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Chemical chaperone-conjugated exendin-4 as a cytoprotective agent for pancreatic β-cells

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ABSTRACT

Endoplasmic reticulum stress has been considered a major cause of pancreatic β-cell dysfunction and apoptosis leading to diabetes. Glucagon-like peptide-1 receptor activation and chemical chaperones have been known to reduce endoplasmic reticulum stress and improve β-cell function and survival. The purpose of this study was to prepare and evaluate the chemical chaperone tauroursodeoxycholic acid-conjugated exendin-4 as a protective agent for pancreatic β-cells. Mono-tauroursodeoxycholic acid-Lys^{27}-exendin-4 conjugate (TUM1-Ex4) showed better receptor binding affinity than other conjugates with strong *in vitro* insulinotropic activity in rat pancreatic β-cells and *in vivo* hypoglycemic activity in type 2 diabetic db/db mice. In INS-1 cells under endoplasmic reticulum stress induced by thapsigargin, TUM1-Ex4 promoted cell survival in a dose-dependent manner. In western blot analysis, TUM1-Ex4 reduced the expression of the endoplasmic reticulum stress marker GRP78 and phosphorylation of the translation initiation factor eIF2α. These results reveal that TUM1-Ex4 accelerates translational recovery and contributes to β-cell protection and survival. The present study indicates that the chemical chaperone-coupled glucagon-like peptide-1 receptor agonist is a feasible therapeutic strategy to enhance β-cell function and survival.

1. Introduction

In pancreatic β-cells, the endoplasmic reticulum (ER) is highly developed owing to the high demand for insulin synthesis and secretion (Schröder and Kaufman, 2005; Fonseca et al., 2011). The ER serves many essential cellular functions, including protein synthesis and modification, lipid synthesis, calcium storage, and protein folding (Marciniak and Ron, 2006). ER stress is an imbalance between the protein folding capacity of the organelle and the functional demand, which results in the accumulation of unfolded/misfolded proteins in the ER lumen (Cnop et al., 2017). Clinical and experimental studies have indicated that severe or prolonged ER stress leads to β-cell death that contributes to the pathogenesis of type 1 and type 2 diabetes (Cnop et al., 2012; Eizirik et al., 2008; Im and Bhang, 2018; O'Sullivan-Murphy and Urano, 2012). Therefore, ER stress pathways and their constituent elements are attractive targets for the development of antidiabetic drugs.

Exendin-4 (Ex4), a glucagon-like peptide-1 (GLP-1) receptor agonist found in the venom of the Gila monster, effectively regulates blood glucose levels *via* glucose-dependent insulin secretion and shows cytoprotective effects on pancreatic β-cells (Aramadhaka et al., 2013; Lee et al., 2016; Park et al., 2016; Salehi et al., 2008). The synthetic Ex4 (exenatide, Byetta®) and biodegradable microspheres product containing exenatide (Bydureon®) have been clinically used for the management of type 2 diabetes (Davidson et al., 2005; Park et al., 2016). Once-weekly injected exenatide microspheres formulation (Bydureon®) can improve the treatment satisfaction of patients compared with twice-daily injected exenatide (Byetta®) (Aroda and DeYoung, 2011). In clinical studies, exenatide was generally well tolerated, but mild-to-moderate gastrointestinal effects including nausea, vomiting, and diarrhea were observed as the most frequent adverse events (Macconell et al., 2012). Long-acting GLP-1 receptor agonists including once-daily liraglutide (Victoza®) and once-weekly albiglutide (Tanzeum®) showed lower rates of gastrointestinal adverse events compared with short-acting exenatide (Pechenov et al., 2017; Pratley et al., 2014). To alleviate gastrointestinal adverse effects, drug molecular properties and formulations to allow the patient to adapt to drug action is recommended (Pechenov et al., 2017).

