Dexamethasone induces pancreatic β-cell apoptosis through upregulation of TRAIL death receptor

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Abstract

Long-term medication with dexamethasone – a synthetic glucocorticoid (GC) drug – results in hyperglycemia, or steroid-induced diabetes. Although recent studies revealed that dexamethasone directly induces pancreatic β-cell apoptosis, its molecular mechanisms remain unclear. In our initial analysis of mRNA transcripts, we discovered the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway may be involved in dexamethasone-induced pancreatic β-cell apoptosis. In the present study, a mechanism of dexamethasone-induced pancreatic β-cell apoptosis through the TRAIL pathway was investigated in cultured cells and isolated mouse islets. INS-1 cells were cultured with and without dexamethasone in the presence or absence of a glucocorticoid receptor (GR) inhibitor, RU486. We found that dexamethasone induced pancreatic β-cell apoptosis in association with the upregulation of TNSF10 (TRAIL) mRNA and protein expression. Moreover, dexamethasone upregulated the TRAIL death receptor (DR5) protein but suppressed the decoy receptor (DcR1) protein. Similar findings were observed in mouse isolated islets: dexamethasone increased TRAIL and DR5 compared to that of control mice. Furthermore, dexamethasone stimulated pro-apoptotic signaling including superoxide production, caspase-8, -9, and -3 activities, NF-κB, and Bax but repressed the anti-apoptotic protein, Bcl-2. All these effects were inhibited by the GR-inhibitor, RU486. Furthermore, knock-down DR5 decreased dexamethasone-induced caspase 3 activity. Caspase-8 and caspase-9 inhibitors protected pancreatic β-cells from dexamethasone-induced apoptosis. Taken together, dexamethasone induced pancreatic β-cell apoptosis by binding to the GR and inducing DR5 and TRAIL pathway.

Key Words

- dexamethasone
- pancreatic β-cells
- TRAIL
- apoptosis
- glucocorticoid receptor

Introduction

Glucocorticoids (GCs) are a group of steroid drugs that exert strong anti-inflammatory and immunosuppressive effects. The synthetic GCs include dexamethasone and prednisolone, which are currently in clinical use for the treatment of several diseases (Clore & Thurby-Hay 2009). Long-term medication with GCs can cause hyperglycemia with or without diabetes (Clore & Thurby-Hay 2009). The time and dosage of GC medication are associated with
an increased risk of new-onset steroid-induced diabetes (NOSID) (Donihi et al. 2006, Fong & Cheung 2013). The pathophysiology of NOSID is still unclear (Di Dalmazi et al. 2012), although it is well known that GCs can induce insulin resistance by increasing hepatic glucose production (Dube et al. 2015) and inhibiting glucose uptake by muscles (Ruzzin et al. 2005). The major effect of GCs on increased hepatic glucose production is the induction of gluconeogenesis (Rizza et al. 1982).

