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Influence of Bushenhuoxue on primary visual cortex's Nissl bodies damage in rat model of chronic elevated intraocular pressure

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Abstract

• AIM: To observe the effect of Traditional Chinese Medicine (TCM) of Bushenhuoxue on primary visual cortex (PVC)'s Nissl bodies damage in rat model of chronic elevated intraocular pressure (EIOP), and explore the mechanism of it initially.

• METHODS: The rat model of chronic EIOP was established by unilaterally cauterizing 3 episcleral vessels, then 30 rats were divided into 3 groups randomly: control group, model group, and treatment group. After given drugs or normal saline for 8 weeks, the rats were put to death. The effect of intraocular pressure (IOP), content of Nissl bodies and ultrastructure of neuron cell in the PVC was observed.

• RESULTS: Unilaterally cauterizing episcleral vessels increased IOP of the rat modle obviously, there was significant difference compared with preoperative ($P<0.01$). Semi-quantitative pathological analysis showed that Nissl body of total area in the model group was (34941 ± 8482.1) S/μm^2, mean optical density was (152.8±27.97), integrated optical density was (11993±3084.8), compared with the control group, total area was (55742±6348.1) S/μm^2, mean optical density was (304.04 ± 100.1), integrated optical density was (18219±3548.9), there were statistically differences (all $P<0.05$). There were statistically difference in Nissl body of total area, mean optical density and integrated optical density between model group and treatment group, total area was (46406±5989.3) S/μm^2, mean optical density was (251.05 ± 77.41), integrated optical density was (16899±4040.6, all $P<0.05$).

• CONCLUSION: TCM of Bushenhuoxue can repair PVC's damage in the rat model of chronic EIOP by enhancing expression of Nissl body, improving ultrastructure of neuron cell.

• KEYWORDS: glaucoma; rat model of chronic elevated intraocular pressure; TCM of Bushenhuoxue; primary visual cortex; Nissl bodies; ultrastructure of neuron cell

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INTRODUCTION

Glaucoma is a group of eye disease that caused damage to the optic nerve atrophy and visual field loss in a characteristic pattern, the pathologic increased pressure is one of major risk factors[1]. In the past, to control the IOP in a safe level (target-level) was the only credible evaluation, but it cannot stop the visual function getting worse and worse. Many domestic and international studies demonstrated that even if controlled the IOP, the patients' visual field defect (about 40%-50%) also developed as chronic and continual advancement[2,3]. Recently years, someone think that this damage will affect the visual center, maybe it is a neurodegenerative disease of the whole visual system. The visual dysfunction was a process which damaged extensive from RGCs to the senior visual center and the injury on each neuron had complex interaction[4,5]. It caused us to rethink the occurrence and development mechanism of glaucomatous optic function damage from the optic path, so that it can provide new ideas for clinical therapy of the glaucoma. The primary visual cortex on cerebral occipital lobe is the center of human visual system. It had been proved that if the visual cortex damaged, the visual function damaged too[6]. However, no report on intervention used by TCM. This experiment used by unilaterally cauterizing 3 episcleral vessels to induce the SD rat model of chronic, moderate EIOP, to observe the effect of Bushenhuoxue on PVC's in rat model of chronic EIOP.

MATERIALS AND METHODS

Materials Thirty, female and male, 8-12 week-old, Sprague Dawley rats (SD rats), weighed about 150-200g, conform the standards of the first class experimental animals,

were fed with whole value grain feedstuff. Rats and the feedstuff were provided by Laboratory Animal Center of Chengdu University of Traditional Chinese Medicine. The raising room temperature was 20℃ - 25℃, fresh air, circulation day and night, relative humidity was 55% -75%, with 12 consecutive hour light exposure, eat and drink freely. Inclusion criteria: 1) without external ocular disease; 2) normal binocular direct light reflex and indirect light reflex; 3) without crooked neck.

Medical and reagent Fufangdansenpian (Batch number: 7122426) and qijudihuangwan (Batch number: 7072153) were bought from Peking Tongrentang Pharmacy Co., Ltd. BDNF polyclonal antibody (Batch number: BA0565) was bought from Bausch & Lomb Biological Engineering Co., Ltd.

