Induced Locomotor Hyperactivity on Ethanol Withdrawal Syndrome in Rats is Inhibited by Quetiapine

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Eur J Basic Med Sci 2012;2(4):112-117

Received: 08-03-2013

Accepted: 18-03-2013

This study was presented as a poster presentation in the 35. Federation of European Physiological Societies (FEPS) and nominated to daily poster selection of the meeting March 26-29, 2006- Munich, Germany.

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ABSTRACT

Our aim is to investigate the effects of two atypical antipsychotics; quetiapine and olanzapine on locomotor activity that is a sign of ethanol withdrawal syndrome in rats. Adult male Wistar rats were subjects. Ethanol (7.2%, v/v) was given to rats by a liquid diet for 30 days. Control rats were pair fed an isocaloric liquid diet containing sucrose as a caloric substitute to ethanol. Quetiapine (10 mg/kg), olanzapine (5 mg/kg) and saline were injected to the rats intraperitoneally 7 days after ethanol withdrawal syndrome and the last one 30 min before ethanol withdrawal testing. After 2nd hour of ethanol withdrawal, rats were observed for 5 min and withdrawal signs that included locomotor hyperactivity were recorded. We have found increased vertical and horizontal locomotor activity in ethanol withdrawal group to control and reduced vertical and horizontal locomotor activity in quetiapine-injected rats. In olanzapine injected rats were seen no reduced locomotor activity. Significant inhibitory e□ects were produced by quetiapine on the signs of ethanol withdrawal. Our results suggest that acute quetiapine treatment has some beneficial efects on ethanol withdrawal in rats. Thus, this drug may be useful for treatment of ethanol withdrawal syndrome.

Key words: *Locomotor activity, Ethanol withdrawal syndrome, Quetiapine, Olanzapine, Rat model.*

Alkol yoksunluk sendromlu sıçanlarda artan lokomotor hiperaktivitenin ketyapinle baskılanması

ÖZET

Amacımız iki atipik antipsikotik olan ketyapin ve olanzapinin sıçanlardaki alkol yoksunluk sendromunda lokomotor aktivite üzerine etkilerini araştırmaktır. Erkek wistar sıçanlar seçildi. Etanol (7.2%, v/v) sıçanlara 30 günlük sıvı diyet içinde verildi. Kontrol sıçanları izokalorik diyetle alkolün yerine sükroz konarak beslendi. Ketyapin (10 mg/kg), olanzapine (5 mg/kg) ve salin enjeksiyonları sıçanlara intraperitoneal olarak alkol yoksunluk sendromundan 7 gün sonra etanol yoksunluk testinden 30 dakika önce yapıldı. Alkol yoksunluk sendromunun 2. saatinde sıçanlar 5 dakika gözlemlendi ve yoksunluk işaretleri ve lokomotor aktivite kaydedildi. Ketyapin enjekte edilen sıçanlarda azalmış dikey ve yatay aktivite gözlemlenirken olanzapin enjekte edilenlerde herhangi belirgin inhibitör etki ortaya çıkmadı. sonuçlarımız akut ketyapin tedavisinin alkol yoksunluğu olan sıçanlarda bazı faydalı etkileri olabileceğini ileri sürmektedir bu yüzden bu ilaç alkol yoksunluk sendromunun tedavisinde kullanılabilinir.

Anahtar Kelimeler*: Lokomotor aktivite, Alkol yoksunluk sendromu, Ketyapin, Olanzapin, Sıçan modeli*

INTRODUCTION

Excess alcohol usage is described the greatest drug addiction in the world. Ethanol withdrawal syndrome (EWS) could occur after the cut-off chronic alcohol intake shows physical alcohol dependence (1). EWS findings in human (2) and rats (3,4) are described in detail but the mechanisms underlined of physical dependence to ethanol have not understood completely. Open field locomotor activity is defined to evaluate the effects of ethanol and the other drugs (5). Studies showed that effects of ethanol were excitatory or depressive (6). Disease processes or events that accompany acute alcohol withdrawal may cause significant disturbance and even death. Appropriate treatment of alcohol withdrawal can relieve the patient's discomfort; prevent the development of more serious symptoms. The symptoms of ethanol withdrawal syndrome have been described in experimental studies (7) and human (8). Although the mechanism of physical dependence has not been enlightened decisively, novel drugs for treatment of alcoholic dependence have been sought.

