Neurological Disorders

Fei et al., J Neurol Disord 2013, 1:3
DOI: 10.4172/2329-6895.1000126

Research Article Open Access

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 mesencephic 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 substantial nigra of mesencephalon, and decreasing release of dopamine in striatum. The etiology is not clear yet. On the aspect of therapy, traditional substitutive therapy of dopamine is majority, levedopa 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 reasonablely 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 be 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. Before being used, EGb Suspension should be agitated by BIM-8A ultrasound shoker.

Levodopa parenteral solution

Levodopa shaf powde (Sigma company produce) was dissolved into injection solution containing 0.05% ethanol and 0.1% ascorbic acid. Concentration of Levodopa parenteral solution is 50 mg/ml. It should be used as soon as dispensed and preserved in hypothermia, avoiding light and enclosed.

Reagents and equipments

Apoptosis 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

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Received April 11, 2013; **Accepted** July 28, 2013; **Published** July 31, 2013

Citation: Fei C, Sheng-Gang S, E-Tang T (2013) Experimental Study on the Neuronal Toxical Effect of Levodopa and the Inhibition of Ginkgo Biloba Extract on Parkinson Disease in Rats. J Neurol Disord 2: 126. doi:10.4172/2329-6895.1000126

brain solid positioner (Xi Bei optic instrument factory, China), media mix color pathologic analysis system (Computer Center of Beijing aerospace college produce).

Animal model making

Following the method of Thomas [2], we made uni-lateral 6-hydrodopamine-lesioned Parkinson disease rats model. The site of locus substantial nigra compact part (SNc) and mid-brain ventral tegmental area (VTA) was determined previously. The needle was inserted at the following coordinates with respect to lambda and dura. The coordinate of SNc is: anterior fontanelle posterior (AP) 4.8 mm, Media line Right-Sidedness(ML) 2.0 mm, dura mater vertical(DV) 7.5 mm; The coordinate of VTA is AP4.4 mm, ML1.2 mm, DV7.5 mm. Each of the two injection contained 6-OHDA parenteral solution 3.3 μl. The neurotoxin was injected slowly at 1.0 μl/min and then the needle was left in place for 2-3 min after each injection. The syringe was then withdrawn at 1.0 mm duration and the skin closed.

Behaviour testing

One week following the lesion, the rats were injected abdominally with Apomophine (APO) 0.5 mg/kg to evoke rotational behaviour, and those rats which rotation were more than 210 ring/30 min were thought to be succeed PD rats model.

PD rats groups and administration

Forty-five succeed PD rats model were selective, divided into three groups randomly: control group (n=l5), Levodopa group (n=l5), EGb-Levodopa therapeutic alliance group (E-D group, n=l5). 10 daya, 12 days, 14 days was observative time point. Each group had five rats at each time point. In E-D group, PD rats were perfused stomach with EGb suspension 100 mg/kg/d for seven days, in the seventh day rats were injected abdominally with levodopa parenteral solution 50 mg/kg/d, till to the the terminal time point. Levodopa group were perfused stomach with the same dose of solvent of EGb suspension for seven days, in the seventh day rats were injected abdominally with levodopa parenteral solution 50 mg/kg/d, till to the the terminal time point. The control group were perfused stomach with solvent of EGb suspension for seven days, in the seventh day rats were injected abdominally with the same dose of solvent of levodopa parenteral solution, till to the terminal time point.

Experimental Marker

Praxeology observing

Rotational behaviour of all groups was observed in the day after reservation time point. Each PD rat was injected apomophine 0.5 mg/kg abdominally, and observed continuously two hours. After observation, rats were instillated and fixed with 4% polymerisatum perfusate with through penetrating heart and then break head select mid-brain, embedding with paraffin wax.

Method of apoptosis detecting

Each paraffin tissue mass section was 5 pm thick. According to Seaton's method of apoptosis detecting [3].

Nissl's staining of substantial nigra neuron

Each paraffin tissue mass section was 5 pm thick and stained for 1 to 2 minutes with bamboo peony aqueous solution and then washing, 95% alcohol dischrom for 1 to 2 minutes, desiccation, clearing and sealing. At last each section was observed with microscope.

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 μm^2. All data was indicated by the manner of mean ± standard deviation, hypothesis testing; significance test of infra-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

Group	Number of rats	Number of apoptosis (piece/mm²)		
		10d	12d	14d
Control	15	400 ± 29	388 ± 26	368 ± 20
L-dopa	15	533 ± 39△	690 ± 41△	605 ± 37△
E-D	15	355 ± 26※△	402 ± 37※	379 ± 26※

Note: Comparing with L-dopa group, ※P<0.05;
Comparing with the contral group, △P<0.05

Table 1: The apoptosis number of substantial nigra of rats at each time perfusing stomach (X ± S).

Group	Number of rats	Rotation behavior (ring/30min)		
		10d	12d	14d
Control	15	231 ± 22	219 ± 21	220 ± 19
L-dopa	15	318 ± 19△	340 ± 23△	360 ± 20△
E-D	15	218 ± 10※	222 ± 11※	244 ± 10※

Note: Comparing with L-dopa group, ※P<0.05;
Comparing with the contral group, △P<0.05

Table 2: The rotation behaviour of rats at each time perfusing stomach (X ± S).

Group	Number of rats	Rotation behavior (ring/30min)		
		10d	12d	14d
Control	15	1750 ± 211	1695 ± 207	1499 ± 188
L-dopa	15	1371 ± 204△	917 ± 167△	805 ± 196△
E-D	15	1814 ± 220※	1601 ± 200※	1303 ± 172※

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).

effect of L-dopa in substantial nigra is mainly caused by self-oxidizing. And the toxical effect leads to PCl_2 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 substantial nigra increased with the time of L-dopa using. In 14d, because of the increasing of L-dopa neuronal toxical effect, dead pattern of neurons of substantial 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 toxical effect of L-dopa could make the function decreased or necrosis from 1d to 21d.

Mechanism of EGb inhibiting neuronal toxical effect of L-dopa

Oxidative stress plays an important role in neuronal toxical 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 Fe^{3+}, inhibite its activity [7], inhibiting produce of free radical. EGb can depress the toxical effect of MPTP [8]. EGb can be inhibite 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 praxeology, rotation rings of E-D group are lower notably than rats in L-dopa group. Testimony of praxeology 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 toxical effect of L-dopa was well controlled.

To sum up, we learn that EGb can well control neuronal toxical 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

This project was supported by grant from the National Nature Science Foundation of China.

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