·Basic Research·

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
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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

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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: $Q\mathrm{m} = (C\mathrm{m} \times Q\mathrm{r})/C\mathrm{r}$, where $Q\mathrm{m}$ is the blood flow of a tissue in terms of mL/min/g, $C\mathrm{m}$ is the microsphere counted per mg of tissue, $Q\mathrm{r}$ is the flow rate of blood sample in terms of mL/min, and $C\mathrm{r}$ 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

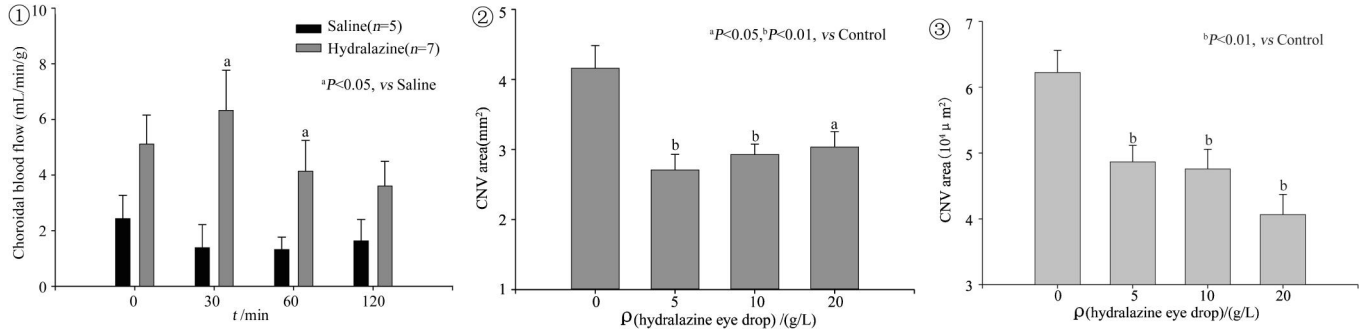

Figure 1 Effect of hydralazine eye drops on rabbits choroidal blood flow

Figure 2 Effect of hydralazine on CNV area measured by fluorescein

Figure 3 Effect of hydralazine on CNV area measured by choroidal flat mount

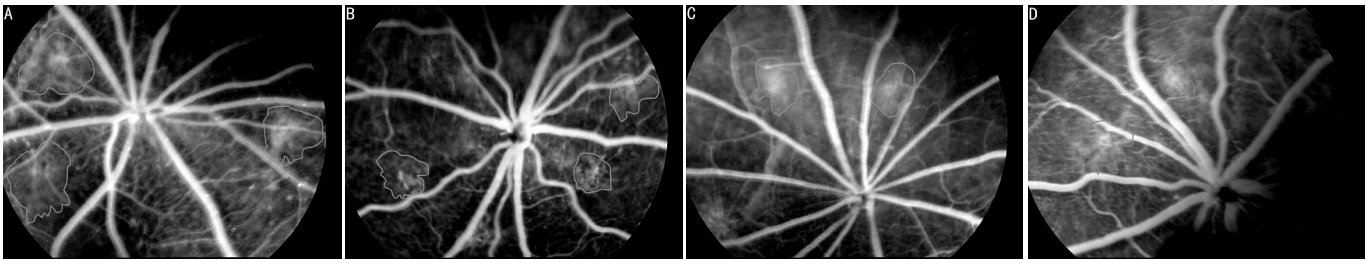

Figure 4 Pictures of fluorescein angiography in rat retinas. Circles show the areas of CNV in different groups A: saline eye drop; B: 5g/L hydralazine eye drops; C: 10g/L hydralazine eye drops; D: 20g/L hydralazine eye drops

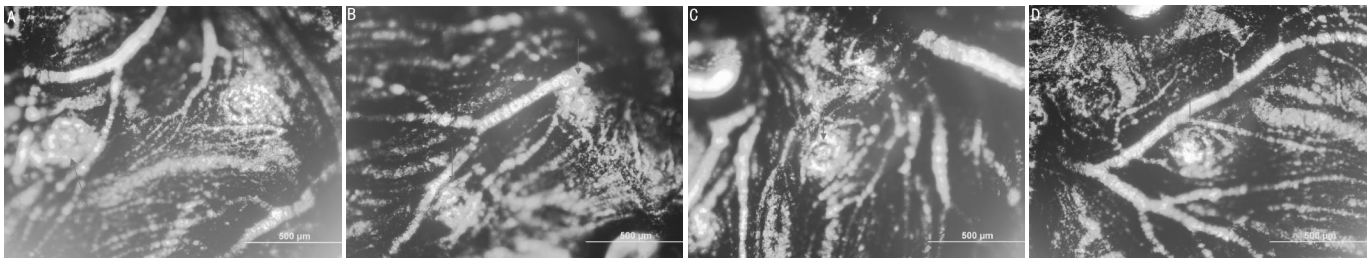

Figure 5 Choroidal flat mounts in different groups. Arrows show the CNV in each choroidal flat mounts A: saline eye drops; B: 5g/L hydralazine eye drops; C:10g/L hydralazine eye drops; D:20g/L hydralazine eye drops

