Antidepressant-Like Effects of Ceftriaxone in Chronic Unpredictable Mild Stress Model in Rats: The Importance of Reuptake Time of Glutamate

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ABSTRACT

Objective: We aimed to indicate the relationship between depression and glutamate, and to reveal the effect of escitalopram, an antidepressant, which is widely used in depression treatment and reuptake parameters of glutamate, and to treat depression with ceftriaxone, one of the beta lactam antibiotics which increased the number and activity of glutamate transporters.

Methods: In CUMS, rats subjected to series of different mild stressors in an unpredictable manner for 40 days. On the day 20 rats were divided in to groups such as CUMS, CUMS+Escitalopram and CUMS+Ceftriaxone. 4 weeks. Treatments were started at 2nd week of CUMS and continued for 21 days. Anhedonia and antidepressant effect were assessed by sucrose preference (SP), locomotor activity (LA), elevated plus maze (EPM) and forced swim test (FST) at the end of the experiment respectively. At the end of the experiment, behavioral tests were made, and glutamate reuptake time in CA3 (cornuammonis 3) brain region which are related with depression were measured by means of in vivo voltammetry technique.

Results: Ceftriaxone treatment had an antidepressant-like effect. Escitalopram and ceftriaxone increased SP and locomotor activity, reduced immobility FST, forced swim and time spent in closed arms in EPM compared to CUMS group. In this in-vivo voltametric study, it was also observed that there was a significant decrease in glutamate reuptake time in depression.

Conclusion: Escitalopram and ceftriaxone demonstrated antidepressant-like effects by reversing behavioral changes in CUMS model. Escitalopram treatment in CA3 region corrected the decrease in glutamate reuptake time which is consistent with the hypothesis that enhanced uptake of glutamate might have antidepressant-like effects.

Keywords: Beta lactam antibiotics, ceftriaxone, depression, voltammetry, glutamate

1. INTRODUCTION

Major depressive disorder (MDD) is a common and chronic, recurring mental illness(1). MDD is affecting nearly 340 million people worldwide and is considered to be an underlying cause in 35–40% of suicides (2). MDD is characterized by depressed mood; anhedonia, appetite changes leading to weight gain or loss, sleep dysregulation, psychomotor changes, loss of energy, feelings of worthlessness and excessive guilt, diminished concentration and recurrent thoughts of death (3).At present, the pathophysiology of many psychiatric disorders is still not fully understood. Current treatment approaches that have been used in psychiatric disorders for many years and usually have a single mechanism-based effect are inadequate to control disease symptoms; modulation of monoamines for depression. Patients who do not respond to the current treatment options in the majority of depression which is affecting 10% of the population and resistance cases constitute about 1/3 of them (4).

The mechanisms driving pathophysiology are complex and remain largely unknown, advances in the understanding of neurotransmitter activities are providing significant clues to their etiology.Moreover, a considerable interest in non-monoaminergic approaches to the treatment of depression and glutamate modifying therapies have become emerging targets which could be more effective and speed up the recovery of patients through fast acting compounds either as monotherapy or add-on treatment.Over the past decade, accumulating evidence has reported a positive relationship between depression and glutamate, which is the most abundant excitatory neurotransmitter in the mammalian central nervous system. Recent studies in rodent models have demonstrated that excitatory amino acid transporters (EAAT) plays a critical role in the regulation of glutamate release however, glutamate cycling rates.

Mental or physical disorders are also known to be triggered by brain anoxia or ischemia which might be due to robust release of glutamate causing the death of neurons. Although the mechanisms underlying this comorbidity are unclear, emerging evidence suggests that maladaptation of the glutamate transporter. Increased levels of glutamate are associated with a number of neurological disorders such as epilepsy, stroke, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and dysfunction of glutamate

transporters has been considered as the starting point of the cascade leading to several brain injuries (5, 6).

Chronic unpredictable mild stress (CUMS) is considered to be one of the most extensively validated animal model of depression that meets good face, construct and predictive validity criteria (7-9). As an environmental stress induced depression model, CUMS paradigm mimics many aspects of depression, especially it is well known for producing anhedonia-like behavior in rodents which can be easily assessed by sucrose preference or sucrose consumption tests (10). Furthermore, CUMS-induced depressive like behaviors only respond to chronic but not acute antidepressant treatments, which makes it one of the more realistic depression models (11-13). Besides, the involvement of neuroinflammation and immune mechanisms in depression has been well studied in CUMS model. In this respect, CUMS-induced inflammation and lately the inflammasome activation has been widely reported by numerous studies (14,15).

