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Neuroprotective Effect of Schisandrin B and Schisandrin C of Schisandra Chinensis on Methyl-4-Phenyl-1,2,3,6 Tetrahydropyridine -Induced Parkinsonian Syndrome in C57BL/6 Mice

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Abstract

It is well known that Schisandra Chinensis (Turcz.) Baill. (Schi. Chinensis) has potential neuroprotective effects on neurodegeneration like Parkinson's Disease. Schisandrin B and Schisandrin C, which are isolated from Schi. Chinensis show better neuroprotective effects in PC12 cells. Based on these reports, this experiment is designed for the following purpose: To investigate the Neuroprotective Effect of Schisandrin B and Schisandrin C of Schisandra Chinensis on MPTP-Induced Parkinsonian Syndrome in vivo. ROS Assay Kit will be used to detect the Reactive Oxygen Species (ROS) level, and Immunohistochemistry will be used to evaluate TH-positive cells. There are two possible results in this experiment: (1) Schi B or Schi C has Neuroprotective Effect. (2) Schi B or Schi C doesn't have Neuroprotective Effect. Each possible result contains several possibilities, which will be revealed in the following part of the text.

Keywords

Schisandrin B; MPTP; ROS; TH; Schisandrin C.

1. Introduction

PD is a common neurodegenerative disorder set in the middle and old age[1], and affects the somatic motor nervous system most[2]. However, the etiology of PD is not clear. Many possibilities, such as mitochondrial dysfunction, oxidative stress, are temporarily inconclusive[3].

MPTP is a famous chemical which is to make Animal Parkinson's Model[4]. MPTP can function through a series of reactions before increasing in ROS[5]. High levels of ROS often imply apoptosis[6].

Schi. Chinensis is a Chinese medicine that has many positive effects. Previous studies have shown its role in antioxidant[7], including neuroprotective effects on PD[8], but it is not sure which substance plays the role. Schi. B and Schi. C are two lignans isolated from Schisandra. A study has demonstrated that they have a neuroprotective effect by the vitro experiments. Some tests confirm that intracellular ROS generation decreased, which is one of the possible causes of Parkinson's disease[9].

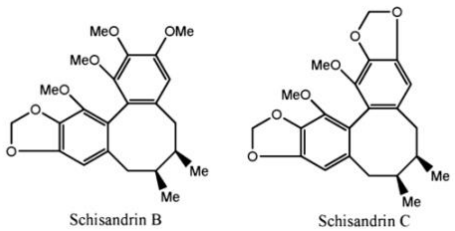

Figure.1 The chemical structures of two tested lignans[9].

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In this work, I made two hypotheses. Schi B and Schi. C are neuroprotective of MPTP-induced PD or not. ROS level will be determined using ROS Assay Kit [10] that will be mentioned in the methods section. TH level will be measured using Immunohistochemistry.

2. Materials and Methods

2.1 Chemicals and animals

2.1.1 Chemicals

In a previous study, the activity level of ROS was decreased in the treatment with Schi. Chinensis significantly [8]. Schi. B, and Schi. C at 5, 10, 25 μM, and 25, 50, 100μM, respectively, can reduce the amount of ROS [9]. According to the past papers, The content of Schi B in Schisandra is about 2.8 mg/g, Schi. C is about 0.6 mg/g [11]. After analyzing the above data, the concentrations of Schi. B in this experiment should be 2.8mg/kg, 5.6 mg/kg and 14.0 mg/kg, and Schi. C should be 0.75, 1.5 and 3.0 mg/kg. Gardenia jasminoides extract is known as a compound that has a neuroprotective effect against MPTP. And the best known effective concentration of this mixture is 100mg/kg, which will be used as positive control[12].

2.1.2 Animals

C57BL/6 mice will be used in this study. The animals should be healthy males. Following the suggestion of Food and Drug Administration guidelines, the dose for mice will be considered differently from a human dose.

All 96 animals will be randomly separated into twelve groups.

