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Exposure to Aroclor 1254 differentially affects the survival of pancreatic β-cells and α-cells in the male mice and the potential reason

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ABSTRACT

Previous works showed that chronic exposure to Aroclor 1254 disrupted glucose homeostasis and induced insulin resistance in male mice. To further observe the different effects of Aroclor 1254 exposure on the pancreatic α-cells and β-cells, male mice were exposed to Aroclor 1254 (0, 0.5, 5, 50, 500 μg/kg) for 60 days, the pancreas was performed a histological examination. The results showed that the percentage of apoptosis cell (indicated by TUNEL assay) was increased in both α-cells and β-cells, as the Aroclor 1254 dose was increased; the proliferation (indicated by PCNA expression) rate of β-cells was elevated while that of α-cells was not affected, resulting in an increased β-cell mass and a decreased α-cell mass in a dose-depend manner. The number of Pdx-1 positive β-cells was significantly increased whereas that of Arx positive α-cells was markedly decreased, indicating an enhanced β-cell neogenesis and a weakened α-cell neogenesis. The drastically reduction of serum testosterone levels in all the treatments suggested an anti-androgenic potency of Aroclor 1254. The up-regulation of estrogen receptors (ERα and ERβ) and androgen receptor in β-cells might be responsible for the increased β-cell mass and neogenesis.

1. Introduction

It has been known that persistent environmental pollutants (POPs) are the risk factors for the prevalence of type 2 diabetes mellitus (T2DM) (Lasram et al., 2014; Ngwa et al., 2015; Song et al., 2016). As a type of POP, polychlorinated biphenyls (PCBs) have been associated with diabetes in human epidemiological studies (Cave et al., 2010; Lee et al., 2014; Singh and Chan, 2017; Vasiliu et al., 2006). Using animal models, the pathological effects and some underlying mechanisms caused by PCBs exposure have been revealed. Treatment with PCB-77 (50 mg/kg) or PCB-126 (1.6 mg/kg) resulted in impaired glucose homeostasis in two months old male mice (Baker et al., 2013). Female C57B/6 mice exposed to 36 mg/kg/wk Aroclor 1254 for 20 weeks showed hyperinsulinemia and insulin resistance (IR) (Gray et al., 2013). Our previous work exhibited that subchronic exposure to Aroclor 1254 (0.5, 5, 50, and 500 μg/kg) for 60 days caused impaired glucose homeostasis and hyperinsulinemia in male mice, the pathological mechanisms included the down-regulation of the insulin receptor signaling pathway in both liver and skeletal muscle (Zhang et al., 2015) and disturbance in the function of β-cells (Xi et al., 2019). Nevertheless, the alteration of α-cells and β-cells is still not well understood.

The islets of Langerhans are mainly composed of four types of functional cells with a principal core of β-cells and surrounded by other functional cells, including α-cells (Bonner-Weir et al., 2015). Insulin and glucagon secreted respectively by β-cells and α-cells play a pivotal part in the regulation of glycaemia. β-cell mass is plastic during the development of T2DM to adapt insulin requirements (Cnop et al., 2011). When β-cells fail to compensate for the metabolic demand, hyperglycaemia and T2DM occur, which is often accompanied by the losses of β-cell mass and function (Rhodes, 2005). However, few studies report the alterations about morphology as well as function of pancreatic α-cells during IR stage in animal and human. In people with T2DM, an increased α-cell mass and hyperglucagonaemia were observed (Ellenbroek et al., 2017). Obesity and IR were associated with

Abbreviations: POPs, persistent environmental pollutants; T2DM, type 2 diabetes mellitus; PCBs, polychlorinated biphenyls; IR, insulin resistance; T1DM, type 1 diabetes mellitus; E_2, 17β-estradiol; ERα, estrogen receptor α; ERβ, estrogen receptor β; T, testosterone; AR, androgen receptor; PCNA, proliferating cell nuclear antigen; Pdx-1, pancreatic duodenal homeobox1; Arx, aristaless-related homeobox; TCDD, 2,3,7,8-tetrachlorodibenzo-pdioxin; TBT, tributyltin chloride; BPA, bisphenol-A

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increased glucagon levels in people without diabetes (Bonora et al., 1990; Færch et al., 2016), while the α-cell mass was decreased in patients with T1DM (Bonnet-Serrano et al., 2018). In obese mice induced by a high-fat diet, pancreatic α-cell area and mass were reduced during islet compensatory stage, hypoglucagonaemia was observed in the fasted and fed mice (Merino et al., 2015). The alteration of α-mass and β-cell mass is associated with the apoptosis, proliferation and size of existing cells, neogenesis from ductal progenitor cells (Chesnokova et al., 2009; Merino et al., 2015). In our previous study, Aroclor 1254 exposure resulted in an increased β-cell mass and a decreased α-cell mass (Zhang et al., 2015), the mechanism for this heterogeneous adaptation of α-cells and β-cells merits further investigation.

Estrogen and androgen play an important regulation in plasma glucose homeostasis and islet cell function. Under normal physiological conditions, estrogen is involved in maintaining insulin sensitivity and is beneficial for β-cell function. However, when estrogen levels are above or below physiological level, IR and T2DM can be promoted (Nadal et al., 2009). Both estrogen receptors (ERα and ERβ) are emerging as key molecules involved in modulating pancreatic β-cell function (Alonso-Magdalena et al., 2013; Liu et al., 2009). 17β-estradiol (E_2) enhances β-cell survival and glucose homeostasis control in various animal models (Contreras et al., 2002; Le May et al., 2006) through ERα (Alonso-Magdalena et al., 2008; Le May et al., 2006) and/or ERβ (Alonso-Magdalena et al., 2013) signaling. Similarly, testosterone (T) concentration below physiological level is a risk factor for the development of T2DM in men (Ding et al., 2006; Fukui et al., 2007). For instance, low testosterone levels may be associated with an increased risk for IR and T2DM by interacting with overall and central obesity in adult men (Li et al., 2017). Some clinical studies have demonstrated that low serum testosterone levels reduce insulin sensitivity and promote IR and T2DM, independent of age and body fat mass (Stellato et al., 2000; Xia et al., 2015; Yeap et al., 2009). Androgen receptor (AR), as a β-cell receptor, enhances the function of β-cells through the cAMP-dependent pathway (Navarro et al., 2016). However, PCBs, as endocrine disruptors, have a possibility to disturb the levels of sex hormones and expression of their receptors. The aim of this study is to investigate the effect of PCBs on the islet cells and the mechanism involved.

2. Materials and methods

2.1. Chemicals

Aroclor 1254 (a complex mixture of multiple PCB congeners) was purchased from SUPELCO (USA). It was dissolved in 100% ethanol and diluted with 0.85% sodium chloride to obtain final concentrations of 0.1, 1, 10 and 100 μg/mL, with a final ethanol concentration of 10% (v/v).

