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Exendin-4 treatment of nonobese diabetic mice increases beta-cell proliferation and fractional insulin reactive area

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Abstract

Objective: The notion of combining immunomodulatory agents with the incretin exendin-4 (Ex-4) has seen considerable favor as a potential therapy for the reversal of type 1 diabetes in man. While the addition of Ex-4 provides modest improvement to the effectiveness of immunological-based monotherapies in reversing hyperglycemia in the nonobese diabetic (NOD) mouse, the mechanism of action underlying this effect remains controversial and formed the basis for this investigation. **Research Design and Methods:** Female NOD mice with new onset diabetes received either Ex-4 (0.2 μg) or saline via daily intraperitoneal injection for 30 days. To maintain viability after diagnosis of diabetes, animals also received subcutaneous insulin pellets. When persistent hyperglycemia returned, animals were sacrificed and histological studies performed to assess beta-cell proliferation (BrdU+/insulin+; Ki67+/insulin+) and fractional insulin reactive area. **Results:** Ex-4-treated animals experienced diabetes reversal rates no better than controls. Despite this, Ex-4-treated mice demonstrated increased fractional insulin area (P=.035) and beta-cell proliferation as evidenced by elevated BrdU (P=.0001) and Ki67 staining (P=.04) with insulin co-localization. Also noteworthy, Ex-4-treated mice had poor weight gain following diagnosis in comparison to saline-treated animals (P=.003). **Conclusions:** Ex-4 monotherapy (0.2 μg daily–10 μg/kg per day) in NOD mice with new onset diabetes increases beta-cell proliferation and fractional insulin area. Ex-4 remains a promising component of combination therapies for type 1 diabetes. Additional studies are needed to identify a dose that maximizes beta-cell proliferation and minimizes potential side effects.

Keywords: Exendin-4; NOD; Proliferation; Beta cell; Type 1 Diabetes

1. Introduction

Successful strategies to prevent and reverse type 1 diabetes will likely utilize combination therapies to ameliorate the autoimmune process and preserve or restore beta-cell mass (Schatz et al., 2003). While the challenges of safely overcoming autoimmunity remain a daunting task, short-term successes in recent clinical trials (e.g., anti-CD3/anti-thymocyte globulin/cytoxan/granulocyte colony stimulating factor) have generated new-found optimism for exploring agents which may function to enhance the preservation or restoration of beta cells via alternative mechanisms (Haller et al., 2007; Herold and Taylor 2003; Voltarelli et al., 2007).

One such agent, the long-acting glucagon-like-peptide-1 (GLP-1) agonist exendin-4 (Ex-4) has garnered significant attention for its potential role in restoring beta-cell mass. Amongst the proposed mechanisms underlying Ex-4's effect on beta cells are stimulation of cellular proliferation, inhibition of apoptosis, and recovery of residual cellular function (Hadjiyanni and Drucker 2007; Hadjiyanni et al.,

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2008). Data supporting these concepts emanates from in vitro work, prevention studies in nonautoimmune rodent models, and a number of studies in the nonobese diabetic (NOD) mouse model of type 1 diabetes (Hadjiyanni et al., 2008; Li et al., 2005; Ogawa et al., 2004; Sherry et al., 2007; Suarez-Pinion and Rabinovitch, 2006; Wang and Brubaker, 2002; Xu et al., 1999; Yang et al., 2006). Still, an inadequate number of investigations have endeavored to explore Ex-4's role as a potential component of combination therapies aimed to reverse diabetes in the NOD mouse. These efforts, to date, suggest Ex-4, when administered with an immunomodulatory agent (e.g., anti-lymphocyte serum, anti-CD3, lisofylline), improves the ability to impart hyperglycemic reversal over administration of the immunomodulatory agents alone (Ogawa et al., 2004; Sherry et al., 2007; Yang et al., 2006). However, these studies have generated conflicting data as to the mechanisms involved in this therapeutic benefit. Furthermore, the highly variable dosing regimens used in published studies do not provide a clear understanding of the optimal Ex-4 dose needed to maximize the potential therapeutic outcome afforded by Ex-4. In addition, in a monotherapy experiment performed by Suarez-Pinion and Rabinovitch (2006), Ex-4 given to NOD mice at the first signs of hyperglycemia (blood glucose >10 mmol) was found to reverse hyperglycemia in up to 50% of animals and resulted in increased pancreatic insulin staining. To further explore the potential benefits of Ex-4 monotherapy and understand the mechanisms involved in Ex-4's ability to improve reversal rates, we investigated the effects of Ex-4 monotherapy on beta-cell proliferation and fractional insulin reactive area when provided to NOD mice at diabetes onset.

