DIABETES MELLITUS

1. DIABETES MELLITUS PROTOCOL

A. Clinical response: Clinical response demonstrates a decrease in progression of disease and evidence of improved symptoms. In addition to physical examinations prior to stem cell graft and 6 months post-procedure, laboratory test results serve as evidence of repair process. Internationally recognized lab tests for monitoring Diabetes Mellitus include:

- Complete Blood Count
- Complete Metabolic Panel
- HbA1c (Glycosylated hemoglobin)
- Fasting Blood glucose
- Oral glucose tolerance tests

B. Objective**:** To provide the patient with a treatment that stimulates his / her immune system, promote cellular regeneration and improve symptoms associated with Diabetes Mellitus. The intra-pancreatic/intravenous Ad-SVF Containing Adult Stem Cell Procedure should serve to compliment the patient's current treatment regimen or to promote healing when current treatment is not responding.

2. PRELIMINARIES

A. Background: Diabetes results from the insufficient production of insulin by the pancreas. This is caused by genetic predisposition as well as sometimes caused by destruction of pancreas by pancreatitis. Insulin allows glucose to enter the cells where it is converted to energy for metabolism. When there is an insulin deficiency, cells do not receive insulin and thus high blood sugar and high urine sugar occurs. The latter causes dehydration. Severe blood sugar is life threatening. Initially, the affected will have a large appetite which will diminish with time. As the disease advances and is not treated, lethargy, loss of appetite, vomiting, dehydration and ultimately coma will occur. Cataracts are a common occurrence and eventually all organs are affected. There are two types of Diabetes: Diabetes type 1 caused by a predisposition to the disease and Diabetes type 2 caused by a pre-existing condition which leads to diabetes [1].

- ***Prediabetes*** is a condition in which blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes. This condition is sometimes called impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Patients with prediabetes are at increased risk of developing Diabetes Mellitus Type 2. Prediabetes usually does not present with symptoms, although some patients may present with acanthosis nigrans.

- ***Diabetes Mellitus Type 1*** is a disease of glucose metabolism secondary to destruction of insulin-producing beta cells in islets of Langerhans. The exact pathophysiology is unknown, but evidence exists for an autoimmune variant and a non-autoimmune variant (also unknown).

- ***Diabetes Mellitus Type 2*** is a group of disorders with insulin resistance, defective insulin secretion, decreased insulin receptors and increased glucose production by liver.

- ***Gestational Diabetes*** is a condition "caused by the hormonal changes and metabolic demands of pregnancy together with genetic and environmental factors" [1].

B. Causes of Diabetes**:**

- ***Diabetes Mellitus Type 1:*** Type 1 diabetes is "caused by a lack of insulin due to the destruction of insulin-producing beta cells in the pancreas" [1].
  - *Genetic Susceptibility*
  - *Autoimmune Destruction of Beta Cells*
  - *Environmental Factors*

- ***Diabetes Mellitus Type 2*** is a group of disorders with insulin resistance, defective insulin secretion, decreased insulin receptors and increased glucose production by liver. [1]
  - *Genetic Susceptibility*
  - *Obesity and Physical Inactivity*
  - *Insulin Resistance*
  - *Abnormal Glucose Production by the Liver*
  - *Metabolic Syndrome*
  - *Cell Signaling & Regulation Issues*
  - *Beta Cell Dysfunction*

- ***Gestational Diabetes***:
  - *Insulin Resistance and Beta Cell Dysfunction*
  - *Family History*
  - *Future Risk of Type 2 Diabetes*

C. Treatment Options: Management of Diabetes is best provided by a multidisciplinary team of health professionals with expertise in diabetes, working in collaboration with the patient and family. Early initiation of pharmacologic therapy is associated with improved glycemic control and reduced long-term complications in type 2 diabetes.

Drug classes used for the treatment of type 2 diabetes include the following:

- ***Biguanides***: Metformin is the only biguanide in clinical use.

- ***Insulins***

- ***Sulfonylureas***: (Glyburide, glipizide, glimepiride) are insulin secretagogues that stimulate insulin release from pancreatic beta cells and probably have the greatest efficacy for glycemic lowering of any of the oral agents. However, that effect is only short-term and quickly dissipates. Sulfonylureas may also enhance peripheral sensitivity to insulin secondary to an increase in insulin receptors or to changes in the events following insulin-receptor binding.

