Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Effect of short-term culture on functional and stress-related parameters in isolated human islets

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Keywords
culture, function, islet, stress, transplantation.

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Received: 8 August 2008
Accepted: 8 September 2008

doi:10.1111/j.1432-2277.2008.00769.x

Summary

The Edmonton protocol for islet transplantation utilizes fresh islet grafts but other protocols increasingly transplant short-term cultured grafts mainly for practical reasons. To improve our understanding of the impact of culture pretreatment of human islets, we assessed post-transplant function by nude mouse bioassay, islet ATP, activity of stress-activated MAP kinases, and expression of stress-related genes by focused cDNA array in freshly isolated and cultured islets. Mean blood glucose levels over 4 weeks after transplantation (2000 IE) of (i) freshly isolated, (ii) cultured and preculture counted (recovery rate; 78 ± 6%), and (iii) cultured and postculture counted islets in diabetic mice were 330 ± 40, 277 ± 65, and 256 ± 52 mg/dl (i versus ii, $P = 0.004$; i versus iii, $P = 0.002$). During culture, islet ATP/DNA and ATP/ADP increased; JNK and p38 MAPK activities decreased. Among 96 genes studied, mRNA expression of heat shock protein 70 genes decreased >twofold during culture in all four pairs; expression of cyclooxygenase-2, superoxide dismutase-2, interleukin-6 and cytochromes P450 1A1 genes increased. Our results show that culturing human islets before transplantation is not disadvantageous in regard of functional recovery from changes induced by nonphysiologic stimuli during islet isolation. The increase in expression of several stress-related genes during culture also shows that improving culture conditions may further enhance post-transplant islet function.

Introduction

The success rate of islet transplants for selected patients with type 1 diabetes increased markedly by transplanting a large islet mass prepared from two to four donor pancreases using the Edmonton protocol [1,2]. However, human islet transplants from a single donor have not been widely successful despite improvements in islet isolation techniques. Many islets are lost prior to stable engraftment during the early stages of transplantation increasing the mass of islets needed to achieve euglycemia [3,4]. Because of the shortage of donor pancreases, reliably obtaining large numbers of islets or enhancing islet viability and functional capacity after isolation would be of great benefit for human islet allotransplantation.

The Edmonton protocol for clinical islet transplantation utilizes freshly prepared islets immediately after harvesting to minimize the risk of islet injury during culture [2]. In culture, a reduction of the islet mass has been consistently observed [5]. However, short-term culture of islets before transplantation is often employed to allow sufficient time for functional assessment of islets and recipient preparation [6–8]. Cultured islets have been shown to have immunologic advantages compared with freshly isolated islets [9–11]. Ultrastructural integrity of human and monkey islets was shown to be maintained during culture for 1 week [12,13]. With respect to postgraft function, it has been shown that rat islets cultured for 1 week were not less effective than the fresh islets when assessed by transplantation into isogeneic

diabetic rats [14]. On the other hand, two recent studies with mouse islet syngeneic transplantation showed superior function of fresh islet grafts to cultured (for 3 or 4–7 days) islet grafts [15,16]. So far, no study has directly compared the postgraft function of fresh and cultured human islets.

Human islets are exposed to a variety of cellular stresses induced by nonphysiologic stimuli during organ preservation and islet isolation resulting in ischemic, mechanical, osmotic, and oxidative stress for islets [17]. Variables such as donor characteristics, organ preservation conditions and cold ischemia time may contribute to the susceptibility of islets to the injury triggered during isolation. The islet grafts are further exposed to a local nonspecific inflammatory and hypoxic microenvironment in the very early stage post-transplant [4,18–20]. Notably, damage associated with nonspecific inflammation occurs not only after transplantation but also during the separation of the islets from the surrounding tissue matrix [17,21]. Thus, optimized pretransplant culture of human islets might help them to recover from stress-related changes and reduce early post-transplant loss.

In this study, we studied the effect of short-term culture on functional and stress-related parameters in human islets isolated from deceased donors. In freshly isolated and cultured islets, we assessed post-transplant function using nude mouse transplant bioassay, islet ATP/ADP content, stress-activated MAP kinase activity, and stress-related gene expression.

Materials and methods

Islet isolation

Human pancreases were recovered from brain-dead donors after *in situ* vascular perfusion with cold University of Wisconsin (UW) solution as part of multiorgan procurement. Informed consent had been obtained from the donor's relatives. Pancreases were preserved in cold UW solution before islet processing. On arrival at our laboratory, the pancreas was perfused with a Liberase enzyme (Roche-Boehringer-Mannheim, Indianapolis, IN, USA) through the cannulated pancreatic duct [22]. The distended pancreas was dissociated mechanically using the automated method of Ricordi *et al.* [23] and islets were purified on continuous gradients of iodixanol or Ficoll using a Cobe 2991 cell separator. Islet preparations of high purity (>90%) were used for this study. Islets were cultured in supplemented CMRL 1066 medium (Mediatech, Herndon, VA, USA) with 10% fetal bovine serum at 37 °C for 12–24 h and at 22 °C [24] for an additional 24–48 h before assessment.

