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The peroxisome proliferator-activated receptor pan-agonist bezafibrate suppresses microvascular inflammatory responses of retinal endothelial cells and vascular endothelial growth factor production in retinal pigmented epithelial cells

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ABSTRACT

A randomized clinical trial showed the beneficial effects of the selective peroxisome proliferator-activated receptor (PPAR)-α agonist, fenofibrate, in reducing the progression of diabetic retinopathy independent of serum lipid levels. All subtypes of PPAR (PPAR-α, PPAR-γ, and PPAR-β/δ) have been reported to play a key role in microvascular inflammation and angiogenesis. Therefore, the agonistic function of fenofibrate against the PPAR-α has been suggested to contribute to its medicinal effect. Furthermore, bezafibrate is a fibrate drug commonly used as a lipid-lowering agent to treat hyperlipidemia and acts as a pan-agonist of all PPARs subtypes. However, the effects of bezafibrate in diabetic retinopathy remain unclear. Therefore, the purpose of this study was to investigate the effects of bezafibrate on retinal microvascular inflammation. Bezafibrate was not cytotoxic against human retinal microvascular endothelial cells (HRMECs) and human retinal pigment epithelial cells (ARPE-19 cells) treated with < 100 and 200 μM bezafibrate, respectively. In HRMECs, the expression levels of tumor necrosis factor (TNF)-α-induced monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 were significantly suppressed by bezafibrate in a dose-dependent manner. TNF-α-induced nuclear translocation of nuclear factor (NF)-κB p65 and cell migration were also significantly inhibited in bezafibrate-treated HRMECs. Furthermore, bezafibrate treatment significantly suppressed interleukin (IL)-1β-induced vascular endothelial growth factor (VEGF) production in ARPE-19 cells. These results suggest that bezafibrate has beneficial effects on retinal microvascular inflammation. Our study demonstrates the therapeutic potential of bezafibrate for managing diabetic retinopathy.

1. Introduction

Diabetic retinopathy is a common complication of diabetes, and its progression is a leading cause of blindness by irreversible retinal microvascular changes [1]. Bezafibrate is a fibrate drug mainly used in Japan and Europe for ameliorating dyslipidemia, and it mainly has serum triglyceride (TG)-lowering and high-density lipoprotein cholesterol (HDL-C) elevating actions [2]. Bezafibrate is known to regulate the transcription of several genes for lipoprotein and fatty acid metabolism by acting as a pan-agonist of the three peroxisome proliferator-activated receptors (PPARs) including PPAR-α, -γ, and -β/δ. These three subtypes regulate DNA transcription and the expression of related genes once they are ligand-bound [3].

Fenofibrate is a fibrate drug widely used for ameliorating dyslipidemia, and it acts as a PPAR-α agonist. Oral administration of fenofibrate was reported to protect against the progression of diabetic retinopathy in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) eye study [4,5]. Recently, fenofibrate and its metabolic product, fenofibric acid, were shown to have inhibitory effects on microvascular inflammation, vascular permeability, and retinal neovascularization [6–8].

Furthermore, it has been reported that all PPAR inhibit nuclear factor (NF)-kB activation, which is the master regulator of the inflammatory response, and all PPAR subtypes are known to be expressed in the retina [3,9]. Thus, PPARs are potential pharmacological targets in the treatment of diabetic retinopathy. Pharmacologically, fenofibrate is a selective agonist for only PPAR-α while bezafibrate acts as a PPAR-

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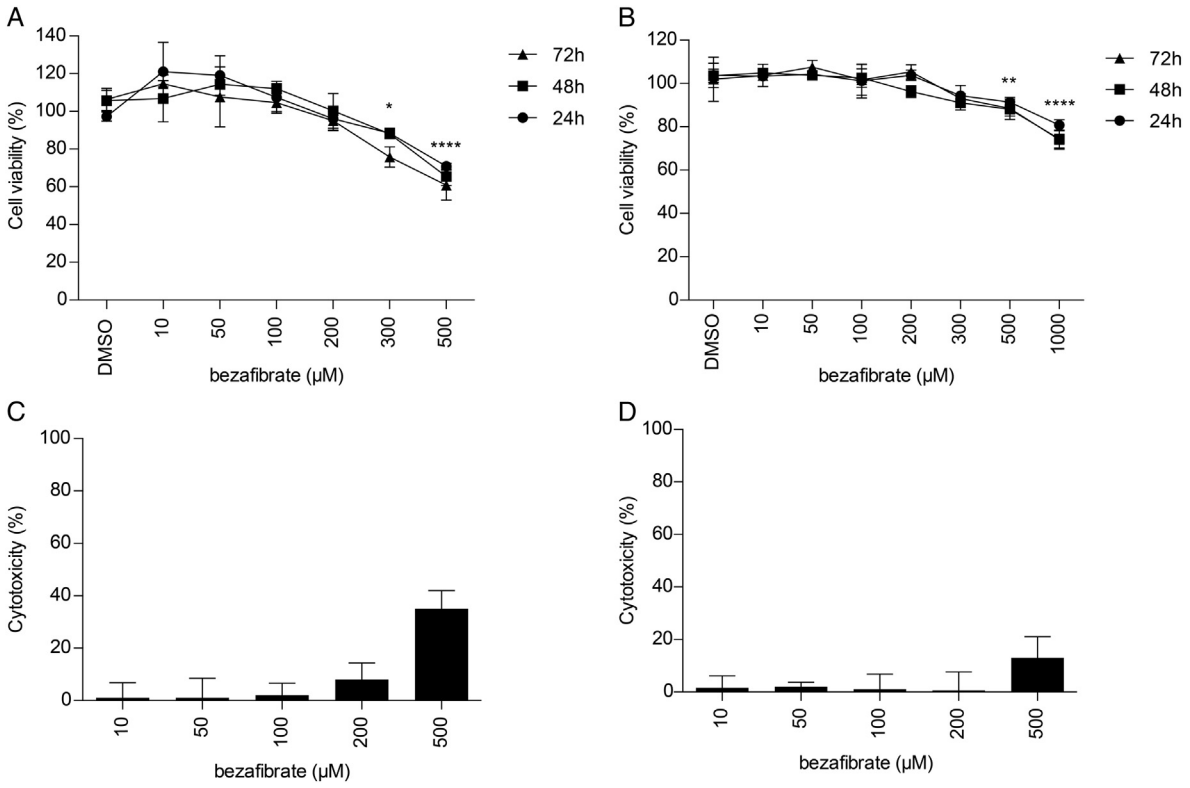

