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Therapeutic Potential of Topical Fenofibrate Eyedrops in Diabetic Retinopathy and AMD Rat Models

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Abstract

Objective: Diabetic retinopathy (DR) combined with age-related macular degeneration (AMD) is the leading cause of blindness in the US. Here, we report the efficacy of topical application of fenofibrate, an anti-lipid drug, in the treatment of retinal inflammation and neovascularization in rat models of DR and wet AMD, and in order to propose its use as a revolutionary therapy for ocular microvascular diseases.

Research design and methods: Fenofibrate was topically administered to rats. Following administration, the bioavailability of fenofibrate and its activated metabolite, fenofibric acid, in the retina, liver, and serum was determined using mass spectrometry. The effects of topical fenofibrate on retinal vascular leakage and inflammation were assessed using vascular permeability and leukostasis assays in streptozotocin (STZ)-induced diabetic rats. The anti-angiogenic effect of topical fenofibrate was evaluated in oxygen-induced retinopathy (OIR) rats and choroidal neovascularization (CNV) rats induced by laser photocoagulation.

Results: Treatment with topical fenofibrate caused no apparent corneal irritation or significant alterations in retinal histology in eyes. Fenofibrate rapidly distributed to the retina when applied topically, and the peak level of fenofibric acid in the retina occurred at 6 hours after administration. The terminal half-life of fenofibric acid in the retina was nearly 12 hours. Fenofibrate or fenofibric acid was not detected in the serum or liver by topical application, whereas oral administration of fenofibrate showed that the concentration of fenofibric acid in liver or serum was over 100-fold of that in the retina. Topical application of fenofibrate reduced the over-expression of soluble intercellular adhesion molecule-1 (sICAM-1) and vascular endothelial growth factor (VEGF), attenuated retinal vascular leakage, and ameliorated inflammation in the experimental diabetic models and prevented retinal neovascularization in the OIR and CNV models.

Conclusion: Topical application of fenofibrate has therapeutic potential in preventing retinal and choroidal NV formation, amelioration retinal inflammation, and reduction of retinal NV leakage.

Keywords: Fenofibrate; Topical application; Diabetic retinopathy; Wet AMD

Introduction

Diabetic retinopathy (DR), an ocular complication of diabetes, and wet age-related macular degeneration (AMD) are the leading causes of blindness in the US [1-3]. Both the pathologic features of DR and wet AMD include oxidative stress-associated inflammation, breakdown of the blood-retina barrier (BRB), vascular leakage, and neovascularization (NV) [4-6]. The pathogenesis of DR and AMD is mediated by several pathogenic factors and signaling pathways, including vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM-1), and tumor necrosis factor-alpha (TNF-α) [7-10].

Fenofibrate is a fibric acid derivative, lipid-soluble, anti-lipid drug [11] that has been widely used to improve the lipid profiles of patients with metabolic disorders and dyslipidemia. In addition to its anti-lipid effects, fenofibrate reduces VEGF receptor 2 (VEGFR2) and platelet-derived growth factor (PDGF) levels, inhibits EC proliferation and migration [12,13], reduces ROS accumulation, induces superoxide dismutase and glutathione peroxide, prevents oxidative stress–associated neuronal death [14-17], reduces intercellular adhesion molecule-1 (ICAM-1) levels, and inhibits tumor necrosis factor-alpha (TNF-α)/ nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling [2,13]. Studies from clinical trials and animal models have demonstrated that fenofibrate significantly prevents DR progression [18-20], which is likely independent of its lipid-modifying effect [19,20]. This study was designed to investigate the potential of fenofibrate eyedrops as a novel treatment for ocular microvascular diseases, including DR and AMD.

Materials and Methods

Animals

Brown Norway (BN) rats were purchased from Charles River (Wilmington, MA). Care, use and treatment of the animals were in strict agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and local ethics committee approval was obtained. All the animals were randomly distributed into experimental groups and control group. Each group includes at least 5 animals. Normal rat experiments include topical fenofibrate treatment group, topical vehicle treatment group, topical PBS treatment group and oral fenofibrate application group. STZ-induced diabetes rat experiments include STZ-induced diabetic rats treated with topical fenofibrat group and STZ-induced diabetic rats treated with topical vehicle group. OIR rat experiments include OIR rats treated with topical fenofibrat group and OIR rats treated with topical vehicle group. CNV rat experiments include CNV rats treated with topical fenofibrat group and CNV rats treated with topical vehicle group. Fenofibrate mixture containing fenofibrate, ethylene glycol, sodium lauryl sulfate, hyaluronic acid and etc. at pH value 6.7, were topically administrated three times a day in diabetic animals (for 1 month following onset of diabetes) and four times a day in OIR animals (for 5 days). Controls were applied with vehicle mixture without fenofibrate.

STZ-induced diabetic rats

Anesthetized BN rats (8 weeks old) were intraperitoneally (IP) injected with STZ (50 mg/kg in 10 mmol/l citrate buffer, pH 4.5) after an overnight fast.

