Effects of hydralazine on ocular blood flow and laser–induced choroidal neovascularization

Wei Jiang, George C Y Chiou

Institute Ocular Pharmacology, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843, USA

Correspondence to: George C Y Chiou. Institute Ocular Pharmacology, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843, USA. chiou@medicine.tamhsc.edu

Received: 2009-09-29 Accepted: 2009-12-11

Abstract

• AIM: To investigate the effect of hydralazine on choroidal blood flow in rabbits and laser-induced choroidal neovascularization (CNV) in rats and on tube formation of human umbilical vein endothelial cells (HUVEC).

• METHODS: Female New Zealand white rabbits were used with raised intraocular pressure (IOP) of the left eye to 40mmHg. Hydralazine (10g/L) eye drops were instilled and ocular blood flow was measured with colored microspheres technique. Male Brown Norway rats were treated with Nd: YAG laser to break Bruch's membrane. Hydralazine (5, 10, 20g/L) eye drops or saline alone was instilled three times a day for 4 weeks after laser treatment. Fluorescein angiography (FA) and choroidal flat mount were used to measure the area of CNV. Tube formation of HUVEC was studied at different concentrations of hydralazine.

• RESULTS: With raised IOP to 40mmHg on rabbits, 10g/L hydralazine eye drops enhanced the choroidal blood flow significantly at 30 and 60 minutes after drug instillation. After 4 weeks of drug treatment, 5, 10 and 20g/L hydralazine eye drops all reduced the CNV formation dramatically measured by fluorescein angiography and choroidal flat mount. When HUVEC was cultured on matrix gel for 48 hours, the tube formation of HUVEC were prevented by hydralazine at 3-30mg/L.

• CONCLUSION: Hydralazine prevents CNV formation in vivo and HUVEC tube formation in vitro and enhances rabbits' choroidal blood flow after ischemia. It is hoped that hydralazine could be used to treat age-related macular degeneration in the future.

• KEYWORDS: hydralazine; choroid; neovascularization; human umbilical vein endothelial cell; age-related macular degeneration

INTRODUCTION

Age-related macular degeneration (AMD) is the leading eye disease to cause blindness among people over 65 years old in the world. Recent studies on the mechanism and etiology of the disease revealed much encouraging information, yet many more questions are still to be answered. Further exploration on new drugs is still in a primitive stage. At present time, there are several anti-vascular endothelial growth factor (VEGF) drugs in the market for the treatment of wet-AMD, like ranibizumab (Lucentis), pegaptanib sodium (Macugen), and bevacizumab (Avastin). The efficiency of those drugs is so different among different clinical reports, some said they are good[1], yet some others reported the opposite results[2,3].

Hydralazine is an antihypertensive vasodilator drug, which is used in the clinics for decades. In addition to treat arterial hypertension, hydralazine can also be used to treat congestive heart failure, pulmonary hypertension in chronic obstructive pulmonary disease, and aortic regurgitation. Since hydralazine can dilate blood vessels and to enhance systemic vascular blood flow[4,5], it might also work on choroidal blood flow, which is related to the development of CNV in AMD patients[6,7]. Thus, it is hoped that hydralazine could improve choroidal blood flow with ischemia insult and laser induced choroidal neovascularization in vivo and the tube formation of endothelial cells in vitro.

MATERIALS AND METHODS

Ocular Blood Flow in Rabbits The method published previously was followed[8]. Briefly, New Zealand white rabbits, weighing 2.5-3.0kg, were used in this experiment. Rabbits were anesthetized with 35mg/kg ketamine and 5mg/kg xylazine intramuscularly and half of the initial dose was given hourly thereafter for keeping the anesthesia. The intraocular pressure of the left eye was raised to 40mmHg by anterior chamber puncture to establish the ocular...
hypertensive model. Injection of microspheres (IMT-Stason Laboratories, Irvine, CA) to left ventricle was accomplished via cannulation through the right carotid artery, and blood sampling was carried out from the femoral artery. One percent hydralazine eye drop (50µL) or saline eye drop (50µL) was instilled topically to the left eye, and the choroidal blood flow of the ocular hypertensive rabbits was measured with colored microspheres at 0, 30, 60, and 120 minutes after drug treatment. At each time point, 0.2mL of different colored microspheres was injected into left ventricle and blood samples were taken from femoral artery for exactly one minute immediately following injection of the microspheres. The volume of blood samples was then recorded. The rabbits were euthanized with an injection of 100mg/kg pentobarbital sodium. Left eyes were enucleated and choroids were taken out carefully. The tissue samples were weighed, digested, and the microspheres in the tissue were counted with hemocytometer. The blood flow of each tissue at a certain time point was calculated from the following equation: \[ \text{Qm} = \frac{(\text{Cm} \times \text{Qt})}{\text{Ct}} \] where \( \text{Qm} \) is the blood flow of a tissue in terms of mL/min/g, \( \text{Cm} \) is the microsphere counted per mg of tissue, \( \text{Qt} \) is the flow rate of blood sample in terms of mL/min, and \( \text{Ct} \) is the total microsphere counted in the referenced blood sample.

