Attenuation of Alcohol Withdrawal Syndrome and Blood Cortisol Level with Forced Exercise in Comparison with Diazepam

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Abstract- Relieving withdrawal and post-abstinence syndrome of alcoholism is one of the major strategies in the treatment of alcohol addicted patients. Diazepam, chlordiazepoxide, and topiramate are the approved medications that were used for this object. To assess the role of non-pharmacologic therapy in the management of alcohol withdrawal syndrome, we analyzed effects of forced exercise by treadmill on alcohol dependent mice as an animal model. A total of 60 adult male mice were divided into 5 groups, from which 4 groups became dependent to alcohol (2 g/kg/day) for 15 days. From day 16, treatment groups were treated by diazepam (0.5mg/kg), forced exercise, and diazepam (0.5 mg/kg) concurrent with forced exercise for two weeks; And the positive control group received same dose of alcohol (2 g/kg/day) for two weeks. The negative control group received normal saline for four weeks. Finally, on day 31, all animals were observed for withdrawal signs, and Alcohol Total Withdrawal Score (ATWS) was determined. Blood cortisol levels were measured in non-fasting situations as well. Present findings showed that ATWS significantly decrease in all treatment groups in comparison with positive control group (P<0.05 for groups received diazepam and treated by forced exercise and P<0.001 for group under treatment diazepam + forced exercise). Moreover, blood cortisol level significantly decreased in all treatment groups (P<0.001). This study suggested that forced exercise and physical activity can be useful as adjunct therapy in alcoholism and can ameliorate side effects and stress situation of withdrawal syndrome periods.

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Keywords: Cortisol; Alcohol; Withdrawal syndrome; Forced exercise

Introduction

Alcohol dependency is a substance related disorder in which an individual is addicted to alcohol either physically or mentally (1). The exact mechanism that describes dependency and withdrawal symptoms of alcohol is not clear (2). A variety of techniques exists for managing alcohol withdrawal, some of them involve pharmacotherapy with sedatives and some of them do not. Drugs with the sedative effect such as diazepam, chlordiazepoxide, topiramate, and naltrexone are used in the pharmacotherapy of alcohol withdrawal syndrome. These medications act in the reduction of withdrawal syndrome severity (3-6). Alcohol withdrawal is characterized by a stressful condition and increased activity of adrenal gland and cortisol level (7,8). Previous studies showed that alcohol dependency increases the expression of CRF mRNA leading to activation of the adrenal gland and increasing of cortisol level as stress hormone (7). Previous data demonstrated that CRF receptors play an important role in the management of ethanol self-administration and attenuation of withdrawal syndrome independent rats. These studies showed that CRF antagonists and drugs that attenuate blood cortisol level can be useful pharmacotherapeutic methods for the treatment of alcoholism in humans (9). Also, these studies indicated that there is an increase in the basal serum cortisol level in the alcoholic subjects compared to the controls (10,11). These studies approved that serum cortisol level can be used to determine the severity of alcohol withdrawal syndrome (11). Exercise plays an important role in the treatment of addictive disorders. Exercise may benefit drug dependent patients attempting recovery from substance problems through a number of different mechanisms of action (12). Recent studies have

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also shown that exercise alleviate mood disturbance and morphine and nicotine withdrawal symptoms (13,14). It has also been shown that exercise can counteract withdrawal symptoms, and physical exercise can reduce the risk of some types of drug addiction (15). In recent years, some studies have found that exercise reduces depressive symptoms and may reduce the risk of relapse of addictive disorder (15). Recent studies have reported the acute effects of exercise on decreased craving for nicotine and morphine consumption and their withdrawal syndrome (13). In the present study, the alleviation of the alcohol withdrawal syndrome and attenuation of stress level were investigated by forced exercise and cortisol was measured as an important parameter of stress.

Materials and Methods

A total of 60 adult male Wistar mice (35-50 grams) were purchased from Iran Razi Institute (Tehran, Iran). All the animals were maintained in standard condition (220 C ± 2 and 12/12 hours dark and light cycle), having free access to food and water. The protocol approved as an undergraduate research and was projected by the Research Council of the Tehran University of medical science.

Drugs

Alcohol and Diazepam were purchased from Sigma–Aldrich Inc (St Louis MO, USA).