The OIR model

The OIR model was induced in BN rats as previously described [21].

Laser photocoagulation-induced CNV model

After anesthetization with ketamine and xylazine, 1% tropicamide was topically applied to adult Brown Norway rats (8 weeks old) for pupil dilation. Four grey bubble lesions were produced in avascular region at a distance of approximately 2 disc diameters to the optic nerve. *In vivo* fundus angiography was used to evaluate the severity of CNV.

Bioanalysis of fenofibrate and fenofibric acid tissue distribution using liquid chromatography-mass spectrometry

Tissue extraction preparation: Freshly prepared samples, blanks, or internal standards (IS) were mixed and incubated with cold acetonitrile on ice for 30 min, followed by centrifugation at 5,000 rpm for 5 min at 4°C. Two hundred microliters of supernatant was lyophilized, reconstituted in a buffer containing formic acid, TFA, and acetonitrile, and was subsequently applied to an high-performance liquid chromatography (HPLC)- mass spectrometry (MS) system.

HPLC-MS system conditions for fenofibrate and fenofibric acid: Two solvent-delivery systems, which included four LC-10AD VP pumps (Shimadzu, Columbia, MD) and an auto-injector (CTC Analytics, Zwingen, Switzerland), were used for the analysis. The loading volume of the injector loop was 40 µl, and the injection volume was set at 10 µl. Chromatographic separations were performed using a Magic MS column (C18, 5 µm, 100 Å, 0.5×150 mm) connected to a guard column (BDS Hypersil C8, 2.1×20 mm, 5 µm). Two mobile phases were used in the chromatography: mobile phase A, consisting of 0.09% formic acid, 0.01% TFA, 2% CH3CN, and 97.9% water, and mobile phase B, consisting of 0.09% formic acid, 0.0085% TFA, 95% CH3CN, and 4.9% water. The mobile phase gradients for the analytical column and guard column were 30% B to 60% B in 1 min, 60% B to 90% B in 10 min, followed by a hold at 90% B for 1 min. The Valco valve was set up with a target mass of *m/z* 300. Analyses were performed using a Bruker Daltonics HCT Ultra Ion trap. MRM transitions monitored included the following: BZFB (used as an IS), *m/z* 362.0→276; fenofibrate acid, *m/z* 319.1→233.1; and fenofibrate, *m/z* 361.1→233.1.

Western blot analysis

A procedure was followed as described previously [22]. Primary antibodies were: rabbit anti-ICAM-1 antibody (Abcam, Cambridge, MA) and rabbit anti-VEGF antibody (Santa Cruz, CA), and rabbit anti-TNFα antibody (Abcam, Cambridge, MA).

Retinal angiography with high molecular-weight fluorescein

Anesthetized rats at postnatal day 18 were intraventricularly perfused with fluorescein via cardiac (50 mg/ml per animal, the molecular-weight isothiocyanate-dextran conjugated fluorescein is 2×10^6) (Sigma, St. Louis, MO) as described by Smith et al. [23]. The 4% paraformaldehyde fixed retina was flat-mounted and the vasculature was examined under a fluorescence microscope (Axioplan2 Imaging, Carl Zeiss, Jena, Germany) by an operator masked to treatment allocation.

Retinal vascular permeability assay

Retinal vascular permeability was conducted following a documented method [24] with modifications by measuring a dye of Evans blue leakage from retinal vessels. Briefly, Evans blue (Sigma-Aldrich, St. Louis, MO), 30 mg/mL in PBS, was injected into the femoral vein (30 mg/kg body weight) and allowed to circulate for 120 min. Evans blue in the circulation was removed by thorough perfusion. The retinas were carefully dissected and homogenized. Evans blue in the retina was extracted and measured with a spectrophotometer. Evans blue concentrations in the retina were normalized by total retinal protein concentrations.

Retinal vascular leukostasis assay

The assay followed a previously described protocol [25]. Briefly, anesthetized rats were perfused with PBS to remove nonadherent leukocytes in vessels. The adherent leukocytes in the vasculature and vascular endothelial cells were stained with FITC-conjugated concanavalin-A (Con-A, 40 µg/ml). The adherent leukocytes in the vasculature of flat-mounted retinas were then counted.

Statistical analysis

Quantitative data were analyzed and compared between fenofibrate treatment groups and control groups. Statistical analysis was performed by Student's *t* test for studies of two groups and by one-way ANOVA for studies of more than two groups. Statistical significance

in multiple groups was determined by Tukey's Post-hoc analysis and statistical significance was set at $p<0.05$.

