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Inorganic arsenic induces pyroptosis and pancreatic β cells dysfunction through stimulating the IRE1α/TNF-α pathway and protective effect of taurine

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ABSTRACT

Low-level inorganic arsenic (iAs) in drinking water is a risk factor for β cells dysfunction. Taurine (Tau) is a kind of semi-essential β amino acid, and beneficial for β cell function. However, the effects of Tau on arsenic trioxide (As_2O_3) induced β cells dysfunction and related mechanisms are still uncertain. Here, we found that Tau relieved As_2O_3-induced endoplasmic reticulum (ER) stress, inflammation and pyroptosis in rat pancreas. In INS-1 cells, with NOD-like receptor family pyrin domain-containing 3 (NLRP3) inhibitor pretreatment, As_2O_3-induced activation of pyroptosis was decreased; with tumor necrosis factor-α (TNF-α) inhibitor pretreatment, As_2O_3-induced activation of NLRP3 inflammasome and pyroptosis were decreased; further, with the inositol-requiring enzyme 1 alpha (IRE1α) inhibitor, As_2O_3-induced induction of TNF-α was decreased. Tau markedly protected As_2O_3-induced β cells dysfunction by reducing the phosphorylation of IRE1α, production of TNF-α, activation of NLRP3 inflammasome and pyroptosis. Our results revealed that ER stress dependent inflammation and pyroptosis are critical pathogenic components of As_2O_3-induced β cell dysfunction. Moreover, TNF-α was a critical signaling node that linked As_2O_3-induced ER stress and pyroptosis. Tau was an effective supplement against As_2O_3-induced β cells dysfunction through the pathway as mentioned above.

1. Introduction

Chronic exposure of arsenic causes cancers of multiple organs and non-cancerous diseases, including diabetes mellitus, which is a major public health problem over the world (Garcia-Esquinas et al., 2013; Paul et al., 2008). Epidemiological evidences indicate that chronic exposure to inorganic arsenic (iAs) suppresses insulin production of pancreatic β cells (Paul et al., 2008). Our previous studies demonstrated that arsenic trioxide (As_2O_3) suppressed insulin production in C57/BL6J mice (Wu et al., 2018b). In addition, exposure to iAs during pregnancy is detrimental to the health of infants and increases the risk of disease development later in life (Rojas et al., 2015).

Impaired glucose homeostasis and insulin deficiency caused by insufficiency and death of β cells are the main etiologies of type 1 diabetes mellitus (Ismail Nagwa et al., 2016). Islet inflammation plays an important role in insulin secretion disorder and β cells dysfunction (Eguchi et al., 2012). Recent findings indicated that NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome was involved in the link of inflammatory response and β cells dysfunction (Oslowski et al., 2012). NLRP3 inflammasome is comprised of cytoplasmic receptor NLRP3, pro-caspase-1 and a bridging adaptor, apoptosis-associated speck-like protein ASC (Gao et al., 2018). It has been

Abbreviations: As_2O_3, arsenic trioxide; FCM, flow cytometry; IAs, inorganic arsenic; IL-1β, interleukin-1β; IRE1α, inositol-requiring enzyme 1 alpha; KRB, Krebs-Ringer Bicarbonate Solution; LPS, lipopolysaccharides; LDH, lactate dehydrogenase; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; NLRP3, NOD-like receptor family pyrin domain-containing 3; PI, propidium iodide; Tau, taurine; TNF-α, tumor necrosis factor-α

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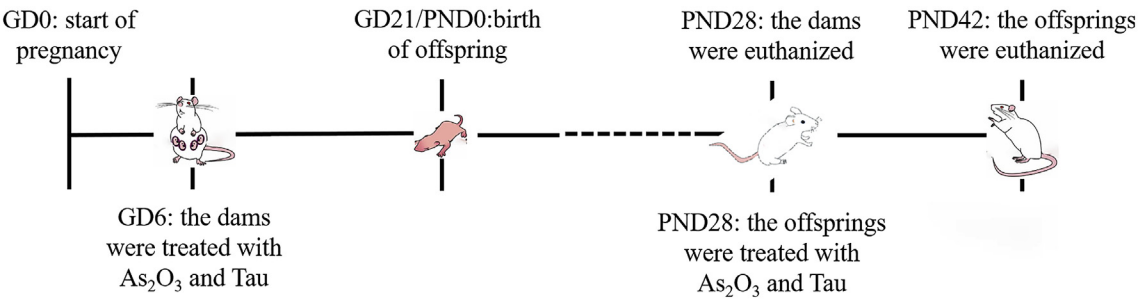

Fig. 1. Experimental design for in vivo experiments. The dams were given As_2O_3 and Tau by gavage once a day from day 6 of gestation (GD 6) until pup postnatal day 28 (PND 28) and the dams were euthanized. The offspring were given As_2O_3 and Tau by gavage once a day from PND 28 until PND 42. At last, the offspring were euthanized.

proved that NLRP3 inflammasome plays a critical role in pyroptosis (Bergsbaken et al., 2009). Pyroptosis is a programmed cell death with characteristics of caspase-1/11-dependent plasma membrane rupture, cell swelling, inflammasome formation and interleukin (IL)-1β secretion (Abe and Morrell, 2016; Chen et al., 2016; He et al., 2015; Wang et al., 2018). However, the mechanism of As_2O_3-induced pyroptosis in the pancreas is largely unknown.

Because of the rapid loss of cell membrane integrity and release of cytosolic inflammatory contents, pyroptosis is regarded to be a process of proinflammation (Miao et al., 2010). Previous studies have reported that the activation of NLRP3 inflammasome required additional proinflammatory factor (Bauernfeind et al., 2016). As a proinflammatory factor, tumor necrosis factor (TNF) α is a predominantly contributor to the pathogenesis of insulin resistance (Lorenzo et al., 2008), which was increased markedly in serum by arsenic through oxidative stress (Huang et al., 2017). In addition, TNF-α is a main inducer of caspase-1 activation in adipose tissue (Furuoka et al., 2016). Although many studies demonstrated the relationship between TNF-α and insulin resistance (Li et al., 2017; Monroy et al., 2009), the mechanisms underlying β cells dysfunction induced by As_2O_3 involved in TNF-α remains poorly understood. Furthermore, whether TNF-α mediates inflammasome formation and pyroptosis requires further studies.

Recent clinical evidences showed that ER stress played a main role in β cell dysfunction and death in patients with type 1 diabetes mellitus (Oslowski et al., 2012). ER stress contributes to NLRP3 inflammasome activation and subsequently cell pyroptosis by induction of lipopolysaccharides (LPS) (Lebeaupin et al., 2015). Experimental evidences suggested that all three ER stress signaling pathways were activated in SKH-1 hairless mice by drinking water containing arsenic (Li et al., 2011). Inositol-requiring enzyme 1 alpha (IRE1α), one of the three classic ER stress signaling pathways, is a key driver of obesity-induced inflammation and TNF-α expression (Bujisic and Martinon, 2017; Shan et al., 2017). However, whether IRE1α mediates NLRP3 inflammasome activation and caspase-1-dependent pyroptosis in pancreas are still unclear.

