Biomedicine & Pharmacotherapy 95 (2017) 129–136

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Telmisartan ameliorates adipoR1 and adipoR2 expression via PPAR-γ activation in the coronary artery and VSMCs

Xuhua Shen*, Hongwei Li*, Weiping Li, Xing Wu, Zhijun Sun, Xiaosong Ding

Department of Cardiology, Beijing Friendship Hospital, Capital Medical University, 95 Yong An Road, West district, Beijing 100050, China

ARTICLE INFO

Article history:
Received 16 July 2017
Received in revised form 5 August 2017
Accepted 7 August 2017

Keywords:
Telmisartan
Diabetic
PPAR-γ
Adiponectin receptors

ABSTRACT

The effects of telmisartan on insulin-resistant properties and expression of adiponectin receptors (AdipoRs) were investigated. A diabetic rat model was established using a high-fat diet and streptozotocin (25 mg/kg) and primary rat coronary vascular smooth muscle cells (VSMCs) were used to elucidate the underlying mechanisms. The diabetic rats were insulin-resistant and exhibited weight gain, elevated blood pressures, and increased plasma triglyceride levels. These manifestations were ameliorated by elmisartan treatment. Four-week telmisartan therapy increased plasma adiponectin and decreased TNF-α expression in the coronary artery. Moreover, telmisartan significantly decreased AdipoR1 and AdipoR2 expression. Using high glucose-treated rat coronary VSMCs, telmisartan and PPAR-γ agonist GW1929 prominently stimulated PPAR-γ and decreased TNF-α expression. Interestingly, telmisartan or GW1929 also prevented hyperglycemia-induced downregulation of AdipoR1 and AdipoR2 expression. Additionally, GW9662 (PPAR-γ antagonist) significantly decreased the effects of telmisartan on AdipoR1 and AdipoR2 expression. These results demonstrated that telmisartan effectively ameliorated coronary insulin resistance and inflammation in diabetic rats and upregulated AdipoR1/R2 expression via activation of PPAR-γ in the coronary artery and VSMCs.

© 2017 Published by Elsevier Masson SAS.

1. Introduction

Adiponectin (APN) is an adipose tissue-derived hormone that is capable of promoting insulin sensitivity. Adiponectin has been to regulate cell migration, proliferation, atherothrombosis and inflammation [1–3]. Interestingly, accumulating evidence suggests that adiponectin possesses protective activities in the myocardium and vasculature [4–6]. Adiponectin attenuated the progression of macrovascular disease in rodent models [7], which is consistent with clinical evidence [8].

Adiponectin functions by binding to adiponectin receptors (AdipoRs), AdipoR1 and AdipoR2 [9]. AdipoR1 is dominantly expressed in the skeletal muscle. However, AdipoR2 is abundantly expressed in the liver [4]. The action of adiponectin could be abolished by disruption of both receptors, which would elevate triglyceride levels and aggravate inflammation and oxidative stress, leading to insulin-resistance and glucose intolerance. We previously established a negative correlation between insulin resistance and AdipoRs in rat coronary arteries and vascular smooth muscle cells (VSMCs) [10]. Moreover, genetic variations in AdipoR1 and AdipoR2 correlated with coronary artery disease [4,11,12].

However, the regulation of AdipoRs in diabetic vascular disease has not been fully elucidated. AdipoR expression was variably regulated upon different stimulations. Chinetti et al. [13] demonstrated that peroxisome proliferator-activated receptor-α (PPAR-α) and PPAR-γ agonists only magnified AdipoR2 expression, while liver X receptor (LXR) agonists increased the mRNA expressions of AdipoR1 and AdipoR2 in human macrophages. Telmisartan is an angiotensin type 1 receptor (AT1R) blocker, which could also activate PPAR-γ[14]. Telmisartan increases plasma adiponectin levels and improves insulin resistance[15]. Although the mechanisms remained unclear, PRAP-γ activation has been shown to prevent type 2 diabetes. However, it still remains to be investigated the mechanisms whereby telmisartan regulates AdipoR expression.

