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DPP IV inhibitor suppresses STZ-induced islets injury dependent on activation of the IGFR/Akt/mTOR signaling pathways by GLP-1 in monkeys

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ABSTRACT

Background: To evaluate the protective effect of the DPP IV inhibitor in STZ-induced islet injury and to identify the molecular events that protect islet against apoptosis.
Methods: 4 diabetic monkeys were treated with streptozotocin (70 mg/kg) in the presence or absence of the DPP IV inhibitor (Sitagliptin), continuing administered for 4 weeks after STZ. The monkeys were evaluated by plasma DPP IV activity, serum active GLP-1 response, blood glucose, insulin and C-P levels, the insulin resistance index (HOMA-IR), and the expression of insulin, caspase-3, IGF receptor (IGFR), p-Akt and p-mTOR in pancreas islets tissues. To test that DPP IV inhibitors might against islets apoptosis via IGFR/Akt/mTOR signaling pathways, the isolated islets from the normal monkeys were pre-treated with or without 10 mM STZ for 1 h, followed by GLP-1 (10 μM) in the presence or absence of NVP-AEW541 or Wortmannin for 24 h, to determined islets function and islet apoptosis.
Results: DPP IV inhibitors treatment showed depressing the degradation of GLP-1 and significantly increased serum GLP-1 levels in DM monkeys. Moreover, treatment of diabetic monkeys with the DPP IV inhibitor or treatment of isolated islets with GLP-1 can decrease islet apoptosis, and enhanced islet function and survival, and the expression of IGF receptor, p-Akt and p-mTOR in islets. When the IGFR/Akt/mTOR signaling pathways was blocked by NVP-AEW541 or Wortmannin, the protective effects of GLP1 on STZ-induced islets injury were inhibited in vitro.
Conclusions: Our data provides evidence that DPP IV inhibitors confer resistance to STZ-induced islet injury. The protective effects of DPP IV inhibitor on STZ-induced islets injury were dependent on activation of the IGFR/Akt/mTOR signaling pathways by GLP-1 in islets of monkeys.

1. Introduction

Glucagon-like peptide-1(GLP-1), an incretin released from the L-cells of the small intestine, targets pancreatic β-cells to release insulin and reduce glucagons production in response to food intake [1]. In addition, GLP-1 has protective effects against inflammation, oxidative injury, and apoptotic β-cell death in various disease models [2,3]. However, the GLP-1 is quickly degraded by the enzyme dipeptidyl peptidase IV (DPP IV) occurs within minutes following GLP-1 secretion [4,5]. Apoptosis is a major cause of β-cell loss during the pathogenesis of type 1 and type 2 diabetes [6]. Thus, we focus on the development of ways to inhibit the degradation of GLP-1 and the treatment strategies protecting β cell from apoptosis.

Highly selective DPP IV inhibitors are quite different from conventional antidiabetic agents and control hyperglycemia by stimulating insulin production via the prevention of the degradation of two major incretins, the GLP-1 and the glucose inhibitory peptide (GIP) [4,5,7,8]. Although the recent studies indicated that DPP IV inhibitor can inhibit the apoptosis of islet in rodent animal [4]. By creating a new-onset diabetes mouse model in which Tacrolimus-induced pancreatic islet injury in transplant recipients, Long's group demonstrated that the DPP IV inhibitor MK-0626 was an effective antidiabetic agent that exerted antioxidative and antiapoptotic effects via enhanced GLP-1 signaling in TAC-induced diabetics [4]. The potential of nonhuman primate (NHP) models in

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diabetes research has been well understood, and NHPs have long been regarded as the "gold standard" for preclinical studies [9]. However, to our knowledge, there are no reports regarding whether the DPP IV inhibitor can improve tolerance of the process of inflammation and oxidative injury, and enhanced islets mass and function in primates. Therefore, we designed this study to assess the effect of a DPP IV inhibitor on STZ-induced diabetes in the non-human primate animal. First, we tested whether the DPP IV inhibitor effectively controlled the degradation of GLP-1 in the non-human primates. Second, we evaluated whether the protective effect of the DPP IV inhibitor was also present in STZ-induced islet injury. Third, to identify the molecular events that protect islet against apoptosis. We expect that the results of our study will provide a rationale for the use of DPP IV inhibitors in diabetic patients.