Alcoholism and depression are often associated in psychiatric patients. Many alcoholic patients have symptoms of depression (9,10). Some antidepressant drugs are of general use in patients with ethanol dependence. They are mainly indicated in the ethanol withdrawal and the treatment of combined psychiatric disorders (11,12). Serotonergic drugs are of particular interest in that point, especially because of the hypothesized links between mood disorders and ethanol consumption. Neurochemical findings from clinical (13) and experimental (14) studies suggested some significant chances in central serotonergic neurotransmission during ethanol consumption and/or withdrawal. Atypical antipsychotics could be of great value in depressive conditions reputed for their resistance to treatment with usual antidepressants (15). Quetiapine and olanzapine are atypical antipsychotics that exhibit antidepressant activity in experimental models (16) and also in clinical trials (15,17). Recently, two psychotropic agents approved for treating bipolar disorder, olanzapine and quetiapine, have also been shown to possess antidepressant activity without destabilizing mood and, as such, are potential mood stabilizers. Antidepressant treatments, such as norepinephrine reuptake inhibitors, SSRIs, and electroconvulsive therapy, induce a reduction of 5-HT (2A) receptors. Both olanzapine and quetiapine not only are antagonists at this receptor but also induce down regulation of 5-HT (2A) receptors (18). The main objective of the present study was to investigate the effects of quetiapine and olanzapine on the locomotor activity, which is a sign of ethanol withdrawal syndrome in rats.

MATERIALS AND METHODS

Animals and laboratory

This study is approved by Gaziantep University Medical School Ethical Committee (B.30.2.GZP.0.01.00.00.211/2017).

All procedures in this study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA). All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male Wistar rats (270–330 g weight at the beginning of the experiments) were subjects. They were housed in a quiet and temperature and humidity-controlled room (22 ±3 C and 65±5%, respectively) in which a 12-h light/dark cycle was maintained (08:00–20:00 h light). Exposure to ethanol and all behavioral experiments involved in ethanol withdrawal syndrome were carried out in the separate and isolated laboratories, which have the same environmental conditions with the colony room.

Ethanol Chronic Administration

The rats were housed individually and ethanol was given in the modified liquid diet as previously described (7). The rats received a modified liquid diet with or without ethanol ad libitum. No extra chow or water was supp-

Table 1. *Locomotor activity: Horizontal*

	n	Mean	Std. D	Std. E	%95 Confidence Interval		Min	Max
					Lower	Upper		
1	10	1157.4900	380.23315	120.24028	885.4876	1429.4924	569.90	1699.70
2	9	827.5556	375.72966	125.24322	538.7442	1116.3669	353.30	1317.80
3	10	1238.8900	537.10890	169.84875	854.6654	1623.1146	722.20	139.30
4	10	1514.2800	356.02461	112.58487	1259.5953	1768.9647	904.50	2181.00
5	10	1394.8900	418.61742	132.37845	1095.4291	1694.3509	853.90	2370.80

***Tablo 2**. Locomotor activity: Ambulatory*

	n	Mean	Std. D	Std. E	%95 Confidence Interval		Min	Max
					Lower	Upper		
1	10	550.9500	231.10413	73.08154	385.6281	716.2719	183.40	861.60
2	9	401.3222	205.84514	68.61505	243.0956	559.5488	137.20	615.30
3	10	635.1100	332.36020	105.10152	397.3538	872.8662	304.90	1189.90
4	10	743.9900	188.34898	59.56118	609.2533	878.7267	430.50	1087.00
5	10	715.4200	253.02500	80.01353	534.4168	896.4232	342.70	1307.90