Nude mouse transplant bioassay

Male athymic nude mice (National Cancer Institute, Hartford, CT, USA) were used in compliance with the guidelines from the Institutional Animal Care Committee at the University of Minnesota. Diabetes was induced by intravenous injection of 240 mg/kg streptozotocin (Sigma, St Louis, MO, USA). Mice were considered diabetic when their blood glucose levels were ≥400 mg/dl on two consecutive days. Immediately after isolation, 2000 fresh IE were transplanted as pellets under the left kidney capsule of diabetic nude mice. After 48–72 h of culture, both preculture-counted and postculture-counted 2000 IE were also transplanted to diabetic nude mice. After transplantation, random blood glucose levels were measured every day for the first week, then 3 days per week. Reversal of hyperglycemia was defined by two consecutive blood glucose levels <200 mg/dl. At 28 days post-transplant, left nephrectomies were performed in cured mice to confirm the return of hyperglycemia.

Islet ATP content and ATP/ADP ratio

The preparation of islet samples and high-performance liquid chromatography (HPLC) analysis for islet ATP and ADP content were performed according to the method of Micheli *et al.* [25]. Nucleotides were immediately extracted from aliquots of 1000 freshly isolated and cultured IE by adding 200 μl of ice-cold 10% trichloroacetic acid. After neutralization with 0.5 M tri-n-octylamine (Sigma) in 1,1,2-trichlorotrifluoroethane (freon) (Sigma), the phases were separated by centrifugation. The top neutral aqueous layer was collected and stored at −80 °C until HPLC analysis and the pellet was stored for DNA determination. ATP content of islets was normalized by DNA content of samples and expressed as pmol ATP/μg DNA. ATP/ADP ratio (ATP/ADP) was calculated.

MAP kinase activity

Aliquots of 5000 freshly isolated and 5000 cultured IE were washed with PBS twice and stored at −80 °C. For ELISA, these islets were lysed with cell extraction buffer (BioSource International, Camarillo, CA, USA) on ice for 30 min. with intermittent vortexing. C-Jun NH2-terminal kinase (JNK) phosphorylated at threonine 183 and tyrosine 185 and p38 MAPK phosphorylated at threonine 180 and tyrosine 182 were measured using ELISA kits purchased from BioSource International. Total protein content of islet lysates was measured by bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). MAP kinase activity of islets was expressed as U of phosphorylated kinase/mg of total protein.

Gene expression analysis

Isolation of RNA

Aliquots of 5000 freshly isolated and 5000 cultured IE were washed with phosphate-buffered saline twice and stored in RNA Later solution (Ambion, Austin, TX, USA) at −80 °C. Total RNA was isolated from these islets using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). The A_{260}/A_{280} ratio of RNA samples was >1.9. The quality of RNA samples was further confirmed by observing the intensity of the 28S/18S rRNA bands on denatured 1% agarose gels.

cDNA Array Assay

To analyze stress-related gene expression, we used a commercial focused cDNA array corresponding to 96 stress- and toxicity-related human genes (Human Stress and Toxicity GEArray Q series; SuperArray, Bethesda, MD, USA). Briefly, 2.5–5.0 μg of total RNA was reverse-transcribed into biotin-16-deoxy-UTP-labeled single-strand cDNA by AmpoLabeling linear polymerase reaction (SuperArray). After prehybridization, array membranes were hybridized with biotin-labeled cDNA at 60 °C overnight. The hybridized probes were detected by chemiluminescent method. Images were obtained using a CCD camera (Xenogen, Alameda, CA) and analyzed with ScanAlyze and GEArray Analyzer software (SuperArray). Signal intensity of each gene was corrected for background and normalized to that of housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and then used to estimate the relative abundance of particular transcripts. A more than twofold change in signal intensity was considered significant.

RT-PCR

To verify changes in the expression of genes observed by array analysis, 2.0–4.0 μg of total RNA from each sample were subjected to reverse transcription followed by the amplification using SingleGene PCR kit (SuperArray). PCR amplifications consisted of 22–30 cycles of denaturing (94 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 45 s). The PCR reaction products were separated on 1% agarose gels containing ethidium bromide. Images were captured using BioRad Gel Doc 1000 and analyzed with BioRad Molecular Analyst Software (Bio-Rad Laboratories, Hercules, CA, USA). The density for each gene product was normalized by the density for GAPDH gene. Changes in the gene expression in islets during culture were expressed as fold increases above the value of fresh islets.

