Redox Biology 20 (2019) 87–97

Contents lists available at ScienceDirect

Redox Biology

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

Fenofibrate improves vascular endothelial function and contractility in diabetic mice

Nan Xu[a], Qin Wang[a], Shan Jiang[a], Qijing Wang[b], Weipeng Hu[a], Suhan Zhou[a], Liang Zhao[a], Lanyu Xie[c], Jianghua Chen[a], Anton Wellstein[d], En Yin Lai[a,*]

[a] Department of Physiology, School of Basic Medical Sciences, and Kidney Disease Center of First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310058, China
[b] Department of Reproductive Endocrinology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou 310058, China
[c] Medical college, Nanchang University, Nanchang 330000, China
[d] Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA

ARTICLE INFO

Keywords:
Diabetes
Fenofibrate
Endothelial dysfunction
Nitric oxide
Oxidative stress

ABSTRACT

Fenofibrate, a peroxisome proliferator-activated receptors α (PPARα) agonist, reduces vascular complications of diabetic patients but its protective mechanisms are not fully understood. Here we tested the hypothesis that fenofibrate improves vascular endothelial dysfunction by balancing endothelium-dependent relaxation and contractility of the aorta in diabetes mellitus (DM). In streptozotocin-induced diabetic mice, eight weeks of fenofibrate treatment (100 mg/Kg/d) improved endothelium dependent relaxation in the macro- and microvessels, increased nitric oxide (NO) levels, reduced renal damage markers and effects of the vasoconstrictor prostaglandin. Levels of superoxide dismutase and catalase were both reduced and hydrogen peroxide was increased in vehicle-treated DM, but these changes were reversed by fenofibrate treatment. Vasodilation of the aorta after fenofibrate treatment was reversed by PPARα or AMPKα inhibitors. Western blots showed that fenofibrate treatment elevated PPARα expression, induced liver kinase B1 (LKB1) translocation from the nucleus to the cytoplasm and activated AMP-activated protein kinase-α (AMPKα), thus activating endothelial NO synthase (eNOS). Also, fenofibrate treatment decreased NF-κB p65 and cyclooxygenase 2 proteins in aortas. Finally, incubation with indomethacin in vitro improved aortic contractility in diabetic mice. Overall, our results show that fenofibrate treatment in diabetic mice normalizes endothelial function by balancing vascular reactivity via increasing NO production and suppressing the vasoconstrictor prostaglandin, suggesting mechanism of action of fenofibrate in mediating diabetic vascular complications.

1. Introduction

The endothelium and factors derived from endothelial cells are known to control vascular function, including the regulation of vascular tone [1,2]. Endothelial dysfunction is a hallmark of diabetes and contributes to macrovascular and microvascular complications associated with diabetes [3,4]. Vascular endothelial dysfunction has been characterized by reduced activity of endothelial nitric oxide synthase (eNOS), decreased generation of nitric oxide (NO) and increased generation of reactive oxygen species (ROS) [3,5]. In manifest diabetes, there is an imbalance between endothelial-dependent vascular relaxation and constriction, which plays an important role in the development of pathologies associated with the disease [4,6,7]. Therefore, understanding the regulation of this imbalance may be important in preventing the development of diabetes-associated vasculopathies.

Fenofibrate is a peroxisome proliferator-activated receptors α (PPAR α) agonist and can improve dyslipidemia [8]. It causes a moderate reduction in total cholesterol (TC), a marked reduction in triglycerides (TG), and an increase in high-density lipoprotein cholesterol (HDL-C) [9]. Emerging clinal evidence has demonstrated that fenofibrate has a therapeutic potential in alleviating diabetes-associated vascular disease independently of its hypolipemic action. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study showed that treatment with fenofibrate (200 mg per day) has promising effects in attenuating the progression of microvascular complications of diabetes, including reduced nonfatal coronary events, decreased need for laser treatment of diabetic retinopathy and delayed progression of diabetic nephropathy [10,11]. The action to control cardiovascular risk

* Corresponding author.
E-mail address: laienyin@zju.edu.cn (E.Y. Lai).

https://doi.org/10.1016/j.redox.2018.09.024
Received 12 June 2018; Received in revised form 11 September 2018; Accepted 27 September 2018
Available online 01 October 2018

in diabetes (ACCORD) study also demonstrated that fenofibrate, when added to statin therapy, slows the progression of diabetic retinopathy in patients with type 2 diabetes [12]. However, the mechanisms underlying vascular benefits of fenofibrate treatment of patients with diabetes are not fully understood.

