

RESEARCH ARTICLE



Berberine protects against palmitate induced beta cell injury via promoting mitophagy

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Abstract

Background Destruction of pancreatic beta cells is the most typical characteristic of diabetes.

Objective We aimed to evaluate the effect of berberine (BBR), a bioactive isoquinoline derivative alkaloid, on beta cell injury.

Methods Rodent pancreatic beta cell line INS-1 was treated with 0.5 mM palmitate (PA) for 24 h to establish an *in vitro* beta cell injury model.

Results BBR at 5 μ M promoted cell viability, inhibited cell apoptosis and enhanced insulin secretion in PA-induced INS-1 cells. BBR treatment also suppressed PA-induced oxidative stress in INS-1 cells, as evidenced by the decreased ROS production and increased activities of antioxidant enzymes. In addition, suppressed ATP production and reduced mitochondrial membrane potential were restored by BBR in PA-treated INS-1 cells. It was further determined that BBR affected the expressions of mitophagy-associated proteins, suggesting that BBR promoted mitophagy in PA-exposed INS-1 cells. Meanwhile, we found that BBR facilitated nuclear expression and DNA-binding activity of Nrf2, an antioxidative protein that can regulate mitophagy. Finally, a rescue experiment was performed and the results demonstrated that the effect of BBR on cell viability, apoptosis and mitochondrial function in PA-induced INS-1 cells were cancelled by PINK1 knockdown.

Conclusions BBR protects islet β cells from PA-induced injury, and this protective effect may be achieved by regulating mitophagy. The present study may provide a novel therapeutic strategy for β cell injury in diabetes mellitus.

Keywords Berberine · Islet beta cells · Antioxidant · Mitophagy

Introduction

Diabetes mellitus (DM) is a systemic metabolic disease characterized by high blood glucose level, causing serious economic and social burden (Padhi et al. 2020; Skyler 2004). The global prevalence of DM is rapidly increasing, and it is estimated to affect 439 million adults (aged 20–79 years) by 2030 (Wang et al. 2018). DM has the second highest mortality among chronic diseases in the world, just following cardiovascular disease. There are two major forms of DM, type 1 (T1DM) and type 2 DM (T2DM) (Forbes and

Cooper 2013). Destruction of islet beta cells is a common characteristic of both T1DM and T2DM (Prentki and Nolan 2006). It is beneficial to investigate the underlying molecular mechanisms of beta cell injury in DM.

Oxidative stress is an important event during the process of the beta cell functional decline (Lacruz et al. 2009). It is caused by excessive production of oxidative free radicals and related reactive oxygen species (ROS) (Newsholme et al. 2016). Mitochondria are the main source of ROS-producing. Excessive ROS production will further lead to mitochondrial dysfunction and cell damage (Wada and Nakatsuka 2016). Notably, mitochondrial dysfunction plays a critical role in the development of DM (Rovira-Llopis et al. 2017). Therefore, maintaining a healthy mitochondrial population is essential for cell survival.

Autophagy is an essential physiological process in the renewal of cellular components, such as aged or damaged proteins, macromolecules, and organelles (Wang and Wang 2019). Mitophagy is a form of selective autophagy which

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is responsible for the clearance of damaged mitochondria. It can target and degrade excess or damaged mitochondria through the lysosomal pathway, enabling them to enter the recycling process of biosynthesis (Pickles et al. 2018). Additionally, mitophagy inhibits the accumulation of damaged mitochondria and stabilizes the cellular level of ROS in injured cells, thus inhibiting oxidative stress and cell death. Recently, it is reported that mitophagy participated in the maintenance of beta cell function (Chen et al. 2017; Guo et al. 2019). Furthermore, mitophagy protects islet beta cells from the influence of inflammatory injury following DM (Sidarala et al. 2020), which indicates that mitophagy might participate in the regulation of beta cell damage in DM.

Berberine (BBR) is a kind of isoquinoline alkaloid mainly extracted from Chinese herb *Coptis* (Zhu et al. 2019). It has shown multiple pharmacological properties, including anti-inflammation and anti-oxidation (Tian et al. 2019). Besides, BBR has been widely used in the treatment of obesity, diabetes and hypercholesterolemia due to its beneficial effects on regulating glucose and lipid metabolism (Wu et al. 2016). However, the underlying mechanisms by which BBR alleviates DM are not fully explored yet. Additionally, previous studies reported that BBR could alleviate cardiac dysfunction by inducing mitophagy (Abudureyimu et al. 2020). Therefore, we hypothesize that BBR may alleviate beta cell dysfunction by promoting mitophagy.

Materials and methods

Reagents and antibodies

INS-1 cells were purchased from Procell life science & Technology Co., Ltd (China). BBR was purchased from Aladdin reagents Co., Ltd (China). Palmitate (PA) was obtained from Solarbio Science & Technology, Co., Ltd (China). Chemiluminescent EMSA kit, nuclear extraction kit, BCA protein quantification assay kit, ATP assay kit, mitochondrial membrane potential assay kit with JC-1 and Annexin V-FITC apoptosis detection kit were purchased from Beyotime Biological Company (China). Mitochondrial protein isolation kit was purchased from BOSTER Co., Ltd. (China). P62 antibody, LC3II/I antibody, Parkin antibody, Mfn1 antibody and Nrf2 antibody were purchased from ABclonal Company (China). PINK1 antibody and Mfn2 antibody were purchased from Affinity Bioscience Company (China). Internal reference β -actin antibody and Histone H3 antibody were purchased from Proteintech Group, Inc (USA). Internal reference COX IV antibody was purchased from ABclonal Company (China). Catalase (CAT) assay kit, Malondialdehyde (MDA) assay kit and superoxide dismutase (SOD) assay kit were purchased from

Nanjing Jiancheng Biological Co., Ltd. (Nanjing Jiancheng Biological Co., Ltd, China). Cell counting kit-8 (CCK-8) detection kit was purchased from Sigma Company (USA).

Cell culture and treatment

The INS-1 cells were cultured in RPMI-1640 Medium with 10% FBS and maintained in an incubator at 37 °C with the atmosphere of 5% CO₂. Cultured cells were pretreated with BBR (5 μ M) for 1 h, and then treated with PA (0.5 mM) for 24 h. Subsequently, cells were collected for testing. Particularly, PA was dissolved in heated 99% ethanol to a concentration of 100 mM and then diluted by serum free RPMI-1640 medium containing 10% fatty acid free BSA to the concentration of 5 mM for storage. The working solution of PA (0.5 mM) was always freshly prepared by further dilution in RPMI-1640 medium.

Cell viability analysis

Cells (4×10^3 /well) were seeded in a 96-well plate. After treatment, cells were subjected to cell viability detection. Briefly, 10 μ L/well of the CCK-8 was added and further incubated for 2 h at 37 °C, 5% CO₂. Cell viability was measured as the absorbance at 450 nm using a microplate reader, and then the results were analyzed.

Insulin secretion assay

Pre-treated INS-1 cells were starved and washed prior to glucose stimulation as previously described (Tian et al. 2020). Insulin secretion level was subsequently detected by ELISA according to the manufacture's instruction (ER1113, FineTest).