Quantitative RT-PCR

Using the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) in combination with TaqMan Universal PCR Master Mix protocol (Applied Biosystems), relative quantitative RT-PCR analysis of RNA was carried out in accordance with manufacturer's recommendations. Specific primers and probes for cyclooxygenase-2 (Cox-2) and macrophage chemoattractant protein-1 (MCP-1) are proprietary to Lark Technologies, Inc. (Houston, TX, USA) and those for 18S rRNA are proprietary to Applied Biosystems. After normalization with 18S rRNA, a comparative threshold cycle (C_T) method was used for relative quantification of gene expression. Changes in the target gene expression in islets during culture were expressed as fold increases above the value of fresh islets.

Statistics

All statistical analyses were performed using MedCalc program (Mariakerke, Belgium). All data are expressed as mean values ± SE. The differences between fresh and cultured islets were considered significant if the *P*-value was <0.05 using a two-tailed paired Student's *t*-test.

Results

Nude mouse transplant bioassay

Transplantation of 2000 freshly isolated human IE reversed the hyperglycemia in only one out of seven diabetic nude mice, while four out of seven recipients that received either postculture-counted or preculture-counted cultured 2000 IE were cured. Mean blood glucose level over 4 weeks after transplantation of postculture-counted cultured 2000 IE in diabetic nude mice was lower than that of freshly isolated 2000 IE in all seven islet preparations (256 ± 52 vs. 330 ± 40 mg/dl, $P = 0.002$, Fig. 1a). Mean blood glucose level over 4 weeks after transplantation of preculture-counted cultured 2000 IE (277 ± 65 mg/dl) was also lower than that of freshly isolated 2000 IE in all seven islet preparations ($P = 0.004$, Fig. 1b) demonstrating superior function of cultured islets even after considering islet loss during culture (IE recovery: 78 ± 6%; range, 58–100%). Blood glucose level at 4 weeks after transplantation of either postculture-counted or preculture-counted cultured 2000 IE in diabetic nude mice was also lower than that of freshly isolated 2000 IE in all seven islet preparations (170 ± 47 vs. 469 ± 63 mg/dl, $P < 0.001$; 226 ± 76 vs. 469 ± 63 mg/dl, $P = 0.003$).

Islet ATP content and ATP/ADP ratio

During culture for 48–72 h, islet ATP content increased in 23 out of 28 islet preparations (85 ± 10 vs. 115 ± 8 pmol/μg, $P = 0.004$, Fig. 2a) and ATP/ADP ratio

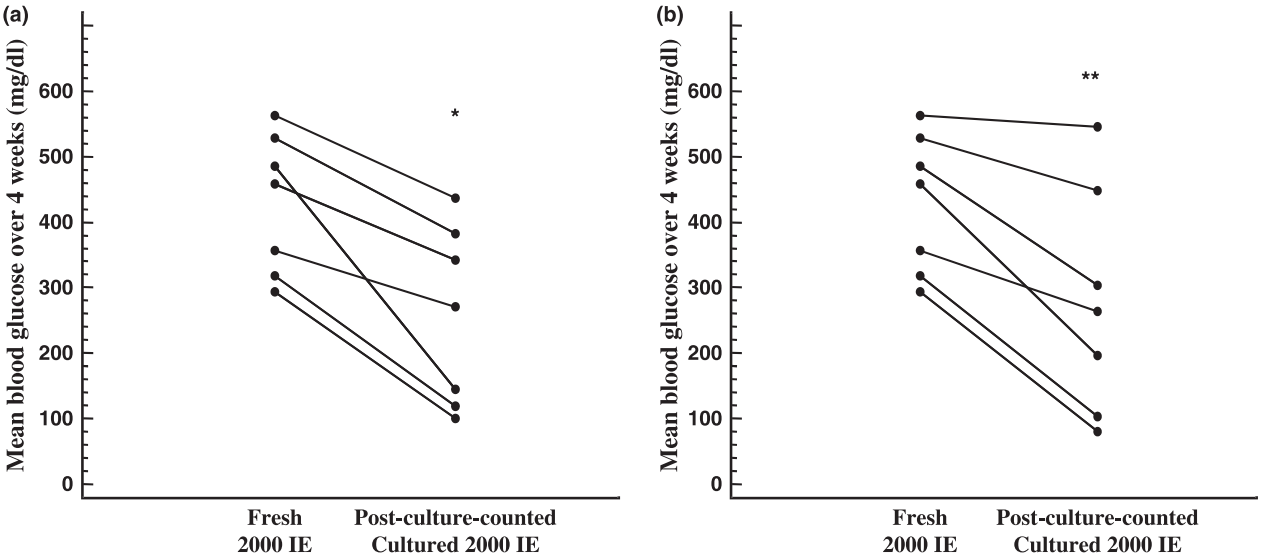

Figure 1 Comparison of mean blood glucose levels over 4 weeks after transplantation of human islets from seven islet preparations in diabetic nude mice. (a) Two thousand freshly isolated IE grafts versus postculture-counted cultured 2000 IE grafts. (b) Two thousand freshly isolated IE grafts versus preculture-counted cultured 2000 IE grafts (recovery rate; 58–100%). *P = 0.002 and **P = 0.004 by paired t-test.

