Duloxetine Attenuated Morphine Withdrawal Syndrome in the Rat

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Abstract

Background: Long term exposure to morphine can induce dependence. The exact mechanisms of dependence are not yet fully understood. Many studies have been conducted to find new drugs that can prevent dependence. This study examined the effects of the chronic administration of duloxetine on the morphine withdrawal syndrome in rats.

Methods: To this end, male Wistar rats (170–220 g) were randomly divided into 5 groups including one saline treated group (non-dependent group) and 4 morphine dependent groups. The experimental groups received additive doses of morphine for 9 days in order to induce dependence according to the following protocol: day 1: 5 mg/kg/12 h, days 2 and 3: 10 mg/kg/12 h, days 4, 5: 15 mg/kg/12 h, days 6 and 7: 20 mg/

kg/12 h and days 8 and 9: 25 mg/kg/12 h. On the ninth day, the morning dose of morphine was only injected. It is worth noting that 30 min before the morning dose of morphine, duloxetine (10, 20, and 40 mg/kg) was injected intraperitoneally. In addition, 2 h after the last injection of morphine, the morphine withdrawal was precipitated by naloxone. The withdrawal signs were recorded for 30 min; these signs included jumping, rearing, genital grooming, abdominal writhing, wet dog shaking, and teeth grinding.

Results: The results of the study revealed that the chronic administration of duloxetine decreased all the withdrawal signs. Besides, it attenuated the total withdrawal scores significantly.

Conclusion: Results indicate that the regulatory effects on serotonergic and noradrenergic parameters might be associated with the amelioration of the withdrawal symptoms.

Introduction

Morphine and other opioids are regularly used to control severe pain in patients with various clinical conditions such as cancer, renal calculus, heart attack, and traumatic injuries. Development of tolerance to the analgesic effect and the physical dependence are two major problems related to the chronic use of morphine and other opioids that obstruct their therapeutics utilizations. Various behavioral symptoms characterize the opioid dependence when the opiate consumption is ceased or the opioid antagonists are administered [1,2]. Because of these problems, many scientists are challenged and numerous studies have been conducted on the experimental animals to identify effective drug therapy for this difficulty. Acute opiate exposure inhibits the adenosine 3',5'-monophosphate (cAMP) pathway in neurons, whereas in the prolonged treatment compensatory up-regulation of the cAMP pathway is observed [3]. In this regard, the up-

regulation of the cAMP pathway is the best established molecular adaption to dependence and addiction [4]. Upon the removal of the opiate agonists, the up-regulation of the cAMP pathway also opposes the acute inhibition of this pathway and thereby represents a form of the opioid dependence. Hence, clonidine (α_2 adrenoceptors agonist) and beta receptor blockers such as propranolol are used for attenuating the withdrawal signs in addicts through their inhibitory effect on adenylyl cyclases activity and the suppression effect on the cAMP production [5]. Besides, the morphine treatment not only changes the signaling pathways but also it modulates auto- and hetero-receptors. A recent study has demonstrated that 5-HT_{1A} auto-receptors and α_2 -adrenoceptors, which regulate serotonin (5-HT) and noradrenaline (NA) synthesis in the central nervous system, respectively, are supersensitive during the chronic morphine treatment and the opiate withdrawal [6]. Hence, the supersensitivity of these regulatory receptors inhibits the 5-HT

