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# Vasoactivity of retinal veins: A potential involvement of endothelin-1 (ET-1) in the pathogenesis of retinal vein occlusion (RVO)

Teruyo Kida[a,*], Josef Flammer[b], Hidehiro Oku[a], Katarzyna Konieczka[b], Seita Morishita[c], Taeko Horie[a], Tsunehiko Ikeda[a]

[a] Department of Ophthalmology, Osaka Medical College, Takatsuki, Japan
[b] Department of Ophthalmology, University of Basel, Basel, Switzerland
[c] Department of Ophthalmology, Osaka Kaisei Hospital, Osaka, Japan

A B S T R A C T

*Purpose:* Whilst the pathogenesis of retinal vein occlusion (RVO) is still unclear, systemic hypertension and increased level of endothelin-1 (ET-1) are known risk factors. Therefore, we studied the influence of ET-1 on the retinal veins in hypertensive rats.

*Methods:* We focused on the behavior of retinal veins in spontaneous hypertensive rats (SHR). To determine whether ET-1 was associated with the blood flow in eyes of SHRs, the chorioretinal blood flow in the rats was assessed using laser speckle flowgraphy (LSFG-Micro, Softcare, Fukuoka, Japan) before and after an intravenous injection of ET-1 under general anesthesia. In addition, retinas from SHRs and age-matched normotensive Wistar-Kyoto rats (WKYs) were removed, and retinal sections were immunostained for the ET-A and ET-B receptors. The protein levels of both ET-1 receptors and hypoxia-inducible factor 1 (HIF-1) in the retinal tissues were also determined by western blot analysis.

*Results:* One of the retinal veins became exceptionally constricted and was nearly occluded, and the chorioretinal blood flow significantly decreased in the retinas of SHRs following the injection of ET-1. Immunoreactivity to ET-A receptor was higher in SHR retinas than in WKY retinas. The protein levels of ET-A receptor and HIF-1 were also significantly higher in SHR retinas than in WKY retinas.

*Conclusions:* An increase of ET-1 in circulating blood leads to the local constriction of retinal veins and this effect is accentuated in hypertensive rats by an upregulation of ET-A receptor. It is plausible that such a constriction of retinal veins increases retinal venous pressure, and may even contribute to the pathogenesis of RVO.

## 1. Introduction

Retinal vein occlusion (RVO) is a common vascular disease of the retina. In general, increased age, hypertension, arteriosclerosis, cardiovascular disorder, dyslipidemia, diabetes mellitus, and cerebral stroke are systemic risk factors of RVO; however, the pathogenesis of RVO is still discussed controversy. Branch RVO (BRVO) commonly occurs at the arteriovenous crossing and it was formerly believed that the diseased artery mechanically compresses the vein. However, it has been reported that the retinal vein runs deep beneath the artery at the arteriovenous crossing in eyes with an arterial overcrossing, and the venous lumen often appears to be preserved, even at the arteriovenous crossing, as shown by optical coherence tomography (Kumagai et al., 2014). In addition, Paques et al. found venous nicking without arteriovenous contact using adaptive optics imaging (Paques et al., 2015). Thus, in this study, we investigated the potential role of a dysregulation of the retinal vein in causing RVO.

It has been postulated that a local constriction of retinal veins, particularly a constriction caused by endothelin-1 (ET-1), increases retinal venous pressure and may even contribute to RVO (Flammer and Konieczka, 2015). In vitro studies have proven the sensitivity of retinal veins to ET-1 (Yu et al., 2016). Systemic hypertension is a well-known risk factor of RVO in humans (Jaulim et al., 2013), and RVO can lead to retinal ischemia. For this reason, we also examined the behavior of retinal veins in spontaneous hypertensive rats (SHR).

## 2. Materials and methods

To examine whether ET-1 was associated with the blood flow in the eyes of SHRs (9–11 weeks of age, $n = 5$), the chorioretinal blood flow in the rats was assessed using laser speckle flowgraphy (LSFG) (LSFG-Micro, Softcare, Fukuoka, Japan) before and after an intravenous injection of ET-1 (2 nmol/kg) under general anesthesia. The methods and principles of LSFG have been described in a previous study (Wada et al., 2016). LSFG imaging provides a relative index of blood velocity represented as MBR (mean blur rate), which is determined by analyzing

* Corresponding author. Department of Ophthalmology, Osaka Medical College, 2-7 Daigaku-machi, Takatsuki, Osaka, 569-8686, Japan.
*E-mail address:* opt038@osaka-med.ac.jp (T. Kida).

