Original Research⚲

Effect of Glibenclamide as a K_{ATP} Channel Blockade in Treatment of Parkinson

Disease in the 6-Hydroxydopamine-Induced Animal Model

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Abstract:

Background: Parkinson disease is the most common neurodegerative disease in the world after Alzheimer disease. The most important cause of Parkinson's is damage to dopaminergic cells in the midbrain area. Glibenclamide is a second-generation sulfonylurea; it has an inhibitory effect on surface and mitochondrial KATP channels. Many reports indicate that glibenclamide can have neuroprotective effects in many neurodegenerative disorders.

Methods: In the present study, we investigated the effect of glibenclamide pretreatment on behavioral symptom in 6-hydroxydopamine-induced animal model. Rats were divided into 4 groups (n=8 per group). Group (I): control without intervention Group (II): Vehicle, Group (III): received as pretreatment glibenclamide (3 mg/kg) i.p from begining of surgery until 4 weeks every day. Group (IV): received as pretreatment glibenclamide (8 mg/kg). In all group except healthy group received 6-OHDA by stereotaxic surgery. Development and severity of Parkinson disease were evaluated by apomorphine-induced rotational and rotarod behavioral tests.

Results: Our result showed that in two behavioral tests, pretreatment with glibenclamide could attenuate severity of Parkinson disease in treatment groups. There was no significant difference between treatment groups with different doses of glibenclamide.

Conclusion: our data showed pretreatment with KATP channel blockers reduces the severity of Parkinson's symptoms caused by the OHDA-6 model in rats in behavioral studies.

Keywords: Parkinson Disease, Glibenclamide, KATP Channel

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease(1, 2). Parkinson is a debilitating neurological disorder that affects about four million people in the worldwide. This disorder determined by selective degeneration of dopaminergic midbrain neurons, especially in the substantia nigra pars compacta (SNpc)(3). Parkinson's is characterized by two or more of the four main symptoms of the disease. Tremor of the limbs at rest, slowness of movement, stiffness of the limbs and body, and impaired posture and balance are the four main symptoms(4). Studies suggest that mitochondrial dysfunction and oxidative stress play important roles in this disease(5). There is currently no known cure for this disease. Glibenclamide is a second-generation sulfonylurea; it has an inhibitory effect on surface and mitochondrial K_{ATP} channels. Glibenclamide suppresses neutrophil migration by suppressing the acute inflammatory response by blocking the K_{ATP} channel (6, 7). Studies have shown Glibenclamide could inhibit pathways that lead to renal ischemic reperfusion injury (7). Many reports indicate that glibenclamide can have neuroprotective effects in many neurodegenerative disorders (8-11). K_{ATP} channels are widely distributed in the brain, especially in the cortex, basal ganglia, hypothalamus and hippocampus. However, there is few studies have evaluated the effect of ATP-dependent potassium channel blockers in the animal model of PD. Therefore, in the present study, we investigated the effect of Glibenclamide pretreatment on behavioral symptom in 6-Hydroxydopamine-induced animal model.

Material and Methods

Animals

Forty rats (weight: 200-250 gr) were used in this study. They were housed under a 12 h light/dark cycle in a room with controlled temperature. Water and food were provided ad libitum. The present study by Tehran University of Medical Sciences (ID: IR.TUMS.MEDICINE.REC.1398.344). Rats were divided into 4 groups (n=8 per group). Group (I): control without intervention Group (II): Vehicle received ethanol intraperitoneally as solvent of glibenclamide, Group (III): rats were received 6-hydroxydopamine by stereotaxic to induce Parkinson in animals with 4 weeks recovery and received as pretreatment glibenclamide (3 mg/kg) i.p from begining of surgery until 4 weeks every day. Group (IV): rats were received 6-hydroxydopamine by stereotaxic to induce Parkinson in animals with 4 weeks recovery and received as pretreatment glibenclamide (8 mg/kg) i.p from beginning of surgery until 4 weeks every day. All pretreatments were started one day before 6-OHDA injection and continued up to 4 weeks after that.

