Title: New Onset Type 1 Diabetes: Role of Exenatide

Background:

Type 1 diabetes mellitus (T1DM) is one of the most common chronic diseases of childhood. In the US, it accounts for about two thirds of newly diagnosed diabetes in patients less than 19 years of age. It is caused by the autoimmune destruction of beta cells in the pancreas resulting in insulin deficiency. The Diabetes Control and Complications Trial demonstrated that improved glycemic control prevents and/or delays long-term complications associated with T1DM. Improved outcomes in glycemic control have been seen in recent years with new insulin analogs and delivery devices. However, blood glucose control still remains imperfect and quality of life is affected by the continued dependence on insulin. Hence, the long term goal is to maintain beta cell mass in the pancreas. Higher residual beta cell mass early in the disease will result in long term benefits. To achieve this, various therapeutic trials have included therapies such as immunosuppression, immunomodulation, antigen specific therapy, islet cell transplant and stem cell transplant. However, these therapies have severe complications requiring further development. Therefore, until such therapies are available, other agents that have received FDA approval must be explored to improve glycemic control. Exenatide, a GLP-1 agonist, is one such agent that is approved for use in adults with type 2 diabetes mellitus (T2DM). Glucagon like peptide-1 (GLP-1) is secreted from the entero-endocrine cells following nutrient ingestion. It regulates glucose metabolism through its effects on the pancreatic islet cells, the gastrointestinal tract, and the central nervous system. The principal mechanisms include stimulation of insulin secretion, inhibition of glucagon secretion, delay in gastric emptying and reduction in food intake. In addition, GLP-1 has been shown to be important in beta cell proliferation and survival. In animal studies, GLP-1 is associated with induction of pancreatic duodenal homeobox 1 (pdx1) gene expression. Pdx1 is a transcription factor, which is primarily expressed in the endocrine pancreas and subsets of entero-endocrine cells in the duodenum. It is highly conserved among different species. Genetic disruptions of the pdx1 gene in mice abrogate normal pancreatic development and formation. In humans, inactivating mutations of the pdx1 gene is associated with pancreatic agenesis. Hence pdx1 is critical for pancreatic development and formation. GLP-1 actions may be mediated through pdx1 and thus influence beta cell proliferation and survival. Furthermore, GLP-1 regulates a subset of genes including pro-insulin, GLUT-2 and glucokinase that are known transcriptional targets of pdx1 action. GLP-1 has anti-apoptotic properties independent of its action on glucose regulation and islet proliferation. Apoptosis related cell

death is a feature of both type 1 and type 2 diabetes mellitus. In mice, with streptozotocin-induced diabetes, there is increased apoptotic related cell death. In these mice, treatment with exendin-4, a GLP-1 receptor analog, resulted in euglycemia significantly longer than those that were not treated with GLP-1. Conversely, mice with targeted disruption of the GLP-1 receptor gene exhibited increased apoptosis after streptozotocin administration. Furthermore, exendin-4 directly reduced cytokine-induced apoptosis in purified rat beta cells exposed to a host of cytokines such as interleukin 1a, tumor necrosis factor alpha, and interferon in vitro. Exenatide is a synthetic analog of exendin-4. It has properties similar to GLP-1 but has only fifty percent homology to native GLP-1. The investigator's brochure for exenatide suggests that in adults with T2DM, it was well tolerated at 0.1mcg/kg/dose and resulted in glucose lowering. GLP-1 is metabolized by di-peptidyl peptidases (DPP-IV) proteolysis and clearance occurs via the kidneys. However, in vitro studies with human placental DPP-IV demonstrated that exenatide is resistant to degradation by this enzyme. No evidence of plasma accumulation with multiple doses was noted in adults with renal dysfunction. Rodent models of hepatic disease with reduced liver function (thioacetamide-and D-galactosamine-induced liver injury) demonstrated no change in the pK role of the liver in the metabolism/ elimination of exenatide, suggesting that the liver does not play a major role in exenatide clearance. Adult studies demonstrate a dose-proportional Cmax and AUC in the 5-10 microgram dose range. The drug is rapidly absorbed with a Tmax of approximately 1-3 hours; mean Cmax values of 0.88-0.99 pmol/L (dose range 5-10 ìgm), and T½ beta of 2-4 hours. Bioavailability of exenatide was comparable whether injected subcutaneously in the arm, thigh, or abdomen. Exenatide has been extensively studied in patients with T2DM. Clinical studies indicate that exenatide is very effective in decreasing postprandial glucose excursions. Limited studies of GLP-1 infusions are reported in adults with T1DM. The principal mechanism of action is increased insulin release from the pancreatic islets, suppression of glucagon leading to decreased hepatic glucose production and decreased immediate postprandial hyperglycemia. In addition, exenatide delays gastric emptying and decreases food intake through centrally mediated mechanisms causing satiety and improved weight control. These effects result in the benefit of further reduction of Hemoglobin A1C (HgbA1C) beyond the reduction observed with insulin alone. Children and adolescents with new onset T1DM demonstrate marked increase in postprandial glucose concentrations despite the use of intense insulin therapy. Use of exenatide would be a novel way to reduce postprandial hyperglycemia and perhaps preserve beta

