Alterations of Blood Glucose and Cortisol Levels after Naloxone Administration in Morphine-dependent Mice

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Abstract—The present study aimed to find a correlation between increased blood glucose and cortisol levels with total withdrawal index (TWI). Sixty four male mice were divided into 2 groups. Control group received saline solution s.c. once daily for 28 days. The second group was treated by morphine once daily with the cumulative dosages of up to 45 mg/kg for 28 days. TWI was determined on 8 animals of each group on days 8, 15, 22 and 29. At the end, animals were euthanized and blood samples were taken for glucose and cortisol measurement. At the end of the weeks 1-4, TWI was significantly higher in the morphine dependent group (P<0.05) and blood glucose levels were also significantly higher in the dependent group (P<0.001). Cortisol was also increased in the morphine dependent animals, however, the change was reached the level of significance only after the week 3 (P=0.005). In the morphine dependent animals, there were direct correlations between the increased levels of glucose with TWI (P<0.001) and between the increased levels of blood cortisol with TWI (P=0.049). These findings show that there is a direct correlation between the elevation of blood glucose and cortisol levels with TWI in the morphine dependent mice after administration of a narcotic antagonist.

Keywords—Glucose, Mice, Morphine, Naloxone

I. INTRODUCTION

Opioid dependence is a great concern for the human society especially in the Eastern world, including Iran, exceeding 20% of some rural population (Bashardoost and Tirani, 2005). For this reason, research activities on this issue are still of a great importance.

Mouse is an animal model used in research protocols to evaluate opioid dependence and withdrawal syndrome (Marshall & Grahame-Smith, 1971). Repeated administration of morphine or other derivatives leads to a real dependence in this animal. Administration of an opioid antagonist (e.g. naloxone) in these animals lead to a series of observable withdrawal signs for each of which a score is given. The total, modified score is taken as so-called Total Withdrawal Index (TWI). TWI is in correlation with the severity of the opioid dependence.

Blood levels of glucocorticoids are long known to be increased during stressful situations. One of the prominent effects of these substances is an elevated metabolism of carbohydrates (Miller et al., 1966) which, in turn, will lead to increased blood glucose levels.

In spite of the studies on the effects of morphine and the symptoms of its withdrawal, no standard experimental study has been performed to evaluate the "intensity" of morphine dependence. In human medicine, non-discriminative routine therapeutic strategies are used for different degrees of morphine dependence. The present study aimed to find a correlation between increased blood glucose and cortisol levels with total withdrawal index (TWI).

II. MATERIALS AND METHODS

A. Animals and Experimental Protocol

Sixty four adult male mice were randomly divided into 2 groups of 32 each. The first group of animals (controls) received saline solution s.c. once daily for 28 days. The second group was treated by morphine once daily with the cumulative dosages of 15-40 mg/kg on days 1-6 and 45 mg/kg on days 7-28.

B. Test Procedure

TWI was determined on 8 animals of each group on days 8, 15, 22 and 29. For this purpose, the modified method of Rasmussen and Vandergriff (2004) applied.

At the end of the experiment, the animals were euthanized with inhalation of diethylether, decapitated and blood samples were taken for the measurement of serum glucose (with a clinical glucometer) and cortisol (with ELISA method).

C. Chemicals

Morphine sulfate was purchased as powder from Tamad Company, Tehran, Iran. Naloxone was an injectable dosage form produced by Daroupakhsh Pharmaceutical Company, Tehran, Iran.
D. Data Presentation and Statistical Analysis

Data are presented as Mean ± SEM throughout this report. For comparison of groups, when they were 2, unpaired Student's t test was used. For comparison of 3 or more groups, they were first analyzed by one-way analysis of variance (ANOVA) and then, when permitted, by the post hoc test of Bonferroni's t test. In addition, correlation-regression analysis was also accomplished and the regression constant (r) was determined between TWI-blood glucose and TWI-blood cortisol levels. When P<0.05, the difference or correlation was considered to be statistically significant.

III. RESULTS

TWI was significantly higher in the morphine dependent group compared with the controls from 3.85 up to 31.72 at the end of the weeks 1-4 (P<0.05, Figure 1). At the end of the weeks 1-4, blood glucose levels were significantly higher (19 to 53 mg/dL) in the dependent group than in the controls (P<0.001, Figure 2). Cortisol was also increased in the morphine dependent animals (2.71-4.9 g/dL) in comparison to the non-dependent mice, however, the change was reached the level of significance only after the week 3 (P<0.005, Figure 2).

Correlation-regression analysis showed that, in the morphine dependent animals, there were direct correlations between the increased levels of glucose with TWI (r=0.985, P<0.001) and between the increased levels of blood cortisol with TWI (r=0.880, P=0.049).

IV. DISCUSSION

These findings show that there is a direct correlation between the elevation of blood glucose and cortisol levels with TWI in the morphine dependent mice after administration of a narcotic antagonist.

There are evidence showing a relation with morphine usage and blood glucose alterations. The effects of acute and chronic morphine treatment on glucose tolerance were investigated in mice. In acute experiments, a single dose of morphine increased the serum and muscle glucose level. In morphine-dependent mice, the fasting serum and muscle glucose levels were similar to those of control (Chan and Dai, 1987). Endogenous opiates modulate the effects of sympathetic nervous system activity in type II diabetes (Surwit et al., 1989). Methadone lowered blood glucose in a dose-dependent manner from the baseline, an effect that was antagonized by naloxone. Morphine failed to lower blood
glucose levels (Faskowitz et al., 2013). Both morphine and beta-endorphin administered i.c.v. acutely increases the blood glucose level in the mouse (Park et al., 2010). For this sort of relationships, we hypothesized that a correlation between naloxone-induced alterations in blood cortisol and glucose levels could reflect the severity of opioid dependence.

Detection of opioid dependence is important from clinical and legal points of view. This is routinely performed by urine analysis for metabolites of these substances although hair and saliva are also rarely used. However, with the observations of the present study, it may be possible in future not only to detect the existence of opioid consumption but also gain more information about the degree of addiction as well.

V. CONCLUDING REMARKS

Finally, from the findings of this research work, the following issues can be concluded:

1. Intensity of opioid dependence is increased in correlation with the duration of drug usage as depicted from TWI values.
2. Increased duration of opioid use leads to an increased blood levels of cortisol (as a stress indicator) after injection of an opioid antagonist.
3. The same happens with blood glucose levels, hence secondary to the increased cortisol concentration.
4. Naloxone increased blood glucose levels even in healthy, non-treated animals. The cause of this effect has to validated by additional experiments in future.

VI. SUGGESTIONS

1. Similar experiments are best to be done, using other animal species to be able to extrapolate these findings to clinical situations.
2. Proper studies are encouraged in humans to find out standardized methods for estimation of the severity dependence and duration of opioid abuse and, accordingly, effective strategies for their treatment and detoxification.
3. Additional studies on the obscure part of our findings (elevation of blood glucose in healthy mice) may lead to understand the mechanisms involved and their implications to clinical situations.

REFERENCES