Inhibitory effect of captoprilon retinal neovascularization in mice

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Abstract

AIM: To study the inhibitory effect of captoprilon retinal neovascularization (RNV).

METHODS: Sixty seven-day-old mice were randomly divided into treated group and control group with thirty mice in each group. These mice were exposed to 750±50mL/L oxygen for 5 days and then to room air. The treated group had been injected captopril (2.7mL/kg), while control group had been injected 9g/L sodium chloride (2.7mL/kg) by intravitreal for 5 days. The mice were sacrificed at the 17th day after birth and the eyes were enucleated. Adenosine diphosphate-ase (ADPase) stained retina flat-mounts was performed to assess the retinal vascular profiles. Hematoxylin Eosin (HE) staining method was applied to count the number of new vascular cell nuclei and the expression of matrix metalloproteinase-2 (MMP-2) and pigment epithelium derived factor (PEDF) was detected by immunohistochemical method.

RESULTS: Comparing with control group, regular distributions, good branch and reduced density of RNV were observed in the treated group. The number of nucleus of new vessels vascular endothelial cells breaking through the internal limiting membrane was less in the treated group than in the control group (P<0.05). Stain of retinal MMP-2 was weaker in the treated group than in the control group and stain of retinal PEDF was stronger in the treated group than in the control group.

CONCLUSION: Intravitreal injection of captopril (2.7mL/kg) may block the RNV in the oxygen-induced mouse model and the method may provide an effective method for preventing RNV.

KEYWORDS: retinal neovascularization; captopril; intravitreal injection

INTRODUCTION

Neovascular diseases of the retina collectively constitute the leading cause of blindness in developed countries[1]. At present, retinal laser photocoagulation appears to be the most effective treatment for retinal neovascularization. However, this procedure can destroy postmitotic retinal neurons and permanently affect visual function [2]. Pharmacologic agents that inhibit angiogenesis without destroying retinal tissue could lead to new treatments for this constellation of diseases. The current researches focus on captopril, the angiotensin-converting enzyme inhibitor that can restrain MMP-2. The research shows that in addition to lowering blood pressure, captopril can also produce anti-tumor blood vessels and corneal neovascularization, so it becomes a new drug candidate to cure vascular proliferative disease[3]. However, it's rarely reported in China that whether captopril has restraining effect on retinal neovascularization (RNV). The research studied the effect of captopril on oxygen-induced mice's RNV by intravitreal injection of captopril.

MATERIALS AND METHODS

Oxygen Induced ROP in Mice ROP was produced in C57BL/6J mice by a method described by Smith et al[4]. Sixty seven-day-old (postnatal day 7) mice were divided into treated group and control group with thirty mice in each group. They and their mothers were placed in an airtight incubator and exposed to an atmosphere of 750±50mL/L oxygen for 5 days. The incubator temperature was maintained at 23±2°C. Then they were returned to room air at postnatal day 12. At the 12th day, the treated group had been injected captopril (2.7mL/kg), while control group had...
been injected 9g/L sodium chloride (2.7mL/kg) by intravitreal for 5 days. The mice were sacrificed at postnatal day 17 and the eyes were enucleated.

**Observation of RNV** At the 17th day, fifteen mice of each group were anesthetized with an intramuscular injection of 100g/L Chloral Hydrate (0.03mL/kg). To evaluate vessel morphology, all eyes were removed and fixed with 40g/L paraformaldehyde in phosphate-buffered saline phosphate buffered saline (PBS) overnight. The cornea, lens, and vitreous were surgically removed and retinas were dissected. Retinas were processed for magnesium activated adenosine diphosphate-ase (ADPase) staining. ADPase-stained retinas were flat mounted on microscope slides with a gelatin-coated cover slip. The vasculature was then examined under microscope.

At the 17th day, fifteen mice of each group were sacrificed and the eyes were enucleated, immersed in 40g/L paraformaldehyde in PBS for at least 24 hours, and embedded in paraffin. Serial 6μm sections from all eyes were cut sagittally parallel to the optic nerve and stained with hematoxylin and eosin according to a standard protocol. The extent of neovascularization was determined by counting neovascular cell nuclei extending through the internal limiting membrane into the vitreous. All counting was done based on a masked protocol. For each eye, twenty intact sections of equal length, each 30μm apart, were evaluated. Ten sections was stained by HE method, the mean number of neovascular nuclei per section per eye was then determined under microscope. Ten percent of the eyes exhibited retinal detachment or endophthalmitis and were excluded from the evaluation.

