to its phosphorylation (24). Recently, it has been documented that protein kinase C and some other kinase enzymes conjugated with G protein and the β adrenoreceptor kinase 1 group are involved in lowering the hyperacute sensitivity of the opioid receptors (25) and what has been reported more frequently is that valproate causes significant effects on the PKC (protein kinase C) signaling pathway and lowers its activity and

isoforms (26). Furthermore, since this agent is widely used for the treatment of epilepsy, it has been easily accessible for its clinical pharmacotherapy of addicted patients and can have a drastic effect on lowering the withdrawal syndrome experienced by the patient.

Overall, this study showed that the treatment with the combination of methadone and valproate acts better as compared to treating with valproate and methadone alone regarding the occurrence of morphine dependence and tolerance. It has been presumed that this is due to the fact that the GABA-system promoting characteristics of valproate acts upon lowering the weakening effects that morphine has on the said system. Further studies should be conducted to reach a conclusion that whether this combination is beneficial in clinical treatment of addicted patients.

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