Surgical Procedures

All animals except of healthy group received 6-OHDA with stereotaxic surgery. To induce Parkinson, rats were anesthetized with ketamine (70 mg/kg) + xylazine (10 mg/kg) and then, were placed in stereotaxic instrument. After shaving and puncturing of the skull, 4 μl of 6-OHDA (4 μg/ml) was injected via Hamilton syringe into four region of right striuatum. Coordinates for injections were anterior/posterior (AP): 1.5 mm, lateral (L): -2.5 mm, dorsal/ventral (DV): -6 mm and AP: 0.8 mm, L: -3 mm, DV: -6 mm, and AP: 0.1 mm, L: -3.2 mm, DV: -6 mm and AP: -0.5 mm, L: -3.6 mm and DV: -6 mm. AP and L were measured from bregma and DV from the surface of skull according to the atlas of Paxinos and Watson . After 3 weeks recovery, animals entered the behavioral study.

Apomorphine-Induced Rotational Test

In this behavioral study, injection of 6-OHDA toxin causes extensive neuronal damage in the midbrain. 2 to 4 weeks after surgery, rats show rotation movements toward the injection site in response to the injection of apomorphine (a dopaminergic receptor agonist). The number of these rotations per unit time is a way to measure of the severity of neuronal damage in the midbrain and the effect of the intervention. To perform this test, the rats were first placed inside a transparent Plexiglas cylinder with dimensions of 28 cm in diameter and 38 cm in height and were given 5 minutes to adapt to the environment. Apomorphine hydrochloride (0.5 mg/kg, ip) was injected to rats, and 1 minute after that, the number of turns toward the injection site (negative number) or other site (positive number) calculated for 1 hour at 10-minute intervals. Contralateral and ipsilateral rotations (away and toward the lesioned side, respectively) were counted as positive and negative scores and the net number of rotations defined as the positive scores minus the negative ones. The rotations was measured in the second, fourth and eighth weeks post-surgery(12).

Rotarod test

In this test, the motor ability, balance and also motor learning of animals are evaluated. The rotarod device consists of a rotating rod that rotation speed increases over time. The length of time the animal stays on the bar is calculated as the function of the animals. In this study, the test duration was 200 seconds, which the rotational speed of the rotating rod started from 5 rpm, and within 120 seconds, it reached a maximum speed of 40 rpm, and the rest of the test, time remained at the maximum speed. The test was performed 2 times a day for 3 consecutive days. In order to prevent fatigue in rats, at least 60 minutes interval between test sessions was considered every day(13). Rotarod test data are presented based on the area under the curve (AUC), which is calculated based on the following formula:

$$\text{AUC} = \text{time on the rod (s)} \times [\text{time on the rod(s)} \times 0.44/2]$$

Note: 0.44 is the acceleration speed per second.

Statistical analysis

To analyze of data, we used the software of GraphPad Prism 7.0. All data were shown with Mean±SEM. We were first analyzed data by Kolmogorov-Smirnov test to assess the normality of the data. Since distribution was not normal, to compare the results of between groups in study we used Kruskall–Wallis nonparametric ANOVA followed by Mann–Whitney U test. A P value≤0.05 was considered statistically significant

Result
Rotational behavior

In All experimental groups, rats showed an asymmetrical rotation toward the injection site. More than 30 rotation toward the injection site is a symptom that rats have Parkinson disease. So treatment with glibenclamide just decrease asymmetric rotational and attenuate the severity of Parkinson disease in treatment groups but they cannot prevent completely from induction of Parkinson disease (Table 1). In treatment group with glibenclamide, net contralateral rotation was significantly more than vehicle group (P<0.05). There is not a significant difference between Low (3 mg/kg) and high glibenclamide dose (8 mg/kg) in attention of asymmetric rotational movement (Figure 1). These data showed high dose of glibenclamide no more improvement is achieved in comparison with lower dose. So, glibenclamide could prevent the development of 6-OHDA-induced model of Parkinson disease in animals.

Rotarod test

In the healthy group, the performance of rats in each session was better than in the previous sessions and the rat reached its maximum performance in sessions 4 to 6. These data showed in healthy group motor learning of rotarod test was completely happened. All 6-OHDA-treated rats showed some degree of motor learning, but no significant differences were observed between 6-OHDA-treated rats and healthy rats. None of the 6-OHDA rats (as expected) reached peak performance even in the last session (vehicle group, Low Glib and high glib groups). In all behavioral tests, there was no significant difference between the low glib and high glib groups, indicating that increasing the dose of glib to more than 3 mg / kg did not potentiate its anti-Parkinson effect (Figure 2).

