Mechanism for antioxidative effects of thiazolidinediones in pancreatic β-cells

Sung Soo Chung, Min Kim, Ji Seon Lee, Byung Yong Ahn, Hye Seung Jung, Hak Mo Lee, and Kyong Soo Park

Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea

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Chung SS, Kim M, Lee JS, Ahn BY, Jung HS, Lee HM, Park KS. Mechanism for antioxidative effects of thiazolidinediones in pancreatic β-cells. Am J Physiol Endocrinol Metab 301: E912–E921, 2011. First published August 16, 2011; doi:10.1152/ajpendo.00120.2011.—Thiazolidinediones (TZDs) are synthetic ligands of peroxisome proliferator-activated receptor-γ (PPARγ), a member of the nuclear receptor superfamily. TZDs are known to increase insulin sensitivity and also to have an antioxidative effect. In this study, we tested whether TZDs protect pancreatic β-cells from oxidative stress, and we investigated the mechanism involved in this process. To generate oxidative stress in pancreatic β-cells (INS-1 and BTC3) or isolated islets, glucose oxidase was added to the media. The extracellular and intracellular reactive oxygen species (ROS) were measured to directly determine the antioxidative effect of TZDs. The phosphorylation of JNK/MAPK after oxidative stress was detected by Western blot analysis, and glucose-stimulated insulin secretion and cell viability were also measured. TZDs significantly reduced the ROS levels that were increased by glucose oxidase, and they effectively prevented β-cell dysfunction. The antioxidative effect of TZDs was abolished in the presence of a PPARγ antagonist, GW9662. Real-time PCR was used to investigate the expression levels of antioxidant genes. The expression of catalase, an antioxidant enzyme, was increased by TZDs in pancreatic β-cells, and the knockdown of catalase significantly inhibited the antioxidative effect of TZDs. These results suggest that TZDs effectively protect pancreatic β-cells from oxidative stress, and this effect is dependent largely on PPARγ. In addition, the expression of catalase is increased by TZDs, and catalase, at least in part, mediates the antioxidative effect of TZDs in pancreatic β-cells.

peroxisome proliferator-activated receptor-γ; reactive oxygen species; catalase

OXIDATIVE STRESS HAS BEEN IMPLICATED in the pathogenesis of type 2 diabetes and its vascular complications. Oxidative stress plays an important role in the development of β-cell dysfunction and insulin resistance, two major pathophysiological abnormalities of type 2 diabetes. It is well known that excessive reactive oxygen species (ROS) cause β-cell dysfunction, such as defects in insulin synthesis and secretion, and apoptosis (9, 23, 32). Pancreatic β-cells are very susceptible to oxidative stress because the expression levels of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), are relatively low in β-cells (12, 39). There are several reports showing that the overexpression of antioxidant enzymes protects pancreatic β-cells from oxidative stress-induced dysfunction (1). Exposure to a high level of glucose and/or free fatty acid (FFA) results in the dysfunction of isolated islet or β-cells, such as a decrease in insulin release and apoptosis (33, 36). An antioxidant N-acetyl-cysteine (NAC) protects β-cells from the high-glucose- or high-fat-induced impairments (37). These results suggest that ROS are involved in the glucose/FFA-induced β-cell dysfunction.

Peroxisome proliferator-activated receptor-γ (PPARγ), a member of the nuclear receptor superfamily, is expressed predominantly in adipose tissue and plays an important role in adipogenesis and lipid and glucose homeostasis. Thiazolidinediones (TZDs) such as rosiglitazone and pioglitazone are synthetic ligands of PPARγ, and they are used for diabetic treatment. TZDs increase insulin sensitivity and have anti-inflammatory effects (3). In addition, TZDs have been proposed to have antioxidant effects in vitro and in vivo, although the mechanism is not well understood (5, 6, 14, 21). In a previous study, we demonstrated that TZDs dramatically increased the expression of glutathione peroxide 3 (GPx3), and the antioxidative effect of PPARγ was mediated primarily by GPx3 in myotubes (4). There are many clinical studies reporting that TZDs preserve islet β-cell function in patients with diabetes or high risk for diabetes (7, 11, 42). In addition, TZDs improve islet β-cell function and reduce oxidative stress in islets of db/db mice (15–17). Although the expression level of PPARγ is quite low in islets, several reports have shown protective effects of TZDs against glucose- or lipid-induced toxicity in islets (27, 35, 41). These reports suggest that TZDs directly provide beneficial effects on islet β-cells, and the reduction of oxidative stress may play an important role in this process. We tested the antioxidative effect of TZDs in pancreatic β-cells and isolated islets and tried to figure out the mechanism involved in this process.

