• Brief Report •

Effect of Qingguang'an II on expressions of OX42 protein and IL-1β mRNA of retinal microglia cells of rats with chronic high intraocular pressure

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Abstract

• The study investigated the effects of Qingguang'an II (a Chinese medicinal preparation) on expressions of OX42 protein and interleukin-1β (IL-1β) mRNA of retinal microglia cells of rats with chronic high intraocular pressure (IOP). SD rats were randomly divided into 6 groups, that were: A: blank group; B: model group; C: Qingguang'an II low dose group; D: Qingguang'an II medium dose group; E: Qingguang'an II high dose group; F: Yimaikang disket (a Chinese medicinal preparation) group. Experimental rats in B, C, D, E, F groups were established the model of chronic high IOP by cauterizing of superficial scleral vein. Tissues of eyes were obtained after intragastric administration for 2 and 4wk. At the time-point of 2wk, OX42 protein and IL-1β mRNA in group B were statistically expressed in higher level comparing with other groups (*P*<0.05). Moreover, at the time-point of 4wk, OX42 protein and IL-1β mRNA in groups C, D and E were statistically expressed in lower level comparing with group F (*P*<0.05). Besides, OX42 protein and IL-1β mRNA in groups C and D were statistically expressed in higher level comparing with group E (*P*<0.05). OX42 protein and IL-1β mRNA in groups C and D were expressed in similar level (*P*>0.05). The study indicated that, in the protection of optic nerve of rats with chronic high IOP, the high dose of Qingguang'an II at the time-point of 4wk was the better choice.

• KEYWORDS: Qingguang'an II; chronic high intraocular pressure; OX42 protein; interleukin-1β mRNA

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INTRODUCTION

Glaucoma is a kind of blind-causing disease with structural injury of optic nerve and defection of visual field. Pathological high intraocular pressure (IOP) is a major risk factor for glaucoma patients, thus it is necessary to protect the optic nerve in time when high IOP appeared. With advancing in research techniques and methods, immune factors are considered to be involved in the process of optic neuropathy in glaucoma[1]. Microglia cells, the immune cells of retina, played a particularly significant role in this process[2-4]. For years of clinical practices and theoretical researches, our team had found that "bloodstasis and water retention" were the pathogenesis of glaucoma on early stage. Therefore, based on Buyang Huanwu Decoction (a Chinese medicinal formula), tutor founded a pure traditional Chinese medicinal preparation: Qingguang'an granule. On the basis of the pathological mechanism of injury of glaucomatous optic nerve in traditional Chinese medicine (TCM) and Qingguang'an granule, Qingguang'an II was be created. The preliminary results have shown that, to a certain extent, Qingguang'an II can ameliorate cell layer atrophy and reduce retinal ganglion cell apoptosis which induced by chronic high intraocular pressure. Thus, the animal experiment was designed to investigate the effect of Qingguang'an II on expressions of OX42 protein and interleukin-1β mRNA of retinal microglia cells in chronic high IOP.

METHODS

A total of 40 healthy male SD rats were included in the study, which were provided by Experimental Center of Human University of Traditional Chinese Medicine of China, with an average weight of 180-200 g.

The rats were randomly divided into 6 groups: Group A: blank group; Group B: model group; Group C: low dose group of Qingguang'an II; Group D: medium dose group of Qingguang'an II; Group E: high dose group of Qingguang'an II; Group F: Yimaikang disket (a Chinese Medicinal preparation) group.

Table 1 Average optical density of OX42 protein of each group mean±SD

Time	Group A	Group B	Group C	Group D	Group E	Group F
2wk	88.16±3.62[a]	132.20±4.28	109.03±4.91[a]	108.18±7.60[a]	107.67±3.00[a]	109.43±1.98[a]
4wk	88.40±2.73[b]	151.96±4.21	98.80±2.62[b,c,d]	98.41±2.41[b,c,d]	94.27±1.94[b,c]	106.15±2.85[b]

[a]P<0.05 compared with group B; [b]P<0.05 compared with group B; [c]P<0.05 compared with group F; [d]P<0.05 compared with group E.

Table 2 The relative expressions of IL-1β mRNA of each group mean±SD

Time	Group A	Group B	Group C	Group D	Group E	Group F
2wk	1.60±0.66[a]	6.52±2.02	4.20±0.57[a]	3.09±0.85[a]	3.43±0.83[a]	3.78±0.60[a]
4wk	1.72±0.53[b]	9.05±1.02	2.60±0.56[b,c,d]	2.51±0.54[b,c,d]	1.63±0.36[b,c]	3.55±0.53[b]

[a]P<0.05 compared with group B; [b]P<0.05 compared with group B; [c]P<0.05 compared with group F; [d]P<0.05 compared with group E.

In addition to group A, the other groups were be modeled. The establishment of animal model of chronic high IOP referenced to Wang *et al*'s[5] methods, that was, cauterizing superficial sclera vein. If the postoperative IOP sustained above 25 mm Hg for 8wk, it would be treated as the successful modeling.

