Progressive Retinal Degeneration in Transgenic Mice with Overexpression of Endothelin-1 in Vascular Endothelial Cells

Xue-Song Mi, Xu Zhang, Qian Feng, Amy Cheuk Yin Lo, Sookja Kim Chung, and Kwok-Fai So

PURPOSE. Endothelin-1 (ET-1), synthesized in vascular endothelial cells, is a potent vasoconstrictor. ET-1-related vascular abnormality has been known to be important in the pathogenesis of glaucoma, especially in normal tension glaucoma. However, the long-term effect of increased vascular ET-1 on the retinal tissue is still unclear.

METHODS. The mice with overexpression of ET-1 in vascular endothelial cells (TET-1 mice) were examined with the profile of intraocular pressure (IOP), retinal layer thickness, numbers of retinal ganglion cells (RGCs), and axonal changes associated with blood vessel changes.

RESULTS. The TET-1 mice exhibited a significant progressive loss of RGCs and decrease of retinal thickness in the inner nuclear layer (INL) and outer nuclear layer (ONL) as early as around 10–12 months. At 24 months, the retinal degeneration became more severe, with around 30% RGC loss associated with thinning of the retinal nerve fiber layer and there was an increase in neuronal loss and thinning of the INL and ONL. In the 24-month-old TET-1 mice, IgG leakage in the blood vessels and decrease in the occludin protein were observed. There was increased glial fibrillary acidic protein expression in the Müller cells. In addition, the astrocytic end-feet on blood vessels were enlarged. The IOP level was normal in all ages (1–24 months) of TET-1 mice.

CONCLUSIONS. These data suggested that TET-1 mice may be a useful model to address endothelial ET-1–related mechanisms in vascular-associated retinal degenerative diseases. (Invest Ophthalmol Vis Sci. 2012;53:4842–4851) DOI:10.1167/iovs.12-9999

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Supported in part by a General Research Fund grant from Hong Kong Research Grant Council, National Basic Research Program of China Grant 2011CB707501, and the Fundamental Research Funds for the Central Universities Grant 21609101.

Submitted for publication April 10, 2012; revised May 28, 2012; accepted June 15, 2012.

 Disclosure: X.-S. Mi, None; X. Zhang, None; Q. Feng, None; A.C.Y. Lo, None; S.K. Chung, None; K.-F. So, None

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Glaucoma, an irreversible blindness-inducing disease affecting individuals worldwide, is a neurodegenerative disease of the eye. Normal-tension glaucoma (NTG), a subtype of primary open-angle glaucoma (POAG), shares similar features of neurodegeneration with POAG, although without the elevation of IOP. Epidemiologic studies showed that NTG could contribute to a large portion of all POAG in some races, suggesting that IOP-independent factors may play key roles in the progressive neurodegeneration of NTG. Clinically, vasospastic disorders, such as migraine headache, Raynaud phenomenon, and ischemic vascular diseases, were shown a higher prevalence among patients with NTG. Together, among these non-IOP-related mechanisms, vascular abnormality may play an important role in the pathogenesis of NTG.

Endothelin-1 (ET-1), a potent vasoconstrictor, has been suggested to be a contributor to the pathophysiology of glaucoma. Clinically, an increased level of ET-1 has been found in circulating plasma of patients with NTG in many studies. Moreover, ET-1 was thought to be a link between vascular dysfunctional symptom and patients with NTG. In animal models, administration of ET-1 to the eye produced chronic optic nerve ischemia. Further comparison of these findings indicated that different delivery methods induced different effects on the retinal neurons; for example, retrobulbar delivery of ET-1 could kill only the retinal ganglion cells (RGCs) whereas intravitreal injection of ET-1 not only induces the selective loss of RGCs but also damages the neurons in the inner nuclear layer (INL). These phenomena may be attributed to the local effects of ET-1. However, there is no proper animal model yet to mimic the clinical conditions of the long-term effects of increased vascular ET-1 in circulation in some population of patients with NTG.