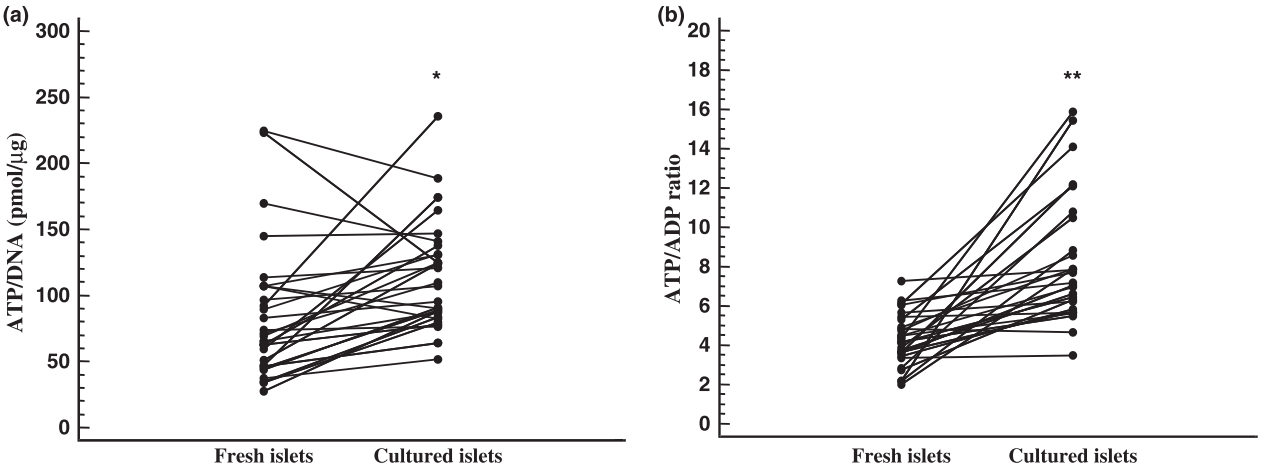

Figure 2 Change in islet ATP content (a) and ATP/ADT ratio (b) during culture in 28 islet preparations. *P = 0.004 and **P < 0.001 by paired t-test.

also increased in 26 out of 28 islet preparations (4.3 ± 0.3 vs. 8.0 ± 0.7, $P < 0.001$, Fig. 2b).

MAP kinase activity

During culture for 48–72 h, phosphorylated JNK/islet total protein decreased in eight out of 10 islet preparations (3.5 ± 0.6 vs. 1.2 ± 0.3 U/mg, $P = 0.008$, Fig. 3a) and phosphorylated p38 MAPK/islet total protein decreased in 12 out of 13 islet preparations (102.3 ± 8.3 vs. 52.0 ± 6.7 U/mg, $P < 0.001$, Fig. 3b).

Gene expression analysis

To analyze stress-related gene expression, mRNA expression in freshly isolated and cultured islets was compared by focused cDNA array in four different islet preparations. Representative membranes from cDNA array are shown in Fig. 4a and b. Among 96 genes studied, mRNA expression of heat shock protein 70 (HSP70) 1A, 1B and 1L genes decreased during culture more than twofold in all four preparations, while the expression of Cox-2, interleukin-6 (IL-6) and cytochromes P450 1A1

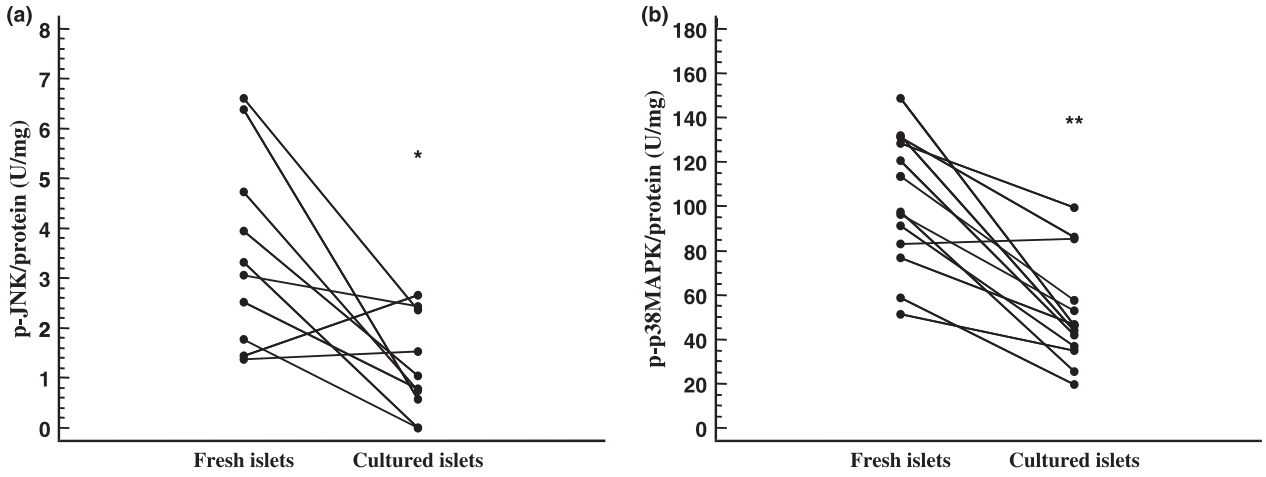

Figure 3 (a) Change in phosphorylated JNK/islet protein during culture in 10 islet preparations. (b) Changes in phosphorylated p38 MAPK/islet protein during culture in 13 islet preparations. $*P = 0.008$ and $**P < 0.001$ by paired *t*-test.