Table1. The treatment groups

Group	Treatment
Ⅰ **(normal control)**	triple-deionized water (1 mL/kg)
Ⅱ	MPTP (25 mg/kg, i.p.)
Ⅲ **(positive control)**	MPTP (25 mg/kg, i.p.) + Gardenia jasminoides extract (100mg/kg, p.o.)
Ⅳ	MPTP (25 mg/kg, i.p.) + Schi. B (2.8mg/kg, p.o.)
Ⅴ	MPTP (25 mg/kg, i.p.) + Schi. B (5.6mg/kg, p.o.)
Ⅵ	MPTP (25 mg/kg, i.p.) + Schi. B (14.0mg/kg, p.o.)
Ⅶ	MPTP (25 mg/kg, i.p.) + Schi. C (0.75mg/kg, p.o.)
Ⅷ	MPTP (25 mg/kg, i.p.) + Schi. C (1.5mg/kg, p.o.)
Ⅸ	MPTP (25 mg/kg, i.p.) + Schi. C (3.0mg/kg, p.o.)
Ⅹ	MPTP (25 mg/kg, i.p.) + Schi. B (2.8mg/kg, p.o.) + Schi. C (0.75mg/kg, p.o.)
Ⅺ	MPTP (25 mg/kg, i.p.) + Schi. B (5.6mg/kg, p.o.) + Schi. C (1.5mg/kg, p.o.)
Ⅻ	MPTP (25 mg/kg, i.p.) + Schi. B (14.0mg/kg, p.o.) + Schi. C (3.0mg/kg, p.o.)

It will take seven consecutive days to finish this experiment. Mice will be administrated with drugs 1 hour before MPTP by intraperitoneal injection. The mice will be killed on the last day, and the striatums will be isolated for immunohistochemistry studies and Western blot analysis [8].

2.2 Assay for ROS in the striatum

To detect the intracellular responsive oxygen level, ROS Assay Kit will be used. This kind of kit uses the change in fluorescence intensity to dye 2,7-Dichlorodi -hydrofluorescein diacetate (DCFH-DA). It could enter the cell and form into DCF, which is fluorescent, and the intensity of green fluorescence is proportionate to the level of ROS. Fluorescence signals will be detected using an enzyme-labeled

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instrument. The actual relative fluorescence unit (RFU) is equal to the sample RFU minus the control RFU. The actual RFU value increases indicate the high ROS content 10.

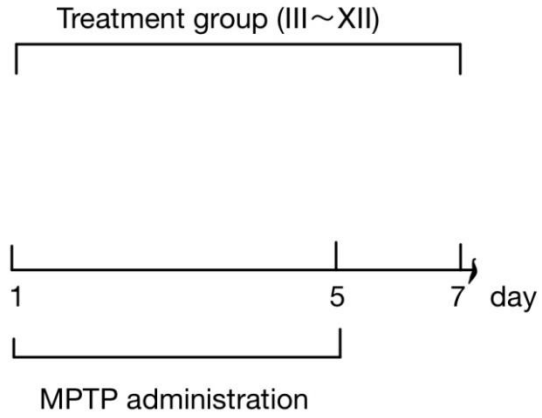

Figure 2. Animals administration

2.3 Immunohistochemistry

To deal with the striatum tissues of mice, 4% paraformaldehyde, 0.1 M phosphate-buffered saline (PBS), paraffin, will be used by steps. Polyclonal goat anti-rabbit immunoglobulins/HRP will be used to incubate. After a series of operations mentioned in the previous papers, the sections will finally be visualized with diaminobenzidine (DAB). Hematoxylin will be used to dye the sections, and the sealing solution will be used to seal at last. After doing the above, images of the stained slices will be captured. According to the following formula, TH-positive cells will be evaluated after the measurement of the number of TH-positive cells:[10]

$$\textbf{IHC Index} = (n1/T) \times 1 + (n2/T) \times 2 + (n3/T) \times 3.$$

2.4 Statistical Analysis

All the data will be expressed as mean ± standard deviation (n=12). All of the possible graphs will be presented by Sigma plot software. To statistical differences over groups, Dunnett's test will be used by ANOVA. The student's t-test will further analyze the data. $p < 0.05$ shows a significant difference [8].

3. Possible results

3.1 Neuroprotective Effect of Schi. B and Schi. C

3.1.1 Schi. B, Schi. C and the mixture of them decrease the generation of ROS and increase TH level in the striatum

Table 2. Several possibilities to support hypothesis 3.1.1

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	√	×	×
①	Ⅴ	√	×	√
	Ⅵ	√	√	√
	Ⅶ	√	×	×
②	Ⅷ	√	×	√
	Ⅸ	√	√	√
	Ⅹ	√	×	×
③	Ⅺ	√	×	√
	Ⅻ	√	√	√

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As an indication of ROS content, the actual RFU value of the striatum in the MPTP group increased compared with the first group. After treatment with drugs, the actual RFU value in the striatum decreased. The above results demonstrate that MPTP can increase striatum ROS content, while Schi B, Schi. C and Schi B + Schi C can reduce increased ROS.