Alcohol withdrawal syndrome protocol

Grouping protocol

Induction of alcohol dependency

To induce alcohol dependency, in 4 groups (of 5); the animals were given alcohol (2g/kg/day) for 15 days (16). Animals in the negative control group received normal saline for 31 days intraperitoneally. The ethanol-dependent mouse (Groups 1, 3, 4, and 5) received alcohol intragastrically.

Treatments

From day 16, the positive control group (group 1) received the same dose of alcohol (2 g/kg/day) for maintenance of dependency for two weeks. Negative control group (group 2) received normal saline for four weeks. Group 3 were treated with diazepam (0.5mg/kg) for two weeks. Group 4 trained by forced exercise that is described below for two weeks; and group 5 received diazepam (0.5mg/kg) concurrent with forced exercise for two weeks. All doses mentioned about alcohol and diazepam were chosen based on previous studies (17).

Evaluation of alcohol withdrawal syndrome

In day 31, animal observed for 4 minutes in the 24 hour period of the ethanol withdrawal. All of the objects were observed, and the camera recorded five major behaviors including stereotyped behaviors, agitation, tail stiffness, abnormal posture, and abnormal gait. After computation of recorded data, behaviors were counted and analyzed, and a digit allocated to each one (Table 1-2). The summation of these digits gives Alcohol Total Withdrawal Score (ATWS) (18).

Table 1-2. Rating scale for some behaviors signs induced by ethanol withdrawal in rats

Signs	Scoring
Stereotyped behaviors*	1: rats showing only one stereotyped behavior 2: two stereotyped behaviors 3: three stereotyped behaviors 4: four stereotyped behaviors 5: all of the stereotyped behaviors
Agitation	1: rats showing mild or moderate agitation 2: very irritable 3: handling vocalization and moderately aggressive 4: handling vocalization and very aggressive 5: spontaneous vocalization and very aggressive
Tail stiffness	1: mild tail rigidity 2: moderate tail rigidity 3: tail rigidity but mildly flexible during ambulation 4: tail rigid and not flexible during ambulation 5: tail very rigid and not flexible during ambulation
Abnormal posture	1: mild head-down, back-hunched 2: moderate head-down, back-hunched 3: prominent head-down, back-hunched 4: in addition hind legs wide apart 5: in addition to limbs apart
Abnormal gait	1–2: mild difficulty ambulating and rearing is normal 3–4: moderate difficulty ambulating and rearing 5: prominent difficulty ambulating and no rearing

*Grooming, sniffing, head weaving, gnawing, and chewing

Treadmill forced exercise protocol

All animal were allowed to run on a motor-driven leveled treadmill (ModelT408E, Diagnostic & Research Instruments Co., Taoyuan, Tai). The objects in group 4 and 5 were trained by the treadmill for 45 minutes/day, 5 days a week for 2 weeks (19).

Measuring the blood cortisol concentration

On the 31nd day after the behavioral signs been

recording, whole blood of animals was collected, their serum was separated and the fasting serum level of cortisol was measured based on µg/dl and by ELISA method.

Statistically analysis

All data expressed as means ± standard error of the mean (SEM). The differences between positive and negative control groups were analyzed by unpaired Student's t test. A difference between treatment groups compared by one-way ANOVA and then, post hoc test of Bonferroni's was used for group-by-group analysis. The value of $P<0.05$ was taken as statistically significant.

Results

Alcohol Total Withdrawal Score (ATWS) results in control and treatment groups

Current data indicate that ATWS in negative control group received saline during protocol process was 14 ± 2 while ATWS in positive control group (dependent group) which was 30 ± 1.6 increased significantly by 53% ($P<0.05$) in comparison with negative control (Figure 1).

Administration of diazepam caused 33% decrease in ATWS (20±2) in comparison with a positive control group ($P<0.05$) (Figures 3-5). Also treatment of the animal by treadmill forced exercise caused 26% decrease in ATWS (22±1.3) in comparison with the positive control group. Moreover, finally combination therapy with diazepam and treadmill forced exercise caused 46% decrease in ATWS and reached the 16±2 score ($P<0.05$) (Figure-2).

