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Intravenous immunoglobulin improves glucose control and β-cell function in human IAPP transgenic mice by attenuating islet inflammation and reducing IAPP oligomers

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by β-cell loss, insulin resistance, islet inflammation and amyloid deposits derived from islet amyloid polypeptide (IAPP). Reducing toxic IAPP oligomers and inhibiting islet inflammation may provide therapeutic benefit in treating T2DM. Intravenous immunoglobulin (IVIg) is an efficient anti-inflammatory and immunomodulatory agent for the treatment of several autoimmune or inflammatory neurological diseases. However, whether IVIg has therapeutic potential on T2DM remains unclear. In present study, we showed that IVIg treatment significantly improved glucose control and insulin sensitivity, and prevented β-cell apoptosis by lowering toxic IAPP oligomer levels, attenuating islet inflammation and activating autophagy in human IAPP transgenic mouse model. These results suggest that IVIg is a promising therapeutic potential for T2DM treatment.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder characterized by insulin resistance, β-cells failure and islet inflammation, as well as pancreatic amyloid deposits derived from islet amyloid polypeptide (IAPP). In 2013, 382 million persons were affected by T2DM, the prevalence of the disease is expected to rise to 592 million by 2035 [1,2]. T2DM has been an urgent health-care problem with serious social and economic consequences. Current therapeutic medicines include insulin [3], metformin [4,5], sulphonylureas, thiazolidinediones, α-glucosidase inhibitors, incretin hormone and sodium-dependent glucose co-transporter inhibitors [6], all of them provide pharmacological interventions to improve glucose regulation, but they only focus on treating hyperglycemia. There are few strategies that halt the progressive deterioration of glucose homeostasis by targeting pathogenesis with long-lasting effects and minimal adverse effects.

The contribution of inflammation in the pathogenesis of T2DM and associated complications is now well established [1,7]. Multiple mechanisms underlying insulin resistance in T2DM include glucotoxicity, lipotoxicity, oxidative stress [8], endoplasmic reticulum (ER) stress [9], and the formation of amyloid deposits in the islets [10], all of which are associated with inflammatory responses. Aggregation and accumulation of IAPP is another important hallmark of T2DM [11]. IAPP is a 37-amino acid polypeptide synthesized in pancreatic β cells and co-secreted with insulin. It is found in approximately 90% of patients with T2DM, and the extent of its deposition correlates negatively with β-cell mass [12]. Human IAPP (hIAPP) induced macrophage IL-1β secretion by stimulating both the synthesis and processing of proIL-1β leading to impair β-cell insulin secretion [13,14]. IL-1β autostimulation further amplified inflammation, engendering a vicious cycle. IL-1β also deteriorated β-cell function, and directly led to insulin resistance and β-cell apoptosis [15]. Therefore, reduction of IAPP levels and inhibition of islet inflammation are proposed as an ideal therapeutic strategy in treating T2DM.

Intravenous immunoglobulin (IVIg) is a preparation of polyclonal serum IgG pooled from thousands of blood donors, which has been proved to be an efficient anti-inflammatory and immunomodulatory adjunctive or first-line therapeutic agent for nearly three decades [16]. FDA-approved indications for IVIg therapy include many autoimmune disorders such as Guillain-Barré syndrome [17], dermatomyositis,

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Kawasaki disease, and some neurological diseases including multifocal motor neuropathy, stiff person syndrome, multiple sclerosis and myasthenia gravis [18]. IVIg therapy is also used for some acute infections and complications of organ transplantation [18]. Moreover, IVIg has been an effective treatment agent for diabetic neuropathy [19–21] and diabetic amyotrophy [22,23]. Lots of diseases associated with diabetes mellitus also showed potentially beneficial responses to IVIg [24,25]. Despite of the widespread use and therapeutic successes of IVIg, its effects on IAPP-related T2DM remains unclear. In this study, we assessed therapeutic efficacy of IVIg on glucose control and β-cell function in a hIAPP transgenic mouse model.

2. Materials and methods

2.1. Mice and IVIg treatment

The hemizygous hIAPP transgenic mice were purchased from the Jackson Laboratory (Stock No: 008232), and self-crossed to generate the homozygous counterpart. 4-week-old male homozygous hIAPP mice and their age-matched WT littermates were housed in a conventional facility at Tsinghua University with a reverse 12-h light/12-h dark cycle and ad libitum access to food and water. All experiments with mice were performed during daylight hours and carried out under the China Public Health Service Guide for the Care and Use of Laboratory Animals. Experiments involving mice and protocols were approved by the Institutional Animal Care and Use Committee of Tsinghua University. IVIG was provided by Shandong Taibang Biological Products Co., Ltd. (Taian, Shandong, China). hIAPP mice were given a 6% fat diet and randomly treated in cohorts (n = 8) with vehicle or IVIg (100 mg/kg body weight) twice a week by intraperitoneal injection for 3 weeks. WT littermates were administered with vehicle.