Recently limited number of studies indicated that ceftriaxone, a β-lactam antibiotic, treatment may enhance the expression of EAATs and prevent neurons in related pathologies. CEF has neuroprotective effects in both in vitro and in vivo models by inhibiting neuronal cell death (16).Based on the above mentioned hypothesis of depression, current study designed to investigate i) possible effect of ceftriaxone in a well-known validated animal model of depression and ii) possible role of ceftriaxone on glutamatergic transmission through EAATs in comparison with selective serotonine reuptake inhibitor escitalopram.

2. MATERIAL AND METHODS

2.1. Animals and drugs

Male adult Sprague-Dawley rats (290-320 g) were used in the present study which were supplied from Ataturk University Medical Application and Research Center (Erzurum, Turkey). Rats were housed in groups (n=4-5 per each cage) under standard laboratory conditions (12 h light-12 h dark cycle; room temperature 21±2 °C). Food and water were provided ad libitum except the duration of CUMS. The study was approved by the Animal Ethics and Care Committee of Ataturk University (AUDHADYEK 42190979-01-02/2236). Rats were divided into 3 experimental groups: Control (non-stressed, saline-treated, 0.1 ml/100 g/rat for 3 weeks), CUMS (saline-treated, 0.1 ml/100 g/rat for 3 weeks), CUMS+Escitalopram (Citoles 20 mg tablet, Abdi Ibrahim Pharmaceuticals, Inc) (10 mg/kg/day for 3 weeks) and CUMS+Ceftriaxone (Iesef 1g, IM vial, Ibrahim EthemUlagayPharmaceuticals, Inc) (200 mg/kg/day for 3 weeks) (n=10/group). Bychronic unpredicted stress model, by applying various stressors to experimental animals for 40 days, depression was formed. On the 20th day of stressor application, the treatment of escitalopram and ceftriaxone were applied for two groups of experimental animals divided as randomized control, depression, escitalopram and ceftriaxone for 20 days.

2.2. Behavioral Experiments

2.2.1. Chronic Unpredictable Mild Stress (CUMS) Model

The CUMS procedure was developed by Paul Willner in the late 1980s to model MDD more accurately, with regard to the human MDD pathogenesis (17). By combining genetic features and socio-environmental chronic stressful events, the unpredictable, chronic mild stress model can be used to study the etiological and developmental components of major depression(18). Human MDD's can only be effectively treated by chronic, but not acute, antidepressant administration (17). Furthermore, CUMS is a model to produce a depressive-like state in rodents, induced by the exposure to some natural and mild environmental stressors over a long period (4–9 weeks) that leads to the development of chronic psychological changes. Psychological changes, evoked by CUMS, are symptoms like decreased reply on rewarding stimuli including e.g. anhedonia and decreased locomotor activity (19). Anhedonia is defined as a decreased ability (the animal is not unable to experience it, but the ability is strongly reduced) to experience pleasure and is the key symptom of depression and depressive disorders. It can be assessed by measuring sucrose preference and coat state (20) of rodents.

2.2.2. Sucrose preference test(SPT)

Anhedonia as a core symptom of depression was evaluated by the SPT.For the adaptation period, rats were first introduced two bottles each filled with 200 ml 1% sucrose solution (w/v) for 24 hours. Then, one of the bottles was taken out and replaced with an identical bottle filled with 200 ml tap water while the other bottle was fulfilled 200 ml of 1% sucrose solution (w/v) and kept still for a second 24 h period. This adaptation period was followed by 24 h period of food and water restriction. Following the last 24 h period, subjects' baseline sucrose consumptions were measured before starting CUMS procedure. This time again there were two identical bottles one of them was filled with 200 ml of tap water and the other one was with 200 ml 1% (w/v) sucrose solution. Rats were allowed to have access to both bottles for 24 h. At the end of 24 h, baseline sucrose preference was calculated according to the following formula:

Sucrose preference: (consumption of sucrose solution/consumption of total liquid) x 100

Sucrose preference test was repeatedly applied 14., 21. and 41. day (time schedule) throughout the CUMS procedure in order to evaluate anhedonia-like behavior of subjects.