In this study, we tested whether TZDs also have antioxidative effects in the β-cell lines BTC3 and INS-1 and in isolated islets. The extracellular/intracellular H2O2 levels and activation of ROS-responsive Ser/Thr kinases were decreased by TZDs in the cells treated with glucose oxidase, which generates H2O2 using glucose as a substrate. These effects seemed to require PPARγ. TZDs also restored glucose-stimulated insulin secretion (GSIS) and cell viability impaired by ROS. In addition, catalase seemed to largely mediate the effects of TZDs in pancreatic β-cells.

MATERIALS AND METHODS

Cell culture. The pancreatic β-cell lines (BTC3 and INS-1) were cultured in RPMI 1640 (Weltgen, Daegu, South Korea) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). When cells were 60% confluent, TZDs were added for the indicated periods.

Isolation and culture of porcine islets. Islets were isolated from adult market pigs (18). In brief, the harvested pancreas was distended intraductally with University of Wisconsin (UW) solution containing 0.15% Liberase PI (Roche Biochemicals, Basel, Switzerland). The distended pancreas was digested in a modified Ricordi chamber, and islets were isolated on a continuous UW/OptiPrep density gradient (1.100 and 1.060 g/cm3) in a COBE 2991 cell separator (Gambo, Lakewood, CO) at 2,200 g for 5 min. The islets were then cultured in...
M199 (GIBCO, Grand Island, NY) supplemented with 10% pig serum.

**Induction of oxidative stress and measurement of extracellular hydrogen peroxide levels in pancreatic β-cells.** To induce oxidative stress, β-cells or isolated islets were incubated with glucose oxidase. Glucose oxidase in the media produces H$_2$O$_2$ using β-n-glucose. To measure the extracellular level of H$_2$O$_2$ after the treatment with TZDs and glucose oxidase, the media were collected (0.4 ml) and transferred to tubes containing 0.1 ml of 50% trichloroacetic acid. The tubes were then inverted several times, chilled on ice, and centrifuged (16,000 g for 5 min). The supernatant (0.2 ml) was mixed with 40 μl of 10 mM ferrous ammonium sulfate and 20 μl of 2.5 M potassium thiocyanate in a 96-well plate, and absorbance was measured at 491 nm (24). To expose βTC3 cells to a high-fat condition, cells were treated for 48 h with an intralipros fat emulsion (100 mg/dl; Fresenius Kabi, Homburg, Germany), which consists of 10% soybean oil and 1.2% egg yolk (25).

**Intracellular ROS measurement.** βTC3 or INS-1 cells were treated with glucose oxidase (75 μM/ml, 45 min) or intralipros (100 mg/dl, 48 h) and washed with PBS three times. The cells were incubated with 2',7'-dichlorohydrofluorescein diacetate (Invitrogen, Carlsbad, CA) in RPMI 1640 supplemented with 10% FBS for 15 min. Fluorescence was measured with an excitation at 485 nm and emission at 535 nm using a Victor 3 1420 multilabel counter (PerkinElmer, Boston, MA).

**Determination of GSIS.** Insulin secreted from β-cells was measured by an insulin immunoassay kit (ALPCO, Salem, NH) according to the manufacturer’s instructions. Cells were treated with TZDs for 72 h in RPMI 1640 (2.5 mM glucose) supplemented with 10% FBS, and then glucose oxidase was added to the medium for 1 h. The medium was replaced with low glucose (2.5 mM) or high glucose (16.7 mM) containing the Krebs-Ringer-HEPES buffer (119 mM NaCl, 4.74 mM KCl, 2.54 mM CaCl$_2$, 1.19 mM KH$_2$PO$_4$, 1.19 mM MgCl$_2$, 10 mM HEPES, 25 mM NaHCO$_3$, and 0.2% bovine serum albumin). After 20-min exposure of low or high concentration of glucose, the supernatants were collected for the measurement of insulin.