Discussion

In the present study, we showed that pretreatment of rats with glibenclamide reduced the severity of 6-OHDA-induced behavioral symptoms of Parkinsonism. There are a lot of evidences that there is a relationship between nigral cell death and the severity of behavioral symptoms in Parkinson disease. The rotational test, which is the most valid 6-OHDA-induced Parkinson's assessment test and also there is a inversive relationship between substantia nigra (SN) lesions, and duration of time which rats spent on the spinning wheel in the Rotarod test.Based on this evidence, we suggest that pretreatment with Glib has a neuroprotective (neuroprotective) effect on SN lesions and reduces the neurotoxic effect of 6-OHDA on SN-dependent dopaminergic (DA) neurons. In confirmation of our results, several studies show that inhibition of KATP channels provides a neuroprotective effect in Parkinson disese. Lee et al.2005 (9) showed that glibenclamide reduced the cytotoxicity of MPP^+ in PC12 cells by suppressing changes in mitochondrial membrane permeability.

Another study on Stroke models in rats showed glibenclamide treatment can reduce cerebral edema, myocardial infarction and mortality by up to 50%(14). Studies on midbrain DA neurons also show that blocking KATP channels protects these neurons from neurodegenerative diseases. In contrast, the evidence presented revealed that opening and activating (rather than blocking) KATP channels protects neurons from neurotoxins. Nagy et al. 2004 (15) reported that K^+ channel opener dioxide protects neurons from the toxicities of amyloid beta peptide, and glutamate. This effect was inhibited by 5-HydroxyDecanoate, a selective mitochondrial KATP channel inhibitor and glibenclamide. Several other studies have shown that the KATP channel opener provides significant neuroprotection in various animal models of Stroke and Parkinson disease (16-18). This difference in the role of KATP channels in neuroprotection may be due to the nature of neuronal action. For example, under physiological conditions, midbrain DA neurons show the onset of spontaneous action potential, and many KATP channels are closed, at least in in vitro brain sections. Activation of KATP channels hyperpolarizes DA neurons, resulting in a complete loss of their normal pacemaker activity. It is suggested that in PD, a chronic decrease in neuronal activity may not be neuroprotective at first step, but it may reduce the effect of some genes that it may result to increase the chance of survival, such as neurotrophins. In this regard, Another study showed that continuous activity of KATP channels may increase nerve damage specically DA neurons(8). Thus, activation of KATP channels can play an unexpected role in the progressive death of DA neurons in chronic diseases. Several mechanisms have been proposed for the neuroprotective effect of glibenclamide. It has been reported that glibenclamide inhibits the activation of endothelial caspase-3. Caspase-3 has been

defined as a major cause of activation of apoptotic pathways. Glibenclamide also suppresses the inflammatory response, by inhibition of proinflammatory cytokines (14, 19). Also, glibenclamide has an antioxidant effect independent of the blocking effect of KATP activity (20, 21). 6-OHDA can inhibit mitochondrial complexes 1 and 4, leading to Mitochondrial dysfunction and ATP deficiency which can produce neurodegenerative effects by keeping the KATP channels active(22, 23). In Conclusion based on our data, pretreatment with KATP channel blockers reduces the severity of Parkinson's symptoms caused by the OHDA-6 model in rats in behavioral studies.

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Tables

Table 1: Net contralateral rotations in three different test days. The tests was measured in the second, fourth and eighth weeks post-surgery