2.2.3. Locomotor activity test

Locomotor activity was measured using an activity recording system (May Act 508 animal locomotor activity meter) consisting of a rectangular plexiglass activity cage (41 x 44 x 32 (h)) attached with 4 blocks on the side walls and an electronic unit. The blocks on the side walls (1 pair of opposing blocks on the opposing side walls for vertical and

1 pair for horizontal activity) contained infra-red radiant light beams and photocells. Therefore, interruptions occurring from either vertical or horizontal movements was detected through the photocells and counted by the counter. On 40th day (after treatment), locomotor activity was measured for 30 min and the surface was cleaned with 70% ethanol and left to dry before testing of each subject.

2.2.4. Forced swim test (FST)

The FST was used as an acute despair model to assess anti-depressant like activity(16). For the habituation, rats were individually placed inside a plexiglass cylinder (46-50 cm height, 20 cm diameter) filled with water (23 – 25°C) to a depth of 30-31 cm and allowed to swim for 15 min. On the testing day (40th day, after treatment), 24 hours following after habituation, rats were again subjected to the same conditions except that for 5 min this time and each swimming sessions was video recorded. The time of immobility, the absence of any movement except the minor movements in order to keep the head above the water, was video scored by an experienced observer. The subjects were immediately removed from the water at the end of 5 min and gently dried by using towel in a warm atmosphere. The water was always refreshed and the temperature was checked before each subject to be tested.

2.2.5. Elevated plus maze (EPM)

Anxiety-like behavior of rats was evaluated by using the EPM test. The maze was a plus shaped platform located 80 cm above the floor consisting of 4 identical arms (50 cm length x 14 cm width); 2 opposing open and 2 opposing closed arms connected with a central open area (dimensions). The wall height of the closed arms was 50 cm each subject was gently placed on the central area facing one of the open arms and video recorded for a 5 min of duration. On 40th day (after treatment), time latency for first entering a closed arm, time spent on open and closed arms and the number of open and closed arm entries was scored by an experienced observer. Subjects were removed from the platform at the end of 5 min and transferred to their home cage in the holding room. The apparatus was cleaned with 70% ethanol and allowed to dry before placing the next subject.

2.2.6. In vivo voltammetry

Platinum microelectrode arrays microelectrodes the real-time monitoring of rapid changes in extracellular levels of glutamate and other neuro-active molecules in the central nervous system were provided by fast analytical sensing technology (FAST). We also used S2 type (for rats), glutamate oxidase and nafioncoated multisite ceramic microelectrodes in this study. FAST and microelectrodes were obtained commercially from Pronexus Analytical AB (Stockholm, Sweden). The microelectrodes have platinum (Pt) recording sites with Pt connecting lines.

Calibration tests were performed with FAST-16 Data Acquisition Unit (Pronexus Analytical AB, Stockholm, Sweden). We used constant amperometric 0.7 voltage for in vivo voltammetry. The ceramic microelectrode amplifies head stage by being attached to a FAST 16 system. An Ag/AgCl commercial electrode also attaches to the head stage and functions as the reference electrode (21).An electrode manipulator that attached to electrode on a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA) was positioned above the rat brain. Our microelectrode had nafion and glutamate oxidase on the platinum surface. Glutamate oxidase converts glutamate to peroxide. On the other hand, the nafion blocks interferents. The peroxide can pass through the nafion barrier and can be detected by the platinum side of microelectrode in voltammetry system. The peroxide level is correlated with the glutamate level. The coated tip of the Ag/AgCl reference electrode is placed into the same rat brain (22).Voltammetric analyses were after all behavioral tests have ended (40th day). CA3 region of hippocampus in the brain was entered rat brain atlas according to coordinates (+3.2 on the x axis, − 3.6 on the y axis, 3.5 on the z axis) stereotaxicallyand voltammetric recordings were taken.

2.2.7. Statistical analysis

Statistical analysis was performed by using GraphPad Prism® 5 program (GraphPad Software Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) followed by post-hoc Tukey's HSD test was used for gene expression analysis of statistics. Two-way ANOVA followed by Bonferroni test was used for analysis of SPT. Data were presented as the means±SD. $p < 0.05$ was regarded to be statistically significant.