cell mass in the pancreas. Our hypothesis is that exenatide can reduce postprandial glycemic excursions by twenty percent compared to insulin mono-therapy in children and adolescents with new onset type 1 diabetes. In addition, we would like to study the safety of this low dose of Exenatide (1.25 micrograms) in new onset type 1 diabetes. The specific aims of this study are to determine the following: 1. The role of exenatide as compared to insulin mono-therapy in reducing postprandial hyperglycemia. 2. The role of exenatide on postprandial glucagon and gastric emptying. 3. The effect of long acting insulin/ rapid acting insulin on a basal rate in an insulin pump on postprandial glucose excursions, glucagon concentrations and gastric emptying. 4. Postprandial glucose excursions, glucagon concentrations and gastric emptying in normal healthy controls.

Study Design:

A randomized, non-blinded trial with a crossover design will be used. Following informed consent and with appropriate subject assent, all subjects will have a screening visit. Following the screening visit, subjects with T1DM will undergo 3 studies: Part A (exenatide and long acting insulin), Part B (rapid and long acting insulin) and Part C (long acting insulin + rapid acting insulin + exenatide). For Type 1 diabetes subjects who are on an insulin pump, for Part A they will be on their usual basal rate of rapid acting insulin and they would receive exenatide, for Part B they will be on their usual basal rate of rapid acting insulin and before they drink the boost milk shake, they will receive an insulin bolus according to their carbohydrate and sensitivity ratio's. For Part C they will be on their usual basal rate of rapid acting insulin and will also get exenatide and bolus of rapid acting insulin before they drink the boost milkshake.The subjects will be admitted to the CRC on three separate occasions, at least 3-4 weeks apart. The three studies will be performed in a random order and the randomization will be done using a computerized system. The healthy controls will undergo a single study visit and will not receive any insulin or study drug. Except for the absence of diabetes, the healthy controls will be identical to the study subjects. Subjects with new onset diabetes will be compared to healthy controls. Inclusion Criteria: 1. Age between 12-18 years of age at the time of enrollment. 2. Diagnosed with antibody positive T1DM in the past 3 months. 3. Otherwise healthy except for their TIDM and treated hypothyroidism. 4. Females must have a negative pregnancy test. 5. Hemoglobin equal to or greater than 12 g/dl before each study. 6. Weight greater than 44 kg.