Results

Characterization and tissue distribution of fenofibrate and fenofibric acid in rodents following topical application of fenofibrate

We first used HPLC-MS to determine the bioavailability of topically applied fenofibrate in the retina. HPLC-MS analysis of fenofibrate exhibited an abundant ion peak at approximately m/z 361.1 ± 0.3 (Figure 1A), representing the *m/z* 361.1 ion corresponding to fenofibrate. The MS spectrum of 0.25 ng of the internal standard (bezafibrate), 1.0 ng of fenofibric acid, and 1.0 ng of fenofibrate obtained using an ion trap mass spectrometer exhibited abundant peaks at *m/z* 362.0 (276.1), *m/z* 319.1 (233.1), and *m/z* 361.1 (233.1), respectively (Figure 1B). The concentrations of fenofibrate and its activated metabolite, fenofibric acid, were determined using standard HPLC-MS analysis in tissue homogenates from rats administered 1 dose of fenofibrate eyedrops. Both fenofibric acid and fenofibrate were detected in the eyecups in the first 3 h following topical fenofibrate application. The majority of the fenofibrate was converted into fenofibric acid at 4 h and reached a maximal concentration at 6 h, which was sustained until 24 h (Figure 1C-1E). In comparison to topical application, no fenofibrate was detected in the rodents following oral fenofibrate treatment (Figures 1F and 1G). The concentrations of fibric acid and fenofibrate in different tissues at 6 h following oral fenofibrate administration (0.2% fenofibrate) or topical fenofibrate administration were compared (Figure 1F). The serum concentration of fenofibric acid was positively correlated with the concentration of oral fenofibrate. However, this correlation was not observed in tissues such as the retina or liver (Figure 1F). The concentrations of fenofibric acid in the serum and liver were over 100-fold higher than that in the retina in animals administered oral fenofibrate. A comparison between the two administration routes indicated that topical ocular fenofibrate administration results in a higher concentration in the retina than oral administration. Both fenofibrate and fenofibric acid were undetected in the liver and serum in animals that were administered fenofibrate topically (Figure 1G). These results demonstrate that topical ocular fenofibrate application penetrates well into the ocular tissues and does not exhibit systemic adverse effects on the serum or liver.

Topical ocular fenofibrate does not cause abnormalities in the cornea or in retina

We next examined whether topical fenofibrate application causes corneal irritation or retinal function abnormalities. Treatment with fenofibrate eyedrops did not cause any retinal cell apoptosis (Figure 2A), and did not affect the corneal transparency and histological alternation compared with the vehicle-treated rats (Figure 2B). Tear production was determined using the Schirmer tear test, which indicated no difference between rats topically administered PBS, vehicle, or fenofibrate eyedrops (Figure 2C and 2D). These results suggest that the topical application of fenofibrate eyedrops may represent a safe and effective treatment.

Fenofibrate reduces retinal vascular leakage and attenuates retinal inflammation in type 1 diabetic models

Next, we evaluated the therapeutic effects of fenofibrate eyedrops on retinopathy. First, because increased retinal vascular leakage is responsible for diabetic edema in DR, we investigated whether topical ocular fenofibrate demonstrates a therapeutic effect on retinal vascular leakage in diabetic models. STZ-induced diabetic rats at 6 weeks of diabetes were treated with topical fenofibrate eyedrops for four weeks, and control rats were treated with vehicle alone. Retinal vascular leakage was evaluated using Evans blue dye as a tracer, with normalization to the total retinal protein concentration. Our results indicated that treatment with fenofibrate eyedrops significantly reduced retinal vascular leakage in STZ-diabetic rats (Figure 3A).

Leukocyte adherence (leukostasis) and inflammation in the retina are crucial steps in the pathological development of DR. We therefore examined the effects of fenofibrate on leukostasis and retinal inflammation in diabetic rats. Multiple adherent leukocytes were observed in the retinal vasculature of diabetic rats treated with the vehicle, which were significantly reduced by topical treatment with fenofibrate (Figure 3B and 3C). Inflammatory markers, such as ICAM-1 and TNF-α, which mediate cell-cell adhesion and recruit inflammatory cytokines, respectively, and VEGF play important roles in the development of inflammation in DR [26]. Topical ocular fenofibrate significantly reduced retinal ICAM-1 (Figure 3D), TNF-α (Figure 3E), and VEGF (Figure 3F) levels in diabetic rats. These findings suggest that topical fenofibrate eyedrops have a therapeutic role in the prevention of retinal vascular leakage and retinal inflammation in diabetes.

Topical application of fenofibrate reduces retinal vascular leakage and attenuates NV in OIR rats and laser-induced CNV rats

Lack of integrity in the neovasculature results in severe vascular leakage and is responsible for retinal edema. Ischemia-induced retinopathy and laser-induced CNV are widely accepted experimental models to evaluate NV. We first investigated whether topical application of fenofibrate alleviates vascular leakage and prevents NV in OIR rats. Following exposure to hypoxia, rats were topically administered fenofibrate eyedrops for 5 days beginning at postnatal day 12 (P12). *In vitro* fluorescein angiography with a 66 KD fluorescein conjugated Dextran leakage assay indicated that topical ocular fenofibrate reduced retinal vascular leakage in OIR rats prevented developing severe retinal NV (Figure 4B) compared with control rats treated with vehicle alone (Figure 4A). In a laser-induced CNV model, following laser coagulation, rats were topically administered fenofibrate eyedrops for 21 days, and *in vivo* retinal angiography was used to evaluate neovasculature leakage and NV. As shown in Figure 4E, laser coagulation induced CNV formation and severe subretinal vascular leakage, which were reduced following topical treatment with fenofibrate (Figure 4F).