Taurine (Tau) is one of semi-essential β amino acid found in most tissues with high concentration (Gaull, 1986). One of the most important physiological functions of Tau is the ER stress regulation, which contributes to its anti-inflammatory effect (Jong et al., 2017). Tau markedly decreased plasma glucose levels in alloxan-induced diabetic rats via the potent insulin effect by protecting pancreatic β cells (Das et al., 2012). In addition, Tau supplementation during pregnancy in rats restored low-protein diet-induced abnormal development of β cell mass and islet vulnerability, rehabilitated insulin like growth factor levels in the endocrine pancreas (Boujendar et al., 2002).

In this study, we hypothesized that ER stress triggered by As_2O_3 induced INS-1 cells pyroptosis via TNF-α, and the activation of NLRP3 inflammasome was involved. Further, we sought to investigate the protective effect and mechanism of Tau on β cells dysfunction induced by As_2O_3.

2. Materials and methods

2.1. Ethics statement

The Animal Ethics Committee of the Institute of Zoology, Dalian Medical University, approved this experimental design. The institute issued an ID [SCKK (Liao) 2002–002] to this animal study and the ethical committee guided the animal use and conduct.

2.2. Animal models

To study the effect of As_2O_3 on offspring rats, all rats were 8 weeks old in our experiment and all the adult Wistar rats weighed 230–250 g were obtained from Dalian Medical University. Day 0 of gestation (GD 0) was designated as the day when sperm plug was confirmed, all the pregnant rats were randomly divided into five groups and were kept in individually house at 20–25 °C and 12 h light-dark cycle and maintained 50–70% humidity with food and water ad libitum. Six pregnant rats in each group, and one offspring from each litter were randomly selected for the experiment. Group 1: Control group. Dams were treated with distilled water. The offspring were treated as their mothers. Group 2: Dams were treated with 2 mg/kg BW As_2O_3(Sigma-Aldrich, USA). The offspring were treated as their mothers. Group 3: Dams were treated with 4 mg/kg BW As_2O_3. The offspring were treated as their mothers. Group 4: Dams were treated with 8 mg/kg BW As_2O_3. The offspring were treated as their mothers. Group 5: Dams were treated with 8 mg/kg BW As_2O_3 and 150 mg/kg Tau. The offspring were treated as their mothers. The schematic diagram has been shown in Fig. 1.

2.3. Cell culture

The INS-1 cells originated from a rat insulinoma cell line were developed and propagated at the China Center for Type Culture Collection (CCTCC, China). All cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (Hyclone, USA), and in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells were trypsinized at 0.25% trypsin solution for 45 s and passaged in complete culture medium. INS-1 cells were treated with 1, 2, 4 μM of As_2O_3 for 24 h. Before treatment of As_2O_3, cells were pretreated with 100 μM Tau, 50 μM IRE1α inhibitor irestatin 9389 (Axon Medchem, Holland) for 6 h, 5 μM NLRP3 inhibitor MCC950 (MCE, China), 1 μg/ml LPS (Sigma-Aldrich, USA) for 4 h, and 5 μM TNF-α inhibitor lenalidomide (MCE, China) were co-treated with As_2O_3 for 24 h.

2.4. Cell viability assay

Cell viability was investigated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Solarbio, USA). Firstly, INS-1 (2×10^4) cells were seeded in 100 μl medium in 96 well culture plate.

Secondly, INS-1 cells were incubated at 37 °C with 5% CO_2 for 24 h. Thirdly, INS-1 cells were treated with different concentrations (0, 1, 2, 4, 8, 16, 32 and 64 μM) As_2O_3 for 24 h. After treatment, 100 μl of MTT solution (0.5 mg/ml) was added each well and cultured for 4 h and then 100 μl DMSO was added each well and cultured 30min. Finally, the viability of the cells was recorded at an absorbance of 570 nm. The cell viability (%) was calculated (A570 of treated sample/A570 of control × 100%).

2.5. Immunofluorescence staining

INS-1 Cells (1×10^5) were cultured in 24 well culture plate and fixed with 4% paraformaldehyde for 25 min, washed twice with PBS, and then permeabilized with 0.1% Triton X-100 for 20 min at room temperature. INS-1 Cells were stained with anti-p-IRE1α (Abcam, USA, 1:200) antibody at 4 °C overnight, and subsequently Alexa Fluor 488-conjugated goat anti-rabbit (Proteintech, China, 1:250) was used in the dark, 37 °C for 1 h. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, Keygen, China) for 10 min. Images were captured using a fluorescence microscope (40 × 10). Data were analyzed by Image-Pro-Plus 7.0 software.

2.6. Western blot

Protein was extracted using RIPA buffer from part of rat pancreas or the INS-1 cells. RIPA containing 1 mM phenylmethylsulfonyl fluoride (PMSF, Keygen, China), 1 mM protease inhibitor (Keygen, China) and 1 mM phosphatase inhibitors (Keygen, China). Protein quantification was done with BCA Protein Assay kit (Thermo, USA). Proteins were separated in 8–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were blotted to PVDF membranes (Merck Millipore, USA). Membranes were blocked for 1 h with 10% powdered skimmed milk by gentle shaking at 37 °C water bath shaker and then incubated with the following primary antibodies: IRE1α (Santa, USA, 1:500), p-IRE1α (Abcam, USA, 1:500), NLRP3 (Wanleibio, China, 1:500), pro-caspase-1 (Abcam, USA, 1:1000), caspase-1 p20 (Abcam, USA, 1:1000), pro-IL-1β (Wanleibio, China, 1:1000), IL-1β p17 (Wanleibio, China, 1:1000), MPO (Abcam, USA, 1:1000), TNF-α (Wanleibio, China, 1:500), β-actin (ZSGB-BIO, China, 1:1000), overnight at 4 °C. After washing with TBST 10 min every time, followed by incubation with HRP-conjugated secondary antibody for 2 h in a shaker room and at room temperature. Protein expression levels were tested using an ECL kit (Beyotime, China), imaged on a Bio-Rad ChemiDocTMMP system, and analyzed by ImageJ software.

2.7. Flow cytometric analysis

Pyroptotic INS-1 cells were examined by flow cytometry (FCM, ImmunoChemistry Technologie, USA). Briefly, INS-1 Cells (5×10^5) in 6 well culture plate, after 24 h exposure to As_2O_3, were trypsinized and centrifuged at 1000 rpm in 1.5 ml microcentrifuge tubes. FLICA 660-YVAD-FMK were directly added to 300 μl of the cell suspension for 30 min at room temperature in the dark to determine the intracellular activation of caspase-1. INS-1 cells were washed once with PBS, centrifuged at 1000 rpm, resuspended, and then propidium iodide (PI, Keygen, China) was added. The pyroptosis cells were measured using an ACEA NovoCyte flow cytometer (NovoCyte, 2040R, USA), and pyroptosis was quantitated the proportion as double positive for caspase-1 and PI.

2.8. LDH release assay

INS-1 Cells (1×10^5) were cultured in 24 well culture plate, LDH levels were measured by the lactate dehydrogenase (LDH) assay kit (Keygen, China). The percentage LDH release was calculated as described in manufacturer's protocol. The absorbance was measured using the microplate reader (Thermo Fisher Scientific, USA) at 450 nm.

2.9. Insulin release assay

INS-1 cells were plated into 6-well plates (5×10^5 cells/well) and pretreated with inhibitors and As_2O_3 as described above. For insulin secretion studies, INS-1 cells were first kept at 37 °C for 30 min in Krebs-Ringer Bicarbonate Solution (KRB), pH 7.4. Cells were then incubated for 2 h at 37 °C in 2 ml of fresh KRB buffer containing 2.8 mM or 17.6 mM glucose. At the end of the incubation period, the supernatant fraction was collected and centrifuged at 1000 rpm for 20 min to remove any floating cells. Insulin was measured with the Rat INS (Insulin) ELISA Kit (Finetest, China).

