Pharmacological Research 111 (2016) 226–236

Contents lists available at ScienceDirect

Pharmacological Research

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

Original research article

TGR5 activation suppressed S1P/S1P2 signaling and resisted high glucose-induced fibrosis in glomerular mesangial cells

Zhiying Yang[a,b,c], Fengxiao Xiong[a], Yu Wang[a], Wenyan Gong[a], Junying Huang[a], Cheng Chen[a], Peiqing Liu[a,b,c], Heqing Huang[a,b,c,*]

[a] Laboratory of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China
[b] National and Local United Engineering Lab of Druggability and New Drugs Evaluation, Guangzhou 510006, China
[c] Guangdong Provincial Engineering Laboratory of Druggability and New Drugs Evaluation, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form 17 May 2016
Accepted 23 May 2016
Available online 16 June 2016

Chemical compounds studied in this article:
INT-777 (PubChem CID: 45483949)
JTE-013 (PubChem CID: 10223146)

Keywords:
TGR5
Renal fibrosis
S1P2
AP-1
Fibronectin

ABSTRACT

Glucose and lipid metabolism disorders and chronic inflammation in the kidney tissues are largely responsible for causative pathological mechanism of renal fibrosis in diabetic nephropathy (DN). As our previous findings confirmed that, sphingosine 1-phosphate (S1P)/sphingosine 1-phosphate receptor 2 (S1P2) signaling activation promoted renal fibrosis in diabetes. Numerous studies have demonstrated that the G protein-coupled bile acid receptor TGR5 exhibits effective regulation of glucose and lipid metabolism and anti-inflammatory effects. TGR5 is highly expressed in kidney tissues, whether it attenuates the inflammation and renal fibrosis by inhibiting the S1P/S1P2 signaling pathway would be a new insight into the molecular mechanism of DN. Here we investigated the effects of TGR5 on diabetic renal fibrosis, and the underlying mechanism would be also discussed. We found that TGR5 activation significantly decreased the expression of intercellular adhesion molecule-1 (ICAM-1) and transforming growth factor-beta 1 (TGF-β1), as well as fibronectin (FN) induced by high glucose in glomerular mesangial cells (GMCs), which were pathological features of DN. S1P2 overexpression induced by high glucose was diminished after activation of TGR5, and AP-1 activity, including the phosphorylation of c-Jun/c-Fos and AP-1 transcription activity, was attenuated. As a G protein-coupled receptor, S1P2 interacted with TGR5 in GMCs. Furthermore, INT-777 lowered S1P2 expression and promoted S1P2 internalization. Taken together, TGR5 activation reduced ICAM-1, TGF-β1 and FN expressions induced by high glucose in GMCs, the mechanism might be through suppressing S1P/S1P2 signaling, thus ameliorating diabetic nephropathy.

1. Introduction

Diabetic nephropathy (DN) is one of the most serious microvascular complications of diabetes and the major cause of death and disability in diabetes mellitus. It is mainly characterized by renal fibrosis, and glomerulus mesangial cells (GMCs) are the main functional cells in the kidney. In diabetes, excessive deposition of extracellular matrix (ECM) proteins, specifically fibronectin (FN), and transforming growth factor-beta 1 (TGF-β1) overexpression lead to mesangial expansion, which accelerates the pathological progress of renal fibrosis [1,2]. The pathogenesis of DN is complicated, hyperglycaemia, hyperlipidaemia, oxidative stress and inflammatory factors are considered closely related to diabetic renal fibrosis. Chronic inflammatory reactions by multiple factors are contributed to diabetic renal fibrosis [3,4]. However, insufficient specific drugs and methods make DN treatment difficult. Thus, exploration of new targets and therapeutic methods for renal fibrosis are significant for preventing and treating DN.