In this study, we used a transgenic mice line (TET-1) with overexpression of ET-1 in the vascular endothelial cells using the tyrosine kinase receptor specific for endothelial cells (the Tie-1 promoter) to examine the long-term effect of endothelial ET-1 on the morphologic changes in the retina and optic nerve. We further tested the hypothesis that the overexpression of endothelial-ET-1 may be a cause or a contributor in the development of neurodegeneration in the retina.

METHODS

Mice

The generation of TET-1 mice was reported previously by microinjection of the ET-1 construct, which contains ET-1 cDNA with SV40 polyA driven by the Tie-1 promoter. TET-1 mice, maintained in the F1 hybrid background (C57BL/6J × CBA), and their non-transgenic littermate control (Non-tg) mice were used in this study. The animals were maintained on a 12-hour light/dark cycle and received food and water without restriction. All animal experimental design and
протоколы проведены в соответствии с ARVO утверждением о выживании животных в офтальмологическом и исследовании зрения, и были одобрены Комитетом по использованию живых животных в обучении и исследовании (CULATR #1664-08) в Гонконге.

**Retinal Sample Preparation**

Животных обезвоживали пентобарбиталом. Глаза удалялись немедленно, обработанные в 4% формальдегиде (ПФА) на ночь при 4°С, дегидратированы с последующим погружением в серию этилового спирта, и последовательно в парафиновый воск. С помощью четких ориентировок на глазу были установлены проходные зоны с помощью линейного кода на каждом надрезе. Повторным окислением с дополнительным окрашиванием и последующим погружением в 1% озонит в 0.1 М фосфатном буфере (ПБ) на 2 часа при 4°С. Сухие ткани были окрашены в 10% козьей сыворотки, а затем инкубированы с первичным антителом. Окончательное инкубирование с вторичными антителами проводилось при комнатной температуре.

**Histologic Analysis on H&E-Stained Retinal Sections**

В предыдущих методах, для стандартизации срезов для исследования, срезы с оптическим нервом были использованы для окрашивания гематоксилином и эозином (H&E). При наличии трех дискретных срезов с оптическим нервом были выбраны. Четыре образца, подвергнутые амплификации в течение 30 минут, были взяты из одного среза в центре сетчатки (200 мкм от оптического нерва) и периферический ретинальный (200 мкм от периферического края) с помощью флуоресцентного микроскопа (Аксиопот 2; Carl Zeiss Microscopy). Интенсивность флуоресценции, отображающая количество зрительных нейронов (РГН), была рассчитана на основании количества РГН в 4% ПФА на 4°С для 1 часа перед извлечением из глаза.

**Morphologic Examination of the Optic Nerve Head**

Наблюдения для окрашивания гематоксилином и эозином (H&E) и микроскопического исследования использовались для исследования оптического нерва. Для исследования оптического нерва, темная ткань и гипертрофия были выбраны. Оптический нерв был готов под наложением на предметное стекло, а затем под микроскопом, чтобы обеспечить равномерную ориентацию.

**Immunocytochemistry (ICC)**

ICC стимулирование проводилось в соответствии с следующим протоколом. Силовые образцы слизьали с ПФА с 0.3% трихлорэтиленом и 10% аммиаком на 1 час. Затем, они были инкубированы с первичными антителами на 4°С на ночь, а вторичные антитела были инкубированы с конъюгированными флуоресцентными пигментами (табл.)

**RGC Counting on Flat-Mounted Retinas**

Счетчик РГН, обработанных в 4% ПФА на 4°С, был проведен следующим образом. Количество РГН подсчитывалось с помощью подсчета на 200 × 200 мкм² в каждую из 24 полей зрения, начиная от периферического края до периферического края, и затем подсчитывалось количество РГН в 4% ПФА на 4°С.

**IgG Extravasations**

Ретинальные срезы были подготовлены по описанным ранее методикам и блокированы с помощью аммиака. Статистика была использована для определения количества IgG с помощью диминобензилдигидроксида (DAB).

**Analysis of Capillary Network and Arteriole Branch Points on Flat-Mounted Retinas**

Используя Иохселтин, обнаружены, что капиллярная сеть оценивает содержание диминобензилдигидроксида (DAB).

