Published in final edited form as:

Pediatr Diabetes. 2010 November ; 11(7): 471–478. doi:10.1111/j.1399-5448.2009.00629.x.

Hyperbaric oxygen therapy improves early posttransplant islet function

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Abstract

Objective—This study investigates the therapeutic potential of hyperbaric oxygen therapy (HBO) in reducing hypoxia and improving engraftment of intraportal islet transplants by promoting angiogenesis.

Methods—Diabetic BALB/c mice were transplanted with 500 syngeneic islets intraportally and received six consecutive twice-daily HBO treatments (n = 9; 100% oxygen for 1 h at 2.5 atmospheres absolute) after transplantation. Dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) was used to assess new vessel formation at postoperative days (POD) 3, 7, and 14. Liver tissue was recovered at the same time points for correlative histology, including: hematoxylin and eosin, hypoxia-inducible factor (HIF1 α), Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL), vascular endothelial growth factor (VEGF), and von Willebrand Factor immunohistochemistry.

Results—HBO therapy significantly reduced HIF-1 α , TUNEL and VEGF expression in islets at POD 7. In the non-HBO transplants, liver enhancement on DCE MRI peaked at POD 7 consistent with less mature vasculature but this enhancement was suppressed at POD 7 in the HBO-treated group. The number of new peri-islet vessels at POD 7 was not significantly different between HBO and control groups.

Conclusion—These results are consistent with a hyperbaric oxygen-mediated decrease in hypoxia that appeared to enhance vessel maturation in the critical days following intraportal islet transplantation.

Keywords

Islet transplantation; HBO treatment; HIF-1 α ; MRI; VEGF

Introduction

Advances in islet transplantation depend upon prolongation of islet graft viability with a robust insulin response. Although intraportal islet transplantation has been the most successful route clinically (1–3), approximately 60% of islets are lost following intraportal infusion (4,5). Therefore, non-invasive monitoring of transplanted islets is critical for understanding islet health and for deciding adjuvant therapeutic strategies.

Recently, non-invasive examination of transplanted islets has been performed by a variety of methods including magnetic resonance imaging (MRI) (6,7). In addition to structural imaging, we have reported the effectiveness of dynamic contrast-enhanced MRI (DCE MRI) for assessment of angiogenesis after renal subcapsular transplantation of islets (8,9). We have also demonstrated that DCE MRI can reflect islet neovascularization after intraportal islet transplantation (10).

A major reason contributing to early graft loss is islet hypoxia (11,12). Transplanted islets are avascular at the time of transplantation and suffer from hypoxia until revascularization occurs at 1–2 wk after transplantation (8,13). In a previous study, we found that hypoxia-inducible factor-1 α (HIF-1 α) expression was increased in islets after renal subcapsular transplantation in association with β cell death and decreased insulin production (14), which was reversible when revascularization was established. Of note, these transient decrements in islet function could be mitigated by hyperbaric oxygen (HBO) treatment (14). HBO has been used therapeutically in various other clinical and experimental conditions, particularly those associated with increased hypoxia.

In the present study, we focus on intraportal islet transplantation and the effect of HBO treatment in reducing islet hypoxia and apoptosis, improving islet graft function and its effectiveness to enhance graft neovascularization.

Materials and methods

Animals

BALB/c female mice (22–27 g, Charles River Laboratories, Inc., Boston, MA, USA) were used as both donors and recipients. The mice were housed under pathogen-free conditions with a 12-h light cycle and free access to food and water. All animal care and treatment procedures were approved by the Institutional Animal Care Use Committee.

Induction of diabetes in recipient mice

Streptozotocin (STZ, 200 mg/kg/mouse, Sigma-Aldrich, St Louis, MO, USA) was injected intraperitoneally and blood glucose levels were measured by Accu-Chek Advantage glucose monitors (Roche, Indianapolis, IN, USA). Diabetes was diagnosed when the blood glucose level was greater than 13.8 mmol/L.

Islet isolation

Murine islets were isolated by collagenase (collagenase V, Sigma-Aldrich) digestion, separation by Ficoll (Sigma-Aldrich) discontinuous gradients and purification as previously described (15).

