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Substance P preserves pancreatic β-cells in type 1 and type 2 diabetic mice

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ABSTRACT

Preservation of pancreatic β-cells is required for the development of therapies for type 1 and type 2 diabetes (T1D and T2D, respectively). Our previous study demonstrated that substance P (SP) preserves β-cell populations in mice with streptozotocin-induced T1D. Here, we demonstrated that chronic systemic treatment with SP restored the mass of β-cells both in nonobese diabetic (NOD) mice with T1D or db/db mice with T2D. SP delayed the onset of T1D in NOD mice via immune modulation. SP inhibited immune infiltration into islets and the salivary glands of NOD mice. In db/db mice, SP treatment rescued glucose intolerance. Moreover, SP inhibited apoptosis, as well as the activation of pancreatic stellate cells in pancreatic islets of db/db mice. SP downregulated the number of α-smooth muscle actin (α-SMA) expressing cells in db/db pancreatic islets. Cleaved-caspase-3 expression was reduced in islets of SP-treated db/db mice compared to that in the control. Therefore, these results suggested that SP may preserve pancreatic β-cells through immune modulation and protection from the stimulated activation of pancreatic stellate cells and apoptosis in T1D and T2D, respectively.

1. Introduction

Dysfunction and loss of pancreatic β-cells are major causes of diabetes. Type 1 diabetes (T1D) is a chronic autoimmune disease, and more than 70% of β-cells are destroyed in T1D [1,2]. Type 2 diabetes (T2D) is characterized by impaired insulin secretion following a decrease in β-cell mass and by insulin resistance [3]. Therefore, the preservation of β-cells is essentially important in therapies for both, T1D and T2D.

Immune cells, such as B cells, macrophages, and dendritic cells, invade into islets in T1D as well as autoreactive T cells that can specifically respond to β-cells and pancreatic nervous tissues [4,5]. The infiltration of immune cells into islets in T1D is called insulitis [6]. Such immune dysregulation plays essential roles in both, defects in insulin production following β-cell deficiency and the onset of T1D [2].

Chronic inflammation in insulin target organs, such as muscles, has been associated not only with insulin resistance in T2D but also with pancreatic β-cell dysfunction in T2D [7]. Islet-associated macrophages are found in animal models as well as patients with T2D [8]. The expression of proinflammatory cytokines such as interleukin-6 (IL-6), C-C motif chemokine ligand 2 (CCL2), and tumor necrosis factor-α (TNF-α) were also elevated in islets in T2D [7,8]. Pancreatic stellate cells (PSCs) also associate with β-cell dysfunction in T2D [9,10]. PSCs are mesenchymal cells found in the periacinar space and are quiescent in healthy adult pancreas [11]. However, PSCs are activated in response to chronic pancreatitis and pancreatic cancers [9,12]. Various factors activate PSCs; IL-6, TNF-α, and transforming growth factor-β (TGF-β). The activated PSCs start to express autocrine factors (such as IL-6 and TGF-β) and extracellular matrix (such as fibronectin and collagen) as well as α-

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smooth muscle actin (α-SMA) [9,11]. Then, the activated PSCs induce islet fibrosis via autocrine factors and the extracellular matrix [11]. Furthermore, islet fibrosis is one of the mechanisms that induce progressive β-cell failure in T2D [9,13].

The dynamics of β-cell mass is dependent on a balanced regulation of replication, apoptosis, transdifferentiation, and neogenesis in β-cells [14,15]. Apoptosis plays important roles in reducing β-cell mass in T2D as well as in T1D [16,17].

Substance P (SP) was identified as a pain transducer in the central nervous system and plays essential roles in the regeneration of various tissues via immune modulation and the activation of endogenous mesenchymal stem cell-like cells in response to injury [18]. Importantly, our previous study demonstrated that chronic systemic treatment of SP preserves β-cell mass in mice with streptozotocin-induced T1D [19]. It has been also shown that acute local treatment of SP reverses insulitis in NOD mice with T1D [20]. Therefore, chronic treatment of SP may preserve β-cell mass both in T1D and T2D. We examined whether chronic treatment of SP would preserve β-cells in NOD mice with T1D as well as db/db mice with T2D.