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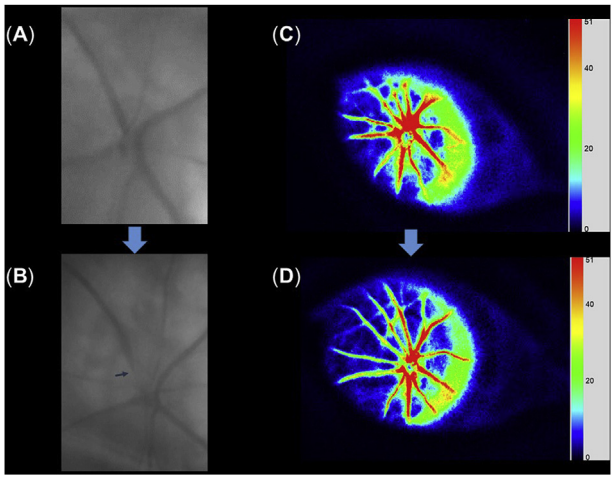


**Fig. 1.** Fundus photos of optic nerve head (A and B) and LSFG imaging of chorioretinal blood flow (C and D) before and after the intravenous injection of ET-1 in SHRs.
One of the retinal veins became exceptionally constricted and nearly occluded (arrow, B). The color mapping scale of the fundus by LSFG shows the volume of blood flow. Red and blue indicate high and low volumes of blood flow, respectively. The chorioretinal blood flow decreased following the intravenous injection of ET-1 (D).

the blurring of the speckle pattern formed through the interference of a laser that is scattered by the movement of blood cells. In addition, retinas from SHRs and age-matched normotensive Wistar-Kyoto rats (WKYs) ($n = 5$ each) were fixed by perfusion under deep anesthesia with a mixture of medetomidine, midazolam hydrochloride, and butorphanol tartrate. The retinal tissues were removed, and retinal sections were immunostained for the ET-A (1:500, Abcam plc, Cambridge, UK) and ET-B (1:500, Sigma-Aldrich, St. Louis, MO, USA) receptors. In addition, we performed immunohistochemical staining of flat mount retina. The retinal flat mounts were immunostained for ET-A, ET-B, and α-smooth muscle actin (SMA) (1:500, Sigma-Aldrich, St. Louis, MO, USA). The processed sections were photographed with a fluorescent microscope (BZ-X700, Keyence, Osaka, Japan). The protein levels of both ET-1 receptors (1:1000) and hypoxia-inducible factor 1 (HIF-1) (1:1000, Santa Cruz, Dallas, TX, USA) in the retinal tissues were also determined by western blot analysis. Samples containing 20 μg of protein were run on 7.5%–10% SDS-PAGE gels and electroblotted onto polyvinylidene difluoride membranes. In addition, the plasma ET-1 concentrations in WKYs and SHRs were measured by ELISA (Quantikine® ELISA Endothelin-1 Immunoassay, R&D Systems, Inc., Minneapolis, MN, U.S.A.). Our experimental protocols conformed to guidelines in Animal Research: Reporting In Vivo Experiments (ARRIVE) and were approved by the Osaka Medical College Committee on the Use and Care of Animals (approval number: 29097).

## 3. Results

One of the retinal veins became exceptionally constricted and was nearly occluded, and the chorioretinal blood flow significantly decreased in the retinas of SHRs 30 min after the injection of ET-1 (Fig. 1). The chorioretinal blood flow decreased after the intravenous injection of ET-1 in all WKYs and SHRs; however, there was a statistically significant difference in the decreases in blood flow from the baseline between WKYs (−7.3 ± 3.0%, mean ± S.D.) and SHRs (−17.3 ± 8.3%) (Student's *t*-test; $P < 0.05$). In the immunostained flat mount retina (Fig. 2), immunoreactivity to ET-A receptor was significantly higher in retinal blood vessels of SHRs (Fig. 2C–D). The immunoreactivity to ET-B receptor in retinal vessels was not specific (data not shown). The immunoreactivity to ET-A receptor was higher in SHR retinas than in WKY retinas (Suppl.Fig. 1A–1B), and choroidal vessels also seemed to be stained by anti-ET-A receptor antibody (Suppl.Figure 1C). The protein levels of ET-A receptor and HIF-1 were significantly higher in SHR retinas than in WKY retinas (Fig. 3, Suppl.Fig. 2A–2D and 2A′-2C′). The plasma ET-1 was significantly greater in SHRs (1.15 ± 0.21 pg/mL, mean ± S.D.) than in WKYs (0.57 ± 0.12 pg/mL) (Student's *t*-test; $P < 0.05$; $n = 5$ each).

## 4. Discussion

We observed a retinal venous constriction that was more pronounced in the SHRs than in the controls, and the chorioretinal blood flow decreased after the intravenous injection of ET-1. To explain this increased sensitivity, we quantified the number of ET-A and ET-B receptors and found a higher concentration of in the retinas of the SHRs. It is known that ET-A receptors are localized in the retinal and choroidal blood vessels (MacCumber and D'Anna, 1994; de Juan et al., 1995), therefore, chorioretinal circulation can be affected by ET-A receptors. It is also known that SHRs have increased plasma levels of ET-1. Systemic hypertension may lead to hypoxia and, therefore, an increase in ET-1 via an increase of HIF-1 in the retina.