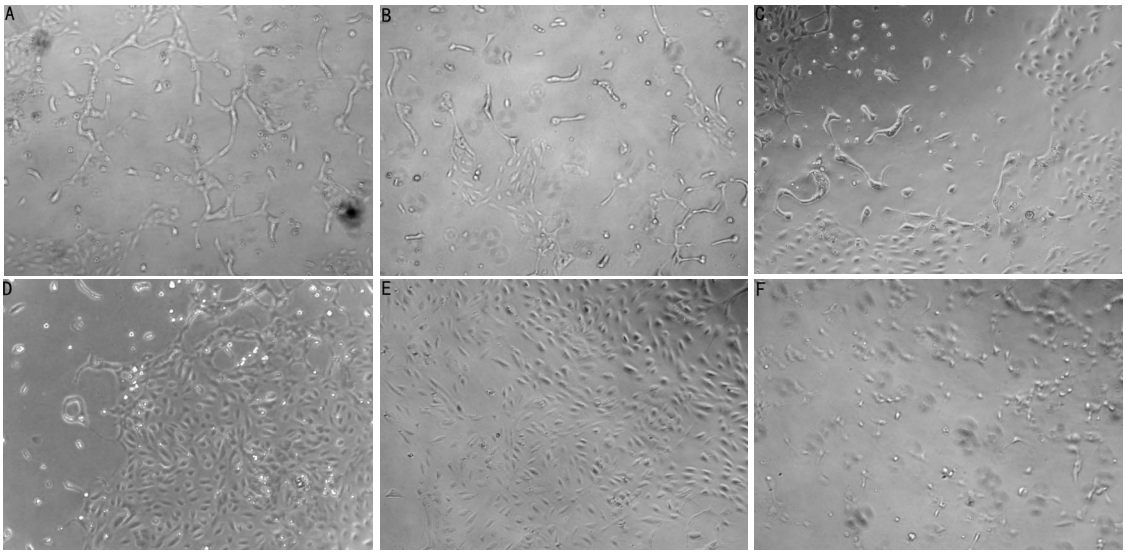

Figure 6 Hydralazine inhibited tube formation on HUVECs A: control; B: hydralazine 1mg/L; C: hydralazine 3mg/L; D: hydralazine 10mg/L; E: hydralazine 30mg/L; F: hydralazine 100mg/L. HUVECs were seeding on 2.5 % matrix gel for 48 hours

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 *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

1 Hussain N, Ghanekar Y, Kaur I. The future implications and indications of anti-vascular endothelial growth factor therapy in ophthalmic practice. *Indian J Ophthalmol* 2007;55(6):445-450

2 Ehlers JP, Fintak DR, Gupta OP, Regillo CD, Fineman MS, Ho AC. Pegaptanib for choroidal neovascularization in treatment-naive exudative age-related macular degeneration. *Ophthalmic Surg Lasers Imaging* 2007;38(5):371-377

3 Geltzer A, Turalba A, Vedula SS. Surgical implantation of steroids with antiangiogenic characteristics for treating neovascular age-related macular degeneration. *Cochrane Database Syst Rev* 2007;17(4):CD005022

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

5 Chelly JE, Doursout MF, Begaud B, Tsao CC, Hartley CJ. Effects of hydralazine on regional blood flow in conscious dogs. *J Pharmacol Exp Ther* 1986;238(2):665-669

6 Grunwald JE, Metelitsina TI, Dupont JC, Ying GS, Maguire MG. Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. *Invest Ophthalmol Vis Sci* 2005;46(3):1033-1038

7 Yamamoto Y. Measurement of blood flow velocity in feeder vessels of choroidal neovascularization by a scanning laser ophthalmoscope and image analysis system. *Jpn J Ophthalmol* 2003;47(1):53-58

8 Xuan B, Xu XR, Chiou GCY. Relationship between nitric oxide production and choroidal blood flow. *J Ocul Pharma Ther* 2003;19(3):247-253

9 Zou YH, Xu XR, Chiou GCY. Effect of interleukin-1 blockers, CK112, and CK116 on rat experimental choroidal neovascularization in vivo and endothelial cell cultures *in vitro*. *J Ocul Pharma Ther* 2006;22(1):19-25

10 Daiber A, Mulsch A, Hink U, Mollnau H, Warnholtz A, Oelze M, Munzel T. The oxidative stress concept of nitrate tolerance and the antioxidant properties of hydralazine. *Am J Cardiol* 2005;96(7B):25i-36i

11 Husain D, Ambati B, Adamis AP, Miller JW. Mechanisms of age-related macular degeneration. *Ophthalmol Clin North Am* 2002;15(1):87-91

12 Shen JK, Dong A, Hackett SF, Bell WR, Green WR, Campochiaro PA. Oxidative damage in age-related macular degeneration. *Histol Histopathol* 2007;22(12):1301-1308

13 Suzuki M, Kamei M, Itabe H, Yoneda K, Bando H, Kume N, Tano Y. Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration. *Mol Vis* 2007;13:772-778

14 Feigl B. Age-related maculopathy in the light of ischaemia. *Clin Exp Optom* 2007;90(4):263-271