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Inhibition of fatty acid translocase cluster determinant 36 (CD36), stimulated by hyperglycemia, prevents glucotoxicity in INS-1 cells

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ABSTRACT

The purpose of the present study was to determine whether exposure of pancreatic islets to glucotoxic conditions changes fatty acid translocase cluster determinant 36 (CD36) and examine the role of CD36 on the induction of glucotoxicity. We measured the changes of CD36 and insulin secretion in high glucose (30 mM) exposed INS-1 cells and CD36 suppressed INS-1 cells by transfection of CD36 siRNA. The intracellular peroxide level of INS-1 cells increased in the high glucose media compared to normal glucose (5.6 mM) media. The mRNA levels of insulin and PDX-1, as well as glucose stimulated insulin secretion (GSIS) were decreased in INS-1 cells exposed to high glucose media compared to normal glucose media, while CD36 and palmitate uptake were significantly elevated with exposure to high glucose media for 12 h. The inhibition of CD36 reversed the decreased GSIS and intracellular peroxide level in INS-1 cells. These results suggest that high glucose may exacerbate glucotoxicity via increasing fatty acid influx by elevation of CD36 expression, and that CD36 may be a possible target molecule for preventing glucotoxicity in pancreatic beta-cells.

1. Introduction

Glucotoxicity in the pancreatic beta-cell is defined as nonphysiological and potentially irreversible cellular damage induced by chronic exposure to supraphysiologic glucose concentrations, which leads to defective insulin secretion and worsening glucose regulation [1–3]. Preservation of beta-cells, through tight glycemic regulation, is an essential component of the treatment strategy for type 2 diabetes. It is well known that one of the main mechanisms of glucotoxicity is chronic oxidative stress [1]. However, tight glycemic control alone often fails to complete preservation of beta-cells in type 2 diabetic patients.

Though free fatty acids (FFA) stimulate insulin secretion, chronically elevated FFA impairs pancreatic beta cell function *in vitro* and *in vivo*, which leads to the induction of lipotoxicity [4,5]. FFAs move into cells through a passive concentration-dependent diffusion, and it has been reported that there are active transport systems to enhance FFA uptake [6]. Fatty acid translocase cluster determinant 36 (CD36), which is part of the FFA transporter system, has been identified in several tissues such as muscle, liver, and insulin-producing cells [7]. Several studies [8,9] have reported that induction of CD36 increases uptake of FFA in INS-1 cells and Caco-2/15 cells, suggesting the functional interplay between glucose and FFA in terms of insulin secretion and oxidative metabolism.

Interestingly, the lipotoxic effect of FFA on normal pancreatic beta-cells is induced only by the combined exposure to hyperglycemic conditions [10–12]. It is also reported that hyperglycemic conditions induce the expression of CD36 in intestinal epithelial cells [9]. However, we do not currently know the regulating mechanism and physiological role of CD36 on glucotoxicity in pancreatic beta-cells.

The purpose of this study was to determine whether hyperglycemia enhances the expression of CD36, and whether the accompanying FFA influx can affect pancreatic beta-cell function, and if inhibition of CD36 reverses the deteriorated beta-cell function.

2. Materials and methods

2.1. INS-1 cell culture

INS-1 cells [13] were grown in 5% CO2-95% air at 37 °C in RPMI-1640 medium (GIBCO, Grand Island, NY) containing 11.1 mM pyruvate, 10 mM HEPES, 50 M 2-mercaptoethanol, 100 U penicillin/mL and 100 g streptomycin/mL. The RPMI-1640 medium used in all

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the experiments contained the supplements described above. The cells were passaged weekly after they had been detached with trypsin–EDTA. All studies were performed using INS-1 cells that were between passages 21 and 29. The glucotoxicity was induced by 3-day treatment of high glucose (30 mM) and confirmed by impaired glucose stimulated insulin secretion (GSIS) in INS-1 cells. CD36 mRNA expression was determined in 12-h exposed INS-1 cells at normal or high glucose condition.

2.2. Evaluation of reactive oxygen species (ROS) with flow cytometry

The intracellular peroxide levels [14] were detected by flow cytometric analysis with using an oxidation-sensitive fluorescein-labeled dye, carboxylated dichlorodi-hydrofluorescein diacetate (carboxy-H2DCFDA, Molecular Probes, Carlsbad, CA, USA). Upon oxidation by intracellular ROS, the non-fluorescent dye is converted into its fluorescent form. The INS-1 cells were labeled with 100 M carboxy-H2DCFDA for 1 h at 37 °C. Following the cell loading of the dye, the cells were washed twice with PBS and then put back into culture conditions for 2 h. The INS-1 cells were then harvested, washed twice with PBS and resuspended in trypsin–EDTA (0.25% trypsin, 2 mM Na4-EDTA, Invitrogen) for 5 min at 37 °C. To disperse the cells into a single cell suspension, INS-1 cells were gently passed 20 times in and out of a 200–1000 μL tip. The cells were then washed twice with ice-cold PBS. The cells were analyzed using a 488 nm argon laser EPICS XL-MCL flow cytometer that was controlled by EXPO 32-ADC software (Beckman Coulter, Fullerton, CA). The ROS values were analyzed based on fluorescence intensity.

