

4-Aminopyridine 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). Although potassium channels affect neural activity and death in this area, little research has investigated the effect of potassium channel blockers, such as 4-aminopyridine in the pretreatment of PD.

Methods: Fifty-six healthy male Wistar rats were selected for this study. They were divided into seven groups according to receiving saline or 4-aminopyridine, receiving a low or high dose of 4-aminopyridine and receiving 4-aminopyridine for short or long periods. Apomorphine-induced rotational test, elevated body swing test and rotarod test were done to examine behavioral performances.

Results: 4-aminopyridine could not completely block behavioral disturbances induced by MPTP, however, it decreased them in all behavioral tests. Long administration of 4-aminopyridine was more effective than short administration in lowering behavioral disturbances. Although high dose of 4-aminopyridine was more effective than low dose in initial trials of each behavioral test, there was no difference between them in the last trial.

Conclusion: Long administration of low dose of 4-aminopyridine is the best way to lessen behavioral disturbances induce by MPTP and also avoiding side effects of high dose of 4-aminopyridine.

Keywords: Parkinson's disease; 4-aminopyridine; behavioral tests.

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INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder which affects seven million people globally and is known by cardinal features of resting tremor, rigidity, bradykinesia, and postural instability¹. Progressive degeneration of dopaminergic neurons in midbrain is thought to be the underlying mechanism of this disorder. However, there is less consensus on the treatment of the disorder. The current treatments are L-DOPA, deep brain stimulation and surgical destruction of the globus pallidus, but none of them could be accounted as a final cure of PD^{2,3}. Although

the antiparkinson medications, L-DOPA and dopamine agonists, improve the early symptoms of PD, eventually they become ineffective and also produce complications such as involuntary writing movements⁴. Accordingly, new medications for PD seems crucial.

Potassium channels play a significant role in regulating neuronal activities. By having different subtypes, they can change the neural firing rate and model which yield various motor and sensory signaling⁵. Besides, potassium channels regulate the death signaling such as caspase cascade. Indeed, decrease in intracellular potassium induce apoptosis through activating caspase-3

6. Moreover, in another neurodegenerative disorder, Alzheimer's disease, Amyloid- β deposition is associated with activation of Calcium-activated potassium channels which causes neurotoxicity. 4-aminopyridine (4-AP) is an organic compound which decrease potassium conductance through its channels and so increases neural excitability and firing rate⁵. This drug has been used in spinal cord injuries due to its properties in increasing motor evoked potentials and conduction velocity⁷. Furthermore, recent research focus on its beneficial effects on multiple sclerosis⁸, Alzheimer's disease⁹ and various types of cerebellar ataxia⁹ which may be partly related to the ability of 4-AP in releasing multiple neurotransmitters including dopamine, noradrenaline and acetylcholine¹⁰. However, there is less information about the effect of 4-AP on PD. This study aimed to investigate the effect of 4-AP on behavioral disturbances in an animal 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 ($55 \pm 5\%$) 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 0.1 ml/kg saline 30 minutes before the first time of injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and eight days after that, twice a day.
- B- A short period 4-AP group which included eight rats and received 0.5 ml/kg 4-AP intraperitoneally 30 minutes before the first time of injection of MPTP and eight days after that, twice a day.
- C- A short period 4-AP group which included eight rats and received 1.0 ml/kg 4-AP intraperitoneally 30 minutes before the first time of injection of MPTP and eight days after that, twice a day.
- D- A long period saline group which included eight rats and received 0.1 ml/kg saline 30 minutes before the first time of injection of MPTP and 16 days after that, twice a day.
- E- A long period 4-AP group which included eight rats and received 0.5 ml/kg 4-AP intraperitoneally 30 minutes before the first time of injection of MPTP and 16 days after that, twice a day.
- F- A long period 4-AP group which included eight rats and received 1.0 ml/kg 4-AP intraperitoneally 30

minutes before the first time of injection of MPTP and 16 days after that, twice a day.

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

MPTP-treated model mice were prepared as described¹¹. Rats were treated with MPTP (25 mg/kg) once a day for five consecutive days. Apomorphine-induced rotational test (AIRT)¹², elevated body swing test (EBST)¹³ and rotarod performance test (RPT)¹⁴ 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 were 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.

Data are presented as mean \pm SD. The differences between results of behavioral tests before and after the administration of MPTP was analyzed using the students' T-test. SPSS software ver.20 was used to perform

statistical analyses and $\alpha=0.05$ was considered as the level of significance.

RESULTS

All groups showed some degrees of rotations in AIRT. Hence, treatment with 4-AP could not completely block the neurodegeneration induced by MPTP. Although treatment with 4-AP did not change number of rotations of first test in short or long period groups, it decreased number of rotations of second and third test in both long and short periods of administrations. In long period administration of 4-AP, both low and high doses of it could decrease rotations of the second test; however, only high doses of 4-AP could decrease number of rotations of the second test when it was administered in short period. Furthermore, number of rotations in the second test was significantly lower in long period group compared to short period group except when the vehicle was administered. In the third test, treatment with 4-AP in both low and high dose decreased number of rotations in both short and long periods. Although there was no difference in the number of rotations between short and long period groups with low dose of 4-AP, a high dose of 4-AP could decrease number of rotations in short period group more than the long period group.