(CYP1A1) genes increased during culture more than two-fold in all four preparations (Fig. 4c). The expression of manganese superoxide dismutase (SOD2) gene increased more than 1.3-fold during culture in all four preparations in spite of high expression in freshly isolated islets (Fig. 4c). The expression of housekeeping genes (GAPDH, β-actin, cyclophilin A and ribosomal protein L13A) remained unchanged.

To confirm the cDNA array results, we performed RT-PCR for HSP70 1B, Cox-2, SOD2 and IL-6 genes in the same four and one additional islet preparations. Representative gels from RT-PCR analysis are shown in Fig. 5a and b. The expression changes of these genes during culture observed in cDNA array were confirmed in all five islet preparations (Fig. 5c).

To further validate the change observed in Cox-2 gene expression and to study the effect of culture on the gene expression of MCP-1, which has been reported to increase during islet culture at the protein level and to be predictive of transplantation outcome [26], mRNA expression in freshly isolated and cultured islets was compared by quantitative RT-PCR for Cox-2 and MCP-1 genes in the same four and an additional three islet preparations. Cox-2 gene expression in islets increased 11.9 ± 2.7-fold during culture (Fig. 6). MCP-1 gene expression was also found to increase 22.2 ± 8.8-fold during culture.

Discussion

Because of the notoriously wide variations that are observed in many functional and stress-related parameters between islet preparations from different donors, each donor served as its own control in this study. In addition, only islet preparations of high purity (>90%) were used and the purity of islets did not change significantly during this short-term (48–72 h) culture.

One of the concerns on culturing islets has been the loss of function and mass of islets during culture [5,27–29]. Our results with nude mouse transplant bioassay show a rather positive impact of short-term culture on post-transplant function of human islets. The finding that the function of preculture-counted 2000 cultured IE grafts (i.e. 1160–2000 cultured IE grafts) was better than that of 2000 freshly isolated IE grafts demonstrates the superior function of cultured islets even after considering islet loss during culture. This suggests that the *in vitro* culture condition used in this study provides a better microenvironment to islets immediately after isolation than *in vivo* avascular graft site with respect to post-transplant functional survival. While we did not directly measure post-transplant islet mass at the graft site, Biarnes *et al.* [3] reported that approximately 60% of transplanted tissue of fresh mouse islets was lost 3 days after syngeneic transplantation and that both apoptosis and necrosis contributed to β-cell death during the early stage of transplantation. Thus, short-term pretransplant culture may reduce cell loss under hypoxic condition prior to stable engraftment. On the other hand, two recent studies with mouse islet syngeneic transplantation showed superior function of fresh islet grafts to cultured (for 3 or 4–7 days) islet grafts [15,16]. This different finding from ours might be attributable to differences in species of islets, isolation procedure, culture protocol/duration, and graft size. In comparison with mouse islets, human islet cells encounter immense stresses caused by brain death, cold storage and long exposure to ischemia, enzymatic digestion and hyperosmolar gradient solutions during organ preservation and islet processing.

