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Ceftriaxone Decreases MPTP-induced Behavioral Disturbances in Animal Model of Parkinson's disease

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ABSTRACT

Background and purpose: Progressive degeneration of dopaminergic neurons in the midbrain is the main mechanism of Parkinson's disease (PD). Recent studies have shown ceftriaxone, a β-lactam antibiotic, to be a neuroprotective in various neurodegenerative disorders. Hence, the present study aimed to investigate the effect of ceftriaxone on behavioral disturbances of PD in an animal model.

Methods: Fifty-six healthy male Wistar rats were selected for this study. They were divided into seven groups according to receiving saline or ceftriaxone, receiving a low or high dose of ceftriaxone and receiving ceftriaxone for short or long periods. Apomorphine-induced rotational test, elevated body swing test and rotarod test were done to examine behavioral performances.

Results: Ceftriaxone can effectively diminish behavioral disturbances induced by MPTP in all behavioral tests. Long administration of ceftriaxone was more effective than short administration in lowering behavioral disturbances. High dose of ceftriaxone was more effective than low dose in initial trials of each behavioral test; however, no difference was observed between them in the last trial.

Conclusion: Results of the current study suggest that ceftriaxone have neuroprotective effects in PD. To obtain a sufficient neuroprotective effect for lowering behavioral disturbances of PD and also preventing side effects of ceftriaxone, long administration of low dose of ceftriaxone seems the best option.

Keywords: Parkinson's disease; Ceftriaxone; Behavioral tests

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INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, affecting seven million people globally. This disease is characterized by resting tremor, rigidity, bradykinesia, and postural instability [1]. The pathophysiology of this disorder is now extensively revealed. Progressive degeneration of dopaminergic neurons in midbrain is thought to be the underlying mechanism of this disorder. The degeneration may be the result of glutamate excitotoxicity and oxidative stress itself which are induced through the inhibition of complex I of the electron transport chain of the mitochondria of the dopaminergic neurons by MPTP [2-4]. However, there is less agreement on how to treat this disorder. Current treatments include L-DOPA, deep brain stimulation and surgical destruction of the globus pallidus. However, none of them could be considered as a satisfactory treatment option for PD [5,6]. The antiparkinson medications, L-DOPA and dopamine agonists, improve the early symptoms of PD; however, they eventually become ineffective and

also produce complications such as involuntary writing movements [7]. Hence, investigating new treatments for PD seems crucial.

Ceftriaxone is a common antibacterial β-lactam which is used in different infections. Previous researches have reported ceftriaxone to have neuroprotective effect in different neurodegenerative disorders [8]. Ceftriaxone can pass blood-brain barrier, thus enters the central nervous system and upregulates expression of glutamate transporter especially glutamate transporter subtype 1 (GLT1) which removes glutamate from extracellular space in the brain [9]. Downregulation of GLT1 protein expression in striatum of mouse models of PD is reported in previous studies [9]. Accordingly, upregulation of GLT1 protein expression may be an effective strategy in treating or preventing PD. Besides the effect of ceftriaxone on upregulation of GLT1 protein expression, we need to assess the effect of ceftriaxone on behavioral disturbances which is made by PD. Therefor the present study aimed to investigate the modulatory effect of ceftriaxone against MPTP-induced behavioral disturbances in mouse model of PD.

MATERIALS AND METHODS

Fifty-six healthy male wistar rats were selected. All these animals were adult and their weight were between 200-300 gr prior to the study. Animals were housed under conditions of constant temperature (23 ± 1 °C) and humidity (55 ± 5%) on a 12-h light–dark cycle. All rats were fed and given water ad libitum and were divided into seven groups as follows:

A- A short period saline group which included eight rats and received 150 ml/kg saline intraperitoneally 30 minutes before the first time of injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and during eight days after that, twice a day.

B- A short period ceftriaxone group which included eight rats and received 150 mg/kg ceftriaxone intraperitoneally 30 minutes before the first time of injection of MPTP and during eight days after that, twice a day, at the given dose.

C- A short period ceftriaxone group which included eight rats and received 200 mg/kg ceftriaxone intraperitoneally 30 minutes before the first time of injection of MPTP and during eight days after that, twice a day, at the given dose.

D- A long period saline group which included eight rats and received 150 ml/kg saline intraperitoneally 30 minutes before the first time of injection of MPTP and during 16 days after that, twice a day.

E- A long period ceftriaxone group which included eight rats and received 150 mg/kg ceftriaxone intraperitoneally 30 minutes before the first time of injection of MPTP and during 16 days after that, twice a day, at the given dose.