Because VEGF is an angiogenic factor for NV, we also evaluated the direct effects of fenofibrate eyedrops on VEGF in both the OIR and laser-induced CNV models. Compared with the vehicle controls, fenofibrate eyedrops remarkably reduced retinal VEGF levels in the OIR rats (Figure 4C and 4D) and eyecup VEGF levels in rats with laser-induced CNV (Figure 4G and 4H), indicating that the topical administration of fenofibrate has inhibitory effects on angiogenesis.

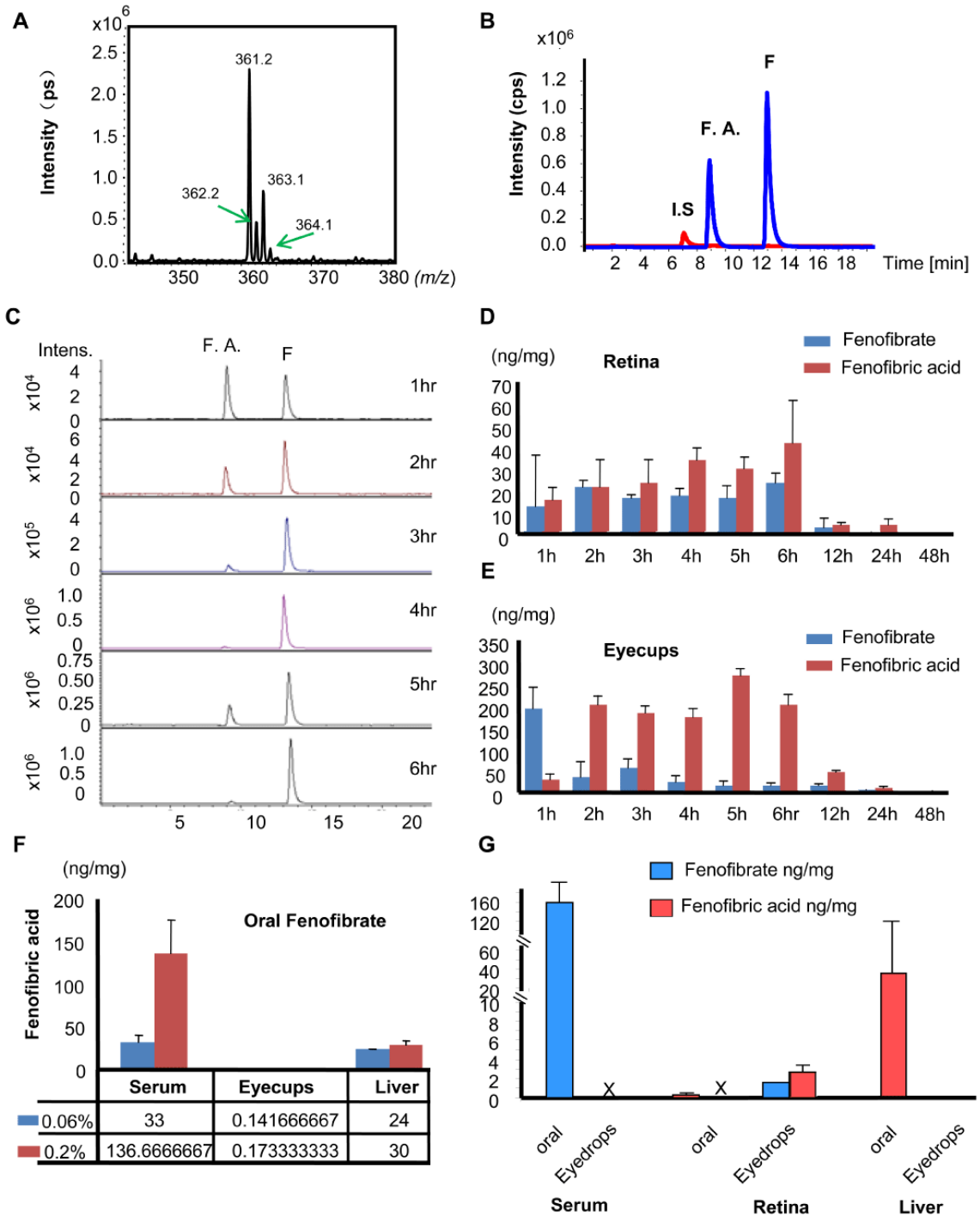

Figure 1: Tissue distribution of fenofibrate in animals following fenofibrate treatment. (A) A representative HPLC-MS spectrum of fenofibrate (20 ng/μl; *m/z* 361.1 ± 0.3) in the retina. **(B)** A representative HPLC-MS spectrum of the extracted ion chromatogram of fenofibric acid (F.A.) and fenofibrate (F); BZFA (I.S.) served as an internal standard. **(C-E)** Normal Brown Norway adult rats were topically administered fenofibrate eyedrops (15 μl/eye). **(C)** A representative HPLC-MS spectrum of the extracted ion chromatogram of fenofibric acid (F.A.), and fenofibrate (F) from eyecup extracts at 0, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h, respectively. **(D,E)** Fenofibrate or fenofibric acid levels in eyecup and retinal extracts at 0, 1 h, 2 h, 3 h, 4 hr, 5 h, 6 h, 12 h, 24 h, and 48 h were determined using HPLC/MS (mean ± SD, *n*=3). **(F)** Normal adult rats were fed fenofibrate (0.06% or 0.2%) for 5 days, and fenofibric acid levels in the serum, retina, and liver extracts were determined using HPLC/MS (mean ± SD, *n*=3). **(G)** Normal adult rats were treated with oral fenofibrate (120 mg/kg/d, 5 days). Fenofibrate or fenofibric acid levels in tissue extracts were determined using HPLC-MS in animals administered oral fenofibrate and in animals administered topical fenofibrate eyedrops (4 times/day, 5 days) (mean ± SD, *n*=5).

