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The effect of FFAR1 on pioglitazone-mediated attenuation of palmitic acid-induced oxidative stress and apoptosis in βTC6 cells

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ABSTRACT

Objective. We sought to determine whether free fatty acid receptor 1 (FFAR1), a receptor for free fatty acids on the β-cell membrane, can mediate the pioglitazone (PIO)-attenuating effect on lipoapoptosis in β cells and its relationship to oxidative stress.

Methods. The glucose-sensitive mouse beta pancreatic cell line βTC6 was used to investigate the effect of FFAR1 on PIO-attenuating palmitic acid (PA)-induced oxidative stress and apoptosis.

Results. (1) PIO reduced PA-induced lipoapoptosis in β cells and upregulated the expression of FFAR1 at the mRNA and protein levels in a dose- and time-dependent manner. Silencing of FFAR1 expression was shown to weaken the protective effect of PIO on PA-induced lipoapoptosis in βTC6 cells; while lentiviral-mediated overexpression of FFAR1 was shown to enhance the protective effect of PIO against lipoapoptosis in β cells. (2) Downregulation of FFAR1 expression reduced the attenuating effect of PIO on the expression of NAPDH oxidase subunit $p47^{phox}$, Bax, cleaved caspase 3, and the production of reactive oxygen specific (ROS) induced by lipotoxicity, thereby preventing the upregulation of the expression of bcl-2. Inducing the overexpression of FFAR1 enhanced the anti-oxidative stress effect of PIO. Similarly, these effects of FFAR1 on PIO were reproduced under conditions of oxidative stress and apoptosis in βTC6 cells that were induced by H_2O_2. (3) PIO was found to increase the expression of PLCγ, ERK1/2, and PPARγ in lipotoxic β cells. Silencing FFAR1 expression reduced the PIO-mediated increases in the expression of above proteins; while inducing FFAR1 overexpression showed the opposite effect. Use of an inhibitor of PLCγ, ERK1/2, PPARγ was shown to restrict the protective effect of PIO on oxidative stress and lipoapoptosis of β cells.

Conclusions. FFAR1 can mediate PIO suppression of β-cell lipoapoptosis through anti-oxidative stress, which may be related to the activation of the PLCγ-ERK1/2-PPARγ pathway.

Abbreviations: FFAR1, free fatty acid receptor 1; FFAs, free fatty acids; PIO, pioglitazone; PA, palmitic acid; ROS, reactive oxygen specific; PLCγ, Phospholipase C γ; ERK1/2, extracellular signal-regulated kinase 1/2; PPARγ, peroxisome proliferator activated receptor gamma; GPR40, G protein-coupled receptor 40; PDX-1, duodenal homeobox gene-1; GLUT-2, glucose transporter protein-2; MOI, multiplicity of infection; qRT-PCR, quantitative real-time reverse transcription- polymerase chain reaction; NADPH, nicotinamide adenine dinucleotide phosphate; GSIS, glucose-stimulated insulin secretion; IP3, inositol 1,4,5- triphosphate; BC, no-treatment control; NC, negative control; $FFAR1^{(+)}$, FFAR1-overexpressing transfected cells; DCFH-DA, dichloro-dihydro-fluorescein diacetate.

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1. Introduction

Long-term exposure of pancreatic β cells to high levels of free fatty acids (FFAs) can induce lipotoxicity [1], an important factor in the decline of β-cell function. FFAR1, also known as G protein-coupled receptor 40 (GPR40), is a specific receptor for long-chain FFAs that is highly expressed on the pancreatic β-cell membrane in response to FFA-mediated increases in glucose-stimulated insulin secretion [2]. However, there is no unified conclusion as to whether FFAR1 can mediate the damage of long-term lipotoxicity