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Influence of simvastatin on dopaminergic neurons of lipopolysaccharide-induced rat model of Parkinson's disease

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ABSTRACT

Objective: To investigate the neuroprotective effects of simvastatin on lipopolysaccharide (LPS)-induced rat model of Parkinson's disease (PD) and the mechanisms involved. **Methods:** Hemiparkinsonian rat models were induced by stereotaxieal injection of LPS in the right substantia nigra compacta. After 2 weeks of simvastatin treatment, rotational behavior test was performed after the intraperitoneal injection of apomorphine. Expression of tyroxine hydroxylase (TH) and glial fibrillary acidic protein were analyzed through immunohistochemical staining of substantia nigra and striatum, and the level of TNF-α was evaluated using enzyme-linked immunosorbent assay. **Results:** Comparing with untreated group, behavioral symptoms of the rats were significantly less in the rats that received simvastatin treatment. The TH positive cell count in substantia nigra and striatum were significantly increased ($P<0.05$) and TNF-α expression was significantly decreased ($P<0.05$) in simvastatin group compared to untreated group. **Conclusions:** Simvastatin could effectively inhibit the activation of astrocytes, reduce TNF-α expression, and exert anti-inflammatory effects, and thus protect the dopaminergic neurons in substantia nigra and striatum of the rat model of PD.

1. Introduction

Parkinson's disease (PD) is a degenerative disease of the central nervous system, which has become one of the most important diseases that severely influence humans especially aged people. Pathological characteristics of PD include absence of substantia nigra dopaminergic neuron and formation of Lewy body, which could occur several years before the appearance of typical motor symptoms[1]. Conventional treatment with levodopa could remit clinical symptoms but could not prevent the disease progression; in addition, long-term drug therapy could also induce severe adverse effects[2]. Statins is widely used in clinical practices in treating hyperlipemia. Clinical studies demonstrated that statins could remit motor and non-motor symptoms of patients with PD, and delay the progression of disease[3]. In the present study, the protective effect of simvastatin (simv) on neurons of rats with lipopolysaccharide (LPS)-induced PD was investigated.

2. Materials and methods

2.1. Animals

Forty-five male adult Sprague-Dawley (SD) rats, weighing 230-250 g (Laboratory Animal Centre of Tongji Medical

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College, Huazhong Science and Technology University, China), were divided into 3 groups, namely control group, LPS group, and LPS+simv group (15 rats in each group).

2.2. Regents and equipment

LPS and apomorphine were purchased from Sigma-Aldrich (America), enzyme-linked immunosorbent assay (ELISA) Kit for tumor necrosis factor-alpha (TNF-α) was purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China), monoclonal antibody of tyrosine hydroxylase (TH) was purchased from Abcam (America), and glial fibrillary acidic protein (GFAP) polyclonal rabbit anti rat antibody was purchased from Bioss Biotechnology Co., Ltd. (Beijing, China). Rat stereotactic instrument was purchased from Ruiward Company (Shenzhen, China).

2.3. Model induction and treatment

Two μL of LPS (5 μg/μL) was stereotaxically injected into the right substantia nigra pars compacta (SNpc) to induce hemiparkinsonian rat model. Intraperitoneal injection of simvastatin (5 mg/kg) was performed for the rats in the LPS+simv group at 5 pm each day (1 hour before the operation), while intraperitoneal injection of same volume of normal saline was performed for the rats in the control and LPS groups. Intraperitoneal injection of 10% chloral hydrate (3.5 mL/kg) was given for anesthesia, and then the rats were horizontally fixed in the stereotactic instrument with the ear bar parallel to the line connecting bilateral ears, and the height of incisor bar of -3.3 mm. Bregma was considered as origin of coordinates, and the coordinate of substantia nigra was A-5.0 mm, R-2.0 mm, and DV-7.6 mm. For the rats in the LPS and LPS+simv groups, 2 μL of LPS was stereotaxically injected into the right SNpc, while 2 μL of normal saline was injected into the right SNpc for the rats in the control group; the injection speed was 0.4 μL/min, and the needle was retained for 10 min and then retreated slowly.

2.4. Behavioral test

Apomorphine (0.05 mg/kg) was subcutaneously injected. Two weeks later, the time taken to rotate the head to the side contralateral to destruction was recorded and the rotation cycles were recorded every 5 min for 30 min.

2.5. Immunohistochemical examination

Striatum and substantia nigra were collected according to Paxinos and Franklin atlas. Paraffin sections of the tissues were performed of 5 μm thickness. One section was selected from each 6 continuous sections, and 2 sections were selected for each rat. The TH (1:200 dilution) and GFAP (1:100 dilution) staining were performed. Six visual fields of striatum or substantia nigra were selected on the left and right sides of each section (with 3 visual fields on each side), observed, and photographed at same amplification factor (× 20/100/200/400) and at same light intensity. Image-Pro Plus 6.0 software was used to analyze the mean optical density (OD) of TH positive regions of striatum, TH positive cell count at substantia nigra, and mean OD of GFAP positive regions of substantia nigra.