2. Materials and methods

2.1. Animals

Rhesus monkeys (age, 3–5 year) were obtained from Chengdu Ping'an Experimental Animal Reproduction Center (Sichuan, China). The animals were cared for in accordance with the guidelines of the Experimental Animal Center, Sichuan University, which have been approved by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.2. Drugs and analytical measurements

4 monkeys were given a single dose of STZ (70 mg/kg) [9]. Meanwhile, the DPP IV inhibitor (Sitagliptin, 20 mg/kg) were administered once daily before feeding. The monkeys were evaluated by the following parameters: (1) blood glucose and glycosylated hemoglobin (HbA1C); (2) radioimmunoassay to analyze insulin and C-P levels (Beijing North Institute of Biological Technology, Beijing, China); (3) the insulin resistance index (HOMA-IR) was estimated using the homeostasis model assessment [10]; and (4) biopsies of the pancreas: immunohistochemistry to analyze insulin, caspase-3, IGF receptor (IGFR), p-Akt and p-mTOR in he pancreas islets tissues.

2.3. Measurement of the Levels of DPP-IV and GLP-1

After 4 weeks of treatment, the animals were fasted for approximately 24 h. Blood samples were drawn from tail veins at 0, 5, 10, 30 and 60 min after glucose loading (2 g/kg). To determine the activity of GLP-1 (uncleaved, 7–36 amide or 7–37) in the serum, blood samples were collected into tubes containing a DPP IV inhibitor (50 mM), centrifuged, and frozen at −80 °C. To measure plasma DPP-IV activity, blood samples were collected into prechilled, EDTA-treated tubes, centrifuged at 4 °C, separated, and frozen at −80 °C. The level of active GLP-1 (Millipore Corporation, Billerica, MA) and DPP IV activity (Merck KGaA, Darmstadt, Germany) were measured using a commercially available ELISA kit, according to the manufacturer's recommendations.

2.4. Islet isolation and culture

To harvest the pancreas, the pancreatic duct was cannulated with a 20 G angiocath and the pancreas transported to the laboratory for islet isolation (cold ischemia time < 1 h). The pancreas was distended with Liberase HI (Roche, Indianapolis, IN) and digested for 10 min at 37 °C in a water bath. The islets were purified by density gradient centrifugation. The islets were collected and washed with Hank's solution containing 1% bovine serum albumin (BSA; Roche), then stained with dithizone (DTZ; Sigma) and counted under a microscope. The islets were cultured in RPMI-1640 medium (Gibco, Invitrogen) supplemented with 10% FBS, 100 U/ml of penicillin, and 100 U/ml streptomycin.

2.5. In vitro injury model induced by STZ

To mimic the inflammation of islets, we utilized the STZ–induced islets injury test. We pretreated islet cultures for 1 h in the presence of STZ (10 mM, Sigma) [11] and subjected them to GLP-1 (10 μM, PIERCE) or vehicle control treatment for 24 h. In other experiments, we preincubated the islets with the IGFR inhibitor NVP-AEW541 (25 mM, Novartis), the mTOR phosphorylation inhibitor Wortmannin (10 μM, Sigma) or vehicle for 1 h before GLP1 treatment.

2.6. Determination of islet cell apoptosis

The cell apoptosis rate was determined by the FCM assay by using the apoptosis detection kit (Keygen, China). Islet cells were dissociated into single cells by incubation with trypsin–EDTA for 7 min. The dissociated islet cells were incubated with annexin V-FITC (annexin V) for 15 min at room temperature. Next, the samples were analyzed via flow cytometry (FCM; Beckman Elite EXP) to determine the number of apoptotic cells (annexin V positive cells). In addition, the activity of Caspase-3 in islets was examined by Western blot analysis.