lied. The composition of the modified liquid diet with ethanol is: cow milk 925 ml (Mis Süt, Turkey), 25–75 ml ethanol (96.5% ethyl alcohol; Tekel, Turkish State Monopoly), and sucrose 17 g (7). This mixture supplies 1000.7 kcal/L. At the end of the exposure to the 7.2% ethanol-containing liquid diet, diet with ethanol was withdrawn and replaced with isocaloric ethanol free diet at 10:30h. Then liquid diet with 2.4% ethanol was administered for 3 days. The ethanol concentration was increased to 4.8% for the following 4 days and finally to 7.2% for 23 days. Liquid diet was freshly prepared daily and presented at the same time of the day (10:30 h). The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as g per kg per day. Ethanol-dependent rats were then assigned into four groups (Group 2, 3, 4, 5) randomly (n = 10 for each group). Group 2_Quetiapine (10 mg/kg), Group 4_olanzapine (5 mg/kg), and Group 5_saline %0.9 were injected into the rats 30 min before ethanol withdrawal evaluation. Group 1_Control rats (n=10) receiving liquid diet without ethanol were also evaluated for ethanol withdrawal signs parallel to ethanol-dependent groups. Group 3_Alcoholic rats (n=10) receiving liquid diet with ethanol were also evaluated for ethanol withdrawal.

Drugs used in the study

Quetiapine (seroquel) and olanzapine (zyprexa), the drugs were dissolved in saline. Quetiapine (10 mg/kg), olanzapine (5 mg/kg) and saline were injected to the rats intraperitoneally at a volume of 1 ml/200 g body weight. Drug solutions were prepared freshly in the morning of each part of the experiment.

Evaluation of ethanol withdrawal syndrome

At the 2nd, 4th and 6th hours of ethanol withdrawal, rats were observed for 5 min, and withdrawal signs including locomotor hyperactivity, stereotyped behaviors, abnormal posture and gait, tail stiffness and agitation were recorded or rated as previously described (7,19,20). Locomotor activities of the rats were recorded using an open-field locomotor activity test apparatus (Opto Varimex Minor, Columbus, OH, USA) as a total of horizontal, vertical and ambulatory activities and expressed as mean±S.E.M. Grooming, sniffing, head weaving, gnawing and chewing were observed as major stereotyped behaviors during the ethanol withdrawal in the study. Each group received a second injection of its original drug 30 min before the 6th hour of observation. After 6 h of withdrawal testing, rats were exposed to an audiogenic stimulus (100 dB) for 60 s in a separate and soundproof place in the laboratory. The incidence and latency of the audiogenic seizures were recorded. Control rats receiving liquid diet without ethanol were also evaluated for ethanol withdrawal signs parallel to ethanol-dependent groups. All experiments were carried out during the light period. A naive observer who was blind to the treatments that the rats received scored all ratings.

Statistics

Changes in locomotor activities and body weights of ethanol-dependent rats as compared with ethanol

***Tablo 3**. Locomotor activity: Vertical*

	n	Mean	Std. D	Std. E	%95 Confidence Interval		Min	Max
					Lower	Upper		
1	10	246.3900	177.56869	56.15215	119.3650	373.4150	28.90	560.80
2	9	96.8778	81.30153	27.10051	34.3839	159.3717	10.40	238.90
3	10	166.8900	102.62293	32.45222	93.4780	240.3020	52.00	345.00
4	10	175.8000	62.20316	19.67037	131.3025	220.2975	82.10	238.20
5	10	264.0500	124.80730	39.46753	174.7682	353.3318	139.00	531.00

Tablo 4. *Locomotor activity: Total*

	n	Mean	Std. D	Std. E	%95 Confidence Interval		Min	Max
					Lower	Upper		
1	10	1954.8300	767.96125	242.85067	1405.4636	2504.1964	782.20	3065.40
2	9	1325.7556	639.25458	213.08486	834.3810	1817.1301	502.50	2172.00
3	10	2040.8900	893.44095	282.53083	1401.7608	2680.0192	1142.10	3563.80
4	10	2434.0700	570.57651	180.43214	2025.9042	2842.2358	1467.40	3499.10
5	10	2374.3600	759.64459	240.22071	1830.9430	2917.7770	1335.60	4209.70

non-dependent control rats were analyzed by unpaired (between groups) Students' t-test. Analysis of variance (one-way ANOVA) followed by duncan test was used in evaluation of the e□ects of quetiapine and olanzapine on the locomotor activities. All analyses were made using the SPSS statistical software package. The level of significance was set at $p < 0.05$ levels.

