Protective effects of hydrogen gas in a rat model of branch retinal vein occlusion via decreasing VEGF-α expression

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Research Article

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Abstract

Background: Oxidative stress (OS) is an essential factor in the pathogenesis of branch retinal vein occlusion (BRVO). Lines of studies have demonstrated the role of hydrogen gas in the regulation of OS. This study was designed to evaluate the effect of hydrogen gas on BRVO rat model which was established by modified laser photocoagulation method.

Methods: 24 BRVO rats were randomly divided into two groups: hydrogen gas (H) group (42% H2, 21% O2, 37% N2) and model (M) group (21% O2, 79% N2). Rats in H group were inhaled with hydrogen gas for 8 hours every day continued to 30 d post-occlusion. 12 age-matched healthy rats were served as control (C) group. Retinal function and morphology were detected at 1, 7, 14 and 30 d post-occlusion. Furthermore, the expression of vascular endothelial growth factor (VEGF-α) was detected by immunofluorescent staining.

Results: Full-field Electroretinography (FfERG) revealed that the amplitude of the d3.0 (dark-adaptation 3.0 response) b wave and OPs2 (oscillatory potentials) wave achieved quick recovery at 7 d post-occlusion in H group compared with M group (p<0.05; p<0.05). Retinal edema, especially in outer nuclear layer (ONL) at 1 d and 3 d post-occlusion in H group was slighter than M group (p<0.05). The reopen time of occlusive retinal vessel in H group was 2.235 ± 1.128 d, which was sooner than that in M group 4.234±2.236 d (p<0.05). Moreover, the flow velocity of ear vein blood was increased in H group compared with that in M group (p<0.05). The expression of VEGF-α in H group was dramatically decreasing compared with M group at 1 d, 7 d and 14 d post-occlusion (p<0.05), while there was no significant difference at 30 d post-occlusion (*p>0.05*).

Conclusions: Our findings demonstrate that inhalation of hydrogen gas could alleviate retina edema, shorten reopen time and improve retinal function via decreasing VEGF-α expression.

Key words: Hydrogen gas; branch retinal vein occlusion; electroretinography; vascular endothelial growth factor

Background

Retinal vein occlusion (RVO), could be classified with central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO) according to the occlusive vein sites. It is well-known that the sight-threatening ophthalmic vessel disease most commonly affects middle-aged to older people [1,2]. Compared with CRVO, BRVO is featured with higher morbidity and larger therapeutic window of opportunity, which should be optimized management [3]. Currently, the first-line therapeutic methods, including glucocorticoid, anti-VEGF, laser photocoagulation, traditional Chinese medicines (most in eastern countries), all didn't receive satisfying results [4]. Specifically, glucocorticoid is convenient, but inevitably meet various adverse side effects, such as increasing susceptibility to infection, damage to optic nerve, cataract and so on [5,6]. Although adverse events following anti-VEGF therapy are rare, it

would be relevant to meet challenges such as frequent visits, need for frequent injections, recurrent macular edema, anti-VEGF non-responders and high expenses related to the agents. So, it is urgent to explore effective measures against BRVO with evident therapeutic effects and less adverse side effects. However, conventional medicine could not get satisfied results because of the blood-retina barrier and the unique retina structure.

Recently, hydrogen gas, as a novel inert gas, has aroused much interest in medical area. A remarkable achievement is a clinical research confirmed that inhalation hydrogen gas could alleviate brain ischemia/reperfusion (I/R) injury via anti-inflammation and antioxidation [7]. More experimental evidences, including studies on brain, heart, liver, lung, kidney and eye, have confirmed the beneficial role of inhalation hydrogen gas. Furthermore, our laboratory previously applied hydrogen gas in several ophthalmic diseases, including retina injury induced by a great intense light [8], retinitis pigmentosa induced by methylnitrosourea (MNU) [9], dry eyes and endotoxin-induced uveitis [10], which verified the potential value of hydrogen gas on ophthalmic disease.

In this study, we proposed the hypothesis whether hydrogen gas could be a new therapeutic method for BRVO, collected the therapeutic evidences of hydrogen gas and explored the potential mechanism.

Methods

Animals

42 adults male Sprague-Dawley (SD) rats (6-8 weeks, 180-220 g) were obtained from the Laboratory Animal Center of Air Force Medical University (license No.2014270138S). The rats were raised under clean environment (room temperature 23°C ± 3°C, humidity 45-65%, 12 h light/12 h dark) with food and water free intake. In this study, All the experimental protocols were approved by the ethical committee of the Animal Care and Experimental Committee of Air Force Medical University. All experiments were accordance with the Association for Research in Vision and Ophthalmology (ARVO) statements for ophthalmic research animal using. Rats were performed with euthanasia with lethal sodium pentobarbital (Sigma, St Louis, MO, USA) at each detecting point.

BRVO model

30 Rats were anesthetized by intraperitoneal injection (IP) with 1% sodium pentobarbital (0.1 mL/100 g) and sumianxin II (50 uL) (Jilin Shengda Animal Pharmaceutical Co., Ltd., Jilin, China). 50 mg/mL Rose Bengal (Chengdu aikeda reagent co. LTD, China) was injected into rat tail vein 1 min before laser applied. Additionally, right eye was dilated with 0.5% tropic amide (Shenyang Xingji Corporation, Shenyang, Liaoning Province, China). Then bifurcation of retinal vein secondary vascular were found at Micron IV Retinal Imaging Microscope (Lumenis, Inc. USA) and 50 laser spots were applied with a setting parameter (power: 80 mW, duration: 100 ms; spot size: 100 μm).

