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An increase in GABA content in Hippocampus of Albino rats exposed to chronic restraint model and treated by Quetiapine for 3 weeks

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Abstract

Quetiapine is a novel antipsychotic drug. However, there is limited clinical evidence regarding prescribing patterns for quetiapine when used as maintenance treatment for bipolar disorder.

Thirty-six albino rats were divided into 3 equal groups: control normal group ^[1] without exposure to chronic restraint for 6 hours daily/21 days, group ^[2] received DMSO 5% (v:v), as a solvent of quetiapine, with exposure to chronic restraint for 6 hours daily/21 days and group ^[3] received quetiapine 10 mg/kg/day ip for 3 weeks during exposure to chronic restraint for 6 hours daily/21 days.

Intraperitoneal (ip) administration of quetiapine at a dose of 10 mg/kg/day for 3 weeks significantly ($p < 0.05$) reduces the duration of immobility recorded by the forced swimming test (FST) and significantly ($p < 0.05$) increases the contents of GABA neurotransmitter in hippocampus homogenates.

The present study adds a positive implication of quetiapine, as an antipsychotic drug, on both the immobility and the reduction of GABA content in hippocampus of albino rats exposed restraint model for 21 days.

Keywords: Restraint model - hippocampus – quetiapine - forced swimming test – GABA- albino rats.

1. Introduction

Quetiapine (QUE) is a novel atypical antipsychotic drug that is widely used to treat schizophrenia and other psychotic disorders e.g. post-traumatic stress disorder ^[1, 7]. It possesses neuroprotective effects; it seems to have a protecting action against cognitive impairments ^[8].

It was found that atypical antipsychotics are capable to increase the action of the extracellular signal-regulated protein kinase (ERK), a member of the mitogen-activated protein kinase family ^[3]. The inhibition of this kinase enzyme, in prefrontal cortex and hippocampus, increases the liability to stressful stimuli and leads to cognitive and depressive disorders ^[9]. Thus, ERK may be involved in the pathogenesis of stress and anxiety as well as the therapeutic effects of these atypical antipsychotic drugs e.g. quetiapine ^[12].

GABA plays an important role in relieving depressed mood. As it is a major inhibitory neurotransmitter in the brain, it has an important role in diminishing the activity of its target neurons e.g. neurons of limbic system that is involved in affective disorders. It interacts with the activity of several neurotransmitters including dopamine, serotonin, and norepinephrine with a regulating effect on mood status ^[11].

Research and development of new antipsychotic and antipsychotic drugs is not only dependent on the monoamine hypothesis of depression but also on hypothesis of neuroplasticity that focuses on neurons that carry the functions of injured or damaged neurons in affective disorders and in psychosis. Actually, there is a problem in explanation of the pathogenesis and hence in strategic treatment plan of a large number of patients with affective disorders who fail to reach complete cure of depressed mood or any other affective disorder. So, there is a developing need to ameliorate plans of drug therapy of cases suffering from treatment-resistant depression by more development of new drugs with novel mechanisms of action such as antipsychotic drugs acting on GABA transmission and its related pathways, depending on powerful understanding of pathogenesis of different forms of affective disorders ^[12].

This study is addressed to investigate whether quetiapine modulates both the duration of immobility and GABA content in hippocampus in an albino rat model of restraint for 21 days.

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2. Drug & Chemicals

Quetiapine powder (AstraZeneca Pharmaceuticals, Macclesfield, UK) was dissolved in 5% DMSO (v:v). The selected dose of quetiapine (10 mg/kg/day ip) [He *et al.*, 2006]^[8]. Quetiapine (C₂₁H₂₅N₃O₂S) is a white crystalline powder whose molecular weight: 383.5099.

Gamma aminobutyric acid (GABA), norvaline standards, phenylisothiocyanate [PITC] and glacial acetic acid (Sigma chemicals Co), ethanol, triethylamine (TEA), [HPLC grade, MERCK] hydrochloric acid (32%, MERCK), acetonitrile [MERCK], and sodium acetate anhydrous [MERCK].