synthesis and increases the NA synthesis in the brain during the prolonged treatment of opiate and its withdrawal [6]. Moreover, the Locus Coeruleus (LC), a bilateral nucleus in the brain stem, is the major noradrenergic nucleus that regulates the attention states and the activity of the autonomic nervous system in the brain. The LC is also implicated in the somatic opiate withdrawal signs [7]. In this regard, pharmacological and behavioral studies have indicated that the hyperactivity of the LC plays an important role in the precipitation of the physical signs of the opiate withdrawal [8,9]. This finding is in accordance with the results of other study that demonstrated that lesion to the LC reduces the somatic signs of the opioid withdrawal [10]. The LC also innervates heavily through serotonergic fibers and terminals [11]. Furthermore, from an anatomical perspective, the LC contains high levels of both 5-HT reuptake and its binding sites. This dense innervation through the serotonergic fibers emphasize that the serotonin plays an important role in controlling the noradrenergic LC neurons discharge. It is worth nothing that the serotonergic neurons denervation and the 5-HT synthesis inhibition are two conditions that increase the NA synthesis in the LC. Moreover, microdialysis studies on rats have shown that following administration of the 5-HT₂ receptor agonists reduces the spontaneous noradrenergic activity of the LC [12]. In this respect, Done and Sharp used the microdialysis technique and determined the NA concentration in the rat hippocampus. They reported the inhibitory influence of the 5-HT agonists on the NA synthesis in this nucleus [13]. In addition, the latter study suggested that the 5-HT₂ receptors mediate the 5-HT inhibitory effect on the NA synthesis [13]. Duloxetine is also a 5-HT and NA reuptake inhibitor [14] that inhibits the 5-HT reuptake 5-fold greater than the NA [15]. Besides, it inhibits the reuptake of dopamine weakly, and is also inactive as a ligand to opioid, dopaminergic, histaminergic, and beta and alpha-adrenergic as well as serotonergic and muscarinic receptors [16]. It is worth noting that due to the unimportant affinity of duloxetine for binding up the ion channels like glycine and GABA_A, and transporters such as choline and gamma-aminobutyric acid transporters [17], it also has a mild side effects and is used for the treatment of major depressive disorder (especially in patients with urinary incontinence) [18], generalized anxiety disorder [17], pain [19], and fibromyalgia as well [20]. Fuller, et. al. further reported that duloxetine antagonize the depletion of the brain's 5-HT through *p*-chloroamphetamine and the depletion of the heart's NA by means of 6-hydroxydopamine in mice [21]. Moreover, Rueter, et. al revealed that the chronic administration of duloxetine in the rats produced the regulatory effects on both the serotonergic and the noradrenergic parameters through the desensitization of the 5-HT_{1A} auto-receptors and the α_2 -adrenergic hetroceptors on nervous terminals [22]. The purpose of the present study is to investigate the effects of various doses of duloxetine on the naloxone induced morphine withdrawal signs in the rats.

Materials and Method

Animals

Male Wistar rats (170–220g) were purchased from the Pasteur Institute in Tehran, Iran. They were housed in the cages in the laboratory temperature (20±3 °C) and the humidity (60%) under a 12-h light-dark cycle. Food (lab chow) and water were available *ad libitum*. 2 days before the experiment, the rats were habit-

uated to the testing environment including transfer to the experimental laboratory, weighing, and handling in order to adapt them. All the procedures for the humane treatment of the rats were approved by the Research Committee of the Medical Sciences in Tabriz University. These procedures were performed based on the Guide for Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Drugs

The following drugs were used in the present study: morphine sulfate (Temad Company, Iran), naloxone hydrochloride (Daroupankhsah Company, Iran), and duloxetine hydrochloride (Sigma Aldrich, USA).

Morphine sulfate and naloxone hydrochloride were dissolved in 0.9% physiological saline, and injected subcutaneously and intraperitoneally, respectively. Duloxetine hydrochloride (10, 20, and 40 mg/kg) was dissolved in the sterile water and administered intraperitoneally. Needless to say that the chosen dose of duloxetine was selected based on the previous studies [23–25]. All the solutions were prepared freshly on the experimentation day and exactly before the administration.

Induction of morphine dependence

Additive doses of morphine were administered subcutaneously for 9 days in order to induce dependence. The procedure of the administration is as follow: day 1: 5 mg/kg/12h, days 2 and 3: 10 mg/kg/12 h, days 4 and 5: 15 mg/kg/12 h, days 6 and 7: 20 mg/kg/12 h, and days 8 and 9: 25 mg/kg/12 h. On the ninth day, the morning dose of morphine was only injected. This morphine administration protocol showed a high degree production of dependence in the rats [26]. Under the same condition, the rats in non-dependent groups received only saline.