Hydrogen gas administration

The mixed gas was produced by ASM-H01 hydrogen-oxygen nebulizer (Asclepius, Shanghai, China), which contain 67% H_2 and 33% O_2 from water by electrolysis. Furthermore, N_2 was applied to modulate the concentration of O_2 at 21% equaled with the conventional condition. Rats in hydrogen gas (H) group inhaled a gas mixture (21% O_2, 42% H_2, 37% N_2) at 3 L/min speed for 8 h (once/day, 30 d). Additionally, the concentration of hydrogen gas was monitored by thermal trace GC ultra-gas chromatography (Thermo Fisher, MA, USA), which maintained at a 42% concentration throughout the study. The rats were placed into a special closed gas chamber and moved freely. Rats in model (M) group and control (C) group did not receive any treatment.

Electroretinography

FfERG measurement was performed as international electrophysiological standard (ISCEV) including dark-adapted 0.01 ERG, dark-adapted 3.0 ERG, dark-adapted oscillatory potentials, light-adapted 3.0 ERG and light-adapted flicker ERG at 1, 7, 14 and 30 d post-occlusion. In brief, rats were adapted in a dark environment for 12 h for dark adaptation. All operation procedures were performed in a dim red-light room. Rats were anesthetized as previous method. The pupils were dilated with 0.5% tropicamide ahead of ERG operation. FfERG was recorded using the full-field (Ganzfeld) stimulation with a computer system (RETI port; Roland Consult GmbH, Brandenburg, Germany). The recording electrode was a custom-made silver chloride electrode and softly placed on the center of the cornea. Stainless steel needle electrodes were placed in the cheek and tail to serve as the reference and ground electrode, respectively. Gatifloxacin eye gel (Shenyang Xingji Corporation, Shenyang, Liaoning Province, China) was used three times a day after ERG testing to avoid infection.

OCT, fundus photograph and ear microcirculation detection

OCT images detection were a pplied on 1, 7, 14 and 30 d post-occlusion. The detection procedure complied with operator manual. Right eye had previously been dilated with 0.5% tropic amide. Then gatifloxacin eye gel was used as coupling gel to protect rat cornea from injuring. Fundus and OCT images were captured from 20 positions for each eye using Retinal Imaging System (OPTO-RIS, OptoProbe, Canada) and 4D-ISOCT Microscope Imaging System (ISOCT, OptoProbe, Canada). And the thickness of the retinal layers was calculated with an OCT Image Analysis Software (Optoprobe, Version 2.0, Canada). Furthermore, BRVO rat model ear microcirculation was detected by microcirculation detector (Xindi, Inc, Shanghai, China).

Immunofluorescence staining

Immunofluorescence staining was performed according to manufacturer's instructions at 1, 7, 14 and 30 d post-occlusion (n=3). Eye paraffin sections were deparaffinized in dimethylbenzene and dehydrated in gradient ethyl alcohol. Then the sections were washed in phosphate buffer saline (PBS; 0.1 mM, pH 7.2) 3 times for 5 min. Antigen retrieval solution (9 mL 0.1 mmol/L citric acid, 41 mL 0.1 mmol/L sodium citrate, 450 mL dd$H_2$0) was performed with a medium baking temperature for 10 min. Next the sections were washed in PBS 3 times for 5 min. 10% goat serum was applied for 2 h, then sections were incubated with

anti-VEGF-α (GeneTex, GTX102643, USA) at 1:100 dilution overnight at 4 ℃. Slides incubated without primary antibody served as control slide. The slides were washed with PBS 3 times for 5 min and incubated with IgG (H+L), Cy3 fluorescence secondary antibody (Zhuangzhi, EK022, Xi'an, Shaanxi province, China) at 1.200 dilution for 1 h at room temperature. Slides were washed in PBS 3 times for 5 min. DAPI (100 ng/mL) was used to stain nuclear for 10 min. Images of slides were captured on fluorescence microscope (BX53, Olympus, Japan).

Statistical analyses

Analysis of variance (ANOVA) followed by Bonferroni's post-hoc analysis was performed to examine the statistical differences among the all groups unless otherwise specified, the values are presented as mean ± standard deviation (SD), with $p \leq 0.05$ considered as statistically significant.

Results

BRVO rat model

BRVO model was confirmed by OCT and Fundus photography. 24 BRVO rats were constructed successfully, whose successful rate was 80% (24/30). Moreover, 24 BRVO rats were randomly divided into 2 groups: hydrogen gas (H) group (n=12) and model (M) group (n=12). 12 age-matched male rats were served as control (C) group.

Effects of hydrogen gas on retinal function

To evaluate retinal function of BRVO treated with or without hydrogen gas, ffERG was performed. As shown in Fig 1, the amplitude of b wave (dark adaptation 3.0 response) and OPs2 wave in H and M group were decreased compared with C group at 1, 7 and 14 d post-occlusion (all $p<0.05$), while there was no significant difference among H, M and C group at 30 d post-occlusion ($p>0.05$). In addition, the rats in H group had higher amplitude of b wave (dark adaptation 3.0 response) and OPs2 wave compared with M group at 7 d post-occlusion (all $p<0.05$).

Effects of hydrogen gas on branch retinal vein recovery

To explore the evolution process of the occlusive vein, fundus photograph was applied at 1, 3, 5, 7, 14 and 30 d after BRVO model established with laser photocoagulation. The obvious occlusive vein and non-perfusion was observed in both H group and M group at 1 d post-occlusion. As shown in Fig 2A, the reopen time in H group was 2.235 ± 1.128 d, which was sooner than that in M group 4.234 ± 2.236 d ($p<0.05$).