2.7. Glucose-stimulated insulin secretion (GSIS) and insulin content measurement

Handpicked islets were subjected to a static glucose challenge in Krebs–Ringer bicarbonate buffer (KRBB) (pH 7.35) containing 10 mmol/l HEPES and 0.5% BSA (Sigma). After conditioning, the islets were incubated in KRBB containing low (2.8 mmol/l) and high (20 mmol/l) glucose concentrations for 1 h. At the end of the glucose challenge, the insulin levels were measured by an enzyme-linked immunosorbent assay (ELISA) (Linco Research, USA). The stimulation index (SI) for each experimental condition was calculated as the ratio of insulin released in high versus low glucose concentrations.

2.8. Western blot analysis

Whole-cell lysates were resolved on 10% SDS–PAGE gels followed by electrophoretic transfer to nitrocellulose membranes. After transfer to membranes, we performed an immunoblot analysis with the indicated primary antibodies at a 1:800 dilution. This was followed by incubation with the secondary antibody conjugated with horseradish peroxidase at a 1:3000 dilution. We used the ECL-PLUS Western Blotting Detection kit (Amersham Pharmacia Biotech) for detection. Primary antibodies were an anti-caspase-3 monoclonal antibody, anti-IGF receptor polyclonal antibody, anti-phospho Akt kinase monoclonal antibody, anti-phospho mTOR monoclonal antibody (all antibodies supplied by Abcam), and anti-GAPDH monoclonal antibody (Biogenesis).

2.9. Statistical analysis

All data are expressed as the mean ± SD. The differences between mean values were determined by the Student's t test for singular comparisons and by the one-way ANOVA for multiple comparisons. All statistical analyses were performed with SPSS using $P < 0.05$ to achieve significance.

3. Results

3.1. The DPP IV inhibitor treatment increases circulating levels of GLP-1

DPP IV inhibitors can prevent the degradation of the GLP-1 [4,5]. Therefore we evaluated the effect of DPP IV inhibitor on plasma DPP IV activity, serum active GLP-1 response, and plasma insulin excursions in glucose-loaded DM monkeys after 24 h fasting (Fig. 1). After treatment with 20 mg/kg of DPP IV inhibitors, plasma DPP IV activity was significantly decreased (Fig. 1A). And serum GLP-1 levels was significantly increased (5.65 pM in the STZ-DPP IV inhibitors group vs. 3.07 pM in the STZ group at 5 min, $P < 0.05$) than that observed in the STZ group within 30 min after glucose loading (Fig. 1B). Concomitantly, the high level of insulin profile was responded in the STZ-DPP IV inhibitors groups compared with the STZ group (Fig. 1C).