2. Methods

2.1. Animals

Eight-week-old female NOD mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and housed at the University of Florida. Studies were approved by the Institutional Animal Care and Use Committee. After 9 weeks of age, blood glucose was measured three times per week until greater than 13.2 mmol/l (240 mg/dl). Mice were monitored daily thereafter and diabetes was diagnosed when glucose was greater than 13.2 mmol/l (240 mg/dl) on two consecutive days as previously described (Serreze et al., 2000).

2.2. Treatment

Mice diagnosed with diabetes received 30 days of Ex-4 (n=10, 0.2 μg/mouse, 100 μl; Eli Lilly, Indianapolis, IN, USA) or saline (n=8, 100 μl) via daily intraperitoneal injection. The dose of 0.2 μg/mouse per day (~10 μg/kg per day) was chosen carefully after reviewing available literature related to Ex-4 therapy in rodent diabetes models. To date, Ex-4 has been used as prevention agent in STZ-induced C57BL/6 mice at a dose of 0.96 μg/mouse per day (Li et al., 2003), to study islet regeneration db/db mice at a dose of 0.008 μg/mouse per day (Stoffers et al., 2000), in the type 2 diabetes Sprague-Dawley rat model at 4 μg/rat per day (Xu et al., 1999), as a monotherapy in the NOD mouse at doses of approximately 0.3 to 10 μg/mouse per day (Suarez-Pinion and Rabinovitch 2006), and to study combination therapy approaches to reverse diabetes in the NOD mouse at doses as high as 48 μg/mouse per day (Ogawa et al., 2004) and as low as 0.007 μg/mouse per day) (Yang et al., 2006). Given the greater than 4000-fold difference between the doses used in the NOD mouse, the variable use of insulin in the above protocols, and the fact that Ex-4 alone failed to reverse diabetes at the lower dose, we felt a dose of 0.2 μg/mouse per day was justified in an attempt to maximize potential benefit while limiting the expected hypoglycemic and anorexic side effects associated with Ex-4 and insulin therapy. Small pilot studies in which larger doses of Ex-4 were provided in combination with insulin resulted in marked hypoglycemia and death, and as such led us to believe that 0.2 μg/mouse per day is a near-maximal dose when given concurrently with insulin pellets.

In order to simulate treatment of new onset type 1 diabetes patients, mice receiving either Ex-4 or saline therapy received a single subcutaneous insulin pellet at diagnosis (LinShin, Toronto, Canada). One insulin pellet typically provides 2 weeks of normoglycemia before pellet exhaustion and recurrence of hyperglycemia. All mice received a second insulin pellet when hyperglycemia recurred [glucose >13.2 mmol/l (240 mg/dl) on two consecutive days]. Following this, when severe hyperglycemia developed [glucose>22 mmol/l (400 mg/dl) on two consecutive days], mice were sacrificed and histological studies were performed. Animals were weighed weekly.

2.3. Histology

Necropsy was performed and the pancreas was fixed in 10% formalin solution overnight, transferred into PBS solution, and embedded in paraffin such that the surface area was maximized. Four-micrometer-thick sections were cut at 100-μm intervals, deparaffinized, and stained with hematoxylin and eosin. Additional sections were stained with polyclonal guinea pig anti-insulin and anti-Ki67 (DakoCytomation, Denmark), and anti-BrdU antibodies (AbD Serotec, UK).

2.4. Beta-cell proliferation, regeneration, and fractional insulin reactive area

Ki67 and BrdU indices were used to quantify beta-cell proliferation and regeneration. Mice were injected with BrdU (50 mg/kg ip) 12–16 h prior to sacrifice. Modification of the techniques used by Menge et al. (2008) and Sherry et al. (2007) was used to calculate BrdU and Ki67 indices,