Primary antibodies used for immunostaining included: rabbit anti-insulin, mouse anti-glucagon and mouse proliferating cell nuclear antigen (PCNA) antibody (all from Abcam, UK), rabbit anti-glucagon and rabbit pancreatic duodenal homeobox1 (Pdx-1) antibody (all from Cell Signaling Technology, USA), Mouse anti-insulin (Santa Cruz Biotechnology, CA), rabbit aristaless-related homeobox (Arx) antibody (Affinity, China). Secondary antibodies included goat anti-rabbit Alexa 488 (Abcam, UK), goat anti-mouse Alexa 488 (Proteintech Group, China), goat anti-rabbit Alexa 647 and goat anti-mouse Alexa 647 (Affinity, China). Primary antibodies used for western blotting included those for rabbit ERα (Santa Cruz Biotechnology, CA), rabbit AR and ERβ (Biosynthesis biotechnology, China), mouse β-actin (Sigma-Aldrich, USA). Appropriate horseradish peroxidase-conjugated secondary antibodies were used to detect the primary antibody/antigen complexes. The In Situ Cell Death Detection Kit, TMR red was purchased from Roche (USA).

2.2. Animals and treatments

All experimental procedures conducted in this study were approved by the Xiamen University Institutional Animal Care and Use Committee. Forty male C57BL/6 mice (21 days of age) were purchased from the center of experimental animals, Xiamen University, China. Mice were housed in micro-isolator cages in a temperature-controlled room (22 ± 1 °C) under a 12:12 h light − dark cycle with free access to water and a diet with natural ingredients (Keao Xieli Feed company, Beijing, China). After two days of adaptation, 40 male mice (without significant difference in weight and adverse clinical symptoms) were divided randomly into 5 groups. These animals were orally gavaged with Aroclor 1254 (0.5, 5, 50, and 500 μg/kg) once every 3 days for 60 days; control mice received an equal volume of the vehicle (5 μl/g) (10% ethanol in 0.85% sodium chloride). Individuals were weighed before gavage, and actual dosing volumes were calculated.

2.3. Tissue sampling and histological examination

Mice were fasted overnight for 14 h and then euthanized by cervical dislocation. Blood samples were collected from the eye socket and allowed to clot at room temperature for 1.5 h, then centrifuged, and the serum was stored at −80 °C for the analysis of serum T and E_2. Four pancreases from each treatment group were fixed (10% neutral-buffered formalin), embedded in paraffin, and sectioned at 5 μm for immunohistochemistry experiments. Other four pancreases from each group were frozen in liquid nitrogen immediately and then stored at −80 °C for Western blot analysis.

2.4. Immunostaining and the calculation of positive cells

Immunohistochemistry experiments, including PCNA and TUNEL, were performed following the methods previously described (Zuo et al., 2014).

For immunofluorescence assessment, sections (5 μm) were deparaffinized and washed in PBS, then immersed in antigen retrieval buffer (0.01 M citric acid, pH 6.0) and heated in a microwave oven for three sequential 2-min cycles at 100 °C. Subsequently, the slides were permeabilized with 0.5% Triton X-100 and blocked in 5% goat serum. Double label staining of PCNA, Pdx-1 and Arx with insulin or glucagon was performed to co-localize islet cells. Primary antibodies were incubated overnight at 4 °C and the appropriate secondary antibodies were used with the following dilutions (Table S1, Supporting Information).

For double label staining of TUNEL with insulin or glucagon, the section was stained with the anti-insulin or anti-glucagon antibody, then the apoptotic cells were identified using an In Situ Cell Death Detection Kit, TMR red based on the manufacturer's instructions. DAPI was used to stain the nucleus, and the staining signals of apoptosis were evaluated using fluorescence microscopy (Leica DMI4000B, Germany). Negative control sections that were not incubated with the primary antibodies were run in parallel.

For each individual, 20 areas from four distinct sections were chosen at random for analysis under a light microscope at a magnification of × 200. Four individuals were analyzed in each group. Relative α-cell and β-cell area and their mass were measured following the method described (Rafacho et al., 2010). The average numbers of the positive cells in the α-cells and β-cells were measured based on the reported method (Bruin et al., 2012).

2.5. Determination of serum hormone

The concentrations of T and E_2 in serum were determined radioimmunologically (RIA) using a commercial radioimmunoassay kit (Furui Biological Engineering Co., Beijing, China; FR-006). The lowest limits of sensitivity for T and E2 assays were 50 pg/mL and 2 pg/mL,

respectively. The intra- and inter assay coefficients of variation for both analyses were 10% and 15%.

2.6. Western blot analysis

The whole pancreas of three animals from each group was individually analyzed. Protein was extracted from the frozen tissue and 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was performed based on our previous methods (Zhang et al., 2015). After blocking with 5% nonfat dried milk, the membranes were incubated with primary antibodies overnight at 4 °C. The following antibodies were used: antibodies against ER-α (1:800), ER-β (1:800), or AR (1:1000). The membranes were then washed three times in TBST (TBS, 0.05% Tween-20) for 15 min and incubated with the respective horseradish peroxidase–conjugated secondary antibodies (1:10000) for 1 h at room temperature. The antibody-reactive bands were visualized using enhanced chemiluminescence (Sigma, UK), and a densitometry analysis was quantified using Quantity One software (Bio-Rad, U.S.A.).

2.7. Statistical analysis

The data were analyzed using SPSS 20.0 software (IBM, Chicago, IL, USA) and expressed as the mean ± standard error (SE). For all the obtained results, the Kolmogorov–Smirnov test demonstrated normal distribution, and Levene's statistic test validated the assumptions of the homogeneity of variances. The differences among treatments were examined using one-way ANOVA followed by the Duncan's test. Significant differences between groups were assumed at $P < 0.05$.

3. Results

3.1. Aroclor 1254 increased β-cell mass and decreased α-cell mass

The insulin-specific antibody immunofluorescence staining showed that insulin positive cells were located in the central region with high frequency in the pancreatic islet, and the glucagon labeling cells was in a discontinuous ring-like staining (Fig. 1A). Relative β-cell area (1.74, 1.82 and 2.05-fold) and β-cell mass (1.87, 2.09 and 2.12-fold) in the pancreas were significantly increased in a dose-dependent manner in the 5, 50 and 500 μg/kg groups, while relative α-cell area (25% and 25%) and α-cell mass (16% and 25%) were significantly decreased in the 50 and 500 μg/kg groups (Fig. 1B and C).

3.2. Aroclor 1254 increased α-cell and β-cell apoptosis

After exposure to Aroclor 1254 for 60 days, the percentage of TUNEL-positive cells significantly elevated in the islets (1.27, 1.51, 1.57 and 1.6-fold) in the 0.5, 5, 50 and 500 μg/kg treatments (Fig. S1).

