Biochemical and Biophysical Research Communications 441 (2013) 133–138

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effect of Liraglutide on endoplasmic reticulum stress in diabetes

Linlin Zhao [a], Hao Guo [a], Hong Chen [a], Robert B. Petersen [b,c,d], Ling Zheng [e], Anlin Peng [f], Kun Huang [a,g,*]

[a] Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan 430030, PR China
[b] Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA
[c] Department of Neuroscience, Case Western Reserve University, Cleveland, OH 44106, USA
[d] Department of Neurology, Case Western Reserve University, Cleveland, OH 44106, USA
[e] College of Life Sciences, Wuhan University, Wuhan 430072, PR China
[f] The Third Hospital of Wuhan, Wuhan, Hubei 430060, PR China
[g] Centre for Biomedicine Research, Wuhan Institute of Biotechnology, Wuhan, Hubei 430074, PR China

ARTICLE INFO

Article history:
Received 4 October 2013
Available online 12 October 2013

Keywords:
Liraglutide
ER stress
INS-1 cells
Akita mouse
Diabetes

ABSTRACT

Endoplasmic reticulum (ER) stress is associated with the development of diabetes. The present study sought to investigate the effect of Liraglutide, a glucagon like peptide 1 analogue, on ER stress in β-cells. We found that Liraglutide protected the pancreatic INS-1 cells from thapsigargin-induced ER stress and the ER stress associated cell apoptosis, mainly by suppressing the PERK and IRE1 pathways. We further tested the effects of Liraglutide in the Akita mouse, an ER-stress induced type 1 diabetes model. After administration of Liraglutide for 8 weeks, p-eIF2α and p-JNK were significantly decreased in the pancreas of the Akita mouse, while the treatment showed no significant impact on the levels of insulin of INS-cells. Taken together, our findings suggest that Liraglutide may protect pancreatic cells from ER stress and its related cell death.

1. Introduction

The prevalence of diabetes mellitus is increasing dramatically; the number of diabetic patients may reach 300 million by 2030 globally [1]. Pancreatic β-cells possess a highly developed endoplasmic reticulum (ER) system for the biosynthesis, process and secretion of insulin, and β-cell dysfunction plays a key role in the pathogenesis of diabetes [2]. Accumulating evidence suggests that ER stress contributes to β-cell loss and insulin resistance, thus playing an important role in the pathogenesis of diabetes [3]. Many stimuli can trigger ER stress in β-cells including hyperglycemic and/or hyperlipidemic conditions, amyloid deposits, and inflammatory cytokines [4]. Upon ER stress, cells induce the unfolded protein response (UPR) *via* three signaling transducers: double-stranded RNA-activated protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF-6), and inositol requiring enzyme-1 (IRE1) [5]. The three signaling transducers are activated by luminal binding protein (Bip) mediated dissociation and initiate the three corresponding downstream signaling pathways. For the PERK branch, PERK phosphorylates eukaryotic translation initiation factor 2α (eIF2α) to attenuate the translation of cell cycle proteins and enhance the translation of ATF4, which further induces C/EBP homologous protein (CHOP) and ATF3 resulting in cell apoptosis [6]. In the other two pathways, activated IRE1 induces JNK phosphorylation and contributes to ER stress-mediated apoptosis [7,8]; whereas phosphorylated ATF-6 activates the transcription of ER chaperones, including Bip and protein disulfide isomerase (PDI), to increase the level of ER proteins that facilitate protein folding [9,10]. The unsolved ER stress contributes many diseases, including diabetes [11].

Glucagon-like peptide 1 (GLP-1) is a gut hormone that can stimulate insulin release, inhibit glucagon secretion and gastric emptying to decrease blood glucose. GLP-1 also reduces β-cell apoptosis and promotes their regeneration [12,13]. To study whether GLP-1 affects ER stress in pancreatic β-cells, Liraglutide, an acylated GLP-1 analogue with prolonged half-life [14], was added to thapsigargin (TG) treated INS-1 cells (a rat insulinoma cell line). TG is a specific inhibitor of a Ca^{2+}/ATPase pump that is used as an ER stress inducer [15]. We also administered Liraglutide to Akita mice, a model of type 1 diabetes [16], which has the accumulation of misfolded insulin in the ER lumen inducing ER stress in the β-cells due to a mutation in the insulin 2 gene (*Ins2*, Cys96Tyr) [17]. Our