2.6. TNF-α expression at substantia nigra

TNF-α expression at substantia nigra was determined using ELISA. In brief, the rats were decapitated and the brains were collected, and then the substantia nigra was collected on ice, homogenated, and centrifuged at 4 ℃, 12 000 rpm for 10 minutes. Supernatant was collected, and then the TNF-α level was determined according to the instructions of radioimmunoassay kit.

2.7. Statistical analysis

The SPSS software, Version 17.0, was used for the statistical analyses. Data were described as means and standard divisions, and compared using t-test or analyses of variances. A $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of simvastatin on rotational behavior of the rats

Two weeks after the intraperitoneal apomorphine administration, no rotational behavior was found for the rats in the control group, while increased rotation cycles were found for the rats in the LPS group [mean: (146.8± 7.2) cycles/30 min, $P<0.01$]. However, in LPS+simv group (rats received 14 continuous days of treatment with 5 mg/kg simvastatin), the rotational behavior of the rats significantly decreased as compared with the rats in the LPS group [(79.5± 9.4) cycles/30 min, $P<0.05$].

3.2. Immunohistochemical staining result of TH

The TH positive cell count in the injection region of substantia nigra or striatum, as well as OD in striatum in the control group was not significantly different from LPS group or LPS+simv group ($P>0.05$). In contrast, TH positive neurons

were significantly decreased in the substantia nigra of the rats in LPS group, protuberances and fiber connection of the cells were also found reduced, attenuated, and shortened significantly; TH positive reaction in striatum and staining was also significantly reduced (Figure 1B). The TH positive cell count in substantia nigra was found significantly increased in LPS+simv groups (P<0.05); TH positive response (OD value) and staining in striatum was also increased significantly (Figure 1C). Figure 2 shows the percentage of dopaminergic neuron loss in the 3 groups. The 78.6% of the dopaminergic neurons in the damaged side was found lost as compared with the undamaged side in LPS group (P<0.01), while TH positive neurons in LPS+simv group recovered to the level of 59.8% of the undamaged side (P<0.05).

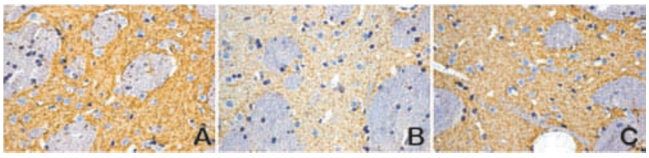

Figure 1. A, B, and C shows TH positive cells in the striatum of control, LPS, and LPS+simv groups (×400).

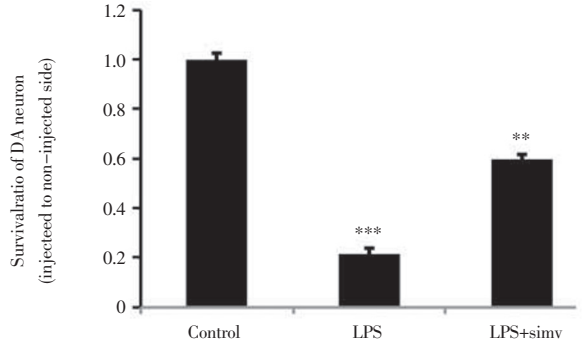

Figure 2. Ratio of dopaminergic neuron in the damaged side to non-damaged side in the 3 groups.

3.3. Changes in GFAP positive cells of substantia nigra

The GFAP positive cell count was found decreased in the undamaged substantia nigra of the rats in control, LPS, and LPS+simv groups, the star-shaped cell body was found with shallow staining (Figure 3A), and the difference among these 3 groups was not statistically significant (P>0.05). The count of GFAP positive cells increased significantly, protuberances shortened, and staining increased after 2-weeks of administration of LPS into substantia nigra (Figure 3B), with significant difference in mean OD as compared with control group (P<0.05); whereas, the count of activated GFAP positive cells were significantly decreased in the LPS+simv group as compared with LPS group (Figure 3C), and the OD difference was statistically significant (P<0.05) (Figure 4).

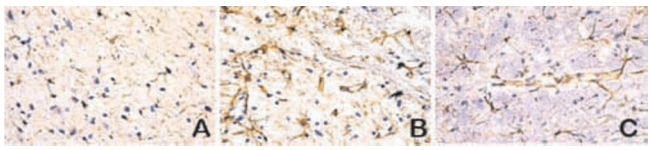

Figure 3. GFAP expression (*400×) A: control group; B: LPS group; C: LPS+simv group.

