Cell Transplantation, Vol. 17, pp. 1295–1304, 2008
Printed in the USA.

0963-6897/08 $90.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

Combined Treatment of Intrapancreatic Autologous Bone Marrow Stem Cells and Hyperbaric Oxygen in Type 2 Diabetes Mellitus

Esteban J. Estrada,* Fabian Valacchi,* Eduardo Nicora,* Sergio Brieva,* Claudio Esteve,* Laura Echevarria,* Tatiana Froud,† Karina Bernetti,† Shari Messinger Cayetano,† Omaida Velazquez,¶ Rodolfo Alejandro,† and Camillo Ricordi†‡§

*Stem Cell Argentina, Buenos Aires, Argentina
†Diabetes Research Institute, University of Miami, Miami, FL, USA
‡Wake Forest Institute for Regenerative Medicine, Wake Forest University, Winston-Salem, NC, USA
§Karolinska Institute, Stockholm, Sweden
¶Department of Surgery, University of Miami Hospital, Miami, FL, USA

The objective of this study was to determine whether the combination therapy of intrapancreatic autologous stem cell infusion (ASC) and hyperbaric oxygen treatment (HBO) before and after ASC can improve islet function and metabolic control in patients with type 2 diabetes mellitus (T2DM). This prospective phase 1 study enrolled 25 patients with T2DM who received a combination therapy of intrapancreatic ASC and peri-infusion HBO between March 2004 and October 2006 at Stem Cells Argentina Medical Center Buenos Aires, Argentina. Clinical variables (body mass index, oral hypoglycemic drugs, insulin requirement) and metabolic variables (fasting plasma glucose, C-peptide, HbA1c, and calculation of C-peptide/glucose ratio) were assessed over quartile periods starting at baseline and up to 1 year follow-up after intervention. Means were calculated in each quartile period and compared to baseline. Seventeen male and eight female patients were enrolled. Baseline variables expressed as means ± SEs were: age 55 ± 2.14 years, diabetes duration 13.2 ± 1.62 years, insulin dose 34.8 ± 2.96 U/day, and BMI 27.11 ± 0.51. All metabolic variables showed significant improvement when comparing baseline to 12 months follow-up, respectively: fasting glucose 205.6 ± 5.9 versus 105.2 ± 14.2 mg/dl, HbA1c 8.8 ± 0.2 versus 6.0 ± 0.4%, fasting C-peptide 1.5 ± 0.2 versus 3.3 ± 0.3 ng/ml, C-peptide/glucose ratio 0.7 ± 0.2 versus 3.5 ± 0.3, and insulin requirements 34.8 ± 2.9 versus 2.5 ± 6.7 U/day. BMI remained constant over the 1-year follow-up. Combined therapy of intrapancreatic ASC infusion and HBO can improve metabolic control and reduce insulin requirements in patients with T2DM. Further randomized controlled clinical trials will be required to confirm these findings.

Key words: Stem cell; Hyperbaric oxygen; Type 2 diabetes; Autologous transplant

INTRODUCTION

A combination of insulin resistance and pancreatic β-cell defects appear to be involved in the development of type 2 diabetes mellitus (T2DM). Moreover, there is growing evidence showing that the β-cell is central in the development and/or progression of T2DM (35). It has been demonstrated that glucotoxicity and lipotoxicity lead to increased β-cell apoptosis and affect insulin secretion associated with an increase in oxidative stress and inflammation (45). This concept has stimulated the search for new approaches of β-cell protection/regeneration in T2DM treatment.

Several new therapies have appeared in the last 10 years for the treatment of T2DM, but the complication rates have not decreased, and optimal glycemic control is still elusive (19). New therapies that prevent β-cell damage and/or lead to β-cell regeneration together with achievable life style changes may prevent or ameliorate T2DM and therefore decrease the incidence and progression of chronic complications and improve the general health and quality of life of patients with T2DM (9).

Autologous stem cell therapies (ASC) are an emerging set of therapies with promising results and low side effect profiles. They are easily accessible and have the advantage of avoiding histocompatibility issues while

Received September 24, 2008; final acceptance December 16, 2008.
Address correspondence to Esteban Estrada, M.D., Stem Cell Argentina, Edison 1789 - (1640) Martínez, Buenos Aires, Argentina. Tel: 01154-11-4-836-2003; Fax: 0054-11-4-717-4996; E-mail: info@stemcellargentina.com or drestradaesteban@yahoo.com.ar *and* Camillo Ricordi, M.D., Diabetes Research Institute, University of Miami Miller School of Medicine, 1450 N.W. 10 Avenue, Miami, FL 33136, USA. Tel: 305 243-6913; Fax: 305 243-4404; E-mail: ricordi@miami.edu

maintaining the potential for differentiation into multiple cell types. Recently, several observations suggest that multipotential adult stem cells are capable of producing a whole spectrum of cell types, regardless of whether or not these tissues are derived from the same germ layer, highlighting the opportunity to manipulate stem cells for therapeutic use (26). Some adult stem cell therapies are already in clinical trials for a variety of conditions, including Crohn's disease (20), myocardial infarction (17, 43,47), and graft-versus-host disease (15,44).

In the setting of diabetes, embryonic and adult stem cells have been induced to form insulin producing cells and /or islet clusters in vitro (25,26,42,54,55). It has also been shown that islet precursor cells exist within the pancreas (6,10,23,33) and they can be induced to differentiate into β-cells under the right circumstances (32).

Hyperbaric oxygen therapy (HBO) is a safe and noninvasive modality that has been used in a variety of maladies but primarily in the treatment of carbon monoxide poisoning, air embolism, and enhancement of wound healing (1,21,50). Recently, HBO has been shown to increase mobilization of stem cells and release of endothelial progenitor cells via a nitric oxide-dependent mechanism (22,48).

We hypothesized that ASC infusion accompanied by peritransplant HBO could be a therapeutic option in T2DM by leading to β-cell differentiation from existing stem cells or providing stem cells to replace those that are not differentiating adequately and reducing inflammation.

The primary objective of this pilot clinical trial was to evaluate the safety of ASC–HBO combined therapy in patients with T2DM and to determine whether this treatment could result in an improvement of metabolic control with a subsequent reduction in HbA1c and improved β-cell function (C-peptide), leading to a decrease in oral agents and/or insulin requirements.