Abbreviations: ER, endoplasmic reticulum; UPR, unfolded protein response; PERK, double-stranded RNA-activated protein kinase (PKR)-like ER kinase; ATF-6, activating transcription factor 6; IRE1, inositol requiring enzyme 1; Bip, luminal binding protein (also known as GRP78); eIF2α, eukaryotic translation initiation factor 2α; GLP-1, glucagon-like peptide 1; CHOP, C/EBP homologous protein; TG, thapsigargin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

* Corresponding author at: Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan 430030, PR China.
E-mail address: kunhuang2008@hotmail.com (K. Huang).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.026

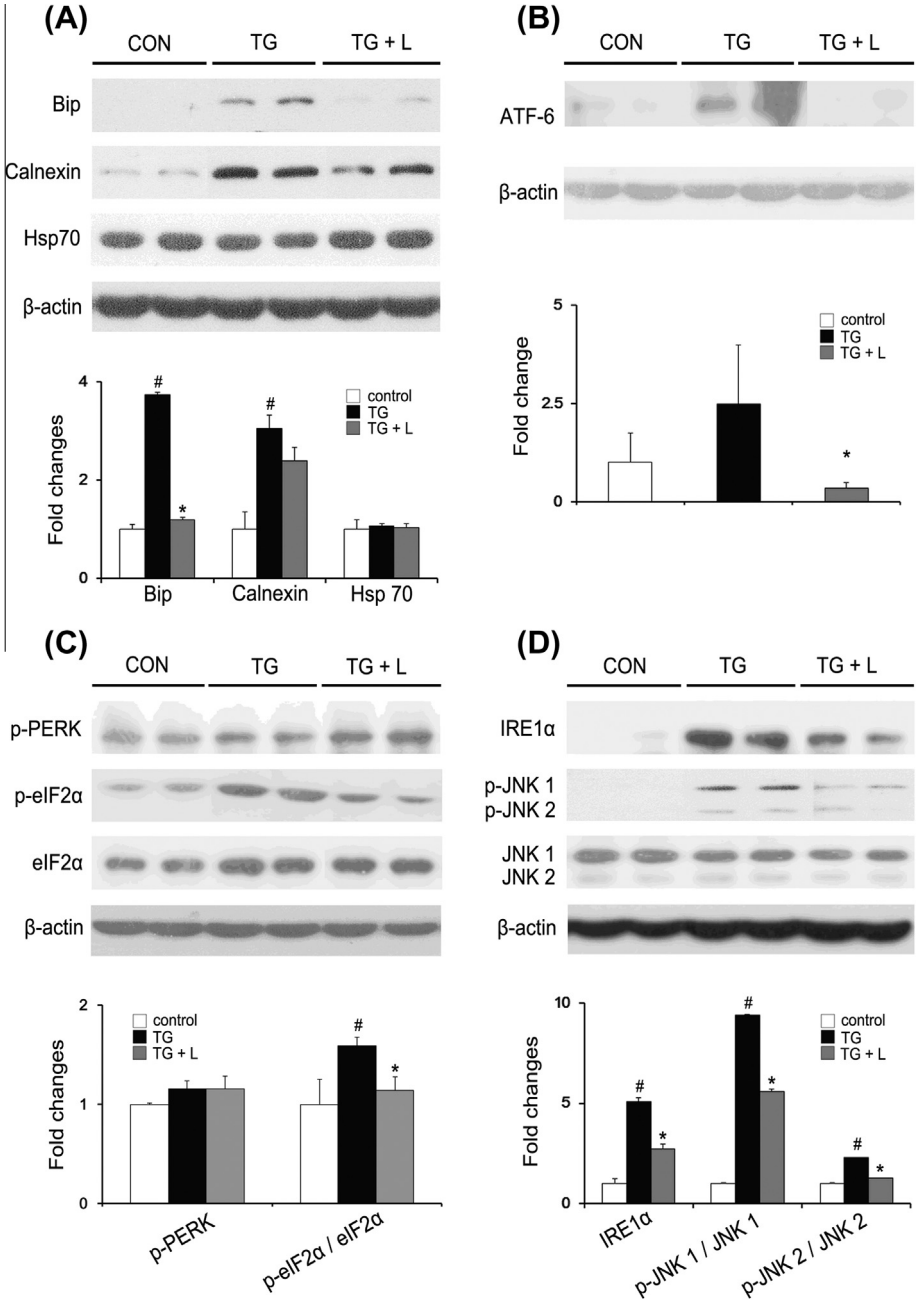

Fig. 1. Changes in ER stress pathways by Liraglutide-treatment of INS-1 cells. (A) Western blot analysis of chaperone protein levels in different experimental groups. (B) Western blot analysis of protein levels of ATF-6 in different experimental groups. (C) Western blot analysis of protein levels of PERK signaling pathway in different experimental groups. (D) Western blot analysis of protein levels of IRE1 signaling pathway in different experimental groups ($n = 3$ in each group, CON, control group, non-treated cells; TG, cells treated with TG; TG + L, cells treated with TG and Liraglutide; $^{\#}p < 0.05$ compared with control group; $^{*}p < 0.05$ compared with TG group, the protein levels were normalized against β-actin, eIF2α and JNK, respectively).