The reason for the upregulation of the ET-1 receptors is not yet known. These receptors are internalized after the ET-1 binding and then recycled. Therefore, a short-term elevation in ET-1 reduces the

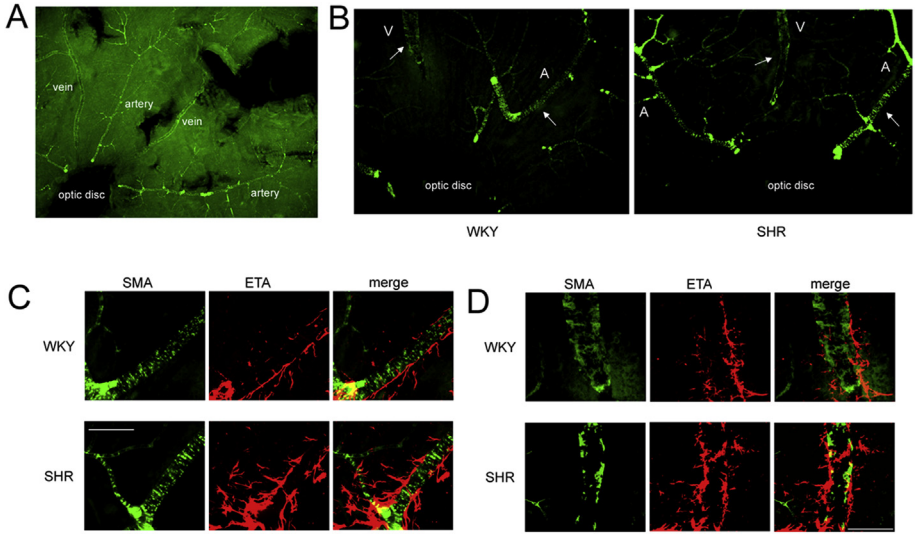


**Fig. 2.** The flat mount of retinas in WKY and SHR.
(A) The orientation of retinal vessels and optic nerve head in the flat mount immunostained for SMA. (B) The flat mount of retinas in WKY and SHR immunostained for SMA (A: artery, V: vein). (C) Retinal artery (A with arrow in Fig. 2B) immunostained for ET-A receptor and SMA. The immunostained for ET-A was observed along with the vessel wall stained for SMA, and the immunoreactivity to ET-A was higher in SHR compared to WKY. (D) Retinal vein (V with arrow in Fig. 2B) immunostained for ET-A and SMA. The immunostained for ET-A was observed along with the vessel wall stained for SMA, and the immunoreactivity to ET-A was higher in SHR compared to WKY.

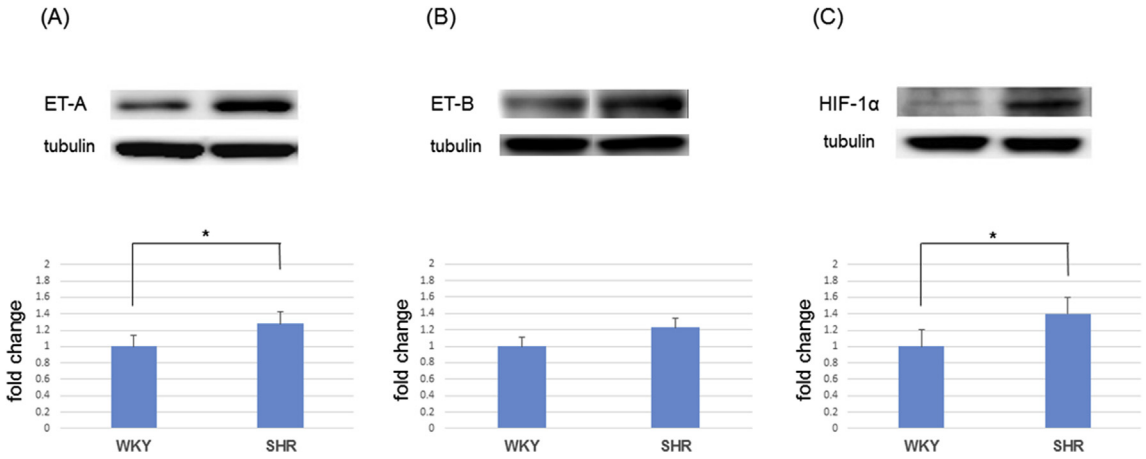


**Fig. 3.** Protein levels of both types of ET receptors and HIF-1 by western blot. The protein levels of ET-A receptor and HIF-1 were significantly higher in SHR retinas than in WKY retinas (Student's *t*-test; $P < 0.05$; $n = 5$ each).

concentration of ET-1 receptors, while a long-term increase in ET-1 also increases the density of the receptors due to the autocrine and paracrine function of ET-1; this may be the case in SHRs.

Overall, SHRs have high blood pressure, increased ET-1 sensitivity in the retinal veins, and an increased density of retinal ET-1 receptors. This supports the hypothesis that ET-1 can constrict retinal veins, thus increasing retinal venous pressure, and that ET-1 may even contribute to the pathogenesis of RVO.

Clinically, RVOs take numerous clinical courses. Vein congestion can lead to local hypoxia, thereby increasing the vascular endothelial growth factor (VEGF). Anti-VEGF therapy is the standard treatment for RVO-related macular edema at present; however, the early detection and treatment by internal medicine specialists of cardiovascular diseases that are known risk factors of RVO are still important and helpful to ophthalmologists.

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## Disclosures

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.exer.2018.07.016.

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