Biochemical assays

MDA level, CAT activity and SOD activity were respectively assessed using commercial assay kits following the manufacturer's instructions (Nanjing Jiangcheng Bioengineering institute). The principle to detect MDA level was based on the derivatization of MDA with thiobarbituric acid (TBA). Briefly, MDA in samples could react with thiobarbituric acid (TBA) to generate a MDA-TBA adduct, which could be quantified colorimetrically at OD 532 nm. CAT activity in each sample was determined by the ammonium molybdate colorimetry. CAT could catalyze the decomposition of H₂O₂, which reacts with ammonium molybdate to form a stable yellow complex. It can be detected at OD 405 nm and negatively correlated with CAT activity. SOD activity was detected by hydroxylamine method. SOD catalyzes the dismutation of the superoxide anion, which reacts

with hydroxylamine to generate nitrite. Nitrite was further visualized as violet red by chromogenic agent and could be detected at OD 550 nm.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts from INS-1 cells were prepared using nuclear extraction kit. With these protein samples, the Nrf2 activity was determined using Chemiluminescent EMSA Kit (GS009, Beyotime) with specific probe (GS013B, Beyotime). The consensus oligo sequence for probe was listed as follows:

5'-ACT GAG GGT GAC TCA GCA AAA TC-3'
3'-TGA CTC CCA CTG AGT CGT TTT AG-5'

Real-time PCR analysis

Total RNA was extracted from cultured cells using TRIpure lysis buffer (BioTeke, Beijing). Extracted RNA was reversely transcribed into cDNAs by BeyoRT II m-MLV reverse transcriptase (Beyotime, China). Then real-time PCR reaction system was prepared according to instructions in SYBR Green (Solarbio, China) kit. The constructed PCR reaction system was put into Exicycler TM 96 fluorescence quantifier (Bioneer, Korea) for fluorescence quantification. β -actin was used as the internal reference and method of $2^{-\Delta\Delta CT}$ was used to analyze the relative expressions of target genes. The primer information was listed as follows: PINK1 forward: GGACCGCTACCGCTTCTT, PINK1 reverse: CCCTGC-CAACGTCGTGT; Nrf2 forward: CCATTGAGGGCTGT-GAT, Nrf2 reverse: TTGGCTGTGCTTTAGGT; beta-actin forward: GGAGATTACTGCCCTGGCTCCTAGC; beta-actin reverse: GGCCGGACTCATCGTACTCCTGCTT.

Western-blot analysis

Cells were collected by trypsinization and lysed in RIPA lysis buffer. After centrifugation at 10,000×g for 10 min, the sample was quantified with a BCA protein quantification assay kit. Then the protein samples were separated by SDS-PAGE, transferred to PVDF membranes, incubated with antibodies, and scanned by a chemiluminescence system. Finally, the target band optical density was analyzed by Gel-Pro-Analyzer software (Liuyi, Beijing). The information of primary antibodies were listed as follows: p62 antibody (A19700, ABclonal), LC3II/I antibody (A19665, ABclonal), Parkin antibody (A0968, ABclonal), PINK1 antibody (DF7742, Affinity), Mfn1 antibody (A9880, ABclonal), Mfn2 antibody (DF8106, Affinity), Nrf2 antibody (A0674, ABclonal), β -actin antibody (60008-1-Ig, proteintech), Histone H3 antibody (17168-1-AP, proteintech) and COX IV antibody (A11631, ABclonal).

Apoptosis detection by flow cytometry

Cell apoptosis was assessed using flow cytometry. In brief, cells were collected after centrifugation at 300×g for 5 min, and then cells were stained with ANNEXIN-V-FITC/PI according to the manufacturer's protocols. Subsequently, apoptotic cells were analyzed under a flow cytometer (ACEA Biosciences, US).

Mitochondrial membrane potential assay

Mitochondrial membrane potential (MMP) was detected by JC-1 staining. Cells were washed with PBS, and then 0.5 mL JC-1 staining solution was added. Cells were incubated with JC-1 staining solution for 20 min at 37 °C in an incubator. Finally, changes of JC-1 monomers and JC-1 aggregates were respectively detected by a flow cytometer (ACEA Biosciences, US). The transition from JC-1 aggregates (red) to JC-1 monomers (green) indicated the reduction of MMP. Specially, JC-1 monomers are with the maximum excitation at 514 nm and maximum emission at 529 nm. JC-1 aggregates are with the maximum excitation at 585 nm and maximum emission at 590 nm.

Mitochondria functional analysis

ATP contents, total ROS (t-ROS) level and mitochondrial ROS (Mito-ROS) production were respectively measured to analyze mitochondrial function following different treatments. Accordingly, ATP contents were detected using the ATP assay kit according to the manufacturer's protocols. For total ROS and mitochondrial ROS detection, cells were respectively probed with H2DCFDA (maximum excitation at 504 nm and maximum emission at 529 nm) and MitoSOX (maximum excitation at 510 nm and maximum emission at 580 nm). The contents of t-ROS and Mito-ROS were finally assessed by flow cytometry (ACEA Biosciences, US).

Statistical analysis

Data were presented as mean \pm standard deviation (SD). Commercial GraphPad Prism 8 was used to perform the significance analysis of experimental data with one-way ANOVA followed by Tukey's test ($P < 0.05$).

Results

BBR reduces PA -induced beta cell injury

Cell morphology was assessed by light microscope. Results showed that number of injured cells in BBR group

was decreased compared to PA group (Fig. 1 A). Additionally, BBR significantly facilitated insulin secretion in PA-induced INS-1 cells (Fig. 1B). The analysis of CCK-8 assay showed that cell viability in BBR group was significantly

increased than that in PA group ($P < 0.01$, Fig. 1 C). As depicted in Fig. 1D and E, the apoptotic cells were evidently decreased in PA group after the treatment with BBR. Results