Methods

Modeling After 3 days adaptive feed, measured the IOP and estimated the normal pressure region which the average was from 9mmHg to 18mmHg. Then, 30 SD rats were randomly assigned to control group, model group and treatment group. Model group and treatment group were operated to monocular (right eye) model, The left eye was not given any treatment. Methods as follows: Rats were anesthetized with injection of pentobabitone sodium (3%, 1.5mL/kg) after weighed and fixed. Then given the rats topical anesthesia of proxymetacaine (0.5%). Cut out the upper bulber and separated fascia far away from corneoscleral limbus 1-2mm on 10 o'clock to 2 o'clock. Then exposed the three episcleral vessels which nearby the equator 3mm-4mm on 10 o'clock, 12 o'clock and 1 o'clock. Next, cautuizing the 3 episcloral, reconstructured bulbar conjunction and coated chiortetracycline eyepaste. When the rats revived , sent back to the cage, dropped chioromycetin (0.25%) twice a day. The control group had been done the same operation except cauterizing the vessels. Four rats died for mal-intragastric administration.

Grouping and medicine administration Each groups were given medicine 8 weeks, methods as follows: Control group, model group were administrated with equivalent 3mL sodium chioride every day. The treatment group were treated by fufangdansenpian (0.96g/kg daily) and qijudihuangwan suspension (3.0g/kg daily, as 20 times of the adult daily dose). The gastric perfusion was given 8 weeks in the same time once a day, weighed the rats every two weeks and adjusted the dosage.

IOP detection In this experiment, the IOP measurement was taken at the same time everyday (2:00 p.m. -5:00 p.m.). Used the handheld tonomecer (TONO-PEN) to measure the IOP 3 days before operation. Taken the average as the normal pressure. To measure the IOP during post - operation immediately, week 1, 2, 4, 6 and 8. This experiment persistence 8 weeks.

Histopathological observation After operation 8 weeks killed the rats by cervical vertebra dislocation, opened skull, peeled brain tissue and took it into the complex stationary liquid immediately. Then injected the liquid into the middle of interhemispheric. The specimen was fixed 72 hours, took the rat cerebral location stereogram as example to open and locate it[7]. Taken the 17 distribution of left occipital lobe (PVC), dehydrated in an ethy alcohol series, xylene transparent, embedded in paraffin, sectioned at 10μm, dryed and stained it by Nissl immunohistochemical method (toluidine blue staining). The Nissl body colored dark blue (toluidine blue). Each groups measured the 6 rats' total area, mean opticoldensity and intergrated optica in every visual field randomly (Each randomly selected from 5 perspectives).

Ultrastructure of primary visual cortex Randomly selected one SD rat each group, fixed, anetheticed, decapitated, and cut PVC into $2mm^3$ pieces. Next, put it into the liquid with glutaraldehyde (3%), refrigerated it 2 hours at 4℃ and fixed it 30 minutes with osmium acid, the ultrastructure of primary visual cortex was observed. This observation process finished in the Electron microscopy room of west China medical center of Sichuan university.

Statistical Analysis Analysis of variance was used by SPSS 13.0 software for Windows statistical software in our study. Paired t test was used for before and after comparison, while one-way analysis of variance was conducted for comparison between groups. Data were presented as mean ± standard deviation. Statistically significant difference was set at $P < 0.05$ or $P<0.01$.

RESULTS

IOP compared between each group As shown in Table 1, the IOP had no significant difference between groups before operation ($P>0.05$). Immediately modeling, the IOP of model and treatment group were highly significant different compared with control group ($P<0.01$), it indicated modeling success. While model group and treatment group had no significant difference ($P>0.05$), there were equilibrium between groups. After 8 weeks modeling, we found the IOP of model group and treatment group had significant difference compared with control group and pre-modeling ($P<0.01$), it showed that the elevate intraocular pressure maintained good, while model group and treatment group had no significant difference ($P>0.05$).