**Table. Reagent Utilization**

<table>
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<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Host</th>
<th>Company</th>
<th>Catalog Number</th>
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<td>Rabbit</td>
<td>Peninsula</td>
<td>T-1050</td>
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<td>GS</td>
<td>1:600</td>
<td>Mouse</td>
<td>Millipore</td>
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<td>GFAP</td>
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<td>Mouse</td>
<td>Sigma</td>
<td>G3893</td>
</tr>
<tr>
<td>GFAP</td>
<td>1:1000</td>
<td>Rabbit</td>
<td>Dako</td>
<td>Z0554</td>
</tr>
<tr>
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<td>Covance</td>
<td>MMS-435P</td>
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<tr>
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<td>002114</td>
</tr>
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**http://rsbweb.nih.gov/ij/**
from 0 to 255, then the mean optical density was calculated to represent the average capillary density of each retina. For the quantification of vascular branch points, 3 of 10 images were used. In each retinal quadrant, the counting was performed from only one side of branches in the main retinal artery within a single pair of main arteriole and main venule. The number of points in four quadrants of each retina was summed and then divided by the total number of primary branches counted in retinal arterioles and the value obtained was the branch point density of the retina.

**Semiquantification of GFAP Staining on Retinal Cross-Sections**

The method to collect retinal sections was as described earlier. Three slides (including at least 12 sections) with the same code number and not adjacent to each other were used to ensure the comparison in similar retinal areas among different groups. For image analysis, at least five photographs per section at ×400 amplification were taken randomly under the same fluorescence exposure setting. ImageJ software (http://rsbweb.nih.gov/ij/) was used to obtain the fluorescence intensities and the average value was used to represent the labeling intensity of each retina. This method was based on the criteria published previously.

**Western Blotting**

The level of occludin in the retina was measured using Western blotting. The retinas (24-month-old of TET-1 and Non-tg mice) were homogenized in lysis buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% protease inhibitor cocktail, and 1% phosphatase inhibitor cocktails) and centrifuged (2000g, 5 minutes, 4°C). The supernatant was measured by protein assay kit (Bio-Rad Laboratories, Hercules, CA). A 40 μg aliquot of proteins from each individual animal was subjected to 12.5% SDS-polyacrylamide gel electrophoresis and transferred into a polyvinylidene difluoride membrane. The blot was incubated with antibody against occludin (1:1000; Invitrogen, Carlsbad, CA) and β-actin (1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibody was horseradish peroxidase–conjugated secondary antibody (Dako Japan Co. Ltd., Kyoto, Japan). Signals were visualized by emitter-coupled logic (ECL; Amersham, Buckinghamshire, UK) and quantitated using ImageJ software. The ratio of occludin expression for TET-1 retina over Non-tg counterparts was determined after normalizing the individual β-actin levels.

**Intraocular Pressure (IOP) Monitoring**

IOP was measured using a noninvasive rebound tonometer (TONOLAB; Colonial Medical Supply, Franconia, NH). TET-1 mice and their littermates were grouped by their ages as young (1–2 months), adult (10–12 months), and aged (21–24 months). Both male and female animals were used. Animals were anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg). Proparacaine hydrochloride 0.5% (Alcaine; Alcon, Ltd., Fort Worth, TX) was also applied to desensitize the cornea. All IOP measurements were taken consistently at approximately 8 to 10 PM to avoid diurnal variations and 10 readings were averaged for each measurement of both right and left eyes. The animals were returned to their cages after the measurements and kept warm for recovery.

**Figure 1.** Loss of RGCs in TET-1 mice. (A–D) Hematoxylin–eosin-stained retinal sections of 24-month Non-tg and TET-1 mice. (A) The central retina of Non-tg mice. (B) The central retina of TET-1 mice. (C) The peripheral retina of Non-tg mice. (D) The peripheral retina of TET-1 mice. (E, F) β-Tubulin III–labeled RGC staining on retinal flat-mounts. (G) The analysis of neuronal loss in the central area of GCL (n = 7, *P < 0.001). (H) The analysis of neuronal loss in the peripheral area of GCL (n = 7, *P < 0.001). (I) The analysis of β-tubulin III–labeled RGCs on retinal flat-mounts of 24-month Non-tg and TET-1 mice (n = 7, *P < 0.001). All scale bars: 50 μm.