Effects of hydrogen gas on retinal structure

To evaluate the protection effect of hydrogen gas on retinal structure after BRVO in vivo, OCT was performed. As for OCT, low-reflective band contained inner nuclear layer (INL) and outer nuclear layer

(ONL) which were composed with nuclear. Moreover, high-reflective band contained innerplexiformlayer (IPL), outerplexiformlayer (OPL), the photoreceptor inner segment/outer segment (IS/OS) junction line and retinal pigment epithelium (RPE) layer. As shown in Fig 2B, OCT found that the thickness of retina at BRVO area in M group was remarkable increased for laser-induced edema at 1 d, while significantly decreased at 7, 14 and 30 d post-occlusion compared with C group (all $p<0.05$). Furthermore, there were no significant difference between H and C group at 1 d post-occlusion ($p>0.05$). At the same time, the thickness of total retina was more oedematous in M group compared with that in H group at 3 d post-occlusion ($p<0.05$). Interestingly, we found the total retina thickness in M group was dramatically decreased compared with that in H group at 30 d post-occlusion ($p<0.05$).

Effects of hydrogen gas on microcirculation

Microcirculation was a predicted role to evaluate occlusive vein prognosis. Microcirculation detector found that the flow velocity of ear vein blood was decreased in M group compared with H and C group at 7 d post-occlusion (all $p<0.05$), while there existed no significant difference between H and C group (all $p>0.05$) (Fig 3).

Effects of hydrogen gas on the expression of retina VEGF-α

To evaluate the expression of VEGF-α treated or untreated with hydrogen gas, immunofluorescence was applied. As shown in Fig 4, VEGF-α expressed mainly in ganglion cell layer (GCL). Furthermore, the expression of VEGF-α in H and M group at 1 d post-occlusion was dramatically increased compared with C group (all $p<0.05$), while the expression of VEGF-α in H group was less than M group ($p<0.05$). At 1, 7 and 14 d post-occlusion, the expression of VEGF-α in H group was significantly less than M group (all $p<0.05$). However, there existed no significant difference among each group at 30 d post-occlusion (all $p>0.05$).

Discussion

In this study, hydrogen gas via inhalation administration could protect BRVO rat retinal function recovery and retinal structure integrity. We found hydrogen gas could shorten reopen time and improve ear vein microcirculation. Interestingly, hydrogen gas could lighten the damage site retinal edema in the early stage. More importantly, hydrogen gas could decrease the expression of VEGF-α to improve hypoxia condition in the early stage.

BRVO is one of the most common vessel-associated ophthalmology diseases and damages visual sight in different degree [11]. It was difficult for people to manage and treat for BRVO itself and the troubled complications. An essential reason is lacking a suitable BRVO animal model. In the history, people established BRVO model by laser photocoagulation [12], diathermic cauterization, intravitreal injection of PD032590 [13], thrombin [14], NPe6 [15], or endothelin-1(ET-1) [16]. We have previously showed that a modified laser photocoagulation could superbly mimic BRVO disease. In this study, BRVO rat model was established by a modified laser photocoagulation method, which featured with stabilization and

homogeneity. The typical features of BRVO, such as retinal edema, non-perfusion, reperfusion and neovascular, were both observed in the experiment. Retinal edema was observed at occlusive site of both inner retinal neural layers and outer retinal layers at 1 d post-occlusion. We found retinal edema mainly existed in the nerve fiber layer (NFL), outer plexiform layer (OPL) and ONL which was accordance with the previous study [17]. Additionally, retinal atrophy and photoreceptor cell loss were observed at 14 and 30 d post-occlusion, which was associated with BRVO complication retinal edema [18].

Hydrogen gas has been received more and more attention in many disorders since Ohsawa demonstrated that hydrogen gas could remarkably suppressed I/R brain injury by buffering the effects of oxidative stress. Moreover, hydrogen gas could activate NF-E2-related factor 2 (Nrf2) cell signal pathway [19], which reduced OS by up-regulating a variety of antioxidant enzymes [20]. However, the specific mechanisms of hydrogen gas have not been totally illustrated, which was urgent for further explanation. Now Many studies have clarified and confirmed the varying degrees effect of hydrogen gas and the potential advantages have been revealed: 1) hydrogen gas could rapidly diffuse across cell membranes and directly target to eliminate intracellular reactive oxygen species (ROS), such as hydroxide radical (•OH) and peroxynitrite (ONOO•); 2) hydrogen gas is a mild deoxidizer, which have little effect on normal ROS associated cell signal pathway [21]; 3) hydrogen gas is obtained easily and be safe even at high concentration [22,23]. Considering the specific effect, hydrogen gas could easily pass through phospholipid bilayer, and could be administrated in BRVO to eliminate free radical.