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Ex4 stimulates cAMP production by binding to the GLP-1 receptor on the surface of the cell and improves β-cell function and survival during ER stress (Lee et al., 2014; Oh et al., 2013). Ex4 controls one arm of the unfolded protein response, specifically double-stranded RNA-dependent protein kinase-like ER kinase; thus, GLP-1 receptor signaling decreases the phosphorylation of eIF2α, which enhances insulin biosynthesis and upregulates its protein expression (Yusta et al., 2006). Chemical chaperons have been known to facilitate the trafficking of mutant proteins and improve protein conformation stability in the ER (Ozcan et al., 2006; Welch and Brown, 1996). Among them, tauroursodeoxycholic acid (TUDCA) has been known to be an effective inhibitor of ER stress in several experimental models (Chen et al., 2016; Keestra-Gounder et al., 2016; Lee et al., 2010). TUDCA is a safe molecule that has been used as a hepatoprotective agent for cholestatic liver diseases (Ozcan et al., 2006; Poupon et al., 1999).

In this study, we conjugated TUDCA to Ex4 to obtain a novel chaperone using a combination of a chemical chaperone and a GLP-1 receptor agonist. Assays for evaluating *in vitro* and *in vivo* biological activities of TUDCA-conjugated Ex4 (TUDCA-Ex4) as a GLP-1 receptor agonist, ER stress-induced cell death assay, and western blot analysis were performed. We hypothesized that TUDCA-Ex4 might serve an advanced function in ER because Ex4 and TUDCA can induce fast translational recovery and prevent β-cell apoptosis.

2. Materials and methods

2.1. Materials and animals

Ex4 was obtained from American Peptide, Inc. (Sunnyvale, CA, USA). TUDCA, Lys-C, α-cyano-4-hydroxycinnamic acid, and RPMI-1640 medium were supplied from Sigma-Aldrich Co. (St. Louis, MO, USA). All other reagents, unless indicated, were supplied from Sigma-Aldrich Co. and were used as received.

Male C57BL/6 db/db mice (6–8 weeks old) were supplied from the Korea Research Institute of Bioscience and Biotechnology (Daejon, Korea). Animals were maintained on a 12-h light/dark cycle with free access to food and water. Animal care was carried out according to the guidelines issued by the National Institutes of Health (NIH) for the care and use of laboratory animals (NIH publication 80-23, revised in 1996). This animal experiment was approved by the Ethical Committee on Animal Experimentation at SungKyunKwan University.

2.2. Preparation and characterization of TUDCA-Ex4

TUDCA was conjugated to Ex4 by a coupling reaction between *N*-hydroxysuccinimide (NHS) ester of TUDCA (TUDCA-NHS) and Ex4 (Fig. 1). TUDCA-NHS was synthesized as described previously (Lee et al., 2005; Son et al., 2009). Ex4 at a concentration of 5 mg/ml in dimethylsulfoxide (DMSO) was reacted with TUDCA-NHS at a concentration of 3.588 mg/ml in DMSO containing 3% triethylamine at molar ratios of 1:1–1:4 (Ex4:TUDCA-NHS). The reaction mixtures were gently agitated at 25 °C for 2 h. To stop the reaction, 100 µl of water containing 1% trifluoroacetic acid (TFA) was then added to the reaction mixture.