- ***Meglitinide derivatives***: Meglitinides can be used alone in treating diabetes, (repaglinide, nateglinide) are much shorter-acting insulin secretagogues than the sulfonylureas are, with preprandial dosing potentially achieving more physiologic insulin release and less risk for hypoglycemia. Although meglitinides are considerably more expensive than sulfonylureas, they are similar in their glycemic clinical efficacy.

- ***Alpha-glucosidase inhibitors***

- ***Thiazolidinediones (TZDs)***: TZDs (pioglitazone, rosiglitazone) act as insulin sensitizers; thus, they require the presence of insulin to work. They must be taken for 12-16 weeks to achieve maximal effect. These agents are used as monotherapy or in combination with sulfonylurea, metformin, meglitinide, DPP-4 inhibitors, GLP-1 receptor agonists, or insulin. They are the only antidiabetic agents that have been shown to slow the progression of diabetes (particularly in early disease).

- ***Amylinomimetics***: Pramlintide acetate is an amylin analog that mimics the effects of endogenous amylin, which is secreted by pancreatic beta cells. This agent delays gastric emptying, decreases postprandial glucagon release, and modulates appetite.

- ***Other treatment options include***: bile acid sequestrants, dopamine agonists, Glucagonlike peptide–1 (GLP-1) agonists, Dipeptidyl peptidase IV (DPP-4) inhibitors

- Treatments currently being researched include ***pancreas transplant*** and ***transplant of islet cells***. The adverse effects of these investigational treatments include high risk of infection, organ injury and tissue rejection. Transplant of the pancreas is usually reserved to patients' with diabetes that is difficult to control with standard treatments.

- ***Infusion of adipose derived stem cells*** to patients with Type 2 Diabetes Mellitus has been studied in phase 1 and phase 2 clinical trials. This investigational treatment option has fewer risks involved than pancreas transplant and transplant of islet cells. Adipose derived stem cells have the potential to repair insulin producing cells in the pancreas as well as repairing other tissues damaged by the diabetes disease process.

3. AD-SVF CONTAINING ADULT STEM CELLS TREATMENT OPTION

A. Ad-SVF Containing Adult Stem Cells Procedure

- ***Initial patient evaluation***: A physician revises the medical information, lab work, and diagnostic imaging provided by the patient in order to determine the stage of the medical condition and any other secondary conditions.

- ***Pre-op Evaluation / post-op medical consultation***: A medical specialist to the specific condition to be treated provides a medical consultation at the location where the procedure will be performed. During pre-op evaluation informed consent is obtained from all patients and medical records are updated, including patient's most recent physical exam, most up-to-date lab results and imaging studies. Physician then performs surgical risk assessment. On the morning prior to procedure history and physical are performed by physician.

- ***Harvesting of adipose tissue***: Adipose tissue acquisition can be summarized as three step process:

  - ***Application of anesthetic / injection of tumescent solution***
  - ***Waiting time***
  - ***Acquisition of adipose tissue:*** An area of the body with sufficient adipose tissue is selected; this is usually the periumbilical area. With the patient supine, the physician infiltrates a small amount of local anesthetic. A tissue sample is then obtained using 60 cc syringe(s) to aspirate 50 to 100 cc of adipose tissue. Immediately following lipo-aspiration, adipose tissue sample is processed (minimally manipulated) to separate stem cells for use as graft.

- ***Autologous implant of Ad-SVF***: The stem cells obtained from the adipose tissue sample are resuspended in 5-10 cc of normal saline and applied to the patient using appropriate protocol for their condition. Autologous Ad-SVF containing adult stem cells are infused via catheter into the pancreas or intravenous infusion.

B. Risks: There are possibilities for unwanted effects related to the local anesthesia, harvesting procedure, and injection of stem cells. Even with the most established protocol, adequate technique, and careful administration; a medical team may encounter uncontrollable events. Although there is no guarantee of perfect results, excellent results can be attained. The surgeon provides services in the most responsible, professional and diligent manner, always considering that surgeries imply risks. The risks of complications of adipose tissue harvesting and stem cell infusion are very low. Possible risks include but are not limited to:

- Vascular spasm
- Vascular obstruction
- Pseudo-aneurysms
- Lymphadenopathy
- Bruising
- Nerve or muscle injury
- Allergic reaction
- Dizziness

- Nausea / Vomiting
- Allergic reaction
- Pain at site of injections
- Bleeding at injection site
- Malaise
- Low-grade fever
- Hot flashes
- Itching at injection site