2.10. Statistical analysis

Data was reported as mean ± standard deviation than or equal to three independent experiments and analyzed with the one-way analysis of variance (ANOVA) or *t*-test. *P* values were all two-sided and a *P* value < 0.05 was considered as a statistically significant.

3. Results

3.1. Effects of Tau on As_2O_3-caused ER stress, pyroptosis and inflammation in pancreas of rat offspring

The effects of Tau on pancreas of rat offspring exposed to As_2O_3 were measured by H&E and Western blot (Fig. 2). As shown in Fig. 2A, compared with control group, the structures of pancreas in As_2O_3-treated groups were irregularly arranged and unclear, with glands swelling. After Tau treatment, improvements on the morphology of pancreas were observed. The acini were well arranged and organized in an orderly fashion and the outline was clear. Moreover, gland swelling was relieved. The IRE1α, phosphorylated IRE1α (p-IRE1α), TNF-α, NLRP3 inflammasome, pro-caspase-1, cleaved caspase-1 p20 and IL-1β, MPO expression in the pancreas of rat offspring were measured by Western blot (Fig. 2). As shown in Fig. 2B, p-IRE1α level was significantly increased in the As_2O_3 contamination group at 8 mg/kg ($P < 0.05$) and TNF-α were significantly increased under As_2O_3 exposure at 4 mg/kg and 8 mg/kg (both $P < 0.01$). Correspondingly, the expression of NLRP3 and caspase-1 p20 were markedly augmented by As_2O_3 at concentration of 4 mg/kg and 8 mg/kg, although the expression of ASC was not altered. Moreover, expressions of IL-1β and MPO, but not pro-IL-1β p35, were significantly higher in As_2O_3-treated pancreas than control group (Fig. 2C). However, the expressions of p-IRE1α, TNF-α, NLRP3 inflammasome, IL-1β and MPO were all significantly decreased in Tau treated group compared with only As_2O_3-treated group (all $P < 0.05$) (Fig. 2B and C).

3.2. As_2O_3 induced the activation of IRE1α ER stress pathway, up-regulated the level of TNF-α and pyroptosis in INS-1 cells

INS-1 cells treated with As_2O_3 showed an obvious dose-dependent growth inhibition effect (Fig. 3A), thus As_2O_3 at the concentrations of 1 μM, 2 μM and 4 μM were chosen to treat the INS-1 cells. To investigate the effect of As_2O_3 on the activation of IRE1α, the immunofluorescence staining of p-IRE1α was used. As in Fig. 3B, the fluorescence intensity was significantly increased in As_2O_3-treated groups (2 μM, 4 μM) compared with the control group (both $P < 0.01$). Simultaneously, TNF-α expression was significantly elevated by 4 μM of As_2O_3 ($P < 0.05$) (Fig. 3C). Further, As_2O_3 evoked pyroptosis evidenced by increased expression of NLRP3, caspase-1 p20 and IL-1β p17, but not pro-caspase-1, pro-IL-1β and ASC. (Fig. 3D).

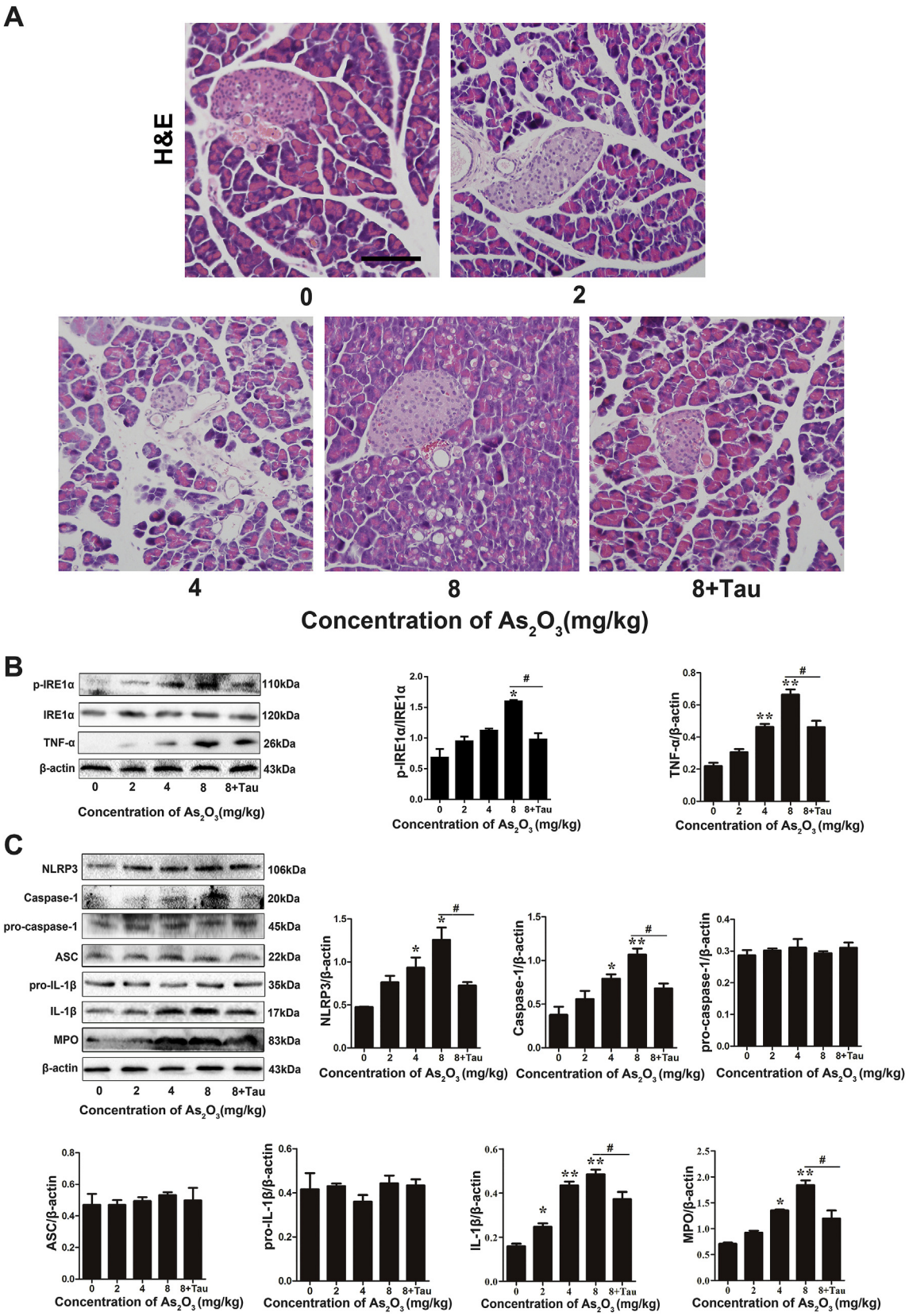

Fig. 2. The effect of As_2O_3 on ER stress, pyroptosis and inflammation in rat pancreas. The rats were treated with 2 mg/kg BW to 8 mg/kg BW As_2O_3. The rats of group 5 were co-treated with 150 mg/kg Tau and 8 mg/kg BW As_2O_3. The cytosolic fractions were analyzed by Western blot. β-actin was used as an internal control. (A) H&E staining of offspring's pancreas sections after As_2O_3 administration and pretreatment with Tau (scale bar = 50 μm). (B) The protein level and densitometric analyses of p-IRE1α/IRE1α ratio and TNF-α expressed in pancreas tissues. (n = 6). (C) NLRP3, ASC, caspase-1, IL-1β and MPO expression and densitometric analyses in pancreas tissues. (n = 6). The bar represents the means ± SD; $^*P < 0.05$ vs. control; $^{**}P < 0.01$ vs. control; $\#P < 0.05$ vs. 8 mg/kg group.

