Experimental Eye Research 174 (2018) 152–160

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Impact of fenofibrate on choroidal neovascularization formation and VEGF-C plus VEGFR-3 in Brown Norway rats

Jian-Feng Zhao, Hai-Rong Hua, Qian-Bo Chen, Meng Guan, Jin-Hui Yang, Xiao-Ting Xi, Yan Li**, Yu Geng*

Ophthalmology Department, The First Affiliated Hospital of Kunming Medical University, Kunming 650031, Yunnan province, PR China

A R T I C L E I N F O

Keywords:
Brown Norway rats
Choroidal neovascularization
Fenofibrate
VEGFC
VEGFR-3

A B S T R A C T

Objective: This study aims to explore the possible role of fenofibrate in inhibiting choroidal neovascularization (CNV) in Brown Norway (BN) rats.

Methods: BN rats underwent binocular retinal laser photocoagulation to induce CNV. On day one, fenofibrate was injected into the vitreous cavity of rats in the control and experimental groups. Fundus fluorescein angiography (FFA), isolectin B4-FITC staining, immunofluorescence staining, qRT-PCR and western blot were performed at 1, 2, 3 and 4 weeks to observe the morphological changes of CNV and the expression of the vascular endothelial growth factor C (VEGF-C) and the vascular endothelial growth factor receptor-3 (VEGFR-3).

Results: CNV with the spontaneous gradual regression and scarring phenomenon appeared in BN rats. In neovascularization, VEGF-C was mainly distributed in the ganglion cell layer, while VEGFR-3 was mainly expressed in the choroid. In the control group, choroidal VEGF-C initially increased, and subsequently decreased, while VEGFR-3 level maintained a constant level after the decrease. Both had a decreasing expression in the retina. The early formation of CNV was significantly weakened in the experimental group, but there was no difference in the later period. VEGF-C and VEGFR-3 expression in the choroid and retina were lower than in the control group. Furthermore, VEGFR-3 protein was not expressed in the retina. However, this gradually increased in the early period and declined in the terminal stage in the choroid.

Conclusion: VEGF-C and VEGFR-3 participated in the laser-induced CNV formation in BN rats. Fenofibrate could inhibit CNV formation.

1. Introduction

Choroidal neovascularization (CNV) is a chorioretinal disease that causes severe vision loss. CNV is the main cause of persistent hemorrhagic damage in the macular area of the posterior retina. Retinal pigment epithelial (RPE) cells can synthesize and secrete VEGF on pathological status to promote CNV formation (Oh et al., 1999). The process of CNV formation requires complex regulation with dependence on VEGF and VEGFR concentration gradients (Padera and Jain, 2008).

In adults, VEGF-C participates in tumor angiogenesis (Wang et al., 2016). The blocking of VEGFR-3 could lead to tumor sprouting and blood vessel branching (Tammela et al., 2008). Furthermore, it is relatively independent of the role for promoting vascular budding growth (Benedito et al., 2012). In the past, VEGF signals were considered to be mediated by its receptor VEGFR-2 in endothelial cells, while the Notch signal pathway regulated the balance between VEGF and VEGFR-2 (Su et al., 2008). In the process of neovascularization, masses of anti-neovascularization studies have focused on the canonical pathway, because VEGFR-2 was generally considered as a VEGF basic signaling receptor. However, the formation of new blood vessels may not merely come from this pathway.

Fenofibrate is a highly selective peroxisome proliferator-activated receptor alpha (PPARα) agonist. Oral fenofibrate significantly improves retinal vascular leakage in patients who suffer from type-2 diabetes (Steiner, 2008). Fenofibrate inhibits oxidative damage-induced retinal neovascularization (Valentin et al., 2009; Bogdanov et al., 2015), as well as RPE cells under hypoxia VEGF-C and VEGFR-3 expression, and vascular endothelial cell tube and migration activities (Zhao et al., 2015). Furthermore, fenofibrate is able to downregulate VEGF and hypoxia-inducible factor-1 (HIF-1) expression (Walker et al., 2012; Chen et al., 2013). The introduction above suggests that fenofibrate may have an inhibitory impact on CNV formation. We established a BN

