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Pioglitazone prevents hyperglycemia induced decrease of AdipoR1 and AdipoR2 in coronary arteries and coronary VSMCs

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ABSTRACT

Background: Adiponectin receptors play an important role in inflammatory diseases like diabetes and atherosclerosis. Former studies revealed that the regulation of adiponectin receptors expression differs in the receptor responses to pioglitazone. However, expression of AdipoRs has not been investigated in the coronary arteries or the coronary vascular smooth muscle cells (VSMCs). In the present study we investigated the effect of pioglitazone on the adiponectin receptors both in vitro and in vivo.

Methods: Male Sprague–Dawley rats were randomly divided in three groups. One of them fed with regular chow (the Control group) and two of them fed with high-fat diet and then received low-dose Streptozotocin once by intraperitoneal injection (the DM groups). Rats in one of the DM groups were further treated with pioglitazone (the PIO group). Blood pressure, serum adiponectin, fasting blood glucose, fasting serum insulin, cholesterol, triglyceride, AdipoR1 and AdipoR2 expression, and TNF-α expression in coronary arteries of these groups were investigated. For the in vitro study, the rat coronary VSMCs maintained under defined in vitro conditions were treated with either PIO or the PIO+ GW9662 (PPAR-γ antagonist), and then stimulated with high glucose. AdipoR1 and AdipoR2 expression, TNF-α expression and PPAR-γ expression were investigated.

Results: Compared to the DM group, treatment with PIO in vivo significantly attenuated cholesterol level, triglyceride level, fasting serum insulin and TNF-α overexpression ($p < 0.05$). PIO also increased AdipoR1 and AdipoR2 expression in coronary arteries, which were reduced notably in the DM group ($p < 0.05$). Consistently, in the study with rat coronary VSMCs, PIO prominently downregulated TNF-α expression and induced PPAR-γ expression, as well as prevented hyperglycemia induced decrease of AdipoR1 and AdipoR2 expression ($p < 0.05$). And pretreatment of PIO + GW9662 did not manifest the prevention effect.

Conclusion: In this study, we showed that treatment with PIO could ameliorate coronary insulin resistant and upregulate the expression of AdipoR1/R2. PIO showed an anti-atherogenic property via the activation of PPAR-γ, suppression of TNF-α overexpression in coronary and coronary VSMCs.

1. Introduction

Diabetes mellitus is an important risk factor for the development of atherosclerosis. The risk for cardiovascular disease in patients with diabetes is two to sixfold higher than in people without diabetes. The clustering of traditional risk factors such as hypertension and hypercholesterolemia cannot explain the excessive cardiovascular burden of patients with diabetes. The UK prospective diabetes study (UKPDS) showed that while intensive control of blood glucose level could not reduce the cardiovascular risk, multifactorial intervention was effective in improving cardiovascular mortality and morbidity in patients with diabetes (UK Prospective Diabetes Study Group, 1998).

Thiazolidinediones (TZDs) are pharmacologic agents that improve glucose homeostasis in type 2 diabetes by increasing insulin sensitivity (Olefsky, 2000). In addition, the pioglitazone (PIO), one of the TZDs, significantly reduces the incidence of major adverse cardiovascular events, strokes, and all-cause mortality in high-risk patients with type 2 diabetes (Dormandy et al., 2005). The PIO is believed to be mediated by their interaction with the nuclear receptor peroxisome proliferator–activated receptor (PPAR-γ). PPAR-γ is a member of the nuclear hormone receptor superfamily of ligand-activated transcriptional factors. Recently, it has been

Abbreviations: PIO, pioglitazone; VSMCs, vascular smooth muscle cells; SD, Sprague–Dawley; STZ, Streptozotocin; DM, diabetic mellitus; APN, adiponectin; FSI, fasting serum insulin; AdipoR, adiponectin receptor; PPAR-γ, peroxisome proliferator-activated receptor; AMPK, 5′-AMP-activated protein kinase.

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demonstrated that PPAR-γ is expressed in endothelium, vascular smooth muscle cells, and monocytes/macrophages, which were considered as important cells for atherosclerosis (Marx et al., 2004; Little et al., 2008). It suggests that PIO may exert direct beneficial effects on the vascular wall.

Adiponectin (Scherer et al., 1995) is a hormone secreted by adipocytes, which function as the key antidiabetic and anti-atherogenic adipocytokine (Scherer, 2006). Plasma adiponectin levels are decreased in obesity, insulin resistance, and type 2 diabetes mellitus. Many studies have shown that the high levels of adiponectin are associated with insulin sensitization, whereas low levels are found in insulin resistance (Satoh et al., 2005).

Recently, two distinct receptors for adiponectin (AdipoR1 and AdipoR2) have been identified (Yamauchi et al., 2003). AdipoR1 was ubiquitously expressed and most abundantly expressed in skeletal muscle, whereas AdipoR2 was most abundantly expressed in mouse liver (Yamauchi et al., 2003). Adiponectin receptors are key innate sensors of endogenous damage signals and play an important role in inflammatory diseases like diabetes and atherosclerosis. Adiponectin receptors mediate the activation of AMPK, PPARα, and fatty acid oxidation, which increases glucose uptake and improves lipid metabolism (Yamauchi et al., 2003). AdipoR1- and AdipoR2-mediated signal transduction has been implicated in steatosis, inflammation, and oxidative stress, all key abnormalities associated with obesity and the metabolic syndrome (Kadowaki, 2006).