In this study, we hypothesized that telmisartan could ameliorate coronary insulin resistance through regulating AdipoR expression. Both *in vivo* and *in vitro* experiments were carried out to evaluate the mechanism underlying the effects of

* Corresponding authors.
E-mail addresses: shenxuhua102@126.com (X. Shen), lhw19656@sina.com (H. Li).

http://dx.doi.org/10.1016/j.biopha.2017.08.041
0753-3322/© 2017 Published by Elsevier Masson SAS.

telmisartan on AdipoR expression in rat coronary arteries and VSMCs.

2. Materials and methods

Telmisartan and GW9662 were purchased from Sigma Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), TRIzol reagent and Sample Reducing Agent were obtained from Invitrogen Life Technologies (Shanghai, China). Anti-GAPDH antibody was obtained from ProMab Biotechnologies Inc. (ProMab, USA). AdipoR1 (AHP1824) and AdipoR2 (AHP1900) antibodies were obtained from AbD Serotec (AbD, U.K.). PPAR-γ (B0557) antibody was from Assay BioTech (ABT, USA). Anti-TNF-α and horseradish peroxidase conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.1. Animals

The experimental protocols of this study were approved by the Animal Care Committee of Beijing Friendship Hospital. Rats were first divided into two groups, the control group (n = 6) and the diabetic model group (n = 12). The diabetic group was further divided into the DM group and the DM+ telmisartan (TEL) group (called the TEL group). In the control group, the rats were fed with a regular diet for 4 weeks and then injected with an equivalent volume of citrate buffer (0.1 M). In the diabetic model rat group, after being fed with a high-fat diet (HFD) for 4 weeks, male Sprague–Dawley rats (200 ± 20 g)rats received a single intraperitoneal injection of streptozotocin (25 mg/kg diluted in 0.1 M citrate buffer, pH = 4.5; Sigma, USA). The TEL group was diabetic and received telmisartan at 5 mg/kg per day by gavage for 4 weeks. Telmisartan was dissolved in two drops of Tween and diluted in 0.9% saline.

On day 7 after injection, fasting blood glucose (FBG) and serum insulin (SI) were measured. The insulin sensitivity index (ISI, ISI = $-$In (FBG $\times$ SI)) was calculated. Rats with the ISI ≤ -4.88 were considered diabetic. The diabetic rats were divided into the diabetic model group (DM group) and the TEL group. Rats in the TEL group received telmisartan by gavage at a dose of 5 mg/kg/day for 4 weeks. A normal control group was also set in the experiment. All the rats were housed (three per cage) in a controlled environment at 18 ± 2 °C, 30–70% humidity, 12 h light cycle, and water ad libitum. The rats were weighed and blood samples were collected in the ninth week. In accordance with the protocol, the rats were euthanized after the experiments. The coronary arteries were dissected from the adherent fat and connective tissue on ice, and then frozen in liquid nitrogen and kept at -80 °C for subsequent experiments.

2.2. Measurements of blood pressure

Blood pressure (systolic, mean and diastolic) was recorded at the end of treatment using the tail cuff blood pressure recorder (Gene&I Co., Model No. BP-98A, China). Blood pressure was measured between 9 and 11 am after rats were acclimatized to the heating chamber (24–26 °C) for 20 min. Three parameters were recorded for each rat and the mean was calculated.

2.3. Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed in the ninth week. All rats fasted for 12 h and blood samples were collected from the caudal vein at 0 (immediately after glucose administration, 2 g/kg), 30, 60 and 120 min after glucose administration. Blood glucose was evaluated by the enzymatic glucose oxidase method using a commercial glucometer (Acku-check, Sensor Confort, Roche, China). The area under the curve for glucose (AUCglucose) was integrated, which was calculated by the trapezoid rule.