Fig. 1. Effect of bezafibrate on viability of human retinal microvascular endothelial cells (HRMECs) and human retinal pigment epithelial ARPE-19 cells. Viability of cells treated with bezafibrate at 0–1000 μM for 24, 48, and 72 h was assayed using cell counting kit (CCK)-8. HRMEC and ARPE-19 cell viability was significantly decreased by bezafibrate at concentrations higher than 300 and 500 μM, respectively at any time point. Cytotoxicity of bezafibrate (0–1000 μM) after 48-h treatment was assayed by evaluating lactate dehydrogenase (LDH) elevation. Bezafibrate showed significantly increased cytotoxicity against (C) HRMECs and (D) ARPE-19 cells at concentrations higher than 200 μM. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$.

α,γ,β/δ pan-agonist [2].

Thus, bezafibrate is suggested to have a strong potential to inhibit the inflammatory response by targeting all PPAR subtypes and exerts a therapeutic effect on diabetic retinopathy. However, it is not unclear whether bezafibrate exerts beneficial effects on microvascular inflammation or retinal neovascularization in diabetic retinopathy. Therefore, in this study, we examined the effect of bezafibrate on human retinal microvascular endothelial cells (HRMECs) and retinal pigmented epithelial cells to determine its beneficial effects in diabetic retinopathy.

2. Materials and methods

2.1. Reagents

Bezafibrate was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO). Recombinant human interleukin (IL)-1β and tumor necrosis factor (TNF)α were purchased from Peprotech (Rocky Hill, NJ, USA). Recombinant human vascular endothelial growth factor 165 (VEGF165) was purchased from R & D Systems (Minneapolis, MN, USA).

2.2. Cell culture

Primary HRMECs were purchased from Cell Systems (Kirkland, WA, USA) and cultured in CS-C complete medium with serum (Cell Systems) containing 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO_2 incubator. All tissue culture surfaces were precoated with the attachment factor from the CS-C complete medium kit (Cell Systems), and all experiments were performed on HRMECs within the first five passages. ARPE-19 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM-HamF12 medium (Life Technologies, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin).

2.3. Cell viability and cytotoxicity assays

Cell viability was assessed using the cell counting (CCK)-8 kit (Dojindo, Rockville, MD, USA) according to the manufacturer's protocol. HRMECs or ARPE-19 cells were seeded at 5000 cells/well in medium containing 10% serum in 96-well plates. After a 24-h incubation, the medium was serum-starved with 1% FBS for 6 h, the CCK-8 reagent was added, and the absorbance of the resultant solution was measured at 450 nm by using a microplate reader at three time points, 24, 48, and 72 h after treatment with bezafibrate (0, 10, 50, 100, 200, 500, and 1000 μM).

In addition, the cytotoxicity of HRMECs and ARPE-19 cells was assessed using the cytotoxicity lactate dehydrogenase (LDH) assay kit-water-soluble tetrazolium (WST, Dojindo Kumamoto Japan) according to the manufacturer's protocol. HRMECs or ARPE-19 cells were seeded at 5000 cells/well in medium containing 10% serum in 96-well plates. After a 24-h incubation, the WST reagent was added, and the absorbance was measured at 490 nm using a microplate reader after 48 h treatment with bezafibrate (0, 10, 50, 100, 200, 500, and 1000 μM).

2.4. Quantitative polymerase chain reaction (qPCR) assay

HRMECs and ARPE-19 cells (5×10^4 cells/mL) were plated into six-well plates in medium containing 10% serum. After a 24-h incubation of HRMECs, the medium was serum-starved with 1% FBS for 6 h and