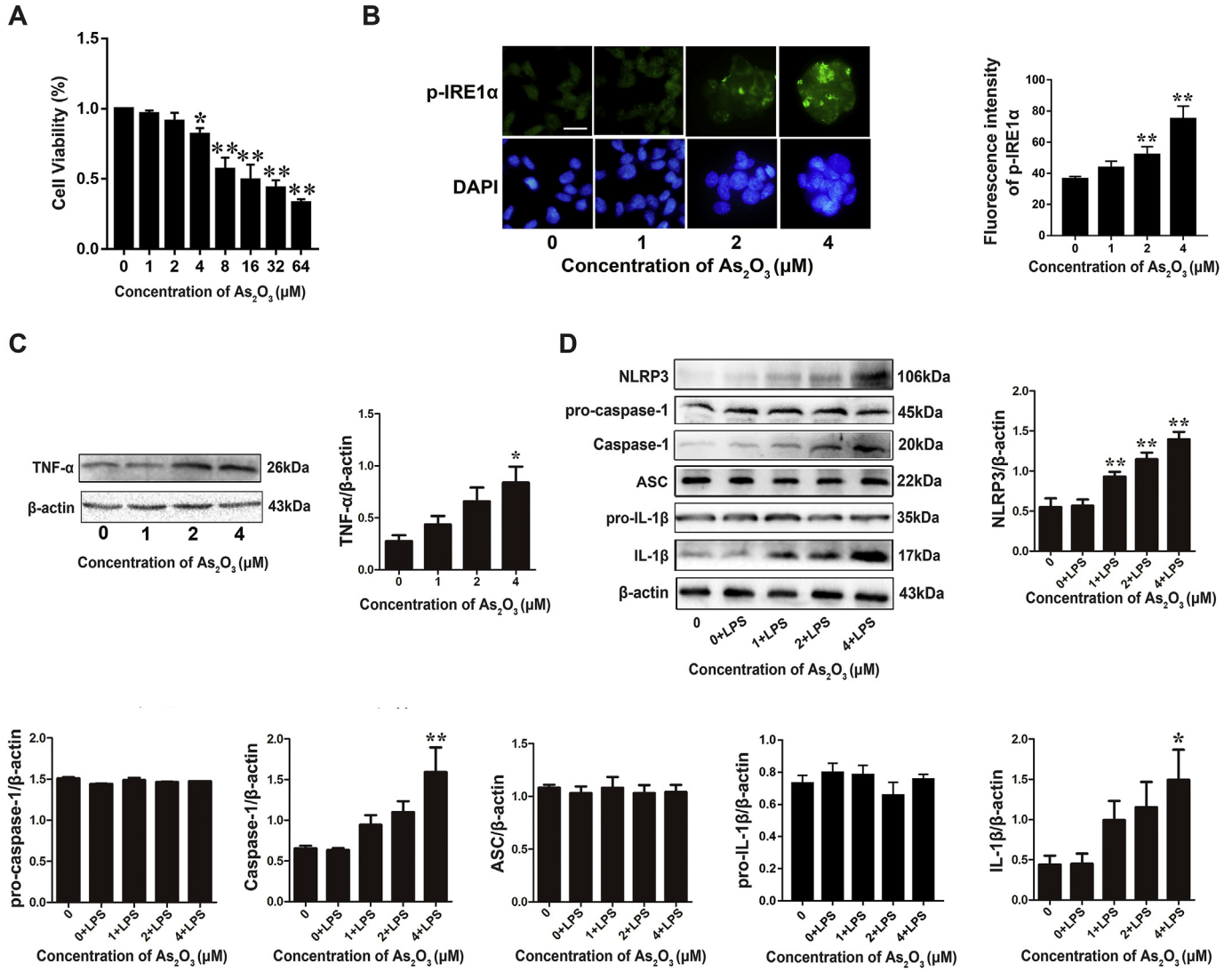

Fig. 3. The effect of As_2O_3 on IRE1α, TNF-α and pyroptosis in INS-1 cells. (A) INS-1 cells were treated with 1, 2, 4, 8, 16, 32, 64 μM As_2O_3 for 24 h. Effects of As_2O_3 on cell viability in INS-1 cells. (B) INS-1 cells were treated with 1, 2, 4 μM As_2O_3 for 24 h. The p-IRE1α were measured by immunofluorescence staining and quantification of green fluorescence intensity were analyzed in INS-1 cells. Magnification: ×400. Scale bar = 20 μm. (C) INS-1 cells were treated with 1, 2, 4 μM As_2O_3 for 24 h. TNF-α expression and densitometric analyses in INS-1 cells. β-actin was used as an internal control. (D) INS-1 cells were pretreated with 1 μg/ml LPS for 4 h, and then treated with 1, 2, 4 μM As_2O_3 for 24 h. NLRP3, ASC, caspase-1, IL-1β expression and densitometric analyses in INS-1 cells. (n = 3). β-actin was used as an internal control. The bar represents the means ± SD; *$P < 0.05$ vs. control; **$P < 0.01$ vs. control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. NLRP3 inflammasome regulated As_2O_3-induced pyroptosis in INS-1 cells

To test whether the As_2O_3-induced pyroptosis depends on activation of NLRP3 inflammasome, the NLRP3-specific inhibitor MCC950 was used. As described in Fig. 4A, MCC950 restored the elevated protein level of NLRP3, caspase-1 p20 and IL-1β induced by As_2O_3 (4 μM) in INS-1 cells. We then took advantage of flow cytometry to further investigate whether pyroptosis occurred and to measure the membrane pore formation. As exhibited in Fig. 4B, double positive for PI and FLICA 660-YVAD-FMK, a caspase-1 specific marker, suggested that pyroptosis was suppressed in cells pretreated by MCC950 under As_2O_3 administration ($P < 0.01$). Moreover, MCC950 diminished the level of LDH release rate in INS-1 cells under As_2O_3 stress ($P < 0.05$) (Fig. 4C), representing that the cell membrane damaged by As_2O_3 was relieved after inhibition of NLRP3. Importantly, MCC950 treatment promoted insulin release suppressed by As_2O_3 ($P < 0.05$) (Fig. 4D). Those consistent data indicated that the NLRP3 inhibitor was beneficial for As_2O_3-induced pyroptosis and improved loss of insulin-secreting capability in INS-1 cells.

3.4. TNF-α regulated As_2O_3-induced NLRP3 inflammasome activation in INS-1 cells

To survey the role of TNF-α in As_2O_3-induced activation of NLRP3 inflammasome and pyroptosis, an inhibitor lenalidomide was used to block TNF-α production. As depicted in Fig. 5A, As_2O_3-induced expression of caspase-1 p20 and IL-1β were obliterated by lenalidomide treatment (both $P < 0.05$). Simultaneously, double positive for PI and FLICA 660-YVAD-FMK rate elevated by As_2O_3 were suppressed by lenalidomide ($P < 0.05$) (Fig. 5B). In addition, the level of LDH release stimulated by As_2O_3 was relevantly decreased by lenalidomide ($P < 0.05$) (Fig. 5C). In contrast, lenalidomide promoted insulin secretion of INS-1 cells under As_2O_3-induced injury ($P < 0.01$) (Fig. 5D). Thus, TNF-α regulated As_2O_3-induced NLRP3 inflammasome activation in INS-1 cells.