2.3. GSIS

Static incubation of the INS-1 rat insulinoma cell line in Krebs–Ringer buffer (KRB) (118 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.18 mmol/L KH_2PO_4, 1.18 mmol/L $MgSO_4$, 25 mmol/L $NaHCO_3$, 10 mmol/L HEPES and 0.1% BSA, pH 7.4) that contained either non-stimulatory or stimulatory concentrations of glucose (5.6 mM or 16.7 mM, respectively) was performed for 1 h [11]. The insulin levels in the KRB media collected from the static incubations from the INS-1 cells by using a 95.5 ethanol: hydrochloric acid solution were measured using a enzyme-linked immunosorbent assay (Rat Insulin ELISA kit; Mercodia, Uppsala, Sweden).

2.4. Construction of siRNA for CD36

INS-1 cells were plated at a density of 1×10^6 cells per well in a 6-well plate and subcultured for 2 days in INS-1 medium. The cells were transiently transfected with CD36 siRNA oligos in Lipofectamine™ 2000 Transfection Reagent (Invitrogen, Carlsbad, CA). The cells were incubated for 4 h, washed to remove siRNA, and then cultured in media containing the indicated glucose concentration (11.1 or 30 mM). Cells were harvested 24 h after transfection. The siRNA sequences are as follows: CD36 (siRNA#1, siRNA#2 and siRNA#3-scramble) siRNA#1, CUG AGU AGG UUU UUC UCU U (sence); AAG AGA AAA ACC UAC UCA G (antisence) and siRNA#2, GUU CUU UUC CUC UGA CAU U (sence); AAU GUC AGA GGA AAA GAA C (antisence) and siRNA#3, CAU CAA UUU CUG CAG AAC U (sence); AGU UCU GCA GAA AUU GAU G (antisence).

2.5. Real time PCR

Total RNA was obtained from the INS-1 cells by using Trizol Reagent (Bio Science Technology, Korea). cDNA was synthesized using 1 μg total RNA with oligo-(dT) primers and Prime RT Premix (GENET BIO, Korea). Real-time RT-PCR was performed in the LightCycler (Roche, Germany) as previously described. The following primers were used: for insulin, 5′-ACC CAA GTC CCG TCG TGA AGT-3′ (forward) and 5′-CCA GTT GGT AGA GGG AGC AGA TG-3′ (reverse); for PDX-1,5′-GGC TTA ACC TAA ACG CCA CA-3′ (forward) and 5′-GGG ACC GTC CAA GTT TGT AA-3′ (reverse); for CD36, 5′-GTG GCT AAA TGA GAC TGG GAC C-3′ (forward) and 5′-AGA CCA TCT CAA CCA GGC CC-3′ (reverse); for β-actin, 5′-TAC TGC

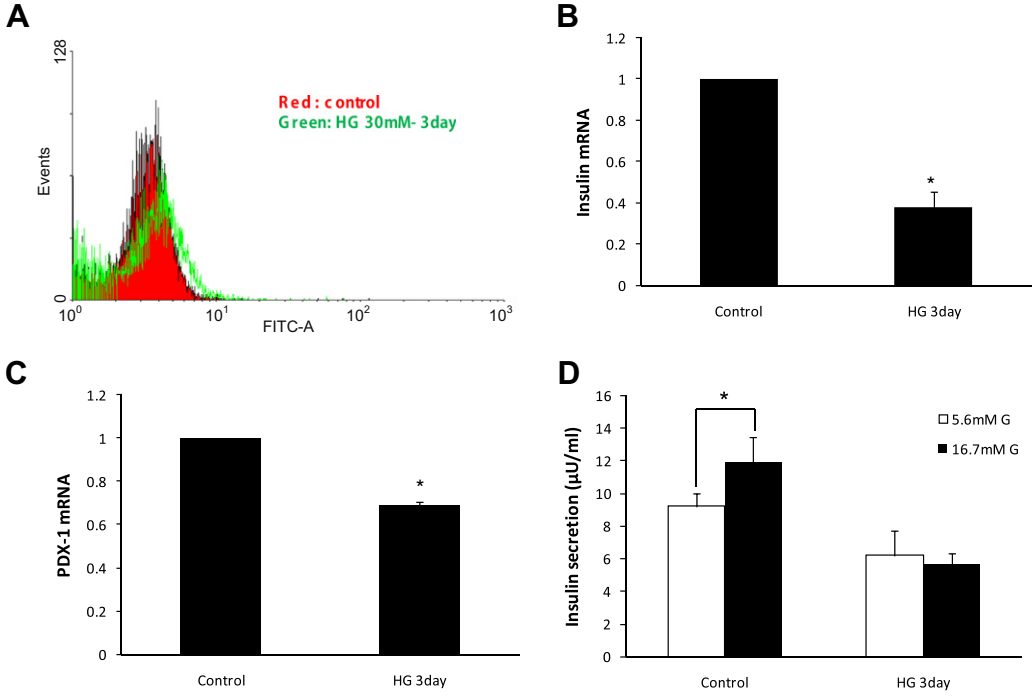

Fig. 1. Effect of 3 day-treatment with high glucose (30 mM, HG 3 day) or normal glucose (5.6 mM, Control) on intracellular peroxide level (A), mRNA expression of insulin (B) and PDX-1 (C), and glucose stimulated insulin secretion (D) in INS-1 cells. *$P < 0.05$.