**Measurement of pancreatic β-cell viability.** Cells were plated on 24-well plates and treated for 72 h with troglitazone (10 μM), rosiglitazone (20 μM), or pioglitazone (20 μM), and then glucose oxidase (2 h) was added to the medium. Cell viability was measured by using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan). A cell solution (10 μl) was added to the 100 μl of culture medium. All procedures were performed according to the manufacturer’s instructions. The optical density was measured at 450 nm.

**Northern blot analysis and real-time PCR.** The cDNA fragments of mouse ATF4, CHOP, and GAPDH were labeled with [α-32P]dATP (PerkinElmer) by Klenow polymerase (Ambion, Austin, TX). Total RNAs were isolated using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The RNAs (20 μg) were used for Northern blotting. To determine the effect of TZDs on the expression level of antioxidant genes, total RNAs were prepared after βTC3 cells were treated with TZDs in 1% lipoprotein-deficient bovine serum (Biomedical Technologies, Stoughton, MA) for 48 h. Real-time PCR was performed with the SYBR green I-based Prime Q-Mastermix (PerkinElmer) by Klenow polymerase (Ambion, Austin, TX). Total RNAs were isolated using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The RNAs (20 μg) were used for Northern blotting. To determine the effect of TZDs on the expression level of antioxidant genes, total RNAs were prepared after βTC3 cells were treated with TZDs in 1% lipoprotein-deficient bovine serum (Biomedical Technologies, Stoughton, MA) for 48 h. Real-time PCR was performed with the SYBR green I-based Prime Q-Mastermix (PerkinElmer) by Klenow polymerase (Ambion, Austin, TX). Total RNAs were isolated using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The RNAs (20 μg) were used for Northern blotting. To determine the effect of TZDs on the expression level of antioxidant genes, total RNAs were prepared after βTC3 cells were treated with TZDs in 1% lipoprotein-deficient bovine serum (Biomedical Technologies, Stoughton, MA) for 48 h.

**RESULTS**

TZDs reduce H$_2$O$_2$ levels and activation of ROS-responsive kinases. To determine whether TZDs have an antioxidant effect in pancreatic β-cells, βTC3 and INS-1 cells were exposed to a high level of H$_2$O$_2$, which was generated by glucose and glucose oxidase in the media. After incubation with glucose oxidase (75 μM/ml) for 45 min in βTC3 or INS-1 cells, the extracellular H$_2$O$_2$ level was increased three- to fourfold; the H$_2$O$_2$ concentration in the medium reached ~0.3 mM (Fig. 1, A and B). When cells were pretreated with troglitazone, rosiglitazone, or pioglitazone for 72 h before treatment with glucose oxidase, the H$_2$O$_2$ level was decreased significantly (to ~50% of the induced levels). We also used isolated porcine islets, and similar effects of the TZDs were detected (Fig. 1C). In addition to extracellular H$_2$O$_2$ levels, intracellular ROS was measured. Treatment with glucose oxidase increased intracellular ROS, and TZDs effectively reduced the ROS levels close to basal levels in βTC3 and INS-1 cells and isolated islets (Fig. 1, D–F). Because ROS activates the stress-sensitive Ser/Thr kinase systems (9), phosphorylation of JNK, Erk, and p38 was determined by a Western blot analysis. Consistent with the results in Fig. 1, phosphorylation of JNK, Erk, and p38 was markedly increased by treatment with glucose oxidase, and preincubation with TZDs strongly inhibited the activation of these kinases in both β-cell lines and to a lesser extent in

**Western blot analysis.** The cell lysates were subjected to SDS-PAGE. Antibodies against phospho (p)-JNK, p-p38, p-Erk1/2, p-ElF2α, and IKKe/β were purchased from Cell Signaling Technology (Danvers, MA). An antibody against γ-tubulin was purchased from Sigma-Aldrich (St. Louis, MO). An antibody against catalase was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Immunodetection was performed by chemiluminescence (Pierce, Rockford, IL).