C. Benefits: Adipose derived stem cells have the potential to repair insulin producing cells in the pancreas as well as repairing other tissues damaged by the diabetes disease process. The use of mesenchymal stem cells has been shown in cell culture and in animal studies to differentiate into functional islet cells which produce insulin in the pancreas. This differentiation will reduce the need for insulin in the body, thus reducing the need for insulin therapy [3]. In addition, in animal models, it has been found to reduce hyperglycemia by 90% in many rat cases. The stem cells not only proliferate into the islet cells but also stimulate the surrounding cells to differentiate as well causing a general healing response in the pancreas [4]. There are currently a great deal of clinical human trials on the treatment of both type 1 and type 2 diabetes mellitus which study not only the effects of the stem cells on the disease but also the dosing [5].

D. Follow-up Plan: Clinical response demonstrates a decrease of disease activity and improvement of symptoms associated with Diabetes Mellitus. International standards for follow-up:

- ***Pre-Ad-SVF implant***: Clinical evaluation of Diabetes symptoms. Review & record current laboratory results.
- ***3 months after Ad-SVF implant***: Clinical evaluation of Diabetes symptoms. Review & record current laboratory results.
- ***6 months after Ad-SVF implant***: Clinical evaluation of Diabetes symptoms. Review & record current laboratory results. Nerve conduction studies and quantitative neurological exams.

Diabetes – Adult Stem Cells Schedule of Events

1. **Initial Patient Evaluation:** A physician reviews the medical information, lab work, and diagnostic imaging provided by the patient in order to determine the stage of the medical condition and any other secondary conditions.

   A. Pre-Examination:
   - You will have a physical exam, which will include measuring your blood pressure, temperature and heart rate (vital signs).
   - Your doctor will discuss your medical history and any medications that you are taking.
   - Your doctor will assess how well you can perform your daily activities
   - If needed, you will have a urine or blood pregnancy test.
   - Blood will be taken.

   B. Additional Tests: should be done during or soon after this visit

   - Complete Blood Count
   - Complete Metabolic Panel
   - HbA1c (Glycosylated hemoglobin)
   - Fasting Blood glucose
   - Oral glucose tolerance tests

   C. Review Results: After your doctor has reviewed the results of these tests, he or she will assess whether you are a good candidate for stem cell therapy. If you decide to obtain this therapy you will sign a consent form. A medical specialist to the specific condition to be treated provides a medical consultation at the location where the procedure will be performed. During pre-op evaluation informed consent is obtained from all patients and medical records are updated, including patient's most recent physical exam, most up-to-date lab results and imaging studies. Physician then performs surgical risk assessment.

2. **Pre-Operation / Stem Cell Procedure:**

   A. Two Weeks Before Procedure:

   - No Aspirin or medicines that contain aspirin or Ibuprofen since it interferes with normal blood clotting. You may take Tylenol or generic forms of this drug.
   - Discuss with your primary physician to discontinue anticoagulant drugs at least 1 week before the procedure.
   - Please discontinue all herbal medications as many have side effects that could complicate a surgical procedure by inhibiting blood clotting, affecting blood pressure, or interfering with anesthetics.
   - Please discontinue all diet pills whether prescription, over-the-counter or herbal.
   - NO SMOKING because nicotine reduces blood flow to the skin and can cause significant complications during healing.
   - Purchase a compressive garment to wear after the lipoaspiration procedure.

B. Morning of the Procedure:

- Have a light breakfast.
- Take your regular prescribed medications
- Wear comfortable, loose-fitting clothes that do not have to be put on over your head.

3. Stem Cell Procedure:

- Preparation & Harvesting of Adipose Tissue:
  - ***Application of anesthetic / injection of tumescent solution***
  - ***Waiting time (~15 – 20 minutes)***
  - ***Acquisition of blood sample***
  - ***Acquisition of adipose tissue:*** An area of the body with sufficient adipose tissue is selected; this is usually the periumbilical area. With the patient supine, the physician infiltrates a small amount of local anesthetic. Immediately following lipo-aspiration, adipose tissue sample is processed (minimally manipulated) to separate stem cells for use as graft.

- Autologous implant of Ad-SVF: The stem cells obtained from the adipose tissue sample are resuspended in 5-10 cc of normal saline and applied to the patient using appropriate protocol for their condition. Autologous Ad-SVF containing adult stem cells are infused via catheter into the pancreas or intravenous infusion.