RESULTS

Daily ethanol consumption of the rats in control, quetiapine, and olanzapine treated groups during the exposure to ethanol (7.2%) is no significant di□erence between the groups was observed. Body weights of the rats increased progressively during the study.

Horizontal locomotor activity is shown in Table 1, ambulatuar locomotor activity is shown in Table 2, vertical locomotor activity in Table 3 and total activity in locomotor system is shown in Table 4. There was increased vertical and horizontal locomotor activity in ethanol withdrawal group to control ($p<0.001$). Post-hoc analysis of data indicated that reduced vertical and horizontal locomotor activity in quetiapine injected rats ($p<0.002$). In Olanzapine injected rats were seen no reduced locomotor activity ($p>0.05$).

DISCUSSION

Alcoholism is one the most important major worldwide public health problem. Disulfiram (an aldehyde dehydrogenase blocker), naltrexone (an opioid antagonist) and acomprosate (a functional glutamate antagonist) were approved for the treatment of alcohol dependence, but these medications are effective on ethanol craving and drinking rather than treatment of withdrawal syndrome. Although attenuating the severity of ethanol withdrawal symptoms is also important, treatment choices are very limited except benzodiazepines. New approaches and new drug choices are necessary for treatment of ethanol withdrawal syndrome (21). Dopaminergic and serotonergic systems play a crucial role in the development of ethanol dependence (22,23). There may be potential beneficial effects of new atypical antipsychotic drugs in the treatment of the signs of ethanol withdrawal as well as blocking the craving effects of ethanol on human (24) and experimental studies (25-29).

In a previous study, it is observed some significant reductions in striatal 5-HT levels in rats during early ethanol withdrawal (30) and chronic ethanol consumption (31). Depending on this data, it was speculated some beneficial effects of 5-HT enhancers such as fluoxetine (32) and venlafaxine (33) ethanol withdrawal syndrome agents that can increase central serotonergic neurotransmission might be useful for treatment of ethanol abuse and/or dependence and also clinical studies have indicated that fluoxetine at antidepressant doses; it was able to prevent relapses in alcoholics (34). Schizophrenia is four times more frequent among alcoholic than non-alcoholic subjects. Thus, co-morbid substance use in schizophrenic patients is common, and an important factor affects the outcome of disease (35). It is reported that olanzapine did not produce any significant change on locomotor hyperactivity at 4th and 6th h of ethanol withdrawal and did not cause any significant change on locomotor activity of the naive (no ethanol dependent) rats (27). In that study (27), they used 0.5, 1 and 2 mg/kg for olanzapine treatment. In our study, we couldn't find any change on locomotor activity although we used higher dose (5 mg/kg) for treatment.

Marked inhibitory effect of quetiapine on locomotor hyperactivity may be explained by their dopamine D2 receptor antagonistic activity. (29) Antagonism of 5-HT2 receptors, especially the 5-HT2C subtype, reduced anxiogenic behaviors during ethanol withdrawal (36-38). Quetiapine has antagonistic activity at the 5-HT2A and 5-HT2C receptors. It was shown that Quetiapine signifi-

cantly affected Ethanol Withdrawal Syndrome-induced locomotor hyperactivity (8 mg/kg and 16 mg/kg) (29). In our study we have given 10 mg/kg to see the effect on locomotor hyperactivity and our study supported to those findings.

CONCLUSION

Quetiapine produced significant inhibitory effects on the signs of ethanol withdrawal but not olanzapine. Our results suggest that acute quetiapine treatment has some beneficial e□ects on ethanol withdrawal in rats. Thus, this drug may be useful for treatment of ethanol withdrawal syndrome.

Acknowledgements

This study was supported by Gaziantep University Research Foundation (GÜAF_Proje No: TF.03.14). The authors would like to thank Prof. Ecir Ali Çakmak PhD, Hakan Kayır MD, and Ayşe Karaduman for his technical assistance during the study.

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