G alginate (1.25 wt% LVG). The inner LVM core of the microcapsules was chelated with 55mM of sodium citrate for 2 min. A marginal mass of the encapsulated islets (~2000 islets/kg) was transplanted in an omentum pouch made in each of 5 STZ-diabetic Lewis rats whose blood glucose, plasma C-peptide, and body weights were monitored for 90 days along with those of a control group (n=5) which received empty capsules (no islets). The control group received daily insulin injections to keep blood glucose <500 mg/dL during follow-up. Although normoglycemia was not achieved with the marginal mass, the islet recipients had a 12% reduction in their mean blood sugar levels compared to controls ($p<0.001$), and increased their body weight from the diabetic baseline in contrast to the control group. Also, C-Peptide (Mercodia ELISA kit) increased from a non-detectable level to a range of 200-600 pmol/L in the islet recipients, but not in the control group during the 3-month period. These data show for the first time that a marginal mass of encapsulated islet transplants have long-term function in an omentum pouch making it a possible alternative site for encapsulated islet transplantation in large animals and humans with abundant omental tissue.

Supported by: RO1DK080897

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Islet Allograft Rejection Recognizes a P2X7R-Mediated Mechanism

ANDREA VERGANI, CARMEN FOTINO, SARA TEZZA, RUTH MOLANO, MICHELE PODETTA, ANTONIO SOLETI, FABIO GRASSI, CAMILLO RICORDI, MOHAMED SAYEGH, ANTONELLO PILEGGI, PAOLO FIORINA, *Boston, MA, Milan, Italy, Miami, FL, Torino, Italy, Bellinzona, Switzerland*

ATP, released during cell damage/activation, is sensed by P2X7 receptor (P2X7R) on lymphocytes and promotes T-cell activation. Novel P2X7R inhibitors are available (e.g. periodate-oxidized ATP [oATP]), rendering P2X7R target a potential therapy. We demonstrated that P2X7R is induced in murine islet allograft-infiltrating lymphocytes. P2X7R targeting on murine CD4+ T-cells by oATP inhibited Th1 and Th17 differentiation and anti-CD3- anti-CD28-Ig-induced IFNγ production (number of spots, ELISPOT: oATP=164±14 vs. Control=401±12, n=5; p=0.0002); oATP was not effective on P2X7R-/- cells, confirming drug specificity. WB analysis revealed inhibition of STAT3 phosphorylation; the use of Colivelin restored STAT3 phosphorylation, thus mitigating oATP effects. Short-term oATP treatment (250 µg day 0 to day 15 i.p.) in allogeneic islet transplantation (BALB/c into hyperglycemic C57BL/6 mice), reduced the frequency of peripheral and intra-graft Th1/Th17 cells, reduced T-cell STAT3 phosphorylation, inhibited alloantigen-specific T-cell activation, thus prolonging graft survival (Mean Survival Time [MST]: oATP treated=22 days vs. Untreated=14 days, n=10; p=0.0001) with indefinite graft survival achieved in 3 mice out of 10. Treated mice were immunocompetent (as assessed by Ovalbumin rechallenge). Graft survival prolongation was observed in P2X7R-/- recipient, although compensatory P2X1R/P2X4R expression limited the benefit of P2X7R genetic targeting. Tor-inhibitors increased P2X7R expression on murine and human T-cells and the use of Rapamycin synergized with oATP preventing murine CD4+ T-cells activation (IFNγ, number of spots: Rapamycin(-)/oATP(-)=348±29, Rapamycin(+)/oATP(-)=111±16; Rapamycin(+)/oATP(+)=49±2, n=3; p<0.01 vs. all) and promoting indefinite graft survival in 5 out of 7 recipients (p<0.01 vs. Rapamycin). These data demonstrate the central role of purinergic system in priming Th1/Th17 response during islet rejection possibly through STAT3 phosphorylation.

NOVEL AGENTS FOR DIABETES MANAGEMENT

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Targeting Insulin Resistance via the Immune Modulation of Cord Blood-Derived Multipotent Stem Cells by the Stem Cell Educator Therapy

YONG ZHAO, ZHAOSHUN JIANG, MINGLIANG YE, ZHAOHUI YIN, HENG LI, YALIN DIAO, MARK HOLTERMAN, THEODORE MAZZONE, *Chicago, IL, Jinan, China, Peoria, IL*