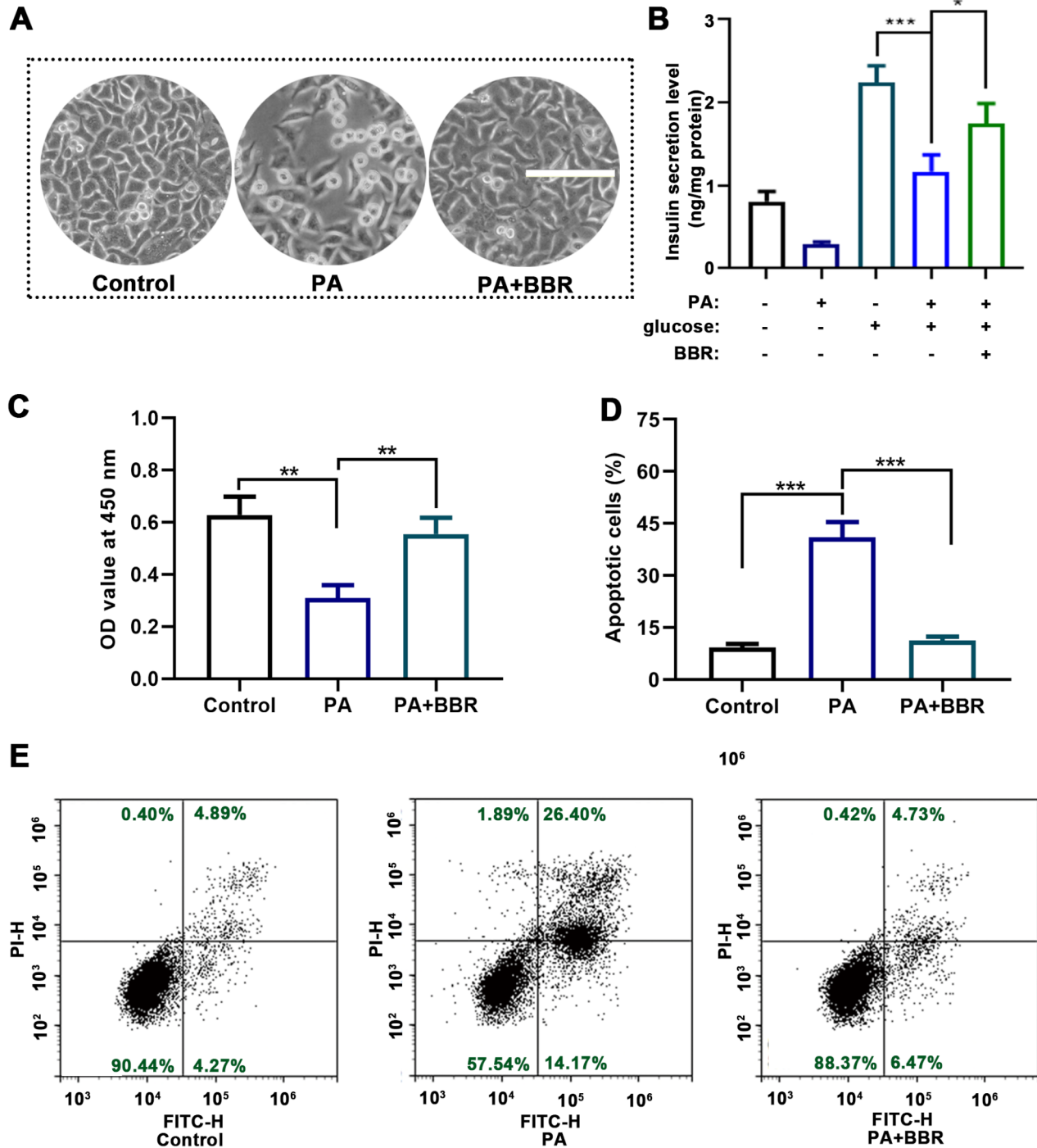


Fig. 1 Effects of BBR on PA-induced beta cell injury. (A) Cell morphology under the magnification of 100 \times . (B) Insulin secretion was detected by ELISA. (C) Cell viability was determined by CCK-8 (OD: 450 nm). (D-E) Cells apoptosis was determined by flow cytometric analysis. Data are expressed as mean \pm standard deviation (N = 3/group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

presented above demonstrated that BBR protects against PA-induced beta cell injury.

BBR alleviates oxidative stress in PA-induced islet beta cells

Accumulating evidence suggests that the occurrence

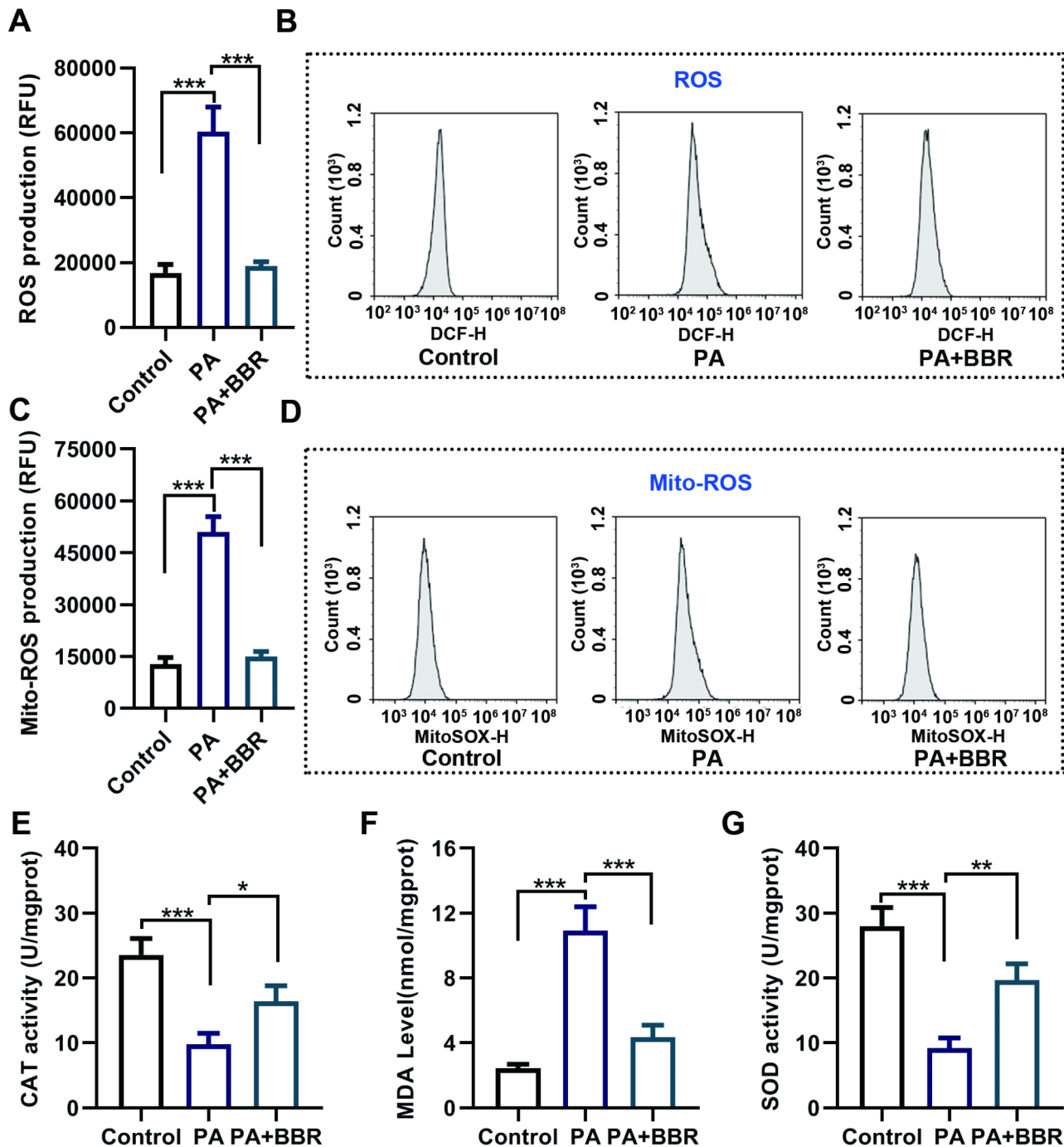


Fig. 2 Effects of BBR on oxidative stress in PA-induced islet beta cells. Intracellular total ROS production (A-B), mitochondrial ROS production (C-D), CAT activity (E), MDA level (F) and SOD activity (G) were determined by indicated assay kits. Data are expressed as mean \pm standard deviation (N=3/group). * P<0.05; ** P<0.01; *** P<0.001; ****P<0.0001. ROS: reactive oxygen species; CAT: catalase; MDA: malondialdehyde; SOD: superoxide dismutase

of DM is closely related to oxidative stress. Therefore, in this study, we respectively detected the level of total ROS, mito-ROS, MDA together with activity of CAT and SOD in different groups. As shown in Fig. 2, BBR pretreatment induced a significant decrease in the levels of MDA, total ROS and mito-ROS, while led to an obvious increase in the activity of CAT and SOD when compared with PA group ($P < 0.001$ or $P < 0.01$). These results indicated that BBR alleviated oxidative stress in PA-induced islet beta cells.