4. Recommended Post-Operation / Stem Cell Therapy Schedule:

A. Post-Op Medical Instruction - **(Please follow these instructions closely!)**

- ***Post-op medication*** will be given to you the day of your surgery. They will consist of an antibiotic and a painkiller:
  - *Antibiotic*: Cephalexin/Cipro, please take as directed beginning the day after surgery
  - *Painkiller:* Please take as directed and only as needed for pain
    * If you are unable to take any of these medications, please contact your patient coordinator so we can arrange for other medications.
- ***Resume previous medication*** as directed by the physician
- ***Report any symptoms of feeling unwell***: fever, pain, etc. Patients should be seen promptly by an ophthalmologist for full evaluation should any of the above symptoms be encountered.
- It is recommended that the ***patient have a companion stay with him or her*** for at least 24 hours after discharge.
- You should ***expect some of blood-tinged anesthetic solution to drain from the incision sites*** during the first 24 to 48 hours. This will vary from patient to

patient. Maxi-pads are recommended for bandages over your incision sites. You may take a shower 24 hours after the procedure.

- ***Compressive garments should be worn*** 24 hours a day for the first week and 12 hours a day for the second week.
- ***Do not shower for the first 24 hours***. ***Do not submerge yourself in any water*** (i.e. taking a bath or swimming) for the 1st week.
- ***If you experience nausea or vomiting it is probably due to the medication***. Please try to take it with food. If it persists, please contact our office.
- ***Diet-meals are not restricted.***
- ***Drink plenty of clear fluids***. We recommend 8 glasses of water or fruit juice every day.
- ***Do not drink any alcohol*** for 48 hours and limit alcohol intake for the first week.

B. Post-Op Medical Consultation Schedule: 3 Months & 6 Months

- Review of medical history
- Review of medication history
- Review of any adverse events since the previous visit
- Clinical evaluation of Diabetes symptoms
- Review & record current laboratory results
- Nerve conduction studies and quantitative neurological exams (6 months only)

Your doctor will contact you by phone within the first week to follow up then future follow up visits will be arranged through your patient coordinator. If you need assistance before do not hesitate to contact us.

DIABETES – Supporting Studies

Autoimmunity. 2008 Dec;41(8):666-72. doi: 10.1080/08916930802200208.

Autologous stem cell transplantation for early type 1 diabetes mellitus.

Couri CE, Voltarelli JC.

Source
Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.

Abstract
Type 1 diabetes mellitus (T1DM) is the result of the autoimmune response against pancreatic insulin producing beta cells. This autoimmune response begins months or even years before the first presentation of signs and symptoms of hyperglycemia and at the time of clinical diagnosis near 30% of beta-cell mass still remains. In daily clinical practice, the main therapeutic option for T1DM is multiple subcutaneous insulin injections that are shown to promote tight glucose control and reduce much of diabetic chronic complications, especially microvascular complications. Another important aspect related to long-term complications of diabetes is that patients with initially larger beta-cell mass suffer less microvascular complications and less hypoglycemic events than those patients with small beta-cell mass. In face of this, beta-cell preservation is another important target in the management of type 1 diabetes and its related complications. For many years, various immunomodulatory regimens were tested aiming at blocking autoimmunity against beta-cell mass and at promoting beta-cell preservation, mainly in secondary prevention trials. In this review, we summarize some of the most important studies involving beta-cell preservation by immunomodulation and discuss our preliminary data on autologous nonmyeloablative hematopoietic stem cell transplantation in newly-diagnosed T1DM.

DIABETES – Supporting Studies

JAMA. 2009 Apr 15;301(15):1573-9. doi: 10.1001/jama.2009.470.

C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus.

Couri CE, Oliveira MC, Stracieri AB, Moraes DA, Pieroni F, Barros GM, Madeira MI, Malmegrim KC, Foss-Freitas MC, Simões BP, Martinez EZ, Foss MC, Burt RK, Voltarelli JC.

Source
Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.

Abstract
CONTEXT:
In 2007, the effects of the autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT) in 15 patients with type 1 diabetes mellitus (DM) were reported. Most patients became insulin free with normal levels of glycated hemoglobin A(1c) (HbA(1c)) during a mean 18.8-month follow-up. To investigate if this effect was due to preservation of beta-cell mass, continued monitoring was performed of C-peptide levels after stem cell transplantation in the 15 original and 8 additional patients.