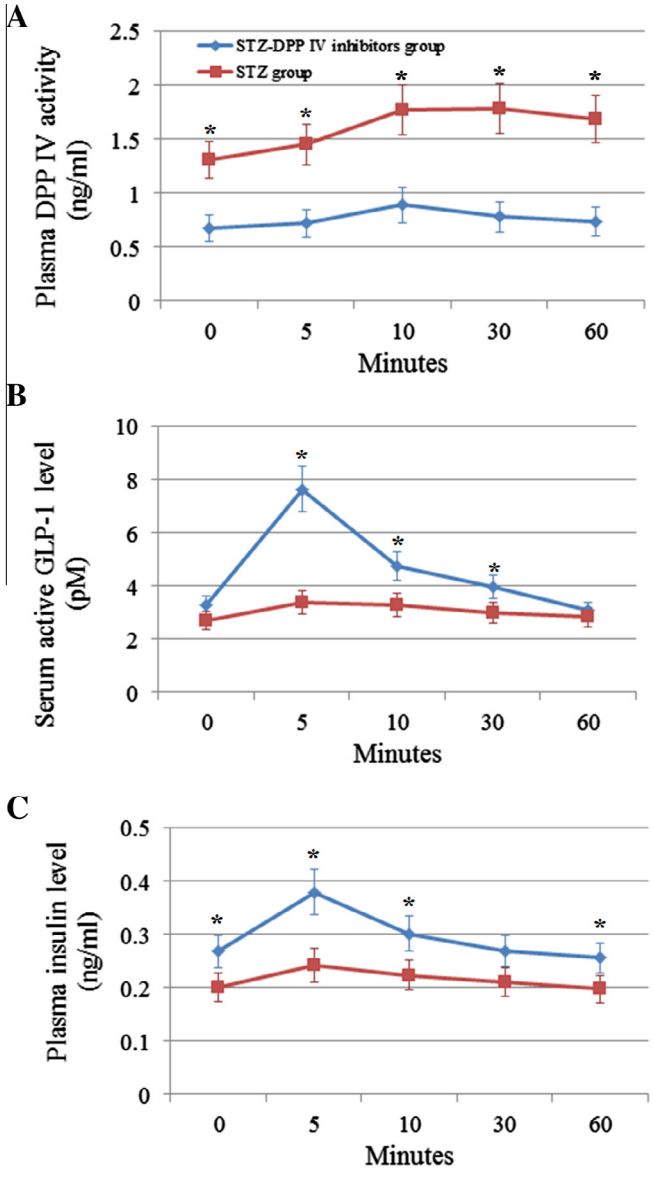

Fig. 1. Changes in the level of DPP IV, GLP-1, and insulin after glucose loading in DM monkeys after 4 weeks of DPP IV inhibitors treatment. DPP IV inhibitors-treated monkeys were given glucose at 0 min, followed by the measurement of plasma DPP IV activity (A), serum active GLP-1 level (B), and plasma insulin level (C) at 0, 5, 10, 30, and 60 min. $N = 2$ monkeys per group. $^*P < 0.05$ vs. STZ group vs. STZ-DPP IV inhibitors groups.

3.2. The DPP IV inhibitor treatment attenuates STZ-induced islet injury

As treatment of rodents with GLP-1 agonists leads to increased islet mass in association with islets proliferation and neogenesis [12,13], we hypothesized that the DPP IV inhibitor might also enhance islets mass via protection from cellular apoptosis. To test this hypothesis, 4 diabetic monkeys were treated with low-dose streptozotocin, a chemical known to induce islets apoptosis [14], in the presence or absence of the DPP IV inhibitor, continuing administered for 4 weeks after STZ. The levels of plasma GLP-1 were significantly greater in the DPP IV inhibitor treated monkeys compared with the STZ group (Fig. 1). The levels of blood glucose (Fig. 2A; $P < 0.05$ for day 16–28) were significantly lower in the STZ-DPP IV inhibitors groups compared with the STZ group. The levels of plasma C-p (Fig. 2B; $P < 0.05$) and the HOMA-IR index (Fig. 2C; $P < 0.05$) remained significantly greater in the STZ-DPP IV inhibitor group after a 4-week treatment with DPP IV inhibitor. Furthermore, we performed immunohistochemistry and quantitative analysis of insulin and caspase-3 in islets tissues (Fig. 3A and B). The number of insulin-positive cells within the islets was significantly higher in the STZ-DPP IV inhibitor group compared with the STZ group (Fig. 3A and B). The number of the active form of caspase-3 in the islets was reduced by addition of STZ-DPP IV inhibitor compared with those observed in the STZ group (Fig. 3A and B).

3.3. The DPP IV inhibitor enhanced IGF-1R, p-Akt, p-mTOR expression in islets

We found that treatment of diabetic monkeys with the DPP IV inhibitor enhanced the expression of IGF receptor in islets tissues (Fig. 3A and B). We also examined the PI3K/Akt/mTOR signaling, a well-characterized downstream target of IGFR [15]. We found that p-Akt, p-mTOR expression of islets tissues was enriched in the STZ-DPP IV inhibitor group compared with the STZ group (Fig. 3A and B), suggesting that IGF1 receptor signaling might be activated by DPP IV inhibitor. Next, we determined whether treatment of islets (pre-treated with 10 mM STZ for 1 h) with GLP-1 (2 and 10 μM, 24 h) could alter the expression of IGF1 receptor, p-Akt,