BBR alleviates mitochondrial damage in PA-induced islet beta cells

Cellular ATP is an important parameter of mitochondrial function. As shown in Fig. 3 A, ATP content in BBR group was higher than that in PA group ($P < 0.05$). Analysis of JC-1 staining further indicated that BBR treatment alleviated the reduction of mitochondria membrane potential (Fig. 3B). These results indicated that BBR alleviated mitochondrial damage in PA-induced islet beta cells.

BBR promotes mitophagy in PA-induced islet beta cells

Additionally, we further explore the effect of BBR on mitophagy. As shown in Fig. 4 A-C, we observed that BBR treatment significantly increased the expression of LC3II/I and decreased expression of p62 ($P < 0.001$ or $P < 0.01$). Besides, PINK1 and Parkin expression was significantly depressed in PA group, while BBR treatment increased their expression levels ($P < 0.001$, Fig. 4D-H and J). These results suggest that BBR may advance PINK1/Parkin-mediated

mitophagy. Additionally, results of western blot analysis (Fig. 4I and K) showed that BBR treatment led to an obvious increase in Mfn1 and Mfn2 expression ($P < 0.001$). Furthermore, BBR facilitated mitochondrial translocation of Parkin (Fig. 4L), further validating that BBR treatment promoted mitophagy in PA-induced islet beta cells.

BBR promotes the activation of Nrf2 signaling pathway in PA-induced islet beta cells

In order to further explore the mechanism by which BBR mediates PA-induced beta cell injury, Nrf2 signaling pathway was investigated. As depicted in Fig. 5 A, the expression of nuclear Nrf2 in PA+BBR group was significantly increased compared with PA group ($P < 0.001$). EMSA analysis showed that BBR treatment increased DNA-binding activity of Nrf2 (Fig. 5B). Meanwhile, immunofluorescence results also indicated that BBR treatment promoted Nrf2 nuclear translocation in PA-induced INS-1 cells (Fig. 5G). Moreover, the mRNA level of PINK1 was found to be significantly increased after the treatment with BBR ($P < 0.01$, Fig. 5C), while Nrf2 knockdown reversely reduced PINK1 mRNA level in BBR treated INS-1 cells (Fig. 5D-F). These results indicated that BBR up-regulated the expression level of PINK1 via activating Nrf2 signaling pathway.

BBR alleviates PA-induced beta cell injury through mitophagy

To further confirm the role of mitophagy in beta cell injury, the siRNA of PINK1, a key marker for mitophagy, were transfected into islet beta cells (Fig. 6 A). The

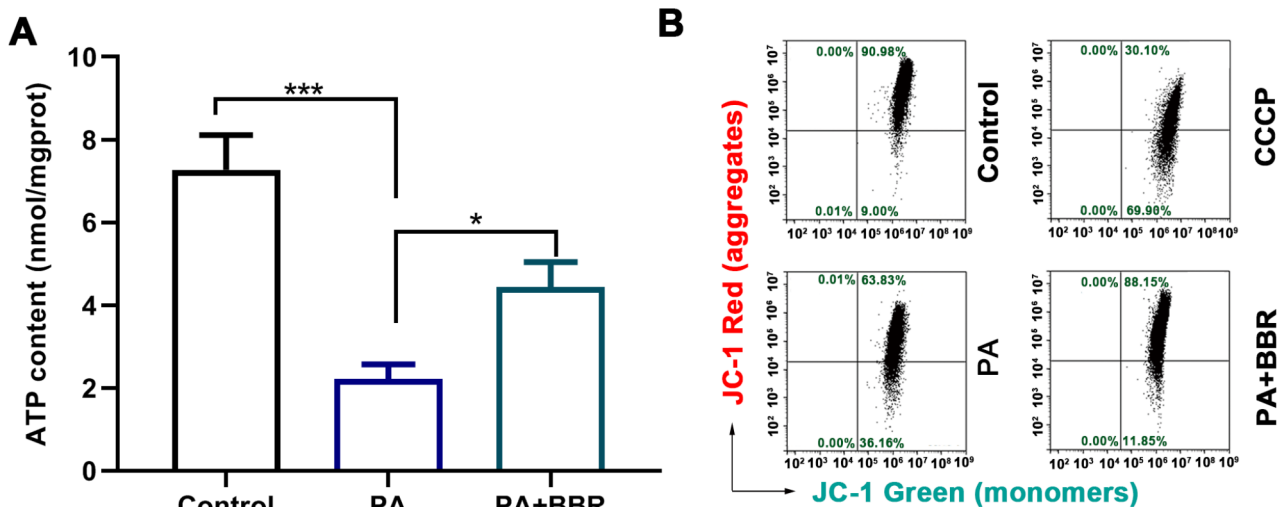
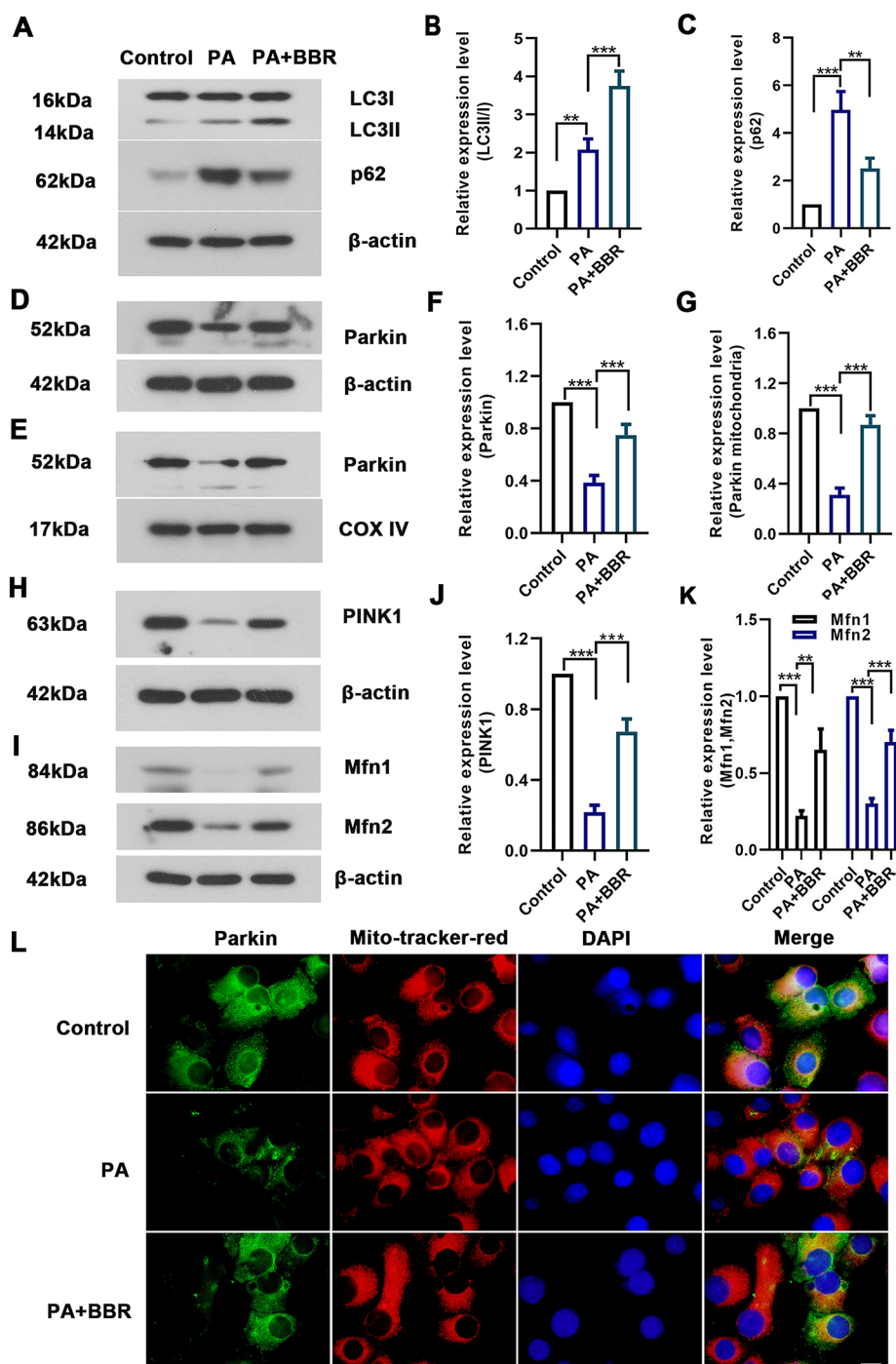