The prevalence of type 2 diabetes (T2D) is increasing worldwide, highlighting the need for a better understanding of the pathogenesis of the disease and the development of innovative therapeutic approaches for the prevention and cure of the condition. Mounting evidence points to the involvement of immune dysfunction in insulin resistance in T2D, suggesting that immune modulation may be a useful tool in treating the disease. We developed an innovative procedure for Stem Cell Educator therapy in which a patient's blood is circulated through a closed-loop system that separate lymphocytes from the whole blood and briefly co-cultures them with adherent human cord blood-derived multipotent stem cells (CB-SCs), and returns the educated lymphocytes (but not the CB-SCs) to the patient's circulation. In an open-label, phase1/phase 2 study, patients (n = 25) with T2D received one treatment with the Stem Cell Educator. Median age was 50 years (range, 29 to 66), and median diabetic history was 9 years (range, 1 to 25). Notably, we found that T2D patients achieve improved metabolic control and reduced inflammation. Median glycated hemoglobin (HbA_1C) was significantly reduced from 8.47% ± 0.99 at baseline to 7.87% ± 1.07 at 4 weeks post treatment ($p = 0.022$), and to 7.1% ± 0.6 at 12 weeks post treatment ($p = 1.6E-05$). More than 80% of subjects achieved the <7% standard recommended by the ADA. Homeostasis model assessment of insulin resistance (HOMA-IR) and HOMA-pancreatic islet beta-cell function (HOMA-B) demonstrate that insulin sensitivity have been improved post treatment. Mechanistic studies revealed this therapy can correct the immune dysfunction, as demonstrated by balancing the Th1/Th2/Th3 cytokine productions. Thus, Stem Cell Educator therapy is safe, and in individuals with moderate or severe T2D, a single treatment produces lasting improvement in metabolic control, without the safety and ethical concerns related to conventional stem cell-based therapies.

Supported by: JDRF, UIC CCTS Grant, Chinese Government Funding

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FGF21-Adnectin-Pharmacokinetic Enhancer (FGF21-AdPKE): A Novel Protein Candidate With Uniquely Extended Pharmacokinetic Profile for the Treatment of Diabetes and Dyslipidemia

RANJAN MUKHERJEE, PAUL MORIN, JOSEPH TAYLOR, DENNIS FARRELLY, DEBORAH HAGAN, BIN HE, DANIEL COHEN, JOHN KRUPINSKI, MARK KIRBY, MICHAEL GOSSELIN, HONG SHEN, CHRISTINE HUANG, BRADLEY ZINKER, *Pennington, NJ*

FGF21 treatment has been shown to improve the diabetic, insulin resistant and dyslipidemic state in several animal models of diabetes and obesity without inducing hypoglycemia, mitogenesis or edema. Its short half life (30 minutes in mice) would require multiple, daily injections to be used as a therapeutic agent. We hypothesized that extending the half life of FGF21 would lead to improved efficacy and require less frequent dosing. Adnectins are a new family of proteins based on the 10th type III domain of human fibronectin that can be designed to bind to targets of interest with high affinity and specificity. We have created a novel protein by fusing human FGF21 to an adnectin pharmacokinetic enhancer (FGF21-adPKE) which binds to human and monkey but not mouse serum albumin. This novel FGF21-adPKE retains the property of inducing ERK phosphorylation in β-klotho-expressing cells and glucose uptake in adipocytes. In ob/ob mice injected q.d., s.c., for 7 days with FGF21-adPKE (1 mg/kg) or native FGF21 (0.3 mg/kg), non-fasting glucose was lowered by 29% and 34% respectively. When the FGF21-adPKE was co-injected with human albumin, glucose lowering was 41% and the increased efficacy was associated with prolonged exposure. After 14 days of q.d. co-dosing of FGF21-adPKE with human albumin, vehicle subtracted HbA1c was lowered by 0.94%. In cynomolgus monkeys where FGF21-adPKE bound to endogenous monkey serum albumin, the half life was 97 hours, significantly greater than native human FGF21 (4 hours). FGF21-adPKE is produced in E.coli, has low viscosity and displays preclinical pharmacokinetic properties that would support convenient dosing regimens (including once weekly) in humans. In conclusion, FGF21-adPKE represents a promising candidate for the treatment of diabetes and attendant dyslipidemic disorders.

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SRT3025, a Novel SIRT1 Activator, Reverses Metabolic Dysfunction Induced by a High Fat Diet through Transcriptional and Post Transcriptional Modulation of Multiple Metabolic Pathways

VIPIN SURI, YONG QI, MEGHAN L. DAVIS, QING NIE, MARC O. JOHNSON, ANGELA M. COTE, ELDEN LAINEZ, GEORGE P. VLASUK, JAMES L. ELLIS, *Cambridge, MA*

Activation of the protein deacetylase SIRT1 through transgenic overexpression in mice confers resistance to body weight gain, insulin resistance, dyslipidemia and hepatic steatosis induced by a high fat diet. We report here the discovery of SRT3025, a novel small molecule activator of SIRT1, which reverses several aspects of metabolic dysfunction induced by a high fat diet. SRT3025 enhanced the deacetylase activity of SIRT1 by 4-fold, with half maximal activation at 200 nM. Diet Induced Obese (DIO) mice treated with 100 mg/kg SRT3025 for 7 weeks weighed 13% less than vehicle treated mice and showed significant reductions in fed glucose (-17%) and insulin (-77%), fasted glucose (-23%) and insulin (-62%), and serum (-41%) and hepatic lipids (-37%). Indirect calorimetry indicated that SRT3025 reduced body weight by increasing energy expenditure through stimulation of both carbohydrate and lipid oxidation. Euglycemic-Hyperinsulinemic clamp studies showed that SRT3025 increased hepatic insulin sensitivity as well as insulin-stimulated

For author disclosure information, see page 797.