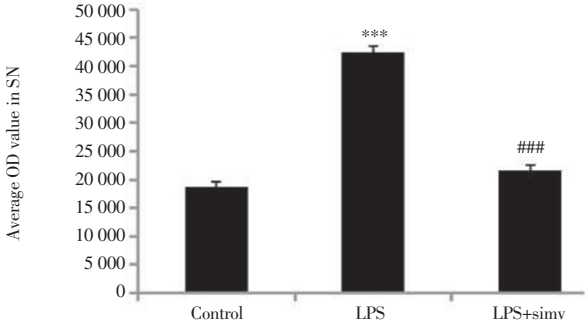

Figure 4. Mean OD value of GFAP in the 3 groups.
***P<0.01, compared with the control group; ###P<0.05, compared with the LPS group.

3.4. TNF-α change in substantia nigra

TNF-α expression was significantly increased in LPS group (LPS injected in substantia nigra) as compared with control group (P<0.01), while treatment with simvastatin effectively reduced the TNF-α expression (P<0.05) (Figure 5).

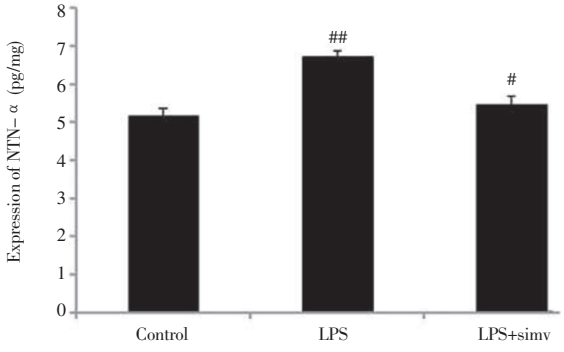

Figure 5. TNF-α expression in damaged substantia nigra of the rats. ##P<0.05, compared with the control group; #P<0.05, compared with LPS group.

4. Discussion

Etiology and pathogenesis of PD is not clearly understood to date. However, previous studies suggested that PD is caused by multi-factors including genetic factors, environmental factors, oxidative stress, and energy metabolism abnormalities[4]. Although no effective treatment is available for PD to date, neuroprotective treatments have been widely used to manage PD.

Santiago *et al*[5] found that the level of dopamine was significantly increased after treatment with simvastatin in inflammatory rat model (induced by LPS injection into striatum)[5], suggesting that simvastatin could result in neuroprotective effects. Mutez *et al*[6] retrospectively analyzed data from 419 PD patients with or without lipid-lowering therapy (statins or fibrates), and found that the time of PD onset delayed 9 and 8.7 years, respectively, in the patients with lipid-lowering therapy than those without lipid-lowering therapy. The findings of the present study showed that simvastatin treatment could significantly reduce rotational behavior induced by apomorphine as compared with the rats in the control group ($P<0.05$), suggesting that simvastatin could effectively improve rotational behavior induced by apomorphine. In addition, TH positive cells in substantia nigra and striatum were increased significantly (both $P<0.05$), TH positive response (OD) and staining in striatum increased, and TH positive neurons recovered to 59.8% level of the undamaged side ($P<0.05$), suggesting that simvastatin could effectively protect cell body of dopaminergic neurons in both substantia nigra and striatum, and the improvement of motor symptoms could be the results of protective effects on dopaminergic neurons in substantia nigra.

Recent studies showed that inflammation play an important role in PD[7]. As dopaminergic neurons are susceptible to inflammations and oxidative damages, neuroinflammation mediated by glial cells has attracted more and more attention. Previous studies revealed abnormalities in astrocytes and microglia's in patients with PD[8], suggesting the involvement of these two cells in the development of early stage PD. The exact effects of abnormal changes of astrocytes in patients with PD are not clearly understood. Recent studies showed that astrocytes could release soluble molecules including glutathione and neurotrophin, which could ensure the survival and normal function of neurons; in addition, astrocytes could also release cytokines and chemokines to prevent neurons from damages[9]. Increased expression of several inflammatory factors including interleukin (IL)-1, IL-6, and TNF-α could be found in such pathological processes[10]. TNF-α is a pro-inflammatory factor which could stimulate astrocytes to release more cytokines and chemokines, accelerate and expand inflammations, and thus induce neurocyte injuries[11]. The GFAP could only be found in astrocytes, and thus could be used as a specific biomarker in identifying astrocytes. The present study showed that rats with LPS induced lateral substantia nigra and striatum destructions had increased number of astrocytes and OD ($P<0.05$), suggesting the increased immunoreactivity; GFAP positive cells and OD were significantly decreased in simvastatin treatment group as compared with LPS group ($P<0.05$). Moreover, TNF-α secretion and expression decreased significantly in simvastatin treatment group as compared with LPS group ($P<0.05$). These results suggested that simvastatin could reduce the number and inhibit the activation of GFAP positive astrocytes in LPS induced rat models and decrease the TNF-α release. Hence, the author hypothesize that simvastatin could reduce the expression of TNF-α, inhibit astrocyte activation, and thus reduce the release of cytotoxic substances from activated astrocytes, which results in protective effects on dopaminergic neurons in substantia nigra. However, further studies are needed to investigate the mechanism of protective effects of simvastatin on PD.

Conflict of interest statement

We declare that we have no conflict of interest.

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