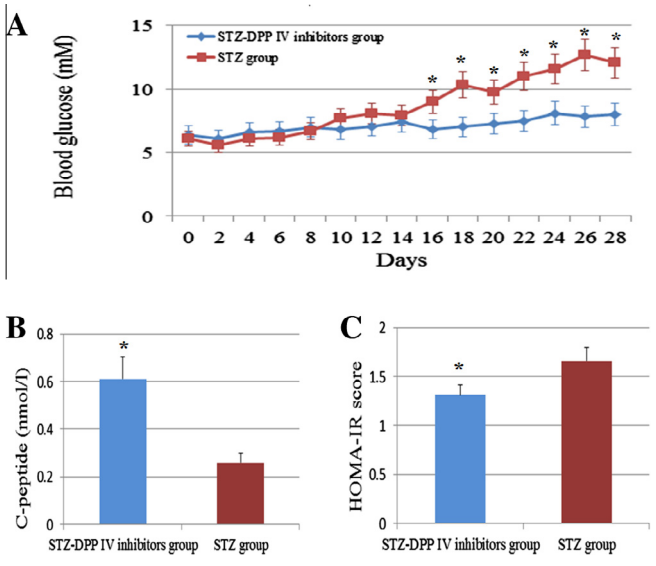

Fig. 2. Effect of DPP IV inhibitor on STZ-induced pancreatic islet dysfunction. (A) Blood glucose monitoring are recovered after treatment with DPP IV inhibitor. (B) Plasma C-peptide level after 4-week treatment with DPP IV inhibitor (C). Homeostatic model assessment of insulin resistance (HOMA-IR) index after a 4-week treatment with DPP IV inhibitor. $N = 2$ monkeys per group. $^*P < 0.05$ STZ group vs. STZ-DPP IV inhibitors groups.

