·Basic Research·

Protective effect of Lycium barbarum polysaccharide on retinal ganglion cells *in vitro*

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Abstract

• AIM: To observe the effect of Lycium barbarum polysaccharide (LBP) on rat retinal ganglion cells (RGCs) *in vitro*.

• METHODS: Retinal cells of neonatal Sprague-Dawley rats were collected 1 to 3 days after birth, and co-cultured with different concentrations of LBP for 24 hours. Absorbance values (OD) were recorded using MTT assay for calculating survival rates.

• RESULTS: All the test groups had protective effects on RGCs. The group with 10mg/mL concentration of LBP had the most significantly difference of OD value compared with that in control group (P<0.01).

• CONCLUSION: LBP can increase the survival rate and promote the growth of mixed cultured rat RGCs.

• KEYWORDS: Lycium barbarum polysaccharide; *in vitro*; retinal ganglion cells

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INTRODUCTION

Lycium barbarum can nourish liver and kidney, enrich essence and blood and improve eyesight according to Traditional Chinese Medicine. It's widely used for the treatment of ocular disorders. Lycium barbarum polysaccharide (LBP), extracts from Lycium barbarum, is an important water-soluble component which has protective effect in promoting survival and prolonging growth of RGCs.

MATERIALS AND METHODS

Animals Sprague-Dawley rats (1-3 days old with weight of 6-8g) supplied by Animal Research Center of Chengdu University of TCM were used in this study, both female and male rats with breast-feeding were contained.

Main reagents and equipments Mouse anti-rat monoclonal antibody (Sigma, USA), DMEM (Sigma, USA), methylthiazolyte-trazolium (MTT, Sigma, USA), trypsin (Gigco, USA), HEPES (Sigma, USA), bovine serum (Biochemical Products Factory, Huaxi, China), fetal bovine serum (Sijiqing Company, Hangzhou, China). Inverted phase contrast microscope (HB050 type, Zeiss, Germany)

All experimental protocols and procedures for isolating and culturing RGCs have followed the methods of Wu *et al*[1].

Mixed cultivation of RGCs Twenty SD rats with 1-3 days old were scarified by decapitation and eyeballs were immediately enucleated. Tissues were soaked in 40mL sodium chloride with 1 million units of penicillin; the retinas were then isolated under microscope and digested by 0.125% trypsin for 20 minutes. After sterile filtration with 220-mesh, bovine serum was used for termination of the digestion, and followed with centrifugation (1 000r/min) for 5 minutes. The suspension was collected by removing supernatant and repeated pipetting with 20% DMEM culture medium.

Cell purification Petri dishes of 100mm diameter were blocked with goat anti-mouse IgG primary antibody (1:100, PBS diluted, IR-2080) for 24 hours at room temperature.

After washed by culture medium 3 times, the dishes were blocked with mouse anti-rat Thy-1.1 single monoclonal secondary anti-body (1:150, PBS diluted, M-7898) at room temperature for 24 hours, and then washed as before.

The retinal cells suspension and 20% DMEM medium were added to the coated dishes and incubated for 30 minutes at 37℃ for fully integration between RGCs and Thy-1.1 monoclonal antibody. After repeated washing to remove the 0.125% trypsin for 10 minutes, and then terminated by fetal bovine serum. Supernatants were removed followed 1000r/min centrifugation for 5 minutes. And finally, 20% DMEM culture medium were added into dishes and followed by repeated pipetting, the cell suspension were made for cell counting and cultivation.

MTT Using MTT assay, the protective effects of LBP on the survival of RGCs were observed. The suspension of the purified RGCs were added into 96-well plates in 1 000cells/mm^2 density, and incubated with 5% CO_2 for 24 hours at 37℃ for completely adherence. After washed by serum-free culture medium 3 times, the cells were divided randomly into 8 groups with 7 holes in each. The final concentrations of LBP in test groups were different, including 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064mg/mL. Blank plates were used as control group. All groups were incubated with 5% CO_2 for 24 hours at 37℃, followed by incubation with 100μL MTT for 4 hours, and then terminated by 150μL DMSO medium. OD values were measured for evaluation of survival rate.

