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Brief Report

Attenuation of Withdrawal Signs, Blood Cortisol, and Glucose Level with Various Dosage Regimens of Morphine after Precipitated Withdrawal Syndrome in Mice

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What's Known

- Many previous basic and clinical data and treatment approaches were done on the application of a secondary drug to manage opioid dependency. However, these drugs have side effects and are often not associated with a favorable outcome.
- There was a need to introduce a new approach for opioid withdrawal syndrome management.

What's New

- This study considered the influence of altered morphine dosage regimens on detoxification and attenuation of its withdrawal signs.
- Morphine itself was used against morphine-induced dependency and altered morphine dosage regimen was used for opioid withdrawal syndrome management.

Abstract

Morphine withdrawal usually results in unsuccessful outcomes. Despite partial benefits from alternative substances such as methadone, its use may not lead to the desired result due to the lack of mental tranquility during the withdrawal period. In this study, by means of an animal model, morphine itself was used to manage morphine dependence. Forty mice were divided into 5 groups, in which 4 groups became dependent by increasing daily doses of morphine for 7 days (15-45 mg/kg). Afterwards, the animals received morphine for 14 days by either of the following regimens:

- Once daily 45 mg/kg (positive controls)
- Increasing the interval (each time 6 hours longer than the previous interval)
- Irregular interval in every 36, 12 and 24 hours until the 21th day
- 12, 24, 36 hours decreasing doses (each time 2.5 mg/kg less than the former dosage).

Negative controls received saline solution only. On day 22, total withdrawal index (TWI) was determined by injecting 3 mg/kg of naloxone. Thereafter, blood samples were taken for the measurement of cortisol and glucose levels. TWI significantly decreased in all test groups in comparison with the positive control animals ($P<0.001$). Cortisol levels significantly decreased when either the dosage or the administration frequencies were decreased on a regular and gradual basis ($P<0.005$). Blood glucose levels significantly decreased in animals that received decreasing doses of morphine ($P<0.005$). This study suggests that no other measures may be required in clinical practice except for changing the dosage regimen of morphine for the cessation of self-administration.

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Keywords ● Morphine ● Cortisol ● Glucose ● Substance-Related Disorders ● Withdrawal syndrome

Introduction

The usage of opioids has long been accepted as a standard pain relief method in patients with cancer and acute pain.[1] One of the major problems with long-term use of opioids such as morphine is drug dependency. It is characterized by physical dependence

and withdrawal syndrome resulting in sudden discontinued usage of opioid agonist or usage of opioid antagonist such as naloxone.[2,3] Clinical approach to manage the withdrawal syndrome is mostly based on pattern detoxification of the opioid substance using medication or a weak, long-acting opioid agent instead of the main drug.[4-6] Despite the immense benefits of drugs used to treat dependency and due to inadequate sedation of these drugs, the use of mentioned agent is often not associated with a favorable outcome.[7-9]

Morphine withdrawal is characterized by an increase in the hypothalamus-pituitary–adrenocortical (HPA) axis activity that causes an increase in corticosterone and blood cortisol levels. These hormones are the main hormones involved in stress in rodent. They are responsible for stress and anxiety of withdrawal period and probably glucose levels.[10] Chronic use of opioids causes key changes in pituitary–adrenal axis, which induces changes in physiological status of anxiety and stress situation.[11] Based on previous studies, blood glucose levels changed in dependent mice. This study showed that stable and fixed dose of opioid can alter the blood glucose and other endocrine factors.[12]

The aim of the present study was to evaluate possible influence of altered morphine dosage regimens on detoxification and attenuation of its withdrawal signs. In addition to observe the signs of morphine abstinence, blood cortisol and glucose concentrations were also measured. These factors were measured as parameters involved in the stress situation and confirmation of our protocol's effectiveness in the management of withdrawal syndrome.

Materials and Methods

Animals

Forty male adult Balb/c mice were obtained from Pasteur institute of Iran (Tehran, Iran). The average weight and age were 27.5 g and 8 weeks, respectively. The mice were held at room temperature with free access to commercial chow and tap water. Light and dark periods were supplied with 12 h cycles and controlled temperature (22±2ºC). The protocol was approved as an undergraduate research project by the Research Council of the University of Tehran, Faculty of Veterinary Medicine. The animals were kept and treated according to the university animal care guideline.

Induction of Morphine Dependency (Days 1-7)

Animals were divided randomly into 5 groups of 8, and were treated once a day at 8 a.m. subcutaneously with either saline solution (SHAM) or morphine sulphate (morphine 15, 20, 25, 30 35, 40, and 45 mg/kg in 7 coming days, respectively).