In order to further detecting the apoptosis of β-cells and α-cells, immunofluorescence co-localization of TUNEL with insulin or glucagon was used. Representative islets showing TUNEL/insulin and TUNEL/glucagon staining in islets of control, Aroclor 1254 treated C57BL/6 mice are shown in Figs. 2 and 3. Aroclor 1254 treatment not only significantly increased the number of apoptotic β-cells in the 5, 50 and 500 μg/kg groups (1.17, 1.34 and 1.31-fold) but also significantly elevated the rate of apoptotic α-cells in the 500 μg/kg groups (1.17-fold).

3.3. Aroclor 1254 increased the proliferation of β-cells but not α-cells

In immunohistochemistry, PCNA positive cells were shown by brown staining. Aroclor 1254 treatment for 60 days caused an increase of the percentage of PCNA-positive cells in the islets in a dose-dependent manner, and reached a significance difference (by 1.78, 2.03, 2.07 and 2.31-fold) in all treatment groups compared to the control (Fig. S2).

To further measure β-cell or α-cell proliferation, we performed co-immunostaining of insulin (green)/glucagon (green) and PCNA (red) with DAPI (blue) in pancreas sections (Figs.4 and 5). PCNA positive β-cells increased in a dose-dependent manner and reached a significant difference (2.09 and 2.45-fold) in the 50 and 500 μg/kg groups compared to the control (Fig. 4), whereas PCNA positive α-cells exhibited no significant changes between the treated mice and the control (Fig. 5).

3.4. Aroclor 1254 induced the up-regulation of Pdx-1 in pancreatic β-cells

To examine the effect of Aroclor 1254 on Pdx-1 levels, double-labeling immunofluorescence staining was performed using the pancreatic sections. A significant increase in the expression of Pdx-1 was observed in all the treatments (8.14, 9.88, 11.11 and 13.58-fold) compared to the control (Fig. 6A and B).

3.5. Aroclor 1254 inhibited the expression of Arx in pancreatic α-cells

The expression of Arx in α-cells was significant decreased by 41%, 33% and 62% in 5, 50 and 500 μg/kg groups compared to the control (Fig. 6C and D).

3.6. Aroclor 1254 disrupted sex hormones levels

The serum E_2 levels showed no significant alteration after exposure to Aroclor 1254 for 60 days, except a significant reduction in the 0.5 μg/kg group (by 16%) (Fig. 7A). However, serum testosterone concentrations were significantly decreased by 87%, 94%, 89% and 89% in the all treatments compared to the control (Fig. 7B).

3.7. Aroclor 1254 increased the expression of estrogen and androgen receptors in the pancreas

As revealed by western blotting, Aroclor 1254 treatment resulted in an significant elevation of ERα (5.06, 4.50 and 5.03-fold) and ERβ (1.83, 1.78 and 1.7-fold) expression in the 5, 50 and 500 μg/kg groups. The expression of AR was significantly up-rgulated (1.97, 2.4 and 1.85-fold) in the 0.5, 5, and 50 μg/kg treatments (Fig. 7 C and D).

4. Discussion

Murine islets of Langerhans are mainly composed of β-cells (secreting insulin), surrounded by α-cells (secreting glucagon) (Migliorini et al., 2014). Under normal physiological conditions, α-cells and β-cells co-regulate blood glucose level. Hyperglycemia activates β-cells to release insulin and stimulate glucose uptake in liver, muscle and adipose tissue. Conversely, α-cells release glucagon in response to hypoglycemia and stimulate glycogenolysis in the liver (Röder et al., 2016). β-cell mass in the adult is plastic, and adjustment in β-cell growth and survival maintains a balance between insulin supply and metabolic demand (Rhodes, 2005). Through the expansion of β-cell mass and the enhancement of insulin secretion, islets have the functional flexibility to adapt rapidly to environmental changes (Tritschler et al., 2017). Exposure to environmental pollutants can cause changes in β-cell mass. Male mice exposed to Aroclor 1254 caused IR demonstrated by increased β-cell mass and hyperinsulinemia (Zhang et al., 2015). In young rodents, hyperglycemia, insulin resistance, obesity and pregnancy all contribute to larger β-cell mass associated with increased demand for insulin (Cnop et al., 2011). In the present work, the increased β-cell mass seemed to compensate for the increased metabolic load and IR.

In contrast to β-cells, the effect of pollutants on α-cells has not been extensively studied. In our previous work, we found that the serum glucagon levels were decreased accompanied by a progressive decrease in α-cell mass in male mice treated with Aroclor 1254 for 60 days (Zhang et al., 2015). A decreased α-cell mass would disrupt the glucose homeostasis, and lead to a failure of the α-cells counter-regulation