Survival rate= (OD of test group---OD of control group)/OD of control group×100%

Statistical Analysis Analysis-of-variance was used by SPSS 16.0 software for Windows statistical software in our study. Data were presented as mean ±standard deviation. Statistically significant difference was set at P<0.01.

RESULTS

Cells adherence was observed after cultivation for 3-4 hours under phase contrast microscope. The adherent cells were round and dark in central with obvious halo. Followed by 24-48 hours incubation, cellular processes extended from surface of cells, resulted in polygonal cell shape. Most of the cells had strong refraction and trended to aggregation growth. The processes were increased and elongated after 72 hours.

OD values were measured by MTT assay (Figure 1), the difference between each groups were illustrated as below (Table 1).

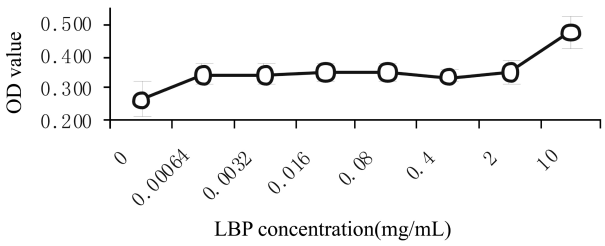

Figure 1 OD values of each group

Table 1 OD values of RGCs and the survival rate

Groups (LBP concentration, mg/mL)	Mean±SD	Survival rate (%)
1(10)	0.48±0.0520[1]	208
2(2)	0.35±0.0234[1]	86
3(0.4)	0.34±0.0382[1]	72
4(0.08)	0.35±0.0231[1]	83
5(0.016)	0.35±0.0393[1]	84
6(0.0032)	0.35±0.0308[1]	74
7(0.00064)	0.35±0.0342[1]	76
8 control group	0.27±0.0527	52

[1]the OD values in test groups were significantly different from control group

DISCUSSION

Lycium barbarum (Gouqizi, Wolfberry), which is sweet in taste and neutral in nature, was first described in "Shen Nong's Herbal Classic". It can nourish kidney, marrow and liver, and improve eyesight, hence it is widely used for the treatment of various eye diseases. According to the researches in recent years, Lycium barbarum contains betaine, polysaccharides, crude fat, crude protein, riboflavin, carotene and other chemical components. However, investigations on the therapeutical essential components and the target cells in various eye diseases were scarce. LBP is water-soluble polysaccharide and is the major functional component which is derived from extraction of Lycium barbarum. Modern medical studies shows that LBP is the main active ingredient for modulating immunity and anti-aging, it has effects of improving fatigue, poor appetite and blurred vision in elderly, and lowering blood-fat, anti-fatty liver, *etc* [2-6].

It was reported that LBP has protective effect on rat retinal cells, especially in the first 8 weeks [7]. It has been shown to protect visual functions in light-induced phototoxicity[8]. The researches by University of Hong Kong in 2006-2011 demonstrated that LBP has neuroprotective effects on RGCs in glaucoma [9,10], which is the first *in vivo* report showing LBP can significantly decrease the apoptosis of RGCs in rat with high intraocular pressure[11].Liu *et al* [12,13] found that this effect was probably by protecting RGCs from apoptosis and

expressing of Caspase-2 at the early stage in retinal degeneration without toxicity.

The present study focused on the co-cultivation of LBP and RGCs for 24 hours, and evaluated the survival rate using MTT assay. The results showed that all of the 7 groups treated with LBP in different concentrations can promote the survival rate of RGCs *in vitro*, which indicated the protective effect on RGCs. The group with 10mg/mL concentration of LBP had the most significantly difference of OD value compared with control group (P<0.01). The neuroprotective effect of LBP may provide a new way for the treatment of nerve protection in glaucoma, but further study is still needed for investigating the mechanism of the protective effect of LBP on RGCs *in vitro*.

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