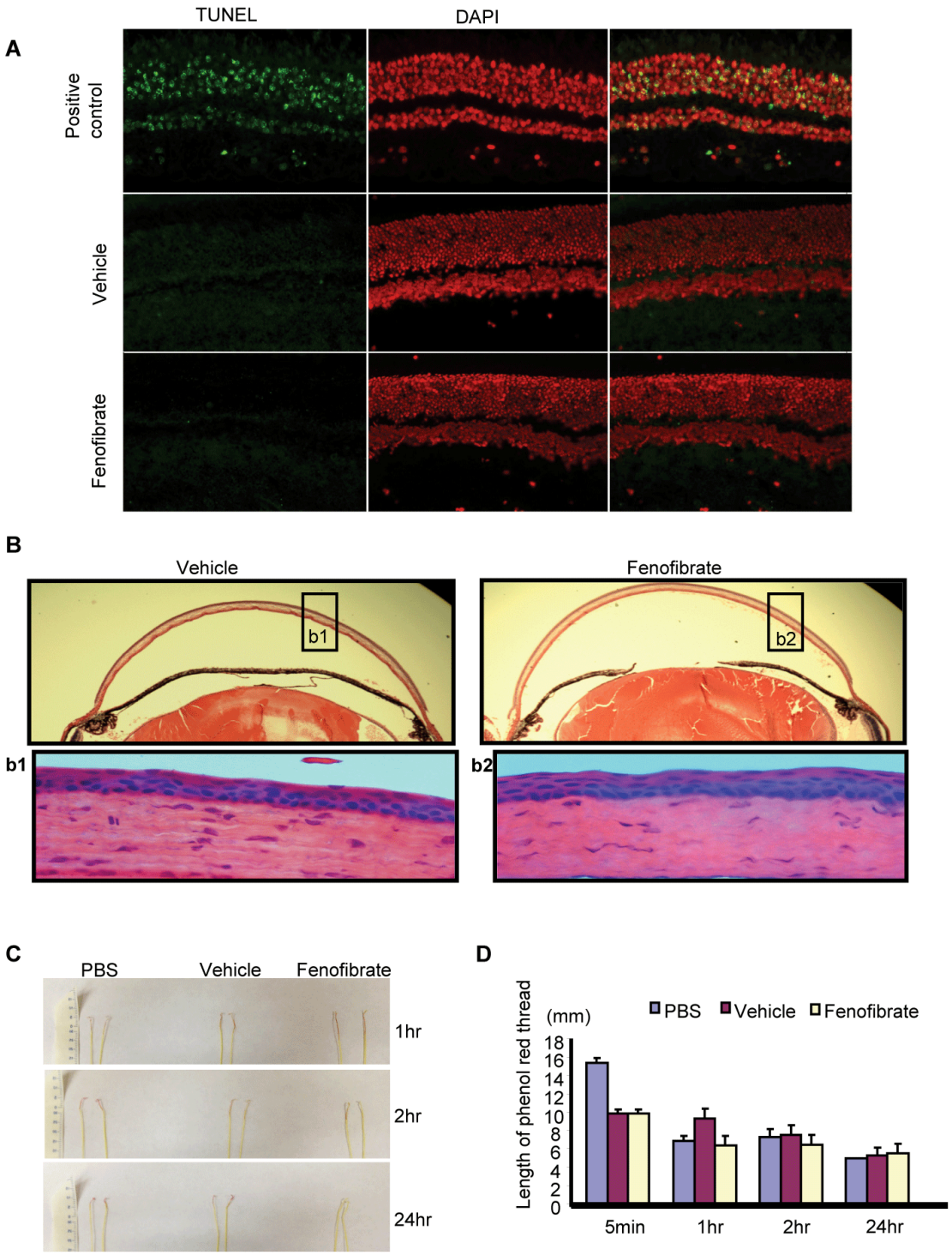

Figure 2: Topical fenofibrate does not cause abnormalities in the anterior or posterior segment in rats. Normal Brown Norway rats were treated with the vehicle or fenofibrate eyedrops (30 µl/eye, 4 times/day for 4 weeks). **(**A**)** Representative TUNEL assay analysis of retinal cells apoptosis. Ocular section of human DR patient served as positive control. **(**B**)** Representative ocular sections of anterior segments of eyes. **(**C**)** Normal adult rats were treated with the vehicle or fenofibrate eyedrops (30 µl/eye, 1 dose), and PBS served as the control. The Schirmer tear test was performed at 0, 1 h, 2 h and 24 h following eyedrop application. **(D)** The lengths of the phenol red threads were averaged and compared. The Schirmer tear test was performed at 0, 1 h, 2 h and 24 h following eyedrop application (mean ± SD, *n*=7).