* Corresponding author. Ophthalmology Dept., The First Affiliated Hospital of Kunming Medical University, No. 295 of Xichang Street, Kunming 650031, PR China.
** Corresponding author. Ophthalmology Dept., The First Affiliated Hospital of Kunming Medical University, No. 295 of Xichang Street, Kunming 650031, PR China.
E-mail address: gengyu@mail2.sysu.edu.cn (Y. Geng).

https://doi.org/10.1016/j.exer.2018.05.030
Received 16 January 2018; Received in revised form 24 April 2018; Accepted 29 May 2018
Available online 30 May 2018
0014-4835/ © 2018 Elsevier Ltd. All rights reserved.

rat model of CNV and performed the intravitreal injection of fenofibrate to explore its impact on CNV formation and the regulation effects of VEGF-C and its receptor VEGFR-3, providing experimental evidence for clinical application.

2. Materials and methods

2.1. Experimental animals

All experimental animals were approved by the Animal Ethics Committee of Kunming Medical University, and abided by the Animal Criterion of Animal Feeding and Use Committee of the Experimental Animal Center, Kunming Medical University, Kunming, China. Brown-Norway (BN) rats were provided by the Experimental Animal Center of Kunming Medical University. All BN rats were intraperitoneally injected with 10% chloral hydrate 0.5 ml for the experiment and tissue cutting.

A total of 104 healthy 10-week-old male BN rats were used for the present study. Rats were divided into two groups: control group ($n = 52$) and experimental group ($n = 52$). All subgroups were observed for 1, 2, 3 and 4 weeks, and were drawn materials at the above time spots.

CNV model of BN rats was established. Both pupils were dilated by tropicamide. Surface anesthesia was induced with buprenorphine hydrochloride, and krypton laser (wavelength = 659 nm) was used, with a laser power of 360 mW, laser spot diameter of 50 μm, and exposure time of 0.05 s. This was performed around the optic disc, with a distance of approximately 1000 μm from the optic disc. Eight laser spots were made, which broke through Bruch's membrane.

In the right eye of BN rats, 5 μl of fenofibrate lysate (DMSO + BSS) and 5 μl (2.26 μg) of fenofibrate were injected into the posterior vitreous cavity of the eyeball at one day after the operation.

2.2. Fundus fluorescein angiography (FFA)

In experimental group, the eyes were given dilated pupils three times at an interval of five minutes. Then, topical anesthesia with buprenorphine hydrochloride was applied and an intraperitoneal injection of 0.5 ml of sodium fluorescein was administered. FFA photography was immediately performed after 5 and 10 min, respectively. The fluorescent leakage area and intensity of the fundus were measured at the same time point using Image-Pro Plus.

2.3. Choroidal membrane and isolectin-B4-FITC staining

BN rats in the control and experimental groups were perfused with 4% paraformaldehyde solution (4% paraformaldehyde + 0.1 M of PBS solution) at 1, 2, 3 and 4 weeks, and fixed in formaldehyde solution for 30 min. The eyeball was cut along the limbus, the lens was removed, the retina was stripped to the papilla, the optic nerve is cut off from the papilla, and the sclera was flattened on a glass slide with the sclera facing down and the choroid facing up. Isolectin B4-FITC (10 μg/ml) (Alexis, Switzerland) was added dropwise to the specimen with 0.01 M of PBS solution (pH 7.2) three times for five minutes, and incubated in a humid chamber overnight at 4 °C. Then, the samples were rinsed with 0.01 M of PBS liquid for three times, per five minutes. then observed under the fluorescence microscope after being mounted.