Maintenance of Morphine Administration (Days 8-21)

On the 8th day, group 1 (SALINE-NEG CONTROL) received saline and the remaining four groups were treated in four different ways. On the last day, group 2 received morphine at doses of 45 mg/kg, at fixed intervals of 24 h up to the 21th day (MOR45-POS CONTROL). Group 3 (MOR45-INC INTERVAL) received morphine (45 mg/kg) at increasing intervals of 6 hours (i.e. 30 h, 36 h, 42 h, 48 h, 54 h, 60 h, and 66 h) in the coming days. Group 4 (MOR45-IRREG INTERVAL) received morphine (45 mg/kg) at irregular intervals of 36 h, 12 h, 36 h, 12 h, 36 h, 12 h, 24 h, 36 h, 12 h, 36 h, 12 h, 36 h, 12 h, and 24 h. The last group (MOR-DEC DOSE) received morphine at 24 h intervals in decreasing dosage manner of 42.5, 40, 37.5, 35, 32.5, 30, 27.5, 25, 22.5, 20, 17.5, 15, 12.5, and 10 mg/kg, respectively.

Evaluation of Withdrawal Syndrome (Day 22)

On the 21st day, all animals received 3 mg/kg of naloxone sulfate at 8 a.m. Then, they were placed in chambers having 15×20×50 cm (w×d×h) dimensions, and were monitored for 30 minutes to record the signs of withdrawal syndrome. 14-scale behavior was recorded and the rate of each behavior was divided by a weighing factor (Table1) and the results were added to derive the total withdrawal index (TWI) for each animal.

Measuring Blood Cortisol and Glucose

After behavioral studies, the whole blood was collected and the levels of serum cortisol and glucose were measured based on µg/dl and mg/dl, respectively.

Measuring Weight Changes in Animals

On the first day before the injection of morphine and on the 22nd day before the injection of naloxone, all animals were weighed.

Table 1: Weighing factors (WFs) of different withdrawal signs of morphine in the mouse

Behavior	WF	Behavior	WF
Jumping	4	Body grooming	10
Head shake	5	Face wipes	10
Wet dog shake	5	Swallowing	10
Paw tremor	5	Teeth chattering	10
Writhing	5	Dysphoria	10
Walking sniffing	5	Rearing	20
Sniffing	5	Chewing	20
Penile licking	5	-	-

The weight change of animals was calculated as a percentage by the following formula.

$$\text{Difference of the mice weight as percent} = \frac{\text{Mice weight at the first of period-Mice weight at the last of period}}{\text{Mice weight at the first of period}}$$

Drugs and Chemicals

Morphine sulphate was a product of Temad Company (Tehran, Iran). Naloxone was purchased from Tolid-e Darou Company (Tehran, Iran).

Statistical Analysis

Data were averaged in every experimental group and expressed as mean±standard error of the mean (SEM). Then, differences between the control and morphine-dependent groups were evaluated by unpaired Student's t-test. Differences among groups receiving various dosage regimens of morphine were first compared by one-way ANOVA and then group-by-group with Bonferroni post hoc t-test. P values <0.05 and <0.001 were considered statistically significant.

Results

Total Withdrawal Index (TWI)

TWI increased by 67% from 17.8±0.5 in the controls (SALINE-NEG CONTROL group) to 54.8±1.1 in the morphine dependent (MOR45-POS CONTROL) animals (P<0.001). In comparison with MOR45-POS CONTROL animals, TWI was decreased by 44%, 33%, and 41% in MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups, respectively. The difference reached the level of significance in MOR45-INC INTERVAL and MOR-DEC DOSE groups (P<0.05) (Figure 1).

Blood Cortisol Levels (BCL)

BCL increased by 57% from 5.4±0.9 mg/dL in SALINE-NEG CONTROL group to 12.7±1 mg/dL in MOR45-POS CONTROL animals (P<0.001). In comparison with MOR45-POS CONTROL animals, BCL was decreased by 37%, 11%, and 41% in MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups, respectively. The difference reached the level of significance in MOR45-INC INTERVAL and MOR-DEC DOSE groups (P<0.05) (Figure 2).

Blood Glucose Levels (BGL)

BGL increased by 30% from 84.6±2.6 mg/dL in the SALINE-NEG CONTROL group to 121.5±2.6 mg/dL in the MOR45-POS CONTROL animals (P<0.001). In comparison with MOR45-POS CONTROL animals, BGL was decreased by 12%, 1%, and 28% in MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups, respectively. The difference reached the level of significance only in the MOR-DEC DOSE group (P<0.05) (Figure 3).