PATIENTS AND METHODS

Study Design

Between March 2004 and October 2006 a prospective, phase I, single arm study was conducted in patients with T2DM at Stem Cells Argentina Medical Center in Buenos Aires, Argentina. Patients underwent a combined intervention treatment that consisted of autologous intrapancreatic stem cell infusion and hyperbaric oxygen treatment before and after the stem cell infusion. All patients provided informed consent before participating in the study and the study protocol was approved by the local institutional review board.

Inclusion and Exclusion Criteria

Patients of both genders, older than 18 years old, diagnosed with T2DM according to the American Diabetes Association criteria (13), were included. Patients were otherwise healthy with no contraindications for hyperbaric oxygen treatment such as: claustrophobia, lung disease, upper respiratory tract disease, or seizure disorder and with no contraindications for pancreatic arteriography and/or bone marrow harvest such as: symptomatic cholecystitis, pancreatitis, or coagulopathy.

Clinical Assessment and Follow-up

Patients were assessed in a diabetic clinic over a period of 1–3 months before the intervention. All patients were recommended a 1500 calories diet and an exercise routine (walking or similar for 45 min three times a week for the duration of the follow-up). Follow-up for 1 year after study intervention was performed every 3 months. Age, gender, diabetes duration, number of medications taken (including insulin, metformin, combination of both, or other oral hypoglycemic drugs), body weight and height, and insulin requirements were recorded at baseline and during follow-up every 3 months. Body mass index (BMI) was calculated by the formula weight/height^2 (kg/m^2). Similarly, fasting plasma glucose (enzymatic colorimetric method glucose oxidase/peroxidase), C-peptide (chemiluminescence assay), and HbA1c (immunoturbidimetry method) were measured and calculation of C-peptide/glucose ratio (CPGR) by the formula C-peptide × 100/glucose was assayed to evaluate glycemic profile at the different time points.

Intervention Procedures

Hyperbaric oxygen treatment consisted of a total of 10 sessions; five daily sessions were conducted prior to and after the stem cell infusion. During each 1-h session patients were enclosed in a hyperbaric pressure chamber (Multiplace Hypermed-Med, model 302) at a target pressure of 2.3–2.5 atmospheres (minimum 2.0 atmospheres) breathing 100% pure oxygen through a facial mask.

All subjects received a daily dose of vitamin C 1000 mg and vitamin E 200 mg for up to 3 months after the last intervention in order to minimize potential adverse effects of free oxygen radicals secondary to hyperbaric oxygen treatment.

Intrapancreatic autologous stem cells infusion was conducted upon completion of the first five hyperbaric oxygen sessions. Patients underwent bone marrow harvest under local anesthesia, using multiple bone marrow aspirations from both iliac crests. A minimum of 100 ml and a maximum (target) of 375 ml of bone marrow were mixed with anticoagulant (heparin) 20,000 units and preservative in a Quadruple Collection Bag (PL Rivero and Company S.A). If the target volume of 375 ml could not be achieved, the difference was completed to up to 375 ml by adding peripheral blood inside the bag.

Bone marrow or mixed bone marrow and peripheral blood was processed by centrifugation, gravity flow, and the various bags of the quadruple bag system. The red cells were discarded in the second bag, the buffy coat collected in the third bag, and the plasma and fat discarded with the first bag. The buffy coat was washed and resuspended in isotonic normal saline in the third bag for the final product. This was transported for immediate infusion into the main arterial vessel supplying the body and tail of the pancreas. Patients underwent angiography of the celiac axis and splenic artery. In all subjects a large vessel consistent with the dorsal pancreatic artery was identified, the catheter positioned at the origin, and the cells infused by gravity over 20 min. Patency of the vessel was confirmed at the end of the procedure. Patients were monitored for amylase and lipase postprocedure.

Outcomes

The following variables were assessed: glycemic profile measured by plasma glucose levels and HbA1c; β-cell function measured by basal C-peptide levels and changes in insulin requirements and/or oral hypoglycemic agents and BMI during follow-up.

Statistical Analysis

Clinical and laboratory variables were indexed by 3-month periods (quartiles): baseline (data collected between 50 days preinfusion and 7 days postinfusion), first quarter (3 months), second quarter (6 months), third quarter (9 months), and fourth quarter (12 months) following infusion of stem cells. For each variable under consideration, linear mixed models regression was fit to the data to estimate and compare mean response values for all periods. This method of analysis generalizes linear regression techniques, allowing for repeated observations by taking into account correlation that exists within observations from the same patient to more appropriately estimate variances used in tests of significance. With this approach, we are able to estimate and compare differences between time points of interest (baseline vs. postinfusion periods). Results of continuous variables are expressed as mean ± SE. Values of $p < 0.05$ were considered significant. Analysis was performed using SAS 9.1 software (SAS Institute Inc., Cary, NC).

RESULTS

Twenty five Caucasian patients, 68% ($n = 17$) male, between 36 and 74 years old (55.8 ± 2.14), were enrolled in the study. Demographic characteristics and baseline diabetic profile are shown in Table 1. All subjects completed five hyperbaric oxygen daily sessions pre- and postautologous stem cell infusion into main pancreatic artery without complications. BMI was obtained during the entire follow-up in 23 patients. There was a decrease in mean BMI throughout the follow-up but it did not reach statistical significance (BMI baseline 27.1 ± 0.5, 3 months 26.5 ± 0.5, 6 months 26.5 ± 0.6, 9 months 26.8 ± 0.7, and 12 months 27.0 ± 0.7, with a $p > 0.4$ at all time points compared to baseline) (Fig. 1).

Metabolic Parameters

Plasma Glucose. Mean fasting glucose levels showed a progressive reduction during follow-up in all patients with a maximal decrease in the fourth quarter (12 months) after intervention (baseline 205.6 ± 5.9, 3 months 143.4 ± 6.9, 6 months 137.1 ± 7.8, 9 months 115.0 ± 12.6, and 12 months 105.2 ± 14.2, with a $p < 0.0001$ at all time points compared to baseline) (Fig. 2).

HbA1c. Mean HbA1c values also showed a progressive reduction after intervention with a maximal decrease in the fourth quarter (12 months) after intervention (baseline 8.8 ± 0.2, 3 months 7.7 ± 0.2, 6 months 7.1 ± 0.2, 9 months 6.6 ± 0.3, and 12 months 6.2 ± 0.4, with a $p < 0.001$ at all time points compared to baseline) (Fig. 3).