The direct effect of GCs on pancreatic β-cells has been widely investigated (Reich et al. 2012, Guo et al. 2016). Their effects on insulin secretion from pancreatic β-cells depend on the time of exposure (Beaudry & Riddell 2012). The acute effects of GCs have also been associated with decreased insulin secretion both in the pancreatic β-cell lines and mouse pancreatic islets (Hult et al. 2009). GCs reduce insulin secretion through several mechanisms, including decreased glucose uptake and oxidation, membrane depolarization, and calcium-induced insulin exocytosis (Wang et al. 2010, Fransson et al. 2013). On the other hand, the chronic effects of GCs cause a defect of insulin biosynthesis and induce pancreatic β-cell death through endoplasmic reticulum (ER) stress (Lissens et al. 2011). GCs can also induce a defect in pancreatic β-cells through the glucocorticoid receptor (GR). This observation has been supported by evidence in mice, in which diabetes developed after the specific overexpression of the GR in pancreatic β-cells (Davani et al. 2004). In addition, a hyperglycemia clamp study in humans demonstrated that single nucleotide polymorphisms of the GR gene that increases GR sensitivity were associated with a reduction in insulin secretion (van Raalte et al. 2012). However, the molecular mechanisms through which GCs directly induced pancreatic β-cell apoptosis have not yet been well established.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF-ligand family. It is regulated by cytokine, injury, and several transcription factors, including SP-1, NFAT, STAT1, and NF-κB (Rivera-Walsh et al. 2001, Allen & El-Deiry 2012). The physiological role of TRAIL is still unclear (Schafer et al. 2007). The TRAIL protein consists of three domains: cytoplasmic, transmembrane, and extracellular. It is cleaved by aspartic proteinase cathepsin E and matrix-metalloproteinase-2 (MMP-2) to produce a soluble form of TRAIL (Kawakubo et al. 2007, Secchiero et al. 2010). TRAIL is well known to induce apoptosis in a variety of cancer cells (Sadarangani et al. 2007); this induction occurs through the binding of TRAIL to the death receptor (DR) (Pan et al. 1997). Humans and chimpanzees have both DR4 and DR5, whereas mice and other vertebrates have only DR5 (van Roosmalen et al. 2014). TRAIL also binds to its decoy receptors (DcR1, DcR2, and OPG) (Marsters et al. 1997, Emery et al. 1998, Merino et al. 2006). These receptors compete with DR in binding to TRAIL and inhibit TRAIL-induced apoptosis (Merino et al. 2006). Previous studies have shown that TRAIL plays a role in the development of type 1 and 2 diabetes (Cheung et al. 2005, Chang et al. 2011). However, it is unknown whether TRAIL plays a role in dexamethasone-induced pancreatic β-cell apoptosis. Thus, the present study aimed to investigate this aspect.

**Materials and methods**

**Animal studies**

**Animals**

Male ICR outbred 8–12 weeks old mice were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. The animal experimentation protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, Siriraj Hospital, Mahidol University (Approval No: SI-ACUP 001/2559). Male, 8-week-old, ICR mice were acclimatized for 1 week and then randomly assigned to either a treatment or control group, each with a group size of 5–6 mice. The mice in both groups were maintained in a 12-h light:12-h darkness cycle environment at 25°C ± 2°C and 60% humidity. They were housed 5–6 per cage with a wooden chip bedding and were provided chow pellet *ad libitum* (Perfect Companion Group Co., Ltd., Bangkok, Thailand).

**INS-1 cell culture**

INS-1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in humidified air containing 5% CO₂. The culture medium was changed every 2 days. The cells were cultured in the medium either with or without 0.1 μM dexamethasone and in the presence or absence of 1 μM RU486.

**Mouse pancreatic islet isolation and culture**

Pancreatic islets were isolated by collagenase digestion using the modified method of Lacy & and Kostianovsky and Gotoh (Lacy & Kostianovsky 1967, Gotoh et al. 1985). Briefly, pancreases were infused with collagenase-P and digested at 37°C. The islets were separated by using...
a histopaque gradient and manually picked under a stereomicroscope. Isolated islets were cultured in an RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in 5% CO₂. The culture medium was changed every 2 days. All methods were carried out in accordance with ACUC guidelines. The numbers of 300–500 islets were cultured with or without 0.1 µM dexamethasone for 7 days.

**Measurement of intracellular superoxide generation**

Superoxide production was detected using a nitroblue tetrazolium (NBT) assay. Briefly, INS-1 cells were treated with 0.1 µM dexamethasone in the presence or absence of 1 µM RU486 for 48 h. After incubation, the cells were again incubated with NBT for 90 min. The cells were then lysed in potassium hydroxide (KOH). The released insoluble formazan was dissolved in DMSO. The amount of superoxide production was measured as optical density (OD) at a wavelength of 630 nm by a PowerWave microplate scanning spectrophotometer (BioTek Instruments Inc.).

**Analysis of cell apoptosis by annexin V-FITC/PI staining**

Briefly, INS-1 cells were treated with 0.1 µM dexamethasone in the presence or absence of 1 µM RU486. Dexamethasone and RU486 doses were used as those previously reported (Reich et al. 2012). After incubation for 48 h, the cells were collected and stained with propidium iodine (PI), and cell apoptosis was detected with an FITC Annexin V Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA). The apoptotic cells were then analyzed by a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA).