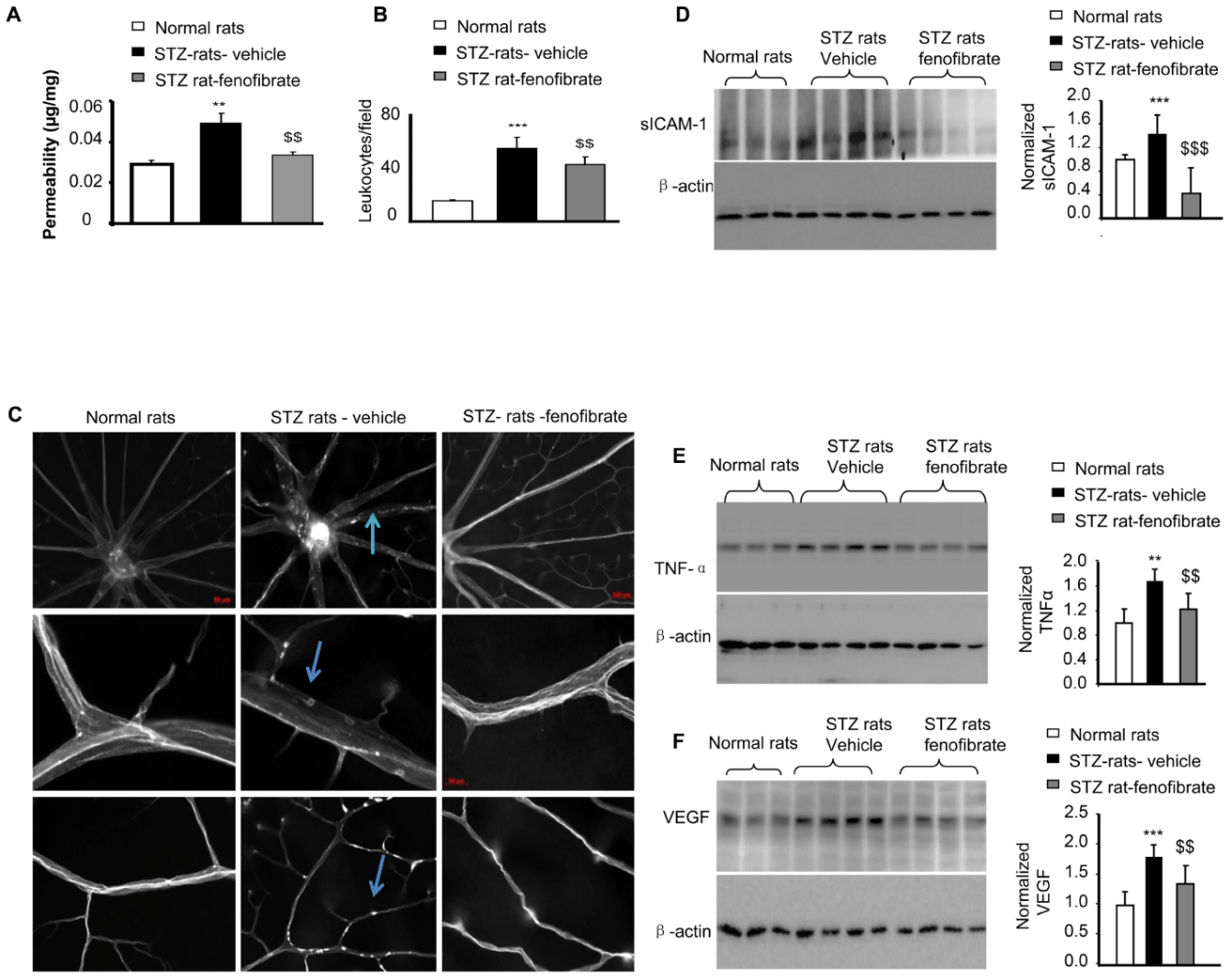

Figure 3: Topical fenofibrate ameliorates retinal inflammation and reduces retinal vascular leakage in type 1 diabetic animals. STZ-induced diabetic rats were topically administered fenofibrate (15 µl/eye, 3 times/day for 4 weeks) 6 weeks after diabetes onset, and an identical volume of the vehicle served as the control. **(A)** Retinal vascular leakage was quantified using a vascular permeability assay (mean ± SD, n=5; ** P<0.01 STZ vehicle vs. nondiabetic rats (NDM); $$ P<0.01 STZ-fenofibrate vs. STZ-vehicle). **(B,C)** The retinal vascular endothelium and adherent leukocytes were stained using FITC-conjugated Con-A following the removal of circulating leukocytes. The retinas were flat mounted, and adherent leukocytes were visualized using fluorescence microscopy. **(B)** Leukocyte quantification indicated that topical fenofibrate significantly reduced adherent leukocytes in the retinal vasculature from diabetic rats (mean ± SD, n=7, *** P<0.001 STZ vehicle vs. nondiabetic rats (NDM); $$ P<0.01 STZ-fenofibrate vs. STZ-vehicle). **(C)** Representative images of retinal flat mounts from nondiabetic rats, STZ-induced diabetic rats treated with vehicle, and diabetic rats treated with fenofibrate eyedrops (30 µl/eye, 3 times/day for 4 weeks) are shown. Scale bar, 100 µm in the first row panel, 50 µm in the second and third row panels. **(D-F)** Western blot analysis of retinal soluble form ICAM-1 (sICAM-1), TNF-α, and VEGF levels in equivalent amounts (50 µg) of retinal proteins. Each lane represents an individual rat (mean ± SD, n=3 or 4; *STZ vehicle vs. non-diabetic rats (NDM); $STZ-fenofibrate vs. STZ-vehicle; * or $ P<0.05; ** or $$ P<0.01; *** or $$$ P<0.001).