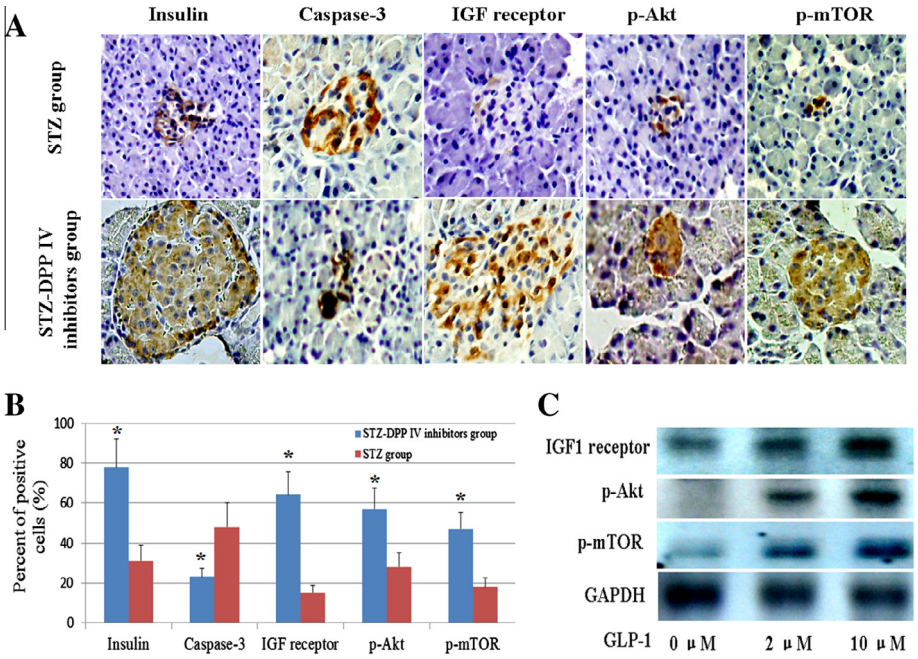

Fig. 3. Effect of PP IV inhibitor or GLP-1 on the expression of IGF receptor, p-Akt, and p-mTOR during STZ-induced islet injury. (A and B) Immunohistochemistry for IGF receptor, p-Akt and p-mTOR, and its quantitative analysis in pancreatic islets of DM monkeys after 1 month treatment with DPP IV inhibitor. $N = 2$ monkeys per group. $^{*}P < 0.05$ STZ group vs. STZ-DPP IV inhibitors groups. (C) Immunoblot analysis of IGF receptor, p-Akt, and p-mTOR in isolated pancreatic islets from normal monkeys. Note that the isolated islets were pre-treated with 10 mM STZ for 1 h, followed by GLP-1 (2 or 10 μM) or vehicle control for 24 h.

p-mTOR compared with untreated islets cells in vitro. IGF1 receptor expression was induced increase after 24 h of treatment with GLP-1 (Fig. 3C), and a dose dependent relationship of the expression of IGF1 receptor with GLP-1 was examined after GLP-1 treatment in the islets (Fig. 3C). Fig. 3C shows that the GLP1-induced IGF1 receptor expression was accompanied by increased expression of p-Akt and p-mTOR by Western blot analysis. Those results show that the effects of DPP IV inhibitor were at least in part dependent on activation of the IGF1 receptor by GLP-1.

3.4. GLP-1 agonists suppress STZ-induced islets apoptosis in vitro

To determine the potential protective effects of GLP-1 on STZ-induced islets cell death, the islets were pre-treated with or without 10 mM STZ for 1 h, followed by GLP-1 (10 μM) or vehicle control for 24 h, islet apoptosis was determined by a flow cytometry assay, the activity of caspase-3 was examined by Western blot analysis, and the islets function was determined by glucose-stimulated insulin secretion. Consistent with previous findings [14], STZ treatment greatly induced islets apoptosis (Fig. 4A and B) and reduced the function of GSIS (Fig. 4C) on islets. Interestingly, treatment of islets with GLP-1 markedly suppressed STZ-induced cleaved caspase-3 expression (Fig. 4B). Flow cytometry revealed a dramatic reduction of STZ-induced apoptosis in islets treated with GLP1 (Fig. 4A). Consistent with a suppressive effect of GLP-1 on STZ-induced apoptosis, glucose-stimulated insulin secretion revealed a dramatic elevation of STZ-induced decreasing the islets function in islets treated with GLP-1 compared to islets treated with STZ alone (Fig. 4C).

3.5. GLP1-induced IGFR expression promote islets survival through activation of IGFR/PI3K/Akt/mTOR signaling pathways

DPP IV inhibitors are quite different from conventional antidiabetic agents and control hyperglycemia by stimulating insulin production via the prevention of the degradation of the GLP-1 [4,5]. As treatment with DPP IV inhibitors or GLP-1 leads to increased IGFR, p-Akt, p-mTOR expression levels in association with islets function and islets survival, we hypothesized that the GLP-1 might against islets apoptosis via IGFR/Akt/mTOR signaling pathways protection from cellular apoptosis. To test this hypothesis, the islets were pre-treated with 10 mM STZ for 1 h, then the IGFR inhibitor (25 mM, NVP-AEW541) (Fig. 4D), the specific inhibitors of PI3K/Akt/mTOR (10 μM, Wortmannin) (Fig. 4D) or vehicle control for 1 h before GLP-1 treatment, followed by GLP-1 (10 μM) for 24 h, to determined islets function, islet apoptosis, and caspase-3 expression. The protective effect of GLP1 on STZ-induced islet injury was greatly inhibited by NVP-AEW541 or Wortmannin treatment (Fig. 4E and F); in the meantime, the inhibitory effect of GLP1 on caspase-3 was counteracted by NVP-AEW541 or Wortmannin (Fig. 4D), indicating that GLP1 protects the islet from STZ -induced injury may through activate IGF receptor and PI3K/Akt/mTOR signaling pathways.