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Statistical Analysis

All data analysis was performed in a blinded manner. The values were present as mean ± SD. Student’s t-test or one-way ANOVA followed by Bonferroni’s post-test was used for analysis of the results, including the IOP measurement, cell counting, retinal layer thickness analysis, and glial fibrillary acidic protein (GFAP) staining intensity. Statistically significant difference was set at \( P < 0.05 \).

RESULTS

Loss of RGCs and Thinning of Retinal Layers in TET-1 Mice

Our previous study has reported the upregulated mRNA and peptide expression of ET-1 in TET-1 mice. Here, in H&E-stained retinal sections, 24-month-old TET-1 mice showed a decrease of cell density in the ganglion cell layer (GCL) in both the central and peripheral retina (Figs. 1B, 1D) when compared with the age-matched Non-tg controls (Figs. 1A, 1C). Quantitative analysis showed a progressive loss of neurons in the GCL in the early age of TET-1 mice when compared with the age-matched Non-tg (\( n = 7, P < 0.001 \)) (Figs. 1G, 1H). At as early as 12 months of age, a significant neuronal loss in the GCL was first shown in the peripheral retina, whereas the cell density in the central GCL showed no significant decrease. At the age of 18 months, the cell density of GCL became smaller not only in the peripheral but also in the central retina. At 24 months, an obvious neuronal loss was shown in the TET-1 retina (32.1% in the central area and 35.7% in the peripheral area; \( n = 7, P < 0.001 \)). These data suggested that in the TET-1 mice, the tendency of neuronal degeneration in the GCL started first from the peripheral retina and then spread to the central retina.

\( \beta \)-Tubulin III can be used as a marker to label RGCs. Here we used it to distinguish RGCs from displaced amacrine cells in the GCL on retinal flat-mounts. Our results showed that there was a remarkable RGC loss at around 33.4 ± 5.7% in the 24-month-old TET-1 mice (2547 ± 218 cells/mm²) compared with the Non-tg controls (3826 ± 152 cells/mm²) (\( n = 7, P < 0.001 \)) (Figs. 1E, 1F, 1I), a ratio similar to that seen in retinal sections.

To check if there is degeneration of other retinal neurons in the TET-1 mice, retinal layer thickness analysis was used. When compared with the age-matched Non-tg controls at each time point (12 months, 18 months, and 24 months), TET-1 mice showed a progressive thinning of the INL and ONL (Fig. 2). As early as the age of 12 months, a significant decrease of retinal thickness was detected in the peripheral INL and ONL (Figs. 2C, 2D); at the age of 18 months, a significant decrease of the INL and ONL was observed in both the peripheral and central retina (Figs. 2A, 2B); at the age of 24 months, the decrease of thickness became more obvious. These findings suggested that TET-1 mice showed a severe neuronal loss not only in the GCL but also in the INL and ONL. Thus, the aged TET-1 mice showed degeneration in many retinal layers.

Degeneration of Optic Nerve in the TET-1 Mice

Associating with the progressive loss of RGCs, 24-month-old TET-1 mice showed the attenuation of the retinal nerve fiber layer (NFL) (Fig. 3B) compared with the same aged Non-tg controls (Fig. 3A). Quantitative analysis showed the significant NFL thinning in the TET-1 mice (41.6%; \( n = 7, P < 0.05 \)) at the central retina (200 μm from the optic nerve head) when compared with the Non-tg mice (Fig. 3C). The transverse section of the myelinated optic nerve showed the morphologic changes in the 24-month-old TET-1 mice, exhibiting the dense staining of the degenerating axons and many vacant holes.

\[ F(2, 24) = 25.8, P < 0.001 \]

\[ F(2, 24) = 22.9, P < 0.001 \]

\[ F(2, 24) = 18.4, P < 0.001 \]
replacing the lost axons (Fig. 3E). The increased expression of GFAP and the disarranged structure of astrocytes suggest the presence of astrocytosis in the TET-1 optic nerve (Fig. 3G) compared with the Non-tg mice (Fig. 3F). This is the corresponding reactivation of glial cells associating with the axonal degeneration of RGCs.