**Transient transfection of plasmids and reporter assays.** A reporter construct containing mouse catalase promoter (−9,054/−8911-SV40-Luc) was a generous gift from Dr. I. Shimomura (Osaka University, Osaka, Japan) (30). βTC3 cells were seeded in 12-well plates and transiently transfected with 0.3 μg of reporter vector, 0.1 μg of expression vectors for PPARγ and retinoid X receptor-α (RXRα), and 0.05 μg of pCMV-β-galactosidase using Lipofectamine and Plus reagent (Invitrogen). Cells were treated with pioglitazone (10 μM) and retinoic acid (1 μM) for 24 h and then harvested for measuring luciferase and β-galactosidase activities (Promega, Madison, WI). Luciferase activity was normalized by β-galactosidase activity.

**Ectrophoretic mobility shift assay.** Double-stranded oligonucleotides of the following sequences were used: the PPAR response element (PPRE) probe in the mouse catalase promoter (DR1–1 WT: 5'-CTCATGAAAACAAAGGTCAATTATG-3'; a PPRE was underlined) (30); a competitor of the PPRE mutant (DR1–1 MT: 5'-CTCATGAAAACAAAGGTCAATTATG-3'); and a consensus PPRE (5'-CAGGGACAGAGCAGGCACACAGGTCATTTATG-3'). The probe was labeled with [α-32P]dATP by Klenow DNA polymerase (Ambion, Austin, TX), and an aliquot of the labeled probe (25,000 counts/min) was incubated with the nuclear extracts (10 μg) of βTC3 in 10 mM HEPES (pH 7.9) containing 50 mM KCl, 0.1 mM EDTA, 0.25 mM dithiothreitol, 0.1 mg/ml poly(dIdC), 0.01% Nonidet P-40, and 10% glycerol at room temperature. competitors were added at a 50-fold molar excess with respect to the labeled probe. DNA-protein complexes were separated on 6% polyacrylamide gels and detected by autoradiography.

**Statistics.** All data were represented as means ± SE. Differences between more than two groups were determined using the one-way ANOVA (GraphPad InStat version 3.05). A P value of <0.05 was considered to be statistically significant.
porcine islets (Fig. 2). These results suggest that TZDs reduce ROS levels in pancreatic ß-cells and ß-cells.

TZDs prevent impairment of GSIS by ROS. To investigate whether ROS affects GSIS, ßTC3 cells were treated with various concentrations of glucose oxidase. As glucose oxidase concentration increased, the extracellular H₂O₂ levels also increased (Fig. 3A). Insulin secretion was increased twofold when cells were treated with a high level of glucose (Fig. 3B). However, insulin levels were decreased by elevated H₂O₂ levels in a dose-dependent manner (Fig. 3B). To examine effects of TZDs on GSIS damaged by oxidative stress, cells were incubated under relatively mild oxidative conditions (10 or 20 mU/ml gluOx) at which cell viability was not apparently affected (data not shown), but GSIS was reduced significantly

Fig. 1. Thiazolidinediones (TZDs) reduce H₂O₂ levels in pancreatic ß-cells. ßTC3 (A and D), INS-1 (B and E), or isolated porcine pancreatic islets (C and F) were treated with troglitazone (tro; 10 μM), rosiglitazone (rosi; 20 μM), or pioglitazone (pio; 20 μM) for 72 h and with 75 mU/ml glucose oxidase (gluOx) for 45 min. The amount of H₂O₂ in the media (A–C) and intracellular reactive oxygen species (ROS; D and E) were measured. The value obtained from the cells treated with gluOx but not with TZDs was set to 100, and the other values were expressed as relative to that. The graph represents the mean ± SE of 5 independent experiments. *P < 0.05 vs. the value of cells treated with gluOx.