Participants

Experimental groups

45 male Wistar rats were divided into 5 experimental groups (n=9) randomly. The rats were included in one saline treated group (non-dependent group) and 4 morphine treated groups (morphine-dependent groups). The non-dependent group or saline treated group (group I) was only treated with saline. 30 min before the morning morphine injection, groups II, III, and IV or in other words the morphine dependent animals received 10, 20, and 40 mg/kg duloxetine intraperitoneally, respectively. Instead of duloxetine with the same condition, the animals in group V (the control group) were treated by 0.25 mL saline intraperitoneally.

Procedures

Induction of the morphine withdrawal and measurement of the withdrawal behaviors

The experiments of the study were carried out in a quiet room during the light phase of the light-dark cycle. The behaviors of each animal were evaluated by an observer who was not aware of the nature of the treatment received by animals. On the ninth day, 2h after the last morphine injection, the rats received naloxone (4mg/kg) intraperitoneally in order to induce the withdrawal signs. Subsequently, the rats were placed in a clear plexiglas cylinder test chamber (measuring: 30 cm diameter and 50 cm height) that was equipped with a digital camera to record the animal behaviors during 30min period following naloxone injection and six distinct behaviors included wet dog shaking,

Table 1 Weighting factor of different withdrawal signs.

Behavior signs	Weighting factor
Jumping	4
Wet-dog shake	5
Abdomen writhing	5
Genital grooming	5
Teeth grinding	10
Rearing	20

teeth grinding, abdomen writhing, rearing, jumping, and genital grooming were recorded. If necessary, the records were repeated for precise scoring and analysis. The score of each behavior was divided by weighting factor attributed to it (Table 1), and the results were added and come to a Total Withdrawal Score (TWS) for each animal. The TWS was used as an index of the withdrawal intensity [27–29].

Data analysis

Statistical comparisons among the experimental groups were made by the one way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. All the results were shown by the mean $\pm$ SEM for 9 rats with the statistical significance set at $p < 0.05$.

Result and Discussion

As it can be seen in Fig. 1, the administration of naloxone increased the TWS (39.2 ± 4.1) in the control group (Morphine + Saline) significantly as compared with the saline treated group (6 ± 1 , $p < 0.001$). In addition, Fig. 2 shows that duloxetine administration (10, 20, and 40 mg/kg) caused a significant reduction in the expression of naloxone-induced TWS when compared with the control group ($p < 0.01$ for 10 mg/kg, and $p < 0.001$ for 20 mg/kg and 40 mg/kg). Based on Fig. 2, the TWS in the rats that received 20 mg/kg and 40 mg/kg duloxetine was significantly ($p < 0.01$) less than the rats that received 10 mg/kg duloxetine; it seems that duloxetine reduced the TWS in a dose dependent pattern.

As expected, the administration of naloxone precipitated jumping in the control group (13.1 ± 2.3), whereas the co-administration of duloxetine (10, 20, and 40 mg/kg) with morphine decreased the number of jumping (9.0 ± 2.1 , $p < 0.05$, 4 ± 1 , $p < 0.001$, and 3 ± 1 , $p < 0.001$, respectively) as compared with the control group (Fig. 3). In addition, naloxone-induction of the withdrawal wet dog shake appeared to be greater in the control group than the saline treated animals. In the morphine dependent animals, which received duloxetine (10, 20, and 40 mg/kg), the naloxone-induced withdrawal wet dog shakings were attenuated (14 ± 4 ($p > 0.05$), 6 ± 2 ($p < 0.001$), and 4 ± 2 ($p < 0.001$), respectively) in comparison with the control rats (Fig. 4). Moreover, the injection of naloxone precipitated a constellation of other withdrawal signs including rearing, teeth grinding, abdominal writhing, and genital grooming. Table 2 represents the attenuated effects of duloxetine on the incidence of these signs in compression with the morphine dependent group that received only saline.

The results of this study indicated that duloxetine attenuated the severity of the withdrawal signs. Besides, it suggested that the chronic administration of duloxetine, a 5-HT/NE reuptake inhibitor, tempered the naloxone-precipitated withdrawal signs.