3. RESULTS

3.1. The effect of CUMS and ceftriaxone treatment in locomotor activity.

As shown in Table 1 rats exposed to 6-week CUMS procedure developedmarked decreases in activity compared to non-stressed control group (p<0,01) in locomotor activity test. Both escitalopram and ceftriaxone treatment increased locomotor activity back to control levels compared to CUMS group significantly (p<0,05) (Table 1).
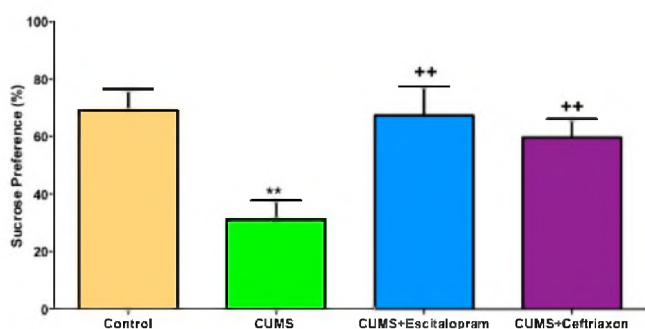
Table 1. The effect of CUMS and ceftriaxone treatment in locomotor activity.

Study groups	n	Mean±SD
Control	10	1710±188
CUMS	10	1156±312**
CUMS+Escitalopram	10	1598±231+
CUMS+Ceftriaxone	10	1515±268+

All data are expressed as distance traveled (cm) mean ± SD (n=10/group).

3.2. The effect of CUMS and ceftriaxone *treatments on sucrose preference in the SPT*

As shown in Figure 1, rats exposed to 6-week CUMS procedure developed anhedonia-like behavior with marked decreases in sucrose preference compared to non-stressed control group (p<0,01) in SPT. Chronic administration of ceftriaxoneand escitalopram during the last 3 weeks of CUMS procedure significantly increased sucrose preference compared to CUMS group (p<0,01). Our results showed that ceftriaxone treatment reversed CUMS-induced anhedonia-like behaviors in rats (Figure 1).

Figure 1. *The effect of CUMS and ceftriaxone treatments on % sucrose preference. Data are expressed as mean ± S.D and **: p<0.01 compared to control and ++: p<0.01compared to CUMS group.*

3.3. The effect of CUMS and ceftriaxone *treatments on immobility time in the FST*

In the FST, the time of immobility was significantly elevated in CUMS group compared to control group (p<0,01). However, when CUMS-exposed rats chronically treated with ceftriaxone(p<0,01) and escitalopram(p<0,01), had significantly shorter duration of immobility showing that higher dose of ceftriaxone was effective in improving CUMS-induced despair-like behaviors of rats (Figure 2).
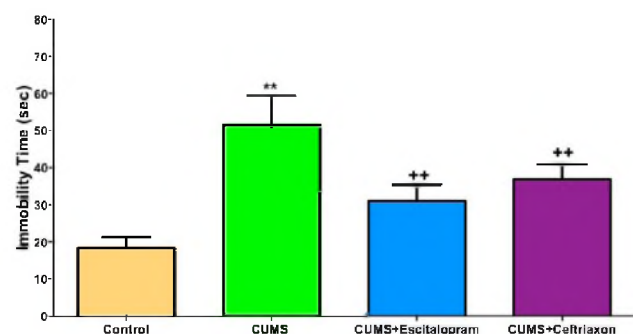
Figure 2. *The effect of CUMS and ceftriaxone treatments on immobility time in the FST Data are expressed as mean ± SD and **: p<0.01 compared to control and ++: p<0.01compared to CUMS group*

3.4. The effect of CUMS and ceftriaxone *treatments the number of open arm entries in EPM test*

Number of open arms entries for 5 minutes is summarized in the table 2. The number of open-arm entries of the depression group reduced significantly when compared to the control group (p<0.05). Escitalopram and ceftriaxone treatment increased significantly according to depression group p<0.05).

Table 2. *The effect of CUMS and ceftriaxone treatment in Evalated Plus Maze Test.*

Study groups	n	Mean±SD
Control	10	1,25±0,4629
CUMS	10	0,333±0,5164[#]
CUMS+Escitalopram	10	1,429±0,7868*
CUMS+Ceftriaxone	10	1,167±0,4083*

All data are expressed as the number of open arm entries mean ± SD (n=10/group).