Fig. 3 Effects of BBR on mitochondrial damage in PA-induced islet beta cells. (A) Level of cellular ATP was determined by ATP assay kit. (B) JC-1 staining was used to detect the change of membrane potential (flow cytometry, CCCP treatment, as positive control). Data are expressed as mean \pm standard deviation ($N = 3$ /group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fig. 4 Effects of BBR on mitophagy in PA-induced islet beta cells. (A) Representative gel blots of LC3I, LC3II, p62 and β -actin. (B) Relative level of LC3II/I and (C) p62. (D) Representative gel blots of Parkin and β -actin. (E) Representative gel blots of Parkin and COX IV. (F) Relative level of Parkin. (G) Relative level of mitochondrial Parkin. (H) Representative gel blots of PINK1 and β -actin. (I) Representative gel blots of Mfn1, Mfn2 and β -actin. (J) Relative level of PINK1 and (K) Mfn1, Mfn2. (L) Representative immunofluorescence image of Parkin (Green) and mitochondria (Mito Tracker Red), magnification: 1000 \times . Data are expressed as mean \pm standard deviation (N = 3/group). ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$



expressions of PINK1 and Parkin were significantly inhibited in PA + BBR + si-PINK1 group (Fig. 6B and C, $P < 0.001$). Results of CCK-8 assay showed that cell viability in PA + BBR + si-PINK1 group was significantly decreased than that in PA + BBR + si-NC group (Fig. 6D, $P < 0.01$). Additionally, PINK1 knockdown markedly increased cell apoptosis in BBR-treated cells (Fig. 6E). Furthermore, PINK1 silencing aggravated mitochondrial dysfunction

in BBR treated cells, which was evidenced by inhibited ATP production, reduced MMP and increased mito-ROS (Fig. 6 F-H). These results indicated that mitophagy inhibition by the transfection of PINK1 siRNA offset the protective effect of BBR on beta cell injury.

Discussion

Islet beta cells are functional cells responsible for insulin biosynthesis and secretion, which are critical for the maintenance of blood glucose homeostasis. The impairment or loss of islet beta cells is considered as the common characteristic in diabetes (Wang et al. 2022). BBR is an isoquinoline alkaloid with a variety of biological activities, including anti-oxidation, anti-inflammation and hypoglycemic effects (Kumar et al. 2015). Consistently in our study, we observed that BBR treatment effectively promoted cell viability, inhibited cell apoptosis and enhanced insulin secretion in PA-induced islet beta cells, which suggests the protective effect of BBR on beta cell injury. However, the molecular mechanism on how BBR alleviating the PA-induced beta cell injury is not fully clarified.

The pathogenesis of DM is complicated, while accumulating evidence indicates that oxidative stress plays an important role in the onset of DM (An et al. 2019; Papanthodorou et al. 2020). Accordingly, oxidative stress is the outcome of excessive production of ROS, which exceeds the endogenous antioxidant capacity of organism (Kayama et al. 2015; Sifuentes-Franco et al. 2017). In addition, excessive and sustained oxidative stress could lead to mitochondrial dysfunction and further cell damages (Vela-Guajardo et al. 2021). In this study, BBR is considered to be an antioxidant compound for its effect on reducing ROS production (total and mitochondrial ROS). Besides, BBR treatment increased the activities of SOD and CAT, two key enzymes that protect cells from oxidative damages (Halim and Halim 2019). The level of MDA, which is well accepted as biomarkers of oxidative stress, is significantly reduced after BBR treatment. These findings indicated that BBR alleviated the oxidative stress in PA-induced beta cell injury.

Mitochondria are believed to be the target and sources of oxidative stress (Choi et al. 2009). Thus, maintaining of healthy mitochondrial network is an important approach to protect islet beta cells from oxidative stress. In this study, ATP level and MMP were found to be remarkably reduced in PA-induced INS-1 cells, which indicate the existence of mitochondrial dysfunction in injured islet beta cells. Actually, it has been determined that impaired mitochondrial function plays a crucial role in DM development. The health of mitochondria, characterized by controlled oxidative stress, undisturbed ATP production, is observed to be damaged in liver and skeletal muscles from T2D individuals (Roszczyc-Owsiejczuk and Zabielski 2021). Mitochondrial dysfunction also activates the mitochondrial apoptotic pathway, resulting in cellular death (Kim and Kim 2018). In our study, BBR treatment promoted ATP synthesis and optimized MMP status in PA-induced INS-1 cells, which

indicated that BBR alleviated mitochondrial dysfunction in PA-induced islet beta cells.