Fig. 1 Naloxone (4 mg/kg)-induced TWS in control group in comparison to saline-saline treated group during 30 min of experiment. Data are expressed as mean $\pm$ S.E.M. ***: $p < 0.001$ compared to the morphine dependent group; N = 9 in each group. TWS: Total withdrawal score.

Fig. 2 Effects of intraperitoneal administration of duloxetine (10, 20, 40 mg/kg) on the expression of naloxone-induced TWS in morphine-dependent rats. Data are expressed as mean $\pm$ S.E.M and were analyzed using a one way ANOVA followed by Tukey's post hoc test. N = 9 rat in each group. TWS: Total withdrawal score. **: $p < 0.01$ and ***: $p < 0.001$ different from morphine-dependent saline treated group. *: $p < 0.01$ compared to the morphine dependent rats treated duloxetine (10 mg/kg).

Fig. 3 Effects of intraperitoneal injection of duloxetine on the expression of naloxone-induced jumping in the experimental groups. Data are expressed as mean $\pm$ SEM and were analyzed using a one way ANOVA followed by Tukey's post hoc test. N = 9 in each group. *: $p < 0.05$ and ***: $p < 0.001$ compared to the morphine dependent group that treated with saline. *: $p < 0.05$ compared to the morphine dependent rats treated duloxetine (10 mg/kg).

Opioid addiction results from adaptations in specific brain neurons that are caused by the repeated exposure to opiate such as morphine [4]. It is worth noting that the pharmacological treatment of the opioid dependence is limited. Methadone maintenance is the mainstay pharmacotherapy for the opioid dependent patients. In addition, clonidine, central alpha-2 adrenergic receptors agonist, and beta receptors blocker such as propranolol are another therapeutic agents for attenuation of the withdrawal signs [30,31]. However, these medicines have their own specific side effects. Thus, many researchers investigated the mechanisms of the withdrawal syndrome to find an effective medical treatment that might improve the ability of addicts to discontinue the opiate intake with less side effects and better quality of efficacy. Hence, alteration in noradrenergic [32], serotonergic [33], dopaminergic [34], purinergic [35], glutamatergic [36], and neurotransmitter systems are reported as possible involvement mechanisms in the morphine withdrawal syndrome. Furthermore, Robert's study suggested that the biogenic amine systems are involved in behavioral and physiological effects of the opiates [37]. Serotonergic and noradrenergic systems are also involved in the physical dependence of the opioid withdrawal [32,37]. In this regard, the up-regulation of the cAMP and the increase in the excitatory amino acid (glutamate and aspartate) produce a hyperactivation of the LC during the morphine withdrawal. The synthesis of 5-HT and the NA in brain are regulated by two presynaptic receptors such as 5-HT_{1A} and α_2 adrenocep-

tor that regulate those amine syntheses negatively [6]. It has also been reported that the acute morphine administration enhanced the 5-HT turnover as evidenced by an increase in the serotonin synthesis, release, and metabolism [38]. However, a decrease in the release of 5-HT was observed by the chronic morphine administration [39]. In contrast, the acute activation of the μ -opioid receptors decreases the NA synthesis [40]. It is interesting to mention that the synthesis of the NA is increased significantly after the administration of naloxone to the morphine-dependent rats [41], whereas the 5-HT synthesis is decreased after the naloxone-precipitated withdrawal [6]. It is worth noting that increasing the noradrenergic activity of the LC mediates the somatic signs of the opiate withdrawal. In this regard, neuroanatomical studies have indicated that the LC is innervated by the serotonergic fibers and terminals. Glutamate also induces the hyperactivity of the LC that is suppressed by the serotonergic mechanisms [11,42]. Besides, Done, et. al. reported that the 5-HT induces the inhibitory effect on the LC hyperactivity that is mediated by the 5-HT₂ receptors [13]. Moreover, antidepressant drugs with the 5-HT and/or the NA reuptake inhibition activity are frequently used for the treatment of pains related to postherpetic neuralgia, diabetic neuropathy, and fibromyalgia [43,44]. Recent studies have also demonstrated that the chronic administration of the antidepressant drug produce an inhibitory effect on the firing rate of the LC. This inhibitory effect has been interpreted as a consequence of the increase in the availability of the NA in the synaptic terminal, and increases the activation of the α_2 adrenoceptors that exert a tonic inhibitory effect on the firing rate of the LC [45–48].