Adenosine monophosphate-activated protein kinase (AMPK) has many biological functions, including regulation of glucose metabolism, lipid metabolism [13], intracellular ROS and eNOS levels [14]. Recent studies suggest that modulating AMPK has a great potential in the treatment of metabolic disorders such as obesity and Type 2 diabetes [15]. There are at least two upstream kinases that phosphorylate AMPK: liver kinase B1 (LKB1) and calcium dependent protein kinase kinase-β (CaMKK-β) [16,17]. Previous studies showed that LKB1 forms a heterotrimeric complex with regulatory proteins, which are required for its activation and cytosolic localization [18]. Experiments in cultured cells demonstrated that fenofibrate plays a role in AMPKα activation [19,20]. However, data are limited on the effect of fenofibrate on the expression of AMPK and LKB1 in vascular reactivity in diabetes.

Endothelium-dependent contractions are mediated by the products of cyclooxygenase (COX) [21,22]. COX metabolizes arachidonic acid (AA) to form the unstable prostaglandin H2 which is further converted into thromboxanes (TxAs) and prostaglandins (PGs), including PGE2, PGD2 and prostacyclin [23]. TxA2 can stimulate TxA2/prostanoid (TP) receptor to induce vasoconstriction. TxA2 and PGE2 has been suggested to act as an endothelium derived contracting factor [24]. The production of TxA2 and PGE2 by the endothelium is increased in diabetes [25]. Therefore, inhibition of COX could provide new insights into the mechanism responsible for endothelial dysfunction.

In the present study, we used a mouse model of streptozotocin-induced diabetes mellitus and investigated whether eight-week fenofibrate treatment (100 mg/Kg/d) could prevent diabetes-related endothelial dysfunction in arteries and evaluated possible signaling mechanisms.

2. Materials and methods

2.1. Animal model and experimental groups

Male adult C57Bl/6 mice (25–28 g) were purchased from SLAC laboratory animal company (Shanghai, China). Animals were treated with standard food and water and housed under climate-controlled conditions with a 12 h light/dark cycle. All the animal handling procedures and experiment protocols were approved by Zhejiang University's institutional animal care and use committee and conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

We induced a diabetes similar to a type I diabetic, according to the protocols of Animal Models of Diabetic Complications Consortium (AMDCC) by the National Institutes of Health. Diabetes was induced by intraperitoneally injection of streptozotocin (STZ, Sigma-Aldrich, St Louis, MO, USA, dissolved in 0.1 M sodium citrate buffer, pH 4.5) at a low-dose (50 mg/kg/day for 5 consecutive days). Hyperglycemia was defined as a random blood glucose level > 16.7 mmol/L three days after last STZ injection. Plasma glucose level was monitored by a contour glucose meter (Roche, Mannheim, Germany) twice a week for eight weeks. Fenofibrate (Sigma, St. Louis, MO, USA), 100 mg/kg/d dissolved in 1% sodium carboxymethyl cellulose, was administered intragastrically to diabetic mice daily for eight weeks. The control mice (Con) and diabetic mice (DM) were randomly divided into four groups: vehicle-treated control group, fenofibrate-treated control group, vehicle-treated diabetic group and fenofibrate-treated diabetic group.