Body Weight Alteration (BWA)

SALINE-NEG CONTROL animals gained an increased body weight of 16.6±3.8% within 21 days of the treatment, whereas the MOR45-POS CONTROL animals lost 2±1.5% of their weights (P<0.001). The weight loss was prevented in the MOR45-INC INTERVAL, MOR45-IRREG INTERVAL and MOR-DEC DOSE animals in comparison with the MOR45-POS CONTROL group, which reached the level of significance in MOR-DEC DOSE group (P<0.05) (Figure 4).

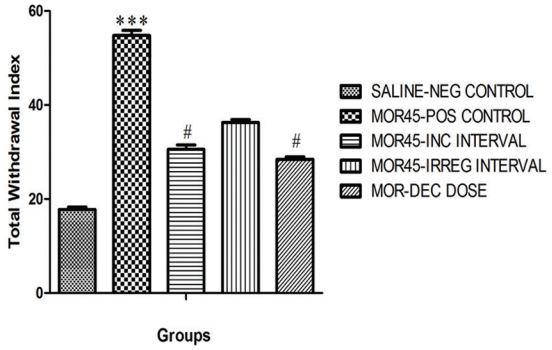

Figure 1: Shows total withdrawal index (TWI) after naloxone injection in the SALINE-NEG CONTROL, MOR45-POS CONTROL, MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups
N= 8 per group
***P<0.001 compared with SALINE-NEG CONTROL group
#P<0.05 compared with MOR45-POS CONTROL group

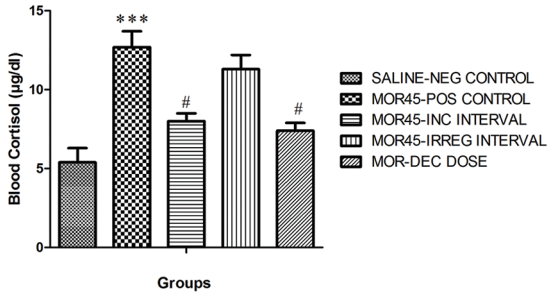

Figure 2: Shows blood cortisol levels after naloxone injection in the SALINE-NEG CONTROL, MOR45-POS CONTROL, MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups
N= 8 per group
***P<0.001 compared with SALINE-NEG CONTROL group
#P<0.05 compared with MOR45-POS CONTROL group

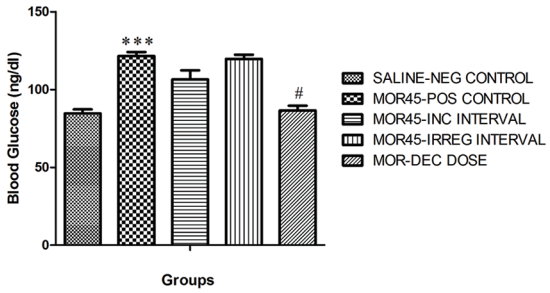

Figure 3: Shows blood glucose levels after naloxone injection in the SALINE-NEG CONTROL, MOR45-POS CONTROL, MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups
N= 8 per group
***P<0.001 compared with the C group
#P<0.05 compared with MOR45-POS CONTROL group

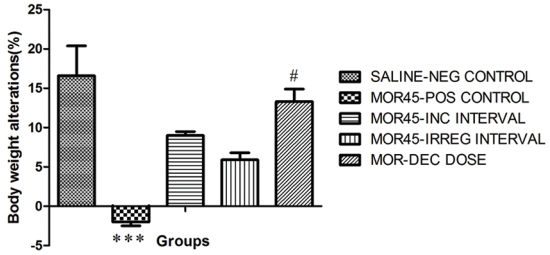

Figure 4: Shows body weight alterations in the mouse within 21 days in SALINE-NEG CONTROL, MOR45-POS CONTROL, MOR45-INC INTERVAL, MOR45-IRREG INTERVAL, and MOR-DEC DOSE groups
N=8 per group
***P<0.001 compared with SALINE-NEG CONTROL group
#P<0.05 compared with MOR45-POS CONTROL group

Discussion

In the present study, a new fascinating area of work for narcotic withdrawal strategies is put forward. It is possible to use the drug of abuse to eliminate the same drug instead of using other replacing substances. Indeed, changes in the pattern of opioid administration, including decreased frequency of application, gradual decrease in drug dosage, and irregular administration intervals may lead to the decreased intensity of morphine withdrawal syndrome. Interestingly, strategies used in this experimental work, especially a gradual decrease in morphine dosage, did not affect the general health of the animals as depicted from the data related to weight gain or loss during the experiments.