OBJECTIVE:
To determine C-peptide levels after autologous nonmyeloablative HSCT in patients with newly diagnosed type 1 DM during a longer follow-up.

DESIGN, SETTING, AND PARTICIPANTS:
A prospective phase 1/2 study of 23 patients with type 1 DM (aged 13-31 years) diagnosed in the previous 6 weeks by clinical findings with hyperglycemia and confirmed by measurement of serum levels of anti-glutamic acid decarboxylase antibodies. Enrollment was November 2003-April 2008, with follow-up until December 2008 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. Hematopoietic stem cells were mobilized via the 2007 protocol.

MAIN OUTCOME MEASURES:
C-peptide levels measured during the mixed-meal tolerance test, before, and at different times following HSCT. Secondary end points included morbidity and mortality from transplantation, temporal changes in exogenous insulin requirements, and serum levels of HbA(1c).

RESULTS:
During a 7- to 58-month follow-up (mean, 29.8 months; median, 30 months), 20 patients without previous ketoacidosis and not receiving corticosteroids during the preparative regimen became insulin free. Twelve patients maintained this status for a mean 31 months (range, 14-52 months) and 8 patients relapsed and resumed insulin use at low dose (0.1-0.3 IU/kg). In the continuous insulin-independent group, HbA(1c) levels were less than 7.0% and mean (SE) area under the curve (AUC) of C-peptide levels increased significantly from 225.0 (75.2) ng/mL per 2 hours pretransplantation to 785.4 (90.3) ng/mL per 2 hours at 24 months posttransplantation ($P < .001$)

and to 728.1 (144.4) ng/mL per 2 hours at 36 months (P = .001). In the transient insulin-independent group, mean (SE) AUC of C-peptide levels also increased from 148.9 (75.2) ng/mL per 2 hours pretransplantation to 546.8 (96.9) ng/mL per 2 hours at 36 months (P = .001), which was sustained at 48 months. In this group, 2 patients regained insulin independence after treatment with sitagliptin, which was associated with increase in C-peptide levels. Two patients developed bilateral nosocomial pneumonia, 3 patients developed late endocrine dysfunction, and 9 patients developed oligospermia. There was no mortality.

CONCLUSION:
After a mean follow-up of 29.8 months following autologous nonmyeloablative HSCT in patients with newly diagnosed type 1 DM, C-peptide levels increased significantly and the majority of patients achieved insulin independence with good glycemic control.

TRIAL REGISTRATION:
clinicaltrials.gov Identifier: NCT00315133.

DIABETES – Supporting Studies

JAMA. 2007 Apr 11;297(14):1568-76.

Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus.

Voltarelli JC, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F, Coutinho M, Malmegrim KC, Foss-Freitas MC, Simões BP, Foss MC, Squiers E, Burt RK.

Source
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Abstract
CONTEXT:
Type 1 diabetes mellitus (DM) results from a cell-mediated autoimmune attack against pancreatic beta cells. Previous animal and clinical studies suggest that moderate immunosuppression in newly diagnosed type 1 DM can prevent further loss of insulin production and can reduce insulin needs.

OBJECTIVE:
To determine the safety and metabolic effects of high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation (AHST) in newly diagnosed type 1 DM.

DESIGN, SETTING, AND PARTICIPANTS:
A prospective phase 1/2 study of 15 patients with type 1 DM (aged 14-31 years) diagnosed within the previous 6 weeks by clinical findings and hyperglycemia and confirmed with positive antibodies against glutamic acid decarboxylase. Enrollment was November 2003-July 2006 with observation until February 2007 at the Bone Marrow Transplantation Unit of the School of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil. Patients with previous diabetic ketoacidosis were excluded after the first patient with diabetic ketoacidosis failed to benefit from AHST. Hematopoietic stem cells were mobilized with cyclophosphamide (2.0 g/m2) and granulocyte colony-stimulating factor (10 microg/kg per day) and then collected from peripheral blood by leukapheresis and cryopreserved. The cells were injected intravenously after conditioning with cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (4.5 mg/kg).

MAIN OUTCOME MEASURES:
Morbidity and mortality from transplantation and temporal changes in exogenous insulin requirements (daily dose and duration of usage). Secondary end points: serum levels of hemoglobin A1c, C-peptide levels during the mixed-meal tolerance test, and anti-glutamic acid decarboxylase antibody titers measured before and at different times following AHST.