2. Materials and methods

2.1. Mice and animal experiments

Five-week-old female NOD mice (Laboratory Animal Resource Center, Korea) and 7-week-old male db/db mice (Narabiotech, Seoul, Korea) were housed under a 12-h light/dark cycle with unlimited access to food and water. All procedures were approved by the animal experiment ethics committee of Kyung Hee University (KHUASP(SE)-13-04). NOD mice were injected intravenously (i.v.) with SP (5 nmol/kg body weight, two consecutive days per week) for 14 or 21 weeks, and db/db mice were injected i.v. with SP (5 nmol/kg body weight or 50 nmol/kg body weight, two consecutive days per week) for 12 weeks. In order to determine the presence of hyperglycemia, blood glucose levels were measured after fasting for 6 h once per week. Hyperglycemia was determined by two consecutive readings of blood glucose greater than 250 mg/dL. Intraperitoneal glucose tolerance tests (IPGTTs) were performed after an intraperitoneal (i.p.) injection of glucose (2 g/kg body weight) into mice who had fasted overnight. Blood glucose levels were measured using an ACCU-CHECK instrument (Roche). At 14 or 21 weeks after the first injection of SP, NOD mice were sacrificed, and the pancreatic and salivary gland tissues were excised. At 20 weeks after the first injection of SP, db/db mice were sacrificed and their pancreatic tissues were excised.

2.2. Tissue processing and immunohistochemical analysis

Tissues were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for 4 h or overnight at 4 °C after washing with PBS. Hematoxylin and eosin (H/E) staining and immunostaining were then performed using paraffin sections of the fixed tissues. Immunohistochemistry was carried out with primary antibodies against insulin (Cell Signaling Technology), glucagon (Cell Signaling Technology), α-smooth muscle actin (α-SMA; DAKO), CD3 (Abcam), and cleaved caspase-3 (Cell Signaling Technology). After incubating the section with the primary antibody, an ABC kit (Vector labs, Burlingame, CA) and DAB kit (Vector labs), or secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 546 (Invitrogen, Carlsbad, CA) were used for staining according to the manufacturer's instructions. When required, nuclei were stained with DAPI (Invitrogen), hematoxylin (Sigma), or Fast-Red (Vector Labs). Images were collected using an Olympus BX41 light microscope or a Zeiss LSM 700 confocal microscope. Insulitis scores were graded as follows: 0, no infiltration; 1, mild infiltration at the periphery of islets (<30%); 2, moderate intra-islet infiltration (30–50%); 3, severe infiltration (>50%). For the insulitis scoring in islets of NOD mice, a total 138 or 280 sections were analyzed for control or SP-injected NOD mice, respectively.

2.3. Statistics

Data were expressed as the mean ± SD or mean ± SEM. Student's *t*-tests, one-way analysis of variance (ANOVA), two-way ANOVA, and log-rank tests were carried out to determine the significance of differences.

3. Results

3.1. SP delayed development of hyperglycemia and preserved β-cells in NOD mice with T1D

We examined whether substance P (SP) could delay diabetic hyperglycemia and preserve β-cells in NOD mice, an autoimmune type 1 diabetic mouse model. NOD mice began to exhibit signs of insulitis at 2–5 weeks of age [21]. SP or buffer injections were initiated at 5 weeks of age and were performed twice (2 consecutive days) per week, with the SP treatment continuing for 21 weeks. NOD mice injected with buffer started to develop hyperglycemia at around 11 weeks of age. Surprisingly, SP delayed hyperglycemia (Fig. 1A). Moreover, SP facilitated the maintenance of glucose tolerance in NOD mice, with mice exhibiting normoglycemia until 26 weeks of age (Fig. 1B–D). Consistent with this, SP preserved β-cells and inhibited the expansion of α-cell in NOD mice at 19 and 26 weeks of age (data not shown and Fig. 1E–G). Therefore, these results suggested that SP delayed hyperglycemia and preserved islets, which contained functional β-cells, in NOD mice.

3.2. SP delayed development of insulitis via immune modulation in NOD mice with T1D

Additionally, we examined the effects of SP on immune infiltration into islets. Paraffin sections of pancreatic tissues were prepared at 26 weeks of age and stained with H/E. Islets were scored as described in the Materials and Methods. Interestingly, SP-injected NOD mice showed lower insulitis scores than age-matched buffer-injected control NOD mice (Fig. 2A–C). Similar results were observed with pancreatic tissues from 19-week-old mice (data not shown). Importantly, T-cell (CD3-positive cells) infiltration into islets was significantly reduced on SP treatment (Fig. 2D and E). NOD mice also develop Sjögren's syndrome, an autoimmune disease regulated differently from diabetes [22]. SP treatment decreased immune infiltration into the salivary glands of NOD mice (Fig. 2M and N). These data suggested that SP may be able to modulate both local and systemic immune responses. Therefore, our data suggested that SP delayed the onset of diabetes in NOD mice by the preservation of β-cells mediated mainly via immune modulation.

3.3. SP delayed development of hyperglycemia and preserved β-cells in db/db mice with T2D

Next, we examined the effects of SP on db/db mice with T2D. SP or buffer injections were initiated when the mice were 8 weeks of age, twice per week (2 consecutive days) with the chronic