findings, both *in vitro* and *in vivo*, suggest that Liraglutide protects pancreatic cells against ER stress and cell death.

2. Materials and methods

2.1. Cell culture

INS-1 were cultured with RPMI-1640 media containing 10% fetal bovine serum, 100 mg/L penicillin–streptomycin and 110 mg/L sodium pyruvate, and incubated at 37 °C in 5% CO_2 atmosphere. 150 nM Liraglutide (GL Biochem., Shanghai, China) was added to the media for 30 min, followed by TG-treatment (5 μM) for 3 h.

2.2. Animals

Nine week old male Akita mice on a C57BL/6 background and their sex- and age-matched wild-type controls were obtained from the Model Animal Research Center of Nanjing University and ABSL-III laboratory of Wuhan University, respectively. The animals were housed in ventilated microisolator cages with free access to water and food. Akita mice were randomly divided into two groups: Akita ($n = 3$) and Liraglutide-treated Akita (50 nmol/kg/day by i.p. injection, from 11 weeks to 18 weeks, $n = 5$). Body weight (BW), fasted blood glucose (FBG) and non-fasted blood glucose (NFBG) were monitored every week with a ONE TOUCH glucometer (Lifescan Canada Ltd.). All animals were sacrificed at 19 weeks of age, the

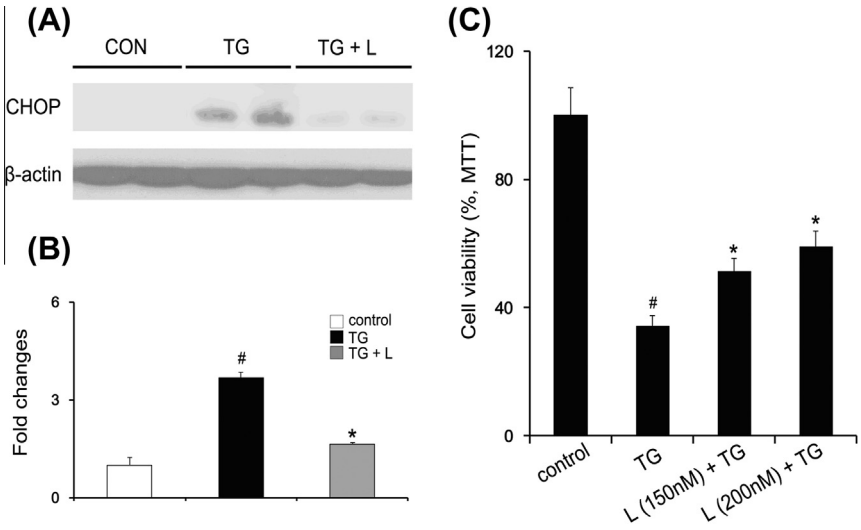

Fig. 2. Liraglutide-treatment suppresses death of INS-1 cells. (A) Western blot analysis of levels of CHOP in different experimental groups, the protein levels were normalized against β-actin. (B) The fold changes of CHOP normalized to β-actin bands ($n = 3$ in each group, $^{\#}p < 0.05$ compared with control group; $^{*}p < 0.05$ compared with TG group). (C) INS-1 cells viability determined by MTT assay, cells co-incubated with TG and different concentrations of Liraglutide ($n = 6$ in each group, $^{\#}p < 0.05$ compared with control group; $^{*}p < 0.05$ compared with TG group).

pancreas was dissected under microscope and immediately frozen in −80 °C until use. All animals were handled according to the guidelines approved by the Committee on Ethics in the Care and Use of Laboratory Animals of College of Life Sciences, Wuhan University.