2.2. Vascular reactivity study

After anesthetizing with 2% isoflurane, the thoracic aortas and mesenteric arteries from mice were rapidly removed, placed in ice-cold Krebs-Henseleit solution, cleaned gently from adherent connective tissue and cut into approximately 2 mm length. The arteries were mounted onto a wire myograph system (model 620 M, Danish Myo Technology, Aarhus, Denmark) in oxygenated (5% CO_2, 95% O_2) and warmed (37 °C) Krebs solution (pH 7.4) with the following composition (mmol/L): NaCl 112, KCl 5, $NaHCO_3$ 25, NaH_2PO_4 1, $MgCl_2$ 0.5, $CaCl_2$ 2.5 and glucose 11.5. Each artery was suspended between two stainless steel wires (diameter, 40 μm) and equilibrated for 90 min at 37 °C before experiments. After that, resting tension was set according to the manufacturer's protocol and vessel viability was assessed by the response to KCl (100 mmol/L). After a wash out, cumulative concentration responses for vasoactive agents were obtained. Endothelium-dependent relaxation in response to acetylcholine (ACh, 10^{-9}–10^{-4} mol/L) and endothelium-independent relaxation by sodium nitroprusside (SNP, 10^{-9}–10^{-4} mol/L) were measured in aortas pre-contracted by norepinephrine (NE, 10 μmol/L). Also, a dose response to NE (10^{-10}–10^{-5} mol/L) was obtained. Some segments were incubated with agonist or antagonist for 30 min before vasoreactivity measurement, include GW 6471 (10 μmol/L, PPARα inhibitor), compound C (1 μmol/L, AMPK inhibitor), 5-aminoimidazole-4 carboxamide-1-β-D-ribofuranosid (AICAR , 1 μmol/L, AMPK agonist), fenofibrate (FF, 100 μmol/L, PPARα agonist), tempol (100 μmol/L, superoxide dismutase mimetic), indomethacin (100 μmol/L, cyclooxygenase inhibitor) and L-nitro-arginine-methyl-ester (L-NAME, 100 μmol/L, NOS inhibitor). For in vitro hyperglycemia experiments, aortas were incubated in normal (11.5 mmol/L) or hyperglycemic (44 mmol/L) Krebs solution for 4 h with or without fenofibrate. Then, after washing the preparation, the relaxant responses to ACh and NE were studied. All the chemicals were from Sigma-Aldrich (St Louis, MO, USA) except AICAR and GW 6471 which were purchased from ApexBio Technology (Houston , USA). Fenofibrate, AICAR, GW 6471 , indomethacin and compound C were dissolved in dimethylsulfoxide (DMSO) and other drugs in distilled water. DMSO (0.1% v/v) did not modify agonist or inhibitor-induced responses.

2.3. Isolation and perfusion of the renal afferent arterioles

To evaluate the effect of fenofibrate on microvessels, isolation and micro-perfusion of afferent arterioles were conducted as described previously [26,27]. In brief, kidneys were removed and sliced along the corticomedullary axis. Slices were placed in ice-cold glucose Dulbecco's modified Eagle's medium (DMEM). Afferent arterioles with attached glomeruli were micro-dissected under a stereomicroscope (SZX16, Olympus, Japan) and then transferred to a temperature-controlled chamber on the stage of an inverted microscope (Axiovert 100TV, ZEISS, Oberkochen, Germany), and perfused using a micromanipulator system with concentric holding and perfusion pipettes. Vessel viability was tested by depolarization using potassium chloride (100 mM), greater than 90% contraction was considered as viable and used in our experiments. Norepinephrine (NE: 1×10^{-6} mol/L) was used to preconstrict afferent arterioles before investigating dilation. Luminal diameter was measured after preconstriction and with increasing doses of ACh (10^{-9} to 10^{-5} mol/L) to test endothelium-dependent dilation in renal afferent arterioles. The arteriolar luminal diameter was calculated as the mean of seven pictures during stable tension

2.4. Western blot analysis

After euthanasia, the aortas were dissected and stored at − 80 °C. Frozen aortic tissue samples were homogenized with lysis buffer (Beyotime, Shanghai, China). Each protein sample was extracted from the isolated aorta of one mouse. The homogenate was centrifuged for 10 min at 13,000 g at 4 °C. Samples containing equivalent amounts of protein were loaded and separated by SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. Membranes were blocked by 5% fat-free milk powder and immunodetected with specific primary antibodies to β-actin, eNOS, p-