Islet function and viability

Islets for *in vitro* assays ($n = 10$ islets) were incubated for 2 h in RPMI 1640 medium containing 3.3 mmol/L glucose (preincubation). Low glucose (3.3 mmol/L) incubation for 30 min was then followed by high glucose (16.7 mmol/L) incubation for an additional 30 min. Supernatants

were collected after each incubation and insulin content was extracted from the islets with acid ethanol. Insulin release and insulin content were measured using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Linco, MO, USA) and the stimulation index (SI) was calculated by dividing the insulin released in high glucose by insulin released in low glucose. The percentage of viable islets were examined by staining 50 islets with SYTO[®] green (Invitrogen, Carlsbad, CA, USA) and ethidium bromide (Sigma-Aldrich), calculating the viability ratio (viable islet cells/(viable islet cells + dead islet cells) × 100) of each islet using Image J (v1.37, National Institutes of Health, Bethesda, MD, USA) and calculating the average (16).

Islet transplantation and hyperbaric oxygen (HBO) treatment

Cultured syngeneic islets 500 islet equivalents, (IEQ = 150 μ m) were transplanted via the portal vein into diabetic mice (17). IEQ's were calculated by microscopically measuring islet size where we collected 133–200 μ m sized islets but rejected islets greater than 267 μ m in size (18). We considered 500 IEQ a marginal islet mass for restoring normoglycemia based on our previous results that showed only 20% of mice achieving normoglycemia with 500 IEQ whereas 75% of the mice with 800 IEQ reached normoglycemia (19). Islet transplanted mice were divided into two groups: (i) no HBO treatment (controls: n = 10), and (ii) HBO treatment (n = 9). HBO treatment with 100% oxygen for 1 h at 2.5 atmospheres absolute (ATA) was initiated immediately posttransplantation and then twice daily for a total of six exposures (0, 12, 24, 36, 48, and 60 h). This HBO protocol was based on our previous study (14). Mice were placed in a hyperbaric chamber (Model 1300B, Sechrist Industries Inc., Anaheim, CA, USA) with a compression rate of 5 psi/min (psi: pound-force per square inch, 1 ATA = 14.223 psi) and oxygen flow at a rate of 22 L/min. Accumulation of CO₂ was prevented by calcium carbonate crystals. No complications because of HBO treatment were observed.

MRI acquisition

MRI was conducted at postoperative days (POD) 3, 7, and 14 according to our previously published methods (8). Briefly, mice were lightly anesthetized using isoflurane and a tail vein catheter was inserted for infusion of gadolinium DTPA contrast [Gadodiamide hydrate (Gd-DTPA-BMA), 0.1 mmol/kg body weight, Omniscan, Amersham Health, Princeton, NJ, USA]. Body temperature was maintained at $36 \pm 1^\circ\text{C}$ and respiration was monitored with an MR-compatible pressure transducer (Biopac MP150, Goleta, CA, USA). Imaging was performed on a Bruker Advance 11.7 T MRI (Bruker Biospin, Billerica, MA, USA) with a 3.0 cm internal diameter (ID) volume radiofrequency coil. The pre/postcontrast T1 was composed of a repetition time/echo time (TR/TE) of 832/10 ms, a 256^2 matrix, 3-cm field of view (FOV), and two averages for a total acquisition time of 14 min. The standard T1 sequences collected 20 coronal slices that were 0.75-mm thick and interleaved by 0.75 mm. The DCE sequence was a rapid image acquisition that acquired one image slice through the right and left liver lobes with the following parameters: TR/TE = 250/6.4 ms, 128^2 matrix, 3-cm FOV, one average for an acquisition time of 32 s/image and a total acquisition time of 32 min with 60 images collected. The contrast agent was injected 2 min after the start of the DCE MRI.