**Laser-induced CNV Rat Model**

The method was described in details previously [9]. Briefly, Brown Norway rats, weighing 150-180g, were anesthetized by injection of ketamine (35mg/kg) and xylazine (5mg/kg) intramuscularly. Pupils were dilated with 10g/L tropicamide and 25g/L phenylephrine. The ocular fundus was visualized with a VOLK super pupil XL biomicroscopy lens (Keeler Instrument Inc., Broomall, PA). A double-frequency Nd: YAG laser (Laserex LP352; Lume-nis Inc., Salt Lake City, UT) was used at 532-nm wavelength. Laser parameters used were 100-µm spot size, 0.15-second exposure, and 200 mw powers. Six lesions were made to the ocular fundus at approximately equal distances to the optic nerves. Only laser spots with bubble formation were included in the study, lesions with much retinal hemorrhage were excluded. Each group had at least five rats. Hydralazine (The powder form compound of hydralazine was purchased from Sigma-Aldrich (St. Louis, MO)) eye drops (5,10,20g/L) or saline eye drops was instilled three times a day for 4 weeks after laser treatment.

**Fluorescein Angiography**

Fluorescein angiography (FA) was performed after 4-week treatment using a digital fundus camera (TRC-50EX; TOPCON, Tokyo, Japan). Ten percent of fluorescein isothiocyanate-Dextran (Sigma-Aldrich, St. Louis, MO) was injected through hypoglossal veins at 1.4mL/kg. Fluorescein pictures were captured within 20 minutes, clearest pictures were chosen for measuring the areas of CNV formation by Imagenet 2000 digital imaging systems (Topcon Medical Systems, Inc., Paramus, NJ).

**Measurement of Choroidal Flat Mounts for CNV**

After fluorescein angiography pictures were captured at the end of 4 weeks treatment, rats were sacrificed and the eyes were enucleated and fixed in 100g/L phosphate-buffered formal-dehyde. The cornea and lens were removed and the entire retina was carefully dissected. Radial cuts (usually 4-6) of the choroids were made from the edge of the choroids to the equator and the eyecup was flat mounted with the choroids facing up. Flat mounts were captured by fluorescence microscopy on an Axioskop microscope (Zeiss, Thornwood, NY) and Image-Pro Plus software (Media cybernetics, Silver Spring, MD) was used to measure the areas of CNV.

**Cell Culture of Human Umbilical Vein Endothelial Cells**

Human umbilical vein endothelial cells (HUVEC) were purchased from ScienceCell (San Diego, CA). The medium was prepared with EBM-2 plus 100g/L fetal cattle serum, EGM-2 SingleQuots (Lonza, walkersville, MD), 2mmol/L glutamine, 100units/mL penicillin and 100mg/L streptomycin. Cells were cultured on 2.5% matrix gel in 50mL/L CO\(_2\), 95% air incubator. Cell morphology was captured by microscopy with camera (Zeiss, Thornwood, NY). In vitro experiment was repeated three times.

**Statistical Analysis**

Data were presented as mean ±SE. Nonpaired Student's \( t \)-test was used for analysis.

**RESULTS**

**Choroids Blood flow**

The choroidal blood flow was significantly increased by 10g/L hydralazine eye drops compared with control at 30 and 60 minutes after treatment (Figure 1).

**Measurement of CNV Area by Fluorescein Angiography**

After 4-week treatment, all the treatment groups of 5,10 and 20g/L hydralazine eye drops reduced the area of CNV significantly (Figure 2, 4).

**Areas of CNV on Choroidal Flat Mounts**

All treatments (5, 10 and 20g/L hydralazine eye drops) reduced the areas of CNV on the choroidal flat mounts after 4 weeks(Figure 3, 5).

**Tube Formation of HUVEC**

From Figure 6, it was clear that hydralazine at 1mg/L was no different from control. With hydralazine at 3mg/L, however, some HUVEC didn't grow into tubes, and it became most apparent for
Hydralazine is one of the widely used antihypertensive drugs. Hydralazine at 30mg/L treatment. Hydralazine at 100mg/L caused apoptosis.

**DISCUSSION**

Hydralazine is one of the widely used antihypertensive drugs...
in the clinics via vasodilation. Further, it has antioxidant effect as well \[10\]. As such, we hoped that hydralazine could be used to treat AMD, whose etiology is related to reduction in choroidal blood flow and oxidative degeneration of RPE and photocells \[11-13\]. Our previous \textit{in vitro} data showed that, hydralazine protected RPE cells degeneration caused by various oxidant compounds. As shown in this study, 10g/L hydralazine eye drops improved the choroidal blood flow significantly in rabbit eyes. Since ischemia could be the central role in both early and late stages of AMD \[14\], hydralazine could be used for the treatment of this disease. Laser-induced CNV model is a commonly used model for exudative AMD and some dry AMD. Our data showed that after one-month treatment, all three concentrations of hydralazine eye drops reduced the area of CNV formation significantly. Further, the tube formation of HUVEC was prevented by hydralazine as a sign of anti-neovascularization. It is concluded that hydralazine could reduce the CNV area formation through the improvement of choroidal blood flow and antiformation of HUVEC tubes. Further, hydralazine is also a direct antioxidative compound which suppressed degeneration of RPE and photocells in AMD.

REFERENCES


4 Loufrani L, Heurion D. Vasodilator treatment with hydralazine increases blood flow in mdx mice resistance arteries without vascular wall remodelling or endothelium function improvement. \textit{J Hypertens} 2005;23(10):1855–1860