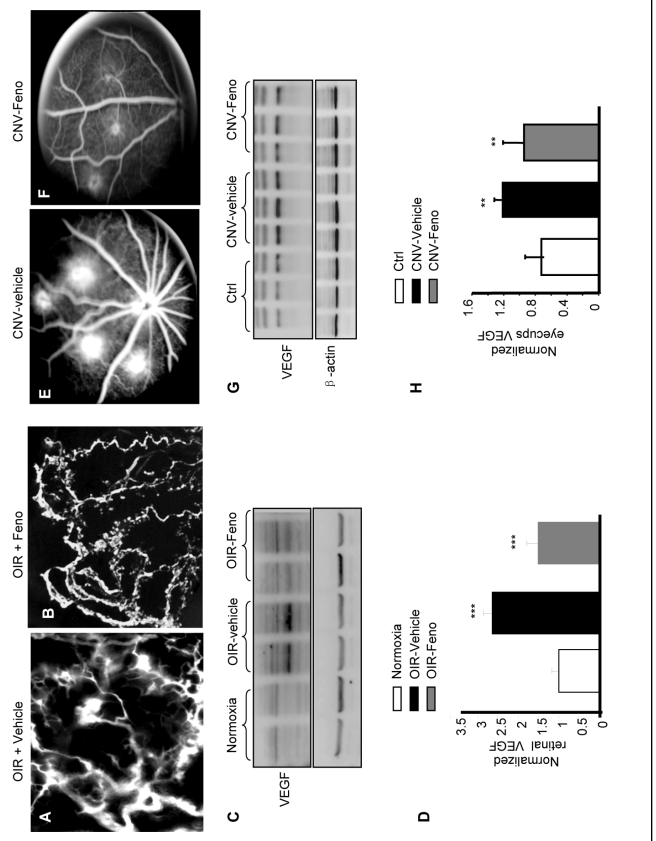

Figure 4: Topical fenofibrate application reduces retinal vascular leakage and prevents NV in OIR and laser-induced CNV rats. (A-D) Rats were exposed to 75% oxygen from P7 to P12. Following a return to normoxic conditions, rats were topically administered fenofibrate (15 µl/eye, 4 times/day for 5 days), and an identical volume of the vehicle served as the control. **(A,B)** At P17, the retinal vasculature and leakage was visualized using fluorescein angiography. **(C,D)** representative Western blot analysis of retinal VEGF levels at P16 in equivalent amounts (50 µg) of retinal proteins using an antibody against VEGF, and β-actin served as a loading control. Each lane represents an individual rat. Compared to the vehicle, fenofibrate eyedrops significantly reduced retinal VEGF levels induced by hypoxia (mean ± SD, n=4; *** P<0.001 OIR-vehicle vs. normoxia; $$$$P$<0.001 OIR-vehicle vs. OIR-fenofibrate). **(E-H)** Adult rats underwent laser coagulation followed by the topical administration of fenofibrate (15 µl/eye, 4 times/day for 21 days), and an identical volume of vehicle served as the control. **(E,F)** A representative fundus angiography was taken at day 21. **(G,H)** Equivalent amounts (50 µg) of total protein from the eyecups were probed using an antibody against VEGF, and β-actin served as the loading control. Each lane represents an individual rat. Compared to the vehicle, fenofibrate eyedrops significantly reduced retinal VEGF levels induced by hypoxia (mean ± SD, n=3, ** P<0.01 CNV-vehicle vs. control; $$$$P$< 0.001 CNV-vehicle vs. CNV-fenofibrate).