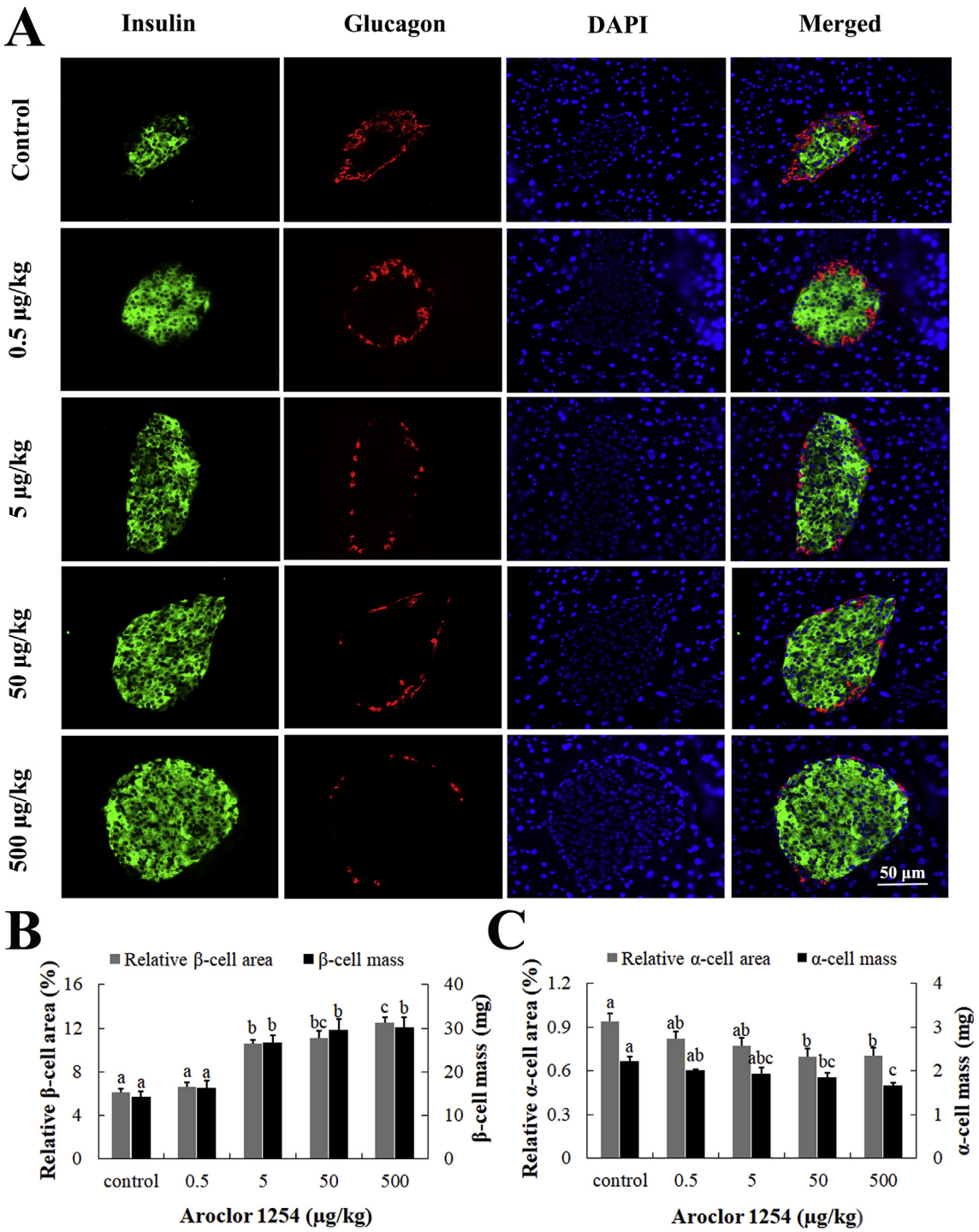

Fig. 1. Relative α-cell and β-cell area and mass in the islet of male mice exposed to Aroclor 1254 for 60 days. (A) Representative images of triple staining for insulin (green), glucagon (red), and DAPI (blue) in pancreas. (B) Relative β-cell area and β-cell mass. (C) Relative α-cell area and α-cell mass. Data are presented as mean ± SE (n = 4). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

response to insulin and inability to maintain normal blood glucose levels. Previous postmortem studies have reported that α-cell mass was unchanged or reduced by about 35% in maturity T2DM compared with non-diabetic controls (Henquin and Rahier, 2011). The decreased α-cells mass would generate failures in the control of glycaemia, which is proximate to the development of diabetes (Dunning et al., 2005).

Apoptosis, the programmed death of eukaryotic cells, is essential for the normal development and homeostasis of organisms. 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD) can induce apoptosis in a number of cell types, such as erythrocytes, hepatocytes and so on (Chopra and Schrenk, 2011). In animal experiments, tributyltin chloride (TBT) exposure inhibited the proliferation and induced the apoptosis of pancreatic islet cells in male mice (Zuo et al., 2014). Pregnant mice treated with low doses of bisphenol-A (BPA) (10 and 100 μg/kg) from day 9 to day 16 of gestation developed glucose intolerance and elevated β-cell apoptosis (Alonso-Magdalena et al., 2015). PCBs is also shown to induce apoptosis in mammalian cell lines such as human microvascular endothelial cells (Lee et al., 2003), human monocytic cells (Shin et al.,

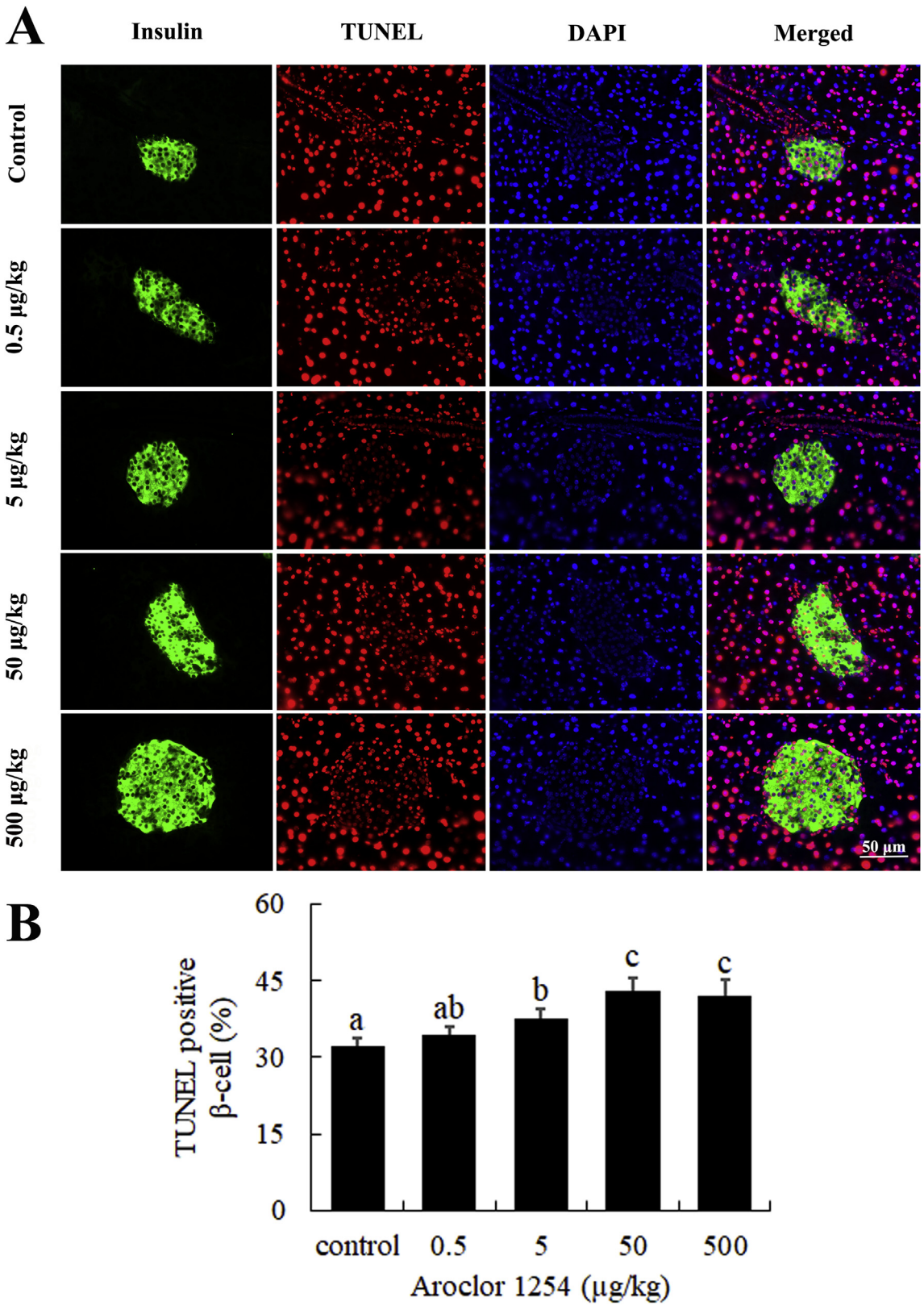

Fig. 2. Cell apoptosis in the islet β-cells of male mice exposed to Aroclor 1254 for 60 days. (A) Representative images of triple staining for insulin (green), TUNEL (red), and DAPI (blue) in pancreas. (B) β-cell apoptosis was quantified by percentage of TUNEL-positive β-cells. Data are presented as mean ± SE (n = 4). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