MRI analysis

DCE analysis utilized the temporal dynamics of contrast enhancement that were quantified using JIM software (Xinapse Systems, Thorpe Waterville, Northamptonshire, UK) as previously described (8). Briefly, regions of interest (20) (right liver, left liver, and muscle) were identified on the DCE images and kinetic analysis used a bi-directional two-compartment model (time and area of enhancement) based on the equations of Tofts (21). DCE MRI tissue Gd concentration curves were extracted and normalized for inter- and intra-animal comparisons and then curve fit. Areas under the curve (AUC) values were calculated by integrating using

the trapezoidal method using SigmaStat (Systat Software, Inc. Chicago, IL, USA). The AUC ratio at POD 7 and 14 vs. POD 3 [= (AUC at POD 7/AUC at POD 3) and (AUC at POD 14/AUC at POD 3)] were used to compare HBO and control groups.

Histological assessment

Liver specimens were acquired from four, five, and six mice at POD 3, 7, and 14, respectively. The fixed livers were embedded in paraffin and cut in 5- μ m thick sections. Specimens were stained for hematoxylin and eosin (H & E) for cellular changes, insulin immunohistochemistry to identify islets, HIF-1 α to determine hypoxia, vascular endothelial growth factor (VEGF) and von Willebrand Factor (vWF) for newly formed blood vessels. For vWF staining, specimens were treated with Proteinase K (Dako, Carpinteria, CA, USA). Primary antibodies were guinea pig anti-insulin antibody (Dako) diluted with 1:100, goat anti-HIF-1 α antibody (Santa Cruz Inc., Santa Cruz, CA, USA) diluted with 1:25, goat anti-VEGF antibody Santa Cruz Inc.) diluted with 1:50 and rabbit anti-vWF (Abcam, Cambridge, MA, USA) diluted with 1:500. After incubating with biotinylated secondary Immunoglobulin G antibody (Vector Laboratories, Burlingame, CA, USA and Santa Cruz Inc.), a peroxidase substrate solution containing 3,3'-diaminobenzidine (DAB, brown for HIF-1 α , Dako) or aminoethylcarbazol (AEC)+ (Red for insulin, Dako) was used for visualization and counterstained with hematoxylin (14). Apoptosis was detected by the Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) method using an *in situ* apoptosis detection kit (Promega, Madison, WI, USA). Sections were treated with proteinase K and incubated with TdT enzyme for 60 min at 37°C. After washing in PBS, the sections were further incubated with streptavidin horseradish peroxidase (HRP) solution and visualized with DAB (14).

Islet cells and surrounding liver tissue were assessed for necrosis (defined as destruction of cell structure with granulation, H & E), apoptosis (TUNEL), and hypoxia (HIF-1 α). Expression of VEGF in islet cells was also examined. Islet cell apoptosis was expressed as the percentage of TUNEL positive islets relative to total islets. The proportion of hypoxic islet cells was expressed as percentage of HIF-1 α positive cells in an islet. Expression of VEGF in islet cells was indicated as percentage of VEGF positive cells in an islet. Necrotic islet cells, hypoxia, apoptosis, and necrosis of the surrounding liver tissue were microscopically scored as zero (absent) or one (present). Newly formed vessels around islets were calculated by counting vWF-positive lumens (brown) adjacent to the islets. The number of islets at POD 3, 7, and 14 were 67 (from 2 mice), 51 (from 3 mice) and 33 (from 3 mice) in HBO group, respectively, and 50 (from 2 mice), 56 (from 2 mice), and 75 (from 3 mice) in the control group at POD 3, 7, and 14.

Blood glucose, percentage and day of normoglycemia, serum insulin levels and glucose tolerance test (GTT)

Blood glucose was measured at POD 0 (pretransplant) and POD 1, 2, 3, 5, 7, 10, and 14. Serum insulin and glucose tolerance test (GTT) were measured at POD 7 and 14. Days to and percentage of normoglycemia were calculated using a blood glucose level of ≤ 8.3 mmol/L as normoglycemia. Intraperitoneal GTT were performed at POD 7 and 14 by overnight fasting for 10 h and then injecting mice with a 2.0 g/kg body weight of glucose solution followed by tail vein blood samples at 0, 15, 30, 60, 90, and 120 min after injection. Glucose level change was expressed as area under the curve (AUC: mmol/L X min). Serum insulin and C-peptide levels were not measured after glucose stimulation.