TGR5 is a novel bile acid receptor and a membrane-typed G protein-coupled receptor (GPCR) consisting of 330 amino acids, including seven transmembrane domains [5,6]. TGR5, also called BG37 or M-BAR, is widely expressed in various human organs, with the highest level found in the spleen and placenta, followed by the kidney, lung, liver, stomach, small intestine, adipose tissue and bone marrow. TGR5 is also expressed in other tissues, such as breast, uterine tissues and skeletal muscles [7]. According to the

Abbreviations: AP-1, active protein-1; DN, diabetic nephropathy; FN, fibronectin; GMCs, glomerular mesangial cells; ICAM-1, intercellular adhesion molecule-1; S1P, sphingosine 1-phosphate; S1P2, sphingosine 1-phosphate receptor 2; Sphk1, sphingosine kinase; TGF-β1, transforming growth factor-beta 1; TGR5, takeda G-protein-coupled receptor 5.

* Corresponding author at: Laboratory of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, 132 WaiHuan East Road, Guangzhou Higher Education Mega Center, Guangzhou 510006, China.
E-mail address: huangheq@mail.sysu.edu.cn (H. Huang).

http://dx.doi.org/10.1016/j.phrs.2016.05.035
1043-6618/

references, TGR5 activation increased GLP-1 levels and regulated glucose metabolism [8,9].GLP-1, a physiologically active substance, promotes insulin incretion, protects islet β cells and lowers body weight [7,10]. In addition to increase GLP-1 levels, TGR5 activation regulates gene expressions related to energy metabolism in peripheral tissues, such as muscles and adipose tissues [11–13]. These observations demonstrate the effects of TGR5 activation on regulating glycolipid metabolism and ameliorating inflammation in diabetes. A recent study showed that, TGR5 restrains kidney disease by inducing mitochondrial proliferation and resisting oxidative stress and lipid accumulation [14].

Sphingosine 1-phosphate (S1P) is a metabolite of phospholipid generated by hyperglycemia, oxidative stress, growth factors and cytokines in various cells [15–18]. S1P acts as an intracellular secondary messenger mediating signal transduction can also be secreted and serves as a ligand of S1P2 to mediate the activation of PKC and MAPK pathways, resulting in cell proliferation, differentiation and migration [17,19,20]. Sphingosine kinase1 (SphK1) is a limited enzyme catalysing the generation of S1P through regulating the nuclear factor AP-1 and NF-κB [21,22]. Recent studies have suggested that S1P signal activation plays a pivotal role in the pathological process of renal fibrosis in DN. Our previous studies confirmed that S1P signal was sustainably activated in the renal tissues of diabetic animals [23]. Under high glucose conditions, S1P2, one of the five subtype receptors of S1P, presents the most abundant expression. S1P2 also promotes AP-1 DNA-binding activity and increases FN expression in GMCs, which is related to the pathological changes of renal fibrosis [20,24,25].

Although TGR5 shows significant effects on glucose and lipid metabolisms and anti-inflammation in obesity and diabetes, its molecular mechanism remains unclear. TGR5 modulates inflammation and fibrosis in the kidney, and its relationship with S1P2, a GPCR similar to TGR5, deserves further study. In the present study, we explored whether TGR5 activation could inhibit the S1P/S1P2 signaling pathway and AP-1 activity in GMCs cultured in high glucose to ameliorate inflammation and fibrosis. Our results provided preliminary experimental evidences supporting TGR5 as a new target in inhibiting renal fibrosis in diabetes.

