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Dipeptidyl peptidase-4 inhibitor, vildagliptin, inhibits pancreatic beta cell apoptosis in association with its effects suppressing endoplasmic reticulum stress in db/db mice☆

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ABSTRACT

Aims. Vildagliptin promotes beta cell survival by inhibiting cell apoptosis. It has been suggested that chronic ER (endoplasmic reticulum) stress triggers beta cell apoptosis. The objective of the study is to explore whether the pro-survival effect of vildagliptin is associated with attenuation of endoplasmic reticulum stress in islets of db/db mice.

Methods. Vildagliptin was orally administered to db/db mice for 6 weeks, followed by evaluation of beta cell apoptosis by caspase3 activity and TUNEL staining method. Endoplasmic reticulum stress markers were determined with quantitative RT-PCR, immunohistochemistry and immunoblot analysis.

Results. After 6 weeks of treatment, vildagliptin treatment increased plasma active GLP-1 levels (22.63 ± 1.19 *vs.* 11.69 ± 0.44, $P < 0.001$), inhibited beta cell apoptosis as demonstrated by lower amounts of TUNEL staining nuclei (0.37 ± 0.03 *vs.* 0.55 ± 0.03, $P < 0.01$) as well as decreased caspase3 activity (1.48 ± 0.11 *vs.* 2.67 ± 0.13, $P < 0.01$) in islets of diabetic mice compared with untreated diabetic group. Further, vildagliptin treatment down-regulated several genes related to endoplasmic reticulum stress including TRIB3 (tribbles homolog 3) (15.9 ± 0.4 *vs.* 33.3 ± 1.7, $\times 10^{-3}$, $P < 0.001$), ATF-4(activating transcription factor 4) (0.83 ± 0.06 *vs.* 1.42 ± 0.02, $P < 0.001$) and CHOP(C/EBP homologous protein) (0.07 ± 0.01 *vs.* 0.16 ± 0.01, $P < 0.001$).

Conclusions. Vildagliptin promoted beta cell survival in db/db mice in association with down-regulating markers of endoplasmic reticulum stress including TRIB3, ATF-4 as well as CHOP.

Abbreviations: ER, endoplasmic reticulum; TRIB3, tribbles homolog 3; ATF-4, activating transcription factor 4; CHOP, C/EBP homologous protein; GLP-1, Glucagon-like peptide-1.

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1. Introduction

Type 2 diabetes is accompanied by growing beta cell damage and defective insulin secretion [1,2]. Apoptosis is a vital contributor responsible for beta cell damage [2]. However, the precise mechanisms underlying beta cell apoptosis are yet to be clarified. Several studies have suggested the role of prolonged ER stress in triggering beta cell death [3]. CHOP is activated under ER stress and functions as an apoptotic modulator [4]. There are mainly three signal pathways involved in the regulation of CHOP expression. Among them, PERK-ATF-4 signaling pathway plays a dominant role [5]. Under ER stress, ATF-4 acts as an upstream activator of CHOP and increases the transcription of CHOP by binding to its promoter [6]. Several lines of evidence have demonstrated that CHOP is considerably elevated in islet beta cells of mice with type 2 diabetes [3]. Moreover, a remarkable decrease of beta cell apoptosis was exhibited in CHOP-deficient db/db mice, which further confirmed that CHOP mediates beta cell apoptosis during ER stress [7]. However, since CHOP was not the final executor of apoptosis, the pro-apoptotic effect of CHOP seemed to be mainly mediated by its downstream targets. TRIB3 was originally described as a kinase-like gene involved in neuron cell apoptosis [8] and subsequently shown to be present in pancreatic beta cells [9]. TRIB3 was identified as an important downstream mediator of CHOP and executed its apoptotic action in response to ER stress [9,10].