As previously discussed, mitochondrial defects contributes to the development of diabetes. Consequently, the quality of mitochondria must be well controlled to protect against from cell injury. There are different mechanisms evolved to maintain their homeostasis, such as proteolytic system, mitochondrial fusion and fission as well as mitophagy (Rovira-Llopis et al. 2017). Accordingly, mitophagy induced the clearance of damaged mitochondria upon injury (Yao et al. 2019), and facilitates the synthesis of fresh mitochondria with the assistance of mitochondrial fusion and fission (Wang and Wang 2019; Youle and Narendra 2011). In this study, BBR promoted mitophagy in PA-induced INS-1 cells, which was evidenced by up-regulating the expressions of LC3II/I, PINK1 and Parkin, while decreasing the abundance of p62. LC3 is the marker of autophagosome, and the transition from LC3I to LC3II occurred following autophagy. It should be noted that PINK1/Parkin-mediated mitophagy is one of the best studied mitophagy-related mechanisms in mammalian cells (Eiyama and Okamoto 2015). PINK1, Parkin and p62 are the major orchestrators of mitophagy. Upon mitochondrial impairments, PINK1 accumulates in mitochondria, and then recruits Parkin to outer mitochondrial membrane (OMM), which further leads to the activation of Parkin. Afterwards, parkin regulates the ubiquitination of OMM proteins, making them tagged for p62 binding (Barazzuol et al. 2020; Rovira-Llopis et al. 2017). Mfn1 and Mfn2 are the mitochondrial substrates of Parkin. As a mitochondrial outer membrane protein, Mfn1/2 participated in mitochondrial fusion and contributed to mitochondrial network maintenance (Chen et al. 2003). BBR treatment could promote the expression of Mfn1 and Mfn2 in PA-induced cells, suggesting that BBR induced the renewal of fresh mitochondria. These findings indicated that BBR promoted mitophagy in PA-induced islet beta cells.

Nrf2 plays an important role in cellular defense against oxidative stress by binding to the antioxidant response element (ARE) sequences of several antioxidant genes (Deng et al. 2019). Meanwhile, Nrf2 is reported to facilitate the transcription of PINK1 (Xiao et al. 2017), which is concordant with our findings. More importantly, PINK1 is an indispensable part in the execution of mitophagy (Barazzuol et al. 2020) (Zheng et al. 2021). Therefore, PINK1 siRNA was used in our study to inhibit BBR-induced mitophagy. It is further determined that the protective effect of BBR on beta cell injury was cancelled by PINK1 silencing, which suggests that BBR attenuates the PA-induced beta cell injury by promoting mitophagy.

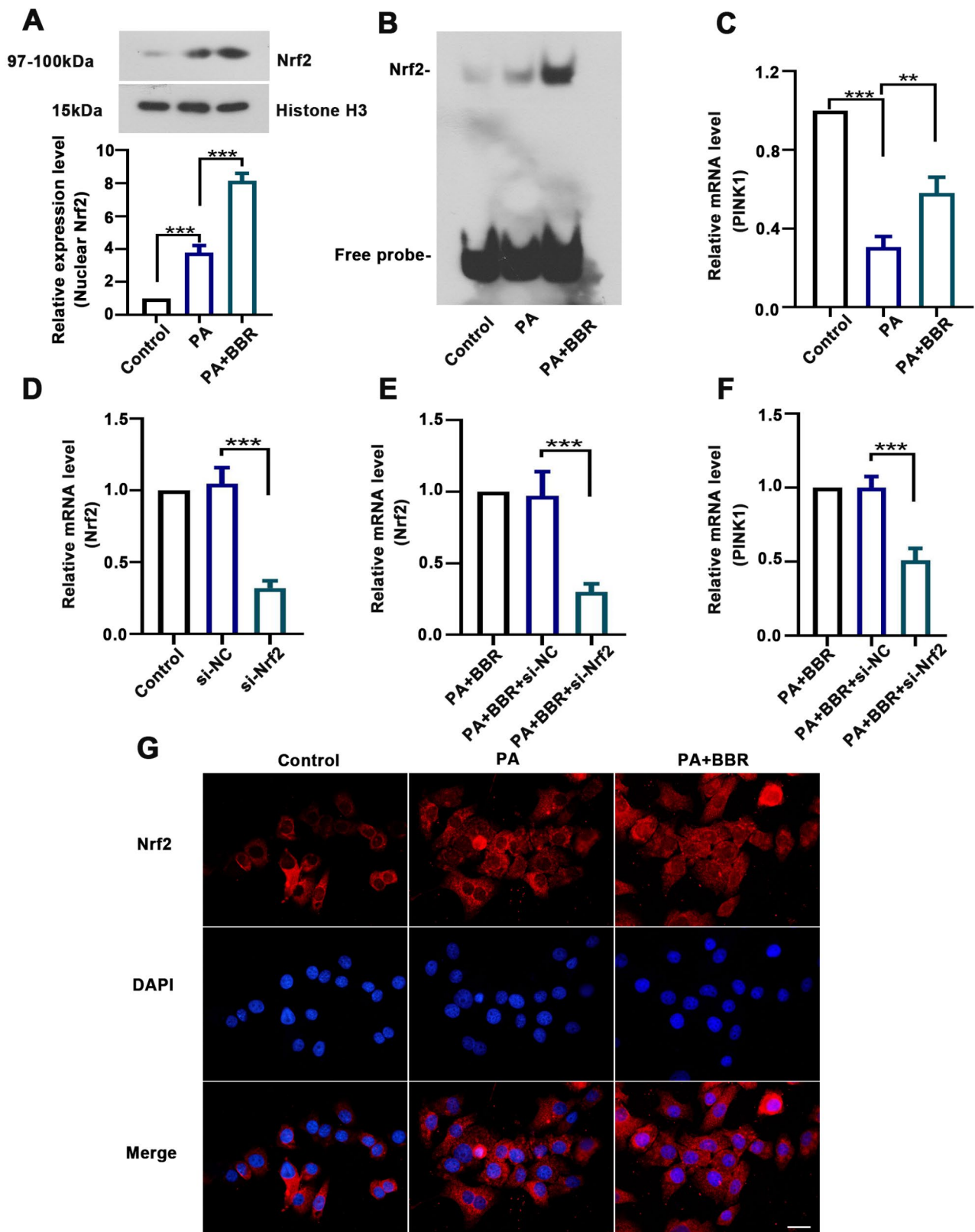


Fig. 5 Effects of BBR on Nrf2 signaling pathway in PA-induced islet beta cells. (A) Nrf2 expression in each group of cells (in nucleus) was determined by western blot. (B) Activity of Nrf2 was detected by EMSA. (C) The PINK1 mRNA levels were detected by real-time PCR. (D) The Nrf2 mRNA level after the transfection of siNrf2 in INS-1 cells. (E-F) The mRNA levels of PINK1 and Nrf2 after the transfection of siNrf2 in BBR treated cells. (G) Immunofluorescence staining to determine the nuclear translocation of Nrf2, magnification: 600 \times . Data are expressed as mean \pm standard deviation (N = 3/group). ** P < 0.01; *** P < 0.001; ****P < 0.0001