Blood glucose levels were measured by Accu-Chek Advantage glucose monitors and serum insulin was measured with a rat/mouse insulin ELISA kit.

Statistical analysis

All the data were expressed as mean $\pm$ standard error of the mean. Analysis of variance was performed for statistical analysis. Significance was designated at $p < 0.05$.

Results

Islet function

In vitro function of isolated islets demonstrated excellent viability ($91.4 \pm 3.4\%$; $n = 5$ isolations) with a mean SI of 11.4 ± 0.3 and mean insulin content of 26.3 ± 0.4 ng/islet.

DCE MRI findings

A sample time sequence of images from a typical DCE scan illustrates the right (transplanted) and left (control) liver lobes (Fig. 1). Peak enhancement in the liver was seen 4 min after contrast injection, with a slow washout of contrast agent where all tissues showed some residual enhancement at the end of the 30-min scan. The time to peak enhancement was significantly earlier in the HBO group, especially at POD 14 (HBO vs. Control: 4.7 ± 0.2 vs. 5.2 ± 0.2 min at POD 3 ($p = 0.07$), 4.8 ± 0.3 vs. 5.4 ± 0.7 min at POD 7 ($p = 0.22$), 4.5 ± 0.1 vs. 5.5 ± 0.2 min at POD 14 ($p = 0.002$)). Normalized temporal enhancement curves between the HBO and control groups at POD 3, 7, and 14 revealed no significant differences between the experimental groups at POD 3 and 14 (Fig. 2A, C). However at POD 7, the DCE in the HBO animals was reduced compared to the control group (Fig. 2B). The DCE ratio at POD 7 compared to POD 3 was significantly ($p = 0.03$) decreased in the HBO group (Fig. 2D) consistent with decreased leakage of the contrast agent at POD 7.

Evaluation of vessels

Some islets associated with vWF-positive staining were found to occlude the portal vein (Fig. 3A) but very few newly formed vessels (vWF positive) around islets were observed at POD 3. The number of vWF stained cells adjacent to islets increased significantly at POD 7 and 14 (Fig. 3B, C). The average number of new vessels seen associated with islets were 0.5 ± 0.1 in the HBO and 0.2 ± 0.1 in the control group at POD 3 (Fig. 3D) which was significantly increased in both groups at POD 7 and 14 ($p < 0.0001$, Fig. 3B–D). There were no significant differences in the number of newly formed vessels between the HBO and control groups at any time point.

Histological findings on islets and liver tissue

Transplanted islets and surrounding liver tissue showed signs of severe hypoxia (HIF-1 α positive), apoptosis (TUNEL positive) and necrosis at POD 3 that was significantly decreased by POD 7 in both groups (Fig. 4). POD 14 showed a return to normoxia in islets and liver tissue with no apoptosis or necrosis (Fig. 4). VEGF was also expressed prominently at POD 3 but was reduced at POD 7 and 14 (Fig. 4).

At POD 3, the percentage of islet cells that stained positive for HIF-1 α was $42.6 \pm 6.1\%$ in HBO ($n = 67$ islets from two mice) and $53.2 \pm 7.1\%$ in control groups ($n = 50$ islets from two mice). There was a significant decrement in islet hypoxia after HBO compared to the non-HBO control group at POD 7 (0.4 ± 0.2 vs. $2.1 \pm 0.7\%$; $p = 0.02$, Figs 4 and 5A). There were also fewer apoptotic islet cells at POD 3 in the HBO group ($43.3 \pm 6.3\%$, $n = 67$ islets) compared to the control group ($51.5 \pm 7.0\%$, $n = 50$ islets). The number of apoptotic islet cells was significantly lower at POD 7 in the HBO group ($1.7 \pm 0.7\%$, $n = 51$ islets) compared to $5.7 \pm 1.5\%$ in the control group ($n = 56$ islets) ($p = 0.01$) (Figs 4 and 5B). VEGF was also prominently diminished in HBO group compared with the non-HBO group at POD 7 (0.01 ± 0.01 vs. $4.7 \pm 1.9\%$; $p = 0.01$, Fig. 5C). Necrotic islets were observed at POD 3 but were not found at POD 7 and 14 (data not shown) but there was no significant difference between groups.