Rats' Nissl body comparison of each groups in PVC As shown in Table 2 and Figure 1A, B and C, the influence of Bushenhuoxue on the expression of PVC's Nissl body as follows: 1) The total area, mean optical density and intergrated optical density of model group were significantly less than control group ($P<0.05$); 2) Compared with the model group, the total area, mean optical density and intergrated optical density of the treatment group were higher ($P<0.05$). The results demonstrated that TCM of Bushenhuoxue can improve the expression of Nissl body in PVC; 3) The total area, mean optical density and intergrated optical density of treatment had no significant difference compared with control group ($P>0.05$). The results demonstrated that TCM of Bushenhuoxue can improve the expression of Nissl body in PVC.

Table 1 Comparison of IOP before and after modeling in each groups $\bar{x}\pm s$

Groups	*n*	Pre-modeling	Immediately modeling	Given medicine 8 weeks
Control group	9	10.36±2.4661	11.42±3.1315	12.04±3.8293
Model group	9	10.54±3.4946	28.14±7.3919[b, d]	27.58±6.3129[b,d]
Treatment group	8	9.52±4.0162	31.74±8.3153[b, d]	25.64±5.5894[b,d]
F		0.253	28.573	22.565
P		0.778	0.000	0.000

[b]$P<0.01$ *vs* pre-models building; [d]$P<0.01$ *vs* control group.

Table 2 Rats' Nissl body comparison of each groups in PVC $\bar{x}\pm s$

Groups	Total area ($S/\mu m^2$)	Mean optical density	Integrated optical density
Control group	55742±6348.1	304.04±100.1	18219±3548.9
Model group	34941±8482.1[a]	152.8±27.97[a]	11993±3084.8[a]
Treatment group	46406±5989.3[c]	251.05±77.41[c]	16899±4040.6[c]
F	13.191	6.312	5.039
P	0.000	0.010	0.021

[a]$P<0.05$ *vs* control group; [c]$P<0.05$ *vs* model group.

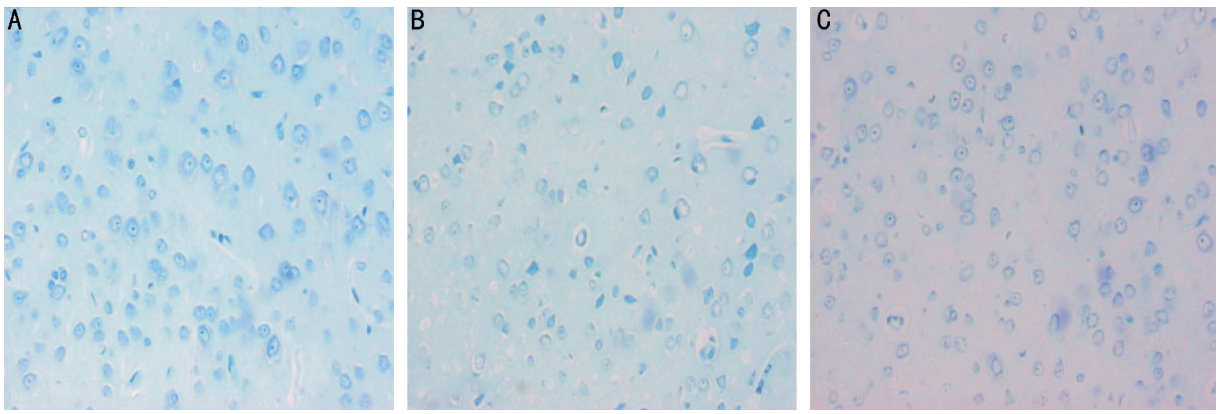

Figure 1 Nissl body stained in PVC (200×) A: control group; B: Model group; C: Treatment group.