3. Ethics

All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act (www.nih.gov).

4. Methods

Chronic restraint stress procedure

Each albino rat of the respective group was placed in a wire mesh restrainer 6 hours daily for 21 days. At the end of the restraint period rats were moved to their cages.

Groups of rats:

After exposure for 3 weeks in a wire mesh restrainer, rats were divided into 2 groups (each group=12 rats) with daily administration of 5% DMSO (v:v) without quetiapine therapy and a 3rd group treated with 10 mg/kg/day for 3 weeks as follows:

Group 1: Control: neither exposed to chronic restraint for 6 hours daily/21 days nor to drug administration.

Group 2: exposed to chronic restraint for 6 hours daily/21 days while not treated with quetiapine but only + ip injection of an 0.5 mL of 5% DMSO (v:v), as a solvent of quetiapine, during the therapeutic period of treated group (3).

Group 3: Rats exposed to chronic restraint for 6 hours daily/21 days and treated with quetiapine 10 mg/kg/day ip dissolved in 0.5 mL of 5% DMSO (v:v).

N.B.: In pilot study, there was no difference in all measured parameters between control normal group injected with 0.5 mL of 5% DMSO (v:v) and control normal group without exposure to chronic restraint for 6 hours daily/21 days or drug administration.

5. Measurement of immobility in rats by the forced swimming test (FST)

At the end of the study, the FST used here was essentially the same as described in detail elsewhere^[4]. Swimming sessions were conducted by placing rats into individual glass cylinders (46 cm height, 20 cm diameter) containing 23-25 °C water 30 cm deep, so that rats could not support themselves by touching the bottom with their paws.

Two training swimming sessions were conducted: an initial 15-min pretest followed 24 h later by a 5-min test. Following each swimming session, the rats were removed from the cylinders, dried with paper towels and returned to their home cages. A single observer, who was blind to the treatment conditions, did all the behavioral scoring.

The immobility is defined as floating in water without struggling, and doing only those necessary movements to keep the head above water; for each rat, the immobility time is calculated in sec. over a period of 5 minutes.

Determination of GABA content in hippocampus homogenates of tested albino rats:

The GABA content in tissue homogenates of the hippocampus was determined according to the method of^[6].

The hippocampus from each rat was isolated & homogenized and samples were centrifuged in a cooling (4°C) centrifuge at 15,000 rpm for 10 minutes. The supernatant was aspirated and transferred to an Eppendorf tube. The pellet was kept at -70°C until assayed for total protein content.

High performance liquid chromatography (HPLC) with pre-column phenyl-iso-thio-cyanate (PITC) derivatization was used for determination of GABA level in homogenates of the hippocampus of the brains of rats from different groups. Data are presented as nmol/mg of tissue protein.

Each sample was derivatized by drying 100 µl of the aspirated supernatant in a centrifuge under vacuum. The residue was dissolved in 20 µl of ethanol-water-triethylamine (2:2:1) and evaporated to dryness under vacuum. Thirty microliters of ethanol-water-triethylamine-phenylisothiocyanate [PITC] (7:1:1:1) was added to the residue and allowed to react for 20 min. at room temperature to form the PITC derivatives of the amino acids. Excess reagent was then evaporated under vacuum. The mobile phase of HPLC consisted of solvents A & B [solvent A: 0.1 M sodium acetate buffer (pH= 5.8); solvent B: acetonitrile: water (60:40, v:v)]. A mixture of 80% solvent A and 20% solvent B was adjusted for the "isocratic" HPLC separations. Flow rate was set at 0.6 ml/min. The injected sample was 20 µl. The peaks were detected at a 254 nm wavelength. Standard curves for GABA and norvaline were plotted using norvaline 2 nmol/20 µl as an internal standard. The ratio of the peak area of each concentration of each standard to the peak area of the internal standard was determined and entered against the concentration of the standard in a simple regression procedure.

