Experimental Study on the Neuronal Toxical Effect of Levodopa and the Inhibition of Ginkgo Biloba Extract on Parkinson Disease in Rats

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Abstract

Objective: To observe neuronal toxical effect of Levodopa and investigate whether using Levodopa together with EGb is an ideal, workable method to treat Parkinson disease.

Methods: In this study, the rat models of Parkinson disease (PD) were made by injecting stereotaxically 6-OHDA to right side of the mesencephalic ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). We used rotational behaviour, TUNEL, immunocytochemical, Nissl’s body staining methods to observe the difference between Levodopa (50mg/kg/d×3d, ×5d, ×7d, L-dopa group) and the combination use of Levodopa and EGb (100 mg/kg/d, E-D group).

Results: The numbers of apoptosis and rotation, bFGF protein expression in the L-dopa group surpassed those in the E-D group (P<0.05). The number of Nissl cells in the L-dopa group was fewer than the E-D group.

Conclusion: Levodopa has neurological toxical effect. EGb may decrease the toxicity of levodopa. The combination use of Ginkgo biloba extract (EGb) and Levodopa is a workable method to treat Parkinson disease and is better than using Levodopa alone.

Keywords: Ginkgo biloba extract (EGb); Apoptosis; L-dihydroxy-phenylalanine (L-dopa)

Introduction

The background of Parkinson disease is degeneration of neuronal degeneration and death of compact part in substantia nigra of mesencephalon, and decreasing release of dopamine in striatum. The etiology is not clear yet. On the aspect of therapy, traditional substitute therapy of dopamine is major, levodopa and its compound agent are major medication. Although having neuronal toxical effect of self-oxidation [1], levodopa cannot be substituted because of its reliable effects. Therefore the problem how to use levodopa reasonably has not been solved. We should confront. The underlying medical effect of Ginkgo biloba extract (EGb) is anti- free radical and anti-palate active factor (PAF). Ginkgo biloba extract has been used to treat cerebrovascular disease because of its reliable effects. Ginkgo biloba extract. Still to now, it has not been reported that treating Parkinson disease using levodopa combining with Ginkgo biloba extract. Using these methods: TUNEL, praxeology, ultrastructural, immuno- histochemistry, we explore to identify if the treatment is feasible. The aim of our research is look for a new method to decrease the toxical effect of levodopa, increase the effect of levodopa.

Materials and Methods

Animal

Select healthy Wistar male rats old as three month, provided by the center of animal, Tong-ji Medical College, Hua-zhong Science and Technology University, weight 180-200 gram. Behaviour of each rat was checked to be normal.

Drug

6-hydroxydopamine (6-OHDA) parenteral solution to provide: 6-hydroxydopamine and Vitmine C shaf powder (Sigma company produce) according to the ratio of 2.5:1, solutes into Sodium chloride solutions, dispensing into 0.3% 6-OHDA solution. It should be used as soon as dispensed and preserved in hypothermia, avoiding light and enclosed.

Ginkgo biloba extract (EGb) Suspension

Material of EGb was offered by Kang Eng-Bei medical conglomerate, Zhe Jang province, China. EGb portion is: 24% flavone, 6% ginkgo lactone. The material of EGb was mixed with carboxymethylcellulose sodium to produce EGb Suspension. Concentration of EGb Suspension is 20 mg/ml. It should be used as soon as dispensed and preserved in hypothermia, avoiding light and enclosed.

Reagents and equipments

Apotoss Test Kit (Boehringer Mannheim produce, German), 5% bamboo peony stain (Boster company produce, China), Anti-rat IgG bFGF 1:100 (Santa Cruz company produce, America). Animal source are credited.

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Analysis of Image and Statistical Method

Use media mix colorful pathological diagram analyse system to analyse diagram. Each section of apoptosis, immune-histochemistry, Nissl’s staining was close neighbour. Measuring window was 262144 μm². All data was indicated by the manner of mean ± standard deviation, hypothesis testing; significance test of intra-group and intra-group was carried by Oxstat statistical program.

Results

Apoptosis result of substantia nigra

From Table 1, we can find that the apoptosis number of substantial nigra in E-D group decreased 33.4%, 40.8%, 35.7% comparing with L-dopa group. And the difference was significant. The apoptosis number of substantial nigra in L-dopa group was 10d<14d<12d (Table 1).