Liver hypoxia assessed by HIF-1 α staining was transiently elevated at POD 3 and was zero at POD 7 and 14 in both groups ($p < 0.0001$). A similar pattern was observed for necrosis (H & E) where the HBO group had some TUNEL positive staining at POD 3 that disappeared at POD 7 and 14, with no significant difference between HBO and control groups (data not shown). The proportion of apoptotic liver tissue in both groups was over 60% at POD 3 and returned to zero by POD 7 ($p < 0.0001$).

In summary, over half of the total islets and the surrounding liver tissue were apoptotic and necrotic at POD 3 but both dramatically decreased by POD 7 in the HBO-treated animals compared to non-HBO controls. There was a significant improvement in HBO group at POD 7 in islet hypoxia and apoptosis and a related decrement in VEGF expression in the HBO group at POD 7.

Blood glucose, serum insulin, and GTT

The serum insulin and GTT data were acquired from all mice included in this study. Serum insulin levels were significantly higher (Fig. 6A; $p = 0.04$) and GTT was significantly lower in HBO-treated mice at POD 7 (Fig. 6B; $p = 0.04$) after HBO treatment compared to controls. However, there were no significant differences in serum insulin and GTT at POD 14 (data not shown), and no significant differences in blood glucose levels between the two groups. Normoglycemia was achieved in 44.4% (4/9 mice) of the HBO and 30% (3/10 mice) of the control group. Days to normoglycemia took an average of 5.3 ± 1.8 d in the HBO group and 6.3 ± 5.3 d in the control group ($p = 0.77$).

Discussion

HBO is a therapeutic option for clinical and experimental conditions associated with increased or acute hypoxia. The mechanisms underlying the therapeutic effectiveness of HBO appear to be related to a reduction in tissue hypoxia, decreased levels of cytokines (TNF- α or IL-1), reduction in the affinity of major histocompatibility complex (MHC) class I specific antibodies, and inhibition of apoptosis (22).

This study on the therapeutic effectiveness of HBO after islet transplants in diabetic mice found: (i) a significant decrease in contrast enhancement within the liver of the HBO group, (ii) a significant decrease in islet hypoxia and apoptosis after HBO therapy compared to controls, (iii) a significant decrease in VEGF expression at POD 7, (iv) that serum insulin production was twofold higher in HBO-treated mice at POD 7, and 5) improved glucose tolerance in HBO-treated mice at POD 7. A significant increase in neovascularization was seen in both HBO and non-HBO groups at POD 7 and 14, despite lack of significant difference between the two groups. In this study, HBO therapy decreased hypoxia and apoptosis and ultimately lead to improved islet function. There was no significant difference in histologically detected neovascularization between the HBO and control groups.

The study evaluated the therapeutic potential of HBO treatment after intraportal islet transplantation on neovascularization. We (8,9) have previously shown that DCE MRI can provide an indirect measure of neovascularization in a variety of animal models. DCE MRI showed peak enhancement at POD 7 in control animals, consistent with islet angiogenesis (10,23). However, this increased angiogenesis was not observed after HBO therapy at POD 7. Our histological data showed decreased HIF-1 α and VEGF staining in the HBO group and no differences between HBO and control groups in the number of peri-islet vWF-positive vessels. These findings would suggest that HBO therapy results in a less permeable peri-islet neovasculature at POD 7 with no net change in the number of new vessels. More importantly, earlier new vessel maturation would result in decreased hypoxia and improved functional outcomes after islet transplant in diabetic mice (see below).

One possible explanation is that a permeable neovasculature is a result of increased VEGF, in part because of upregulation of HIF-1 α , as seen in our control animals (24–26). It is well known that HIF-1 α upregulates VEGF under ischemic conditions and VEGF contributes to a leaky and destabilized vasculature (27,28). However, previous reports have shown that HBO reduces VEGF production in a mouse ischemic hind limb model (29). Thus a potential mechanism underlying our results is that HBO-related HIF-1 α reduction is followed by a strong reduction in VEGF expression. This cascade then leads to decreased permeability in newly formed vessels adjacent to transplanted islets which results in reduced enhancement of DCE at POD 7. In support of this proposed mechanism is a recent report which demonstrated that increased VEGF production delays vessel maturation (27). Thus, our data would suggest that decrements in hypoxia and subsequent decreases in VEGF accelerate the development of mature vessels, which then results in decreased contrast extravasation (DCE MRI).