In the present study, duloxetine, dual 5-HT, and the NA reuptake inhibitor were used in the naloxone induced morphine withdrawal syndrome in rats. The results revealed that the intraperitoneal chronic administration of duloxetine prevented the naloxone precipitated withdrawal signs in the morphine-dependent rats in all used concentrations as compared with the control group (Table 2 and Fig. 2–4). The results of this study also confirm findings of another study that showed the serotonin and the noradrenaline reuptake inhibitor, venlafaxine, attenuated morphine withdrawal signs in the rats [49]. Besides, Rueter, et. al. reported that the chronic administration of duloxetine produced the regulatory effects on both serotonergic and noradrenergic parameters through the desensitization of the somatodendritic 5-HT_{1A} autoreceptors and the α_2 -adrenergic heteroreceptors [22]. It is possibly hypothesized that the intraperitoneal chronic injection of duloxetine produces the regulatory

Fig. 4 The number of wet dog shakes in the experimental groups. Data are shown as mean ± SEM and were analyzed using a one way ANOVA followed by Tukey's post hoc test. N=9 in each group. ***: $p < 0.001$ compared to the morphine dependent group that treated with saline. **: $p < 0.01$ compared to the morphine dependent rats treated with duloxetine (10 mg/kg).

Groups	Signs			
	Rearing	Teeth grinding	Abdomen writhing	Genital grooming
Saline + Saline	8 ± 2	14 ± 3	1 ± 1	16 ± 5
Morphine + Saline	48 ± 8	109 ± 16	10 ± 3	84 ± 20
Morphine + Duloxetine 10	30 ± 7**	65 ± 14**	6 ± 2*	47 ± 9*
Morphine + Duloxetine 20	27 ± 4**	47 ± 11**	2 ± 2**	37 ± 11**
Morphine + Duloxetine 40	22 ± 3**	34 ± 12***	1 ± 1**	29 ± 6**

Data are showed as mean ± SEM and were analyzed by one way ANOVA followed by Tukey's post hoc test

*: $p < 0.05$ compared to the morphine-saline treated group

** : $p < 0.01$ compared to the morphine-saline treated group

***: $p < 0.001$ compared to the morphine-saline treated group

*: $p < 0.05$ compared to the morphine dependent rat treated with duloxetine (10 mg/kg)

** : $p < 0.01$ compared to the morphine dependent rat treated with duloxetine (10 mg/kg)

Table 2 A comparison of the frequency of behavioral manifestations by morphine. Withdrawal precipitated by naloxone between the experimental groups during the 30-min observation.

effects on both serotonergic and noradrenergic parameters, and suppresses the LC hyperactivity during the morphine withdrawal. Moreover, the results of this study indicated that for the first time, duloxetine has a strong potential in the attenuation of the morphine withdrawal, and possibly it is a rational therapeutic choice in the prevention of the opiate dependence and the withdrawal in addicts particularly those suffering from depression. One possible explanation for duloxetine effects on the morphine withdrawal signs is that duloxetine increases the level of the 5-HT at the LC serotonergic nerve terminals through its effects on the 5-HT reuptake, enhances the 5-HT₂ receptor activity, and prevents the LC hyperactivity.

Conclusion

The results of this study indicate that duloxetine attenuate naloxone induced withdrawal syndrome in morphine dependent rats. It seems that, chronic intraperitoneally injection of duloxetine produces regulatory effects on both serotonergic and noradrenergic parameters, suppresses LC hyperactivity and prevents morphine dependence. Further studies are necessary to determine the exact mechanisms of the observed attenuating effect of duloxetine in morphine withdrawal syndrome.

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Conflict of Interest

The authors declare that they had no conflict of interests.

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