F- A long period ceftriaxone group which included eight rats and received 200 mg/kg ceftriaxone intraperitoneally 30 minutes before the first time of injection of MPTP and during 16 days after that, twice a day, at the given dose.

G- A healthy group which included eight rats not receiving MPTP or any other drug.

MPTP-treated model mice were prepared as described [10]. Rats were treated with MPTP (25 mg/kg) once a day for five consecutive days. Apomorphine-induced rotational test (AIRT) [11], elevated body swing test (EBST) [12] and rotarod performance test (RPT) [13] was done to evaluate the behavioral performance of rats. In the long period groups, AIRT and EBST was done three, five and eight weeks after the last administration of MPTP and RPT was done seven weeks after that. In the short period groups, AIRT and EBST was done four, six and eight weeks after the last administration of MPTP and RPT was done seven weeks after that.

Behavioral performance of rats was evaluated as follows:

1- To perform AIRT, animals received apomorphine hydrochloride (0.5 mg/kg, intraperitoneally). After the injection was done, number of rotations of rats in a cylindrical container was counted for 1 h at 10-min intervals. Rotations toward the lesioned side were considered as positive scores while rotations far away the lesioned side were considered as negative scores. Sum of negative and positive scores was considered as the net number of rotations.

2- To perform RPT, a rotarod apparatus with a 3-cm diameter rod set at a height of 63 cm was used. The apparatus was set at a rotation rate of 5 RPM initially which increased to 40 RPM during 180 sec. Then, the apparatus continued to rotate at 40 RPM for 60 sec. The latency of time to fall over this 4 min period was recorded. The test was conducted for three consecutive days, twice a day.

3- To perform EBST, the animal was placed in a cylindrical container and was allowed to habituate for 10 min. Then it was held approximately 2 cm from the base of its tail and elevated 2 cm vertically. During a period of 1 min, swing of animal's head out of the vertical axis to left or right was recorded. Biased swing behavior was calculated using following equations:

L/(L+R)% for left-biased swings and R/(L+R)% for right-biased swing. Between the mentioned swings, the greater number was considered as the net biased swing.

The differences between results of behavioral tests before and after the administration of MPTP was analyzed using the student's t-test. SPSS software ver.20 was used to perform statistical analyses and α=0.05 was considered as the level of significance.

RESULTS

All groups showed some degrees of rotations in AIRT. Hence, treatment with ceftriaxone could not completely block the neurodegeneration induced by MPTP. Treatment with ceftriaxone did not change number of rotations of first trial in short period group; however, when it was administrated in high dose in long period group, it decreased number of rotations compared to the control group significantly (Figure 1). In the second trial of AIRT, both low and high dose of ceftriaxone could decrease rotations when they were administrated in long periods and this reduction was significantly higher in high dose compared to low dose of ceftriaxone. Furthermore, only the high dose of ceftriaxone could reduce the net number of rotations when it was administrated in short period. In the third trial of AIRT, both high and low dose of ceftriaxone reduced number of rotations when they were administrated in a long period; however, when administrated shortly, only the high dose of ceftriaxone could reduce the rotations. In all three trials, long administration of ceftriaxone was more effective in reducing the number of rotations than short administration of it. Although in the second trial, high dose of ceftriaxone lowered number of rotations more than low dose of it both in short and long administrations, this difference did not remain in the third trial. Indeed, the effect of low and high dose of ceftriaxone was only different only when they were administrated for short periods.

The results of the EBST test were Similar to AIRT; however, some differences were revealed (Figure 2). In the first trial, both short and long period administration of high dose of ceftriaxone could reduce the biased swing compared to the control groups. A similar pattern was observed in the second trial; however, in contrast to the first trial, the effect of high dose of ceftriaxone on biased swing was significantly different from the effect of low dose ceftriaxone in both short and long administration. In the third trial, administration of ceftriaxone in short period could alter biased swing neither in low dose nor in high dose. In contrast, both low dose and high dose of ceftriaxone could reduce the biased swing when it was administrated in long period. Similar to the results observed in AIRT test, long period administration of