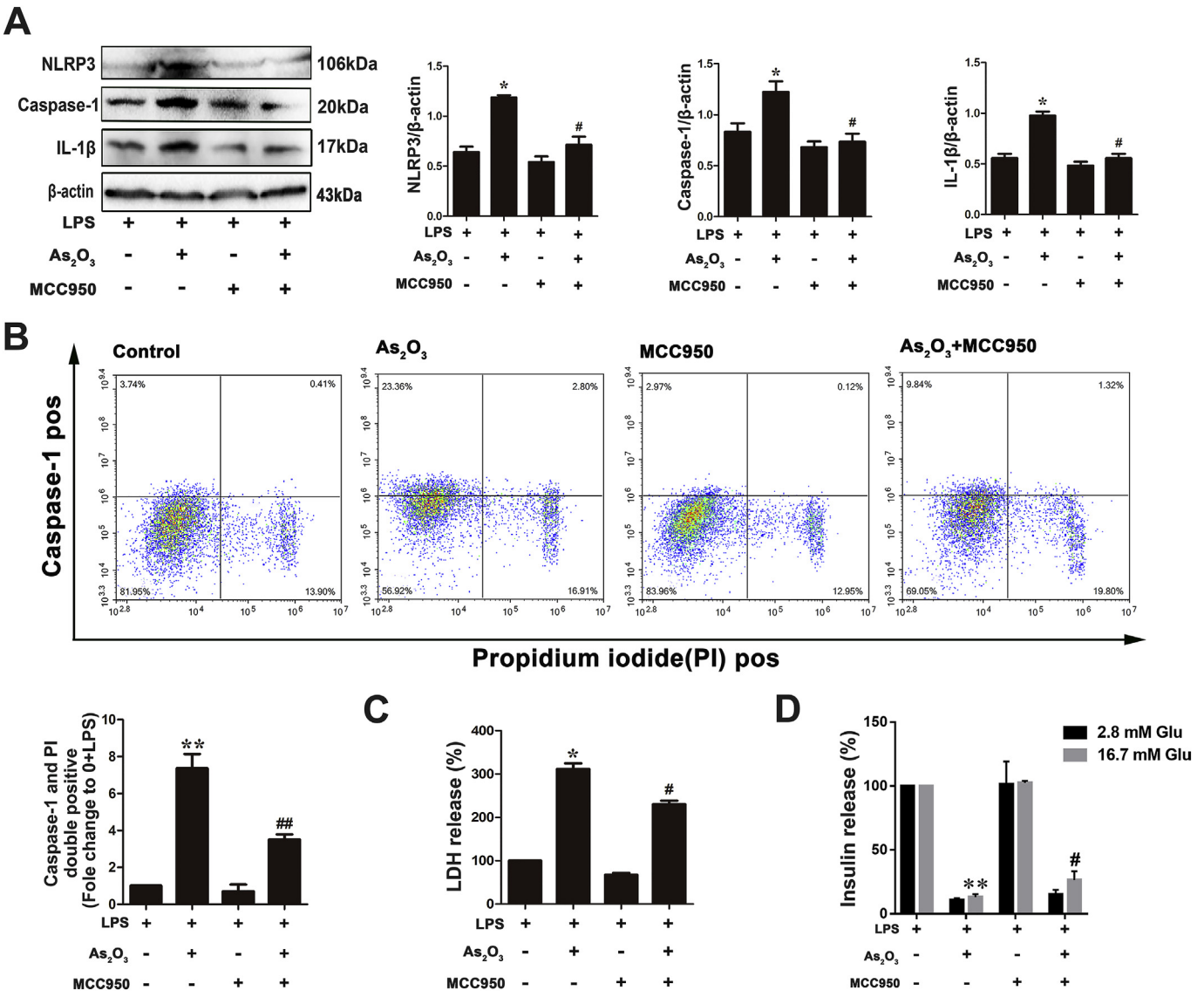

Fig. 4. Pyroptosis induced by As_2O_3 in INS-1 cells was NLRP3 inflammasome-dependent. INS-1 cells were pretreated with 5 μM MCC950, a NLRP3 inhibitor, and 1 μg/ml LPS for 4 h, and then treated with 4 μM As_2O_3 for 24 h. (A) The efficiency of MCC950, and its effect on caspase-1 activation and IL-1β production in As_2O_3-treated INS-1 cell, and densitometric analyses of NLRP3, caspase-1 p20, and IL-1β. β-actin was used as an internal control. (B) Effects of MCC950 on As_2O_3-induced double positivity for PI and activated caspase-1 staining. (C) Effects of As_2O_3 and MCC950 on LDH release rate in INS-1 cells. (D) The INS-1 cells were stimulated with 2.8 mM and 16.7 mM glucose respectively. The relative amounts of insulin secreted were measured using an ELISA kit. The bar represents the means ± SD; (n = 3). *$P < 0.05$ vs. control; **$P < 0.01$ vs. control; #$P < 0.05$ vs. 4 μM As_2O_3 group; ##$P < 0.01$ vs. 4 μM As_2O_3 group.

3.5. Blocking IRE1α decreased As_2O_3-induced production of TNF-α and activation of pyroptosis in INS-1 cells

To corroborate that IRE1α mediated production of TNF-α and pyroptosis, the specific inhibitor of IRE1α, irestatin 9389 was applied. Co-treatment of the cells with IRE1α inhibitor and As_2O_3 modestly attenuated TNF-α expression compared to As_2O_3-treated group under ER stress condition ($P < 0.05$) (Fig. 6A); likewise, irestatin 9389 had a similar inhibition on the expression of caspase-1 p20 and IL-1β upregulated by As_2O_3 (both $P < 0.05$) (Fig. 6A). Notably, IRE1α inhibition blocked LDH release ($P < 0.05$) (Fig. 6C) and decreased the number of FLICA 660-YVAD-FMK and PI double-positive cells in the INS-1 cells ($P < 0.01$) (Fig. 6B). As shown in Fig. 6D, irestatin 9389 increased insulin release compared to As_2O_3-treated group ($P < 0.01$). In brief, these results demonstrated that IRE1α played an important role in the As_2O_3-induced pyroptosis through eliciting TNF-α production.