Basal C-Peptide. Mean C-peptide levels increased progressively with a maximal increase in the last quarter (12 months) after intervention (baseline 1.5 ± 0.2, 3 months 1.7 ± 0.2, 6 months 1.9 ± 0.2, 9 months 2.3 ± 0.3, and 12 months 3.3 ± 0.3, with a $p < 0.04$ in the third and fourth quarters compared to baseline (Fig. 4).

C-Peptide/Glucose Ratio. Mean CPGR levels increased progressively with a maximal increase in the last quarter (12 months) after intervention (baseline 0.7 ± 0.2, 3 months 1.2 ± 0.1, 6 months 1.7 ± 0.2, 9 months 2.1 ± 0.2, and 12 months 3.5 ± 0.3, with a $p < 0.003$ in the second, third, and fourth quarters compared to baseline (Fig. 5).

Insulin Requirements. A progressive reduction in the mean units per day of insulin (U/24 h) was observed in all the patients that were taking insulin ($n = 15$) with a maximal reduction occurring in the last quarter (12 months) after intervention (baseline 34.8 ± 2.9, 3 months 21.6 ± 3.2, 6 months 14.8 ± 3.7, 9 months 7.9 ± 5.6, and 12 months 2.5 ± 6.7, with a $p < 0.004$ at all time points compared to baseline) (Fig. 6).

In 4/15 patients insulin was discontinued; two were concomitantly taking metformin. In one the metformin dose was reduced from 1500 mg/day to 850 mg/day and in the other it was maintained at a stable dose of 1700 mg/day. In 81.8% (9/11) of the remaining patients the reduction in the daily insulin dose was greater than 50% of the preintervention dose. Only three patients had an increase in oral hypoglycemic medications: the first patient reduced insulin dose from 100 U/day to 15 U/day

Table 1. Patients' Demographic and Baseline Diabetic Profile

Variable	*n*	Mean	Min	Max	SE
Clinical					
Age (years)	25	55.8	36	74	2.1
T2DM duration (years)	25	13.2	3	32	1.6
BMI (kg/m^2)	23	27.1	20.5	34.5	0.5
Insulin (U/day)	15	34.8	20	100	2.9
Metformin (mg/day)	13	708.0	850	2500	88.2
No. of oral medications preintervention	25	1.3	0	0	0.1
Laboratory					
Plasma glucose (mg/dl)	25	205.0	116	350	6.7
HbA1c (%)	25	8.8	7.0	11.4	0.23
C-peptide	25	1.5	0.3	2.5	0.2

and metformin 1700 mg/day and glicazide 90 mg/day were added; the second patient reduced insulin dose from 50 U/day to 24 U/day with the addition of repaglimide 2 mg/day and glicazide 90 mg/day; in the third patient insulin dose was decreased from 48 U/day to 10 U/day with glicazide dose unchanged and addition of rosiglitazone 8 mg. Of the other six patients, four were also taking metformin and the dose was not changed, one was taking glibenclamida 10 mg/day and it was discontinued, and in one patient metformin dose was reduced from 2000 mg/day to 1500 mg/day and glibenclamide dose was discontinued.

Oral Hypoglycemic Drugs. At baseline 84% (21/25) of the patients were taking oral hypoglycemic drugs (OHD). Postinfusion, 17/21 (81%) of the patients had a stable, decrease dose or fewer number of OHD. In these 17 patients, the same dose of OHD was maintained and a reduction in insulin dose was observed in five of the patients. The OHD dose was reduced or discontinued without increasing insulin dose in 10 patients. The remaining 2/17 patients were kept only on insulin with discontinuation of all OHD.

Postinfusion, the number or dose of OHD was increased in 4/21 patients. Preintervention, 4/25 patients were only on insulin therapy. Postintervention OHD were added in two of four patients with a decrease in the dose of insulin. In the remaining two patients the dose of insulin was not changed.

Metabolic variables were measured in all patients ($n = 25$) at baseline, with $n = 21$ at 6 months, $n = 12$ at 9 months, and $n = 10$ at 12 months. The loss to follow-

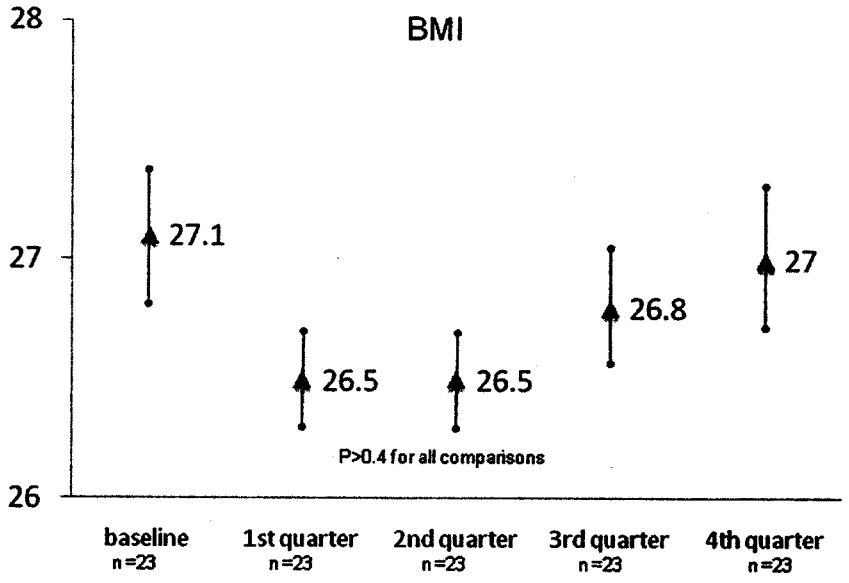

Figure 1. Body mass index changes. No significant changes in BMI over time were observed.