Fig. 2. TZDs reduce ROS-induced serine/threonine kinase activation. Cells (ßTC3 or INS-1) or porcine pancreatic islets were treated with tro, rosi, or pio for 72 h, followed by treatment of gluOx (75 mU/ml) for 45 min, and the cellular proteins were subjected to SDS-PAGE and Western blot analysis.
TZDs partially prevent oxidative stress-induced cell death and endoplasmic reticulum stress. As the concentration of glucose oxidase increased, βTC3 cell viability was significantly decreased (Fig. 4A). However, pretreatment with TZDs partially rescued the cell viability (Fig. 4A). In addition to the effect on GSIS and cell viability, oxidative stress has been known to induce endoplasmic reticulum (ER) stress (26, 40). To test whether TZDs also affect oxidative stress-induced ER stress, βTC3 cells were treated with glucose oxidase in the presence or absence of TZDs, and then the PERK (double-stranded RNA-activated protein kinase-like ER kinase) signaling was monitored. Oxidative stress remarkably increased the phosphorylation of eIF2α (eukaryotic translation initiation factor-2α subunit), a target of PERK, and increased the expression of ATF4 (bZIP-containing activating transcription factor 4) and CCAAT/enhancer-binding protein homologous protein (CHOP; Fig. 4B). However, the induction of ER stress by oxidative stress was hardly detected when cells were pretreated with troglitazone or rosiglitazone. Taken together with the above data, TZDs inhibit various events induced by oxidative stress, such as GSIS malfunction, cell death, and ER stress, in pancreatic β-cells. Therefore, these results suggest that TZDs

Fig. 3. TZDs ameliorate ROS-induced glucose-stimulated insulin secretion (GSIS) impairments. A: βTC3 cells were treated with the indicated concentrations of gluOx for 1 h, and extracellular H2O2 was measured. The graph bar shows the mean ± SE of 4 independent experiments. *P < 0.01 vs. the value of cells treated with high glucose but not with gluOx. B: after the incubation with glucose oxidase for 1 h, the media were replaced by low (2.5 mM) or high (16.7 mM) glucose-media for 20 min, and then secreted insulin levels were measured. Total amount of insulin in the media was normalized to the protein level of the cells. The value of the cells treated with high glucose without gluOx (1.2–1.6 ng/µg) was set to 100, and the other values were presented as relative to that (n = 4). *P < 0.01 vs. the value of cells treated with high glucose but not with gluOx. C: cells were treated with tro (10 µM), rosi (20 µM), or pio (20 µM) for 72 h, and then gluOx was added for 1 h. The value of cells treated with high glucose but not with gluOx was set to 100, and the other values were presented as relative to that (n = 4). *P < 0.05 vs. the value of high-glucose-treated cells. **P < 0.05 vs. the corresponding value of gluOx-treated cells not treated with TZDs.

Fig. 4. TZDs reduce ROS-induced apoptosis and ER stress. A: βTC3 cells were treated with TZDs for 72 h, and then the indicated concentration of gluOx was added for 2 h. Cell viability was measured as described in MATERIALS AND METHODS (n = 5). *P < 0.01 vs. the value of control cells treated with neither gluOx nor TZDs. **P < 0.05 vs. the value of corresponding control cells (30 mU/ml gluOx) not treated with TZDs. †P < 0.01 vs. the value of corresponding control cells (50 mU/ml gluOx) not treated with TZDs. B: after the treatment of TZDs and gluOx, cells were harvested, and Western blot or Northern blot analysis was performed.
Western blot analysis to detect MAPK activation.

**PPARγ is required for the antioxidant effect of TZDs.** To determine whether the antioxidant effect of TZDs in β-cells is mediated by PPARγ activation, GW-9662, a specific antagonist of PPARγ, was added to the cells in the presence of TZDs. After treatment with GW-9662, the basal extracellular level of H2O2 was increased slightly, and H2O2 level remained high in the presence of TZDs (Fig. 5A). In addition, activation of JNK, Erk, or p38 was barely inhibited by TZDs in the presence of GW-9662 (Fig. 5B). These results suggest strongly that the antioxidant effects of TZDs are mediated by PPARγ.