3.5. In vivo voltammetric test

80% of reuptaketime after peak point (t_{80})in the CA3 region is summarized in the table 3. Glutamate reuptake duration of the depression group significantly extended according to the control group. Compared to the depression group, escitalopram therapy markedly reduced the duration of glutamate re-uptake. But, ceftriaxone treatment did not affect the glutamate reuptake time in this region.

Table 3. *Re-uptake time of glutamate on CA3 region of hippocampus.*

Study groups	n	Mean±SD
Control	10	3±0*
CUMS	10	4,8±0,45
CUMS+Escitalopram	10	3,2±0,44*
CUMS+Ceftriaxone	10	5,17±1,94

80 % of reuptake time after peak point (t_{80}) in the CA3 region are summarized (sn) mean ± SD (n=10/group).

4. DISCUSSION

Scientific evidences that glutamate has an effect on depression is rapidly increasing. In 2006 Mitani et al showed a positive correlation between plasma levels of glutamate and the severity of depression (23). Changes in glutamate reuptake time has been shown to play an important role in the pathogenesis of some neurological diseases. Chronic pain and ALS (amyotrophic lateral sclerosis) are just two of them. Glutamate reuptake is associated with exactly glutamate transporter activity. Drugs providing to increase the activity of glutamate transporter have been found useful in these diseases (24, 25). Until now functional magnetic resonance imaging, volume studies, histopathological studies and microdialysis studies were performed to reveal the relationship between depression and level of glutamate changes. But none of the mentioned studies did not show the action of glutamate in synaptic gap

second by second.Microdialysis, which is a very important method for the measurement of neurotransmitters, and if allowed to picomolar levels of substances in a sample to be analyzed in 20 minutes is a significant disadvantage (26). Because neurotransmitters are released and taken back within seconds. In vivo voltammetry technique can detect neurotransmitter movements within one second, which can give exact information about the basal levels and the removal of glutamate parameters. We tested the levels of glutamate change by in vivo voltammetry technique in specific regions of the brain associated with depression. The monoamine hypothesis of depression can be proven to be true only by in vivo voltammetry method. Synaptic glutamate removal range shows the activity of glutamate transporters. Our results suggest that the function of glutamate transporters and glutamate movement can be determined in a selective and sensitive manner with a platinum electrode.

In our study, voltammetric analyzes were performed in the hippocampus region. The hippocampus is the mainly affected area in depression disease. The cornuammonis, one of the basic region of hippocampus, is more sensitive to glutamate toxicity. EAAT2 is one of the major glutamate transporters expressed generally in astroglial cells and is responsible for 90% of total glutamate uptake. Astrocytes compose the greater part of glial cells within the brain and may account for up to 50% of the brain's volume (27-29). In the CA3 region, ceftriaxone wasn't increased the glutamate transporter activity but escitalopram was accelerated carrier activities in this region.

We showed for the first time by this study that escitalopram accelerating the glutamate removal by voltammetry. So we say that as an antidepressant escitalopram is also effective in accelerating the reuptake of glutamate.The hippocampus is the mainly affected area in depression disease. The cornuammonis, one of the basic region of hippocampus, is more sensitive to glutamate toxicity.The CA3 region is associated exactly with depression and only escitalopram had turned to normal prolongation of reuptake period.Therefore, ceftriaxone could not accelerate the reuptake of glutamate in CA3 region (30-32).

In our study depression rat model is formed by chronic unpredicted mild stress application as described by Wilner et al. Then depression model was confirmed with behavioral tests such as locomotor activity test, elevated plus maze test and the forced swimming test (17). In the elevated plus maze test, which is a better indicator of clinical anxiety ceftriaxone treatment showed closer results to control the number of open arm entries but no closer to control of the inactivity time in the forced swimming test, which is more associated with depression. These results showed us that the ceftriaxone had more beneficial effect on anxiety rather than depression.

5. CONCLUSION

We showed that 80% of glutamate removal time (t80) was extended in hippocampus region in depression formed rats.

When investigating the cause of this situation, we found that the glutamate transporter activity-enhancing drugs such as ceftriaxone not show the expected improvement in the CA3 region of t80 period butescitalopram treatment turned back the expression levels in the same brain area. In this study it was showed that a portion of the clinical antidepressant effect of escitalopram was associated with changing the speed of glutamate reuptake and glutamate transporter expression levels.

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