6. Quantification of total tissue protein

Total protein was measured according to the method of [Bradford, 1976]. The aim of this procedure was to correlate glutamate and GABA concentrations to the total tissue protein amount.

Analysis of the data

The data obtained are presented as mean \pm SD [Standard deviation] and evaluated using one-way ANOVA, followed by Tukey's *post hoc* determination, using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, U.S.A.). Differences at $p < 0.05$ were considered significant.

7. Results

I. Effect of ip administration of quetiapine (10 mg/kg/day) for 21 days on serum corticosterone concentration in restraint model exposed albino rats.

Table (1) chronic restraint stress for 21 days produced significant ($p < 0.05$) elevation of serum corticosterone level from the mean value (X) \pm SD of 6.8 ± 1.27 ng/ml in the control group to 14.7 ± 1.88 ng/ml with the mean % increase of 116.18. Administration of ip quetiapine 10 mg/kg/day for 21 days with exposure to restraint model for 21 days produced a significant ($p < 0.05$) reduction of serum corticosterone level from the mean value (X) \pm SD of 1.8 ± 1.27 ng/ml with the Mean % decrease of -87.76% in corticosterone level in quetiapine-treated group compared to stressed non-treated group.

Parameter	Control non-stressed Group (1)	Control restraint non-treated (Group 2)	restraint model+quetiapine (Group 3)
Mean ± SD	6.8 ± 1.27	14.7 ± 1.88*	1.8 ± 1.27**
Mean % change in serum corticosterone level between restraint group (2) & restraint +quetiapine (3)			-87.76 %

Table (1): Influence of exposure to restraint model on serum corticosterone level [as a marker of stress] in albino rats of the different groups; control, stressed -with and without quetiapine treatment. Data are means± SD from 12 rats per group.

* $p < 0.05$ = significant decrease vs control group 1.
 ** $p < 0.05$ = significant increase versus restrained group 2 for 21 days.

II. Table (2): Shows measurements of the immobility time (seconds) in all groups as elicited by the forced swimming test (FST):

A decrease in immobility time (in the FST) was recorded after treatment of albino rats exposed to restraint model with quetiapine (group 3) compared to restraint model-exposed albino rats without treatment (group 2).

Parameter	Control non-stressed Group (1)	Control stressed non-treated (Group 2)	restraint model+quetiapine (Group 3)
Duration of immobility (sec.)	69.15±2.25	167.5±2.5*	44.35±2.0**
% mean change from control		+ 142.23%	- 35.86%
% mean change from restraint model			- 73.52%

Table (2): shows changes in immobility time after 3 weeks of single daily ip administration of quetiapine for 3 weeks of exposure of rats to restraint model.

- * $p < 0.05$ = significant increase in immobility time (sec.) in group (2) compared to the control non-stressed albino rats group (1)
- ** $p < 0.05$ = significant reduction in immobility time (sec.) in quetiapine-treated group (3) compared to the restraint model –non treated albino rats group (2)

III. Effect of 3-weeks administration of quetiapine upon the GABA levels in the homogenates of hippocampus [N.Ac.] of restraint model)- exposed albino rats.

Figure (1) represents the changes in GABA levels in the homogenates of hippocampus of the control, restraint model, restraint model+ quetiapine- treated albino rats. Restraint model significantly ($p < 0.05$) decreased the GABA levels in the homogenates. GABA levels of restraint model-exposed albino rats were significantly ($p < 0.05$) increased by administration of quetiapine.