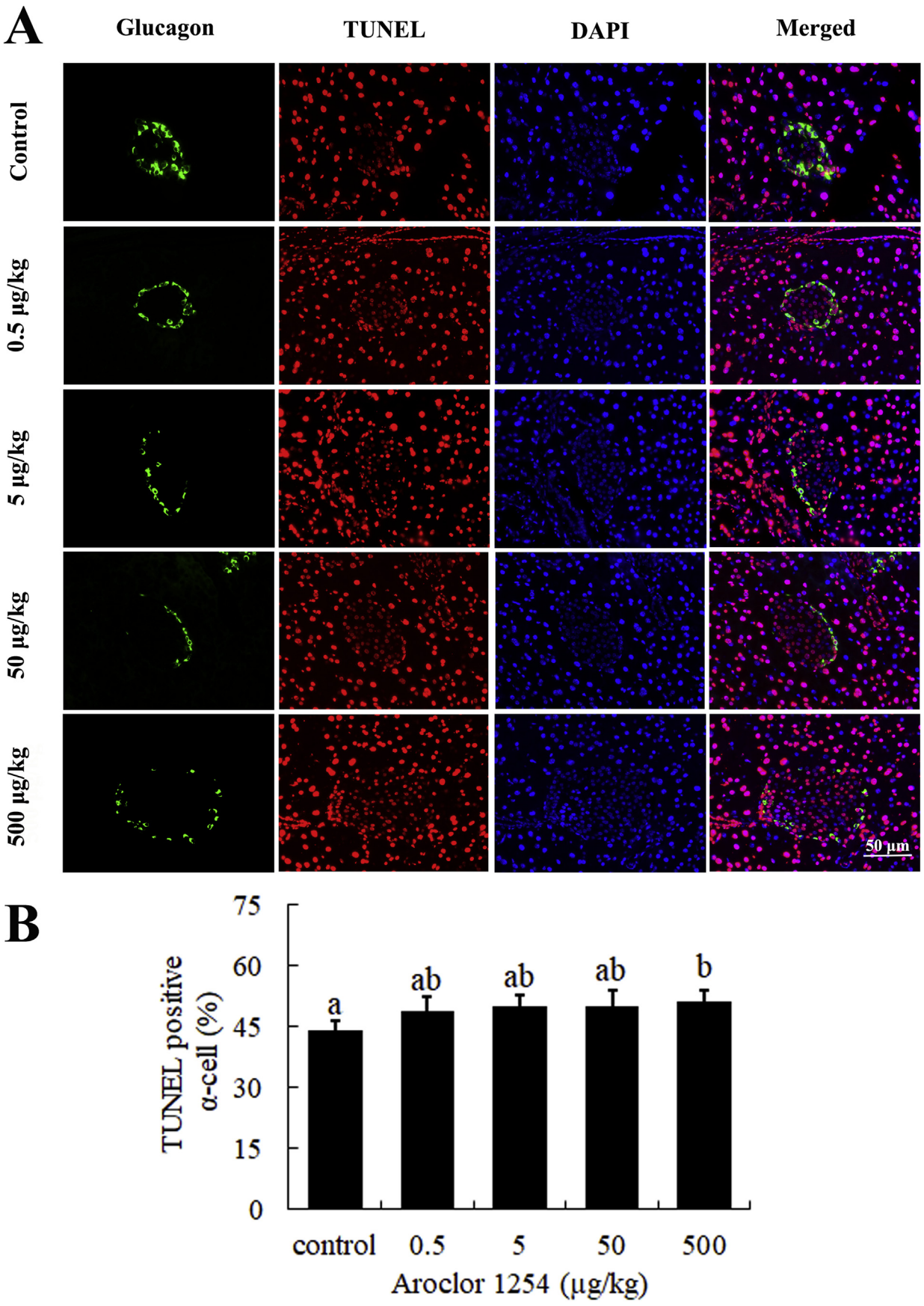

Fig. 3. Cell apoptosis in the islet α-cells of male mice exposed to Aroclor 1254 for 60 days. (A) Representative images of triple staining for glucagon (green), TUNEL (red), and DAPI (blue) in pancreas. (B) α-cell apoptosis was quantified by percentage of TUNEL-positive α-cells. Data are presented as mean ± SE (n = 4). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