HBO therapy led to a significant decrease in islet hypoxia and apoptosis at POD 7 that lead to a significant increase in serum insulin production and improved glucose tolerance. Thus, HBO effectively rescued a subset of transplanted islets from hypoxic injury and apoptosis and as a consequence improved their function. We noted that the effects of HBO therapy on intraportal islets in our current study did not appear to be as robust as those previously reported after renal subcapsular transplants (14). These differences could be related to the vascular (intraportal) route which is known to elicit an instant blood-mediated inflammatory reaction (IBMIR) (30). IBMIR is characterized by activation of platelets and complement when islets are exposed to fresh blood (30). Islet damage related to IBMIR can be detected within an hour (31), thus potentially leading to significant islet damage prior to or during the HBO procedure. Therefore, HBO effectiveness in protecting transplanted islets could be enhanced by the simultaneous treatment of IBMIR. Furthermore, recent reports suggest that embolization of the portal vein by the islets themselves could be a major cause of early islet graft loss (4,18). In our experience (32), we detected near total obstruction of the portal vein with 90% of the surrounding liver tissue exhibiting hypoxia/apoptosis at early posttransplant time points. Islet damage caused by embolization is presumably related to local liver tissue ischemia (4). This local ischemia is also likely reduced in HBO-treated animals providing a healthy environment for the transplanted islets to generate new vasculature to support their survival and function. To reduce the influence of embolization, smaller islets and a larger animal model could also be used thereby improving the efficacy of HBO therapy. Moreover, longer courses of HBO treatment (for example, 7–14 d) might be more effective in promoting islet function. HBO therapy prior to transplantation should also be considered as this may induce endothelial progenitor stem cell differentiation into vascular endothelial cells, further preventing ischemia and improving glycemic control. Actually, some studies revealed that HBO stimulates endothelial progenitor stem cells in animal models of ischemia (25,33).

In conclusion, our data support the potential effectiveness of HBO treatment following intraportal islet transplantation in maintaining a more receptive healthy liver environment and improving islet function. There is a possibility that intensive *more* prolonged HBO would further improve therapeutic outcome. Non-invasive imaging, such as DCE MRI can monitor neovascularization and may indicate enhanced vascular maturation after therapeutic interventions.

Acknowledgments

This work was supported by NIH/NIDDK Grant # 1R01-DK077541 (E. H.), a grant from the National Medical Test Bed (E. H.) and a research fellowship from the Uehara Memorial Foundation (N. S.). We are very appreciative of the microsurgical technical support of the Loma Linda University Microsurgery Laboratory and the kind help in specimen processing by John Hough.