2. Materials and methods

2.1. Reagents and antibodies

INT-777 was purchased from Dalton Pharma Services (Toronto, Canada). S1 P and JTE-013 were obtained from Sigma–Aldrich Co. (St. Louis, USA). 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Beyotime (Haimen, China). DMEM was provided by Life Technologies (Gibico, Carlsbad, CA, USA). Fetal bovine serum was provided by HyClone (South Logan, UT, USA). Antibodies against FN (catalogue: sc-18825), ICAM-1 (catalogue: sc-1511), TGR5 (catalogue: sc-98888) and S1P2 (catalogue: sc-31577) were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Antibodies against TGF-β1 (catalogue: 3709s), p-c-Jun ser63 (catalogue: #2361P), p-c-Jun ser73 (catalogue: #3270P), p-c-Fos ser32 (catalogue: #5348S), c-Jun (catalogue: #9165S), c-Fos (catalogue: #2250S) were all purchased from Cell Signaling Technology (Danvers, USA); α-tubulin (catalogue: sc-18825) and β-actin (catalogue: sc-18825) were purchased from Sigma–Aldrich Co. (St. Louis, USA); GFP (catalogue: AG281-1), rabbit IgG (catalogue: A7016), goat IgG (catalogue: A7007) were purchased from Beyotime (Haimen, China). Goat anti-rabbit IgG (catalogue: #A-11008) labeled with Alexa Fluor 488, donkey anti-goat IgG (catalogue: #A-11058) labeled with Alexa Fluor 594 were purchased from Thermo Fisher Scientific Inc. (Waltham, MA USA); Horseradish peroxidase-conjugated secondary antibodies were supplied by Promega (Madison, WI, USA). Hoechst33342 was purchased from Sigma–Aldrich Co. (St. Louis, USA).

2.2. Cell culture

GMCs were obtained from Sprague–Dawley (SD) rats as previously described [16], cultured in DMEM with 1 × penicillin–streptomycin and 10% fetal bovine serum, grown in an incubator at 37 °C under 5% CO_2 atmosphere, and then subcultured for 10–15 days. The cultured cells were used at confluence between the 6 th and 12 th passages. Upon reaching 80% confluence, cells were serum-starved for 14–16 h before treatment.

2.3. Plasmids, small-interfering RNAs and transient transfection

TGR5 plasmids for overexpression were purchased from OriGene Technologies (OriGene, USA). pAP-1-Luc reporter gene plasmids were purchased from Beyotime (Haimen, China). pRL-TK reporter gene plasmids were obtained from Promega (Madison, WI, USA). p-EYFP-S1P2 plasmids were constructed by TranSheep Biology (Shanghai, China). TGR5 small-interfering RNAs were purchased from GenePharma Co., Ltd. (Suzhou, China). Transient transfection was performed following the manufacturer's instructions for Lipofectamine™ LTX & Plus Reagent (Life Technologies, Carlsbad, CA) and lipofectamine RNAiMAX reagent (Life Technologies, Carlsbad, CA).

2.4. Western blot assay

Western blot assay was performed as previously described [26]. In brief, GMCs were washed twice with cold phosphate-buffered saline, and the total proteins were extracted after treatment. An equal amount of protein samples was subjected to 8%–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to nitrocellulose or polyvinylidene difluoride membranes. After blocking with 5% non-fat milk, the blots were incubated overnight at 4 °C with primary antibodies. After incubation with secondary antibodies, immunoreactive bands were visualized with a GE ImageQuant LAS4000mini (GE healthcare; Waukesha, USA), quantified by densitometry with the Gel Doc XR system (Bio-Rad Laboratories; Hercules, USA), and then analyzed using the Quantity One protein analysis software (Bio-Rad Laboratories; Hercules, USA).

2.5. Dual-luciferase reporter assay

GMCs were seeded in 96-well culture plates and co-transfected with 0.2 μg of pAP-1-Luc and 0.04 μg of pRL-TK using Lipofectamine® LTX & plus reagent (Life Technologies™, Grand Island, NY, USA) according to the manufacturer's instructions for 24 h. After further treatment, cells were lysed and luciferase activity was determined using the Dual-Luciferase® reporter assay system kit (Promega, Madison, WI, USA). Luciferase activity was normalized to the renilla luciferase activity.

2.6. Confocal laser scanning fluorescence microscopy (LSCM)

GMCs were washed with PBS, and fixed with 4% paraformaldehyde for 15–30 min at room temperature. After further washing, the cells were blocked with 10% goat serum. Then cells were incubated with antibodies against TGR5 or S1P2 overnight at 4 °C and with secondary antibodies in the dark at room temperature for 1 h. Cells