2.3. Western blot

INS-1 cells were washed twice with PBS, and collected by centrifugation at 1000*g* for 10 min at 4 °C, protein was extracted by sonication in RIPA buffer (Beyotime Biotech, China). Pancreatic tissue (20 mg) from experimental mice was sonicated in RIPA buffer. Total protein concentration was quantified using a BCA protein assay kit (DBI Bioscience, Ludwigshafen, Germany). 20–50 μg protein samples were separated using 8–12% SDS–PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland). The membranes were first stained with washable Ponceau S solution to establish equal protein loading. After a brief wash, the membranes were blocked with 2% nonfat milk (Bio-Rad, Hercules, CA) in TBST (TBS with 0.1% Tween-20) buffer. Primary antibodies against Hsp70 (1:5000 dilution; BD Bioscience, San Jose, CA), Calnexin (1:5000 dilution; BD Bioscience), PDI (1:1000 dilution; Cell Signaling Technology, Danvers, MA), total eIF2α (1:1000 dilution; Cell Signaling Technology, CST), phosphorylated-eIF2α (1:1000 dilution; CST), JNK (1:1000 dilution; CST), phosphorylated JNK (1:1000 dilution; CST), Bip (1:1000 dilution; CST), Chop (1:1000 dilution; CST), Akt (1:5000 dilution; CST), p-AKT (Ser473) (1:1000 dilution; CST); IRE1α (1:5000 dilution; CST), and phosphorylated PERK (1:5000 dilution; Santa Cruz Biotechnology, CA) were applied overnight at 4 °C. After extensive washing, blots were further incubated with the appropriate HRP-coupled secondary antibody (l:5000; CST) and visualized using ECL reagent (Thermo scientific, Rockland) by exposure to X-OMAT autoradiograph films (Kodak, USA). The developed films were subsequently scanned, and band intensities were quantified using Quantity One Software (Bio-Rad).

2.4. MTT assay

INS-1 cells were plated at a density of 5×10^4 cells/well and incubated at 37 °C in 5% CO_2 for 12 h. Fresh medium containing 150- or 200-nM Liraglutide [18] and 3 μM TG was then added and incubated for 24 h. For the MTT assay, cells were incubated with 10 μl MTT (5 mg/ml) per well for 4 h. 100 μl formazan was added to each well and the absorbance was measured at 570 nm.

2.5. RNA isolation and RT-PCR

RNA was extracted from cultured cells using RNAiso Plus (Takara Biotechnology Co., Dalian, China) as we previously reported [19]. cDNA synthesis was performed using the M-MLV First Stand Kit (Invitrogen, Carlsbad, CA). Primer sequences of target genes are provided in Supplementary Table S1. RT-PCR was performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). PCR products were separated by electrophoresis using 2% agarose gels, and images were photographed by Molecular Imager Gel Doc XR (Bio-Rad, Hercules, CA). Band density was quantified using Quantity One 1-D Analysis Software (Bio-Rad). 18S rRNA was used as an internal control. The data are presented as fold changes compared to the level of NC group, which is set as onefold.

2.6. Insulin ELISA

At 80% cell confluence, the media was replaced with either 5 μM TG media with or without 150 nM Liraglutide, or normal media. Insulin content from INS-1 cells was detected by using an insulin ELISA kit (Millipore, Billerica, MA) according to the manufacturer's instruction.

2.7. Statistical analysis

Data were presented as mean ± SD. Data were analyzed by the nonparametric Kruskal–Wallis test followed by the Mann–Whitney test, where $p < 0.05$ was considered significant.

3. Results

3.1. Liraglutide protected INS-1 cells from TG induced ER stress

To assess the cytoprotective effects of Liraglutide in TG-induced ER stress, INS-1 cells were treated with TG in the presence or absence of 150 nM Liraglutide and the levels of ER stress related chaperones, including Bip, Calnexin and Hsp70, were determined. TG-treatment significantly increased Bip and Calnexin (2.7-fold

and two fold increase respectively, $p < 0.05$, Fig. 1A), indicating elevated ER stress, whereas no significant change in the level of Hsp70 was observed after TG-treatment (Fig. 1A). Liraglutide-treatment significantly decreased the level of Bip (65% lower than TG-treated cells, $p < 0.05$, Fig. 1A), and showed no significant effect on the level of Hsp70 and Calnexin, although a trend toward decreased Calnexin was observed after Liraglutide-treatment. There was a trend toward increased ATF-6 in TG-treated INS-1 cells (1.5-fold increase compared to control, Fig. 1B), whereas Liraglutide-treatment significantly decreased the level of ATF-6 compared to TG-treated cells (86% reduction compared to TG-treated cells, $p < 0.05$, Fig. 1B).