The results also show that plenty of TH positive neurons appeared in the control group. Nevertheless, TH level in the MPTP treatment group decrease, and a substantial neuronal loss caused by MPTP is shown by statistical results. Different results show that those drugs rescue the MPTP-induced loss of TH-positive neurons.

Since it is uncertain about the effective concentration, as long as at least one group in a large group has a positive impact, this hypothesis is valid.

Table 2 shows the drug of this component can reduce the increase in ROS induced by MPTP and increase the level of TH, thereby having a neuroprotective effect. "×" indicates the exact opposite of the "√", indicating that this component of the drug has no effect. Ⅳ Ⅴ Ⅵ, Ⅶ Ⅷ Ⅸ, Ⅹ Ⅺ Ⅻ groups are divided into three large groups. Each group is independent of each other. The seven possibilities in the three groups can all be combined with other groups to form all the possibilities in 3.1.1. For example, possibility 1 in large group ① can combine with possibility 2 in large group ② and possibility 5 in large group ③.

3.1.2 Schi. B and Schi. C decreases the generation of ROS and increase the TH level in the striatum

Schi. B treatment, and Schi. C treatment shows a decrease in the actual RFU value and an increase in TH level. Still, Schi. B + Schi. C treatment group shows nothing about its ability. This result means Schi. B and Schi. C block the neurotoxicity of dopaminergic neurons induced by MPTP in mice independently.

Table 3. Several possibilities to support hypothesis3.1.2

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	√	×	×
①	Ⅴ	√	×	√
	Ⅵ	√	√	√
	Ⅶ	√	×	×
②	Ⅷ	√	×	√
	Ⅸ	√	√	√
	Ⅹ	×		
③	Ⅺ	×		
	Ⅻ	×		

In table 3, the meanings of symbols are the same as those in table 2. Ⅳ Ⅴ Ⅵ, Ⅶ Ⅷ Ⅸ, groups are divided into two large groups. Each of the 7 possibilities of the 2 large groups can be combined with each other to form the final result in 3.1.2.

3.1.3 Schi. B and the mixture of Schi. B and Schi. C decreases the generation of ROS and increase the TH level in the striatum

When the large group ② replaced ③ in 3.1.2 and failed to respond positively to the target indicators, the result of 3.1.3 appeared. This result means Schi. B and the mixture block the neurotoxicity of dopaminergic neurons induced by MPTP in mice.

In table 4, the meanings of symbols are the same as those in table 2. The seven possibilities in ①③ groups can be combined with each other to form all the possibilities in 3.1.3.

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Table 4. Several possibilities to support hypothesis 3.1.3

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	√	×	×
①	Ⅴ	√	×	√
	Ⅵ	√	√	√
	Ⅶ	×		
②	Ⅷ	×		
	Ⅸ	×		
	Ⅹ	√	×	×
③	Ⅺ	√	×	√
	Ⅻ	√	√	√

3.1.4 Schi. C and the mixture of Schi. B and Schi. C decreases the generation of ROS and increase the dopaminergic neurons in the striatum

When the large group ① replaced ③ in 3.1.2 and failed to respond positively to the target indicators, the result of 3.1.4 appeared. This result means Schi. C and the mixture block the neurotoxicity.

Table 5. Several possibilities to support hypothesis 3.1.4

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	×		
①	Ⅴ	×		
	Ⅵ	×		
	Ⅶ	√	×	×
②	Ⅷ	√	×	√
	Ⅸ	√	√	√
	Ⅹ	√	×	×
③	Ⅺ	√	×	√
	Ⅻ	√	√	√

In table 5, the meanings of symbols are the same as those in table 2. The seven possibilities in ②③ groups can be combined with each other to form all the possibilities in 3.1.4.

3.1.5 The mixture of Schi. B and Schi. C decreases the generation of ROS and increases the dopaminergic neurons in the striatum

The mixture treatment shows a decrease in the actual RFU value and an increase in TH level, but nothing positively shows in Schi. B treatment and Schi. C treatment. This result means the mixture blocks the neurotoxicity.

Table 6. Several possibilities to support hypothesis 3.1.5

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	×		
①	Ⅴ	×		
	Ⅵ	×		
	Ⅶ	×		
②	Ⅷ	×		
	Ⅸ	×		
	Ⅹ	√	×	×
③	Ⅺ	√	×	√
	Ⅻ	√	√	√

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In table 6, the meanings of symbols are the same as those in table 2. There are only 7 possibilities in 3.1.5.