Recently, the accurate BRVO mechanism is not totally clear. The studies found VEGF-α played a key role in BRVO prognosis and development [24]. Because it is tightly associated with BRVO complications, such as fragile neovascularization, hemorrhage and retinal edema. So, the agent targeted to VEGF-α is regarded as a promising method. At present, anti-VEGF-α is a direct intervention to reduce the expression of VEGF-α and clinical studies have confirmed it was valid [25,26]. However, anti-VEGF-α agent still inevitably exist some potential disadvantages: 1) molecular targeting drug is expensive for most BRVO patients; 2) Some patients are insensitive to anti-VEGF-α agent and even suffer severe adverse effects; 3) VEGF-α plays an essential role in physical reaction and anti-VEGF-α agent administrated would damage retinal nerve cells [27,28]. It is known that the excessive generation of VEGF-α is accompanied with histiocyte hypoxia, which is associated with further vascular leakage and retinal edema [29]. However, we could not ignore the physical role of VEGF-α, which was indispensable for retina self-repairation. In our study, we found hydrogen gas inhalation could effectively decrease the expression of VEGF-α in the early BRVO stage and shorten BRVO reopen time through indirectly regulating VEGF-α. So, we supposed that hydrogen gas could improve ischemia reperfusion injury and could be applied to those who were insensitive to anti-VEGF-α treatment and covered with severe adverse side. Moreover, compared with anti-VEGF-α agent, hydrogen gas could directly improve retina hypoxia condition and the potential mechanisms could be related to selectively eliminate strong free radicals, inhibit inflammatory factors and active cell survival signals [30]. In the future, we would like to explore the specific mechanisms of hydrogen gas on BRVO treatment.

Interestingly, we found both ERG function, microcirculation and the expression of VEGF-α existed no significant difference in H group compared with M group at 30 d post-occlusion. However, we could see the retinal structure between H group and M group at 30 d post-occlusion. We considered that hydrogen gas could promote occlusive vein reopen. Moreover, ERG showed that hydrogen gas reduced the rapid decline both b wave (d 3.0) and OPs2 response at 7 d post-occlusion when fundus photograph found that the BRVO reopen time closed to. We speculated that hydrogen gas could alleviate retina ischemia-reperfusion injury, which protected retinal structure integrity.

Conclusion

The study showed that hydrogen gas played a specific role in alleviating retinal edema, improving retinal microcirculation, protecting visual function and regulating VEGF-α expression. In a word, the study provided a curative effect support that hydrogen gas was beneficial for clinical application on BRVO disorder as a supplement treatment.

Abbreviations

ARVO: Association for Research in Vision and Ophthalmology; BRVO: branch retinal vein occlusion; CRVO: central retinal vein occlusion; d3.0: dark-adaptation 3.0 response; FfERG: Full-field Electroretinography; Nrf2: NF-E2-related factor 2; OPs2: oscillatory potentials; OS: Oxidative stress; ROS: reactive oxygen species; SD: Sprague-Dawley

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the Animal Care and Experimental Committee of Air Force Medical University, and the procedures conformed to the statements of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and analyzed in the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

JA, TC and ZMZ designed the experiments. WMY, PL and JWL conducted the experiments, analyzed the data, and wrote the manuscript. MSH, ZW and QLZ provided intellectual support and edited the manuscript. MHL and JHX revised the manuscript All authors read and approved the final manuscript.

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References

1. Thapa, R., Bajimaya, S., Paudyal, G., Khanal, S., Tan, S. et al. (2017) Prevalence, pattern and risk factors of retinal vein occlusion in an elderly population in Nepal: the Bhaktapur retina study. Bmc Ophthalmol. 17, 162.
2. Cheung, N., Klein, R., Wang, J. J., Cotch, M. F., Islam, A. F. M. et al. (2008) Traditional and Novel Cardiovascular Risk Factors for Retinal Vein Occlusion: The Multiethnic Study of Atherosclerosis. Investigative Opthalmology & Visual Science. 49, 4297.
3. Ponto, K. A., Elbaz, H., Peto, T., Laubert-Reh, D., Binder, H. et al. (2015) Prevalence and risk factors of retinal vein occlusion: the Gutenberg Health Study. J Thromb Haemost. 13, 1254-1263.
4. Kaldirim, H. E., Yazgan, S. (2017) A comparison of three different intravitreal treatment modalities of macular edema due to branch retinal vein occlusion. Int Ophthalmol.
5. Mitry, D., Bunce, C., Charteris, D. (2013) Anti-vascular endothelial growth factor for macular oedema secondary to branch retinal vein occlusion. Cochrane Database Syst RevD9510.
6. Demir, M., Oba, E., Guven, D., Acar, Z., Cinar, S. (2014) Results of intravitreal triamcinolone acetonide in patients with macular edema secondary to branch retinal vein occlusion. Int J Clin Pharm. 36, 438-442.
7. Ohta, S. (2014) Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. Pharmacol Ther. 144, 1-11.
8. Qi, L. S., Yao, L., Liu, W., Duan, W. X., Wang, B. et al. (2015) Sirtuin Type 1 Mediates the Retinal Protective Effect of Hydrogen-Rich Saline Against Light-Induced Damage in Rats. Invest Ophthalmol Vis Sci. 56, 8268-8279.