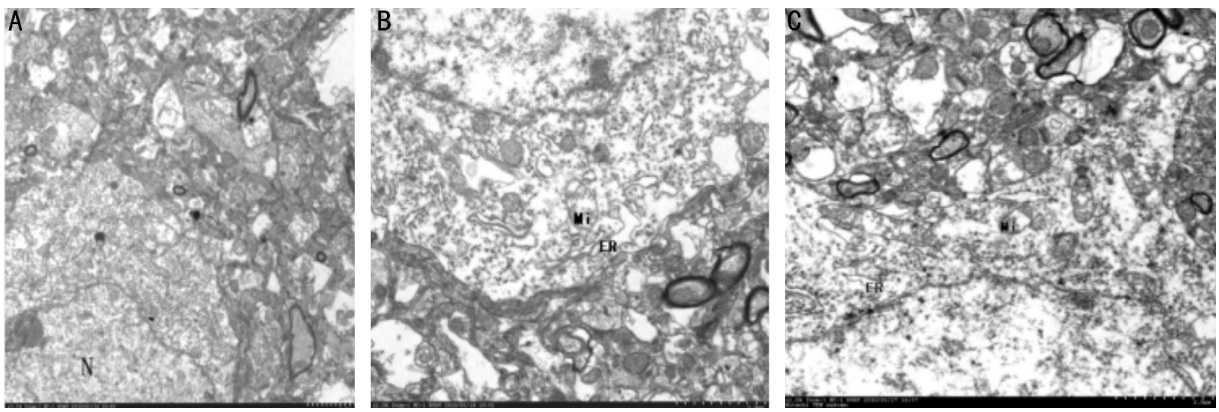

Figure 2 Ultrastructure of each groups in PVC's neuronal cells(12000×) A: Control group; B: Model group; C: Treatment group.

Ultrastructure of each groups in PVC's neuronal cells

The axon lined uniformity and close. The nucleus looks like round or oroid, the organelles were rich in cytoplasm. Mitochondria, spine and the endoplasmic reticulum can be seen clearly (Figure 2A). The dilatation of endoplasmic reticulum looks like vacuole with swelling mitochondria, crista break and vacuolation (Figure 2B). The axon lined uniformity with loose structure and a little edma. The organelle in cytoplasm were light decreased. The mitochondria slight swelled with breaking crista. The endoplasmic reticulum dilated partly (Figure 2C).

DISCUSSION

Glaucoma is a multiple factors disease. The mechanism of visual dysfunction was still unclear. In recent years, the study show that glaucoma is a kind of neurodegenerative disease in visual system, and the man-made damage of PVC can induce the RGCS happened retrograde trans-neuronal degeneration. The mechanism of it was considered to be due to the decrease of the neurotrophic factor what the RGCs get from PVC[8,9]. On the other hand, when the RGCs further reduced, it must

further decrease the visual afferent impulses and aggravated the disuse atrophy degeneration of PVC. The vicious circle existed in RGCs and the damage of PVC maybe played an important role in the progress of visual function damage[5]. However, the Nissl body was a specific basophil granule distributed in the cytoplasm of neuron except for axons and axon hillock. The chemical composition of Nissl body were ribonucleic acid(RNA) and protein. The main function was to synthesize protein what had a close relationship with neuron. In the electron microscope, Nissl body was consist of a large number of parallel endoplasmic reticulum and ribosome. When the neuro degenerated in pathologic conditions, the Nissl body changed obviously. If the axon damaged, the Nissl body will tend to be dissolve or disappear. The nucleus dissolved or disappeared firstly, then developed to surrounding areas. Many vacuoles appeared in the cytoplasm when it dissolved. Such as inflammation, degeneration, poisoning and other lesions, neuronal Nissl's body are reduced in number, until dissolution or disappear. The Nissl body, the protein synthesis and the cell metabolism can be recovered if neuron injury is not serious, or the risk factors were removed[10]. Therefore, the structure change of Nissl body was the symbol of neuron damage[11]. And the Nissl body can be used as the index of the neuron function.

The previous study[12-17] also showed that Bushenhuoxue will contribute to stop the RNFL (retinal nerve fiber layer) and RGCL (retinal ganglion cells layer) getting thinner, improve the ultrastructure of RCGs, recover the rats' total wave of mfERG (multifocal electroretinogram), the response density of total wave and wave P1 on 1, 2, 3, 4 loop, wave N1 on 2, 3 loop, the peak latency of total wave P1 and wave N1 on 3, 4 loop. It also had positive effects on the expression of Bcl-2 and Bax, restrain the apoptosis of gangliocyte. TCM of Bushenhuoxue also can repaired injury LGN in the rat model of chronic EIOP. Take Chinese traditional medicine of Bushenhuoxue after controlled the IOP can improve the function of glaucoma patient.