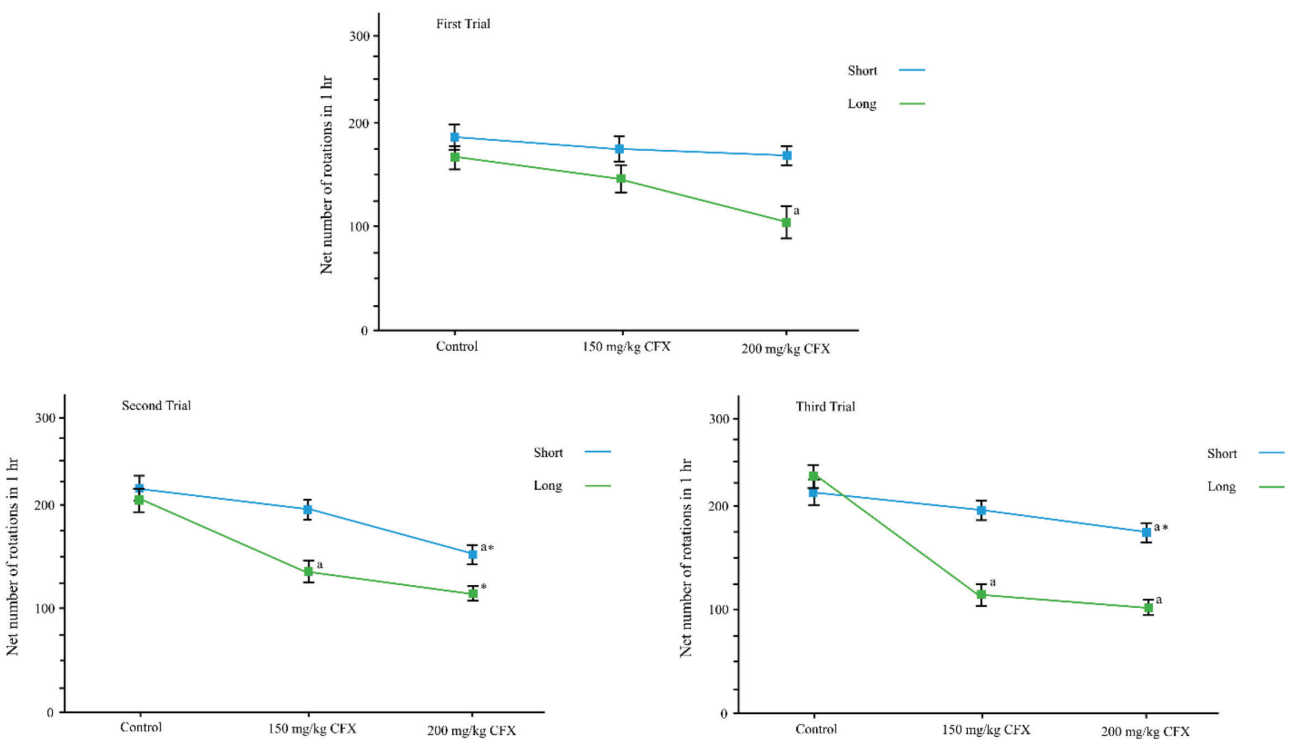

Figure 1. Results of the apomorphine-induced rotational tests. a: p<0.05 for difference between each dose of ceftriaxone and Control group, *: P<0.05 for difference between short and long period of administration of ceftriaxone in each dose. CFX: ceftriaxone