4. Discussion

Our data provide evidence that DPP IV inhibitors confer resistance to STZ-induced islet injury. DPP IV inhibitors treatment showed depressing the degradation of GLP-1, and significantly increased serum GLP-1 levels in DM monkeys. Moreover, DPP IV inhibitors treatment showed improvement in glycemic control and islet function. Additionally, our study suggests that DPP IV inhibitors enhanced islets mass and decreased the number of the active form of caspase-3 in the islets tissues of DM monkeys. When islets were incubated with GLP-1, it maintained the insulin secretion capability of the islet cells and reduced the rate of apoptosis and injury induced by STZ in vitro. However, the pathway and molecular mechanisms underlying the protective effect of DPP IV inhibitors and GLP-1 on islet cells needs further research.

GLP-1, targets pancreatic β cells to leads to increased islet proliferation and function, and reduce inflammation, oxidative injury, and apoptotic cell death [2,3]. Studies have shown that DPP IV

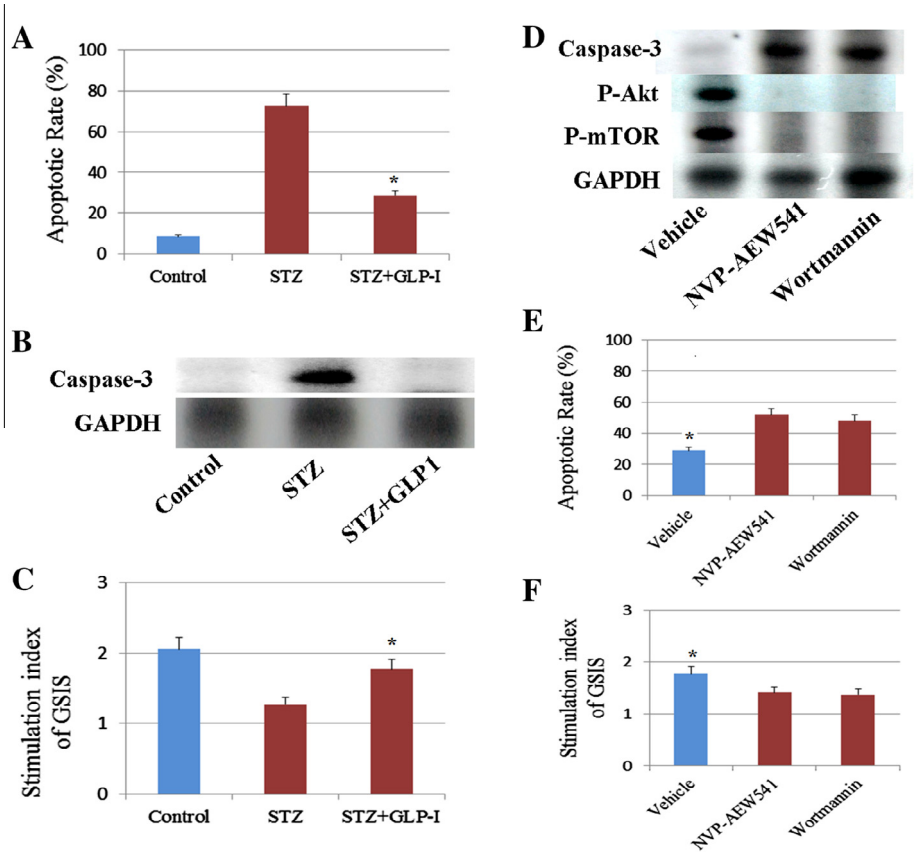

Fig. 4. GLP-1 agonists suppress STZ-induced apoptosis in islets cells, and GLP1-induced IGFR expression promote islets survival through activation of IGFR/PI3 K/Akt/mTOR signaling pathways. (A–C) The islets were pre-treated with or without 10 mM STZ for 1 h, followed by GLP-1 (10 μM) or vehicle control for 24 h, islet apoptosis was determined by a flow cytometry assay (A), the activity of caspase-3 was examined by Western blot analysis (A), and the islets function was determined by glucose-stimulated insulin secretion (C). All treatments in this figure were carried out in triplicate, $^{*}P < 0.05$ STZ vs. STZ-DPP IV inhibitors. (D–F) The islets were pre-treated with 10 mM STZ for 1 h, then NVP-AEW541 (25 mM) or Wortmannin (10 μM) or vehicle control for 1 h before GLP-1 treatment, followed by GLP-1 (10 μM) for 24 h, to determined caspase-3, p-Akt and p-mTOR expression (D), islets apoptosis (E) and islet function (F). All treatments in this figure were carried out in triplicate, $^{*}P < 0.05$ vs. NVP-AEW541 or Wortmannin.