Exclusion Criteria: 1. Any chronic disease: leukemia, inflammatory bowel disease, cystic fibrosis, juvenile rheumatoid arthritis etc, except for diabetes and hypothyroidism. 2. Any medications that may affect glucose metabolism. 3. Abnormal AST, ALT, amylase, lipase, creatinine (more than 3 times normal values). 4. Lack of a supportive family environment as detected by the clinicians and/or social workers. 5. History of substance abuse (evaluated by medical history and CRAFFT questionnaire which will be administered at the screening visit). 6. Positive pregnancy test in females. 7. Lactating and nursing mothers. The study will include 10 subjects with T1DM and 10 healthy controls. The study is designed to detect a 20% difference in mean area under the curve for glucose for a 270 min period (breakfast). From our previous protocols, we have found $r > 0.8$ between repeated measures and that the standard deviation for our excursion measure AUC is approximately 30%. With these specifications, the necessary sample size is 8 subjects. With a 20% drop out rate, we would need 10 subjects with T1DM. We will also recruit 10 healthy controls for comparison. Study Procedure: Screening: After signing a consent form, a screening evaluation will be performed prior to study enrollment. The evaluation will consist of a detailed medical history and physical examination (including height, weight, vital signs and Tanner staging). Baseline laboratory tests will include: CBC, creatinine, AST, ALT, amylase, and lipase. In addition, healthy control subjects will have a diabetes antibody panel drawn. A urine pregnancy test will be performed in all female subjects. The approximate volume of blood drawn is expected to be 3 ml's for subjects with T1DM and 11 ml's for controls. Study: The subject must be NPO (except for water) for 8 hours prior to the study. The subject will be admitted at 0700. Vital signs, stat hemoglobin (if there is no documented hemoglobin in the past 28 days), and pregnancy tests (in females) will be checked. Hemoglobin must be >12 g/dl and female subjects must have a negative urine pregnancy test. An intravenous line will be placed in the forearm. At around 0800 baseline blood samples will be drawn for: glucose, insulin, C-peptide, amylin, GLP-1, and glucagon. In Study A and C, blood for exenatide will also be drawn. If blood glucose values are below 70 mg/dl, IV glucose of 5-15 grams will be administered to achieve euglycemia (90-130 mg/dl). At around 0830 (0 min), the subjects with T1DM will get his/her home dose of long acting insulin (if it has not been administered the night before).Insulin Pump users will be on their usual basal rate of rapid acting insulin. For study part A, Exenatide will be also be given as a subcutaneous injection of 1.25 mcg. For study part B, bolus of rapid acting insulin will also be given based on subjects prescribed regimen. For study

part C, the subject will receive Exenatide 1.25 micrograms and rapid acting insulin (with a 30-50% reduction from the usual dose). No medication will be given to subjects in the control arm. Following this, the subject will be offered a standard liquid meal of Boost High Protein Drink, 12 Oz. (360 calories, 50 g of carbohydrates and 12 g of fat) and asked to consume it within a 10-15 minute period. This will be enriched with 1 gram of [1- 13C] glucose. Blood glucose concentrations will be measured at the bedside using an Analox glucose analyzer at -30, -10, 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 200, 220, 240, 270, 300 min. Blood samples will be collected for hormonal analysis of Insulin, C-peptide, Amylin at -30, -10, 0, 10, 20, 30, 40, 60, 80, 120, 180, 240, 300 min. Samples for GLP- 1 will be collected at -30, 0, 10, 20, 40, 60, 80, 120, 180, 240, 300 min and Glucagon at -30, -10, 0, 10, 20, 30, 40, 60, 80, 100, 120, 140, 180, 240, 300 min. Blood samples for pK exenatide will only be collected in Study A and C at -30, 0, 10, 20, 40, 60, 120, 180, 240, 300 min. A total blood volume of 148.7 ml's will be drawn in study A and study C, and 128.7 ml's in study B and the control study. Expired 13CO2 will be determined in breath samples at 0, 20, 40, 60, 120, 180, 240, 300 min. During the study, if blood glucose values in a subject are less than 55 mg/dl, IV glucose of 5-15 grams will be administered to achieve euglycemia (90-130 mg/dl). 1-2 doses of IV glucose should correct hypoglycemia. If more than 3 doses are required to achieve euglycemia, the study will be terminated, the subject will be offered a meal tray and blood sugar rechecked to ensure euglycemia. If blood sugar at any time is more than 350 with moderate ketones, the study will be terminated. At the end of the study at 300 min, lunch will be provided (consistent carbohydrate meal) and insulin will be given as per the subject's prescribed regimen. The subject will be discharged home with a designated driver due to the risk of hypoglycemia. A subject will be withdrawn from participating in the study if he/she meets any of the following conditions: 1) develops a chronic disease 2) develops anemia 3) becomes pregnant 4) develops a weight loss of greater than 10 pounds for unspecified reasons 5)loss of contact- if we are unable to reach a study subject (within 2 months of screening or completion of the first study) by phone or mail to schedule the next appointment. All study subjects (that are withdrawn from the study) will receive a phone call and a letter notifying them that they have been withdrawn. Blood samples will be kept for 20 years because some of the assays have not been developed for some of the hormone analyses. Since it is difficult to perform clinical studies in pediatrics, we feel it is

better to bank blood samples and perform hormone analyses as newer assays become available rather than repeating the study.