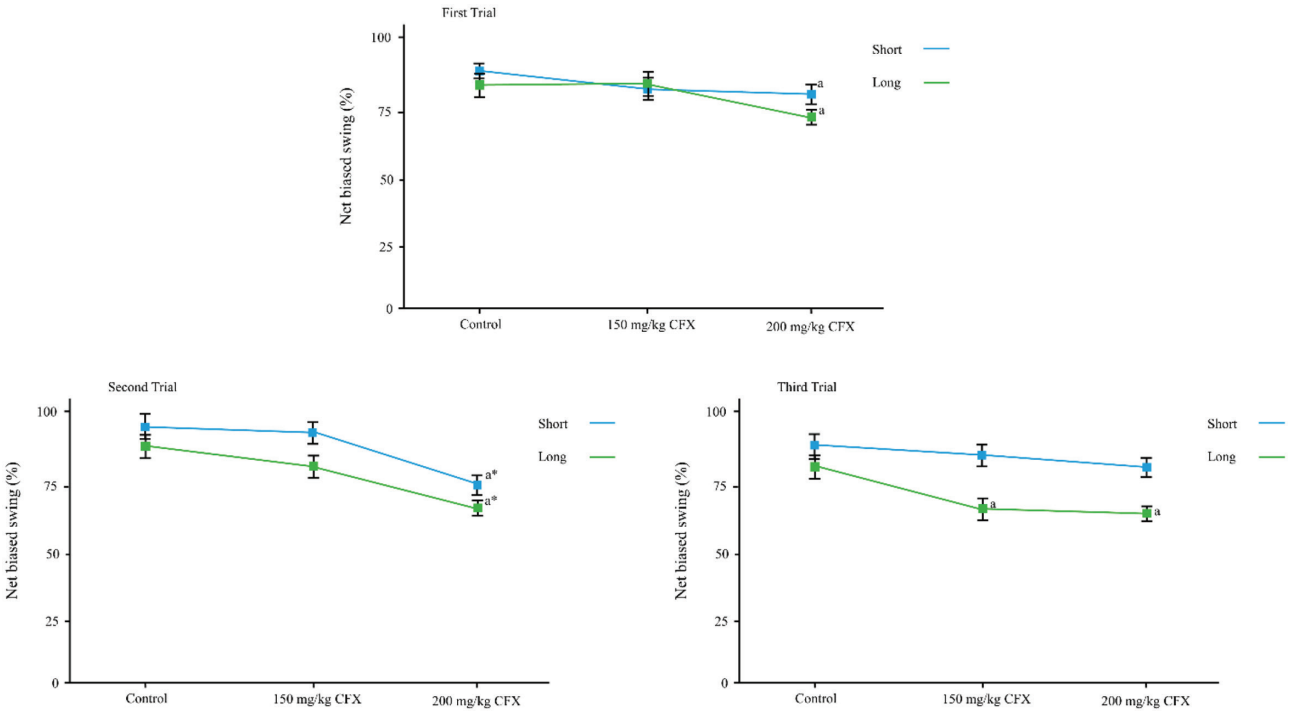

Figure 2. Results of the elevated body swing test. a: p<0.05 for difference between each dose of ceftriaxone and control group, *: P<0.05 for difference between short and long period of administration of CFX in each dose. CFX: Ceftriaxone

ceftriaxone was more effective than short administration of it in each dose. Moreover, in the third trial, there was not a difference between low and high dose of ceftriaxone in reducing biased swing when they were administrated in long period

Results of the rotarod test showed that healthy rats learn to continue to walk on the rotarod in the fourth trial (Figure 3). Although the rotarod performance times are higher in trials 5 and 6 compared to initial trials, they are not significantly higher than the fourth trial. In the control group, the performance of rats remained constant during the trials. Treatment with low dose of ceftriaxone in short period group could not change the performance time; however, when it was administrated in long period, it could increase the performance time in the 5th trial. Treatment with high dose of ceftriaxone could increase the performance time in 5th and 6th trials in both short and long period groups. However, the performance time did not reach the control group in any of the rats treated with ceftriaxone. Moreover, the effect of ceftriaxone in increasing the performance time was clearly different between high dose and low dose of it in a short period administration; however, they were near each other in the 5th trial of long period administration and there was no difference between them in the 6th trial.

DISCUSSION

In the present study we used MPTP to induce behavioral disturbances in rat model of Parkinson's disease which were ameliorated by 8 days and 16 days administration of ceftriaxone in low dose (150 mg/kg twice a day) and high dose (200 mg/kg twice a day) in each subdivided groups.

Rats underwent injection of MPTP and ceftriaxone as described in material and methods and results were evaluated using three behavioral test (AIRT, EBST, and RPT). In AIRT test we did three trials, rats which were treated with long period of ceftriaxone had better behavioral responses compared to short period one. There was no difference in high dose and low dose of ceftriaxone administration in third trials of the test in long period time and these results were observed in two other tests substantially. High dose of ceftriaxone in short period time could diminish behavioral disturbances in second and third trials of AIRT test. Overall, these results showed that during the time, effect of low and high dose of ceftriaxone in decreasing behavioral deficits would be similar when it is administrated in long or short periods. It's a novel approach for substitute high dose of drug with low dose and subside the adverse effect of drug. For short period administration, high doses of drug is needed to observe its effects. This may be related to the