References

1. International Islet Transplant Registry. Newsletter. 2001. p. 9
2. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005;54:2060–2069. [PubMed: 15983207]
3. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343:230–238. [PubMed: 10911004]
4. Yin D, Ding JW, Shen J, Ma L, Hara M, Chong AS. Liver ischemia contributes to early islet failure following intraportal transplantation: benefits of liver ischemic-preconditioning. *Am J Transplant* 2006;6:60–68. [PubMed: 16433757]
5. Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 2002;51:66–72. [PubMed: 11756324]
6. Evgenov NV, Medarova Z, Dai G, Bonner-Weir S, Moore A. In vivo imaging of islet transplantation. *Nat Med* 2006;12:144–148. [PubMed: 16380717]
7. Jirak D, Kriz J, Herynek V, et al. MRI of transplanted pancreatic islets. *Magn Reson Med* 2004;52:1228–1233. [PubMed: 15562474]
8. Hathout E, Sowers L, Wang R, et al. In vivo magnetic resonance imaging of vascularization in islet transplantation. *Transpl Int* 2007;20:1059–1065. [PubMed: 17850231]
9. Hathout E, Chan NK, Tan A, et al. In vivo imaging demonstrates a time-line for new vessel formation in islet transplantation. *Pediatr Transplant* 2009;13:892–897. [PubMed: 19017287]
10. Chan NK, Obenaus A, Tan A, et al. Monitoring neovascularization of intraportal islet grafts by dynamic contrast enhanced magnetic resonance imaging. *Islets* 2009;1. [Epub ahead of print].
11. Moritz W, Meier F, Stroka DM, et al. Apoptosis in hypoxic human pancreatic islets correlates with HIF-1alpha expression. *FASEB J* 2002;16:745–747. [PubMed: 11923216]
12. Lepore DA, Shinkel TA, Fisicaro N, et al. Enhanced expression of glutathione peroxidase protects islet beta cells from hypoxia-reoxygenation. *Xenotransplantation* 2004;11:53–59. [PubMed: 14962293]
13. Jones GL, Juszczak MT, Hughes SJ, Kooner P, Powis SH, Press M. Time course and quantification of pancreatic islet revascularization following intraportal transplantation. *Cell Transplant* 2007;16:505–516. [PubMed: 17708340]
14. Miao G, Ostrowski RP, Mace J, et al. Dynamic production of hypoxia-inducible factor-1alpha in early transplanted islets. *Am J Transplant* 2006;6:2636–2643. [PubMed: 17049056]
15. Gotoh M, Maki T, Kiyozumi T, Satomi S, Monaco AP. An improved method for isolation of mouse pancreatic islets. *Transplantation* 1985;40:437–438. [PubMed: 2996187]
16. Sakata N, Egawa S, Sumi S, Unno M. Optimization of glucose level to determine the stimulation index of isolated rat islets. *Pancreas* 2008;36:417–423. [PubMed: 18437089]
17. Yonekawa Y, Okitsu T, Wake K, et al. A new mouse model for intraportal islet transplantation with limited hepatic lobe as a graft site. *Transplantation* 2006;82:712–715. [PubMed: 16969298]
18. Sakata N, Hayes P, Tan A, et al. MRI assessment of ischemic liver after intraportal islet transplantation. *Transplantation* 2009;87:825–830. [PubMed: 19300184]
19. Sakata N, Tan A, Chan N, et al. Efficacy comparison between intraportal and subcapsular islet transplants in a murine diabetic model. *Transplant Proc* 2009;41:346–349. [PubMed: 19249553]
20. Forleo C, Resta N, Sorrentino S, et al. Association of beta-adrenergic receptor polymorphisms and progression to heart failure in patients with idiopathic dilated cardiomyopathy. *Am J Med* 2004;117:451–458. [PubMed: 15464701]
21. Tofts PS. Modeling tracer kinetics in dynamic Gd-DTPA MR imaging. *J Magn Reson Imaging* 1997;7:91–101. [PubMed: 9039598]
22. Muralidharan V, Christophi C. Hyperbaric oxygen therapy and liver transplantation. *HPB (Oxford)* 2007;9:174–182. [PubMed: 18333218]