9. Yan, W. M., Chen, T., Wang, X. C., Qi, L. S., Zhao, G. H. et al. (2017) The reason for the amelioration of N-methyl-N-nitrosourea-induced retinitis pigmentosa in rats by hydrogen-rich saline. Int J Ophthalmol. 10, 1495-1503.
10. Yan, W. M., Zhang, L., Chen, T., Zhao, G. H., Long, P. et al. (2017) Effects of hydrogen-rich saline on endotoxin-induced uveitis. Med Gas Res. 7, 9-18.
11. Jonas, J. B., Xu, L., Wang, Y. X. (2009) The Beijing Eye Study. Acta Ophthalmol. 87, 247-261.
12. Ham, D. I., Chang, K., Chung, H. (1997) Preretinal neovascularization induced by experimental retinal vein occlusion in albino rats. Korean J Ophthalmol. 11, 60-64.
13. Huang, W., Yang, A. H., Matsumoto, D., Collette, W., Marroquin, L. et al. (2009) PD0325901, a mitogen-activated protein kinase kinase inhibitor, produces ocular toxicity in a rabbit animal model of retinal vein occlusion. J Ocul Pharmacol Ther. 25, 519-530.
14. Tamura, M. (2001) Neovascularization in experimental retinal venous obstruction in rabbits. Jpn J Ophthalmol. 45, 144-150.
15. El-Dessouky, E. S., Moshfeghi, A. A., Peyman, G. A., Yoneya, S., Mori, K. et al. (2001) Toxicity of the photosensitizer NPe6 following intravitreal injection. Ophthalmic Surg Lasers. 32, 316-321.
16. Takei, K., Sato, T., Nonoyama, T., Miyauchi, T., Goto, K. et al. (1993) A new model of transient complete obstruction of retinal vessels induced by endothelin-1 injection into the posterior vitreous body in rabbits. Graefes Arch Clin Exp Ophthalmol. 231, 476-481.
17. Wallow, I. H., Danis, R. P., Bindley, C., Neider, M. (1988) Cystoid macular degeneration in experimental branch retinal vein occlusion. Ophthalmology. 95, 1371-1379.
18. Ieki, Y., Nishiwaki, H., Miura, S., Yamashiro, K., Nishijima, K. et al. (2002) Experimental macular edema induced by macular venule occlusion in monkey. Curr Eye Res. 25, 123-131.
19. Ohta, S. (2014) Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. Pharmacol Ther. 144, 1-11.
20. Johnson, D. A., Johnson, J. A. (2015) Nrf2–a therapeutic target for the treatment of neurodegenerative diseases. Free Radic Biol Med. 88, 253-267.
21. Sauer, H., Wartenberg, M., Hescheler, J. (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem. 11, 173-186.
22. Ohta, S. (2011) Recent progress toward hydrogen medicine: potential of molecular hydrogen for preventive and therapeutic applications. Curr Pharm Des. 17, 2241-2252.
23. Fontanari, P., Badier, M., Guillot, C., Tomei, C., Burnet, H. et al. (2000) Changes in maximal performance of inspiratory and skeletal muscles during and after the 7.1-MPa Hydra 10 record human dive. Eur J Appl Physiol. 81, 325-328.
24. Yao, J., Chen, Z., Yang, Q., Liu, X., Chen, X. et al. (2013) Proteomic analysis of aqueous humor from patients with branch retinal vein occlusion-induced macular edema. Int J Mol Med. 32, 1421-1434.
25. Kida, T., Flammer, J., Oku, H., Morishita, S., Fukumoto, M. et al. (2016) Suppressed endothelin-1 by anti-VEGF therapy is important for patients with BRVO-related macular edema to improve their vision.

EPMA J. 7, 18.

26. Campochiaro, P. A., Heier, J. S., Feiner, L., Gray, S., Saroj, N. et al. (2010) Ranibizumab for macular edema following branch retinal vein occlusion: six-month primary end point results of a phase III study. Ophthalmology. 117, 1102-1112.
27. Huang, Z. L., Lin, K. H., Lee, Y. C., Sheu, M. M., Tsai, R. K. (2010) Acute vision loss after intravitreal injection of bevacizumab (avastin) associated with ocular ischemic syndrome. Ophthalmologica. 224, 86-89.
28. Vertes, D., Snyers, B., De Potter, P. (2010) Cytomegalovirus retinitis after low-dose intravitreous triamcinolone acetonide in an immunocompetent patient: a warning for the widespread use of intravitreous corticosteroids. Int Ophthalmol. 30, 595-597.
29. Campa, C., Alivernini, G., Bolletta, E., Parodi, M. B., Perri, P. (2016) Anti-VEGF Therapy for Retinal Vein Occlusions. Curr Drug Targets. 17, 328-336.
30. Wu, J., Wang, R., Yang, D., Tang, W., Chen, Z. et al. (2018) Hydrogen postconditioning promotes survival of rat retinal ganglion cells against ischemia/reperfusion injury through the PI3K/Akt pathway. Biochem Biophys Res Commun. 495, 2462-2468.