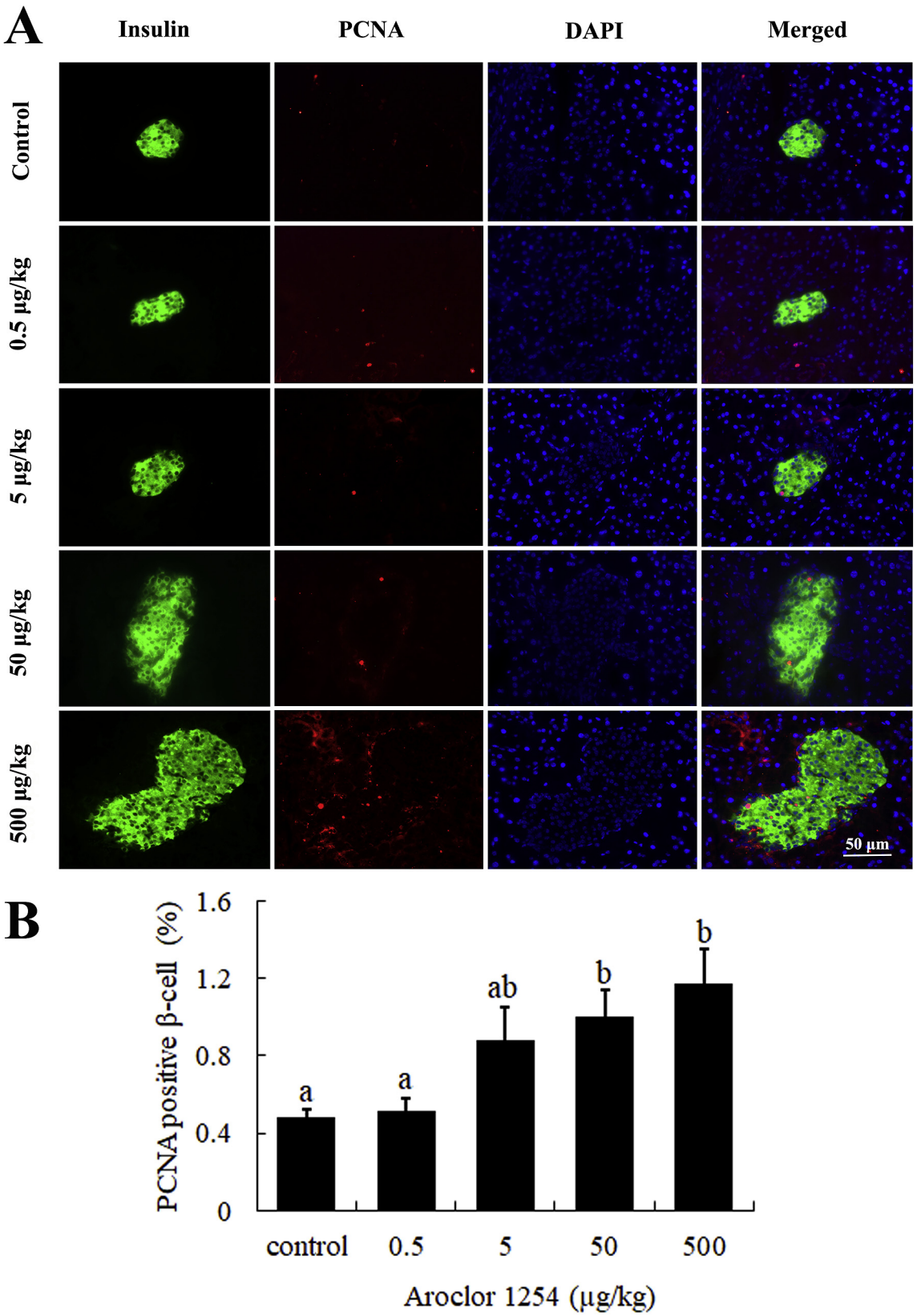

Fig. 4. Cell proliferation in the islet β-cells of male mice exposed to Aroclor 1254 for 60 days. (A) Representative images of triple staining for insulin (green), PCNA (red), and DAPI (blue) in pancreas. (B) β-cell proliferation was quantified by percentage of PCNA-positive β-cells. Data are presented as mean ± SE (n = 4). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

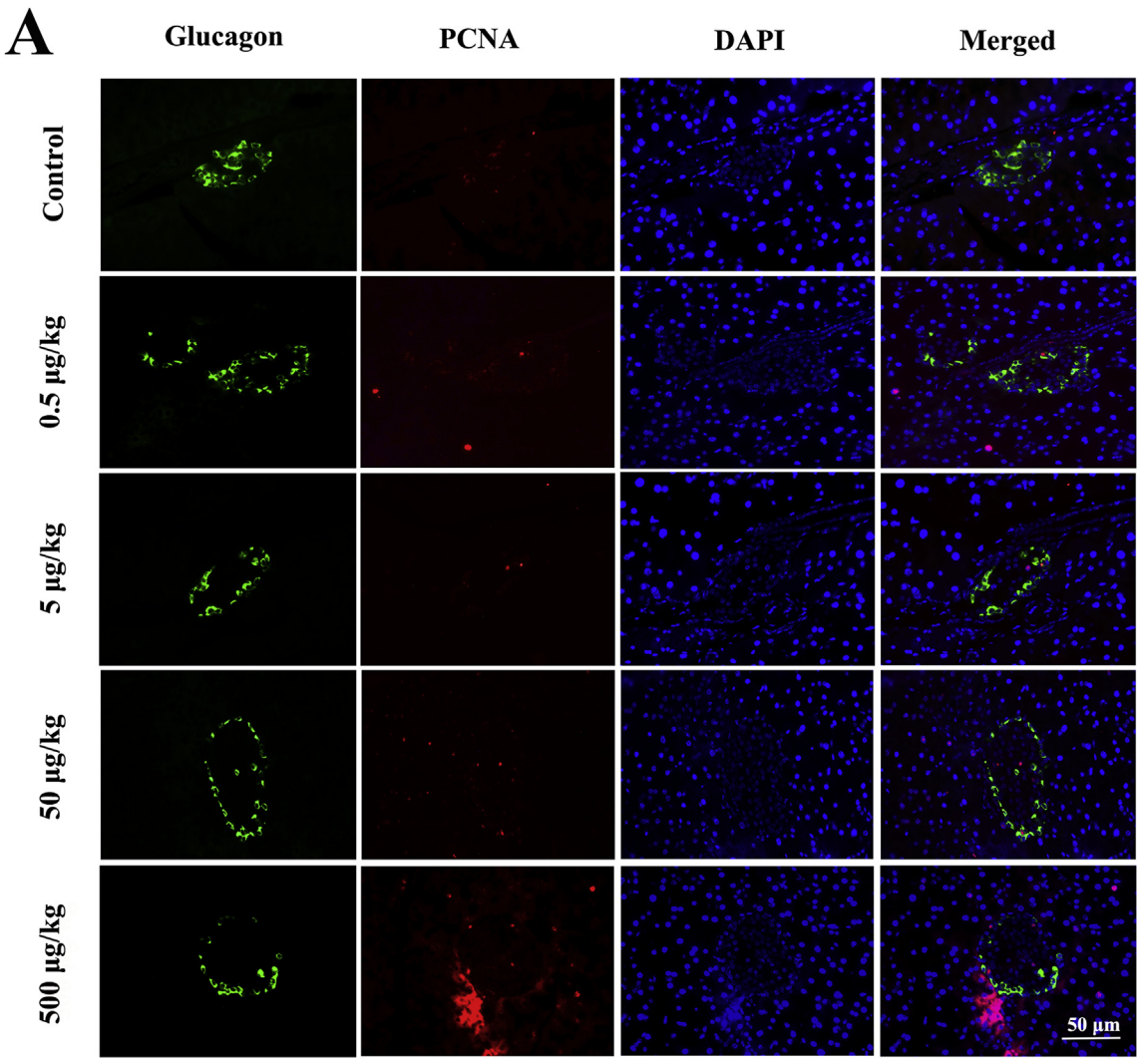

Fig. 5. Cell proliferation in the islet α-cells of male mice exposed to Aroclor 1254 for 60 days. (A) Representative images of triple staining for glucagon (green), PCNA (red), and DAPI (blue) in pancreas. (B) α-cell proliferation was quantified by percentage of PCNA-positive α-cells. Data are presented as mean ± SE (n = 4). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