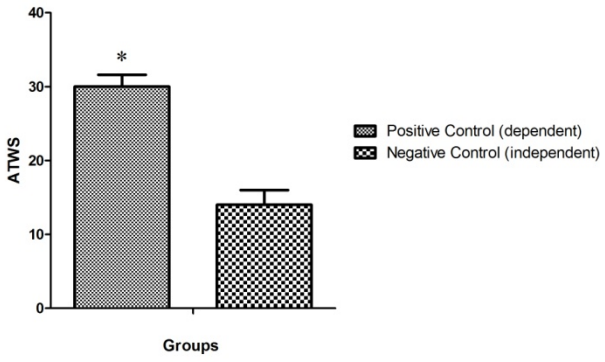

Figure 1. Comparison of the occurrence of the Alcohol Total withdrawal Score between the mice of the negative control group (independent) and positive control (alcohol dependent) group.

*: shows the significant difference ($P< 0.001$) in comparison with the negative control (independent) group. N=8 per group

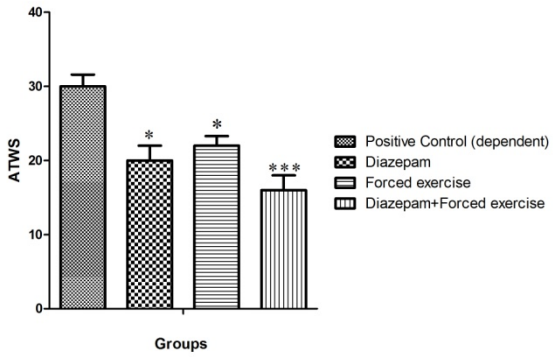

Figure 2. Comparison of occurrence of the Alcohol Total withdrawal Score between the mice of alcohol-dependent group under treatment by Diazepam, by forced exercise and under treatment by combination of Diazepam and forced exercise, in comparison with the positive control (alcohol dependent) group.

***: shows the significant difference ($P<0.001$) in comparison with the positive control (alcohol dependent) group.

*: shows the significant difference ($P<0.05$) in comparison with positive control (alcohol dependent). N=8 per group

Blood cortisol level in control and treatment groups

Blood cortisol level in positive control group was 16 ± 2.1µg/dl after withdrawal period, which was significantly higher than negative control group (7.2 ± 1.3) ($P<0.05$) (Figure -3).

Administration of Diazepam significantly decreased cortisol level (from 16±2.1 to 6.8±1.3 µg/dl or 57% decrease) ($P<0.05$). Treatment of animals by forced exercise caused significant attenuation of cortisol level (50%) in comparison with the positive control group and reached to 7.9 ±1.2 µg/dl. Combination therapy of dependent animals by Diazepam and forced exercise caused 63% decrease in cortisol level and reached 5.8±1.1 µg/dl ($P<0.05$) (Figure 4).

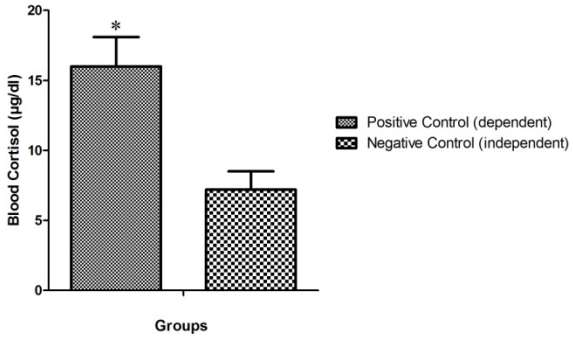

Figure 3. Comparison of blood cortisol levels as µg/dl between the mice of the negative control group (independent) and positive control (alcohol dependent) group.

*: shows the significant difference ($P< 0.001$) in comparison with the negative control (independent) group. N=8 per group

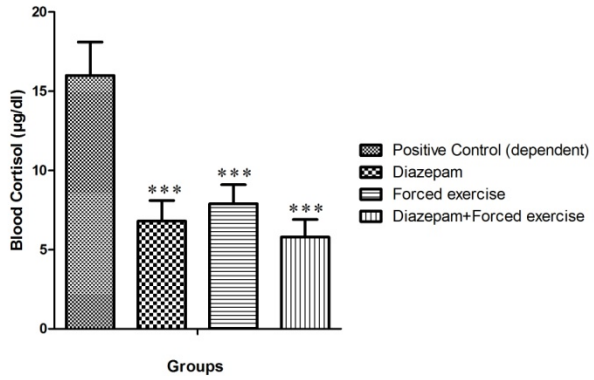

Figure 4. Comparison of blood cortisol levels as µg/dl between the mice of alcohol-dependent group under treatment by Diazepam, by forced exercise and under treatment by combination of Diazepam and forced exercise, in comparison with the positive control (alcohol dependent) group.