inhibitors are quite different from conventional antidiabetic agents and control hyperglycemia by depressing the degradation of GLP-1 [4,5]. Here, we show that DPP IV inhibitors can prevent the degradation of GLP-1, and significantly increase serum GLP-1 levels in DM monkeys. Consistent with increasing serum GLP-1 levels, β cell apoptosis and loss was revealed a dramatic decrease in STZ-induced DM monkeys treated with DPP IV inhibitors. The GLP-1, which bind to specific Gs protein-coupled receptors and activate the cAMP/PKA pathway, protect b-cells against cytokine- or glucolipotoxicity induced apoptosis [16,17]. Apoptosis is a major cause of β-cell loss during the pathogenesis of type 1 and type 2 diabetes [6]. In recent publication showed that GLP-1 protects β-cells against apoptosis through the induction of an IGF receptor autocrine loop [15].

In our study, we found that treatment of diabetic monkeys with the DPP IV inhibitor or treatment of islets with GLP-1 can enhance the expression of IGF receptor in islets. Moreover, we also found that the PI3K/Akt/mTOR signaling, a well-characterized downstream target of IGFR, be activated by DPP IV inhibitor or GLP-1. Our results raise several questions. First, whether the protective effects of DPP IV inhibitor on STZ-induced islets injury were dependent on activation of the IGF receptor by GLP-1 in islets of monkeys. Because STZ is known to induce β-cell destruction in part through activation of apoptotic pathways [14], we examined whether IGF receptor activation influences cell apoptotic in response to STZ. Our data clearly showed that suppressing IGF receptor activation by NVP-AEW541 blocked the anti-apoptotic effect of GLP-1 in primary islets of monkeys. Moreover, the caspase-3, p-Akt and p-mTOR expression was greatly inhibited by NVP-AEW541. A second point concerns the respective role of the PI3K/Akt/mTOR signaling in protection against β cell apoptosis. Our study suggests that treatment with DPP IV inhibitors or GLP-1 leads to increased p-Akt and p-mTOR expression levels in association with islets function and islets survival. When the Akt/mTOR signaling pathways was blocked by Wortmannin, the protective effects of GLP1 on STZ-induced islets injury were inhibited by Wortmannin. These data indicate that the protective effects of DPP IV inhibitor on STZ-induced islets injury were dependent on activation of the IGFR/Akt/mTOR signaling pathways by GLP-1 in islets of monkeys.

In summary, DPP IV inhibitor protects the islet from STZ-induced injury in non-human primates. DPP IV inhibitor protection depends on the suppression of the degradation of serum GLP-1 and the activation of GLP-1–induced IGFR/Akt/mTOR signaling pathways. Here, it is shown that DPP IV inhibitor can improve tolerance of the process of inflammation and oxidative injury, and enhanced islets mass and function, suggesting that the administration of this factor may lead to novel ways to prevent β-cell death and diabetes or to improve the survival of islets used in transplantation therapy of type 1 diabetes. Most significantly, we present the first direct evidence that DPP IV inhibitor protects against STZ-induced islet injury, at least in part by the suppression of the degradation of serum GLP-1 and activation of IGFR/Akt/mTOR signaling pathways via GLP-1.

Author contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; Yi Zhang, Younan Chen, Yanrong Lu and Bole Tian conducted the experiments; Yi Zhang and Bole Tian performed pathological analysis; Yi Zhang and Bole Tian wrote the manuscript.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

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