**Analysis of cell viability by prestoblue assay**

INS-1 was treated with 0.1 µM dexamethasone in the presence or absence of caspase-8 (Z-IETD-FMK) and caspase-9 (Z-LEHD-FMK) (Abcam) at a concentration of 10 µmol/L each and then incubated for 72 h. After incubation, 10 µL of prestoblue® reagent (Invitrogen) was added to each well. Plates were then incubated for 30 min at 37°C after which cell viability was determined by measuring the absorbance at 570/595 nm in a microtiter plate reader (BioTek Instruments, Inc.). Cell viabilities were plotted with GraphPad Prism 5 (GraphPad Software Inc.).

**Caspase-3 activity assay**

A caspase-3 assay was performed in accordance with the manufacturer’s protocol (Promega). Briefly, INS-1 cells were seeded into a 96-well plate and allowed to attach overnight. At 72 h after treatment, 100 µL of caspase-Glo reagent was added to each well and gently mixed on a plate shaker for 30 s. The plates were then incubated at 37°C for 30 min in the dark. A substrate for luciferase (aminoluciferin) was released after caspase-3 enzyme cleavage. The luminescence was measured by a plate-reading luminometer (Synergy H1 Hybrid Multi-Mode Microplate Reader, Bio-Tek). The change in luminescence signal is directly proportional to caspase-3 activity.

**Caspase-8 activity assay**

Caspase-8 assay was performed by following the manufacturer’s protocol (Promega). Briefly, INS-1 cells were seeded into a 96-well plate and allowed to attach overnight. At 48 h after treatment, 100 µL of caspase-Glo reagent was added to each well and gently mixed on a plate shaker for 30 s. The plates were then incubated at 37°C for 30 min in the dark. A substrate for luciferase (aminoluciferin) was released after caspase-8 enzyme cleavage. The luminescence was measured by a plate-reading luminometer (Synergy H1 Hybrid Multi-Mode Microplate Reader, Bio-Tek). The change in luminescence signal is directly proportional to caspase-8 activity.

**RNA isolation and real-time RT-polymerase chain reaction**

The total RNA of INS-1 cells was extracted with a High Pure RNA Isolation Kit (Roche Diagnostic Corporation), following the manufacturer’s instructions. The RNA concentration was measured by a Nano Drop (ND)-1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). RT was carried out using 1 µg of RNA and a reagent of a SuperScript III RT kit with random hexamer primer (Invitrogen), according to the manufacturer’s instructions. A quantitative real-time PCR was then performed using a SYBR Green reaction mix and a Roche LightCycler 480 instrument (Roche Diagnostics). The specific primer sequences for rat TRAIL were forward primer: 5’ TGAATGAGACTCCAGAAATAGC 3’ and reverse primer: 5’ CCAGTTGACTCATATGACTA 3’; for rat β-actin were forward primer: 5’ ATGAAGTGACTGGTACA 3’ and reverse primer: 5’ CCTGAAAGTATTTGCCTGACGATG 3’. The threshold cycles (Ct) of TRAIL and β-actin genes were measured, and the difference between their ΔCt was
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**Western blot analysis**

INS-1 cells and mouse pancreases were lysed in an RIPA buffer (Pierce Biotechnology) and quantified for total protein by a Micro BCA Protein Assay Kit (Pierce). The total proteins were separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Bio-Rad). After that, the membrane was blocked with 5% skimmed milk. The membrane was incubated overnight at 4°C with one of the following primary antibodies: mouse monoclonal anti-TRAIL, goat polyclonal anti-DR5, goat polyclonal anti-DcR1, mouse monoclonal anti-Bax, rabbit polyclonal anti-Bcl-2, mouse monoclonal anti-NF-\( \kappa \)-B, goat polyclonal anti-\( \alpha \)-tubulin, mouse monoclonal anti-\( \beta \)-actin (all of the preceding from Santa Cruz Biotechnology), mouse monoclonal anti-cleaved caspase-9 (Cell Signaling) or mouse monoclonal anti-GAPDH (Cell Signaling). The membranes were then washed and incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology). Protein bands were detected by enhanced chemiluminescence (Pierce). The band intensities of the proteins were analyzed using ImageJ densitometry software (version 1.43; National Institute of Health, Bethesda, MD, USA).