3.1.6 Schi. B decreases the generation of ROS and increases the dopaminergic neurons in the striatum.

When the large group ② replaced ③ in 3.1.5 and failed to respond positively to the target indicators, the result of 3.1.6 appeared. This result means Schi. B blocks the neurotoxicity.

Table 7. Several possibilities to support hypothesis 3.1.6

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	×		
①	Ⅴ	×		
	Ⅵ	×		
	Ⅶ	√	×	×
②	Ⅷ	√	×	√
	Ⅸ	√	√	√
	Ⅹ	×		
③	Ⅺ	×		
	Ⅻ	×		

In table 7, the meanings of symbols are the same as those in table 2. There are only 7 possibilities in 3.1.6.

3.1.7 Schi. C decreases the generation of ROS and increases the dopaminergic neurons in the striatum

When the large group ① replaced ③ in 3.1.5 and failed to respond positively to the target indicators, the result of 3.1.7 appeared. This result means Schi. C blocks the neurotoxicity.

Table 8. Several possibilities to support hypothesis 3.1.7

Large Group	Group	Possibility 1	Possibility 2	Possibility 3
	Ⅳ	√	×	×
①	Ⅴ	√	×	√
	Ⅵ	√	√	√
	Ⅶ	×		
②	Ⅷ	×		
	Ⅸ	×		
	Ⅹ	×		
③	Ⅺ	×		
	Ⅻ	×		

In table 8, the meanings of symbols are the same as those in table 2. There are only 7 possibilities in 3.1.7.

3. 1.8. Schi. B or Schi. C doesn't have Neuroprotective Effect

None of the treatment groups show any decrease in the actual RFU value of the striatum. The level of TH doesn't increase in all of the groups. This result means neither Schi. B nor Schi. C blocks the neurotoxicity of dopaminergic neurons induced by MPTP in mice.

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4. Discussion

To explore the neuroprotective effect, I hypothesize eight different results. There are three possibilities in a similar model. In a large group, three “√” means all of these groups have positive effects. If only one or two “√” appear, the results also support my hypothesis and indicate that other concentrations may not work on MPTP due to the small metering or the first-pass effect or other reasons.

The neuroprotective effects of Schi. B and Schi. C is studied by measuring the actual RFU value of the striatum, and TH level in the striatum. The increase of the actual RFU value means the rise of the ROS level. The severity of PD is reflected by the reduction of TH-positive neurons.

Table9. Results analysis

Result	Efficient medicine			Stands for
	Schi. B	Schi. C	Mixture	
3.1.1		+		Fully supports my hypothesis.
3.1.2	+	+	-	Because both of them exist in an organism at the same time, and the mixture of the two can’t play an ideal role, thus indicating that the active component in Schi. Chinensis extract has other substances
3.1.3	+	-	+	Schi. C may enhance or weaken or doesn't make any efforts in the mixture.
3.1.4	-	+	+	Schi. B may enhance or weaken or doesn't make any effort in the mixture.
3.1.5	-	-	+	Schi. B and Schi. C can’t function alone maybe because one of the substances is necessary for the other to operate.
3.1.6	+	-	-	When Schi. B and Schi. C are shared, Schi. C may inhibit Schi. B activity so that it can’t play a role.
3.1.7	-	+	-	When Schi. B and Schi. C are shared, Schi. B may inhibit Schi. C activity so that it can not play a role.
3.1.8		-		Doesn’t support my hypothesis.

In table 9, “+” means the drug works and makes positive effects no matter the concentration. “-” indicates that the drug doesn’t make any efforts to make positive effects. Second to seventh result partially supports my hypothesis.

5. Conclusion

This study includes some hypotheses about the neuroprotective effect of Schi. B and Schi. C through detecting the actual RFU value of the striatum, and the intensity of TH level by Immunohistochemistry. The results of my research can determine the effect of chemicals of Schi. Chinensis and try to describe the neuroprotective effects of Schi. B and Schi. C against MPTP-induced neurotoxicity in mice. If we know which substances are useful against PD, we can use Schi. Chinensis more efficiently, like using active ingredients as lead compounds for drug development. We can also learn from the methods of Chinese medicine treatment to figure appropriate dosage forms out and find suitable targets to prevent or treat Parkinson's disease or similar diseases continually.

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