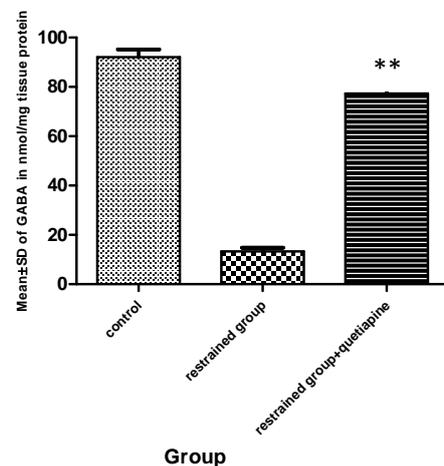


Fig 1: Influence of restraint model with and without administration of quetiapine on GABA levels in homogenates of hippocampus of albino rats of the different groups; control stressed and non-stressed as well as restraint model-treated albino rats

. Data are expressed as the mean ± SD from 12 animals per group.
 * $P < 0.05$ significant reduction versus control-non-stressed group 1.
 ** $P < 0.05$ significant increase versus control stressed non-treated group 2.

8. Discussion

The possible antidepressant action of quetiapine, one of the newer generation of antipsychotics, has been demonstrated in clinical and preclinical studies. Nevertheless, little information is known about its effectiveness in the treatment of stress-related disorder.

In the present study, 3-weeks single daily dose of quetiapine induced a statistically significant increase in serum corticosterone concentration, as a marker of stress, by albino rats exposed to 21 days of restraint model. It also significantly reduced the duration of immobility time in the FST as a behavioral despair screening test of drugs with ability to improve the depressed mood. Additionally, it significantly augmented GABA concentrations of the homogenates of hippocampus [N.Ac.] of the stressed albino rats. This is completely the reverse of what occurs in stressed non-treated group (2).

The hypothesis of GABA mood regulating effect can be explained by its modulating effect of both norepinephrine (NE) and dopamine (DA) systems to the degree that control mood disorders [5].

Nikiforuk, 2013 [10] found that quetiapine augments the action of selective serotonin reuptake inhibitors (SSRIs). This is proven to be beneficial in neuropsychiatric disorders, especially in cases of psychosis associated with depressed mood. An experimental study was conducted on chronically stressed rats to determine the impact of combined administration of quetiapine and escitalopram on their daily activities and their performance. These stressed rats suffered from impairment of extra-dimensional (ED) cognitive acquisition. However *po* administration of quetiapine at a dose of 2.5 mg/kg was given to these rats prior to the restraint sessions. The repetition of administration of quetiapine completely prevented this stress-induced reduction in rats' cognitive performance and ameliorated their physical activities.

Additionally, the previous study demonstrated that single small oral dose administration of quetiapine either 0.63 or 1.25 or 2.5 mg/kg, before exposure to any stressor, reversed any inactivity or reduction in cognitive functions occurring in stressed rats. As well as, co-administration of small doses of quetiapine (0.63 and 0.3 mg/kg in control and stressed rats, respectively) and escitalopram, as an SSRI, (0.3 mg/kg, ip) facilitated all activities and daily performance in either control or stressed rats. Quetiapine administration either prevented or reversed stress-induced impairment in physical and cognitive activities in stressed rats. In addition to that, a beneficial interaction between quetiapine and escitalopram represents by a progressive improvement in cognitive acquisition in stressed rats [10, 15].

Enhancement of GABAergic activity in mood disorders, in parts of limbic system of the brain of stressed albino rats, in addition to, monoaminergic and serotonergic theories, point to the importance of the balance between multiple neurotransmitter systems in strategic plan of mood disorders either alone or in cases of psychosis accompanied by depressed mood. This is evidenced by reduction of GABA levels in plasma and cerebrospinal fluid (CSF) in patients with major depression below normal values [13, 14].

In conclusion, from the changes of GABAergic and glutamate levels in comparison to stressed non-treated rats in addition to the shorter immobility period in the FST- induced by quetiapine, the present study pointed to a possible mood-regulating role of this antipsychotic drug via a reduction in the

duration of immobility in the forced swimming test as well as a significant increase in the level of GABA in the homogenates of hippocampus of albino rats exposed to stress model for 21 days.

9. Disclosure

The authors report no conflicts of interest in this work.

10. Acknowledgment

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