**Secretory protein collection**

Soluble TRAIL in cultured media was collected using a Vivaspin spin column containing an ultrafiltration membrane (GE Healthcare). Briefly, INS-1 cells were treated with 0.1 \( \mu M \) dexamethasone in the presence or absence of 1 \( \mu M \) RU486. At 48 h after treatment, the cultured media were transferred to a 6 mL membrane ultrafiltration tube (5 kDa MWCO) and centrifuged for 20 min. After centrifugation, the solution in the filtrate container was discarded. The concentrated protein sample in the concentrate pocket was collected with a pipette. The sample’s concentration was measured using a Micro BCA Protein Assay Kit (Pierce).

**TNFRSF 10B (DR5) knockdown**

Transfection of siRNA directed against TNFRSF 10B (DR5) (Dharmacon) was performed using Lipofectamine 2000 (Invitrogen) as detailed by the manufacturer. INS-1 cells were seeded into a six-well plate for 24 h before transfection. The double-stranded siRNA was transfected into the cells. After 6 h, the medium was changed to be a complete culture medium. As a control, INS-1 cells were treated with siRNA-control (Dharmacon) under identical conditions. At 24 h after siRNA transfection, the INS-1 cells were treated with dexamethasone for 72 h. Then, the cells were harvested for further examination by Western analysis.

**Statistical analysis**

Data were expressed as mean ± S.E.M.. The differences between the groups of results were determined by one-way ANOVA, followed by Tukey’s post hoc test. A P-value < 0.05 was considered statistically significant.

**Results**

**Dexamethasone induced pancreatic \( \beta \)-cell apoptosis through glucocorticoid receptor**

To examine whether dexamethasone induces pancreatic \( \beta \)-cell apoptosis via the GR, a GR inhibitor – RU486 – was added to INS-1 cells cultured with or without dexamethasone. The results showed that dexamethasone significantly increased pancreatic \( \beta \)-cell apoptosis when compared to control. RU486 with dexamethasone significantly reduced cell apoptosis to be similar to that of the control condition. RU486 alone did not alter cell apoptosis compared to that of the control condition (Fig. 1A). These results suggested that the dexamethasone induced pancreatic \( \beta \)-cell apoptosis via the GR.

**Dexamethasone regulated TRAIL production and secretion via the GR**

To verify that dexamethasone induces TRAIL expression in pancreatic \( \beta \)-cells, INS-1 cells were treated with 0.1 \( \mu M \) dexamethasone in the presence or absence of RU486. TNSF10 (TRAIL) mRNA and protein were determined by real-time PCR and Western blot analysis, respectively. Dexamethasone induced TNSF10 (TRAIL) mRNA and protein expression compared to the control condition (Fig. 1B and C). Addition of RU486 cultured with dexamethasone significantly decreased TNSF10 (TRAIL) mRNA and protein expression when compared to those cultured with dexamethasone alone (Fig. 1B and C). RU486 alone did not have any effect on TNSF10 (TRAIL) mRNA and protein expression. These results indicated that the dexamethasone induced TNSF10 (TRAIL) mRNA and protein expression via the GR.

To determine whether dexamethasone stimulates pancreatic \( \beta \)-cells to secrete TRAIL, the TRAIL protein in culture media was measured by Western blot analysis.
Dexamethasone significantly increased secretory TRAIL protein compared to that of the control condition. The induction of secretory TRAIL protein was blocked by RU486, while RU486 alone did not change the level of secretory TRAIL protein compared with control condition (Fig. 2A). These results indicated that the dexamethasone induced secretory TRAIL protein via the GR.