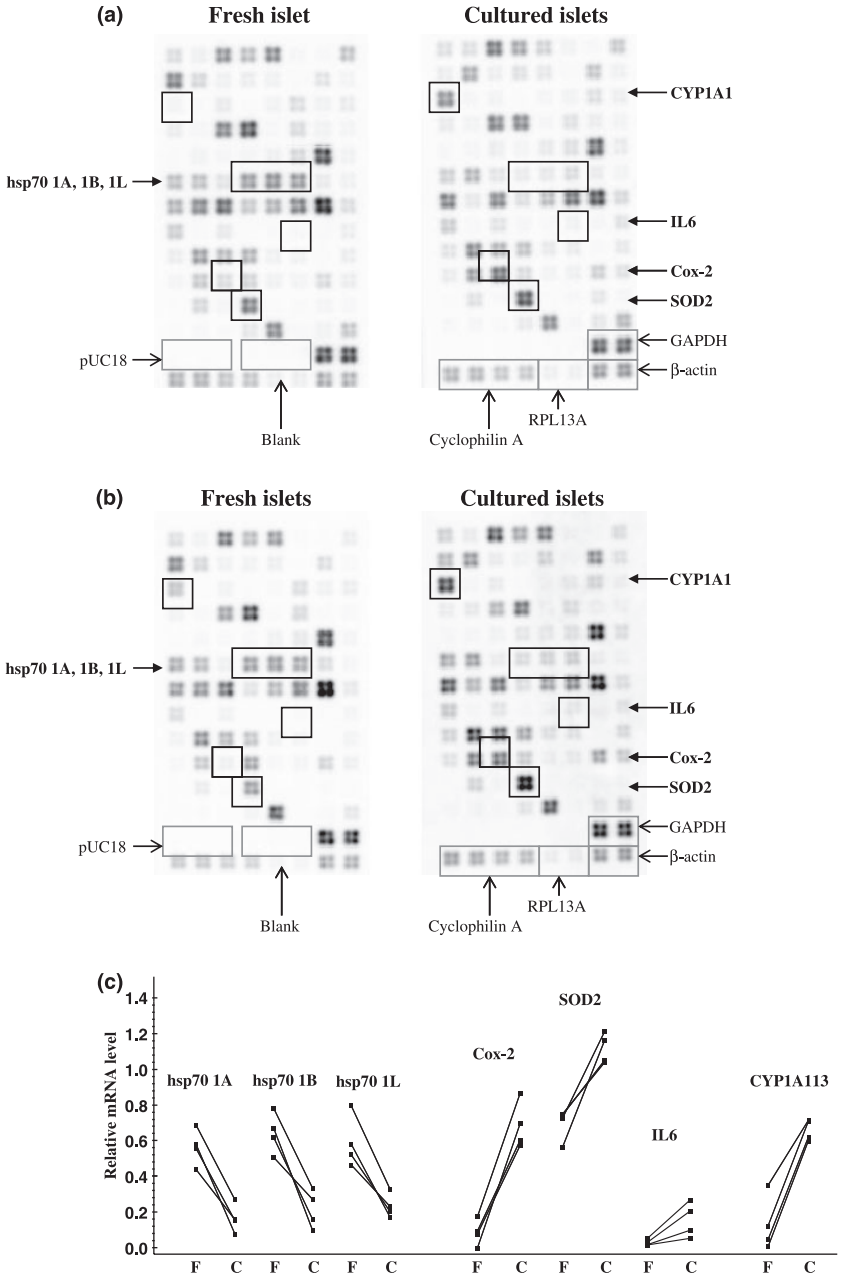

Figure 4 Focused cDNA array analysis for 96 cell stress- and toxicity- related genes. (a and b) cDNAs that were arrayed onto nylon membranes were hybridized with labeled cDNA probes synthesized from RNAs isolated from fresh islets and cultured islets. The spots that are boxed with solid lines represent genes that are decreased or increased during culture greater than twofold in all four islet preparations. The spots that are boxed with dashed lines represent blank, negative control pUC18 and positive controls GAPDH, ß-actin, cyclophilin A and ribosomal protein L13A. The list of 96 genes arrayed is available at the manufacturer's web site (http://www.sabiosciences.com). The two membrane pairs shown in (a) and (b) are representative of four separate experiments using four different islet preparations. (c) The change in the relative abundance of gene transcripts of islets during culture in cDNA array analysis. Signal intensity of each gene was corrected for background and normalized to that of GAPDH. F, freshly isolated islets; C, cultured islets.

Mitochondrial ATP production is required to recover from the stresses and maintain cell homeostasis and function; its content can be indicative of islet viability. This possibility is supported by previous reports showing that graft function and survival correlate significantly with ATP content of transplanted tissue, such as heart, liver, kidney, pancreas and islets in both experimental and clinical transplantations [30–33]. In our results, ATP content of cultured islets was higher than that of freshly isolated islets indicating some recovery of islets in metabolic function and/or functional viability during culture. This observation is in accordance with a previous study by Brandhorst *et al.* [34] which showed that human islet ATP content measured by luminometric method was higher after 5 days of culture than immediately after isolation. Pancreatic β-cells probably use ATP not only as a source of energy as other cells but also as an intracellular second messenger during stimulus-secretion coupling for insulin secretion. There is a positive correlation between the increase of ATP or ATP/ADP ratio and insulin secretion in response to glucose [35]. In our results, not only ATP content but also ATP/ADP ratio of isolated islets