23. Menger MD, Jaeger S, Walter P, Feifel G, Hammersen F, Messmer K. Angiogenesis and hemodynamics of microvasculature of transplanted islets of Langerhans. *Diabetes* 1989;38 (Suppl 1):199–201. [PubMed: 2463196]
24. Sepulveda P, Martinez-Leon J, Garcia-Verdugo JM. Neoangiogenesis with endothelial precursors for the treatment of ischemia. *Transplant Proc* 2007;39:2089–2094. [PubMed: 17889103]
25. Milovanova TN, Bhopale VM, Sorokina EM, et al. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo. *J Appl Physiol* 2009;106:711–28. [PubMed: 19023021]
26. Dai Y, Xu M, Wang Y, Pasha Z, Li T, Ashraf M. HIF-1alpha induced-VEGF overexpression in bone marrow stem cells protects cardiomyocytes against ischemia. *J Mol Cell Cardiol* 2007;42:1036–1044. [PubMed: 17498737]
27. Greenberg JI, Shields DJ, Barillas SG, et al. A role for VEGF as a negative regulator of pericyte function and vessel maturation. *Nature* 2008;456:809–813. [PubMed: 18997771]
28. Ozawa CR, Banfi A, Glazer NL, et al. Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. *J Clin Invest* 2004;113:516–527. [PubMed: 14966561]
29. Asano T, Kaneko E, Shinozaki S, et al. Hyperbaric oxygen induces basic fibroblast growth factor and hepatocyte growth factor expression, and enhances blood perfusion and muscle regeneration in mouse ischemic hind limbs. *Circ J* 2007;71:405–411. [PubMed: 17322643]
30. Bennet W, Groth CG, Larsson R, Nilsson B, Korsgren O. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. *Ups J Med Sci* 2000;105:125–133. [PubMed: 11095109]
31. Johansson H, Lukinius A, Moberg L, et al. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes* 2005;54:1755–1762. [PubMed: 15919797]
32. Sakata N, Obenaus A, Chan N, Mace J, Chinnock R, Hathout E. Factors affecting islet graft embolization in the liver of diabetic mice. *Islets* 2009;1:26–33. [PubMed: 20428330]
33. Goldstein LJ, Gallagher KA, Bauer SM, et al. Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. *Stem Cells* 2006;24:2309–2318. [PubMed: 16794267]

Fig. 1. Dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) enhancement after intraportal liver islet transplantation. Liver DCE MRI in an islet transplanted mouse at POD 7 after HBO therapy demonstrates enhancement after gadolinium (Gd) injection. Liver images prior (0 min) and after (1, 2, 4, 20, and 30 min) Gd injection demonstrated peak enhancement at 4 min. The increased concentration of contrast agent in the intravascular space can be visualized by enhancement of the portal vein (arrows).

Fig. 2. Quantification of dynamic contrast-enhanced magnetic resonance imaging. (A–C) Normalized DCE enhancement curves at POD 3 (A), 7 (B), and 14 (C). HBO inhibited the increase in contrast enhancement found in controls (no HBO) at POD 7. (D) The DCE ratio at POD 7 or 14 compared to POD 3 revealed a significant decrease in enhancement at POD 7 after HBO therapy. * $p < 0.05$.

Fig. 3.

Islets stained with von Willebrand Factor (vWF) for new vessel formation. At POD 3 islets (dotted line) were stained with vWF (indicated as arrow) but there were few newly formed vessels (A). At POD 3 vWF stained areas were considered as coagula. Vessel formation around islets (arrows) were more prominent at POD 7 (B) and 14 (C) (see expanded insets). Figures are from control mice. (D) Quantified vWF staining was significantly increased at POD 7 and 14 compared to POD 3 in both groups (* $p < 0.0001$ in HBO group and ** $p < 0.0001$ in control group, Dunnet test). Calibration bars = 100 μ m.

Fig. 4.

Histological findings at POD 3, 7, and 14. Insulin staining revealed the location of the islets in liver tissues. Necrosis (H & E), hypoxia (HIF-1 α) and apoptosis (TUNEL) of the islets and surrounding liver tissues were prominent at POD 3 but decreased after POD 7. Vascular endothelial growth factor (VEGF) was also prominent at POD 3 and decrement at POD7 and 14. Figures are from control mice. Arrows indicate apoptotic cells. Calibration bar = 100 μ m.

Fig. 5. Quantification of histological assessment at POD 7. (A) Hypoxic islet cells (HIF-1 α positive staining) were significantly decreased in animals undergoing HBO therapy. (* $p = 0.02$). (B) Apoptotic islet cells (TUNEL positive) were also significantly decreased after transplantation of islets in mice that underwent HBO therapy. (* $p = 0.01$). (C) Vascular endothelial growth factor (VEGF) positive islet cells were prominently decreased in HBO group at POD 7 (* $p = 0.01$). Decreased HIF-1 α may contribute to a decrease in VEGF expression.

Fig. 6. HBO improves functional outcomes after intraportal islet transplant. (A) A significant increase in serum insulin levels was observed in HBO-treated animals at POD 7. (* $p = 0.04$). (B) Glucose tolerance test (GTT) was also significantly improved after HBO therapy at POD 7. (* $p = 0.04$). The GTT insert illustrates a typical time course response.