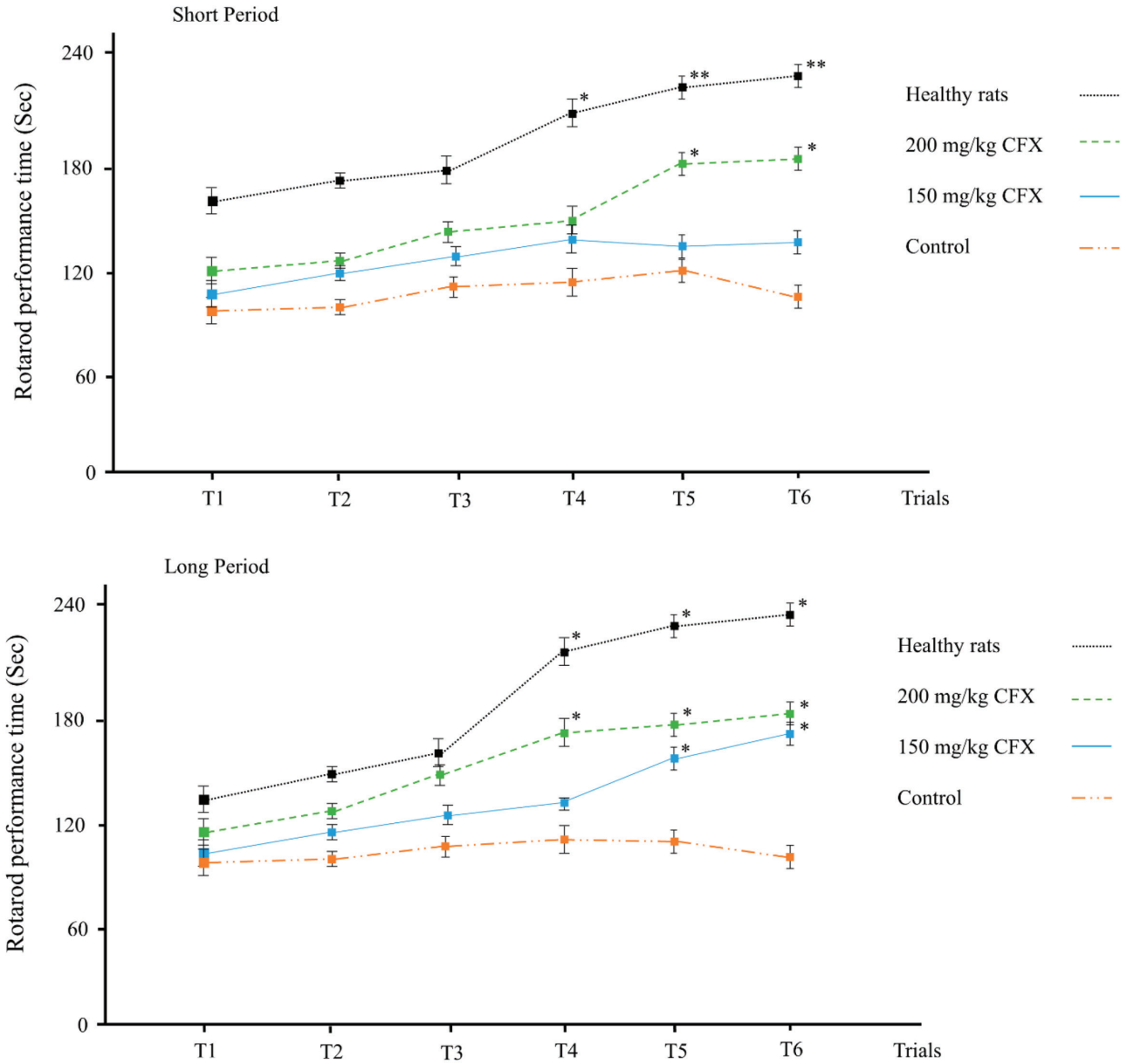

Figure 3. Results of the Rotarod performance test. Results of the trials 1-6 is shown in each panel.* p<0.05 for difference between each trial of the test and the first trial, ** P<0.01 for difference between each trial of the test and the first trial. CFX: Ceftriaxone

long life and clearance of the drugs that lasts more days in the body or the maximal effect in cell function such as up regulation of GLT1 expression that last more days with high dose vs low dose.

EBST test results was similar to AIRT somewhat. It is important to notice that long period administration was more effective than short period and in third trial there was no difference between high and low dose of ceftriaxone. In rotarod test results of 5th and 6th trials were similar to pervious tests which showed that there are no differences in behavioral response and swing test when high or low dose of ceftriaxone administered in long period of time. All these results consequently agree with this concept that long period use of ceftriaxone with low dose is the best option for treatment of Parkinsonian like behaviors and subside the adverse effect of high dose administration of drug for long time. In third trials of AIRT and EBST, due to late evaluation of behavioral change after passing the time, low and high dose of ceftriaxone had similar effects in behavioral enhancement. We hypotheses that long time administration of ceftriaxone for 16 days in low or high dose cause delayed changes in maximum level of activation of downstream pathways such as GTL1 up-regulation. According to our results, high

dose administration of ceftriaxone in long time had more beneficial effects in first and second trials compared to low dose administration; however, in the third trial, effect of low and high dose of long administration was similar. We hypothesize that activation of downstream targets (e.g. GTL1 up regulation) in delayed manner due to cumulative dose of drug when it is used in low dose and activation of target genes with passing the time may be a possible mechanism.