Result of praxeology of rats

Rats rotated toward uninjured side obeying the hour-hand of a clock, head connecting tail, taking hindlimb as fulcrum. The average time of starting was five to eight minutes, cost ten minutes to reach the highest rotation behaviour. The velocity of rotation was fifteen to eighteen. Interval of rotation behaviour was equal. Difference was significant. But there was no difference between E-D group with control group. The average time of starting and the time reaching highest rotation velocity were three to five minutes later than L-dopa group. The highest rotational velocity was seven to ten ring/min; Interval of rotation behaviour was different. In 10d, 12d, 14d, the mean rotation velocity decreased 31.4%, 34.7%, 32% comparing with L-dopa group. The number of rotation in L-dopa group was 10d<12d<14d.

Result of Nissl’s neurons of substantial nigra

The number of Nissl’s neurons of substantial nigra in E-D group was higher than L-dopa group at each time point. The difference is significant. The number of Nissl’s neurons in L-dopa group was 10d<12d<14d (Table 2).

Discussion

Neuronal toxical effect of L-dopa in substantial nigra

Neurons in substantial nigra of patients with PD are easy to be attacked by free radical [3]. Melamed [4] think that neuronal toxical
effect of L-dopa in substantia nigra is mainly caused by self-oxidizing. And the toxic effect leads to PCl₂ neuron apoptosis and necrosis.

Mitochondria diplo-chain DNA (mtDNA) in nuclei is major object attacked by free radical. In our experiment, we find that number of apoptosis in substantia nigra increased with the time of L-dopa using. In 14d, because of the increasing of L-dopa neuronal toxic effect, dead pattern of neurons of substantia nigra changed from apoptosis pattern to necrosis pattern [5]. This opinion is the same as Mayo [6]. Nissl body is mark of stock cell. We found that the number of Nissl neuron was lower notably than the contral group. The finding, Nissl dody lysis from center or extinction, proved that neuronal toxic effect of L-dopa could make the function decreased or necrosis from 1d to 21d.

Mechanism of EGB inhibiting neuronal toxic effect of L-dopa

Oxidative stress plays an important role in neuronal toxic effect of L-dopa. EGB effective background is cleaning free radical and anti-platelet active factor (PAF). Flavonoid glycoside has phenol hydroxy terrapin peroxidase prosthetic group Fe³⁺, inhibit its activity [7], inhibiting produce of free radical. EGB can depress the toxic effect of MPTP [8]. EGB can be inhibit Protease C, decrease velocity of apoptosis [9]. EGB and ginkgo lactone can affect function of dopaminergic system by means of membrane effect of platelet active factor. EGB and ginkgo lactone can inhibit dopaminergic metabolism of striatum and limbic system [10]. EGB prevents not only injury of mitochondria DNA, but also mitochondria function and morphologic change [11]. In our study, we found that number of apoptosis in E-D group was lower 33.4%, 40.8%, 35.7% than L-dopa group in 10d, 12d, 14d. Number of Nissl’s neurons in E-D group was higher than L-dopa group. And difference was significant. What said above states that EGB has neuronal protection of PD rats.

Rotation rings of rats are often used as a marker to reflect degree of dopaminergic neurons [12]. In the respect of praxology, rotation rings of E-D group are lower notably than rats in L-dopa group. Testimony of praxology states that EGB has neuronal protection of PD rats. Effect of therapeutic alliance of EGB and L-dopa is better than using L-dopa alone and neuronal toxic effect of L-dopa was well controlled.

To sum up, we learn that EGB can well control neuronal toxic effect of L-dopa and enlarge the range of L-dopa using safely [13]. EGB has little side effect. Therapeutic alliance of EGB and L-dopa will be useful in the treatment of PD.

### Acknowledgment

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### References


<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Rotation behavior (ring/30min)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10d</td>
<td>12d</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>1750 ± 211</td>
</tr>
<tr>
<td>L-dopa</td>
<td>15</td>
<td>1371 ± 204</td>
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<tr>
<td>E-D</td>
<td>15</td>
<td>1814 ± 220</td>
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Note: Comparing with L-dopa group, *P<0.05; Comparing with the contral group, △P<0.05

Table 3: The number of Nissl’s neurons of substantial nigra at each time perfusing stomach (X ± S).