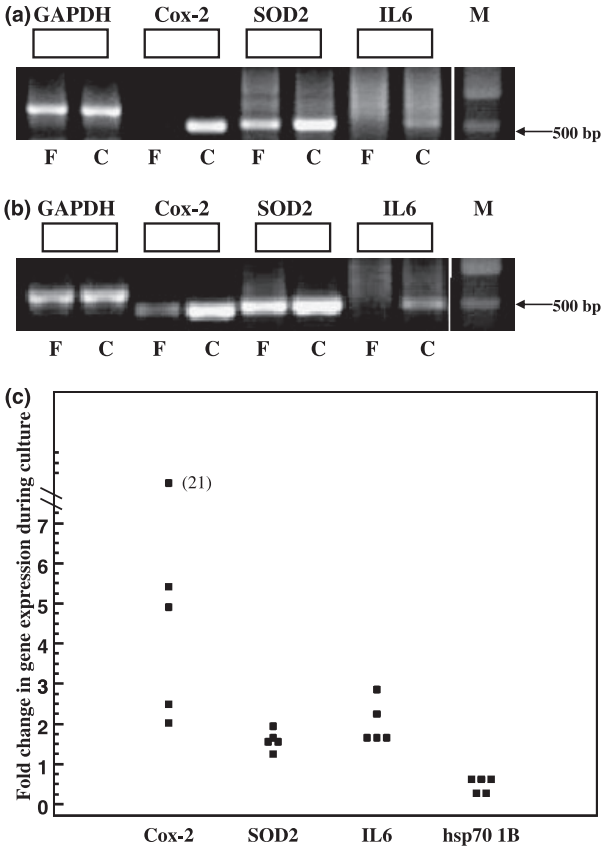

Figure 5 RT-PCR of Cox-2 (492 bp), SOD2 (507 bp) and IL-6 (504 bp) fragments after amplification of RNAs isolated from fresh islets (F) and cultured islets (C). (a, b) The two gels shown in (a) and (b) are representative of five separate experiments using five different islet preparations. M, molecular size markers;. F, freshly isolated isltes; C, cultured islets. (c) Change in Cox-2, SOD2, IL-6 and HSP701B mRNA expressions in islets during culture by RT-PCR analysis. Data are shown as fold change in gene expression during culture in five islet preparations.

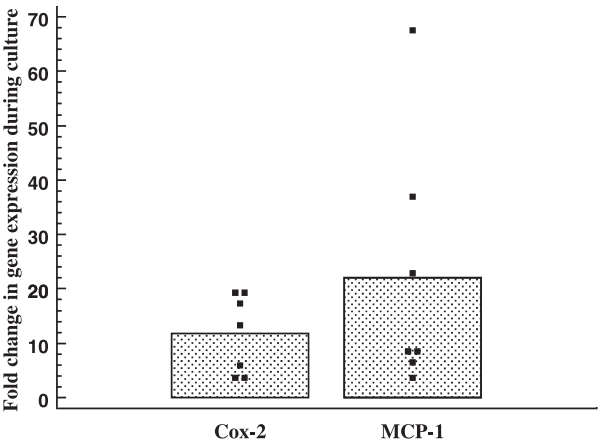

Figure 6 Quantitative RT-PCR analysis of change in Cox-2 and MCP-1 mRNA expression by islets during culture. Data are shown as fold changes in gene expression of cultured islets relative to that of freshly isolated islets in seven islet preparations. The columns denote mean values.

increased in most preparations during culture supporting the premise that functional viability of islets could be recovered during culture thus enhancing post-transplant function of islets.

A variety of extracellular stimuli present during the process of organ preservation and islet isolation can elicit biochemical responses that either promote cell survival or lead to cell death. A major component of the cell response is the activation of protein kinases that phosphorylate numerous substrates, including transcriptional factors. Recently, apoptotic cell loss has been shown to occur in human islets after isolation and purification under standard culture conditions [36]. Signaling pathways for apoptosis involve the stress-activated protein kinases including JNK and p38 MAPK. Activation of these enzymes can be initiated by thermal, hypoxic, osmotic, oxidative and cytokine-mediated insults, all of which may affect islets during isolation process and culture. In our results, although the activity of JNK and p38 MAPK varied widely in freshly isolated human islets among preparations from different donors, it decreased in most preparations during culture. Paraskevas *et al.* [37] have reported a similar temporal change of JNK activity of canine islets during culture, but they observed that p38 MAPK activity increased progressively during culture. This difference in p38 MAPK activity change might be caused by differences in species of islets, isolation and culture protocol, and assay method.

The gene expression of HSP70, an acute stress-induced protein, in islets also showed the same temporal change as the activity of stress-related protein kinases during culture. HSPs are a set of conserved proteins protecting vital cell components against injuries induced by elevated temperatures and a number of other types of cellular stresses, including physical, metabolic, or oxidative stress, and cytotoxic cytokines [38]. Among 18 heating stress-related genes included in our focused cDNA array, mRNA expression of only HSP70 1A, 1B, and 1L genes showed a consistent pattern of change during culture. It has been reported that HSP70 has a special protective potential among HSPs playing a crucial role in the cellular defense against radical-induced or oxidative injury [39,40]. Thus, our finding suggests that oxidative stress to islets is involved during isolation process. This is supported by the previous report that addition of antioxidant SOD mimic during isolation allowed for the survival of a significantly higher islet cell mass [21].