**MMP –2 and PEDF Expression** Five unstained 6μm slices were selected randomly from two groups respectively, and were under immunohistochemical detection by the means of Streptomycin avidin-biotin complex (SABC). The primary and the secondary antibody was provided by Wuhan Boster Biotechnology Company, and the working concentration of antibody was 1:100. The primary antibody was replaced by PBS to make negative control, with diaminobenzidine (DAB) chromogenic. The matrix metalloproteinase-2 (MMP-2) and pigment epithelium derived factor (PEDF) expressed positive cells were cytoplasm or canary and tan particles in nucleus. Select five incontinuous highest possible frequency (400 times) randomly from each slice, use MetaMorph/ Evolution MP5.0/BX51 to do grayscale scanning, determine the positive cells' integral optical density (IOD) of MMP-2 and PEDF, and use their average as the indicator.

**Statistical Analysis** All values were expressed as mean±SD. All analysis were performed with appropriate software SPSS 13.0. P<0.05 was considered as statistically significant.

**RESULTS**

**Retina Vessels** In ADP enzyme stained retina flat-mounts the retina vessels of the control group had an obvious dilation pattern that extended from the optic nerve, bigger non-perfusion area and increased neovascularization (Figure 1A). The retina vessels of the treated group had a fine radial branching pattern that extend from the optic nerve to the periphery, neovascularization were rarely seen (Figure 1B). There were new vessels vascular endothelial cell nuclei breaking through the internal limiting membrane into vitreous of both the control group and the treated group in HE staining sections. In the control group, there were an average of 30.43±0.55 nuclei in the each slice (Figure 2A), significantly more than that nuclei (5.39±1.32) in the treated group (Figure 2B) (t=8.248, P<0.05).
MMP-2 and PEDF Expression  In the control group, the expression of MMP-2 was mainly showed in the ganglion cell layer, the inner plexiform layer, the inner nuclear layer and the neovascularization breaking through the internal limiting membrane (Figure 3A). There was weak protein expression of PEDF in the nerve fiber layer, the ganglion cell layer, the inner plexiform layer, the photoreceptor cells layer and the retinal pigment epithelium (RPE) layer in the control group (Figure 4A). In the treated group, there were no expression of MMP-2 in the inner nuclear layer and the outer nuclear layer and little expression only in intracytoplasm of some ganglion cell layer and the inner plexiform layer (Figure 3B). PEDF protein was mainly expressed in the nerve fiber layer, the ganglion cell layer, the inner nuclear layer, the photoreceptor cells layer and the RPE layer in the treated group (Figure 4B). In the control group, IOD of MMP-2 and PEDF were 16.25±3.75, 8.44±2.61. In the treated group, IOD of MMP-2 and PEDF were 9.26±1.13, 17.63±3.52. Compared with the control group, the protein expression of MMP-2 and PEDF of the treated group was significantly different (τ=4.15, τ=5.23, \(P<0.05\)).

DISCUSSION

RNV threatens the eyesight seriously. Because of its multi-factors, cross-links and uncertain pathogenesis, clinically there is no radical means of curing the RNV. People have been looking for the inhibitor that can prevent the RNV. Matrix metalloproteinase is regarded as the necessary condition of the process of RNV, which is mainly affected by gelatinase A (MMP-2) vascular basement membrane-degradation and extracellular matrix protein. Therefore, the application of artificial matrix metalloproteinase inhibitor to the RNN treatment has become a significant curative means [5]. Captopril observed by the institute is the drug widely used in treating the high blood pressure. It used free sulphydryl to form a chelate with angiotensin-converting enzyme active site Zn\(^{2+}\) to inhibit Matrix metalloproteinase activity. Volpert et al [6] research proved that captopril with specificity could directly act on the capillary endothelial cells to inhibit endothelial cell migration and proliferation, and this inhibition restrained not the effect of angiotensin conversion enzyme (ACE) but the effect of MMP-2 activity. The experiment used this characteristic to observe the effect of captopril on oxygen-induced mice's RNV. The experiment used this characteristic to observe the expression of MMP-2 protein and RNV by intravitreal injection of captopril. The result showed that captopril could reduce but not completely prevent the RNV. The reason was...
perhaps that captopril's local concentration was not high enough to prevent the RNV completely. The drug concentration and dose could be increased to make captopril's local concentration higher \(^7\). Meanwhile, the result showed that though MMP-2 protein expression of the treated group was lower than that of the control group, it couldn't be inhibited completely. So the does used in the experiment was not enough to completely inhibit MMP-2 expression. Further, comparing with the control group, the protein expression of PEDF of the treated group is significantly different. The result showed that captopril inhibited the RNN through inhibiting matrix metalloproteinase activity and up-regulation of PEDF expression. Through the oxygen-induced mice's RNV model, the experiment used intravitreal injection of captopril to inhibit matrix metalloproteinase activity to inhibit the mouse's RNV effectively. The new technology has a higher prospect of application and research, which provides a brand-new method for the prevention and treatment of the RNN disease.

REFERENCES