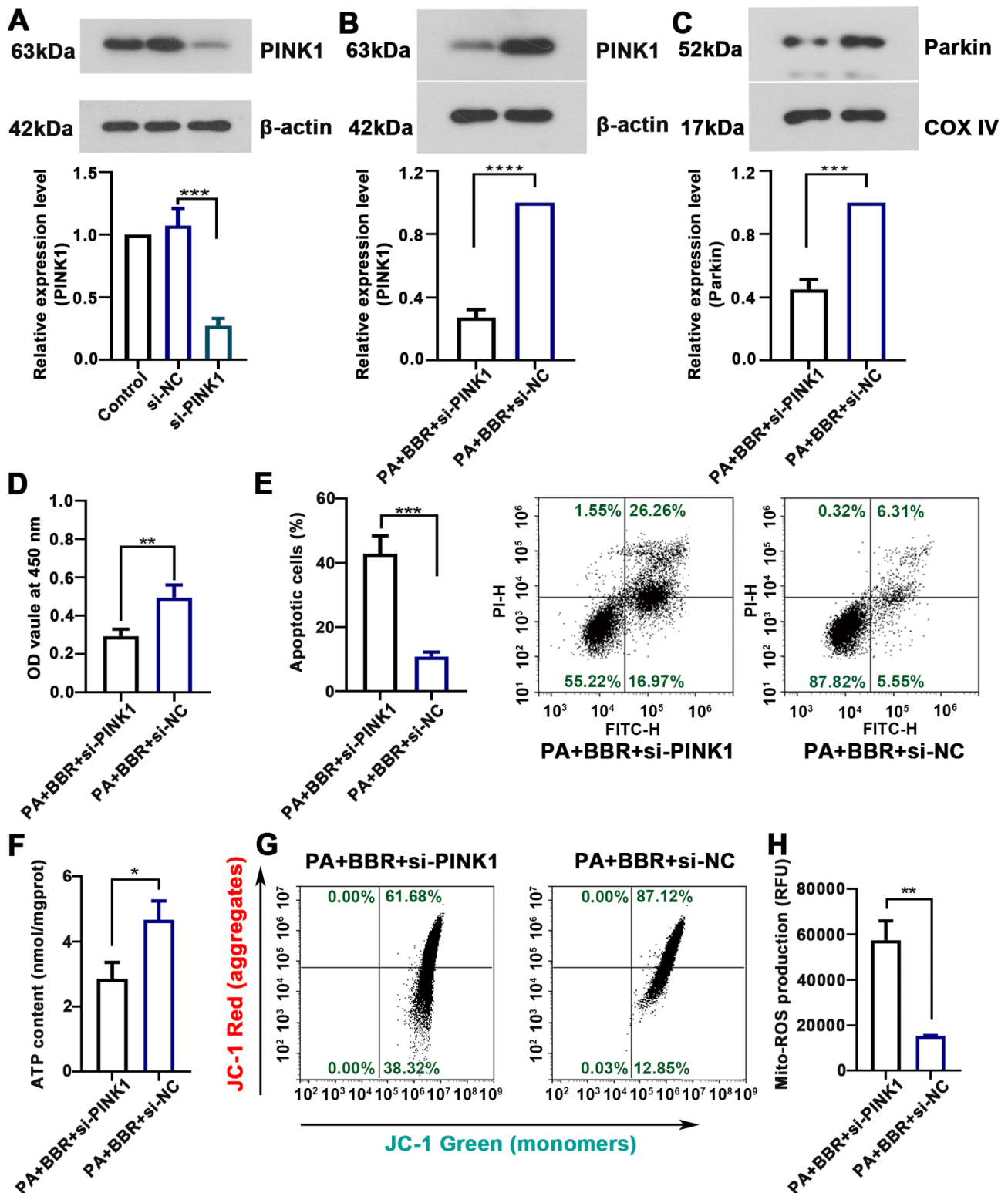


Fig. 6 BBR alleviated PA-induced beta cell injury via mitophagy. (A) siRNA targeting PINK1 was transfected into INS-1 cells, and the protein level of PINK1 was detected by western-blot 48 h later. INS-1 cells were transfected with siRNA targeting PINK1 for 48 h and subsequently treated with BBR (5 μ M) for 1 h, followed with PA (0.5 mM) stimulation for 24 h. (B) The protein levels of PINK1 and (C) mitochondrial Parkin in each group were detected by Western-blot. (D) Cell viability was detected by CCK-8 assay. (E) Cell apoptosis was detected by flow cytometry. Quality changes of mitochondria in BBR treated INS-1 cells after PINK1 silencing was evaluated by ATP assay (F), JC-1 staining (G) and mito-SOX staining (H). Data are expressed as mean \pm standard deviation (N = 3/group). ** P < 0.01; *** P < 0.001; ****P < 0.0001

Conclusions

This study indicated that BBR treatment alleviated PA-induced beta cell injury, as evidenced by the promoted cell viability, inhibited cell apoptotic rates and enhanced insulin secretion in INS-1 cells. It was further demonstrated that BBR treatment regulated beta cell functional injury via facilitating mitophagy. In summary, this study may provide further evidence for the application of BBR in treating DM.

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Availability of data and materials All data generated or analyzed during this study are included in this article.

Declarations

Conflict of interest Author Mo Li, author Jiang She, author Louyan Ma, author Li Ma, author Xiaorui Ma and author Jiajia Zhai declare that they have no conflict of interest.