Normal IOP in the TET-1 Mice

To determine whether the IOP was affected in the TET-1 mice, IOP was measured at around 8 to 10 PM, near the peak of IOP in mouse eyes as reported previously. No significant differences in the IOP level were detected between TET-1 mice and Non-tg mice in various ages, including the young (1–2 months), adult (10–16 months), and aged group (21–24 months). Different categories of sex (male and female) also displayed no significant changes in the IOP ($n = 10–12$ per sex per age; $P = 0.791$ by one-way ANOVA) (Fig. 4).

Increase of IgG-Leaked Blood Vessels in the TET-1 Mice

To detect whether increased endothelial ET-1 in the TET-1 mice has the effect on damaging the permeability of the microvessels, IgG staining was examined in the retinal sections. IgG leakage can be used to detect the damage of the blood-retinal-barrier (BRB), which has been used in previous studies. Our study showed that a greater number of leaky blood vessels with immunopositive IgG signals located outside the endothelial vessel lining was observed in the 24-month-old TET-1 retina (Fig. 5B), when compared with the Non-tg control (Fig. 5A). Quantitative analysis showed there were significant differences both in the GCL and INL ($n = 7$, $P < 0.001$) (Fig. 5C). Western
blotting data showed there was a significantly decreased level of the occludin protein in the 24-month-old TET-1 retina compared with the Non-tg control \((n=6, P<0.001)\) (Fig. 5D).

**Remodeling of Retinal Microvasculature in TET-1 Mice**

Using Isolectin B4, the main retinal arteries and veins were identified morphologically.\(^{26}\) Here, we detected whether overexpression of endothelial ET-1 affects the retinal blood vasculature. The capillary networks were sparse in 24-month-old TET-1 mice (Fig. 6D), whereas it was abundant in Non-tg mice (Fig. 6A). The high-magnification micrographs showed more details of the sparse capillary network around the optic nerve head in TET-1 mice (Fig. 6E) compared with Non-tg mice (Fig. 6B). The sparse capillary networks were detected in the entire retina of TET-1 mice (Fig. 6F) compared with Non-tg mice (Fig. 6C). Further quantification (Figs. 6G vs. 6H) showed a significant decrease of capillary density in TET-1 retinas compared with Non-tg retinas (Fig. 6I). The branch points of retinal arteries were also significantly decreased in the superficial retina of TET-1 mice compared with Non-tg mice (Fig. 6J). The vascular loops were observed in TET-1 retinas (Fig. 6H), whereas no obvious looping formation was observed in vessels of Non-tg retina (Fig. 6G).

**Reactivation of Astrocytes around Blood Vessels in the TET-1 Mice**

Under normal conditions, GFAP is mostly expressed in the astrocytes of the GCL. Under stress, the expression of GFAP will be increased not only in astrocytes but also in the processes of the Muller cells seen clearly in retinal sections.\(^{15,19,24,30}\) Thus, the increased expression of GFAP has been considered as an indication of retinal gliosis. Here, we showed that this change was also present in the TET-1 retina. In the 24-month-old TET-1 mice (Figs. 7B, 7D), the expression of GFAP was increased in the retinal sections when compared with the Non-tg mice (Figs. 7A, 7C). Semiquantitative analysis of GFAP immunointensity confirmed this tendency (Fig. 7E). Moreover, the 24-month-old TET-1 mice also showed the specific morphologic change in astrocytes on retinal flat-mounts, with complicated processes and enlargement of the...
Relationship of TET-1 Mice with NTG and Other Vascular-Related Retinal Diseases

Elevated levels of ET-1 in plasma have been reported in the TET-1 mice in our previous study, which is similar to clinical findings reported in some population of NTG. Many lines of evidence indicated that ET-1 was involved in the pathogenesis of neurodegeneration of NTG both in clinic and in nonhuman animal studies. Here, we investigated the morphologic changes of retina induced by overexpression of endothelial ET-1. TET-1 mice showed progressive loss of RGCs and degeneration of their axons, which are two key phenotypes of NTG. However, some phenotypes were also observed that were different from clinical NTG findings, such as no flat-bottomed cupping and the presence of neuronal loss in the INL and ONL.