Our cDNA array results show that the current short-term culture leads to increased expression of several stress- and toxicity-related genes despite our very strin-

gent criteria for the increase. The mRNA contribution from the small amount of nonislet cells, such as acinar cells, macrophages and endothelial cells, present in the islet preparations cannot be excluded. The increase in expression of SOD2, CYP1A1, IL6 and Cox-2 genes during culture implies the involvement of oxidative and metabolic stresses and inflammatory reactions. Although it is not clear whether these gene expressions were mostly triggered by stimuli during isolation process or during culture, it is likely that improving culture media or conditions may further enhance post-transplant islet function. For example, tissue factor and MCP-1 expression in human isolated islets could be modulated in culture by adding nicotinamide [41].

Because cultured islets were associated with better post-transplant function than freshly isolated islets in nude mouse transplant bioassay, at least some of the induced genes seem to have protective effects, which are important for the survival of isolated islets. The upregulation of SOD2 expression in cultured islets compared to freshly isolated islets may improve cellular defense by scavenging mitochondrial superoxide radical. mRNA levels of antioxidant enzymes have been shown to correlate with enzyme activities [42]. Overexpression of SOD2 in islet cells was protective against cytokine-induced damage and extended islet graft function [43,44]. CYP1A1, a xenobiotic-metabolizing enzyme, has been shown to be expressed in islet cells and inducible by chemical exposure [45,46]. The precise role of CYP1A proteins in endogenous metabolism has not been clearly defined, although they have been shown to metabolize arachidonic acid to metabolites with stimulating effect on insulin exocytosis [45]. It is supposed that the induction of CYP1A1 gene expression in cultured islets is beneficial by inactivating chemicals to which metabolically active β-cells have been exposed during isolation and culture.

Interleukin-6, a pleiotropic cytokine with differentiation and growth-promoting effects, has been shown to be expressed in mouse islets and inducible by interferon-γ and tumor necrosis facto-α [47]. Although the brain death has been shown to induce IL-6 expression in many tissues including pancreas [48], IL-6 expression in freshly isolated human islets in this study was negligible either by cDNA array or RT-PCR and increased during culture. The expression of IL-6 in isolated human islets at the second day of culture has been previously reported in cDNA microarray analysis [49]. The role of IL-6 induced in islets for transplantation is not clear. IL-6 is known to mediate upregulation of Reg (regeneration) protein from isolated human islets, which has the potential to act as an autoantigen [50]. On the contrary, IL-6 has been shown to protect β-cells from cytokine-induced apoptosis by inhibiting NO production [51].

In our results, Cox-2 gene expression in isolated human islets markedly increased during culture. This inducible form of enzyme is expressed during stressful processes, such as inflammation. Although previous study has shown that isolated islets and β-cell lines express Cox-2 continually and dominantly over constitutive Cox-1 [52], this temporal change in Cox-2 expression during culture has not been reported. Cox-2 expression in islets was shown to be further increased by IL-1β resulting in an increase in intra-islet PGE2 and a decrease in glucose-stimulated insulin secretion [52,53]. High Cox-2 expression was observed in rat islet xenografts in NOD mouse 8 h after transplantation and was related to early islet graft failure [54]. Thus, increased Cox-2 expression in islets at the time of transplantation may contribute to early graft damage by insulin secretory dysfunction and nonspecific inflammation.

The secretion of MCP-1, a main monocyte chemoattractant, has been shown to increase during culture of human islets and the secreted level *in vitro* before transplantation correlated with the outcome of clinical islet transplantation [26]. Its importance in islet allotransplantation is further supported by a recent report that the blockade of MCP-1 by monoclonal antibody has profound effects on mouse islet allograft survival [55]. Our quantitative RT-PCR analysis shows the increase of MCP-1 gene expression in human islets during culture. Considering the result of our nude mouse transplant bioassay, it seems that the expression of proinflammatory genes, such as Cox-2 and MCP-1, on islet cells might be already triggered during the islet isolation process rather than during culture. Further study on the effect of inhibiting Cox-2 or MCP-1 gene expression or activity during isolation and/or culture of islets on post-transplant function is needed.

In summary, our results show that culturing human islets before transplantation is not disadvantageous in regard of functional recovery from changes induced by nonphysiologic stimuli during pancreas preservation and islet isolation. The increase in expression of some toxicity- and stress-related genes during culture also shows that improving culture media or conditions may further enhance post-transplant islet function. Continued research on pretransplant human islet culture seems to be warranted and may result in clinically significant post-transplant functional survival.

Authorship

SHI: performed research, collected data and wrote the paper. IM: performed research and collected data. HJZ and JDA: performed research. BJH: designed research and wrote the paper.

Acknowledgements

This research was supported in part by grants from the Juvenile Diabetes Research Foundation to B.J.H. (JDRF #4-1999-841), the National Institutes of Health to B.J.H. (National Center for Research Resources (U42 RR016598-01), the National Institute for Diabetes, Digestive, and Kidney Diseases (R01 DK56963-01), and the National Institute for Allergy and Infectious Diseases (N01-AI95380, U01 AI65193)), and the Innovative Research Institute for Cell Therapy, Republic of Korea (A062260) to S.I.

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