Phosphorylated eIF2α (p-eIF2α), a downstream molecule in the PERK pathway, was increased in TG-treated INS-1 cells (1.6-fold of the control, $p < 0.05$, Fig. 1C), indicating that the PERK pathway was activated in agreement with a previous report [20]. Liraglutide-treatment significantly decreased the levels of p-eIF2α compared to TG-treated cells (reduced by 29%, $p < 0.05$, Fig. 1C).

IRE1 has two homologs, IRE1α and IRE1β, which have been identified in the murine and human genomes. IRE1α is constitutively expressed in all cells and tissues, while IRE1β is selectively expressed in gut epithelial cells [21,22]. Therefore, only IRE1α was analyzed in this study. TG-treatment not only significantly

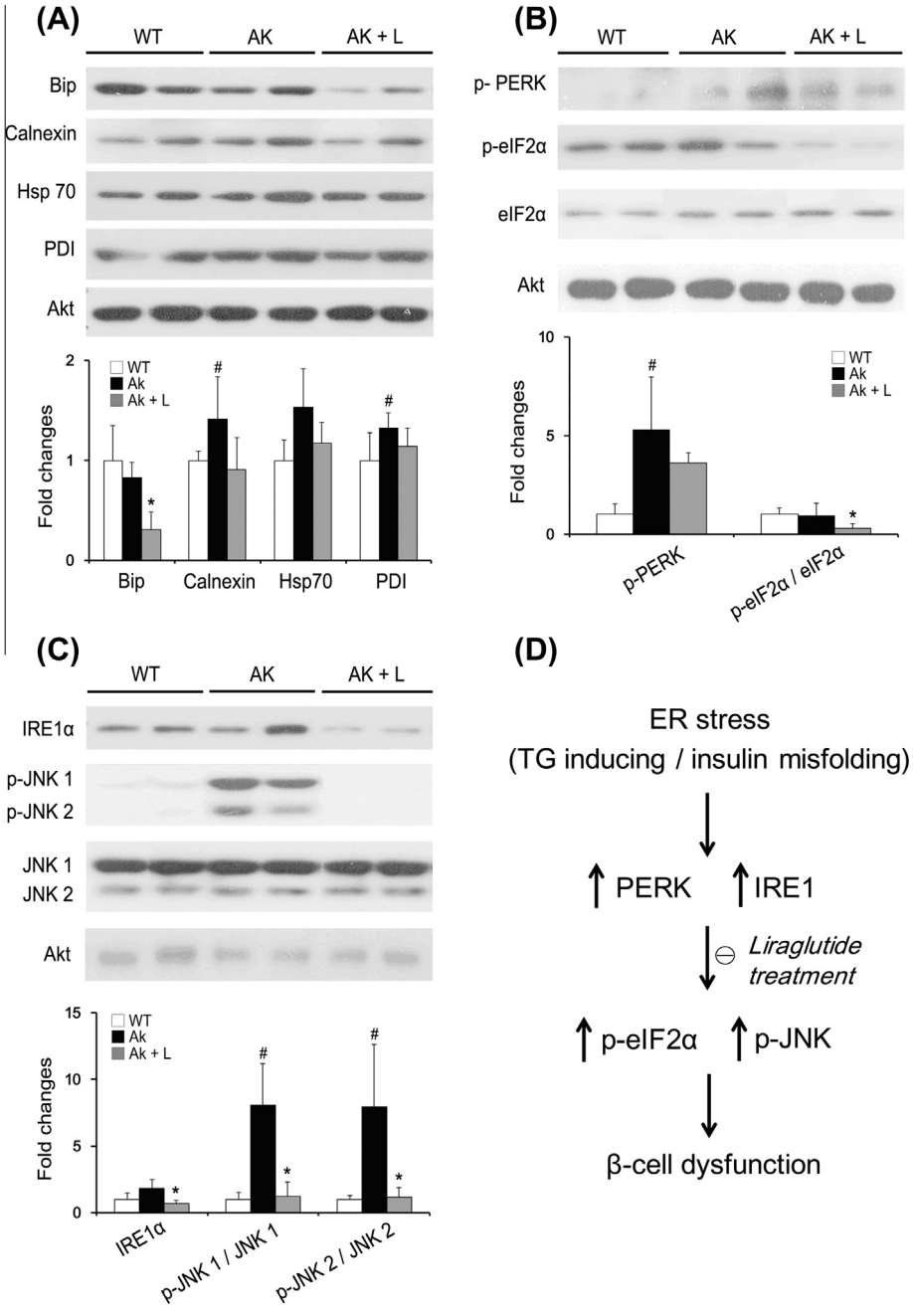

Fig. 3. Liraglutide impact on ER stress in the pancreas of Akita mice. (A) Western blot analysis of chaperone protein levels in different groups. (B) Western blot analysis of PERK signaling pathway in different groups. (C) Western blot analysis of IRE1 signaling pathway in different groups (WT, wild type, $n = 6$; AK, Akita mouse, $n = 3$; AK + L, Akita mouse treated by Liraglutide, $n = 5$; $^{\#}p < 0.05$ compared with WT group; $^{*}p < 0.05$ compared with Akita group, the protein levels were normalized against Akt, eIF2α and JNK, respectively). (D) Possible molecular mechanisms of Liraglutide-treatment on ER stress. Accumulation of unfolded protein triggers ER stress, which dissociates Bip from ATF-6, IRE1 and PERK activating these molecules, further activates the corresponding signaling pathways resulting in cell apoptosis, Liraglutide suppresses ER stress *via* regulation of the IRE1 and PERK pathways.

increased IRE1α (4.1-fold increase, $p < 0.05$, Fig. 1D), but also significantly increased phosphorylated JNK (p-JNK) compared to the controls (7.3-fold, $p < 0.05$, Fig. 1D), demonstrating the activation of the IRE1 pathway. Liraglutide treatment significantly decreased the levels of IRE1α and p-JNK (46% and 43% reduction compared to the TG-treated cells, respectively, $p < 0.05$, Fig. 1D).

To investigate the effects of Liraglutide on insulin levels in INS-1 cells, the mRNA levels of *Ins1* and *Ins2* were first measured. Compared to the control, TG significantly decreased mRNA levels of *Ins1* and *Ins2*, whereas Liraglutide treatment showed no significant effect on the levels of insulin genes (Supplementary Fig. S2A and B). Consistently, the insulin ELISA measurements also showed similar insulin levels among different experimental groups (Supplementary Fig. S2C).