**Dexamethasone upregulated TRAIL death receptor but suppressed TRAIL decoy receptor**

To determine the effects of dexamethasone on TRAIL receptors, TRAIL receptors were examined by Western blot analysis. Dexamethasone significantly induced TRAIL death receptor (DR5) protein expression but reduced TRAIL decoy receptor (DcR1) protein expression in INS-1 pancreatic β-cell when compared to those in control conditions (Fig. 2B and C). RU486 diminished the effects of dexamethasone on DR5 and DcR1 expression, whereas RU486 alone did not change DR5 and DcR1 expressions compared to control conditions. These results indicated that the dexamethasone induced DR5 and suppressed DcR1 expression via the GR.

**DR5 knockdown decreased dexamethasone-induced cleaved caspase 3**

To confirm that dexamethasone increased pancreatic β-cell apoptosis via TRAIL and DR5, DR5 knockdown...
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Dexamethasone increases NF-κB activation and TRAIL protein expression

To determine whether dexamethasone induces NF-κB activation, phosphorylated NF-κB p65, and total NF-κB p65 proteins were determined from experimental conditions by Western blot analysis. Dexamethasone significantly increased phosphorylated NF-κB p65 when compared with control. Co-incubated RU486 with dexamethasone brought phosphorylated NF-κB p65 back to the control level. RU486 alone did not have any effect on phosphorylated NF-κB p65 when compared to control (Fig. 3B). These results suggested that dexamethasone increased NF-κB activation.

Dexamethasone upregulated TRAIL and DR5 protein expression in mouse pancreas

To examine the effects of dexamethasone on TRAIL and DR5 expressions in mouse pancreas, TRAIL and DR5 expressions in dexamethasone-injected mouse pancreases were determined by Western blot analyses. Similar to the results of the studies in INS-1 pancreatic β-cell, dexamethasone significantly increased TRAIL and DR5 protein expressions in mouse pancreata compared to pancreata from the control mice (Fig. 4A and B). These data indicated that the dexamethasone induced TRAIL and DR5 expressions in vivo.

Dexamethasone increased oxidative stress in pancreatic β-cells

To determine whether dexamethasone induces pancreatic β-cell apoptosis by increasing ROS, intracellular superoxide production was determined by an NBT assay. Dexamethasone significantly augmented superoxide production compared to that in the control condition. RU486 co-treatment with dexamethasone reduced superoxide production when compared with the control condition, but RU486 alone did not have any effect on the superoxide level (Fig. 4C). This result indicated that dexamethasone increased the oxidative stress in the pancreatic β-cells through the GR.

Dexamethasone triggered the extrinsic apoptotic pathway in pancreatic β-cells

Our previous results demonstrated that dexamethasone induced secretory TRAIL and DR5. To confirm whether
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TRAIL induces pancreatic β-cell apoptosis through the extrinsic pathway, caspase-8 and caspase-3 activities were measured by luminescence assay. Dexamethasone significantly amplified caspase-8 and caspase-3 activities compared to control. Addition of RU486 to dexamethasone significantly reduced caspase-8 and caspase-3 activities when compared to dexamethasone alone (Fig. 5A and B). RU486 alone did not have any effect on caspase-8 and caspase-3 activities compared to those of the control conditions.

**Dexamethasone activated mitochondrial (intrinsic) apoptotic pathway**

To examine whether apoptosis was mediated by mitochondrial (intrinsic) apoptotic pathway, caspase-9 protein expression was measured by Western blot analysis. Dexamethasone significantly increased cleaved caspase-9 and co-treatment RU486 with dexamethasone significantly decreased cleaved caspase-9 (Fig. 5C). This study further evaluated mitochondrial apoptotic pathway, a pro-apoptotic protein – Bax– and an anti-apoptotic protein – Bcl-2 – were determined by Western blot analysis. Dexamethasone increased Bax protein expression compared to the control condition. Addition of RU486 treated cells with dexamethasone reduced Bax when compared to dexamethasone alone (Fig. 5C). On the contrary, dexamethasone significantly decreased Bcl-2 protein expression compared to the control condition, and this effect was reversed by the addition of RU486 with dexamethasone. RU486 alone did not change the Bax and Bcl-2 protein expressions (Fig. 6A and B). Together, these results indicated that dexamethasone-induced pancreatic β-cell apoptosis is mediated through the mitochondrial apoptotic pathway.
Caspases 8 and 9 inhibitors decreased dexamethasone-induced pancreatic β-cell apoptosis