TZDs ameliorate oxidative stress induced by high levels of lipids. High levels of lipids are known to be one of the major ROS-inducing factors in pancreatic β-cells (28). Therefore, we tested whether TZDs protect cells from lipid-induced oxidative stress. When βTC3 cells were incubated with intralipos (a fat emulsion) for 48 h, the intracellular ROS was increased substantially, and cell viability was decreased. However, when cells were pretreated with TZDs, ROS was only slightly increased by intralipos, and the effect of TZDs was comparable with that of NAC, a representative antioxidant (Fig. 6A). Similarly, cell viability was restored by TZDs in the presence of intralipos (Fig. 6B). Furthermore, the activation of ROS-responsive kinase signaling and ER stress signaling by intralipos was inhibited when cells were pretreated with TZDs (Fig. 6C). These results consistently reveal an antioxidative function of TZDs. To test whether TZDs restore the GSIS impaired by high levels of lipids, GSIS was measured after the incubation with intralipos in the absence or presence of TZDs. GSIS was reduced significantly after the treatment of intralipos; however, in the presence of TZDs GSIS was almost recovered to the level of the control cells (Fig. 6D). This result demonstrates clearly that TZDs preserve β-cell function in high-lipid conditions.

**PPARγ increases catalase expression by directly binding to a PPRE in the catalase promoter.** Our previous study showed that expression of GPx3 was greatly increased by TZDs, and GPx3 mediates mainly the antioxidative effect of TZDs in myotubes (4). Therefore, we tested whether GPx3 expression is also regulated by TZDs in pancreatic β-cells. Contrary to our expectations, expression of GPx3 was only slightly affected by the treatment of βTC3 cells with TZDs (Fig. 7A). Interestingly, the mRNA and protein levels of catalase were increased significantly by TZDs (Fig. 7, A and B). The expression of other antioxidant genes, such as peroxiredoxins and glutathione peroxidases, was only slightly affected by TZDs (data not shown). In the case of myotubes and adipocytes, expression of catalase was increased by TZDs (4, 30), and PPARγ directly increased transcription of catalase in adipocytes by binding to functional PPREs in the mouse catalase promoter (30). To test whether PPARγ regulates catalase expression through the PPREs in pancreatic β-cells, luciferase reporter assays were performed in βTC3 cells using a construct (−9,054/−8,911-SV-Luc) containing the PPREs. Luciferase activity was increased significantly by activation of PPARγ (Fig. 7C). In addition, eletrophoretic mobility shift assay was performed with βTC3 nuclear extracts, and specific binding was detected with one of the PPREs (DR1-1; Fig. 7D). These results suggest that PPARγ positively regulates transcription of catalase by directly binding to the catalase promoter in pancreatic β-cells as well as in adipocytes.

**Catalase plays an important role in the antioxidative effect of TZDs in pancreatic β-cells.** To determine whether catalase mediates the antioxidant effect of TZDs in βTC3 cells, the expression of catalase was knocked down by siRNAs against catalase. Expression of catalase was reduced to ~40% of the control by siRNA as being confirmed in its mRNA and protein levels (Fig. 8, A and B). When catalase expression was knocked down, glucose oxidase-induced H2O2 levels remained high in the presence of TZDs, although the antioxidant effect of TZDs was not completely abolished (Fig. 8C, left). In contrast, knockdown of GPx3 (Fig. 8A) did not affect the antioxidative function of TZDs (Fig. 8C, right). In addition, the protective effect of pioglitazone on the stress-induced signaling pathway almost disappeared when catalase was knocked down by specific siRNAs (Fig. 8D). Similarly, insulin secretion was not recovered by pioglitazone after the knockdown of catalase (Fig. 8E). These results suggest that catalase is involved primarily in the antioxidant effect of TZDs in pancreatic β-cells.

**DISCUSSION**

In this study, H2O2, generated by glucose oxidase and glucose in the media, increased intracellular ROS, activated...
JNK/MAPK signaling, inhibited glucose-stimulated insulin secretion, and decreased cell viability in pancreatic β-cells. A relatively low level of H$_2$O$_2$ is sufficient to induce β-cell dysfunction compared with the higher H$_2$O$_2$ levels required to induce malfunction in myotubes in the previous study (4), which supports the common opinion that pancreatic β-cells are more sensitive to ROS than other cell types. Viability of pancreatic β-cells was decreased as concentration of glucose oxidase and the incubation period were increased; the treatment of 30 mU/ml glucose oxidase for 2 h significantly reduced cell viability (Fig. 4A), but the treatment of 75 mU/ml glucose oxidase for 45 min did not induce an apparent cell death. TZDs effectively alleviated the glucose oxidase-induced oxidative stress by reducing ROS levels in β-cells. These results suggest that TZDs regulate oxidative stress in several tissues where PPARγ is expressed because the antioxidant effect of TZDs depends largely on PPARγ.