Similar to AIRT, treatment with 4-AP could not change net biased swing of the first test compared to vehicle group in both short and long period groups. In the second test, only high doses of 4-AP could decrease biased swing compared to vehicle group but there was not a significant difference between short and long period groups in the level of decrement. Both low and high dose of 4-AP could decrease biased swing in both short and long period groups compared to vehicle. Moreover, long period groups had lower biased swing compared to short period groups in both high and low dose of 4-AP.

Results of the rotarod test showed that healthy rats learn to continue to walk on the rotarod in the fourth trial. 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 vehicle group, rats had a higher performance time in the fourth trial compared to initial trials, however, their performance decreased to initial levels in the 5th and 6th trials. Treatment with low dose of 4-AP in short period group could not change the performance time; however, when it was administered in long period, it could increase the performance time after the 4th trial. Treatment with high dose of 4-AP could increase the performance time in 5th and 6th trials in both short and long period groups.

Although both treatment with 4-AP, especially in high dose, increased the performance time in both short and long period groups, performance time in these rats could not reach the healthy rats.

DISCUSSION

The results of the current study show that 4-AP can block the neurodegeneration in substantia nigra in an animal model of Parkinson's disease. Long period treatment with 4-AP is the most effective way to halt behavioral changes in our models of Parkinson's disease. Long period treatment with 4-AP could lower MPTP-induced behavioral disturbances in AIRT and EBST more than short period administration of it. Moreover, low dose of 4-AP seems to be a better choice than high dose of it to treat behavioral disturbances of PD; indeed, there were significant difference between low and high dose of 4-AP in the results of AIRT and RPT but not EBST. On the other hand, the beneficial effects of 4-AP could not completely block MPTP-induced neurodegeneration and some degrees of behavioral disturbances was observed in PRT compared to healthy rats.

The beneficial effects of K⁺ channel blockers, such as 4-AP, in the treatment of several neurological disorders such as multiple sclerosis⁸ and Alzheimer's disease⁹ have been shown before. 4-AP is a rapid blocker of K⁺ channels which can block KV1 and KV3 subfamilies⁵. These channels have a central role in induction of apoptosis in dopaminergic neurons of substantia nigra¹⁵ and blockage of them can decrease dopaminergic neural death which is the main mechanism of PD. Moreover, selective activation of K⁺-ATP channels in substantia nigra in response to MPTP induces the degeneration of dopaminergic neurons in this area¹⁶. These channels can be blocked by 4-AP which suggest a possible mechanism for neuroprotective effects of 4-AP. Furthermore, A-type K⁺ channels of substantia nigra activate silent neuron, so blockage of them by 4-AP causes firing frequency in this area¹⁷. Another mechanism in this relation may be the effect of 4-AP on activation of NMDA receptors. Indeed, 4-AP abolishes neuronal loss by releasing endogenous glutamate and activating NMDA receptors and subsequently increases dopamine release^{18,19}.

Our results showed that high dose of 4-AP and long administration of it are the most effective ways to lessen behavioral disturbances induced by MPTP. Although 4-AP was more effective in high doses, it can produce side effects such as seizure²⁰. Hence, use of 4-AP in high doses may be limited due to its side effects. Interestingly, in last trial of all behavioral tests, long administration

of low dose of 4-AP lowered behavioral disturbances compared to vehicle group. Parkinson's disease is a chronic disorder and the effect of low dose of 4-AP on PD in the last trial of each test shows the beneficial effect of low dose of 4-AP in the pre-treatment of PD. Consistently there were no difference between the results of the last trial of behavioral test with low and high dose of 4-AP when it was administrated for long time, i.e. 16 days. Therefore, since side effects like seizure are less probable with low dose of 4-AP, long use of low dose of 4-AP may be a better way than use of its high dose in the pre-treatment of PD. However it should be noted that using low dose of 4-AP in short periods has a lower effect on PD than using high dose of 4-AP in the same time. Using high dose of 4-AP for short periods has less side effects than using it in long periods^{20,21}. Hence, using high dose of 4-AP in short periods may be considered as an alternative for using low dose 4-AP in long periods and beneficial effects of both these approaches on PD is similar.

In conclusion, the results of the current study show that long treatment of low dose of 4-AP can decrease behavioral disturbances of MPTP-induced PD. Although high dose of 4-AP had a stronger effect than low dose of 4-AP, side effects of high dose of 4-AP such as seizure can limit its use. Moreover, in last trial of all behavioral tests, there was no difference between low dose and high dose of 4-AP in reducing the behavioral disturbances. Since PD is a chronic disease, it can be concluded that although low dose of 4-AP has a weaker effect than high dose in initial trials, no difference was observed between them in last trial which is more similar to PD. Further research to investigate the 4-AP derivatives without convulsive properties, like 4-AP-3-methanol, and with a higher efficacy in reducing behavioral disturbances of PD is recommended.

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