Two mono-TUDCA-Ex4 conjugates [TUDCA-Lys^{27}-Ex4 (TUM1-Ex4) and TUDCA-Lys^{12}-Ex4 (TUM2-Ex4)] and one di-TUDCA-Ex4 conjugate [TUDCA-Lys^{12,27}-Ex4 (TUDi-Ex4)] were isolated from their reaction mixtures by semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC) with a Capcell-pak RP-18 column (250 × 10 mm id, 5 µm; Shiseido, Japan) at 25 °C. Separation was performed by gradient elution at a flow rate of 5.0 ml/min with the mobile phases consisted of 0.1% TFA in water (mobile phase A) and 0.1% TFA in acetonitrile (mobile phase B). A linear gradient of 35–90% (v/v) mobile phase B for 30 min was applied. UV absorbance was monitored at 215 nm. Each fraction corresponding to TUDCA-Ex4s was collected and their molecular masses were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Microflex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) in the linear and positive-ion mode with an acceleration voltage of 20 kV as described previously (Na et al., 2003; Park and Na, 2016). As a matrix, a saturated solution of α-cyano-4-hydroxycinnamic acid in acetonitrile:water (7:3, v:v) containing 0.1% TFA was used and it was mixed with the analyte in a ratio of 1:1 (matrix:analyte, v:v). One µl of the analyte-matrix solution was applied on the sample plate and air-dried. The purity of TUDCA-Ex4s was checked by analytical RP-HPLC using a Capcell-pak RP-18 column (250 × 4.6 mm id, 5 µm) under the above-mentioned gradient condition. The purified conjugates were stored at −70 °C until use.

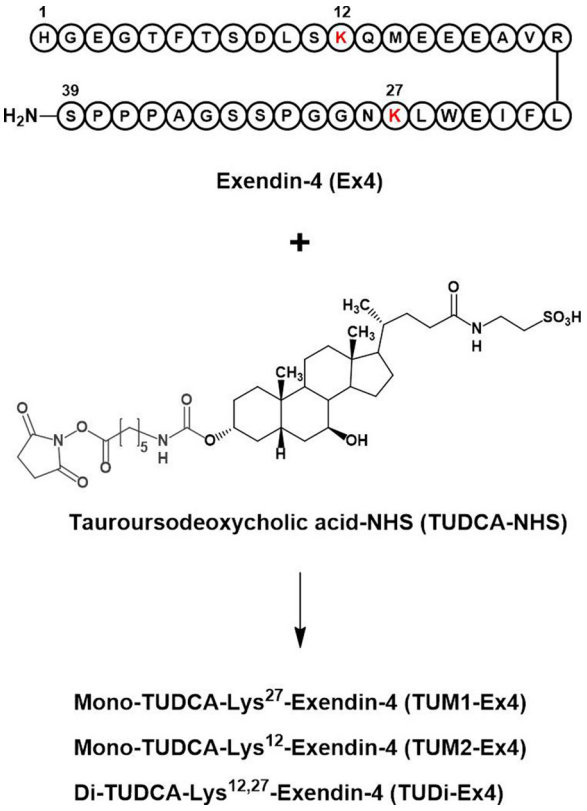

Fig. 1. Chemical structures of Ex4 and TUDCA-NHS, and their conjugates.

The conjugation site of TUDCA in each TUDCA-Ex4 conjugate was identified by the peptide mapping analysis of Ex4 and TUDCA-Ex4s using MALDI-TOF MS as described previously (Na and Lee, 2004; Na et al., 2006). Briefly, 5 µl of Lys-C at a concentration of 10 µg/ml in 50 mM Tris-HCl buffer (pH 8.0) was added to 10 µl of each TUDCA-Ex4 solution at a concentration of 500 µg/ml in the same buffer. The enzyme to substrate ratio was 1:100 (w:w). After the digestion for 5 h at 37 °C, the digested samples were analyzed by MALDI-TOF MS as described above.

2.3. Receptor binding assay

GLP-1 receptor binding assay was performed using rat insulinoma RIN-m5F cells (ATCC, Manassas, VA, USA) as described previously (Son et al., 2009). Briefly, RIN-m5F cells were cultured in RPMI-1640 supplemented with 22.2 mM glucose, 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, and 10 mM HEPES in a humidified 5% CO_2 incubator at 37 °C. The cells were seeded in 12-well plates at 3 × 10^5 cells per well and grown for 2 days. They were washed twice with binding buffer composed of 5 mM potassium chloride, 120 mM sodium chloride, 1.2 mM magnesium sulfoxide, 13 mM sodium acetate, 1.2 g/l Tris, 2 g/l