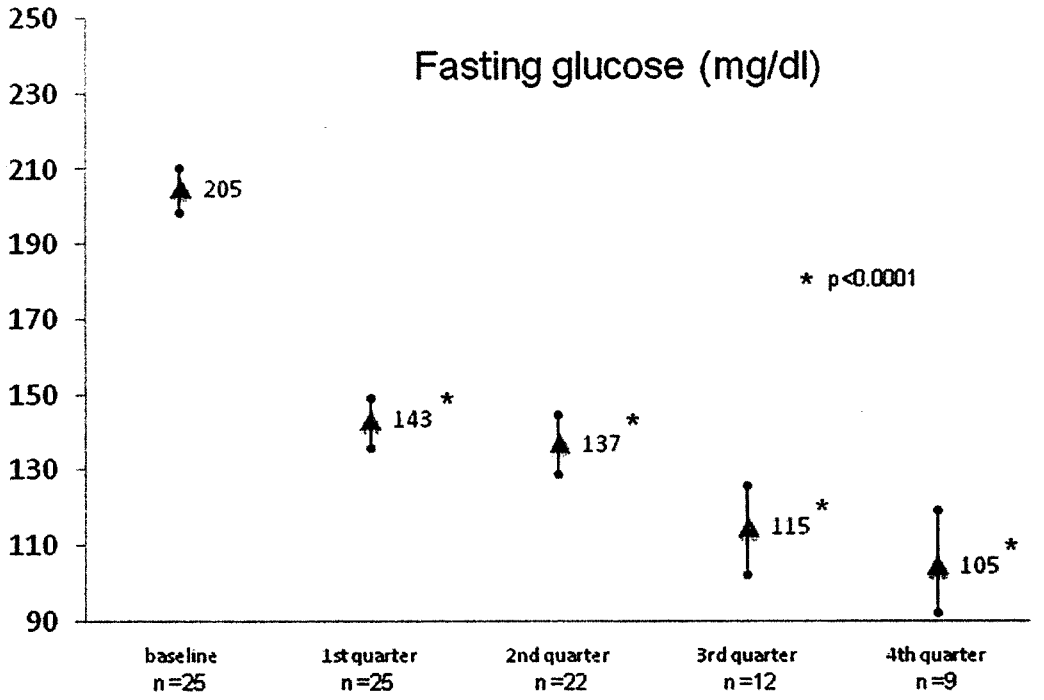

Figure 2. Changes in fasting glucose over time. *$p < 0.0001$ in all comparisons between baseline and first, second, third, and fourth quartiles.

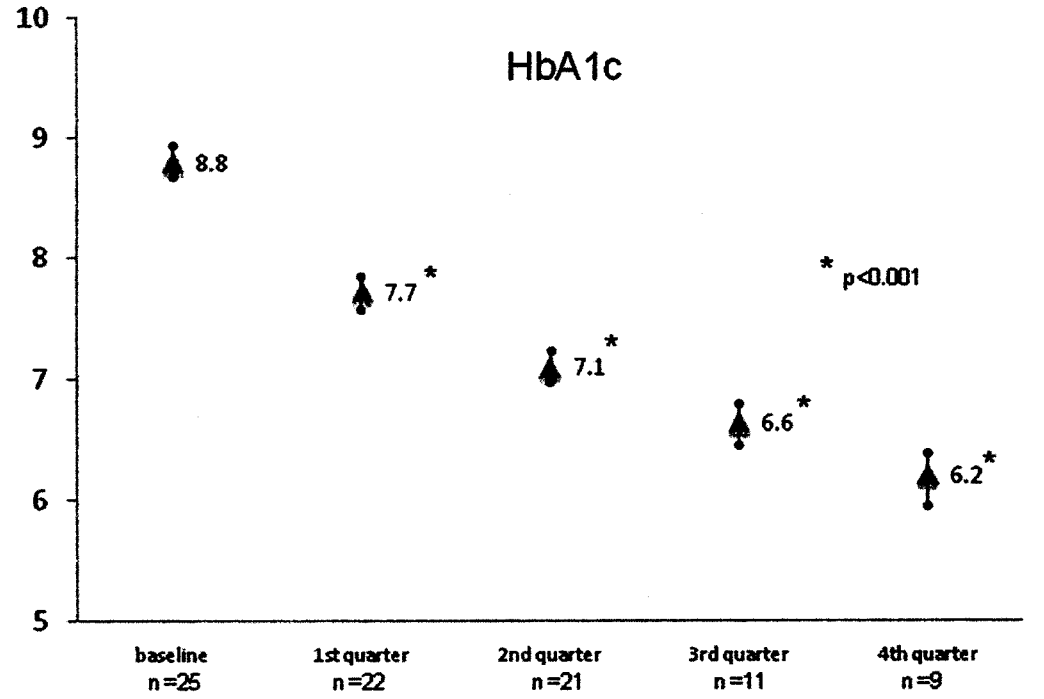

Figure 3. Changes in hemoglobin A1c over time. *$p < 0.001$ in all comparisons between baseline and first, second, third, and fourth quartiles.

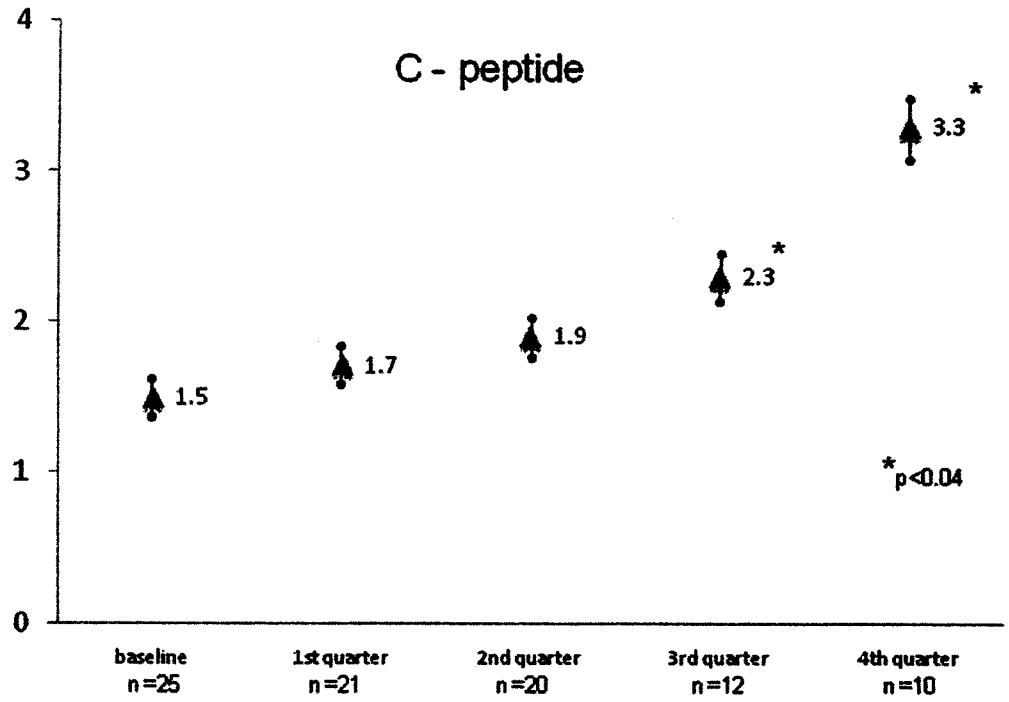

Figure 4. Changes in C-peptide levels over time. $*p < 0.04$ in comparison between baseline and third and fourth quartiles.