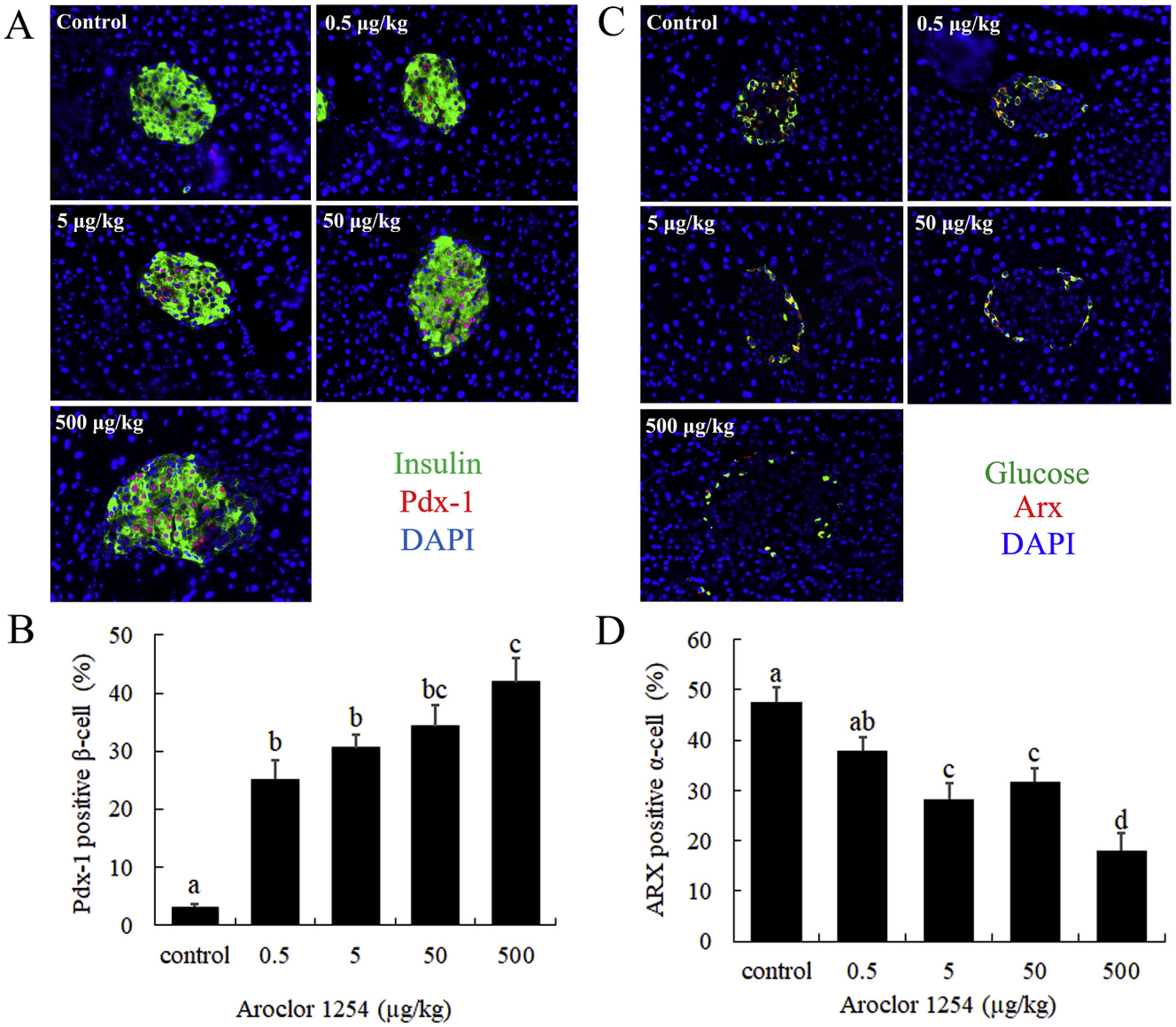

Fig. 6. Immunofluorescence analysis of Pdx1 and Arx expression in islet cells of male mice exposed to Aroclor 1254 for 60 days. (A) Representative images of triple staining for insulin (green), Pdx-1 (red), and DAPI (blue) on fixed, paraffin-embedded pancreas sections. (B) Percentage of Pdx-1 positive data are presented as mean ± SE (n = 3). (C) Representative images of triple staining for glucagon (green), Arx (red), and DAPI (blue) on fixed, paraffin-embedded pancreas sections. (D) Percentage of Arx positive data are presented as mean ± SE (n = 3). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2000), rabbit chondrocytes (Lee and Yang, 2012) and mouse splenocytes (Yoo et al., 1997). As our knowledge, the current study showed for the first time that PCBs exposure induced the apoptosis of islet cells in male mice, including both α-cells and β-cells.

PCNA is an intranuclear polypeptide whose expression and synthesis are linked with cell proliferation (Kubben et al., 1994). After exposure to TCDD (100 ng/kg) for 10 weeks, PCNA positive cells were elevated in pancreatic exocrine cells (Yoshizawa et al., 2005). Maternal exposure to BPA (10 and 100 μg/kg) during pregnancy increased pancreatic β-cell proliferation during early life in male mice offspring (García-Arévalo et al., 2016). In contrast, PCNA positive cells decreased in the islets after exposure to TBT (50 μg/kg) for 45 days (Zuo et al., 2014). Cell mass is a reflection of the dynamic balance between rates of cell generation and death. The result in the present study showed that Aroclor 1254 exposure increased β-cell proliferation, which could make up the decrease of β-cell number caused by apoptosis. However, the proliferation of α-cells was not affected, which resulted in the decrease of α-cell mass.

Pdx-1 plays essential roles in maintaining β-cell function and survival (Chan et al., 2012; Kaneto and Matsuoka, 2015; Pedica et al., 2014). In Pdx-1 haploinsufficient mice, diabetes develops due to an increase in β-cell death leading to reduced β-cell mass (Sun et al., 2016). The expression level of Pdx-1 is directly bound up with the β-cells mass (Fujimoto et al., 2010; Sun et al., 2016). Mice lacking Pdx-1 develop β-cell failure, suggesting that pdx-1 is required to maintain an adequate β-cell mass (Fujimoto and Polonsky, 2009). In the present study, the up-regulated expression of Pdx-1 was associated with the increased β-cell mass.