2.4. Cell culture and treatment

Coronary VSMCs were isolated from the rats (n = 6, in each group) and cultured in DMEM (Gibco, USA) supplemented with 15% fetal calf serum (Gibco, USA), 100 U/mL penicillin G, and 100 mg/mL streptomycin. DMEM had normal glucose (NG, 5.5 mmol/L D-glucose). Coronary VSMCs were incubated with TEL (10 μmol/L) + high glucose (HG, 23 mmol/L D-glucose), or TEL (10 μmol/L) + GW9662 (5 μmol/L) + HG, or GW9662 (5 μmol/L) + HG, or GW1929(20 μmol/L) + HG, or 0.1% dimethyl sulfoxide vehicle in DMEM HG or DMEM NG for 24 h at 37 °C before each assay.

2.5. Real-time (RT)-PCR

Total RNA was isolated from rat coronary arteries and coronary VSMCs using TRIzol reagent (Invitrogen, China). Complementary DNA synthesis was performed using a RevertAid^{TM} H Minus First Strand cDNA Synthesis Kit (Fermentas, China) from 1 μg of extracted total RNA. Then, RT-PCR was performed with specific primers and SYBR green dye using an Applied Biosystems 7500 Real-Time PCR System. The forward (fwd) and reverse (rev) primer sequences were as follows: AdipoR1 (fwd: 5′-CCTGGGACTTGGCTT-GAGT- 3′; rev: 5′-GGAATCCGAGCAGCATAAA −3′), AdipoR2 (fwd: 5′- ACGAATGGAAGAGTTTGTTTG-3′; rev: 5′-GGCGAAACATATAAAA-GATCC −3′), TNF- α (fwd: 5′- GAACAACCCTACGAGCACCT − 3′; rev: 5′-GGGTAGTTTGGCTGGGATAA-3′), GAPDH (fwd: 5′-CCTGCCAAG-TATGATGACATCAAG − 3′; rev: 5′-GTAGCCCAGGATGCCCTTTAGT − 3′). After initial denaturation at 95 °C for 10 min, PCR was run for forty cycles, each consisting of 60 °C for 1 min, melting curve 95 °C for 15s, 60 °C for 30 s, and 95 °C for 15 s. The product was confirmed by melting curve analysis. The ratio of the amounts of target mRNA to those of the internal standard (GAPDH) mRNA was determined as an arbitrary unit.

2.6. Western blotting assays

Western blot analyses (n = 6, in each group) were conducted as previously described (Shen et al., 2012) to assess the expression of AdipoR1, AdipoR2, TNF-α and PPAR-γ. In brief, the coronary arteries and serum-starved coronary VSMCs cells were homogenized in a cold Tris–HCl buffer (150 mM NaCl, 1.0%, Triton X-100, 50 mM Tris–HCl, pH 7.5, 10% glycerol, 1 mM Na_3VO_4, 1 mM EDTA, and protease inhibitor cocktail) for 30 min at 4 °C. Total protein was quantified using a BCA protein assay kit as required (ProMab, No. SJ- 200501, China). Equal amounts of protein (40 μg) were separated by SDS-PAGE. Then, the corresponding primary antibodies, goat AdipoR1 polyclonal antibody (1:1000 dilution; AbD Inc., U.K.), goat AdipoR2 polyclonal antibody (1:1000 dilution; AbD Inc., U.K.), rabbit PPAR-γ polyclonal antibody (1:400 dilution; ABT, USA), goat TNF-α polyclonal antibody (1:500 dilution; Santa, USA), or with polyclonal anti-Mouse GAPDH (1:1000 dilution; ProMab, USA) were applied. The membranes were incubated with the diluted antibody preparations overnight at 4 °C. After washing, rabbit anti-goat peroxidase conjugated antibody was added (1:2000 dilution; Sigma, Shanghai, China) and the bands were visualized by ECL. Target proteins were quantified and normalized relatively to GAPDH (1:800, SANTA, China).

Download English Version:

https://daneshyari.com/en/article/5552534

Download Persian Version:

https://daneshyari.com/article/5552534

Daneshyari.com