3.2. Liraglutide inhibits ER stress-induced β-cell death

The transcription factor CHOP has been implicated as a critical molecule in the ER stress response and cell apoptosis [23]. We found that CHOP was significantly increased in TG-treated INS-1 cells (2.7-fold increase, $p < 0.05$, Fig. 2A), while the Liraglutide treatment significantly decreased CHOP (56% reduction compared to TG-treated cells, $p < 0.05$, Fig. 2B).

MTT assays were performed to determine the effect of Liraglutide on ER stress induced β-cell death. Compared to untreated cells, TG-treated cells showed significantly decreased cell viability (34% of the control, $p < 0.05$, Fig. 2C). Liraglutide attenuated TG-induced cell death. Cells treated with 150 nM and 200 nM Liraglutide showed increased cell viability, 50% and 72%, respectively, compared to TG-treated cells ($p < 0.05$, Fig. 2C).

3.3. Effect of Liraglutide on Akita mice

Previous studies have shown that Liraglutide has multiple beneficial effects on streptozocin-generated diabetic mice [24]. We found that blood glucose levels of Akita mice were significantly higher than the wild type controls, which is in accord with the Akita mouse being a model of type 1 diabetes [25]. Liraglutide administration showed a trend toward decreased FBG, but showed no obvious effects on NFBG and BW (Supplementary Fig. S1B–D). However, Liraglutide has significant effect to increase insulin sensitivity in nondiabetic mice demonstrated by glucose tolerance test (Supplementary Fig. S1A).

3.4. Liraglutide attenuates ER stress in vivo through p-eIF2α and p-JNK

In Akita mice, Calnexin (40% increase, $p < 0.05$) and PDI (30% increase, $p < 0.05$, Fig. 3A) were significantly increased compared with the WT controls (Fig. 3A), while similar levels of Bip were observed between Akita mice and the control C57 mice (Fig. 3A). Liraglutide-treatment not only significantly decreased Bip (70% reduction, $p < 0.05$, Fig. 3A), but also showed a trend in decreasing the levels of Calnexin, Hsp70 and PDI in the pancreas of Akita mice (36%, 24% and 14% reduction, respectively, Fig. 3A).

There was a 5.3-fold increase of p-PERK ($p < 0.05$, Fig. 3B) in the pancreas of Akita mice compared with the wild-type, while Liraglutide-treatment decreased p-PERK (31% reduction) in Akita mice. The level of p-eIF2α was also reduced by 63% in Liraglutide-treated Akita mice (Fig. 3B).