Group A and group B were lavaged saline water for 12 mL/kg. Group C were 6.75 g/kg·d (it was converted according to the equivalent dose "human-animals' body surface area ratio table" and equivalent to adults' dosage). Group D was 13.5 g/kg·d (it was equivalent to 2 times of the adults' dosage). Group E was 27 g/kg·d (it was equivalent to 4 times of the adults' dosage). Group F was lavaged 0.22 g/kg·d.

The rats were anesthetized and enucleated eyeball after lavaging for 2wk or 4wk. In each group, 5 eyeballs were selected and saved in 4% paraformaldehyde solution, for immunohistochemical detection. The remaining eyeballs were peeled off the retina and preserved in the refrigerator of -80℃ for quantirative polymerase chain reaction. The experiment extracted total RNA by Trizol. RNA reverse transcriptions proceed by 30 μL reaction system and then searching target gene sequence in the NCBI. Primer synthesis was completed by Genscript Corporation of Nanjing. IL-1β-F: 5'-TGTGATGTTCCCATTAGAC-3', IL-1β-R: 5'-AATACCACTTGTTGGCTTA-3', product length: 131 bp. Method of $2^{-2\text{-}\Delta\Delta Ct}$ calculated the relative expressions.

RESULTS AND DISCUSSION

The Expressions of OX42 Protein of Retinal in Chronic High Intraocular Pressure In retina, there were small amount of positive expressions of OX42 protein in nerve fiber layer, ganglion cell layer and inner plexiform layer. At the time-point of 2wk: the expressions of OX42 protein in group B were statistically expressed in higher level comparing with other groups (P<0.05). Moreover, at the time-point of 4wk: OX42 protein in groups C, D and E was statistically expressed in lower level comparing with group F (P<0.05). Besides, OX42 protein in groups C and D was statistically expressed in higher level comparing with group E (P<0.05). OX42 protein in groups C and D was expressed in similar level (P>0.05) (Table 1).

The Relative Expressions of IL-1β mRNA of Retinal in Chronic High IOP There are little expressions of IL-1β mRNA in normal retina. At the time-point of 2wk, the expressions of IL-1β mRNA in group B was statistically expressed in higher level comparing with other groups (P<0.05). Moreover, at the time-point of 4wk, IL-1β mRNA in groups C, D and E was statistically expressed in lower level comparing with group F (P<0.05). Besides, IL-1β mRNA in groups C and D was statistically expressed in higher level comparing with group E (P<0.05). IL-1β mRNA in groups C and D was expressed in similar level (P>0.05) (Table 2).

Persistent high IOP may cause chronic and progressive loss of nerve cells. Activated microglia cells have been recognized as a pathological feature of neurodegenerative diseases of the retina [6-8]. OX42 protein was the symbol protein of the activated microglia[9] and had little positive expressions in resting state. When meeting stimulation, they gradually transformed into ameboid cell, which were much closer to the phenotype of phagocyte[10]. IL-1β has been a pleiotropic and highly active cytokines[11-12], which can mediate the occurrence and development of systemic inflammatory, degenerative and ischemia reperfusion injury disease[13-14]. As a kind of potential neurotoxicity to promote apoptosis, it is mainly composed by glial cells especially activated microglia cells in the retina[15]. In the study we found that the positive expressions of OX42 and the release of IL-1β mRNA increased gradually in state of continuous high IOP.

Theories of TCM think that the main pathogenesis of optic nerve injury by chronic high IOP is deficiency of liver and kidney, deposition of aqueous humor, disharmony of qi and blood, impassability of context and blockage of sweat pores. The main effects of Qingguang'an II were tonifying, focusing on supplying yin of the liver and kidney. Deficiency of qi and yin for a long time may lead to blood stasis, thus on the basis of tonifying, herbs of activating blood circulation, alleviating water retention and moving qi were used at the same time. On one hand, qi movement promotes transportation of water and blood, on the other hand the whole prescription were tonify

without causing stagnation. The whole prescription combined tonic with dredging, which making eye unobstructed and harmonizing qi and blood, and all symptoms were resolved.

In this study, the method of cauterizing superficial sclera vein was adopted to make rats in a state of chronic high IOP. Then we observed the functions of Qingguang'an II on the expressions of OX42 protein and IL-1β mRNA in different periods. The low, medium and high dose of Qingguang'an II and Yimaikang disket could inhibit the activation of retinal microglia cells and secretion of IL-1β after chronic IOP in rats with optic nerve injury. The inhibitory effects in low, medium and high dose of Qingguang'an II were better than that in Yimaikang disket. Among them, the high dose of Qingguang'an II was optimal. This animal experimental study preliminarily clarified the relationship between retinal microglia cells and optic nerve injury due to chronic high IOP. We thought that Qingguang'an II has a protective effect on optic nerve injury caused by chronic high IOP and one of the mechanisms may be inhibit the expressions of OX42 protein and IL-1β mRNA of retinal microglia cells.

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