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Reversal of Type-1 Diabetes in NOD Mice with Combination of Exendin-4 and Lisofylline

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Exendin-4 (Ex-4) is a long-lasting analog of glucagon-like peptide-1. Ex-4 stimulates pancreatic beta-cell neogenesis, reduces beta-cell apoptosis and enhances insulin secretion. However, Ex-4 alone could not reverse diabetes in non-obese diabetic (NOD) mice. Lisofylline (LSF), a novel anti-inflammatory compound, has previously demonstrated its ability to prevent autoimmune diabetes. In this study, we investigated the efficacy of combining Ex-4 and LSF for treatment of diabetes in NOD mice.

For *in vitro* studies, freshly isolated BALB/c pancreatic islets were incubated at 37°C with either LSF (50µM) alone, Ex-4 (20nM) alone, or with LSF/Ex-4 combined. After 3 days of culture, islets were treated with or without pro-inflammatory cytokines (10ng/mL IL-1β, 20ng/mL TNF-α, and 100ng/mL IFN-γ) for additional overnight. Apoptotic islets were quantitatively assessed. MTT assay was used to evaluate cell metabolism. For *in vivo* studies, overtly diabetic NOD mice (20-25 weeks old, 3-6/group) were used when blood glucose (BG) >250 mg/dL. Treatment was delivered via subcutaneous osmotic pumps containing saline, Ex-4 (18nM/day), or Ex-4 and LSF (27mg/kg/day) combined. BG levels were monitored daily thereafter. After 28 days of treatment, a glucose tolerance test (GTT) was performed prior to pancreas histology studies.

Ex-4 alone and Ex-4/LSF treatment enhanced β-cell metabolism (2.2-2.5 fold), and reduced apoptosis in response to inflammatory cytokines (30-40%). Combined therapy of Ex-4 with LSF completely reversed diabetes in NOD mice (n=6, 100%). In contrast, individual treatment with saline or Ex-4 alone (n=4 / group) had no effect and animals remained diabetic. The combined therapy of LSF/Ex-4 restored glucose homeostasis detected by a GTT. Pancreas immunohistochemistry showed insulin-positive islet-like cluster cells in the combined therapy group. This study provides evidence that the combination of Ex-4 and LSF is able to enhance beta-cell function, reduce apoptosis, and successfully reverse autoimmune diabetes.