References

- Abudureyimu M, Yu W, Cao RY, Zhang Y, Liu H, Zheng H (2020) Berberine Promotes Cardiac Function by Upregulating PINK1/Parkin-Mediated Mitophagy in Heart Failure. *Front Physiol* 11:565751
- An Y, Zhang H, Wang C, Jiao F, Xu H, Wang X, Luan W, Ma F, Ni L, Tang X et al (2019) Activation of ROS/MAPKs/NF- κ B/NLRP3 and inhibition of efferocytosis in osteoclast-mediated diabetic osteoporosis. *Faseb j* 33:12515–12527
- Barazzuol L, Giamogante F, Brini M, Cali T (2020) PINK1/Parkin Mediated Mitophagy, Ca(2+) Signalling, and ER-Mitochondria Contacts in Parkinson's Disease. *Int J Mol Sci* 21:1772
- Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* 160:189–200
- Chen L, Liu C, Gao J, Xie Z, Chan LWC, Keating DJ, Yang Y, Sun J, Zhou F, Wei Y et al (2017) Inhibition of Miro1 disturbs mitophagy and pancreatic β -cell function interfering insulin release via IRS-Akt-Foxo1 in diabetes. *Oncotarget* 8:90693–90705
- Choi K, Kim J, Kim GW, Choi C (2009) Oxidative stress-induced necrotic cell death via mitochondria-dependent burst of reactive oxygen species. *Curr Neurovasc Res* 6:213–222
- Deng Y, Tang K, Chen R, Nie H, Liang S, Zhang J, Zhang Y, Yang Q (2019) Berberine attenuates hepatic oxidative stress in rats with non-alcoholic fatty liver disease via the Nrf2/ARE signalling pathway. *Exp Ther Med* 17:2091–2098
- Eiyama A, Okamoto K (2015) PINK1/Parkin-mediated mitophagy in mammalian cells. *Curr Opin Cell Biol* 33:95–101
- Forbes JM, Cooper ME (2013) Mechanisms of diabetic complications. *Physiol Rev* 93:137–188
- Guo T, Liu T, Sun Y, Liu X, Xiong R, Li H, Li Z, Zhang Z, Tian Z, Tian Y (2019) Sonodynamic therapy inhibits palmitate-induced beta cell dysfunction via PINK1/Parkin-dependent mitophagy. *Cell Death Dis* 10:457
- Halim M, Halim A (2019) The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). *Diabetes Metab Syndr* 13:1165–1172
- Kayama Y, Raaz U, Jagger A, Adam M, Schellinger IN, Sakamoto M, Suzuki H, Toyama K, Spin JM, Tsao PS (2015) Diabetic Cardiovascular Disease Induced by Oxidative Stress. *Int J Mol Sci* 16:25234–25263
- Kim SH, Kim H (2018) Inhibitory Effect of Astaxanthin on Oxidative Stress-Induced Mitochondrial Dysfunction-A Mini-Review. *Nutrients* 10:1137
- Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK (2015) Current knowledge and pharmacological profile of berberine: An update. *Eur J Pharmacol* 761:288–297
- Lacraz G, Figeac F, Movassat J, Kassis N, Coulaud J, Galinier A, Leloup C, Bailbé D, Homo-Delarche F, Portha B (2009) Diabetic beta-cells can achieve self-protection against oxidative stress through an adaptive up-regulation of their antioxidant defenses. *PLoS ONE* 4:e6500
- Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI Jr (2016) Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J* 473:4527–4550
- Padhi S, Nayak AK, Behera A (2020) Type II diabetes mellitus: a review on recent drug based therapeutics. *Biomed Pharmacother* 131:110708
- Papachristoforou E, Lambadiari V, Maratou E, Makrilakis K (2020) Association of Glycemic Indices (Hyperglycemia, Glucose Variability, and Hypoglycemia) with Oxidative Stress and Diabetic Complications. *J Diabetes Res* 2020:7489795
- Pickles S, Vigié P, Youle RJ (2018) Mitophagy and Quality Control Mechanisms in Mitochondrial Maintenance. *Curr Biol* 28:R170–r185
- Prentki M, Nolan CJ (2006) Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116:1802–1812
- Roszczyk-Owsieczuk K, Zabielski P (2021) Sphingolipids as a Culprit of Mitochondrial Dysfunction in Insulin Resistance and Type 2 Diabetes. *Front Endocrinol* 12:635175–635175
- Rovira-Llopis S, Bañuls C, Diaz-Morales N, Hernandez-Mijares A, Rocha M, Victor VM (2017) Mitochondrial dynamics in type 2 diabetes: Pathophysiological implications. *Redox Biol* 11:637–645
- Sidarala V, Pearson GL, Parekh VS, Thompson B, Christen L, Gingerich MA, Zhu J, Stromer T, Ren J, Reck EC et al (2020) Mitophagy protects β cells from inflammatory damage in diabetes. *JCI Insight* 5:e141138
- Sifuentes-Franco S, Pacheco-Moisés FP, Rodríguez-Carrizalez AD, Miranda-Díaz AG (2017) The Role of Oxidative Stress, Mitochondrial Function, and Autophagy in Diabetic Polyneuropathy. *J Diabetes Res* 2017:1673081
- Skyler JS (2004) Diabetes mellitus: pathogenesis and treatment strategies. *J Med Chem* 47:4113–4117
- Tian H, Kang YM, Gao HL, Shi XL, Fu LY, Li Y, Jia XY, Liu KL, Qi J, Li HB et al (2019) Chronic infusion of berberine into the hypothalamic paraventricular nucleus attenuates hypertension and sympathoexcitation via the ROS/Erk1/2/iNOS pathway. *Phyto-medicine* 52:216–224
- Tian X, Zhang Y, Li H, Li Y, Wang N, Zhang W, Ma B (2020) Palmitate ameliorates high fat diet induced impaired glucose tolerance. *Biol Res* 53:39–39
- Vela-Guajardo JE, Garza-González S, García N (2021) Glucolipototoxicity-induced Oxidative Stress is Related to Mitochondrial Dysfunction and Apoptosis of Pancreatic β -cell. *Curr Diabetes Rev* 17:e031120187541
- Wada J, Nakatsuka A (2016) Mitochondrial Dynamics and Mitochondrial Dysfunction in Diabetes. *Acta Med Okayama* 70:151–158
- Wang S, Zhao Z, Feng X, Cheng Z, Xiong Z, Wang T, Lin J, Zhang M, Hu J, Fan Y et al (2018) Melatonin activates Parkin translocation

- and rescues the impaired mitophagy activity of diabetic cardiomyopathy through Mst1 inhibition. *J Cell Mol Med* 22:5132–5144
- Wang R, Wang G (2019) Autophagy in Mitochondrial Quality Control. *Adv Exp Med Biol* 1206:421–434
- Wang H-L, Wei B, He H-J, Huang X-R, Sheng J-Y, Chen X-C, Wang L, Tan R-Z, Li J-C, Liu J et al (2022) Smad3 deficiency improves islet-based therapy for diabetes and diabetic kidney injury by promoting β cell proliferation via the E2F3-dependent mechanism. *Theranostics* 12:379–395
- Wu YS, Chen YT, Bao YT, Li ZM, Zhou XJ, He JN, Dai SJ, Li CY (2016) Identification and Verification of Potential Therapeutic Target Genes in Berberine-Treated Zucker Diabetic Fatty Rats through Bioinformatics Analysis. *PLoS ONE* 11:e0166378
- Xiao L, Xu X, Zhang F, Wang M, Xu Y, Tang D, Wang J, Qin Y, Liu Y, Tang C et al (2017) The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. *Redox Biol* 11:297–311
- Yao N, Wang C, Hu N, Li Y, Liu M, Lei Y, Chen M, Chen L, Chen C, Lan P et al (2019) Inhibition of PINK1/Parkin-dependent mitophagy sensitizes multidrug-resistant cancer cells to B5G1, a new betulinic acid analog. *Cell Death Dis* 10:232
- Youle RJ, Narendra DP (2011) Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* 12:9–14
- Zheng Y, Huang C, Lu L, Yu K, Zhao J, Chen M, Liu L, Sun Q, Lin Z, Zheng J et al (2021) STOML2 potentiates metastasis of hepatocellular carcinoma by promoting PINK1-mediated mitophagy and regulates sensitivity to lenvatinib. *J Hematol Oncol* 14:16–16
- Zhu X, Bian H, Wang L, Sun X, Xu X, Yan H, Xia M, Chang X, Lu Y, Li Y et al (2019) Berberine attenuates nonalcoholic hepatic steatosis through the AMPK-SREBP-1c-SCD1 pathway. *Free Radic Biol Med* 141:192–204

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