To further examine the roles of caspases 8 and 9 in dexamethasone-induced pancreatic β-cell apoptosis, caspase 8 and 9 inhibitors, Z-IETD-FMK and Z-LEHD-FMK, respectively (at 10 μM each), were used in the inhibition experiments. As shown in Fig. 6C, Z-IETD-FMK or Z-LEHD-FMK significantly inhibited the effect of dexamethasone on cell viability. Z-IETD-FMK or Z-LEHD-FMK alone had no effect on cell viability when compared to that of the control condition (Fig. 6C). These findings indicated that dexamethasone induced apoptosis of INS-1 cells via both mitochondria-dependent and death receptor-mediated pathways.

Discussion

In the present study, we demonstrated that dexamethasone increased pancreatic β-cell apoptosis and that this effect could be inhibited by the GR inhibitor, RU486. This result is in accordance with previous studies which reported that dexamethasone increased cell apoptosis and decreased cell viability in INS-1 cells and mouse islets (Ranta et al. 2006, Guo et al. 2016). Additionally, dexamethasone has been found to induce osteoblastic cell apoptosis (Liu et al. 2011).
The GR inhibitor has been reported to decrease cell apoptosis in MIN6 cells, INS-1 cells, and mouse islets treated with dexamethasone (Ranta et al. 2006, Reich et al. 2012). This finding was also observed in other cells in which the pre-treatment of the GR inhibitor before a dexamethasone injection decreased neuronal cell apoptosis in neonatal rats (Sze et al. 2013). All this evidence indicates that dexamethasone induces pancreatic β-cell apoptosis through the GR.

It has been shown that glucocorticoids modulate through many pathways to induce pancreatic β-cell apoptosis. However, in the current study, we identified a novel apoptotic pathway in that dexamethasone increased pancreatic β-cell apoptosis, which is the TRAIL pathway. Our initial analysis of mRNA transcripts by an RT-PCR array demonstrated that dexamethasone markedly up-regulated the TRAIL pathway, including TRAIL, DR5, caspase-8, caspase-3, Bax, and NF-κB (unpublished data). The results of our subsequent investigations confirmed that dexamethasone significantly increased TRAIL production and secretion, and this effect was inhibited by the GR inhibitor. These results are consistent with those of previous studies in which TRAIL induced apoptosis in a variety of cancer cells, including human colorectal, bladder, and pancreatic cancer cells (Jalving et al. 2006, Szliszka et al. 2009, Stadel et al. 2010). TRAIL can also induce apoptosis of normal hepatic and prostatic epithelial cells (Jo et al. 2000, Nesterov et al. 2002). However, TRAIL has been shown to promote the survival of human spermatozoa and enhance human renal epithelial cell proliferation (Nguyen et al. 2009, Giorgio Zauli 2014). Moreover, it has been demonstrated that a supplement of TRAIL alone does not increase apoptosis of INS-1, MIN6, and pancreatic islet cells (Mi et al. 2003, Kang et al. 2010). TRAIL has also been shown to improve immunity to prevent pancreatic β-cell apoptosis (Mi et al. 2003, Zauli et al. 2010). These data suggested that TRAIL might have a role in the protection of pancreatic β-cell apoptosis. It is possible that the protective effects of TRAIL for β-cells might be attributable to the balance of the TRAIL death receptor (DR5) and TRAIL survival receptor (DcR1) in normal pancreatic β-cells. Thus, TRAIL alone may not be able to induce pancreatic β-cell apoptosis. The induction of DR5 and the repression of DcR1 might be a key role in TRAIL-induced apoptosis. It was found in the present study that although dexamethasone induced TRAIL, it not only increased DR5 but also decreased DcR1. Dexamethasone may, therefore, increase pancreatic β-cell apoptosis by alteration of both TRAIL and the TRAIL receptors. This study confirmed this notion in dexamethasone-injected mouse pancreata that TRAIL and DR5 were upregulated. This notion is supported by the results of earlier studies that demonstrated diabetes patients have a higher serum level of TRAIL than that found in normal patients (Chang et al. 2011) and that the loss of the pancreatic β-cell mass by TRAIL was prevented by DR4 and DR5 antagonists (Ou et al. 2002). Furthermore, dexamethasone, ibuprofen, and inostamycin promoted TRAIL-induced apoptosis in cancer cells by increasing DR5 expression (Wu et al. 1999, Yamamoto et al. 2012, Todo et al. 2013). Knockdown DR5 reduced TRAIL-induced apoptosis in human colon cancer cells (Yamamoto et al. 2012). Downregulation of DcR1 also has a role in TRAIL-induced apoptosis. Another study demonstrated that TRAIL-induced apoptosis in normal prostate epithelial cells was better than that in aorta smooth muscle cells (Nesterov et al. 2002) because the former has fewer DcR1 and DcR2 than normal aorta smooth muscle cells.

It was hypothesized in the present study that secretory TRAIL binds to DR5. This study showed that knocking down DR5

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Figure 6
Effect of dexamethasone on Bax and Bcl-2 in INS-1. (A) A representative Western blot analysis of the pro-apoptotic protein, Bax, normalized to β-actin protein in INS-1 cells at 48 h. (B) A representative Western blot analysis of the anti-apoptotic protein, Bcl-2, normalized to β-actin protein in INS-1 cells at 48 h. (C) INS-1 cells were treated with 0.1 μM dexamethasone in combination with caspase-8 and caspase-9 inhibitors (Z-IETD-FMK, Z-LEHD-FMK) at 72 h and then cell viability was measured by PrestoBlue assay. Data are expressed as mean ± SEM of three independent experiments. **P < 0.01; ***P < 0.001 vs dexamethasone; "++"P < 0.01; "+++",P < 0.001 vs control.

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Dexamethasone induces β-cell apoptosis. Again, this result reiterated that DR5 played an important role in dexamethasone-induced pancreatic β-cell apoptosis. Also, this study further investigates that TRAIL activates apoptosis through the extrinsic pathway. To confirm this notion, caspase-8 and caspase-3 activities were measured. The results showed that dexamethasone increased caspase-8 and caspase-3 activities. This finding corresponds with that of a previous study which reported that dexamethasone induced apoptosis by increasing caspase-8 activation in thymocytes (Marchetti et al. 2003). Mutant caspase-8 reduced the cell apoptosis in human embryonic kidney cells containing an overexpression of DR5 (Kim et al. 2003). Moreover, siRNA caspase-8 caused resistance to TRAIL-induced apoptosis in human colon cancer (Zhang et al. 2005). TRAIL is also known to activate the mitochondrial apoptotic pathway (Deng et al. 2002). This study demonstrated that dexamethasone activated the mitochondrial apoptotic pathway via upregulation of the pro-apoptotic protein, Bax, and the downregulation of the anti-apoptotic protein, Bcl-2. These findings are supported by the results of previous studies in which dexamethasone was found to induce acute lymphoblastic leukemia (ALL) and myeloma cells (MM) by upregulating Bax and downregulating Bcl-2 (Deng et al. 2002, Laane et al. 2007). It has also been demonstrated that an overexpression of Bcl-2 could protect lymphoma cell apoptosis from dexamethasone (Zhang & Insel 2001). The knockdown of Bcl-2 in these cells resulted in them becoming sensitized to dexamethasone-induced apoptosis (Banuelos et al. 2016).

On the other hand, siRNA Bax blocked dexamethasone-induced apoptosis in the human chondrocyte cell line (Zaman et al. 2012). This study showed that caspase 8 and 9 inhibitors partially reversed the effect of dexamethasone-induced pancreatic β-cell death. These results indicated that dexamethasone induced pancreatic β-cell death via both extrinsic and intrinsic apoptotic pathways.

In addition, this study found that dexamethasone significantly stimulated phosphorylated NF-κB and TRAIL expression. It is possible that dexamethasone increases NF-κB phosphorylation, and that the phosphorylated NF-κB promotes TRAIL expression. This notion is supported by the results of another study which found that dexamethasone induced apoptosis by increasing caspase-8 activation in thymocytes (Marchetti et al. 2003).

Mutant caspase-8 reduced the cell apoptosis in human embryonic kidney cells containing an overexpression of DR5 (Kim et al. 2003). Moreover, siRNA caspase-8 caused resistance to TRAIL-induced apoptosis in human colon cancer (Zhang et al. 2005). TRAIL is also known to activate the mitochondrial apoptotic pathway (Deng et al. 2002). This study demonstrated that dexamethasone activated the mitochondrial apoptotic pathway via upregulation of the pro-apoptotic protein, Bax, and the downregulation of the anti-apoptotic protein, Bcl-2. These findings are supported by the results of previous studies in which dexamethasone was found to induce acute lymphoblastic leukemia (ALL) and myeloma cells (MM) by upregulating Bax and downregulating Bcl-2 (Deng et al. 2002, Laane et al. 2007). It has also been demonstrated that an overexpression of Bcl-2 could protect lymphoma cell apoptosis from dexamethasone (Zhang & Insel 2001). The knockdown of Bcl-2 in these cells resulted in them becoming sensitized to dexamethasone-induced apoptosis (Banuelos et al. 2016). On the other hand, siRNA Bax blocked dexamethasone-induced apoptosis in the human chondrocyte cell line (Zaman et al. 2012). This study showed that caspase 8 and 9 inhibitors partially reversed the effect of dexamethasone-induced pancreatic β-cell death. These results indicated that dexamethasone induced pancreatic β-cell death via both extrinsic and intrinsic apoptotic pathways.

In addition, this study found that dexamethasone significantly stimulated phosphorylated NF-κB and TRAIL expression. It is possible that dexamethasone increases NF-κB phosphorylation, and that the phosphorylated NF-κB promotes TRAIL expression. This notion is supported by the results of another study which found that the TRAIL promoter contains two NF-κB binding sites (Allen & El-Deiry 2012). It has been shown that NF-κB enhances the TRAIL expression in human virus-induced T-cell leukemia (Takehiro Matsuda 2011). Prostaglandin repressed TRAIL transcription by inhibiting the binding of NF-κB on the TRAIL promoter (Fionda et al. 2007). How dexamethasone induces NF-κB is still unknown since there are no glucocorticoid response elements on the NF-κB promoter. It might be possible that dexamethasone induces ROS (Reich et al. 2012, Guo et al. 2016), and in turn, the ROS stimulates NF-κB expression (Almeida et al. 2010). The NF-κB expression then stimulates TRAIL production and, vice versa, TRAIL promotes NF-κB expression (Grunert et al. 2012). This may be a vicious cycle in which dexamethasone produces pancreatic β-cell apoptosis. This hypothesis is supported by the results of previous studies that demonstrated dexamethasone enhanced ROS production in osteoblasts, testicular germ cells, and pancreatic β-cells to induce apoptosis (Reich et al. 2012, Mukherjee et al. 2015, Guo et al. 2016, Liu et al. 2018). Increased ROS production then stimulated NF-κB activation (Almeida et al. 2010).

In summary, we have discovered that dexamethasone induces TRAIL and DR5, but it reduces DcR1 to produce pancreatic β-cell apoptosis. Dexamethasone also activates the TRAIL downstream effectors, including caspase-8, caspase-3, Bax, and NF-κB. These effects are able to be inhibited by the GR inhibitor. Taken together, dexamethasone induces pancreatic β-cell apoptosis via its binding to the GR and its activation of the TRAIL pathway.
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