3.6. Tau attenuated As_2O_3-induced pyroptosis via reducing activation of IRE1α and production of TNF-α

To explore whether Tau protected INS-1 cells from As_2O_3-induced pyroptosis through activation of IRE1α and TNF-α, we first detected the expression of p-IRE1α. As Fig. 7A show: there was obvious positive staining for p-IRE1α in As_2O_3-treated group compared with control group; however, there was much weaker fluorescence staining for p-IRE1α in the Tau and As_2O_3 co-treated group ($P < 0.01$). We then analyzed the protein expressions of TNF-α and pyroptosis related proteins with Western blot. As demonstrated in Fig. 7B, pretreatment of Tau diminished As_2O_3-induced expressions of TNF-α, NLRP3, caspase-1 p20 and IL-1β in INS-1 cells (all $P < 0.05$). Similar results were observed in caspase-1-positive cell staining (Fig. 7C) and LDH release rate (Fig. 7D), demonstrating that pyroptosis was significantly reduced by Tau. Importantly, Tau significantly increased the insulin release level of the As_2O_3-treated INS-1 cells ($P < 0.01$) (Fig. 7E). These findings suggested that Tau protected INS-1 cells against As_2O_3-induced

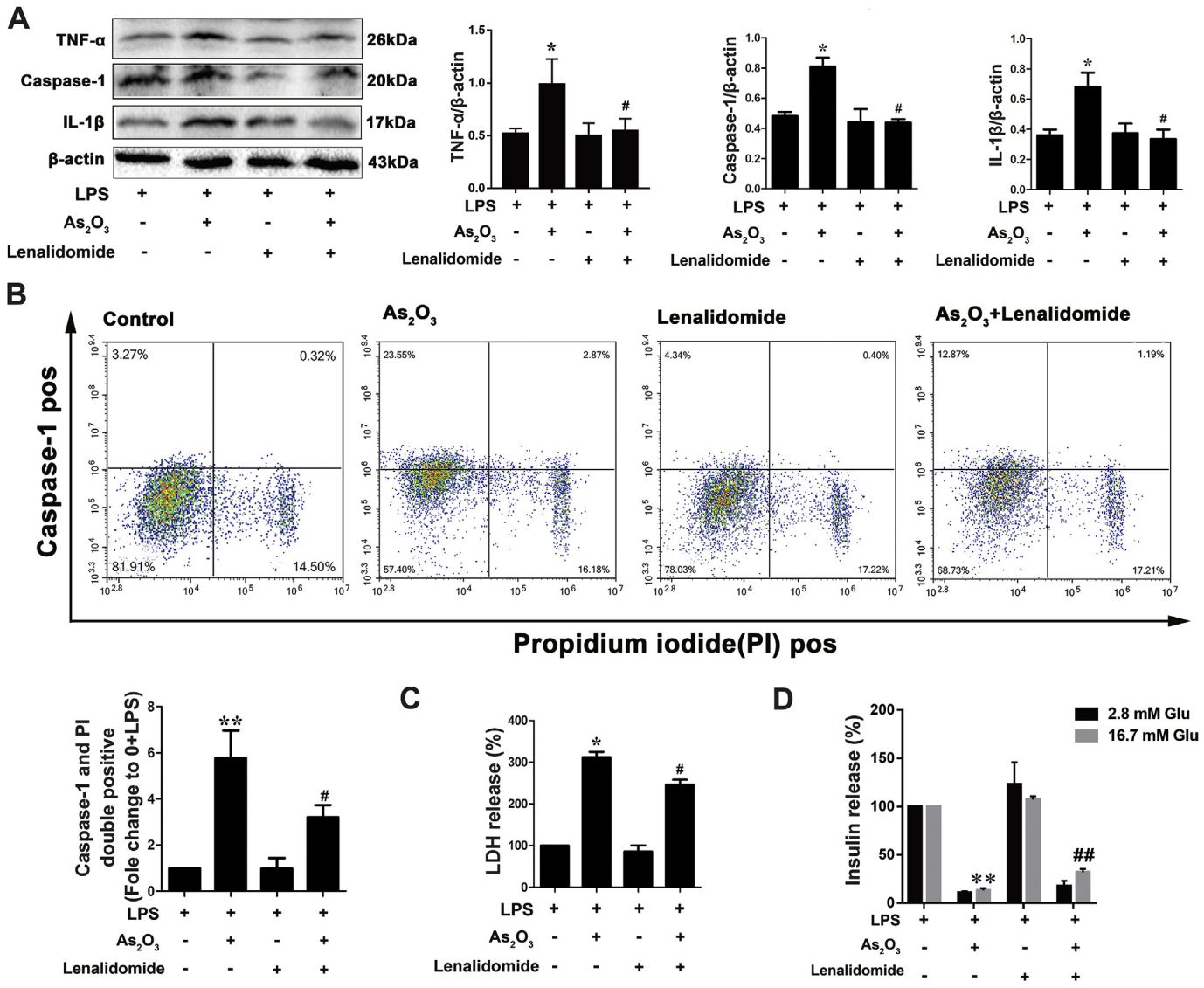

Fig. 5. TNF-α mediated the As_2O_3-induced activation of NLRP3 inflammasomes and pyroptotic cell death. INS-1 cells were pretreated with 1 μg/ml LPS for 4 h, and then co-treated with 5 μM lenalidomide and 4 μM As_2O_3 for 24 h. (A) The efficiency of lenalidomide, and its effect on caspase-1 activation and IL-1β production in As_2O_3-treated INS-1 cells, and densitometric analyses of TNF-α, caspase-1 p20, and IL-1β. β-actin was used as an internal control. (B) Effects of lenalidomide on As2O3-induced double positivity for PI and activated caspase-1 staining. (C) Effects of As_2O_3 and lenalidomide on LDH release rate in INS-1 cells. (D) The INS-1 cells were stimulated with 2.8 mM and 16.7 mM glucose respectively. The relative amounts of insulin secreted were measured using an ELISA kit. The bar represents the means ± SD; (n = 3). *$P < 0.05$ vs. control; **$P < 0.01$ vs. control; #$P < 0.05$ vs. 4 μM As_2O_3 group; ##$p < 0.01$ vs. 4 μM As_2O_3 group.

pyroptosis and dysfunction through modulating IRE1α and TNF-α.

4. Discussion

Our study demonstrated that As_2O_3 upregulated the level of IRE1α, TNF-α and triggered NLRP3 inflammasome activation, leading to INS-1 cells pyroptosis and dysfunction. However, these effects could be improved by Tau. Importantly, we also demonstrated that As_2O_3-induced pyroptosis was depended on the TNF-α mediated activation of NLRP3-inflammasome.

Inorganic arsenic (iAs) has been particularly concerned due to its impact of long-term exposure during pregnancy and newborns. It has been suggested to increase the risk of adverse health effects on the fetus (Morello-Frosch et al., 2000). Therefore, we chose offspring of rats as the study subjects. Wistar rat is an ideal animal model to investigate pancreatic function, it has been used in previous studies from other groups (Peschke et al., 2008; Sadeghi et al., 2017). In addition, considerable amount of studies used Wistar rats as animal model to investigate β cells dysfunction (Koulajian et al., 2013; Tang et al., 2012). Therefore, we chose Wistar rats as in vivo experimental model in the present study. An epidemiology study identified pregnant mothers from at least 20 different countries, such as India, China, Romania and the United States, where the iAs concentrations exceeded 10 μg/L in drinking water from public and private wells (Kile et al., 2015). Metabolism and clearance of iAs are faster in rats than in human (Styblo et al., 1999), therefore the iAs with higher concentrations in vivo than in the environmentally was used. The concentrations of As_2O_3 we used on Wistar rats were in the range of sub-chronic exposure concentrations reported for other group (Durappanavar et al., 2018). Therefore, the As_2O_3 concentrations of 2 mg/kg, 4 mg/kg and 8 mg/kg were used in our vivo exposure.

In our study, we pre-treated INS-1 cells with 1 μg/ml LPS, an activator of inflammasome response (Bandera et al., 2018). We found that LPS at 1 μg/ml largely enhanced the sensitivity of cellular inflammatory responses. However, LPS at this concentration did not increase the activation of NLRP3 inflammasome.