Compared with the wild-type mice, Akita mice showed a significant increase in the levels of p-JNK (8-fold increase, $p < 0.05$, Fig. 3C) and a trend toward increased IRE1α (1.8-fold increase). Administration of Liraglutide significantly decreased both p-JNK (85% reduction, $p < 0.05$, Fig. 3C) and IRE1α (65% reduction, $p < 0.05$, Fig. 3C) in the pancreas of Akita mice. No significant difference was observed for the levels of total Akt and p-Akt (Ser 473) among the experimental groups (Supplementary Fig. S3).

4. Discussion

Due to the important roles that ER stress plays in pathogenesis of diabetes, attenuating ER stress may provide a new approach to alleviate or cure diabetes under certain conditions. As the most critical cell type for normal pancreatic function, β-cells can be triggered to death by severe ER stress *in vitro* [26]. A recent study demonstrates that ER stress related proteins are over-expressed in human umbilical vein endothelial cells when challenged with high glucose, while Liraglutide is capable of down-regulating this high glucose induced ER stress [27]. However, the exact mechanism(s) by which Liraglutide treatment affect pancreatic ER stress remains unclear in animal model. In the present study, the ER stress pathways were significantly increased in TG treated pancreatic INS-1 cells, as well as in the pancreas of Akita mice. We have previously shown that apelin-13, a newly identified bioactive adipokine, alleviates ER stress in Akita pancreases [28]. Interestingly, we found that although Liraglutide showed a trend of decreasing ER stress associated FBG in Akita mice, the NFBG and BW of Akita mice were not significantly changed. However, Liraglutide not only decreased the ER chaperone Bip, but also showed inhibitory effects on ER-stress related pathways mainly through decreasing p-eIF2α and p-JNK. We also found that Liraglutide protected TG-treated INS-1 cells against ER stress-induced cell apoptosis, with attenuated cell-apoptosis associated transcription factor CHOP. The beneficial effects of Liraglutide on ER stress are summarized (Fig. 3D).

In conclusion, our study provides both *in vitro* and *in vivo* evidence that Liraglutide alleviates ER stress mainly via regulating p-eIF2α and p-JNK levels. Therefore, our results provide new angles for treating ER-stress associated type 1 diabetes as well as other ER stress related diseases.

Acknowledgments

This work was supported by the Natural Science Foundation of China (Nos. 31271370, 81100687, 81172971 & 81222043), the Program for New Century Excellent Talents in University (NECT11-0170), the Municiple Key Technology Program of Wuhan (Wuhan Bureau of Science & Technology, No. 201260523174) and the Health Bureau of Wuhan (WX12B06). The authors also thank Mr. Chengyu Liu for assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.026.

References

[1] E.G. Holliday, Hints of unique genetic effects for type 2 diabetes in India, Diabetes 62 (2013) 1369–1370.
[2] S.J. Marciniak, D. Ron, Endoplasmic reticulum stress signaling in disease, Physiol. Rev. 86 (2006) 1133–1149.
[3] H.P. Harding, D. Ron, Endoplasmic reticulum stress and the development of diabetes: a review, Diabetes 51 (Suppl. 3) (2002) S455–S461.
[4] D.L. Eizirik, A.K. Cardozo, M. Cnop, The role for endoplasmic reticulum stress in diabetes mellitus, Endocr. Rev. 29 (2008) 42–61.
[5] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[6] P. Pirot, F. Ortis, M. Cnop, Y. Ma, L.M. Hendershot, D.L. Eizirik, A.K. Cardozo, Transcriptional regulation of the endoplasmic reticulum stress gene chop in pancreatic insulin-producing cells, Diabetes 56 (2007) 1069–1077.
[7] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science 287 (2000) 664–666.