RESULTS:
During a 7- to 36-month follow-up (mean 18.8), 14 patients became insulin-free (1 for 35 months, 4 for at least 21 months, 7 for at least 6 months; and 2 with late response were insulin-

free for 1 and 5 months, respectively). Among those, 1 patient resumed insulin use 1 year after AHST. At 6 months after AHST, mean total area under the C-peptide response curve was significantly greater than the pretreatment values, and at 12 and 24 months it did not change. Anti-glutamic acid decarboxylase antibody levels decreased after 6 months and stabilized at 12 and 24 months. Serum levels of hemoglobin A(1c) were maintained at less than 7% in 13 of 14 patients. The only acute severe adverse effect was culture-negative bilateral pneumonia in 1 patient and late endocrine dysfunction (hypothyroidism or hypogonadism) in 2 others. There was no mortality.

CONCLUSIONS:
High-dose immunosuppression and AHST were performed with acceptable toxicity in a small number of patients with newly diagnosed type 1 DM. With AHST, beta cell function was increased in all but 1 patient and induced prolonged insulin independence in the majority of the patients.

DIABETES – Supporting Studies

PLoS One. 2012;7(2):e31887. doi: 10.1371/journal.pone.0031887. Epub 2012 Feb 22.

Acute response of peripheral blood cell to autologous hematopoietic stem cell transplantation in type 1 diabetic patient.

Zhang X, Ye L, Hu J, Tang W, Liu R, Yang M, Hong J, Wang W, Ning G, Gu W.

Source
Shanghai Key Laboratory for Endocrine Tumors, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, School of Medicine, Shanghai Jiaotong University, Ruijin Hospital, Shanghai, People's Republic of China.
Abstract

OBJECTIVE:
Autologous nonmyeloablative hematopoietic stem cell transplantation (AHST) was the first therapeutic approach that can improve β cell function in type 1 diabetic (T1D) patients. This study was designed to investigate the potential mechanisms involved.

DESIGN AND METHODS:
We applied AHST to nine T1D patients diagnosed within six months and analyzed the acute responses in peripheral blood for lymphocyte subpopulation as well as for genomic expression profiling at the six-month follow-up.

RESULTS:
We found six patients obtained insulin free (IF group) and three remained insulin dependent (ID group); C-peptide production was significantly higher in IF group compared to ID group. The acute responses in lymphocytes at six-month follow-up include declined CD3(+)CD4(+), CD3(+)CD8(+) T cell population and recovered B cell, NK cell population in both groups but with no significant differences between the two groups; most immune-related genes and pathways were up-regulated in peripheral blood mononuclear cell (PBMC) of both groups while none of transcription factors for immune regulatory component were significantly changed; the IF group demonstrated more AHST-modified genetic events than the ID group and distinct pattern of top pathways, co-expression network as well as 'hub' genes (eg, TCF7 and GZMA) were associated with each group.

CONCLUSIONS:
AHST could improve the islet function in newly diagnosed T1D patients and elimination of the islet specific autoreactive T cells might be one of the mechanisms involved; T1D patients responded differently to AHST possibly due to the distinct transcriptional events occurring in PBMC.

TRIAL REGISTRATION:
ClinicalTrials.gov NCT00807651.

DIABETES – Supporting Studies

World J Stem Cells. 2013 Oct 26;5(4):217-28. doi: 10.4252/wjsc.v5.i4.217.

Insulin producing cells established using non-integrated lentiviral vector harboring PDX1 gene.

Boroujeni ZN, Aleyasin A.
Source
Zahra Niki Boroujeni, Ahmad Aleyasin, Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran 14155-6343, Iran.

Abstract
AIM:
To investigate reprogramming of human adipose tissue derived stem cells into insulin producing cells using non-integrated lentivirus harboring PDX1 gene.

METHODS:
In this study, human adipose tissue derived stem cells (hADSCs) were obtained from abdominal adipose tissues by liposuction, selected by plastic adhesion, and characterized by flow cytometric analysis. Human ADSCs were differentiated into adipocytes and osteocytes using differentiating medium to confirm their multipotency. Non-integrated lentiviruses harboring PDX1 (Non-integrated LV-PDX1) were constructed using specific plasmids (pLV-HELP, pMD2G, LV-105-PDX1-1). Then, hADSCs were transduced with non-integrated LV-PDX1. After transduction, ADSCs(PDX1+) were cultured in high glucose DMEM medium supplement by B27, nicotinamide and βFGF for 21 d. Expressions of PDX1 and insulin were detected at protein level by immunofluorescence analysis. Expressions of PDX1, neurogenin3 (Ngn3), glucagon, glucose transporter2 (Glut2) and somatostatin as specific marker genes were investigated at mRNA level by quantitative RT-PCR. Insulin secretion of hADSCs(PDX1+) in the high-glucose medium was detected by electrochemiluminescence test. Human ADSCs(PDX1+) were implanted into hyperglycemic rats.