First, in animal studies, plate-bottomed glaucomatous cupping was observed in the high-IOP-induced glaucoma model, such as nonhuman primates and DBA/2J mice, which is consistent with the finding of clinical glaucoma. However, this classical optic nerve excavation is absent in recently reported NTG-related mouse model, such as glutamate/aspartate transporter deficient (GLAST/−/−) mice, OPTN gene mutated mice, and WD repeat-containing protein 36 (WDR36) transgenic mice. In our study, the thinning of NFL found in the aged TET-1 mice was in agreement with the findings in those gene-mutated normal-IOP mice.

The development of optic nerve excavation, the specific glaucomatous cupping shape with a flat bottom in patients with NTG may be related to elevation of IOP. This is supported in reports that lowering IOP treatment is effective in patients with NTG. Moreover, thinner values of central corneal thickness (CCT) were found in patients with NTG than the mean corneal thickness in that population, resulting in underestimation of the IOP reading and masking the sign of elevated IOP presented in patients with NTG. Together, it is believed that glaucomatous excavation is closely related to elevation of IOP, which made the cupping shape found in NTG-related animals different from that in high-IOP-induced animals.

Second, the presence of neuronal loss in the INL and ONL in the TET-1 mice may be related to the action sites of ET-1. Previous reports using ET-1 retroglobal delivery showed only the loss of RGCs in retina. However, intravitreal injections of ET-1 induced degeneration not only in RGCs but also in neurons of the INL. In our case, endothelial ET-1 could exert bioeffects along with the distribution of retinal blood vessels and aggravate the retinal degeneration by the leakage of BRB. Thus, the long-term effects and broad action sites of ET-1 in the TET-1 mice could be exhibited not only on RGCs but also on other retinal neurons. This pattern of many layer retinal degeneration is in agreement with that of previously reported NTG-related mouse model of OPTN mutated mice and WDR36 mutated mice. Our data showing BRB damage in the TET-1 mice suggested that overexpression of endothelial ET-1 may be a cause of BRB damage found in the patients with NTG and POAG. In addition, dysfunction of photoreceptors was reported recently in some advanced glaucoma patients and in glaucoma animal model of ocular hypertension, indicating loss of RGCs may not be the only neural degeneration in the pathogenesis of glaucoma. Taken together, it may be indicated that NTG-like animals not only contain similar features as clinical NTG but also other different phenotypes due to their specific pathogenesis of gene mutation.

Other vascular-related retinal degenerative diseases, such as age-related macular degeneration (ARMD), were also considered in our study. We carefully checked the histologic sections...
of TET-1 mice and observed that except for a mild increase of autofluorescence in retinal pigment epithelium cells (RPEs), there was no sign of the classic ARMD-related retinopathy in aged TET-1 mice, such as drusen deposition, RPE degeneration, thickening of Bruch’s membrane, and characteristic choriocapillaris (CNV), suggesting exclusion of ARMD-related retinopathy (data not shown). Breakdown of BRB is a key event in ischemic retinopathy, such as diabetic retinopathy (DR). However, we also checked the structure of retinal vasculature using isolectin B4 staining and excluded neovascularization in TET-1 retina (data not shown). Our previous study reported the mild elevation of systolic blood pressure exhibited in TET-1 mice. Thus, in this study, we also performed fundus examination in TET-1 mice during IOP measurement at various ages. However, we did not find obvious signs of hypertensive retinopathy (HR), such as tortuous arteries, artery-vein nipping, hemorrhages, or any formed retinal extrusions. Isolectin B4 staining showed negative HR-related vascular changes in TET-1 retina. Taken together, it is suggested that the presence of IgG leakage and BRB breakdown in normal aging TET-1 mice may not be severe enough to produce formed exudates and neovascularization, as seen in diabetic or other retinopathy. Isolectin B4 staining showed negative HR-related vascular changes in TET-1 retina. Taken together, it is suggested that the presence of IgG leakage and BRB breakdown in normal aging TET-1 mice may not be severe enough to produce formed exudates and neovascularization, as seen in diabetic or other retinopathy. However, the leaky blood vessels in TET-1 retina may indicate exuding of proteins, inflammatory factors, and other deleterious molecules from BRB into retinal tissue, which is detrimental to the survival of retinal neurons. In addition, we cannot exclude that hypertension may play a role in the injury of retinal blood vessels and the neurons in TET-1 mice for its aggravating effects reported in cerebral ischemia.