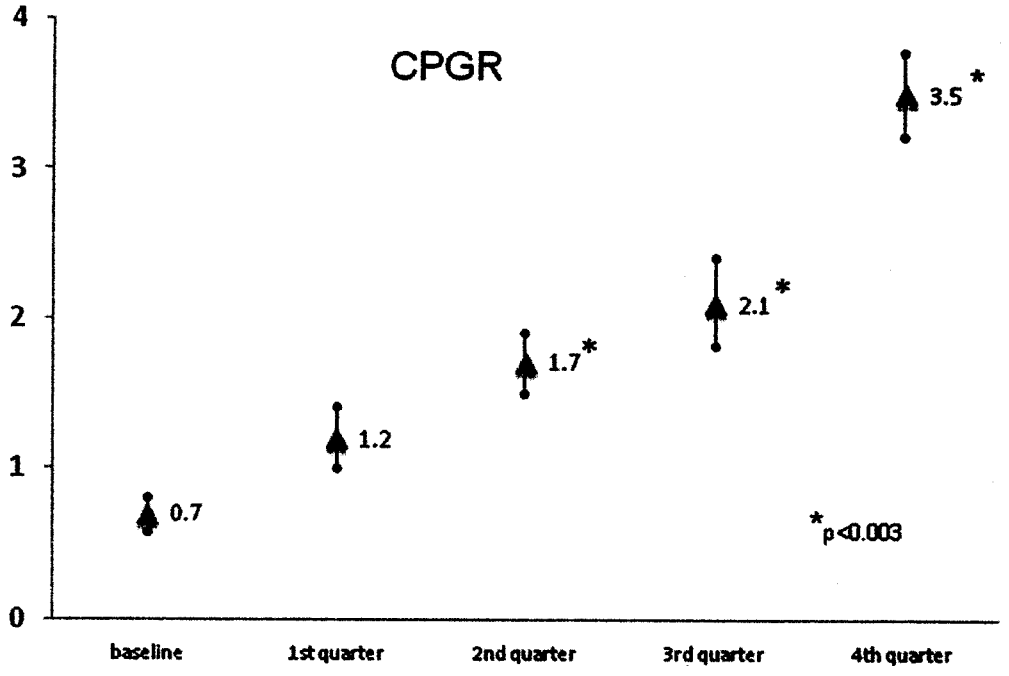

Figure 5. Changes in C-peptide glucose ratio over time. $*p < 0.003$ in comparisons between baseline and second, third, and fourth quartiles.

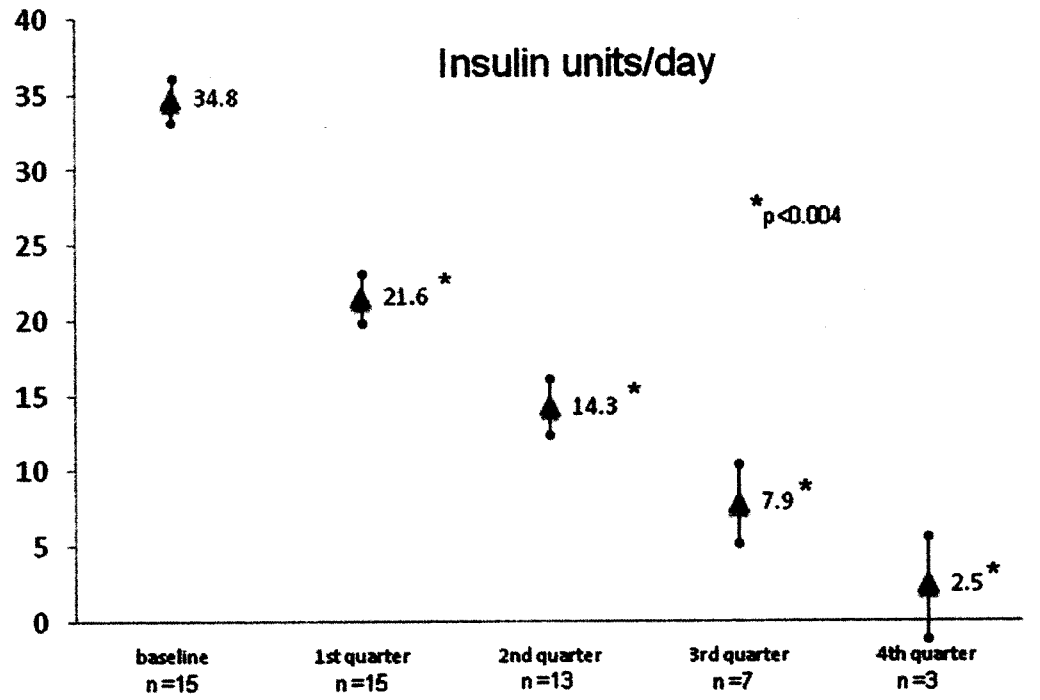

Figure 6. Changes in insulin daily requirements over time. $*p < 0.004$ in all comparisons between baseline and first, second, third, and fourth quartiles.

up observed in last quartile was due to patient nonadherence.

DISCUSSION

This study shows a progressive and consistent reduction in plasma glucose and HbA1c with an increase in C-peptide and CPGR over 1-year follow-up in conjunction with a decrease in the number and dose of oral agents and/or insulin. These findings suggest that autologous stem cell infusion combined with peri-infusion hyperbaric oxygen treatment result in an improvement in metabolic control and β-cell function.

It has been shown that islet precursor cells exist within the pancreas (23). There is ongoing debate as to whether these cells originate from ductal tissue (6), within the islets (33), or from an undefined location (10) and if under the right circumstances they can be induced to form β-cells. In some instances dedifferentiation and redifferentiation into β-cells has been performed (32).