RESULTS:
Human ADSCs exhibited their fibroblast-like morphology and made colonies after 7-10 d of culture. Determination of hADSCs identified by FACS analysis showed that hADSCs were positive for mesenchymal cell markers and negative for hematopoietic cell markers that guaranteed the lack of hematopoietic contamination. In vitro differentiation of hADSCs into osteocytes and adipocytes were detected by Alizarin red and Oil red O staining and confirmed their multilineage differentiation ability. Transduced hADSCs(+PDX1) became round and clusters in the differentiation medium. The appropriate expression of PDX1 and insulin proteins was confirmed using immunocytochemistry analysis. Significant expressions of PDX1, Ngn3, glucagon, Glut2 and somatostatin were detected by quantitative RT-PCR. hADSCs(PDX1+) revealed the glucose sensing ability by expressing Glut2 when they were cultured in the medium containing high glucose concentration. The insulin secretion of hADSCs(PDX1+) in the high glucose medium was 2.32 μU/mL. hADSCs(PDX1+) implantation into hyperglycemic rats cured it two days after injection by reducing blood glucose levels from 485 mg/dL to the normal level.

CONCLUSION:
Human ADSCs can differentiate into IPCs by non-integrated LV-PDX1 transduction and have the potential to be used as a resource in type 1 diabetes cell therapy.

DIABETES – Supporting Studies

Int J Dev Biol. 2010;54(4):699-705. doi: 10.1387/ijdb.092953hk.

Pdx1-transfected adipose tissue-derived stem cells differentiate into insulin-producing cells in vivo and reduce hyperglycemia in diabetic mice.

Kajiyama H, Hamazaki TS, Tokuhara M, Masui S, Okabayashi K, Ohnuma K, Yabe S, Yasuda K, Ishiura S, Okochi H, Asashima M.

Source
Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan.

Abstract
Insulin-dependent diabetes mellitus (IDDM) is characterized by the rapid development of potentially severe metabolic abnormalities resulting from insulin deficiency. The transplantation of insulin-producing cells is a promising approach for the treatment of IDDM. The transcription factor pancreatic duodenal homeobox 1 (Pdx1) plays an important role in the differentiation of pancreatic beta cells. In this study, the human Pdx1 gene was transduced and expressed in murine adipose tissue-derived stem cells (ASCs). To evaluate pancreatic repair, we used a mouse model of pancreatic damage resulting in hyperglycemia, which involves injection of mice with streptozotocin (STZ). STZ-treated mice transplanted with Pdx1-transduced ASCs (Pdx1-ASCs) showed significantly decreased blood glucose levels and increased survival, when compared with control mice. While stable expression of Pdx1 in ASCs did not induce the pancreatic phenotype in vitro in our experiment, the transplanted stem cells became engrafted in the pancreas, wherein they expressed insulin and C-peptide, which is a marker of insulin-producing cells. These results suggest that Pdx1-ASCs are stably engrafted in the pancreas, acquire a functional beta-cell phenotype, and partially restore pancreatic function in vivo. The ease and safety associated with extirpating high numbers of cells from adipose tissues support the applicability of this system to developing a new cell therapy for IDDM.

PMID: 19757377 [PubMed - indexed for MEDLINE]

DIABETES – References

[1] Causes of Diabetes. *National Diabetes Information Clearinghouse.* 2013. Web. 12/11/13

[2] Davidson, J. Clinical Diabetes Mellitus: A Problem-Oriented Approach. Thieme medical Publishers, Inc: New York, 2000.

[3] Chen, L. Jiang, X. Yang, L. "Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells." *World J Gas-troenterol* 2004; 10 (20): 3016-1020.

[4] Fiorina, P. Jurewicz, M. Augello, A. Vergani, A. "Immunomodulatory Function of Bone Marrow-Derived Mesenchymal Stem Cells in Experimental Autoimmune Type 1 Diabetes." *The Journal of Immunology.* 189 (2), 2009, 993-1004.

[5] www.clinicaltrials.gov