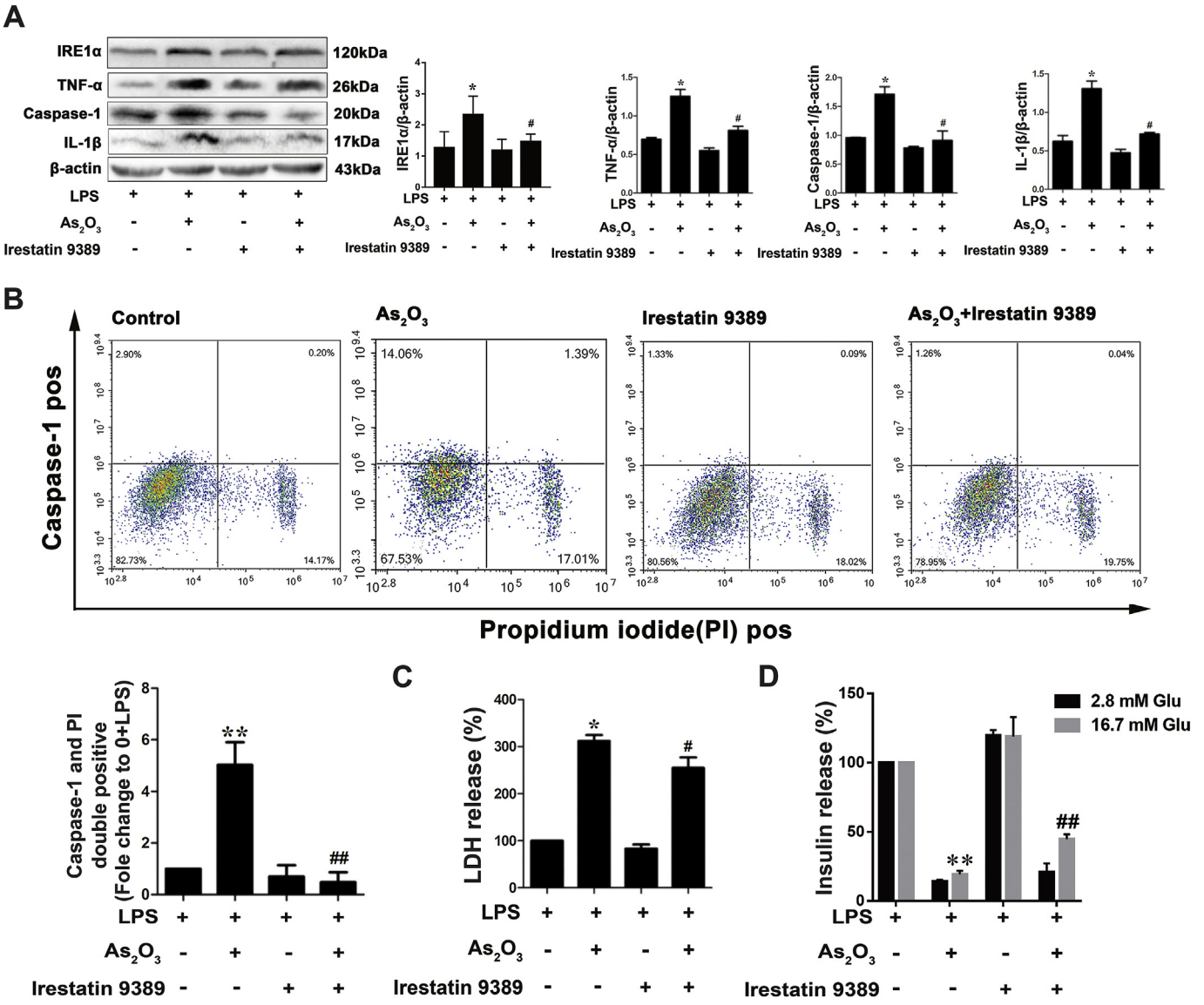

Fig. 6. Effects of irestatin 9389 on TNF-α level, pyroptosis, and INS-1 cells dysfunction. INS-1 cells were pretreated with 1 μg/ml LPS for 4 h, 50 μM irestatin 9389, an IRE1α inhibitor, for 6 h, and then treated with 4 μM As_2O_3 for 24 h. (A) The efficiency of irestatin 9389, and its effect on TNF-α, caspase-1 activation and IL-1β production in As_2O_3-treated INS-1 cells, and densitometric analyses of IRE1α, TNF-α, caspase-1 p20, and IL-1β. β-actin was used as an internal control. (B) Effects of irestatin 9389 on As_2O_3-induced double positivity for PI and activated caspase-1 staining. (C) Effects of As_2O_3 and irestatin 9389 on LDH release rate in INS-1 cells. (D) The INS-1 cells were stimulated with 2.8 mM and 16.7 mM glucose respectively. The relative amounts of insulin secreted were measured using an ELISA kit. The bar represents the means ± SD; (n = 3). *$P < 0.05$ vs. control; **$P < 0.01$ vs. control; #$P < 0.05$ vs. 4 μM As_2O_3 group; ##$P < 0.01$ vs. 4 μM As_2O_3 group.

In our previous studies, we reported that As_2O_3 induced the development of ER stress in the pancreas of C57/BL6J mice and INS-1 cells (Wu et al., 2018b). IRE1α has been proved to be closely related to inflammatory response during ER stress (Wang et al., 2017). In this study, we further showed that inhibition of IRE1α suppressed As_2O_3-induced production of TNF-α. It suggested that activation of IRE1α played an important role in the As_2O_3-induced TNF-α production in INS-1 cells.

Most studies have investigated that high concentrations of TNF-α (> 20 ng/ml) induced apoptosis (Mena et al., 2018). A previous in vitro study showed that the number of apoptotic endothelial progenitor cells were markedly increased by 20 ng/ml TNF-α treatment for 24 h (Du et al., 2014). We also found that As_2O_3-induced production of TNF-α regulated occurrence of pyroptosis, in turn caused INS-1 cells dysfunction. To verify the regulative mechanism underlying the relation between As_2O_3-induced TNF-α and pyroptosis, lenalidomide, an inhibitor of TNF-α, was used. The results demonstrated that TNF-α played a critical role in both As_2O_3-induced pyroptosis and β cells dysfunction. Our results provided direct evidence of the effect of TNF-α in pyroptosis.

Previous results showed that NLRP3 was dispensable for caspase-1 activation in 3T3-L1 cells with treatment of TNF-α (Furuoka et al., 2016). However, Wang et al. (2013) found the NLRP3 regulated the promotion of TGF-β signaling independently of inflammasomes. Bruchard et al. (2015) also found NLRP3 independently of inflammasomes regulated T helper type 2 differentiation. We found NLRP3 and NLRP3 inflammasome were indispensable in As_2O_3-induced pyroptosis in INS-1 cells. To further illustrate the regulative relationship between NLRP3 and pyroptosis, the NLRP3 inhibitor was used to inhibite As_2O_3-induced NLRP3 inflammasome activity. Our data showed that inhibition of NLRP3 inflammasome decreased the activation of caspase-1 and the release of LDH, demonstrating that As_2O_3-induced pyroptosis was regulated by NLRP3 inflammasome rather than other inflammasomes.