2.4. Immunofluorescence staining

The BN rats were perfused and fixed. The eyeballs were placed in 4% paraformaldehyde solution and fixed for four hours. Then, the eyeballs were placed in 10% → 20% → 30% sucrose solution for gradient dehydration (to avoid retinal detachment). After the tissues sunken, the eyeball was cut along the limbus, and the lens was removed. The frozen section was 5–10 μm. Then, oven-baking at 55 °C for one hour, rinsed with 0.01 M of PBS for five minutes × three times, 50 μl of 0.1% Triton X-100 (Beyotime, China) punch for 30 min, rinsed with 0.01 M of PBS for five minutes × three times, and added with mouse polyclonal anti-VEGF-C (1:1000; Abcam, UK) and mouse polyclonal anti-VEGFR-3 (1:500; Abcam, UK) overnight at 4 °C. Next, rinsed with 0.01 M of PBST for five minutes × three times, incubated with goat anti-rabbit IgG (1:500; Abcam, UK) for one hour at 37 °C, rinsed with 0.01 M of PBST solution for five minutes × three times, and observed under a fluorescence microscope.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

The BN rats were anesthetized and sacrificed. Retinal and choroidal tissues were removed and placed in an enzyme-eluting tube. Total RNA was extracted from the retina and choroid by column chromatography using a Purelink RNA Mini Kit (Life Technologies, USA). RNase-free water dissolved the RNA, and each group was adjusted to 1 μg/μl of RNA. The reverse transcription reaction system: 2 μl of total RNA, 4 μl of PrimeScript™ RT Master Mix (TaKaRa, Japan), and 14 μl of RNase-free water. Reverse transcriptase cms: 37 °C, 15 min → 85 °C, 5 min → 4 °C. The qRT-PCR amplification system: 1 μl of cDNA template, 0.5 μl of forward primer, 0.5 μl of reverse primer (Table 1), 10 μl of SYBR qRT-PCR mixer (Bio-Rad, USA), and 8 μl of nuclear-free water were added to a 96-well plate (Roche, Switzerland). Amplification system reaction conditions: 96 °C (five minutes) → 60 °C (30 s) × 40 times. All experiments were repeated three times. Relative expression intensity was calculated using $^{2\text{-}\triangle\triangle}$Ct, and the results were analyzed.

2.6. Western blot

This was prepared similar to the sample size and methods performed for the qRT-PCR. Total protein was extracted: Retinal and choroidal tissues were lysed on ice using RIPA cell lysis solution (Beyotime Biotechnology, China), homogenized, and homogenized to no obvious tissue mass. The supernatant was harvested by centrifugation (12,000 g for five minutes) at 4 °C, and the total protein concentration was 5 mg/ml. The protein was wet-transferred onto the PVDF membrane (Thermo, USA) by 10% SDS-PAGE. The membrane was blocked with 5% non-fat dry milk and 0.01 M of PBS for one hour. Then, the membrane was incubated with mouse polyclonal anti-VEGFR-3 and mouse polyclonal anti-VEGF-C overnight at 4 °C (VEGFR-3, 1:200; VEGF-C, 1:500; Abcam, UK), incubated with Pierce ECL substrate (Thermo, USA) for one minute at room temperature, and the goat anti-rabbit lgG H&L (HRP, 1:2000; Abcam, UK) was incubated for 2 h at room temperature. Meanwhile, the process images were collected by BIO-RAD, the strip gray value was determined by Image J.

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (x ± S), and statistically analyzed using SPSS 18.0 (SPSS Inc., USA) statistical software package. The differences between groups were analyzed by

Table 1
Quantitative real-time polymerase chain reaction (qRT-PCR).

Rat gene	Forward primer	Reverse primer
VEGFC (76bp)	5′-TTGATAATGAGTGGAGAA-3′	5′-TTAAAGAAGGTGTTTGTG-3′
VEGFR-3 (75bp)	5′-GTAGAAGGCTCGGAAGATA-3′	5′-GAAGAAGACTGCGATGAC-3′
GAPDH (80bp)	5′-CATTCTTCCACCTTTGAT-3′	5′-CTGTAGCCATATTCATTGT-3′

Download English Version:

https://daneshyari.com/en/article/8791931

Download Persian Version:

https://daneshyari.com/article/8791931

Daneshyari.com