Bioactive incretins protect islet beta cells from apoptosis [11]. Evidence is emerging that activation of GLP-1(glucagon-like peptide-1) receptors down-regulates expression of ATF-4, CHOP and other ER stress markers in islets of diabetic animal models, thereby inhibiting beta cell apoptosis [12–15]. However, both bioactive GLP-1 and GIP are quickly transformed into inactive form by DPP-4(dipeptidyl peptidase-4) [16]. Vildagliptin, a DPP-4 inhibitor, hinders degradation of bioactive incretins and amplifies their biological action. Clinical studies have reported that treatment with vildagliptin not only controls glucose fluctuations, but also enhances beta cell function in patients with type 2 diabetes [17–19]. Rodent studies have further provided evidence that vildagliptin's effect on beta cell function correlates with decreased beta cell apoptosis [20–23]. Additionally, some studies have demonstrated that treatment with vildagliptin promotes beta cell survival by down-regulating ER stress makers such as CHOP in diabetic animal models [22,23]. However little is known about whether vildagliptin treatment alleviates beta cell apoptosis in db/db mice via regulating other ER stress markers, ATF-4 and TRIB3.

In our study, we explored whether vildagliptin ameliorates islet beta cell apoptosis is associated with the pathway ATF-4, TRIB3 as well as CHOP.

2. Materials and methods

2.1. Animals

Male db/db mice (BKS) and wildtype mice were purchased from Nanjing University (Nanjing, China) at 8 weeks old, housed 3–5 per cage under controlled temperature(20 ± 2 °C) and humidity (60 ± 10%) conditions, alternating between 12-hour light and dark periods. Mice were provided water and laboratory chow diet during the whole experimental period. After a 2-week adaption, db/db mice were randomly allotted to vildagliptin-treated group (n = 20) and diabetic group (n = 20). Non-diabetic wildtype mice were placed in the normal group (n = 20). The original vildagliptin powder was provided by Novartis Pharma AG (Switzerland). Vildagliptin was administered (35 mg/kg body weight) once daily by oral gavage. After 6 weeks of treatment, cervical dislocation was conducted to kill the mice (four from every group). The pancreas was dissected rapidly into two parts; one part, for measuring pancreatic insulin content and caspase3 activity, was placed in liquid nitrogen quickly and kept at −80 °C till analysis, whereas the other part was fixed with 10% neutral formalin.

2.2. Ethics statement

All animal experiments complied with rules of Experimental Animal Care and Use Center in Tianjin Medical University. The protocol was approved by Experimental Animal Ethical Committee of Tianjin Medical University (Permit Number: TMUaMEC 2012017).

2.3. Determination of weights and blood glucose

Body weight and blood glucose were examined each week. Non-fasting blood glucose was monitored before and during the 1st, 2nd, 3rd and 4th week of treatment (1 hour after the dark period). 5-hour fasting blood glucose was measured during the 5th and 6th week of treatment (5 hours after the beginning of the light period). Blood samples were obtained by cutting tail tips. A Biosen5030 glucose analyzer (EKF Diagnostics, Germany) was used to monitor blood glucose.

2.4. Oral glucose tolerance tests (OGTT)

During the 6th week of treatment, four mice in each group were fasted for 5 hours and gavaged with glucose (2 g/kg body weight). Blood glucose was monitored at baseline and every 30 minutes after glucose load. Quantification of area under the curve ($AUC_{0–120\ min}$) was achieved according to trapezoidal rules.

2.5. Determination of plasma HbA_{1c}, insulin and active GLP-1 concentrations

After 6 weeks of treatment, blood samples were collected by cardiac puncture. HbA_{1c} levels were analyzed using automatic biochemistry analyzer (Roche, Germany). To detect plasma insulin level, mice were fasted overnight, and plasma insulin levels were determined by ELISA method (Millipore, MO, USA). To detect plasma GLP-1 levels, mice were fasted for 10 hours, blood sample were collected one hour after feeding. Plasma GLP-1(active form) concentrations were detected by ELISA method (Millipore, MO, USA).

2.6. Islet isolation

After 6 weeks of treatment, mice were anesthetized with 6% chloral hydrate (300 mg/kg body weight) by intraperitoneal