Discussion

Here, for the first time, we provide the first evidence that topical application of fenofibrate has dramatic therapeutic effects on retinal microvascular diseases. We demonstrate that fenofibrate penetrates well into ocular tissue and is undetected in systemic tissue, suggesting a lack of systemic toxicity following treatment with fenofibrate eyedrops. Regarding the fenofibrate mechanism of action, we have shown that the anti-inflammatory effect of topical fenofibrate application results from the downregulation of ICAM-1 and TNF-α in the diabetic retina. Topical ocular application of fenofibrate reduces retinal VEGF overexpression, which may attribute to its anti-NV effect. Moreover, the topical application of fenofibrate eyedrops does not produce apparent corneal irritation or retinal dysfunction, suggesting the tolerance and safety of fenofibrate eyedrops for ocular treatment.

The pathogenic processes of DR and AMD are mediated by multiple pathogenic factors in oxidative stress-related inflammation and angiogenesis [4-6]. The current treatment for DR and AMD focuses on VEGF antagonism alone and is associated with a high risk of trauma and infection [7,8]. The later stage requirements for very frequent injections result in high cost and a reluctance to treatment. Two recent independent large clinical studies demonstrated substantial protective effects of fenofibrate against diabetic eye complications in type 2 diabetic patients. In our recent study, we reported that oral fenofibrate exhibits protective effects against DR in animal models [18-20]. Fenofibrate represents the first oral drug with proven clinical efficacy on retinal NV and macular edema and has thus captured the interest of clinicians, researchers, and pharmaceutical companies seeking new treatments for retinal inflammation and NV. In addition to the recently reported DR protection, fenofibrate, which is a PPARα agonist, exhibits multiple antioxidant, anti-inflammatory, and anti-angiogenic functions. In our study, ocular fenofibrate treatment exhibited both anti-inflammatory and antiangiogenic effects in the eyes of DR and AMD animal models, suggesting it could be used as a novel treatment for retinopathy.

As an anti-lipid oral drug, fenofibrate is primarily used in patients with metabolic disorders and dyslipidemia, including type 2 diabetic patients, and is typically taken once daily [27]. Most patients on fenofibrate have also taken statins to modify their cholesterol levels. However, when fibrates and stains are administered in combination, oral fenofibrate is associated with several side effects. For example, acute renal necrosis induced by rhabdomyolysis has been reported in rats [28,29]. One explanation for this occurrence is the increase in the levels of serum statins by fenofibrate. Nevertheless, the administration of fenofibrate alone is also associated with several side effects, such as myopathy, gallstones, renal insufficiency, and skin reactions [30-32]. Fenofibrate is contraindicated for patients who are hypersensitive to fenofibrate. In addition to these side effects, the renal clearance of fenofibrate decreases in elderly patients with increasing age, which results in higher levels of fenofibrate and its metabolic accumulation [33,34]. Therefore, the fenofibrate dose must be decreased in moderate to severe renal disease patients and in the elderly. Both DR and AMD occur predominately in elderly patients, most of whom exhibit poor renal function. Thus, a safe, effective, and ideally superior substitute for oral fenofibrate is necessary for the treatment of retinal diseases, such as DR and AMD. Fenofibrate exhibits several properties that are suitable for ocular topical application: it is a small, lipid-soluble, stable molecule and has a specific receptor (PPARα) that is expressed in the retina, RPE, and vascular cells [35-38]. Moreover, our recent study

indicated that local ocular fenofibrate delivery into the vitreous cavity reduced vascular leakage and prevented NV. These results indicate that topical fenofibrate application for the treatment of DR and AMD is feasible and necessary. In this study, topical fenofibrate application did not produce detectable corneal stimulation or conjunctivitis. In comparison with a study using oral fenofibrate administration, the fenofibrate eyedrops penetrated well into the ocular tissues and fluids to reach effective doses, as indicated by HPLC-MS analysis. Moreover, neither fenofibrate nor its active metabolite fenofibric acid was detected in the liver or serum, indicating that topical fenofibrate application is a safe and tolerable treatment. Furthermore, topical administration of fenofibrate is effective in reducing vascular permeability, inhibiting angiogenesis, and downregulating VEGF in the retina of retinal NV, CNV, and diabetes animal models. We believe that our study lays a foundation for future clinical studies to optimize a delivery regimen for fenofibrate in DR and AMD patients and will revolutionize the treatment of DR and AMD to benefit millions of patients.

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