New applications of the infusion of autologous bone marrow are currently developed either to tackle autoimmunity (7,8,11,53) or to induce regeneration in diseases like diabetes (5,52). Recent demonstrations have shown important advances in bone marrow stem cells plasticity and their differentiation to β-cells. Animal studies have shown that bone marrow-derived stem cells are capable of inducing endogenous pancreatic tissue regeneration in the mouse model of streptozotocin-induced diabetes (24). In a similar study, multipotent stromal cells from human bone marrow infused into mice treated with streptozotocin resulted in higher levels of mouse insulin and an increase in mouse islets and β-cells. It is not clear if this was due to cell protection or new cell formation (31). Autologous stem cell infusion into the pancreatic artery has been described in diabetic rats in order to obtain greater effect acting directly in the site of injury (2).

Studies in humans and mice have demonstrated that HBO increases bone marrow nitric oxide synthase, which leads to stem cell mobilization and release of endothelial progenitor cells (EPC) (22,51). It is believed that these cells are attracted to sites of inflammation due to local factors (cytokines, chemokines, etc.) and may be important for pancreatic islets repair after injury. If the pathologic mechanisms that affect the islets in diabetes are compared to a type of maladaptive repair in the setting of chronic injury, a potential mechanism of action for the observed effects could be postulated. This mechanism of action could center on EPC biology. The diabetic phenotype may be associated with multiple sites of impairment in the EPC-mediated cascade of healing or repair responses. For example, endothelial nitric oxide synthase phosphorylation within the bone marrow of diabetic mice is impaired, potentially accounting for decreased release of EPC from the bone marrow and

reduced circulating EPC. In addition, those circulating EPC may not be able to home to needed sites of repair because of a diabetes-associated decreased in crucial homing signals such as SDF-1a (18,22,37), and also the circulating EPC may be intrinsically impaired in crucial biological functions, such as migration and proliferation (28,48,49). Thus, we speculate that our strategy to deliver reparative stem cells intra-arterially and to activate these cells via systemic in vivo HBO may be targeting more than one crucial reparative step that counteracts the chronic injuries that attack both the EPC and the islets in the diabetic phenotype. There is strong evidence to suggest that HBO can potentially reverse at least one of the three diabetic-related EPC impairments (i.e., EPC mobilization from the bone marrow by NOS activation) (18,22). Moreover, there is evidence that bone marrow stem cells have an increased colony-forming capacity (CFC) when recovered subsequent to an in vivo HBO (22,51), suggesting an activation effect on a key cellular intrinsic biologic behavior (CFC), induced by the HBO (51).

Numerous studies have shown that insulin resistance precedes the development of hyperglycemia in subjects that eventually develop T2DM (36) and that T2DM only develops in insulin-resistant subjects with the onset of β-cell dysfunction, who are unable to sustain the β-cell compensatory response (30,39,41).

Chronic hyperglycemia can result in a browning reaction between glucose and the free amino acids on proteins and other large biological molecules, resulting in the formation of advanced glycation end products (AGEs) (16,46). AGEs are known to cause tissue damage through activation of inflammatory mediators such as IL-1β, IL-6, and TNF-α (4,38). In addition, AGEs have been implicated in diabetes complications but their role in islet cell damage has not been established (3,27).

There is evidence that nonimmune-mediated inflammatory pathways of β-cell damage occur in vitro in human islets when exposed to hyperglycemia. Furthermore, chronically elevated glucose has been documented to induce IL-1β production and expression of Fas receptor in human islets in association with cell death (12,34). However, this observation has not always been confirmed and it remains to be evaluated by in vivo experiments whether nonimmune islet inflammation contributes to progressive β-cell demise (40,41). Recently, the treatment with interleukin-1 receptor blocker (anakinra) has shown improvements in glycemia and β-cell secretory function, as well as reduction of systemic inflammation markers in T2DM patients (29).

As described above, it is well known that in T2DM there is ongoing inflammation in the pancreas. We believe that at the site of the lesion, mobilized stem cells/EPCs will cause angiogenesis and release of factors that will result in the local differentiation of progenitor cells. It also could simply be an anti-inflammatory effect that prevents the loss of β-cells and allows for recovery of their function. It is not known if the hyperbaric treatment itself could be responsible for this by simply increasing oxygen tension at the site of the lesion.

While HOT may result in low numbers of stem cells finding their way to the pancreas, it would seem logical that infusing stem cells into the pancreatic artery would increase the local level of stem cells/EPCs, resulting in maximization of the effect described above. Diabetes has also been shown to impair progenitor cell mobilization (14), another reason why local stem cell infusion may be important to overcome this problem.

It is still too early to judge the effectiveness of these therapies, and at this point there is a need to generate randomized controlled studies. The fate of infused cells is uncertain, as they could either differentiate into the desired tissues or, as preliminary evidence suggests, simply provide the damaged tissues with trophic signals that may promote their self-regeneration (9).

Our hypothesis is that the differentiation of local progenitor cells or bone marrow stem cells into β-cells or the decrease of inflammation in the pancreas leads to increase C-peptide production and therefore improved metabolic control assessed by HbA1c measurement with a secondary reduction in insulin and oral hypoglycemic drugs requirements.

It could be argued that a close follow-up, including diet, exercise, and intensive diabetes management, might have had a positive effect on its own. However, no significant changes in BMI were observed during follow-up. Overall these results show that a close follow-up with intensive diabetic management alone could not be the only cause of the positive, progressive, and consistent outcomes obtained in this trial over 1-year follow-up.

No complications were observed during or after bone marrow harvest, and autologous stem cell infusion and normal pancreatic enzymes results were obtained after the procedure in all patients. During HBO sessions aural barotrauma occurred in three patients, resolving in all of them by compensation maneuvers. No other reversible complications secondary to oxygen toxicity were observed.

In conclusion, in this pilot study involving 25 subjects with T2DM, significant benefits were noted with improvements in glycemic control and C-peptide levels and reduction in insulin requirements. If these benefits persist, the long-term complications of diabetes may be avoided, decreasing the morbidity and mortality of the disease.

This phase I clinical trial of ASC in conjunction with HBO in patients with T2DM has resulted in encouraging preliminary results that requires confirmation in a controlled, randomized prospective trial.