Although TZDs have similar antioxidant effects in pancreatic β-cells and myotubes, the mechanisms by which TZDs show these effects seem to be distinct. Although GPx3, whose expression is increased dramatically by TZDs in myotubes, primarily reduces ROS levels and restores the metabolic function of myotubes, its expression is only slightly increased by TZDs in pancreatic β-cells, and GPx3 does not seem to mediate the antioxidative effect of TZDs (Figs. 7A and 8C). Instead, the expression of catalase is increased significantly by TZDs in β-cells, and catalase may play an important role in reducing the ROS level (Figs. 7 and 8). However, considering the observation that treatment of TZDs still decreased ROS levels (albeit less effectively) after knockdown of catalase (Fig. 8C), it is possible that other oxidant enzymes may also mediate the effect of TZDs in addition to catalase. Taken together, it is quite interesting that TZDs reduce ROS levels by inducing different antioxidant enzymes in a cell type-specific manner.

Pancreatic β-cells contain a highly developed ER, which is responsible for the processing and export of newly synthesized insulin (13), and the activation of the ER stress responses is closely related to β-cell dysfunction in diabetes (20). It has been suggested that ER stress and ROS are closely linked, although the mechanisms involved in the relationship between them have not been well investigated. Thus we tested the effect of ROS on ER stress by monitoring the activation of the PERK-eIF2α signaling pathway. The results showed that increased ROS induced ER stress responses and that treatment of TZDs could protect β-cells from ROS-induced ER stress (Fig. 4B). PERK-eIF2α signaling occurs in pancreatic β-cells in response to ER stress,
and interestingly, it is known that the PERK-eIF2α pathway reduces oxidative stress (26). It will be worthwhile to examine whether TZDs also affect the ER stress responses directly rather than indirectly by reducing ROS. Recently, a report demonstrated that TZDs may directly reduce ER stress by inhibiting CHOP expression (8), and further study is required to answer the question.

It is well known that hyperglycemia impairs insulin secretion, induces apoptosis, and decreases the expression of important genes for β-cell function, including insulin, GLUT2, and pancreatic duodenal homeobox-1 (10, 19, 34). In addition to hyperglycemia, high levels of lipids also induce β-cell dysfunction (2). Treatment with an antioxidant such as NAC or overexpression of antioxidant enzymes prevents β-cell dysfunction that is induced by high levels of glucose and/or lipids (37, 38). Therefore, oxidative stress may be the central mechanism mediating glucotoxicity or lipotoxicity in pancreatic β-cells (31). Interestingly, TZDs also inhibit β-cell dysfunction induced by high levels of glucose or lipids (35, 41, 43). In this study, we found that
high levels of lipids resulted in the increase in intracellular ROS, and TZDs protected β-cells from lipotoxicity by inhibiting the induction of ROS as effectively as NAC (Fig. 6A). Therefore, our study strongly suggests that the antioxidative function of TZDs is very important in protecting pancreatic β-cells from lipotoxicity.

As mentioned above, pancreatic β-cells have a very low level of antioxidant enzymes, and therefore, they are vulnerable to oxidative stress. During islet transplantation in particular, isolated islets are exposed to high levels of ROS generated by the hypoxic conditions, which is at least one factor inducing apoptosis and necrosis (22). It has been reported that overexpression of a combination of antioxidant enzymes improves islet grafting (29). In addition, there are many clinical data to demonstrate beneficial effects of TZDs on pancreatic β-cell function (7, 11, 42). Considering these clinical reports and TZD’s antioxidant and anti-inflammatory effects, TZDs may be useful in protecting islets during transplantation.

In conclusion, TZDs effectively protect pancreatic β-cells from oxidative stress, and catalase, the expression of which is increased significantly by TZDs, may at least in part mediate the antioxidative effects of TZDs in pancreatic β-cells.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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