As a marker transcription factor for the α-cell lineage, Arx is required for α-cell development (Bramswig and Kaestner, 2011) and directly maintains α-cell mass (Courtney et al., 2013). Arx mutant mice display a loss of mature α-cells and exhibit severe hypoglycemia (Collombat et al., 2007). Mice with Arx null mutations in the germ-line, pancreatic progenitors, or endocrine progenitors all display a complete

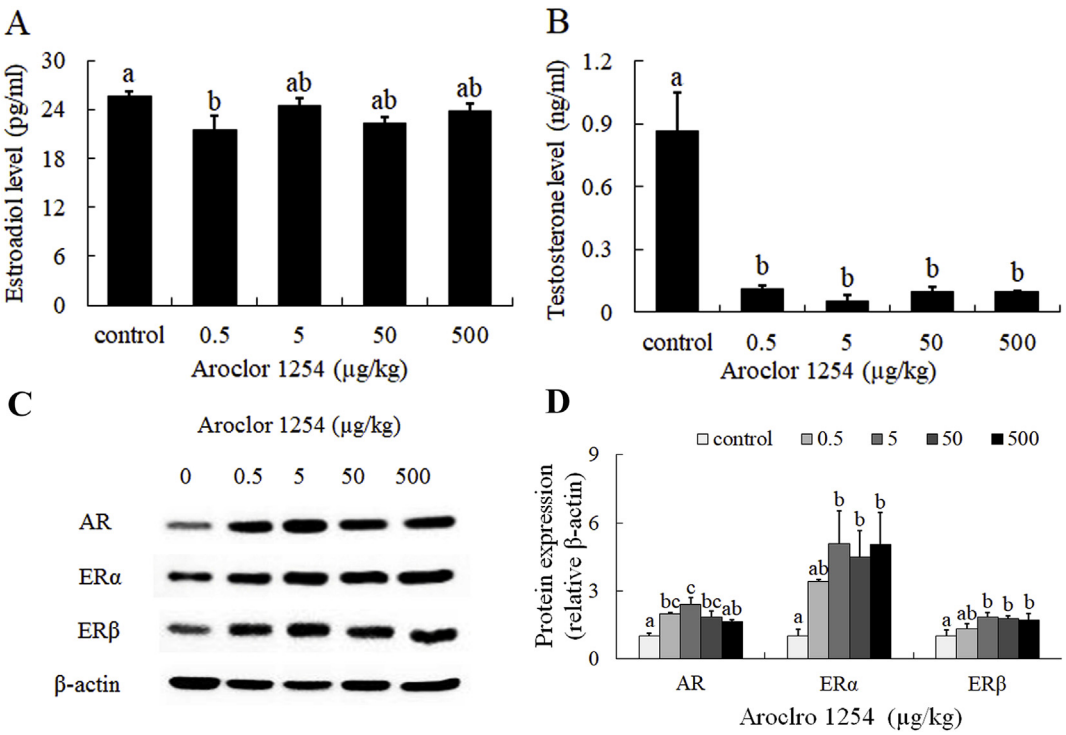

Fig. 7. The serum levels of sex hormones and Western blot analysis of estrogen receptors and androgen receptor in the pancreas of male mice treated with Aroclor 1254 for 60 days. (A) The serum levels of estradiol. (B) The serum levels of testosterone. The data are expressed as mean ± SE (n = 6). (C) A representative chemiluminescent detection of protein expression was shown. (D) Intensities of protein bands were quantified by densitometry, respectively. Results were expressed as folds of optical density of target protein and the β-actin determined in control. The mean protein expression from the control was designated as 1 in the graph. Values (mean ± S.E.) are representative of data obtained in three independent experiments (n = 3). Means of exposures not sharing a common letter are significantly different at $P < 0.05$ as assessed by one-way ANOVA followed by the Duncan test.

loss of α-cells with a concurrent increase in β-cells and δ-cells in the pancreas (Collombat et al., 2007; Mastracci et al., 2011). In the present study, the down-regulated expression of Arx was consistent with the decreased α-cell mass.

Some previous studies have illustrated that both anti-androgenic and weak estrogenic activities of non-dioxin-like PCBs are the most prominent in vitro effects (Hamers et al., 2011; Takeuchi et al., 2017). For instance, the female rats treated with a reconstituted mixture PCBs during pregnancy and lactation decreased the levels of estradiol and testosterone in female offspring, while only serum testosterone reduced in the exposed male offspring (Kaya et al., 2002). Similarly, gestational exposure to PCB126 or PCB169 resulted in reduced levels of serum testosterone in weanling male offspring rats (Yamamoto et al., 2005). As previously reported, greater concentrations of serum PCBs were associated with reduced testosterone levels in adolescent (Schell et al., 2013) and adult males (Goncharov et al., 2009). The present study demonstrated a moderate decrease of estradiol levels and a dramatic decrease of testosterone levels in male mice exposed to Aroclor 1254, this result is consistent with the anti-androgenic effects indicated by the previous studies.

Earlier studies suggest that E_2 can protect β-cell survival though ERα in various animal models (Alonso-Magdalena et al., 2008). Yuchi et al. (2015) revealed that ERα activity contributes to the increase of pancreatic β-cell proliferation in mice induced by partial duct ligation. However, up to now, evidence points to ERβ as another main mediator. Alonso-Magdalena et al. (2013) proposed that an ERβ agonist acted as a regulator of pancreatic β-cell growth and promoted an elevation of β-cell mass under pathological conditions. Moreover, E_2 protects β-cell survival through ERα and ERβ via estrogen response element independent extra-nuclear mechanisms (Liu et al., 2009). In the present study, the expression of ERα and ERβ were significantly increased in the pancreas of male mice, suggesting that exposure to Aroclor 1254 increased β-cell mass via the up-regulation of ERα and ERβ. However, ERα and ERβ are absent from α-cells (Le May et al., 2006; Liu et al., 2009), resulting in no significantly increase in α-cell proliferation and α-cell mass.

Low blood testosterone levels have been confirmed to be one of the risk factors for the development of T2DM in men (Ding et al., 2006; Fukui et al., 2007), such as androgen deprivation in prostate cancer patients, which increased risk of T2DM (Keating et al., 2006; Pelley et al., 2006). In this study, the reduction of androgen levels might be a cause for the IR in male mice exposed to Aroclor 1254 (Zhang et al., 2015). Although the role of AR in obesity and insulin sensitivity in male mammals is known, the role of AR in islet function has not been fully investigated (Mauvais-Jarvis, 2016; Navarro et al., 2015). Adult male mice with β-cell selective AR deficiency exhibited a reduction in glucose-stimulated insulin secretion, resulting in glucose intolerance (Navarro et al., 2016). Harada et al. (2017) showed that AR in β-cells was involved in androgen signaling-mediated enhancement in β-cell mass. Moreover, the AR is located in male rat β-cells (Harada et al., 2012), but was not observed in α-cells (Li et al., 2008; Navarro et al., 2016). Thus, significantly higher expression of AR in pancreatic tissue of male mice exposed to PCBs might be one of the reasons for the increase in β-cell mass.

In summary, subchronic exposure to Aroclor 1254 increased pancreatic β-cell mass but decreased α-cell mass. The up-regulation of pancreatic ERα, ERβ and AR expression might be responsible for the enhancement of pancreatic β-cell proliferation and mass. The results indicated that pancreatic β-cells would have a compensatory adaptation during the stage of IR caused by PCBs exposure, while decreased pancreatic α-cells would arise a defect in response to hypoglycemia.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109875.

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