REFERENCES

1. Abidia, A.; Laden, G.; Kuhan, G.; Johnson, B. F.; Wilkinson, A. R.; Renwick, P. M.; Masson, E. A.; McCollum, P. T. The role of hyperbaric oxygen therapy in ischemic diabetic lower extremity ulcers: A double-blind randomized controlled trial. Eur. J. Vasc. Endovasc. Surg. 25: 513–518; 2003.
2. Alvarez, S. S.; Jimínez, L. M.; Murillo, A. Z.; Gómez, I. G.; Ligero, J. M.; Gómez-Pineda, A.; Rollán-Landeras, E.; Cuevas, P.; Jara-Albarrán, A. A new approach for bone marrow-derived stem cells intrapancreatic autotransplantation in diabetic rats. Microsurgery 26(7):539–542; 2006.
3. Aronson, D. Hyperglycemia and the pathobiology of diabetic complications. Adv. Cardiol. 45:1–16; 2008.
4. Avogaro, A.; Fadini, G. P.; Gallo, A.; Pagnin, E.; de Kreutzenberg, S. Endothelial dysfunction in type 2 diabetes mellitus. Nutr. Metab. Cardiovasc. Dis. 16:39–45; 2006.
5. Baharvand, H.; Jafary, H.; Massumi, M.; Ashtiani, S. K. Generation of insulin-secreting cells from human embryonic stem cells. Dev. Growth Differ. 48(5):323–332; 2006.
6. Bonner-Weir, S.; Taneja, M.; Weir, G. C.; Tatarkiewicz, K.; Song, K. H.; Sharma, A.; O'Neil, J. J. In vitro cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. USA 97(14):7999–8004; 2000.
7. Burt, R. K.; Cohen, B.; Rose, J. Hematopoietic stem cell transplantation for multiple sclerosis. Arch. Neurol. 62(6): 860–864; 2005.
8. Burt, R. K.; Marmont, A.; Oyama, Y. Randomized controlled trials of autologous hematopoietic stem cell transplantation for autoimmune diseases: the evolution from myeloablative to lymphoablative transplant regimens. Arthritis Rheum. 54(12):3750–3760; 2006.
9. Caplan, A. I. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J. Cell. Physiol. 213(2):341–347; 2007.
10. Choi, Y.; Ta, M.; Atouf, F.; Lumelsky, N. Adult pancreas generates multipotent stem cells and pancreatic and nonpancreatic progeny. Stem Cells 22(6):1070–1084; 2004.
11. Domínguez-Bendala, J.; Ricordi, C. Stem cell therapies in reparative medicine. Cell Transplant. 12(4):329–334; 2003.
12. Donath, M. Y.; Ehses, J. A.; Maedler, K.; Schumann, D. M.; Ellingsgaard, H.; Eppler, E.; Reinecke, M. Mechanisms of β-cell death in type 2 diabetes. Diabetes 54:108–113; 2005.
13. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 26:5–20; 2003.
14. Fadini, G. P.; Sartore, S.; Schiavon, M.; Albiero, M.; Baesso, I.; Cabrelle, A.; Agostini, C.; Avogaro, A. Diabetes impairs progenitor cell mobilization after hindlimb ischemia-reperfusion injury in rats. Diabetologia 49(12): 3075–3084; 2006.
15. Fang, B.; Song, Y. P.; Liao, L. M.; Han, Q.; Zhao, R. C. Treatment of severe therapy-resistant acute graft-versus-host disease with human adipose tissue-derived mesenchymal stem cells. Bone Marrow Transplant. 38(5):389–390; 2006.
16. Fu, M. X.; Wells-Knecht, K. J.; Blackledge, J. A.; Lyons, T. J.; Thorpe, S. R.; Baynes, J. W. Glycation, glycoxidation, and cross-linking of collagen by glucose. Kinetics, mechanisms, and inhibition of late stages of the Maillard reaction. Diabetes 43(5):676–683; 1994.
17. Galiñanes, M.; Loubani, M.; Davies, J.; Chin, D.; Pasi, J.; Bell, P. R. Autotransplantation of unmanipulated bone marrow into scarred myocardium is safe and enhances cardiac function in humans. Cell Transplant. 13(1):7–13; 2004.
18. Gallagher, K. A.; Jun Liu, Z.; Xiao, M.; Chen, H.; Goldstein, L. J.; Buerk, D. G.; Nedeau, A.; Thom, S. R.; Velazquez, O. C. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alfa. J. Clin. Invest. 117(5):1249–1259; 2007.
19. Gangaram-Panday, S. T.; Faas, M. M.; de Vos, P. Towards stem-cell therapy in the endocrine pancreas. Trends Mol. Med. 13(4):164–173; 2007.
20. Garcia-Olmo, D.; Garcia-Arranz, M.; Herreros, D.; Pascual, I.; Peiro, C.; Rodriguez-Montes, J. A. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis. Colon Rectum 48(7):1416–1423; 2005.
21. Gill, A. L.; Bell, C. A. N. Hyperbaric oxygen in uses, mechanisms of action and outcomes. QJM 97:385–395; 2004.
22. Goldstein, L. J.; Gallagher, K. A.; Bauer, S. M.; Bauer, R. J.; Baireddy, V.; Liu, Z. J.; Buerk, D. G.; Thom, S. R.; Velazquez, O. C. Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. Stem Cells 24(10):2309–2318; 2006.
23. Hao, E.; Tyrberg, B.; Itkin-Ansari, P.; Lakey, J. R.; Geron, I.; Monosov, E. Z.; Barcova, M.; Mercola, M.; Levine, F. β-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat. Med. 12(3):310–316; 2006.
24. Hess, D.; Li, L.; Martin, M.; Sakano, S.; Hill, D.; Strutt, B.; Thyssen, S.; Gray, D. A.; Bhatia, M. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat. Biotechnol. 21(7):763–770; 2003.
25. Hori, Y.; Rulifson, I. C.; Tsai, B. C.; Heit, J. J.; Cahoy, J. D.; Kim, S. K. Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. Proc. Natl. Acad. Sci. USA 99(25):16105–16110; 2002.
26. Kim, D.; Gu, Y.; Ishii, M.; Fujimiya, M.; Qi, M.; Nakamura, N.; Yoshikawa, T.; Sumi, S.; Inoue, K. In vivo functioning and transplantable mature pancreatic islet-like cell clusters differentiated from embryonic stem cell. Pancreas 27(2):34–41; 2003.
27. King, G. L.; Brownlee, M. The cellular and molecular mechanisms of diabetic complications. Endocrinol. Metab. Clin. North Am. 25(2):255–270; 1996.
28. Kuki, S.; Imanishi, T.; Kobayashi, K.; Matsuo, Y.; Obana, M.; Akasaka, T. Hyperglycemia accelerated endothelial progenitor cell senescence via the activation of p38 mitogen-activated protein kinase. Circ. J. 70:1076–1081; 2006.
29. Larsen, C. M.; Faulenbach, M.; Vaag, A.; Volund, A.; Ehses, J. A.; Seifert, B.; Mandrup-Poulsen, T.; Donath, M. Y. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N. Engl. J. Med. 356(15):1517–1526; 2007.
30. Leahy, J. L. Pathogenesis of type 2 diabetes mellitus. Arch. Med. Res. 36(3):197–209; 2005.
31. Lee, R. H.; Seo, M. J.; Reger, R. L.; Spees, J. L.; Pulin, A. A.; Olson, S. D.; Prockop, D. J. Multipotent stromal

cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc. Natl. Acad. Sci. USA 103(46):17438–17443; 2006.
32. Lipsett, M. A.; Castellarin, M. L.; Rosenberg, L. Acinar plasticity: Development of a novel in vitro model to study human acinar-to-duct-to-islet differentiation. Pancreas 34(4): 452–457; 2007.
33. Lipsett, M.; Finegood, D. T. β-cell neogenesis during prolonged hyperglycemia in rats. Diabetes 51(6):1834–1841; 2002.
34. Maedler, K.; Spinas, G. A.; Lehmann, R.; Sergeev, P.; Weber, M.; Fontana, A.; Kaiser, N.; Donath, M. Y. Glucose induces β-cell apoptosis via upregulation of the Fas receptor in human islets. Diabetes 50(8):1683–1690; 2001.
35. Marchetti, P.; Del Prato, S.; Lupi, R.; Del Guerra, S. The pancreatic beta-cell in human type 2 diabetes. Nutr. Metab. Cardiovasc. Dis. 16 (1):3–6; 2006.
36. Martin, B. C.; Warram, J. H.; Krolewski, A. S.; Bergman, R. N.; Soeldner, J. S.; Kahn, C. R. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: Results of a 25-year follow-up study. Lancet 340(8825):925–929; 1992.
37. Mathews, V.; Hanson, P. T.; Ford, E.; Fujita, J.; Polonsky, K. S.; Graubert, T. A. Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. Diabetes 53:91–98; 2004.
38. Miyata, T.; Inagi, R.; Iida, Y.; Sato, M.; Yamada, N.; Oda, O.; Maeda, K.; Seo, H. Involvement of β2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis. Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor-alpha and interleukin-1. J. Clin. Invest. 93(2):521–528; 1994.
39. Poitout, V.; Robertson, R. P. Secondary β-cell failure in type 2 diabetes a convergence of glucotoxicity and lipotoxicity. Endocrinology 143(2):339–342; 2002.
40. Prentki, M.; Joly, E.; El-Assaad, W.; Roduit, R. Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in β-cell adaptation and failure in the etiology of diabetes. Diabetes 51(3):405–413; 2002.
41. Prentki, M.; Nolan, C. J. Islet β cell failure in type 2 diabetes. J. Clin. Invest. 116(7):1802–1812; 2006.
42. Ramiya, V. K.; Maraist, M.; Arfors, K. E.; Schatz, D. A.; Peck, A. B.; Cornelius, J. G. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. Nat. Med. 6(3):278–282; 2000.
43. Ramos, G. A.; Hare, J. M. Cardiac cell-based therapy: Cell types and mechanisms of actions. Cell Transplant. 16(9):951–961; 2007.
44. Ringden, O.; Uzunel, M.; Rasmusson, I. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation 81(10):1390–1397; 2006.
45. Robertson, R.; Zhou, H.; Zhang, T.; Harmon, J. S. Chronic oxidative stress as a mechanism for glucose toxicity of the beta cell in type 2 diabetes. Cell Biochem. Biophys. 48(2–3):139–146; 2007.
46. Robertson, R. P. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet β cells in diabetes. J. Biol. Chem. 279(41):42351–42354; 2004.
47. Schuleri, K. H.; Boyle, A. J.; Hare, J. M. Mesenchymal stem cells for cardiac regenerative therapy. Handb. Exp. Pharmacol. 180:195–218; 2007.
48. Segal, M. S.; Shah, R.; Afzal, A.; Perrault, C. M.; Chang, K.; Schuler, A.; Beem, E.; Shaw, L. C.; Li Calzi, S.; Harrison, J. K. Nitric oxide cytoskeletal-induced alterations reverse the endothelial progenitor cell migratory defect associated with diabetes. Diabetes 55:102–109; 2006.
49. Tepper, O. M.; Galiano, R. D.; Capla, J. M.; Kalka, C.; Gagne, P. J.; Jacobowitz, G. R.; Levine, J. P.; Gurtner, G. C. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 106:2781–2786; 2002.
50. Thom, S. R. Hyperbaric-oxygen therapy for acute carbon monoxide poisoning. N. Engl. J. Med. 347:1105–1106; 2002.
51. Thom, S. R.; Bhopale, V. M.; Velazquez, O. C.; Goldstein, L. J.; Thom, L. H.; Buerk, D. G. Stem cell mobilization by hyperbaric oxygen. Am. J. Physiol. Heart Circ. Physiol. 290(4):1378–1386; 2006.
52. Urbán, V.; Kiss, J.; Kovács, J.; Gócza, E.; Vas, V.; Monostori, E.; Uher, F. Mesenchymal stem cells cooperate with bone marrow cells in therapy at diabetes. Stem Cells 26:244–253; 2008.
53. Voltarelli, J. C.; Couri, C. E.; Stracieri, A. B. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 297(14):1568–1576; 2007.
54. Yang, L.; Li, S.; Hatch, H.; Ahrens, K.; Cornelius, J. G.; Petersen, B. E.; Peck, A. B. In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc. Natl. Acad. Sci. USA 99(12): 8078–8083; 2002.
55. Zhao, M.; Amiel, S. A.; Christie, M. R.; Muiesan, P.; Srinivasan, P.; Littlejohn, W.; Rela, M.; Arno, M.; Heaton, N.; Huang, G. C. Evidence for the presence of stem cell-like progenitor cells in human adult pancreas. J. Endocrinol. 195(3):407–414; 2007.