Figures

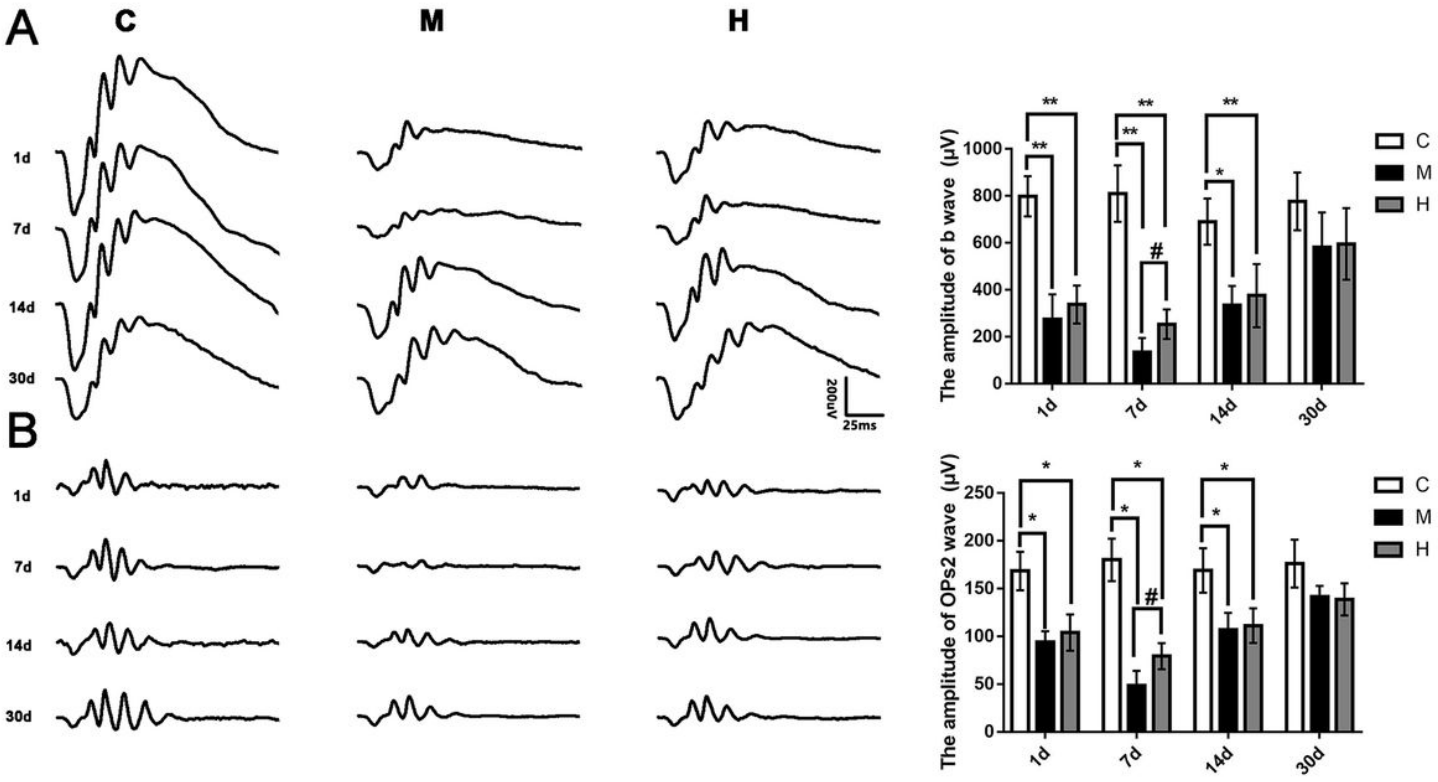

Figure 1

Hydrogen gas improved BRVO rat retinal function at 7d post-occlusion. A: The typical dark-adaptation 3.0 response pictures and the amplification of b (dark-adaptation 3.0 response) wave at 1, 7, 14 and 30 d post-occlusion. B: The typical OPs response pictures and the amplification of OPs2 wave at 1, 7, 14 and 30 d post-occlusion. All analyses were performed in duplicates. The data are expressed as mean ± standard deviation (SD), n = 4-12 rats per group. *, p<0.05, H and M group vs C group; **, p<0.01, H and M group vs C group; #, p<0.05, H group vs M group. C: control group, M: model group, H: hydrogen gas group.