***: shows the significant difference (P<0.001) in comparison with the positive control (alcohol dependent) group. N=8 per group

Discussion

Alcohol abuse disorders are a major public health concern. Despite the efficacy of numerous treatments for alcohol dependency, its withdrawal syndrome remains a major problem (1). Healthy lifestyle changes and pharmacotherapy may contribute to the long-term maintenance of recovery, and interventions targeting physical activity, in particular, may be especially valuable as an adjunct to alcohol treatment (12). The role of exercise as an adjunct to alcohol treatment has been explored by participants receiving inpatient alcohol rehabilitation treatment (12,20,21). Many previous studies were conducted in the field of treatment of alcohol dependency and management of withdrawal syndrome, all of these experimental studies had been conducted by groups of sedative and hypnotic medications such as diazepam, chlordiazepoxide, topiramate (21,22). This study showed that forced exercise with treadmill and its combination with diazepam, as the standard treatment of alcohol dependency, can be effective in alcohol withdrawal syndrome management. Current findings showed that dependent groups under treatment by forced exercise with treadmill or dependent group under treatment by diazepam (0.5mg/kg) alone showed significant decrease in alcohol abandonment sign and its severity (P<0.05). Also, a dependent group under treatment by exercise in combination with diazepam significantly attenuate the withdrawal signs in comparison with positive control (dependent without treatment) (P<0.001). These results show that physical activity by treadmill can ameliorate the severity of withdrawal symptoms. Recent studies showed that exercise can abolish these symptoms by attenuating pain perception, depression and reducing anxiety probably by increasing the release of the opioid-like peptides such as endorphin (23-27). These results can be arguable with this concept that exercise plays an important role in the prevention and treatment of alcoholic addictive disorders. Also we can argue these findings by describing the possible mechanisms of exercise on reducing the rewarding effects of drugs such as cocaine and morphine since recent study has indicated that exercise leads to an increase in the synthesis and release of some neurotransmitters such as dopamine, serotonin, and GABA (27,28). On the other hands, exercise has been shown to result in acute improvement in positive-activated affect and alleviate mood disturbance and withdrawal symptoms in patient attempting to quit alcohol (20). These positive reinforcing properties may be mediated in part by exercise effects on the endogenous opioid system and potentiating of dopaminergic systems linked importantly to the experience of enhanced mood and experienced pleasure (29,30).

The present study indicates that the chronic abuse of alcohol and its abstinence syndrome can increase the activity of the CRF-secreting cells and activate adrenal cortex (7,9). Alcohol dependency increases stress parameters and HPA axis activity by changes in gene expression of CRF in selective neurons of the paraventricular nucleus (9,31,32). The result of present experimental study indicated that alcohol doses in the dependent positive control group cause a significant increase in blood cortisol level in comparison with the independent negative control group during the withdrawal syndrome and cessation period (P<0.05). We can argue this result with the basic concept that the increasing level of stress in animal and consequently increasing the cortisol secretion during the withdrawal period in the mouse. On the other hands, by applying the treatment protocols with diazepam, exercise, and exercise in combination with diazepam, a significant reduction in the blood cortisol level was reached, in comparison with the dependent positive control group was statistically significant (P<0.05). Generally current study results showed that treatment protocols decrease stress level in an animal in the withdrawal syndrome period and consequently cortisol level. We conclude that significant difference presents in withdrawal syndrome and cortisol levels between positive control group and the group treated with diazepam, exercise and diazepam

in combination with exercise.

Application of exercise with alternative medication is the best treatment and is similar to the group that received no alcohol. This study showed that exercise can be an effective adjunct and concurrent therapy with diazepam to reduce symptoms of alcohol withdrawal syndrome respectively and it can assist alcoholic patients to the abandonment of alcohol dependency.

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