Previous researches showed that the up regulation of GLT1 expression by ceftriaxone is short lived [14]. Long-term administration has been suggested to potentiate and prolong its beneficial effects. In clinical application, the dosage of ceftriaxone used to treat bacterial infections and meningitis in a human adult has been reported to be 2 g/day for 2 months, with no side-effects being reported [15]. These results are in concordance with our findings that long time use of ceftriaxone has more beneficial effects than short period one. MPTP is transformed by glial MAO into the active compound MPP+. The latter crosses the neuronal membrane by a specific uptake mechanism. Once inside the cells, MPP+ leads to a major inhibition of the respiratory chain but also to oxidative stress, both triggering cell death [16]. In glutamatergic hyperactivity, glutamate acts as an excitotoxic agent and is involved in the degeneration of DAergic neurons seen in PD [17]. Several studies in GLT1 expression demonstrated its importance in regulating glutamate homeostasis in brain [18]. It was relevant that a selective decrease in GLT1 protein expression was associated with the neuronal loss observed in the sporadic form of amyotrophic lateral sclerosis [19] and contributed to the pathophysiological changes in Alzheimer's disease [20], ischemia, stroke [21], brain tumors [22] and epilepsy [23]. The obstruction of GLT1 expression in parkinsonian models might therefore contribute to the pathophysiological outcomes. Ceftriaxone could upregulate GTL1 expression and redistribution of it by removing extra synaptically released glutamates. It increases not only GLT1 expression but also its function [24,25]. Besides this property of ceftriaxone, the neuroprotection of the dopaminergic neurons in parkinsonian models might be contributed by other characteristics of the drug. One possible neuroprotective mechanism suggested by previous studies was the side chain of ceftriaxone, D-α-amino-adipic acid [9]. This side chain was readily carboxylated upon oxidative damage [26] and prevented the carbonylation of endogenous targets, such as DJ-1 protein which was suggested to be linked to sporadic parkinson's and Alzheimer's diseases [27] leading to the reduction in oxidative stress and apoptosis [28]. Another possible mechanism is based on the N-methyl-D-aspartic acid (NMDA) receptor antagonistic properties of ceftriaxone [29]. D-α-Amino-adipic acid side chain is an antagonist of NMDA receptor. Therefore, ceftriaxone might function as a noncompetitive antagonist of the NMDA receptor and thus might attenuate glutamate exitotoxicity. As previous studies showed, ceftriaxone decrease the MPTP-induced cognitive deficits evaluated with t maze test and object reorganization test. These results are in concordance with our study which is done by tree different test mentioned above. Ceftriaxone also inhibits MPTP-induced dopaminergic degeneration in the nigrostriatal system, microglial activation in the SNc and cell loss in the hippocampal CA1 as it is compatible with our results that ceftriaxone diminished behavioral deficits in rat model of Parkinson disease [30]. Increased GLT1 expression and its co-localization with astrocytes were observed in the stratum and hippocampus in the ceftriaxone-treated animal [31]. Ceftriaxone dose dependently exhibits neuroprotective effect by mediating brain antioxidant defense mechanism and by upregulating dopaminergic pathway and down regulation of glutamatergic pathway [32]. All these previous studies are compatible with our results that high and low dose of ceftriaxone which administered for a long period of time could decrease behavioral deficits and had neuroprotection in MPTP treated rat model of PD. However, it should be considered that our results may be interpreted in the light of the tests used in this study themselves. Indeed, our previous investigation on 4-aminiopyridine showed similar results which may bring up the effect of the used tests in these studies on the results [33].

In conclusion, our findings strongly suggest that administration of ceftriaxone alone contributes significantly against MPTP Induced PD. There is no difference between high dose and low dose administration of ceftriaxone in long period time and it could be a novel finding to subside adverse effects of drug. Long time administration of ceftriaxone with low dose instead of short period of administration with high or low dose is the best option for the treatment of the disease. Further investigations is needed to clarify exact and possible mechanism of drug action for better and additive therapeutic approaches.

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