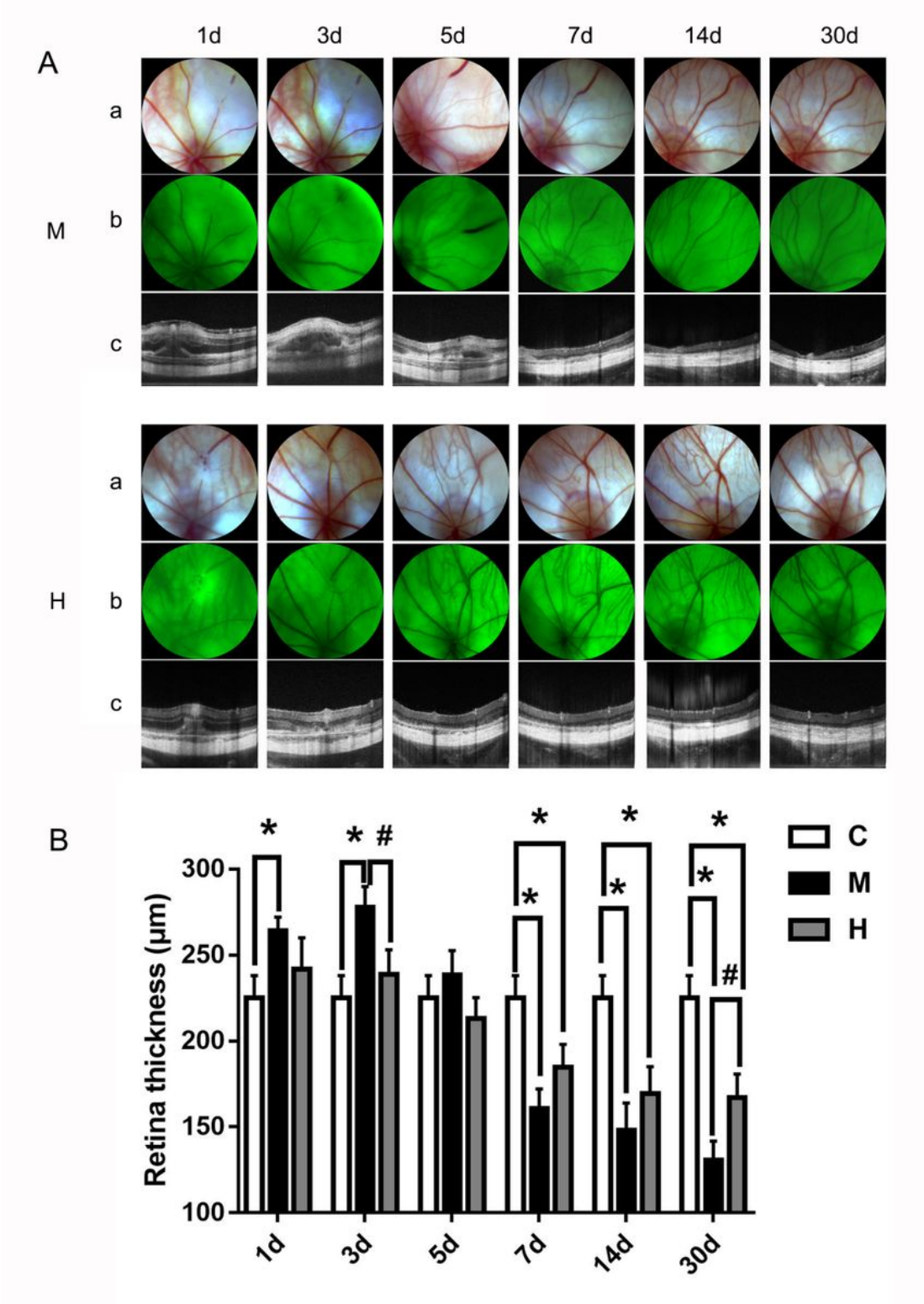

Figure 2

Hydrogen gas attenuated BRVO rat retinal edema and protected retinal structure integral. A: The typical fundus photograph and OCT pictures at 1, 3, 5, 7, 14 and 30 d post-occlusion. B: The thickness of retina of each group at 1, 3, 5, 7, 14 and 30 d post-occlusion. All analyses were performed in duplicates. The data are expressed as mean ± standard deviation (SD), n=4-12 rats per group. *, p<0.05, H and M group vs C group; #, p<0.05, H group vs M group. a: OCT; b: fundus photograph; c: fluorescent fundus color photography; C: control group, M: model group, H: hydrogen gas group.

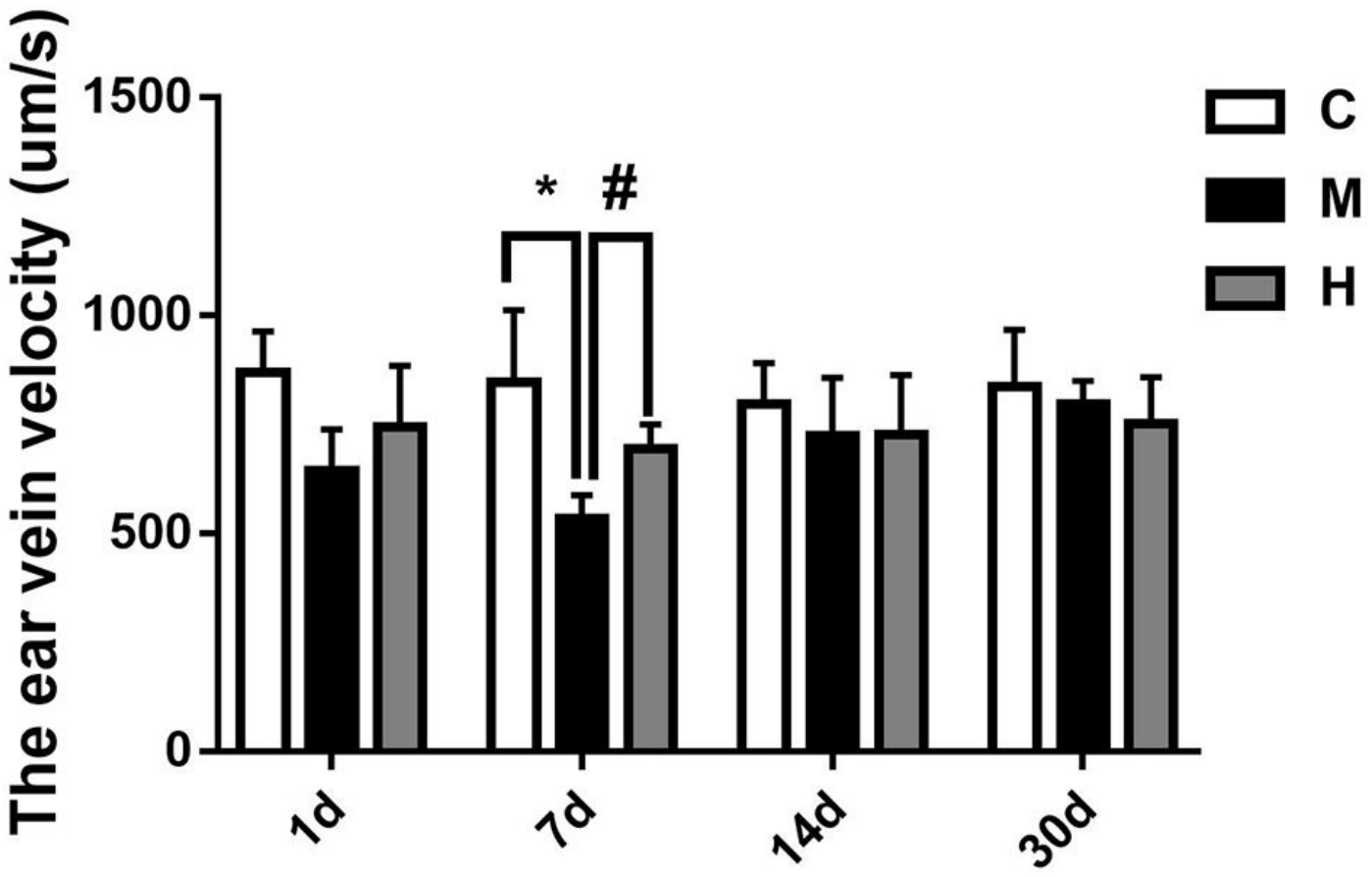

Figure 3

Hydrogen gas improved BRVO rat microcirculation at 7 d post-occlusion. BRVO rats ear vein blood flow velocity at 1, 7,14 and 30 d. All analyses were performed in duplicates. The data are expressed as mean ± standard deviation (SD), n = 4-12 rats per group. *, p<0.05, H and M group vs C group; #, p<0.05, H group vs M group. C: control group, M: model group, H: hydrogen gas group.