Several studies have been performed in the field of morphine withdrawal syndrome, in which various opioids such as naltrexone, dextromethorphan, methadone, tramadol, and buprenorphine were used as morphine replacement. Besides, non-opioid drugs have been applied to decrease the signs of morphine withdrawal by manipulating specific neurotransmitter re-uptake or affecting cerebral amino acids with the advantages like longer duration of action and lesser side effects in comparison with opioid agents.[5,6,9,13-15]

The present study shows that increasing the intervals of morphine injections without changing the dosages and irregular instead of regular injection reduces the symptoms of withdrawal syndrome significantly. We can justify that through increasing the intervals by adding 6 hours to dosage intervals in each time of injection, the central nervous system (CNS) does not encounter regular morphine intake and its award activating system abolishes and can provide a condition to reduce morphine dependence.[14] Due to the regular and continual reduction of morphine doses in the consequential injections (each time 2.5 mg/kg less than the previous time), it is prevented from encountering the opioid receptors with morphine. In fact, sudden termination of the drug dosage is replaced by regular decrease of the drug dosage. This method is similar to the mechanism of the effect of long-term agonist medications such as methadone, tramadol and buprenorphine and antagonist such as nalteroxone.[14]

The morphine withdrawal syndrome is revealed by an increase in the hypothalamus–pituitary–adrenocortical (HPA) axis activity. The origin of this activity is in cellular events, because morphine dependence increase HPA axis activity with changes in gene expression in selective neurons of the paraventricular nucleus.[13] Our study showed that morphine doses in the dependent positive control group caused a significant increase in the blood cortisol in comparison with the independent negative control group during the withdrawal syndrome period. This result is arguable with the increasing level of stress in mice and consequently with increasing the cortisol secretion in the withdrawal period and dependency level in mice.[16]

On the other hand, by applying the treatment protocols, decreased dosage as well as increased intervals did not increase the blood concentrations of cortisol, indicating the lack of a stressful condition for the animals. Actually, increasing intervals of dosage and regular decreasing of morphine dosage in the third treatment group caused a significant reduction in the blood cortisol level in comparison with the dependent positive control group. We established that by using the treatment protocols, the level of stress decreased in the animal during the withdrawal syndrome period and consequently cortisol level was attenuated.[17,18]

Park and colleagues had shown that the level of glucose is elevated in morphine-dependent mice.[19] As a result of stress and glucocorticoid secretion, we expected a rise in blood glucose levels during the withdrawal period. Indeed, this happened in the positive controls. Neither the increased intervals nor irregular morphine application prevented the glucose increase. However, gradual decrease of the morphine dosage significantly prevented glucose increase in the blood. One of the possible mechanisms for the hyperglycemic response after morphine withdrawal syndrome is that stress-induced hypercortisolism may have an opioid effect on the pancreas, stimulating glucagon secretion and thereby causing hepatic glucose output.[20]

Finally, the results of weight changes (by percent) in the control and under treatment groups showed that during opioids consumption in the positive control group, animals encountered a negative balance and even lost weight during the treatment period. However, in the negative control group they had a significant weight increase in comparison with the positive control group. Applying experimental protocols to the test group by regular decreasing intervals of morphine dosages caused an increase in the weight of dependent animals. The increasing intervals of morphine dosages or irregular morphine injection instead of regular injection did not lead to any meaningful weight change in comparison with the positive control animals. We can argue this results with a basic concept that addiction to opioids cause a decrease of appetite, increase saturation hormone, suppress the appetite center in the brain, and also reduced metabolic activity of nutrition in the body, thus it has a negative effect on the body weight gain.[19,20] However, increasing the intervals of morphine dosages or irregular morphine injection instead of the regular injection as well as regular decrease of morphine dosages, causes attenuation of this morphing effect on the metabolism and appetite and thus suppresses morphine induced anorexia.

Conclusion

This study showed that changing the dosage regimen of morphine administration could be useful for the management of withdrawal syndrome and cortisol (as the main marker of anxiety) of the cessation period. This method is recommended as an adjunct treatment of methadone or other standard therapy of dependency. However, in human clinical practice, we would be exposed to more challenges and there is a need for further precise evaluation of our protocol.

Conflict of interest: None declared.

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