Possible Role of Endothelial ET-1 in the Neurodegeneration of Retina

From the preceding discussions, it could be summarized that in the TET-1 mice, endothelial ET-1 induces or contributes to retinal neurodegeneration through four pathways: hypoxia/ischemia damage, BRB damage, retinal gliosis, and inducing RGC death directly.

As a potent vasoconstrictor, ET-1 induced the damage in the optic nerve and retina via the ischemia/reperfusion (I/R) injury. Our previous studies also showed ET-1 could aggravate brain and retina damage in a middle artery occlusion (MCAO)-induced I/R injury by using the TET-1 mice. This study is the first to show endothelial ET-1-induced microvascular remodeling, such as vascular loop formation and decrease of capillary network in the retina of TET-1 mice. As a hallmark of intussusceptive angiogenesis (IA), loop formation was reported in adult mouse retina by confocal microscopy with isolectin-B4–labeled blood vessels (red) and GFAP-labeled astrocytes (blue). Scale bars: (A–D, F–K) 50 μm; (L, M) 10 μm.
hypoxia-adaptation mechanism, which could be able to oppose the diminishing capillary network as reported in our finding. Further work is needed to investigate molecular signaling pathways to evaluate the adaptation/deadaptation mechanism of hypoxia involved in TET-1 retina. The result in this work provided the morphologic evidence that overexpression of endothelial ET-1 leads to altered microvasculature and contributes to retinal degeneration.

ET-1 has the effect of destroying the integrity of BRB by reducing the function of the endothelial tight-junction complex. BRB damage enables the access of circulating molecules in the blood, such as ET-1 and glutamate, which can trigger inflammation, oxidative stress, perivascular edema, and axonal demyelination.

Astrocytes have been suggested as a target of ET-1. ET-1-induced activation of astrocytes in the optic nerve head was considered as a deleterious effect. The mice with overexpression of ET-1 in the astrocytes (GET-1 mice) showed that astrocytic ET-1 has deleterious effects on water homeostasis, cerebral edema, and BRB integrity, which worsened the ischemic brain injury. A previous study reported that the complicated processes of retinal astrocytes was observed as an aging sign of rat retina. However, the enlargement of the end-feet of retinal astrocytes around blood vessels was much more often observed in the aged TET-1 mice than in the aged Non-tg mice, suggesting an effect of overexpressed ET-1 on astrocytes in our study. One of the possibilities of this phenomenon is end-feet edema, which may suggest the functional deficiency of BRB in the TET-1 mice.

ET-1 can affect RGCs directly by three lines of evidences. First, there are ET-1 receptors, ETA and ETB, expressed on RGCs; second, there is evidence shown in the culture system that ET-1 could mediate the apoptosis of RGC-5 cells directly; and third, ET-1 could induce apoptosis of RGCs in the in vivo animal model of ET-1-induced ischemia in the optic nerve head.

In summary, the phenotype of the TET-1 mice involves the major morphologic changes including the loss of RGCs, thinning of INL, ONL, NFL, and the degeneration of optic axons, together with the blood vessel changes and retinal gliosis. Taken together, our transgenic mice with endothelial over-expression using receptor tyrosine kinase tie-1 promoter leads to more severe cerebral damage following transient middle cerebral artery occlusion. J Cardiovasc Pharmacol 2004; 44(suppl 1):S293–S300.

References

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