[8] C. Zhang, J. Kawauchi, M.T. Adachi, Y. Hashimoto, S. Oshiro, T. Aso, S. Kitajima, Activation of JNK and transcriptional repressor ATF3/LRF1 through the IRE1/TRAF2 pathway is implicated in human vascular endothelial cell death by homocysteine, Biochem. Biophys. Res. Commun. 289 (2001) 718–724.
[9] T. Okada, H. Yoshida, R. Akazawa, M. Negishi, K. Mori, Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response, Biochem. J. 366 (2002) 585–594.
[10] K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, Mol. Biol. Cell 10 (1999) 3787–3799.
[11] TEDDY Study Group, The environmental determinants of diabetes in the young (TEDDY) study: study design, Pediatr. Diabetes 8 (2007) 286–298.
[12] M. Zander, S. Madsbad, J.L. Madsen, J.J. Holst, Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study, Lancet 359 (2002) 824–830.
[13] C. Tourrel, D. Bailbe, M.J. Meile, M. Kergoat, B. Portha, Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age, Diabetes 50 (2001) 1562–1570.
[14] T. Vilsboll, Liraglutide: a once-daily GLP-1 analogue for the treatment of type 2 diabetes mellitus, Expert Opin. Invest. Drugs 16 (2007) 231–237.
[15] N. Mukerjee, K.M. McGinnis, Y.H. Park, M.E. Gnegy, K.K. Wang, Caspase-mediated proteolytic activation of calcineurin in thapsigargin-mediated apoptosis in SH-SY5Y neuroblastoma cells, Arch. Biochem. Biophys. 379 (2000) 337–343.
[16] M. Yoshioka, T. Kayo, T. Ikeda, A. Koizumi, A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice, Diabetes 46 (1997) 887–894.
[17] J. Wang, T. Takeuchi, S. Tanaka, S.K. Kubo, T. Kayo, D. Lu, K. Takata, A. Koizumi, T. Izumi, A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse, J. Clin. Invest. 103 (1999) 27–37.
[18] S. Bregenholt, A. Moldrup, N. Blume, A.E. Karlsen, B. Nissen Friedrichsen, D. Tornhave, L.B. Knudsen, J.S. Petersen, The long-acting glucagon-like peptide-1 analogue, Liraglutide, inhibits beta-cell apoptosis *in vitro*, Biochem. Biophys. Res. Commun. 330 (2005) 577–584.
[19] L.L. Wang, H. Chen, K. Huang, L. Zheng, Elevated histone acetylations in Muller cells contribute to inflammation: a novel inhibitory effect of minocycline, Glia 60 (2012) 1896–1905.
[20] X. Lei, S. Zhang, S.E. Barbour, A. Bohrer, E.L. Ford, A. Koizumi, F.R. Papa, S. Ramanadham, Spontaneous development of endoplasmic reticulum stress that can lead to diabetes mellitus is associated with higher calcium-independent phospholipase A2 expression: a role for regulation by SREBP-1, J. Biol. Chem. 285 (2010) 6693–6705.
[21] W. Tirasophon, A.A. Welihinda, R.J. Kaufman, A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells, Genes Dev. 12 (1998) 1812–1824.
[22] X.Z. Wang, H.P. Harding, Y. Zhang, E.M. Jolicoeur, M. Kuroda, D. Ron, Cloning of mammalian Ire1 reveals diversity in the ER stress responses, EMBO J. 17 (1998) 5708–5717.
[23] S. Oyadomari, M. Mori, Roles of CHOP/GADD153 in endoplasmic reticulum stress, Cell Death Differ. 11 (2004) 381–389.
[24] S. Merani, W. Truong, J.A. Emamaullee, C. Toso, L.B. Knudsen, A.M. Shapiro, Liraglutide, a long-acting human glucagon-like peptide 1 analog, improves glucose homeostasis in marginal mass islet transplantation in mice, Endocrinology 149 (2008) 4322–4328.
[25] B.K. Chacko, C. Reily, A. Srivastava, M.S. Johnson, Y. Ye, E. Ulasova, A. Agarwal, K.R. Zinn, M.P. Murphy, B. Kalyanaraman, V. Darley-Usmar, Prevention of diabetic nephropathy in Ins2(+/)(−)(AkitaJ) mice by the mitochondria-targeted therapy MitoQ, Biochem. J. 432 (2010) 9–19.
[26] M. Miani, J. Barthson, M.L. Colli, F. Brozzi, M. Cnop, D.L. Eizirik, Endoplasmic reticulum stress sensitizes pancreatic beta cells to interleukin-1beta-induced apoptosis via Bim/A1 imbalance, Cell Death Dis. 4 (2013) e701.
[27] B. Schisano, A.L. Harte, K. Lois, P. Saravanan, N. Al-Daghri, O. Al-Attas, L.B. Knudsen, P.G. McTernan, A. Ceriello, G. Tripathi, GLP-1 analogue, Liraglutide protects human umbilical vein endothelial cells against high glucose induced endoplasmic reticulum stress, Regul. Pept. 174 (2012) 46–52.
[28] H. Chen, C. Zheng, X. Zhang, J. Li, L. Zheng, K. Huang, Apelin alleviates diabetes-associated endoplasmic reticulum stress in the pancreas of Akita mice, Peptides 32 (2011) 1634–1639.