2.2. Intraperitoneal glucose tolerance test (ipGTT) and intraperitoneal insulin tolerance test (ipITT)

For ipGTTs, 49-day-old mice were i.p. injected with 40% glucose (Beijing Chemicals, Inc., China) at a dose of 2 g/kg body weight after an overnight fast. Blood glucose values were measured using tail-tip blood samples at time points 0, 30, 60, 90 and 120 min via a Glucometer (Freestyle; Roche). Serum insulin levels were measured at time points 0 and 30 min by using a mouse insulin ultrasensitive ELISA kit (ALPCO, Salem, NH). For ipITTs, mice were i.p. injected with 0.75 U/kg body weight of recombinant human insulin (Novolin; Novo Nordisk, Denmark) after 5 h of fasting. Blood glucose was determined at time points 0, 30, 60, 90 and 120 min.

2.3. Homeostatic model of assessment insulin resistance (HOMA-IR)

HOMA-IR was calculated as previously described [26]: fasting insulin (ng/ml) × fasting glucose (mmol/l) = HOMA-IR.

2.4. Immunohistochemistry and immunofluorescence

After an overnight fast, mice were euthanized using isoflurane. Pancreas were isolated and fixed in 4% paraformaldehyde for 24 h at 4 °C, and followed by embedding in paraffin and orienting to make sections cut along the head-tail axis. 5 μm sections of pancreas were then taken through the fixed tissue in the plane of embedding. For immunohistochemistry, the sections were subjected to deparaffination in a series of xylene and ethanol. The endogenous peroxidase in tissues was blocked with 3% H_2O_2 in 80% methanol for 20 min at room temperature. Nonspecific background staining was blocked through incubation in 10% GSA and 0.3% Triton X-100 at room temperature for 1 h. The slides were incubated overnight at 4 °C with anti-insulin antibody (1:100, Abcam, ab7842), anti-IAPP antibody (1:100, Abcam, ab55411), anti-p62 antibody (1:100, Abcam, ab56416), and anti-LC3B antibody (1:100, Abcam, ab48394), respectively. All aforementioned primary antibodies were followed by appropriate secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 594, respectively. The images were acquired with an Olympus IX73 inverted microscope with DP80 camera, and all analyses were performed blind to the genotype and treatment of the mice. Immunofluorescence was quantified using Image J software (National Institutes of Health, USA). β-cell mass was determined by quantifying the cross-sectional β-cell area positive for insulin divided by the cross-sectional area of the total tissue and multiplying by the pancreatic weight.

β-cell apoptosis was analyzed using In Situ Cell Death Detection Kit via TUNEL technique in accordance with the manufacturer's instructions (Roche Diagnostics, 12,156,792,910). The frequency of TUNEL was presented as the percentage of TUNEL-positive β cells among the total number of insulin-positive β cells. For H&E staining, pancreatic sections were dyed by hematoxylin and eosin according to a standard protocol.

2.5. Dot-blot

The mouse pancreas tissues were homogenized in RIPA buffer supplemented with complete protease inhibitor mixture tablets (Roche Diagnostics), and then followed by centrifugation at 16,000 × *g*, 4 °C for 15 min. The supernatants were collected, and protein concentrations were determined using the BCA protein assay (Pierce). The soluble fraction of pancreas lysates was applied to nitrocellulose membrane (Merck Millipore), which were then blocked with 5% milk in PBST and incubated with anti-IAPP (1:100, Abcam, ab55411), W20 (1:100, developed by our lab), OC (1:1000, Merck Millipore, SPC-507D), A11 (1:1000, Invitrogen, AHB0052), or anti-β-actin antibodies (1:1000, MBL, M177-3) at room temperature for 1 h, the bound antibodies were probed with the corresponding HRP-conjugated secondary antibodies. Immuno-reactive blots were visualized with an ECL chemiluminescence kit (Pierce) and quantified by densitometry using Image J software.

2.6. Western-blot

Proteins in the soluble fractions of pancreas lysates were separated by a 4–12% SDS-PAGE gel (Invitrogen) and transferred onto nitrocellulose membrane (Merck Millipore). The membrane was probed with primary antibodies against p-p65 (1:1000, clone 93H1), IKKβ (1:1000, clone L570), and GAPDH (1:1000, clone 14C10) (Cell Signaling, Beverly, MA), respectively, and then followed by appropriate secondary antibodies. Bands in immunoblots were visualized by enhanced chemiluminescence (Pierce) and quantified by densitometry using Image J software.

2.7. Measurement of IL-1β, TNF-α and IL-6

The levels of IL-1β, TNF-α and IL-6 in the pancreas lysates were determined using ELISA kits according to the manufacturer's protocols. Briefly, the pancreas lysates were added to a 96-well ELISA plate and then reacted with the relevant primary antibodies and HRP-conjugated secondary antibodies. 3,3,5,5-Tetramethylbenzidine was used as the substrate, and the absorbance of the mixtures was measured at 450 nm using an MD-M5 microplate reader.

2.8. Statistical analysis

Statistical significance was tested using one-way ANOVA analysis followed by Tukey's post hoc test or student's *t*-test for paired comparisons (GraphPad Prism 6.0). Results are expressed as group means ± SD from three independent experiments except for animal treatment and $P < 0.05$ was considered statistically significant.