The regulation of AdipoR1 and AdipoR2 expression differs in the receptor responses to PIO. AdipoR1 expression is upregulated in adipose tissue, but downregulated in skeletal muscle by long-term treatment of PIO (Sun et al., 2006). On the other hand, PIO did not change AdipoR1 and AdipoR2 expression in peritoneal macrophages and subcutaneous fatty tissue (Tsuchida et al., 2005). However, little is known about its effect on coronary arteries or coronary VSMC which are the key tissue and cells in diabetes, hypertension and atherosclerosis.

In this study, we examined the effects of PIO on AdipoR1 and AdipoR2 expression in rat coronary arteries and coronary VSMC in vivo and in *vitro*. What we found?

2. Materials and methods

2.1. Materials

PIO was donated by Huadong Medicine Co. (Hangzhou, China). GW9662 was purchased from Sigma Co. (Sigma, USA). DMEM, streptomycin, trypsin, fetal bovine serum (FBS), TRIzol reagent, pCR2.1-TOPO vector, LDS sample buffer, and Sample Reducing Agent were from Invitrogen Life Technologies (Shanghai, CN, USA). Anti-GAPDH antibody was from ProMab Biotechnologies Inc. (ProMab, USA). Anti-AdipoR-1 (AHP1824) and AdipoR-2(AHP1900) antibodies were obtained from AbD serotec Corp (AbD, U.K.). Anti-PPAR-γ(B0557) antibody was from Assay bioTech Corp(ABT, USA). Anti-TNF-α and horseradish peroxidase conjugated secondary antibodies were from Santa Cruz Biotechnology (Santa, USA).

2.2. Animals

Male Sprague–Dawley(SD) rats (Vital River; Beijing, China) weighing 250 g were housed in an environmentally controlled room with a 12-h light/dark cycle and given standard rodent chow and tap water ad libitum. The rats were acclimated to handling before randomization and then divided into three groups in the beginning of the study. The control group fed with regular chow for 5 weeks and received the citrate buffer alone for 4 weeks. The other rat received high-fat diet (HFD) for 4 weeks and then a single intraperitoneal injection of STZ (25 mg/kg, in pH 4.5 citrate buffer). After 7 days, blood samples were collected from caudal vein. Fasting Blood Glucose (FBG) and Serum insulin (SI) were estimated, then Insulin Sensitive Index [ISI, ISI = −ln (FBG × SI)] was calculated. The HFD and STZ-treated rats with the ISI $\leqslant -4.88$ were randomized into DM groups and PIO group. DM group received the citrate buffer and the PIO group received PIO at 10 mg/kg per day by gavage. After 4 weeks of treatment, 12 h fasted rat (n = 6–8) was anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The blood samples were collected and the body weight was also measured. The coronary arteries were dissected from the ventricle and placed in a phosphate buffered saline (PBS)-precooled plate. The coronary arteries was dissected from the adherent fat and connective tissue on ice, and then frozen in liquid nitrogen and kept at −80 °C for subsequent analysis. This study was performed in accordance with the guidelines for animal experiments of the Capital University of Medical Sciences.

2.3. Measurement of blood pressure

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

2.4. Oral glucose tolerance test (OGTT)

After 4 weeks of treatment, glucose (2 g/kg) was administered to 12 h fasted rats and blood samples were collected from the caudal vein by means of a small incision at the end of the tail at 0 (immediately after glucose load), 30, 60 and 120 min after glucose administration. Blood glucose lever was estimated by the enzymatic glucose oxidase method using a commercial glucometer (Acku-check, sensor confort, Roche, Germany).The results were expressed as the integrated area under the curve for glucose ($AUC_{glucose}$), which was calculated by trapezoid rule.

2.5. Cell culture and cell treatment

Seven-week-old male SD rats (Vital River; Beijing, China) were anesthetized with pentobarbital sodium (60 mg/kg ip). Rat coronary arteries were dissected from the ventricle and the endothelium in the vessels was denuded with air (Liu et al., 1994). Enzymatic isolation of VSMCs were performed according to published methods (Li et al., 2003). Coronary VSMCs were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 20% fetal calf serum (Gibco, USA), 100 U/mL penicillin G, and 100 mg/mL streptomycin. DMEM was supplemented with normal glucose (NG, 5.5 mmol/L D-glucose). VSMCs were incubated with PIO (10 μmol/L)+ high glucose (HG, 23 mmol/L D-glucose), or PIO(10 μmol/L)+ GW9662(5 μmol/L)+HG, or 0.1% dimethyl sulfoxide vehicle in DMEM HG or DMEM NG for 24 h at 37 °C before each assay. The cells were maintained in a humidified chamber with 5% CO2 at 37 °C.

2.6. Quantitative real-time (RT)–PCR analysis

Total RNA samples were extracted from rat coronary arteries (n = 6–8 in each group) and VSMCs with Trizol reagent (invitrogen, CA), and total RNA was further purified using the RNeasy kitwith RNase-free DNase I treatment according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed with iScript cDNA Synthesis Kit according to the manufacturer's instructions