The experimental result showed that the chronic EIOP can significantly decrease the total area, mean optical density and integrated optical density of PVC's Nissl body in SD rats. TCM of Bushenhuoxue can increase the three of above. This findings indicated that the TCM of Bushenhuoxue can save the Nissl body from death and increase the amount of residual Nissl body. So we can found that the TCM of Bushenhuoxue not only increase the amount of Nissl body but also protect the nerve cell.

Under electron microscope found that: the PVC of chronic EIOP rats damaged obviously. The dilatation of endoplasmic reticulum looks like vacuole with swelling mitochondria (Mi), crista break and vacuolation. After given the TCM of Bushenhuoxue, we found that the conditions such as the line, loose Structure, and edema in axon, and the dilatation of endoplasmic reticulum were all reduced. And the number of organelle in cytoplasm, swelling mitochondria and crista break were all obviously reduced. This all demonstrated that the TCM of Bushenhuoxue can protect the structure and the function of PVC's cell.

Qijudihuangwan is a famous prescription of nourishment for kidney, liver and eyesight in TCM. Fufangdansenpian can resolve blood-stasis and unblock collaterals. Both the two were loaded into the book of Chinese Medicine, used as OTC (over-the-counter) medicine, cheap and good. According to the previous-study and this study, both the two can be widely used as clinical glaucoma optic nerve protection medicine.

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补肾活血中药对大鼠慢性高眼压模型初级视皮质尼氏小体损害的影响

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摘要

目的:观察补肾活血中药对大鼠慢性高眼压(elevated intraocular pressure,EIOP)模型初级视皮质尼氏小体损害的干预作用,并对其作用机理进行初步探讨。

方法:采用烙闭上巩膜静脉法,烙闭大鼠 3 支上巩膜静脉,制作大鼠慢性 EIOP 模型,随机分为 3 组:空白组,模型组,给药组。连续灌胃 8wk,并于 8wk 末处死大鼠,观察其对 EIOP 大鼠眼压(intraocular pressure,IOP),初级视皮质(primary visual cortex,PVC)尼氏小体含量、神经元细胞超微结构影响。

结果:本实验采用的烙闭上巩膜静脉的造模方法使大鼠眼压明显升高($P<0.01$),与造模前比较差异有显著统计学意义($P<0.01$);PVC 病理切片半定量分析表明:模型组尼氏小体总面积 34941±8482.1·S/μm^2、平均光密度 152.8±27.97、积分光密度 11993±3084.8,与空白组(总面积 55742±6348.1·S/μm^2、平均光密度 304.04±100.1、积分光密度 18219±3548.9)比较均有显著统计学意义(均为 $P<0.05$),模型组 Nissl 小体总面积、平均光密度、积分光密度与给药组(总面积 46406±5989.3·S/μm^2,平均光密度 251.05±77.41,积分光密度 16899±4040.6)比较,差异均有统计学意义(均 $P<0.05$)。

结论:补肾活血中药通过增强尼氏小体的表达、改善神经元细胞超微结构而促进 EIOP 大鼠初级视皮质损伤的修复。

关键词:青光眼;大鼠慢性高眼压模型;补肾活血中药;初级视皮质;尼氏小体;神经元细胞超微结构

国际眼科理事会(ICO)发布 2012 年全球眼科医生数量

国际眼科理事会(ICO)调查表明 2012 年全世界 194 个国家和地区共有眼科医生 204,909 名,其中临床实践者(Entering Practice)5,046 名,非实践者(Leaving Practice)3,275 名,手术医生(% Doing Surgery) 61.36%,住院医生(Residents)21,434 名。眼科医生数量较多的前 10 个国家依次为:

1、中国 28,338 名; 2、美国 25,152 名;
3、俄罗斯 14,600 名; 4、日本 13,911 名;
5、巴西 11,350 名; 6、印度 11,000 名;
7、法国 7,000 名; 8、德国 6,638 名;
9、阿根廷 4,500 名; 10、波兰 4,219 名。

其中有 8 个国家和地区眼科医生为 0。

调查结果显示全球眼科医生严重短缺。

(以上信息来自国际眼科理事会网站 www.icoph.org)