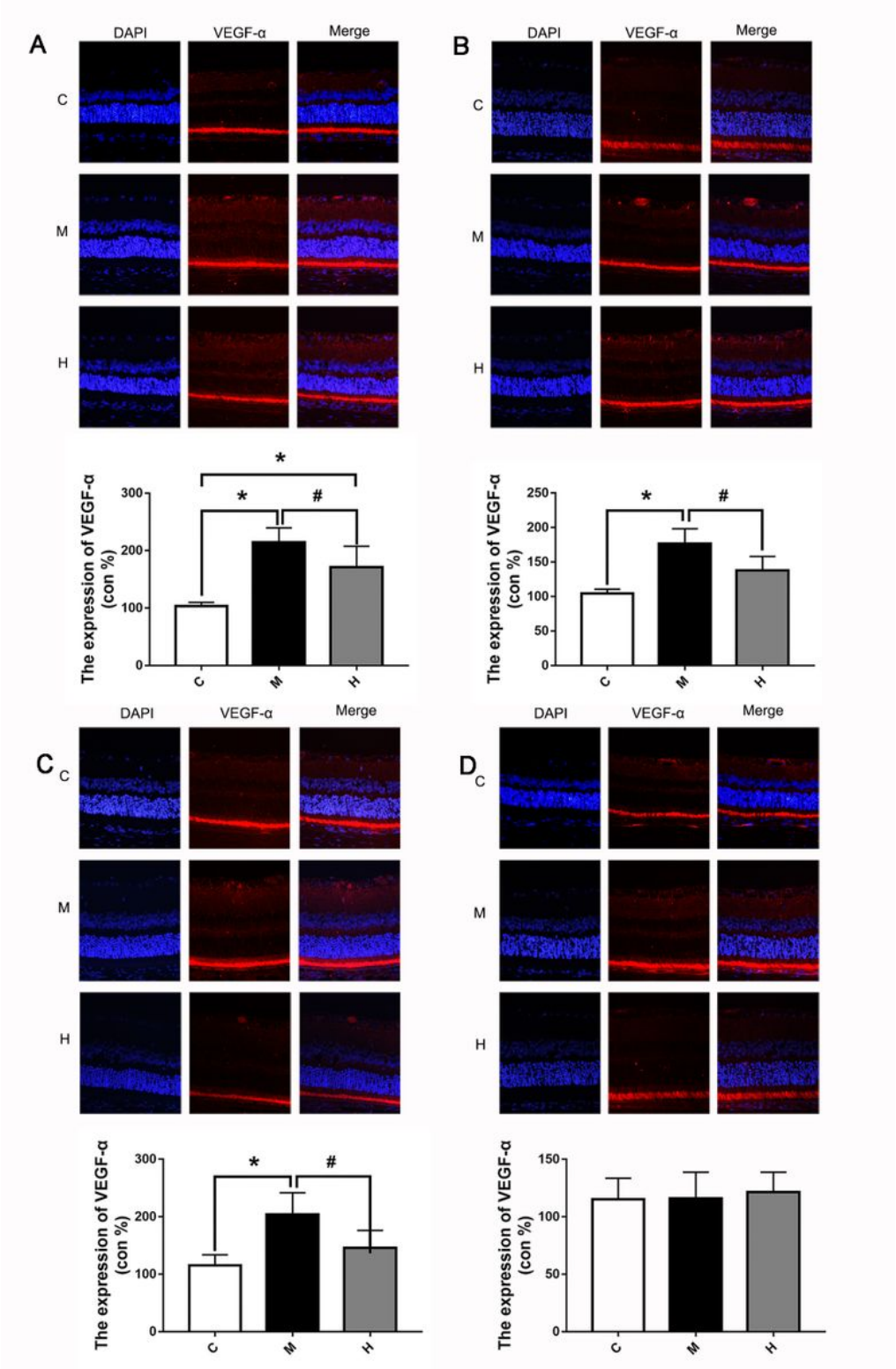

Figure 4

. Hydrogen gas decreased the expression of VEGF-α at 1, 7 and 14 d post-occlusion. A: The typical immunofluorescence staining picture and the expression of VEGF-α at 1 d post-occlusion. B: The typical immunofluorescence staining picture and the expression of VEGF-α at 7 d post-occlusion. C: The typical immunofluorescence staining picture and the expression of VEGF-α at 14 d post-occlusion. D: The typical immunofluorescence staining picture and the expression of VEGF-α at 30 d post-occlusion. All analyses

were performed in duplicates. The data are expressed as mean ± standard deviation (SD), n=3 rats per group. *, $p<0.05$, H and M group vs C group; #, $p<0.05$, H group vs M group. C: control group, M: model group, H: hydrogen gas group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf