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EFFECTS OF XENOESTROGENS ON STREPTOZOTOCIN-INDUCED DIABETIC MICE

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In diabetic mellitus, apoptotic or necrotic deaths of pancreatic β -cells lead to insulin deficiency because plasma insulin is synthesized and released from pancreatic β -cells and involved with blood glucose homeostasis. Since estrogen receptors have been related with glucose metabolism, estrogen-like chemicals (xenoestrogens) including bisphenol A (BPA) and octylphenol (OP) alter the endocrine system, and cause adverse health consequences such as obesity and diabetes. In the current study, levels of plasma glucose were evaluated after administration of BPA and OP using biochemical analysis, and were investigated in insulin and insulin synthesis-related genes in the pancreas and liver of streptozotocin (STZ)-induced insulin-deficient mice. Although the STZ-induced insulin-deficient groups showed an increase in blood glucose compared with control groups, the induced blood glucose level dropped to that of baseline after administration of xenoestrogens. When insulin level and mRNA expression of insulin transcriptional regulators (Pdx1, Mafk, and Neurod1) in pancreatic β -cells were decreased in STZ-induced insulin-deficient groups, they were significantly restored by administration of xenoestrogens. The latter observation is also related to NF- κ B activation for anti-apoptosis effects in pancreatic β -cells. In addition, we observed a complementary convergence in regulation of gluconeogenesis for determination of blood glucose levels. Therefore, the current study may be particularly important for assessment of xenoestrogens under condition of diabetic mellitus or metabolic disorder.

Key words: *estrogen, pancreatic β cell, insulin, diabetes, liver, gluconeogenesis, bisphenol A, streptozotocin, apoptosis*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease related with glucose homeostasis due to lack of insulin production or impairment of insulin response within somatic cells (1, 2). As an indicator of DM, a high level of blood glucose is a classical symptom of polyuria, polydipsia, and polyphagia (3). In a few types of diabetes mellitus, type 1 DM results in failure of insulin production, and the latter was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". Otherwise, type 2 DM leads to development of insulin resistance in which cells fail to use insulin properly, eventually resulting in insulin deficiency for glucose homeostasis. Type 2 DM is generally referred to as either a non-insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". As reported in earlier studies, DM is related to carbohydrate (4, 5), glucocorticoid (6), and compounds (7). In experimental animal models, DM is induced by administration of STZ (7, 8) in which a glucosamine-nitrosourea compound is derived from *Streptomyces achromogenes* and used as a chemotherapeutic agent for pancreatic β -cell carcinoma (9). Following selectivity to β -cells, STZ accumulates within pancreatic β -cells through glucose transporter channels (GLUT2) because this compound has a structural similarity to glucose (10). STZ appears to have the unique cytotoxic property of alkylation corresponding to nitrosourea compounds, and it also elicits a proinflammatory

response related to the release of glutamic acid decarboxylase (9, 11). After infiltration of immune cells in pancreatic islets, damage to pancreatic β -cells not only results in hyperglycemia but is also associated with side effects, including hepatotoxicity and nephrotoxicity.

Estrogen and its metabolites control expression of many genes, and plays a vital role in regulation of various physiological and pathological processes (12, 13). 17 β -estradiol is known to enhance not only the secretion of insulin but also the proliferation of β -cells in a STZ-induced DM model (14). In aromatase or estrogen receptor alpha null mice model, STZ-induced apoptosis in β -cells increased when compared with WT mice, and the latter reveal that 17 β -estradiol induced insulin production and promoted β -cells survival *via* non-genomic signals of estrogen receptor alpha or G protein-coupled estrogen receptor (15, 16). For instance, estrogen-induced increases in second messengers are resistant to transcription and translation inhibitors both of which block steroid effects at the genomic level, and this non-classical mechanism of estrogen leads to the activation of a reporter gene that contains a cAMP response element rather than an estrogen response element (12). Although estrogen increases insulin secretion and promotes insulin sensitivity in β -cell (17), high doses of estrogen and xenoestrogens developed insulin resistance, such as chronic hyperinsulinemia, and alteration of insulin tolerance (18). These findings are particularly intersecting in light of reports indicating

that xenoestrogens could interfere with endocrine in mammals (19, 20). In addition, recent evidence revealed that xenoestrogens have the capacity to mimic endogenous estrogen in various tissues, including uterus (21), ovary (22), duodenum and kidney (23) and *in vitro* (24). According to recent reports, xenoestrogens can be considered a risk factor for development of type 2 diabetes (18, 25, 26). Low doses of bisphenol A (BPA) also increase insulin content in whole islets of the pancreas *via* estrogen receptor α (ER α) (25). In addition, the estrogenic effect of octylphenol (OP) is accompanied by the ER α pathway (27).

We investigated the influence of xenoestrogens under condition of no observed adverse effect level (NOAEL) in which compounds are not observed in the adverse effects in the exposed population compared with the vehicle group. Using a mouse model, early studies revealed that the NOAEL of OP or BPA for systemic toxicity was 12.5 mg/kg/day mice (28) or 5 mg/kg/day (29), respectively. Because BPA and OP are well known compounds associated with the action of estrogen, we set out to characterize these xenoestrogens that exposed within pancreatic β -cells under type I DM states, and determine how the presence of xenoestrogens within these cells might modulate insulin production.

MATERIALS AND METHODS

Chemicals

17 α -Ethinyl estradiol, octylphenol (minimum 90.0% purity), and BPA were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Experimental design and materials

Institutional Animal Care and Use Committee (IACUC) of Chungbuk National University approved all experimental procedures.

Male ICR mice, weighing 25~30 g, six weeks of age, were obtained from Koatech (Pyeongtaek, Republic of Korea). All animals were housed in polycarbonate cages and acclimated in an environmentally controlled room (temperature: 23 \pm 2°C, relative humidity: 50 \pm 10%, frequent ventilation, and a 12-h light/dark cycle). After approximately one week of acclimatization, we used the AMDCC (Animal Models of Diabetic Complications Consortium) protocol for induction of STZ-induced insulin-deficient mice, as previously described (30). Using this protocol, the animals were injected i.p. with a single dose of STZ at 50 mg/kg body weight (b.w.) dissolved in 10 M citrate buffer (pH 4.5), and animals in the control group received injection with vehicle during five days. STZ-induced insulin-deficient mice were exposed to xenoestrogens *via* gavage administration for five days. ICR mice were divided into two main groups: the control (Con) group and the STZ-induced insulin-deficient group. Each main group was divided into four groups; Veh, BPA (5 mg/kg b.w.), OP (12.5 mg/kg b.w.), and EE (0.1 mg/kg b.w.). Each molecule was dissolved in corn oil (Sigma-Aldrich), or corn oil (vehicle) as a control. On day 22 after administration with STZ, all mice were sacrificed using CO₂ in a fume hood.

Plasma glucose and insulin levels

During conduct of the experiments, the body weight of each animal was measured, and the plasma glucose levels were determined on day 0, 5, 10, 13, 16, 19, and 22 using the Accu-Chek® Active (Roche Diagnostics GmbH, Mannheim, Germany). The animals were fasted for 4 hours before performance of blood glucose measurements. On day 22, plasma insulin level was also determined, by using the insulin ELISA kit (SHIBAYAGI, Japan).

Immunohistochemical staining

Pancreatic tissues were embedded in paraffin, cut into sections (5- μ m thick), de-paraffinized in xylene, and hydrated in

Table 1. Blood glucose level in STZ-induced insulin deficient mice.

		STZ		Xenoestrogen				
		(1~5d)	(6~10d)	0d	6d	10d	13d	16d
Con	Veh	161.3	179	171.3	175	164.8	175	179
	(n=7)	\pm 7.0	\pm 21.8	\pm 16.1	\pm 4.2	\pm 7.3	\pm 4.2	\pm 21.8
	BPA	174.5	166	166	171.3	170	171.3	166
	(n=7)	\pm 19.8	\pm 10.5	\pm 6.8	\pm 18.1	\pm 10.8	\pm 18.1	\pm 10.5
	OP	154	162.5	135.8	144.5	159.3	144.5	162.5
(n=7)	\pm 8.0	\pm 5.0	\pm 26.3	\pm 9.5	\pm 26.2	\pm 9.5	\pm 5.0	
EE	184.5	176.8	154.5	160	154.5	160	176.8	
	(n=7)	\pm 16.4	\pm 22.7	\pm 11.7	\pm 13.4	\pm 7.3	\pm 13.4	\pm 22.7
	Veh	161.3	180.5	190.8	289.3	374.0*	414.7*	454.8*
(n=7)	\pm 7.0	\pm 20.2	\pm 25.9	\pm 96.1	\pm 83.5	\pm 133.3	\pm 118.1	
STZ	BPA	174.5	170	170.8	251.5	363.7	403.2*	413.2*
	(n=7)	\pm 19.8	\pm 51.2	\pm 32.1	\pm 133.1	\pm 147.8	\pm 129.0	\pm 118.4
	OP	154	160.8	166	206	250.8	219.2#	237.8#
	(n=7)	\pm 8.0	\pm 41.2	\pm 15.2	\pm 31.3	\pm 48.9	\pm 54.7	\pm 46.9
EE	184.5	142.3	154.3	244.8	305.2	320.2*	339.0*	
	(n=7)	\pm 16.4	\pm 18.9	\pm 10.1	\pm 107.4	\pm 120.1	\pm 127.3	\pm 125.4

* P <0.05 compared with the Con-Veh group, # p <0.05 compared with the STZ-Veh group (unit; mg/dl).

descending grades of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in TBS-T for 30 min. Non-specific reactions were blocked by incubating the sections in 10% normal goat serum (Vector Laboratories, Burlingame, CA, USA) for 1 hour at room temperature. The sections were subsequently incubated overnight at room temperature with antibodies against insulin (1:500, Santa Cruz Biotechnology, CA, USA) diluted in 10% normal goat serum. After washing with TBS-T, the sections were incubated with a biotinylated secondary antibody (goat or rabbit IgG, Vector Laboratories) for 1 hour at 37°C, followed by incubation with ABC Elite (Vector Laboratories) for 30 min at 37°C. Diaminobenzidine (DAB; Sigma-Aldrich) was used as a chromogen. The sections were counterstained with hematoxylin and mounted in Cytoseal*60 (Richard-Allan Scientific Co., Kalamazoo, MI, USA).

Total RNA extraction and quantitative real-time PCR

Pancreatic and liver tissues were rapidly excised and washed in cold, sterile saline (0.9% NaCl). Total RNA was extracted using TRI reagent (Ambion, Austin, TX, USA) according to the

manufacturer's protocol, and the total RNA concentration was determined by measuring the absorbance at 260 nm using an Epoch microplate spectrophotometer (BioTek Instruments, Inc., Winooski, USA). One microgram of total RNA was reverse transcribed into first-strand cDNA using Moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen Co.) and random primers (9-mers; TaKaRa Bio, Inc., Otsu, Japan). The cDNA template (2 μ L) was added to 10 μ L of 2 \times SYBR Premix Ex Taq (TaKaRa Bio, Inc.) containing 10 pmol of primers specific for CaBP-9k and TRPV6. Real-time PCR for TRPV5 was performed using TaqManTM Universal PCR Master Mix (Applied Biosystems, Foster City, USA). Reactions contained cDNA template (2 μ L) added to 10 μ L of TaqManTM Universal PCR Master Mix (Applied Biosystems), 2 μ L of 20 \times Assays-on-DemandTM Gene Expression Assay Mix (TRPV5, Mm01166029 m1; Applied Biosystems), and RNase-free water in a final volume of 20 μ L. PCR was performed for 40 cycles with the following parameters: denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s. The primers for 1A were 5'-CCA GGG TTT GGA ATT ATT TC-3' (sense) and 5'-GAA GAT AAA CCC TAA GGC TC-3'

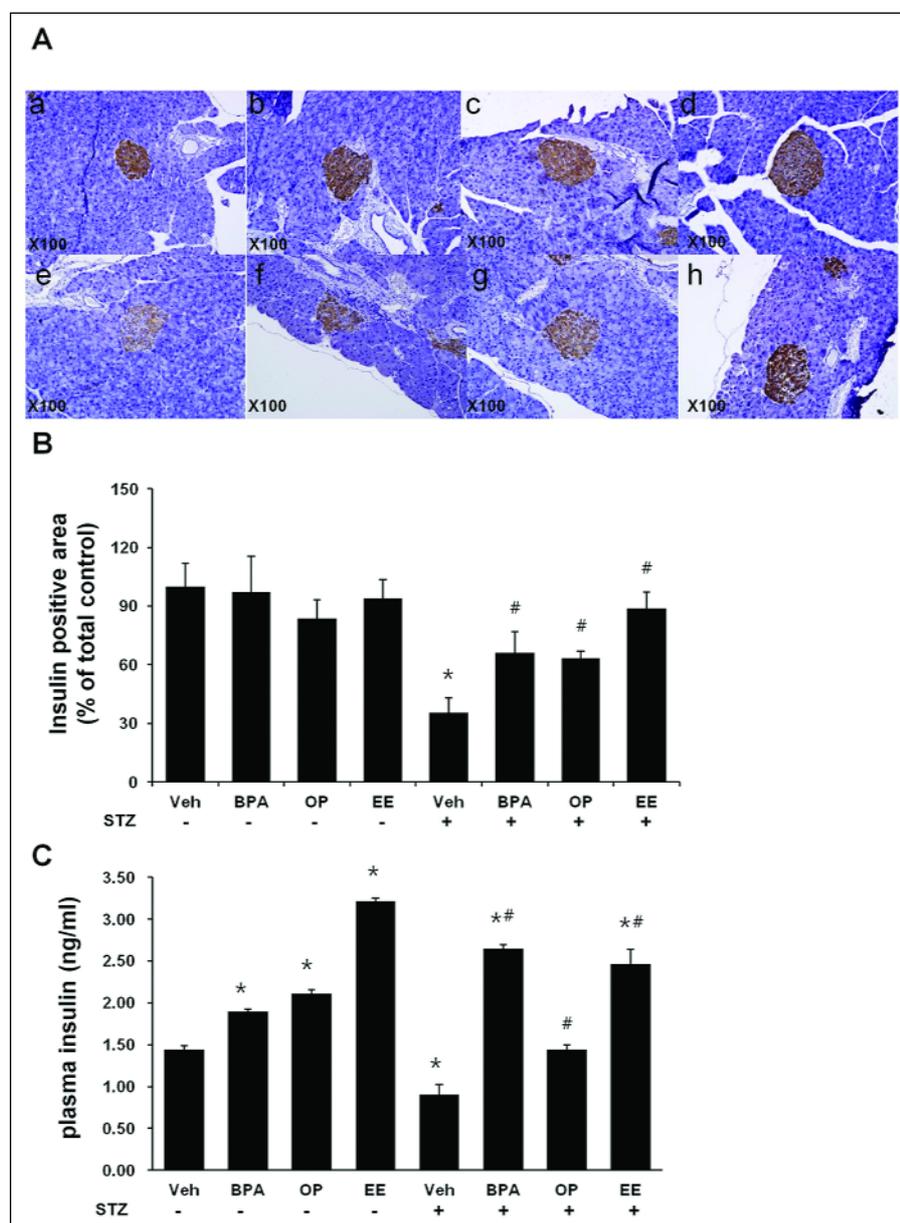


Fig. 1. Localization of insulin-positive β cells in the pancreas and plasma insulin levels of STZ-induced insulin-deficient mice. Insulin expression was observed in pancreatic β -cells. (A): Immunohistochemistry was performed to detect (a. Veh, b. BPA, c. OP, d. EE; control group) and (e. Veh, f. BPA, g. OP, h. EE; STZ group) as described in the Materials and Methods section. (B): Qualification of insulin-positive -cells was performed using NIH Image J software. (C): In control group (Veh, BPA, OP, EE) or STZ group (Veh, BPA, OP, EE), plasma insulin level was examined by insulin ELISA kit. * P <0.05 compared with the Con-Veh group, # P <0.05 compared with the STZ-Veh group.

(antisense). The primers for Pdx1 were 5'-GCT GGA GCT GGA GAA GGAAT-3' (sense) and 5'-GTC ACC GCA CAA TCT TGC T-3' (antisense). The primers for Mafa were 5'-GAG GAG GTC ATC CGA CTG AA-3' (sense) and 5'-CCG CCA ACT TCT CGT ATT TC-3' (antisense). The primers for Neurod1 were 5'-CTC TGG AGC CCT TCT TTG AA-3' (sense) and 5'-AAG ATT GAT CCG TGG CTT TG-3' (antisense). The primers for Esr1 were 5'-TGT GTC CAG CTA CAA ACC AAT G-3' (sense) and 5'-CAT CAT GCC CAC TTC GTA ACA-3' (antisense). The primers for Esr2 were 5'-CTG TGC CTC TTC TCA CAA GGA-3' (sense) and 5'-TGC TCC AAG GGT AGG ATG GAC-3' (antisense). The primers for Bcl-2 were 5'-TGG GGA TGA CTT CTC TCG TC -3' (sense) and 5'-CAT CTC CCT GTT GAC GCT CT-3' (antisense). The primers for Bcl-XL were 5'-AGG CGA TGA GTT TGA ACT GC -3' (sense) and 5'-TGA AGC TGG GAT GTT AGA TCA CT-3' (antisense). The primers for TNF- α were 5'-CCT GTA GCC CAC GTC GTA G-3' (sense) and 5'-GGG AGT AGA CAA GGT ACA ACC C-3' (antisense). The primers for IL-1 were 5'-GGG AGT AGA CAA GGT ACA ACC C-3' (sense) and 5'-TCC AGA TCA TGG GTT ATG GAC TG-3' (antisense). The primers for I B were 5'-TGA AGG ACG AGG AGT ACG AGC-3' (sense) and 5'-TTC GTG GAT GAT TGC CAA GTG-3' (antisense). Fluorescence intensity was measured at the end of the extension phase of each cycle. The threshold value for fluorescence intensity for all samples was set manually. The PCR cycle at which the fluorescence intensity threshold was in the exponential phase of PCR amplification was designated as the threshold cycle (CT). The PCR product of 1A was used as an internal control for normalization.

Western blot analysis

Pancreatic tissues were rapidly excised and washed in cold sterile 0.9% NaCl solution. Total proteins were extracted using 500 μ L Pro-prep (iNtRON Inc., Seoul, Republic of Korea) for each sample according to the manufacturer's instructions. Tissues were homogenized by 20 strokes with a Dounce homogenizer followed by centrifugation at 17,800 \times g for 20 min at 4°C. Protein concentrations were determined using a bicinchoninic acid assay (BCA assay; Sigma-Aldrich). Protein samples (25 μ g for Bcl-2) were separated by electrophoresis in 7.5% and 12.5% sodium dodecyl sulfate (SDS) polyacrylamide gel and transferred onto polyvinylidene fluoride (PVDF) microporous membranes (Millipore, Bedford, MA, USA). The membranes were then blocked for 2 hours at room temperature (RT) with 5% skim milk (Difco™, Sparks, MD, USA) for 2 hours in Tris-buffered saline containing 0.05% Tween-20 (TBS-T). The blots were incubated with the following primary antibodies for 4 hours at RT: anti-Bcl-2 (diluted 1:1000, Santa Cruz Biotechnology). The blots were then incubated in TBS-T containing 5% skim milk with horseradish peroxidase-conjugated secondary antibody (anti-rabbit, 1:5000; Santa Cruz Biotechnology) for 1 hour at RT. After washing three times in TBS-T, binding affinity of the antibodies on the blots was detected with a chemiluminescence reagent (Santa Cruz Biotechnology) and exposure to Biomax™ Light film (Eastman Kodak, NY, USA) for 1–5 min. Signal specificity was confirmed by incubating the blots with secondary antibody in the absence of primary antibody. Band intensities were normalized to β -actin immunoreactive bands on the same

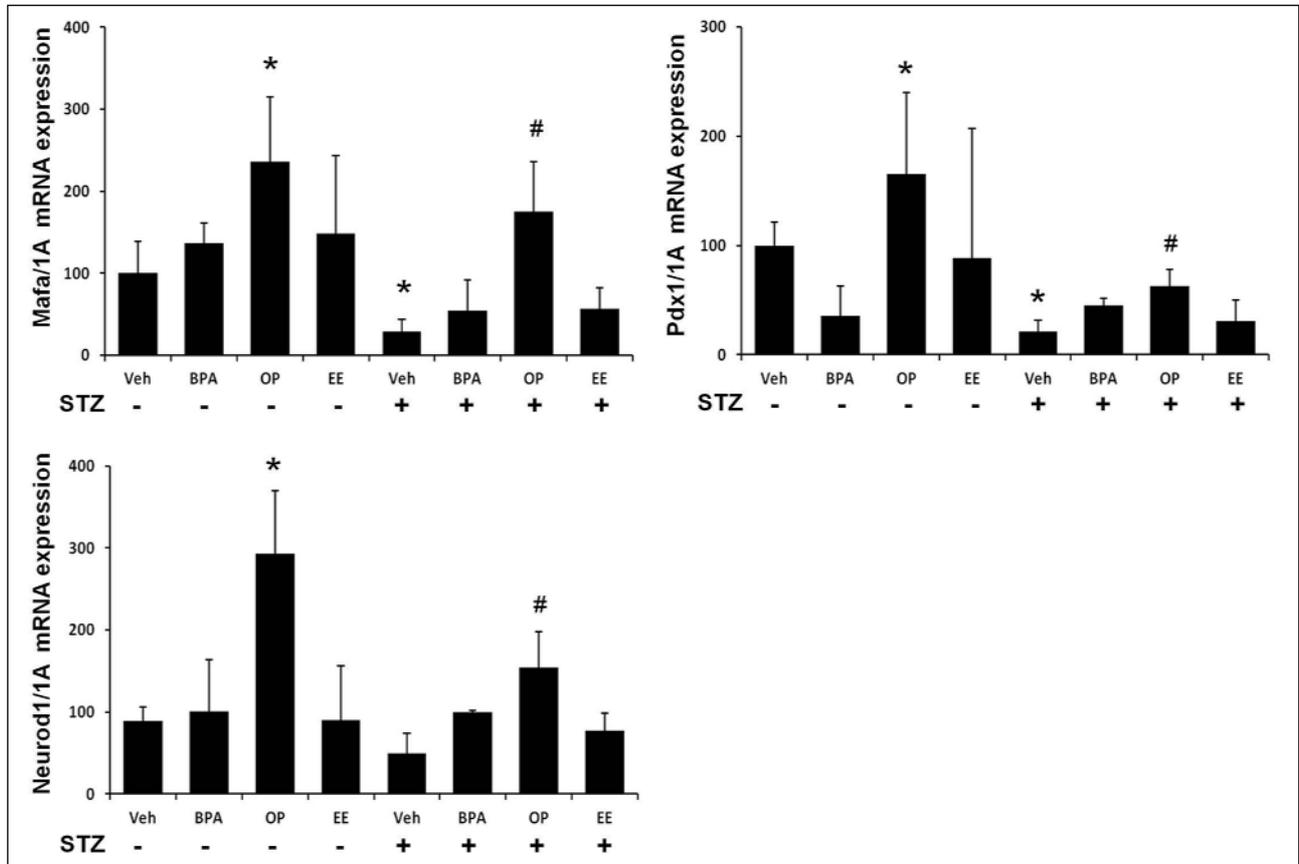


Fig. 2. Patterns of insulin transcriptional factors (Pdx1, Mafa, Neurod1) in the pancreas of STZ-induced insulin-deficient mice. The animals were divided into two groups (control or STZ group). control group (Veh, BPA, OP, EE) or STZ group (Veh, BPA, OP, EE), pancreatic Pdx1, Mafa, Neurod1 mRNA expression was examined by real-time PCR. * $P < 0.05$ compared with the Con-Veh group, # $P < 0.05$ compared with the STZ-Veh group.

membrane visualized after stripping and re-probing. Density for each band was measured using Image J software (National Institutes of Health, Bethesda, USA).

Data analysis

Data were presented as the mean \pm S.E.M. and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical analysis was performed using Prism Graph Pad (v4.0; GraphPad Software Inc., CA, USA). P-values <0.05 were considered statistically significant.

RESULTS

Blood glucose levels in streptozotocin-induced insulin-deficient mice

To determine whether administration of xenoestrogens could affect hyperglycemia in insulin-deficient mice, diabetic symptoms were induced by administration of 50 mg/kg STZ for 5 days. After 5 days treatment with STX, blood glucose levels showed a steady increase from 6 to 21 days, and the induced glucose levels were approximately 3-fold higher than those of control groups, as shown in *Table 1*. Interestingly, the elevated glucose levels were significantly decreased in the OP treatment group but not in other groups (BPA and EE treatment). In control groups, the levels of blood glucose were not significantly changed in the xenoestrogens-treated groups. Mice from all groups had similar weight(s) gain, which did not show response to kind of xenoestrogens and STZ (data not shown).

Measurement of insulin-positive pancreatic β -cells

To investigate the mechanism underlying increased blood glucose levels in STZ-induced insulin-deficient mice (*Table 1*), we examined the expression of insulin in the pancreas of STZ-induced insulin-deficient mice. As shown in *Fig. 1A*, insulin expression was observed in pancreatic β -cells, and its appearance was not different in control groups. In addition, we measured the relative area of insulin-positive β -cells per pancreatic islets using NIH ImageJ software (National Institutes of Health, Bethesda, Maryland, USA), as shown in *Fig. 1A*. In STZ treatment groups, patterns of decrease in insulin-positive β -

cells were detected in pancreatic islets. Their reductions were significantly restored by xenoestrogens (BPA, OP, and EE) compared with STZ treatment alone. The relative numbers of insulin-positive region in STZ groups (35.46 \pm 7.5%), STZ+BPA group (66.06 \pm 10.6%), STZ+OP group (63.28 \pm 3.76%), and STZ+EE group (88.84 \pm 8.3%) were quantified, and each value was normalized to the control groups (*Fig. 1B*).

In control group, plasma insulin level was significantly up-regulated in the BPA (1.90 ng/ml), OP (2.11 ng/ml) and EE (3.22 ng/ml) treated groups, respectively (*Fig. 1C*). While plasma insulin was significantly lower in STZ-induced insulin-deficient mice (0.91 ng/ml) than that of control group, the reduction of plasma insulin in STZ-administrated mice also were restored in BPA (2.65 ng/ml), OP (1.44 ng/ml) and EE (2.47 ng/ml) co-treated groups as shown in *Fig. 1C*.

Insulin transcription factors in the pancreas of streptozotocin-induced insulin-deficient mice

Production of insulin from pancreatic β -cells is critical for maintenance of blood glucose levels. Since the important role of insulin regulators such as Mafa, Pdx1 and Neurod1 has been demonstrated (31), we assessed transcripts of insulin regulators (Mafa, Pdx1, and Neurod1) in the pancreas of STZ-induced insulin-deficient mice. Significant increases of Mafa and Pdx1 transcripts were observed in the STZ-induced insulin-deficient group compared to control (*Fig. 2*). However, change of Neurod1 mRNA expression was not significant. In addition, expression of pancreatic Mafa, Pdx1, and Neurod1 mRNA was elevated by OP administration, and the levels were higher than those of control. These data indicated that OP induces the transcription factors of insulin expression, and may be involved in glucose homeostasis in blood.

Estrogen receptors (alpha and beta) in the pancreas of streptozotocin-induced insulin-deficient mice

We examined the transcriptional levels of estrogen receptors (Esr1 and Esr2) in pancreas of STZ-induced insulin-deficient mice. Pancreatic Esr1 and Esr2 transcripts were decreased by treatment with diabetic group compared with control group. In the xenoestrogens-treated groups, the levels of Esr1 and Esr2 mRNA showed a marked increase compared with the vehicle group in STZ-induced insulin-deficient group (*Fig. 3*). These increases in levels of pancreatic Esr1 and Esr2 mRNA are most

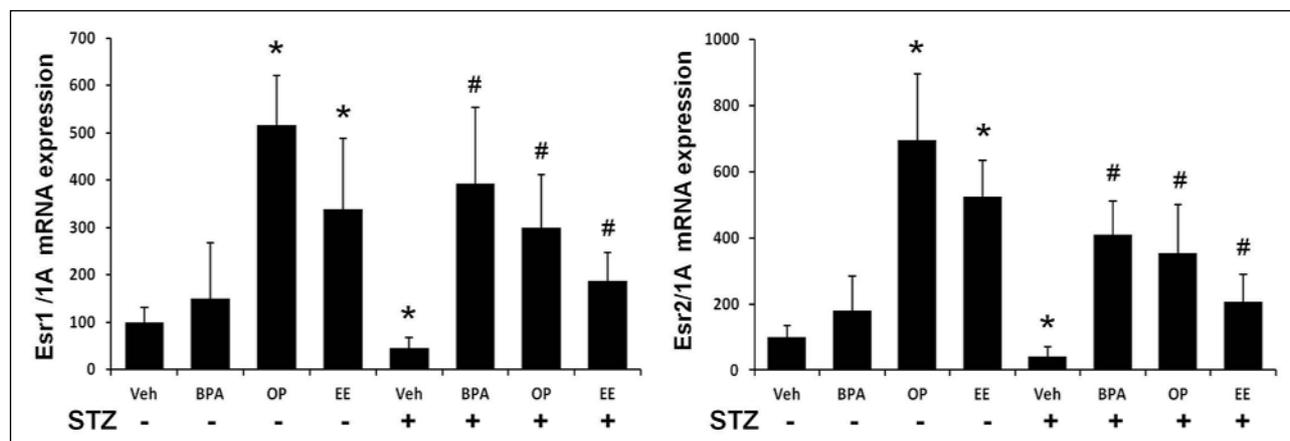


Fig. 3. Expression of estrogen (Esr1 and Esr2) receptors in the pancreas of STZ-induced insulin-deficient mice. The animals were divided into two groups (control or STZ group). In control group (Veh, BPA, OP, EE) or STZ group (Veh, BPA, OP, EE), pancreatic Esr1 and Esr2 mRNA expression were examined by real-time PCR. * $P<0.05$ compared with the Con-Veh group, # $P<0.05$ compared with the STZ-Veh group.

likely regulated through activation of estrogen receptor signaling in these cells, and may be involved in pancreatic β -cell growth and insulin secretion.

E2 and xenoestrogens related inflammation in pancreatic β -cells

To determine whether expression of inflammation-related transcripts are affected by xenoestrogens, we performed real-time PCR for examination of the expression of inflammatory-related transcripts in the pancreas of STZ-induced insulin-deficient mice. The mRNA levels of TNF- α , IL-1, Bcl-2, Bcl-XL, and I κ B α were up-regulated by OP treatment as compared to the control group. In STZ-induced insulin-deficient mice, TNF- α , IL-1 and Bcl-2 mRNA expression were increased in

BPA, OP, and EE-administered groups as compared to mice treated with STZ alone (Fig. 4). However, level of Bcl-XL and I κ B α mRNA was not altered by xenoestrogens. In parallel with transcriptional level, the pancreatic Bcl-2 protein level also increased in xenoestrogens treated groups (BPA, OP, and EE) in STZ-induced insulin-deficient mice, as shown in Fig. 5. These findings suggest that inflammation-related genes were effectively controlled by xenoestrogens, which may be involved in pancreatic β -cell survival.

Insulin related glucose in liver

To determine whether gluconeogenic genes of liver are regulated by administration of xenoestrogens in STZ-induced

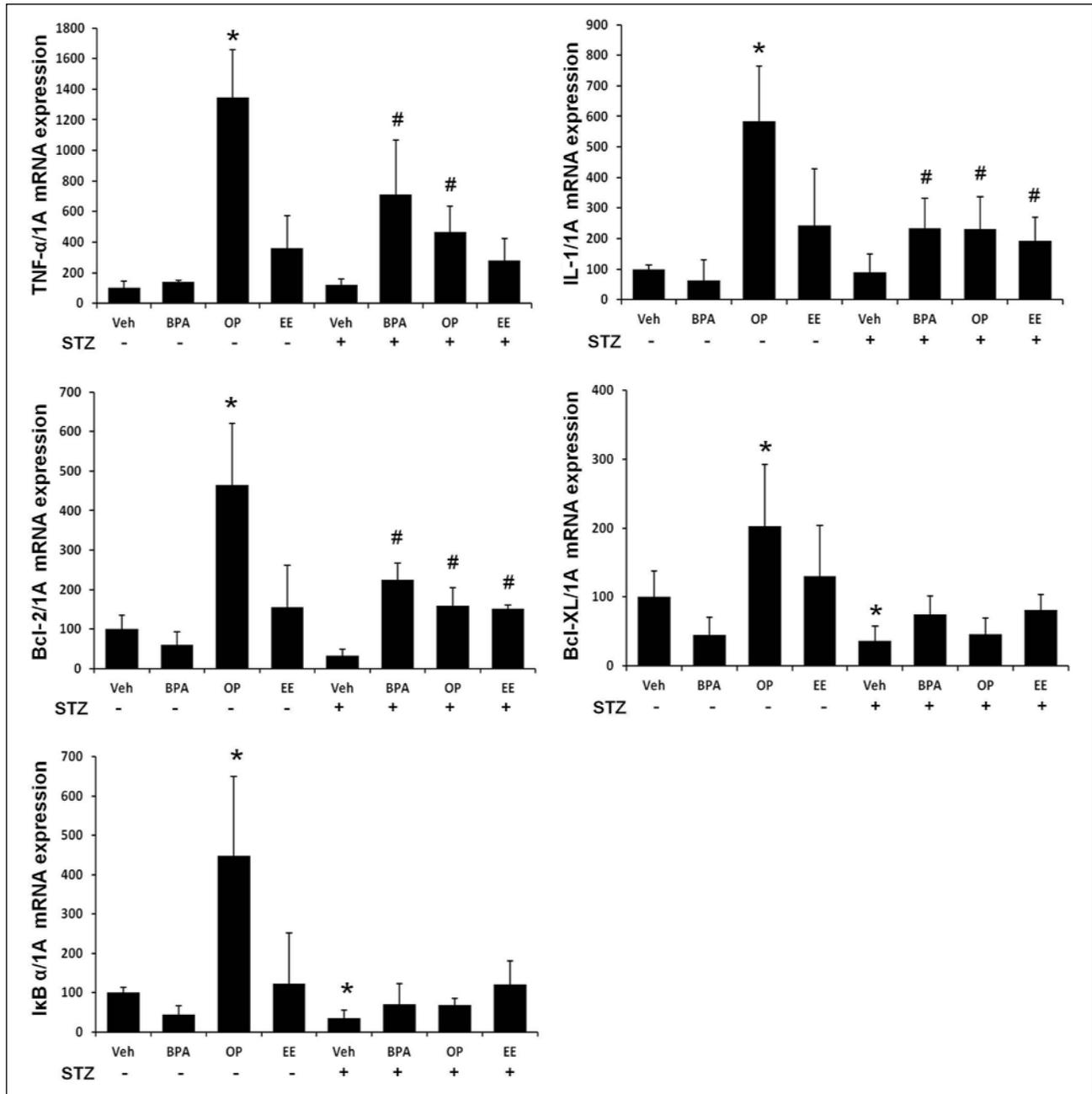


Fig. 4. Inflammation-related genes expression in the liver of STZ-induced insulin-deficient mice. The animals were divided into two groups (control group or STZ group). In control group (Veh, BPA, OP, EE) or STZ group (Veh, BPA, OP, EE), transcripts of hepatic inflammation related genes (TNF- α , IL-1, Bcl-2, Bcl-XL, I κ B α) were examined by real-time PCR. * P <0.05 compared with the Con-Veh group, # P <0.05 compared with the STZ-Veh group.

insulin-deficient mice, we monitored some gluconeogenic genes such as PCK1, Hnf4 α , and Foxo1. Up-regulation of PCK1 and Hnf4 α mRNA occurred primarily by treatment with STZ alone as compared to control. After STZ administration, an induction of PCK1 mRNA was restored in all xenoestrogens cotreatment groups (BPA, OP, and EE) (Fig. 6). Pancreatic Hnf4 α mRNA expression was also decreased in OP or EE treatment groups (Fig. 6). Unlike PCK1 and Hnf4 α transcripts, Foxo1 mRNA were decreased in STZ treated mice, and the decreased level of Foxo1 mRNA was restored by OP or EE treatment. These results indicate that gluconeogenesis of liver was affected by administration of xenoestrogen, and the latter control blood insulin levels in STZ-induced insulin-deficient mice.

DISCUSSION

In order to study the effects of xenoestrogens under condition of diabetic mellitus, we introduced STZ into mice because it is used as an inducer of insulin-deficient model (32). Accumulation of STZ has been reported to occur within pancreatic β -cells through glucose transport channel 2 (GLUT2) because of structural similarity to glucose (33). STZ induces necrosis or/and apoptosis in pancreatic β -cells, finally leading to development of hypoinsulinism and hyperglycemia in mice (34). Of particular interest, 17 β -estradiol is related to glucose homeostasis and insulin resistance in diabetes mellitus and it

also affects induction of insulin resistance in a type-2 diabetic-like model (18). In a STZ-induced insulin-deficient model, the functions of estrogen are related to recovery of an injured pancreas and insulin production from pancreatic β -cells (14).

To determine whether the effects of xenoestrogens lead to altered pancreatic function in STZ-induced insulin-deficient mice, we monitored glucose serum level after administration of BPA or OP. The STZ-induced hyperglycaemic effect was significantly decreased in the OP administration group, and the latter result is similar to that of a report indicating that STZ induced hyperglycemic effect was blocked by 17 β -estradiol (E2), which enhanced the release of insulin in pancreatic islets (14). Because xenoestrogens exhibit estrogen-like activity and interfere with hormone homeostasis, and mimic estrogen action, administration of xenoestrogens results in enhanced proliferation of islet cells and increases pancreatic insulin secretion. In addition, several transcriptional factors (Mafk, Pdx1, and Neurod1) of insulin expression were increased by administration of estrogenic compounds such as BPA, OP, and EE. ER α and ER β play important roles in regulation of glucose homeostasis (25) and protection of pancreatic β -cells from STZ-induced oxidative stress (15, 16). In the current study, we also observed inductions of ER α and ER β transcripts in the pancreas of xenoestrogen-treated mice. These results indicate that oral administration of xenoestrogens promote of insulin production by stimulation of ER-mediated insulin transcription factors in STZ-induced insulin-deficient mice. This finding is particularly

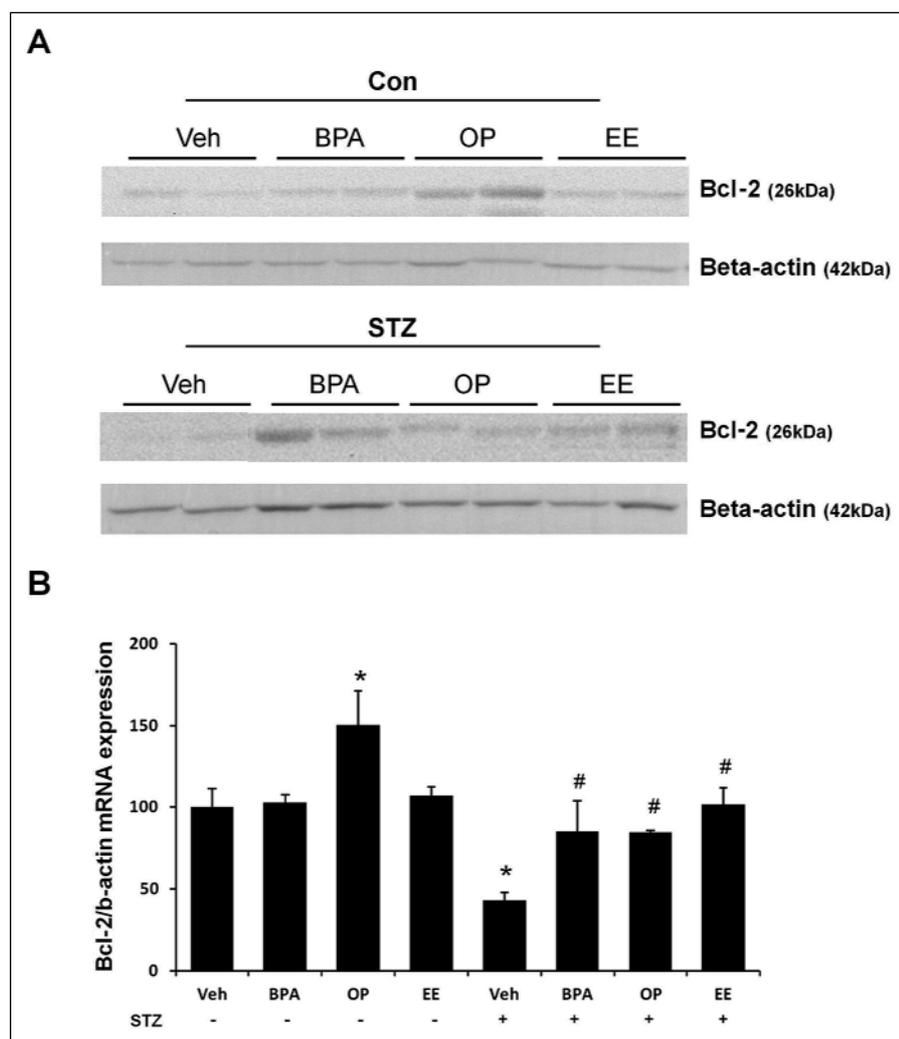


Fig. 5. Inflammation-related protein expression in the liver of STZ-induced insulin-deficient mice. The animals were divided into two groups (control group or STZ group). In control group (Veh, BPA, OP, EE) or STZ group (Veh, BPA, OP, EE), change of hepatic inflammation related protein (Bcl-2) was examined by Western blot. Upper panel blots; down panel, the quantification using by NIH Image J software. * $P < 0.05$ compared with the Con-Veh group, # $P < 0.05$ compared with the STZ-Veh group.

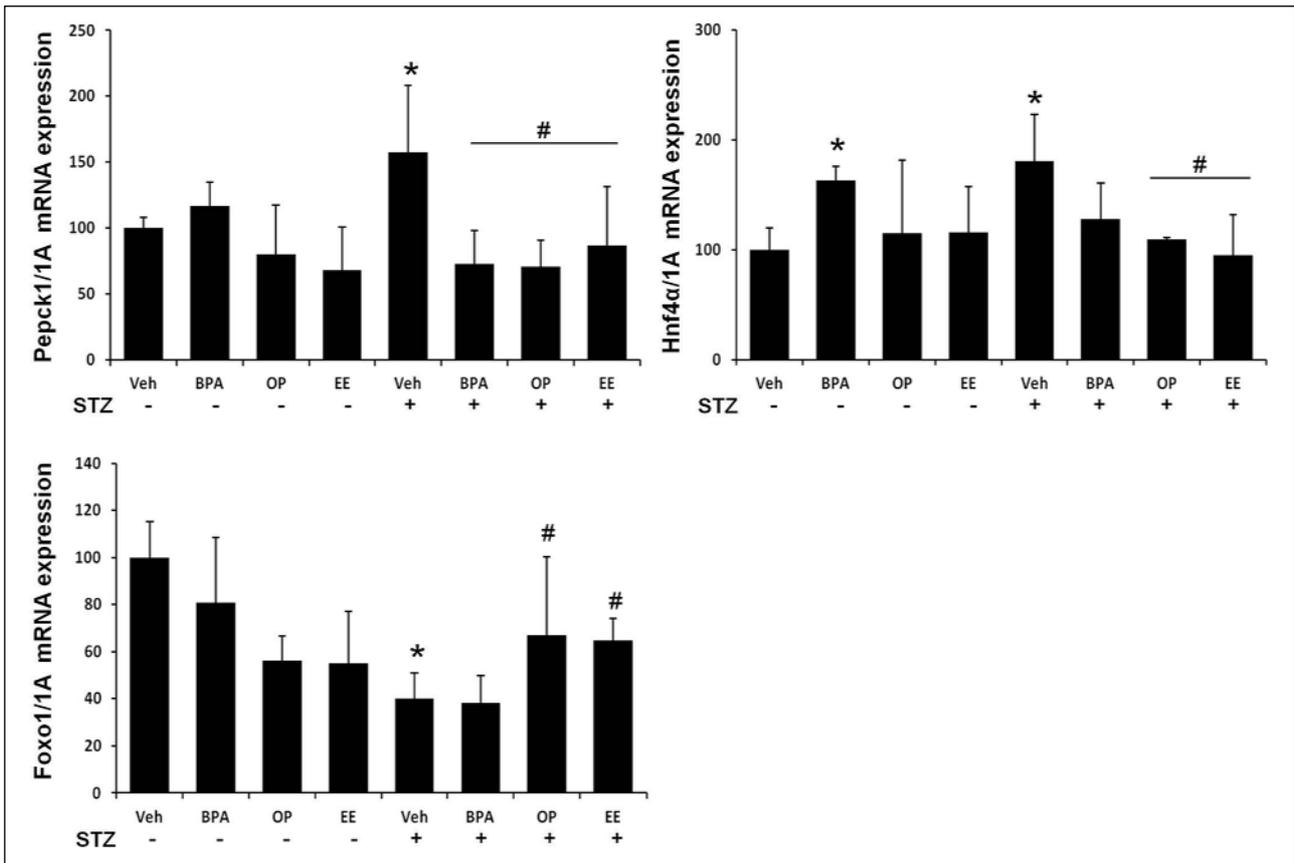


Fig. 6. Gluconeogenesis-related genes expression in the liver of STZ-induced insulin-deficient mice. The animals were divided into two groups (control group or STZ group). In control group (Veh, BPA, OP, EE) or STZ group (Veh, BPA, OP, EE), hepatic gluconeogenic genes (Pepck1, Hnf4 α , Foxo1) were examined by real time PCR. * $P < 0.05$ compared with the Con-Veh group, # $P < 0.05$ compared with the STZ-Veh group.

interesting in report that metabolic syndrome in gestation with type I diabetes is involved with oxidative stress (35).

Insulin is the most critical hormone in regulation of glucose homeostasis (36), and liver, an insulin-responsive tissue, has a central role in maintenance of glucose *via* gluconeogenesis and glycogenolysis (37). Gluconeogenesis is the process of synthesizing glucose from non-carbohydrate sources. Gluconeogenesis occurs mainly in the liver with a small amount also occurring in the cortex of the kidney or other body tissue, and its organs have a high demand for glucose. Insulin normally turns down gluconeogenesis; this hormone represses expression of gluconeogenic genes at the mRNA level (38). Insulin has a direct inhibitory effect on the transcription and activity of key hepatic gluconeogenic enzymes through forkhead box class O-1 (Foxo1) phosphorylation, including phosphoenolpyruvate carboxykinase (Pepck) (39, 40). As a key mediator of gluconeogenesis, Pepck was regulated by serum insulin concentration (40). When there was a lack of insulin (type 1 diabetes) or resistance to its action (type 2 diabetes) under pathological conditions, induction of Pepck was observed in liver and kidney (41, 42). In this study, we observed the induction of PCK1, Hnf4 α , and Foxo1 mRNA in liver of STZ-induced insulin-deficient mice. While hepatic PCK1, Hnf4 α , and Foxo1 mRNA was increased in STZ treated mice, the induced gluconeogenesis-related genes were restored by administration of xenoestrogens. This result is consistent with that of a previous report in which hepatic Pepck, Hnf4, and Foxo1 mRNA expression were increased by treatment with STZ (43, 44). These results indicate that hepatic gluconeogenesis-

related genes were regulated by xenoestrogens, which mimic endogenous estrogen for glucose homeostasis.

Apoptosis of pancreatic β -cells is a critical component in the pathogenesis of autoimmune type 1 diabetes mellitus (45, 46). Exogenous induction of STZ in rodents led to persistent inflammation *via* damage of β -cells (47). Apoptosis signal triggers Bax-associated events, including subsequent caspase activation and progression of apoptotic cell death, however, inhibition of apoptosis by Bcl-2 is blocked by formation of a heterodimer with Bax (48, 49). According to a recent report, NF κ B prevents pancreatic β -cell death and autoimmune type 1 diabetes (50). In addition, apoptosis-related gene, Bcl-2, was down-regulated in STZ-induced DM1 mice (51, 52). We observed a beneficial effect of xenoestrogens on STZ induced damage within β -cells. In parallel with our data, an earlier study reported that estrogen has an anti-apoptotic effect against death in pancreatic islets at five days after administration of STZ (14, 15). In addition, the level of Bcl-2 mRNA was significantly decreased in STZ-induced insulin-deficient mice; however, expression of Bcl-2 was restored by administration of xenoestrogens or EE in STZ-induced insulin-deficient mice. We also found that xenoestrogens or EE increase expression of NF κ B related genes from STZ-induced insulin-deficient mice, and the latter might be involved in NF κ B related anti-apoptosis (50). We suggest that xenoestrogens induce NF κ B signaling against pancreatic β -cell death and autoimmune type 1 diabetes.

In summary, the results of our current study using anti-apoptosis of damaged β -cells in STZ-induced insulin-deficient mice show that oral administration of xenoestrogens or EE

results in protection to STZ-induced apoptosis of pancreatic islets. We demonstrated that β -cells prolong insulin production by regulation of transcription factors associated with estrogen action and/or NF κ B signalling in insulin deficient models. In addition, the increase in insulin level causes a reduction in the glucose level by inhibition of gluconeogenesis in liver and a decrease in blood glucose level in STZ-induced insulin-deficient mice. Our study may be particularly important for assessment of xenoestrogens under condition of diabetic mellitus or metabolic disorder.

Abbreviations: DM, diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; STZ, streptozotocin; BPA, bisphenol A; OP, octylphenol; NOAEL, no observed adverse effect level; EE, 17 β -ethinyl estradiol, Con, control; Veh, vehicle.

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