There are four types of inflammasome activating caspase-1 and further activating pyroptosis: NLRP1, NLRP3, NLRC4, and AIM2,

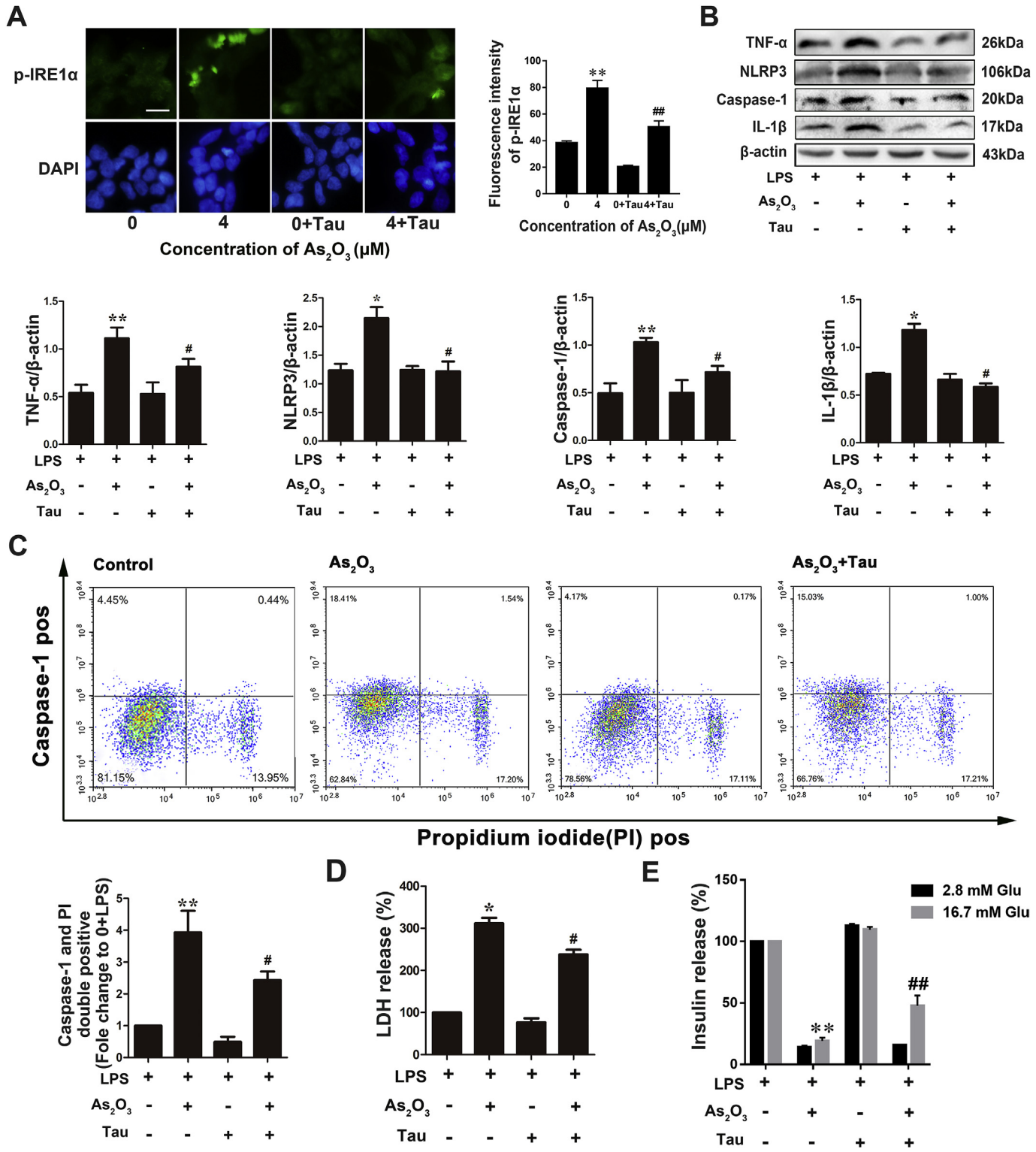

Fig. 7. Tau improves the aberrant activation of ER stress, the secretion of TNF-α, activation of NLRP3 inflammasome, pyroptotic cell death and INS-1 dysfunction caused by As_2O_3. INS-1 cells were pretreated with 100 μM Tau for 6 h, and then were treated with 4 μM As_2O_3 for 24 h. (A) The p-IRE1α were measured by immunofluorescence staining and quantification of green fluorescence intensity were analyzed in INS-1 cells. Magnification: ×400. Scale bar = 20 μm. (B) INS-1 cells were pretreated with 1 μg/ml LPS for 4 h. The effect of Tau on the expression of TNF-α, NLRP3, caspase-1 p20, and IL-1β p17 in As_2O_3-treated INS-1 cells, and densitometric analysis of TNF-α, NLRP3, caspase-1 p20, and IL-1β p17. β-actin was used as an internal control. (C) Effects of Tau on As_2O_3-induced double positivity for PI and activated caspase-1 staining. (D) Effects of As_2O_3 and Tau on LDH release rate in INS-1 cells. (E) The INS-1 cells were stimulated with 2.8 mM and 16.7 mM glucose respectively. The relative amounts of insulin secreted were measured using an ELISA kit. The bar represents the means ± SD; (n = 3). *$P < 0.05$ vs. control; **$P < 0.01$ vs. control; #$p < 0.05$ vs. 4 μM As_2O_3 group; ##$p < 0.01$ vs. 4 μM As_2O_3 group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

among which NLRP3 inflammasome is most thoroughly studied (Wu et al., 2018a). We have shown that activation of caspase-1 p20 and IL-1β depended largely on the NLRP3 inflammasome in INS-1 cells. Therefore, we used the change of caspase-1 p20, IL-1β and LDH to assess pyroptosis in INS-1.

The fetus mainly uptakes Tau from maternal blood (Ditchfield, 2015). One study has shown that Tau was severely reduced in both maternal and fetal plasma as well as in fetal islets by low protein diet. The same authors illustrated that maternal Tau supplementation improved glucose metabolism in rat offspring and prevented the decrease in β cell mass (Tang et al., 2013). However, how exactly Tau improves β cells function in the offspring is unclear. In the previous research, a rat model (200–250 g) of acute hepatic failure was treated with Tau at 250, 500 and 1000 mg/kg BW. Their results have revealed that Tau is a potential protective agent in acute hepatic failure (Jamshidzadeh et al., 2017). In addition, in order to explore the protective effect of Tau on brain injury, the female Wistar rat model was pretreated with Tau at 100–200 mg/kg BW (Owoeye et al., 2018). In our research, we discovered that Tau (150 mg/kg BW) improved β cells dysfunction via down-regulating the level of IRE1α, TNF-α and pyroptosis.

In conclusion, ER stress and pyroptosis are critical pathogenic components of As_2O_3-induced β cell dysfunction. Our data found that TNF-α was a critical signaling node that linked As_2O_3-induced ER stress and pyroptosis. Tau markedly protected As_2O_3-induced β cells dysfunction by reduced the phosphorylation of IRE1α, secretion of TNF-α and activation of pyroptosis.

Author contributions

P.P., X.Y. and X.S. designed the studies. P.P., X.Y., T.Q., L.Y. and N.G. assisted with the animal experiments. P.P., L.J., Z.W., Y.T., S.W. and X.J. participated in the cell experiments. P.P. wrote the manuscript. X.Y., N.W. and X.S. revised the manuscript. All authors reviewed the manuscript. X.S. is the guarantor of the article.

Conflicts of interest

The authors declare that there are no conflicts of interest in the present work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.01.015.

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