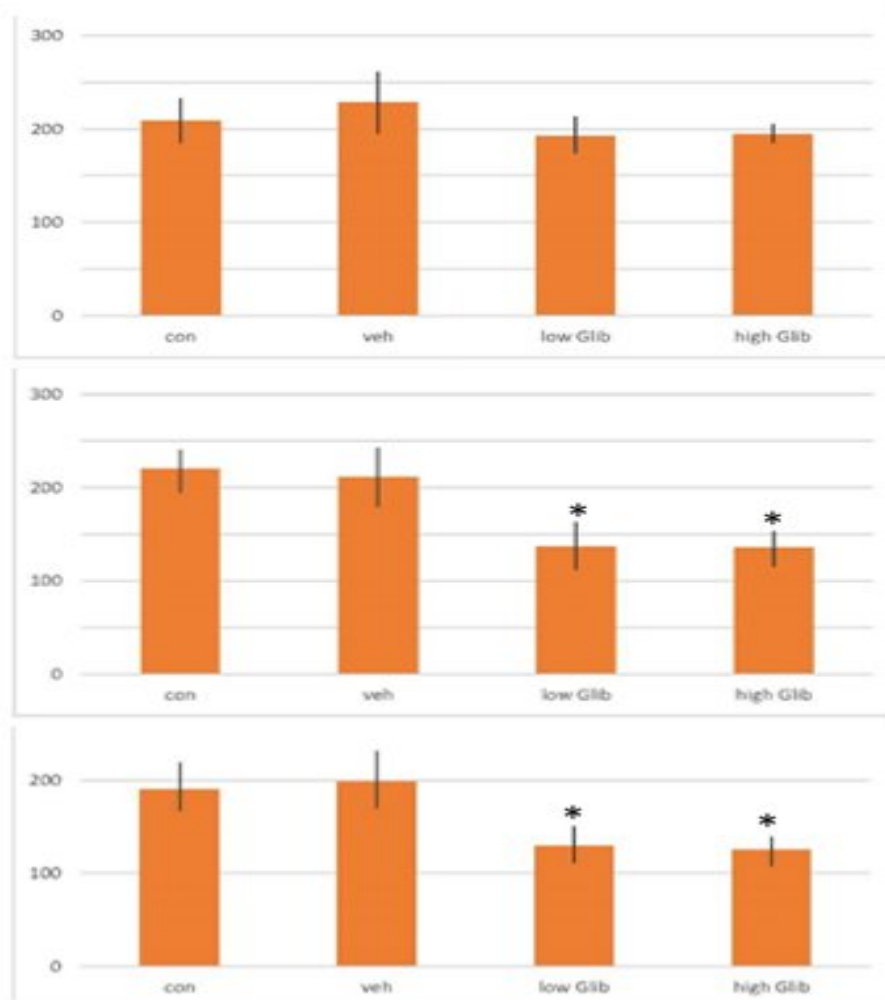|	control	vehicle	Low Glib	High Glib
Test-1	209	228	193	194
Test-2	220	211	137	136
Test-3	190	198	130	125

Figure 1. Apomorphine-induced net contralateral rotations of different experimental groups in the second (upper plot), fourth (middle plot) and eighth (lower plot) weeks post-surgery.data are expressed as means±S.E of animals in each group.

*, $P < 0.05$ compared to vehicle group; Kruskall-Wallis nonparametric test followed by Mann-Whitney U test.
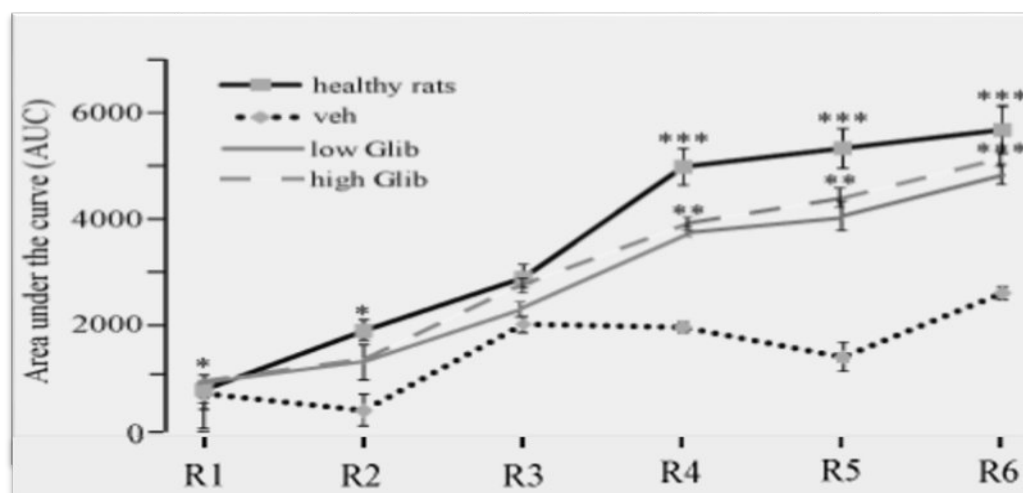
Figure 2. Motor performance of different groups of rats in rotarod test was examined in three consecutive days and two sessions a day. Since control and vehicle groups of rats showed almost similar results, only data of vehicle group are shown.

*, $P < 0.05$; **, $P < 0.01$, *** $P < 0.001$ compared to vehicle group; Kruskall-Wallis nonparametric test followed by Mann-Whitney U test; AUC, area under the curve; R1 - R6, sessions of the test; R1, first session and R6, last session