Brain Death Significantly Reduces Isolated Pancreatic Islet Yields and Functionality In Vitro and In Vivo After Transplantation in Rats

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Although ∼1 million islets exist in the adult human pancreas, current pancreas preservation and islet isolation techniques recover <50%. Presently, cadaveric donors remain the sole source of pancreatic tissue for transplantation. Brain death is characterized by activation of proinflammatory cytokines and organ injury during preservation and reperfusion. In this study, we assessed the effects of brain death on islet isolation yields and functionality. Brain death was induced in male 250- to 350-g Lewis rats by inflation of a Fogarty catheter placed intracranially. The rats were mechanically ventilated for 2, 4, and 6 h before removal of the pancreas (n = 6). In controls, the catheter was not inflated (n = 6). Shortly after brain death induction, a significant increase in tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 mRNA was noted in the pancreas. Brain death donors presented lower insulin release after glucose stimulation assessed by in situ perfusion of the pancreas. Islet recovery was reduced in brain death donors compared with controls (at 6 h 602.3 ± 233.4 vs. 1,792.5 ± 325.4 islet equivalents, respectively; P < 0.05). Islet viability assessed in dissociated islet cells and in intact cultured islets was reduced in islets recovered from brain death donors, an effect associated with higher nuclear activities of NF-κB p50, c-Jun, and ATF-2. Islet functionality evaluated in vitro by static incubation and in vivo after intraportal transplantation in syngeneic streptozotocin-induced diabetic rats was significantly reduced in preparations obtained from brain death donors. In conclusion, brain death significantly reduced islet yields and functionality. These observations may lead to strategies to reduce the effects of brain death on pancreatic islets and improve the results in clinical transplantation. Diabetes 52: 2935–2942, 2003

Application of a new steroid-free immunosuppressive protocol has markedly improved outcomes in pancreatic islet transplantation (PIT). However, large numbers of islets are required to achieve insulin independence (1,2). Disparity between human islet supply and potential demands of millions of diabetic patients mandates that improved methods for achieving and maintaining functional islet mass be developed (3). Although ∼1 million islets exist in the adult human pancreas, current pancreas preservation and islet isolation techniques recover <50% of the islets (4,5). Therefore, improved methods to obtain higher islet yields will be instrumental in facilitating PIT as a cure for type 1 diabetes.

Organ transplantation outcomes are influenced by antigen-dependent and -independent events. Despite progressive improvements in immunosuppressive agents, the success rate of kidneys from cadaveric donors, over both the short and the long term, remains significantly inferior to those from living donors regardless of their genetic relationship to the recipient (6). The major difference between cadaveric and living donors is brain death. Acceptance of this well-defined clinical diagnosis enables the removal of appropriate functioning organs while the circulation is still sustained. These tissues/organs experience profound physiological derangements that may be activated by the central catastrophe, in addition to the potential injury secondary to the later effects of storage and reperfusion (7). Presently, cadaveric donors remain the sole source of pancreatic tissue for PIT. Several studies demonstrated that deterioration of organs follow brain death by multiple interrelated events, including the effect of massive acute cerebral injury, hypotension, and circulating factors. Brain death is characterized by extensive cortical necrosis that stimulates multiple cell types to release proinflammatory cytokines (PICs), such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and interferon-γ (7–12). The mRNA expression of these factors also has been demonstrated in peripheral tissues (11). PICs have a profound impact on pancreatic β-cell function...
and death during type 1 diabetes and PIT (13). To date, studies have not been conducted to determine the impact of brain death on islet isolation, culture, and transplantation. Furthermore, virtually all experimental studies in islet transplantation use young, healthy, living animals as donors. We report herein that PIC release associated with brain death significantly reduced islet yields, viability, and function in vitro and in vivo after transplantation.

**RESEARCH DESIGN AND METHODS**

**Animals.** Inbred 250- to 300-g male Lewis rats (Harlan Sprague-Dawley, Indianapolis, IN) were assessed by enzyme-linked immunosorbent assay (ELISA) following procedures previously described (15). Briefly, the whole pancreas was extracted and washed in ice-cold PBS, minced, passed through a 21-G needle, suspended in 5 ml of acid/ethanol (0.18 mol/l HCl in 70% ethanol), sonicated for 30 s, and extracted at −20°C. Tubes were centrifuged at 3,000 rpm for 10 min at 4°C, and the supernatant was stored at −20°C for future insulin determination. After neutralization, the insulin content in the extracts was measured by ELISA as described above. Insulin concentration was expressed as islet total protein (Bio-Rad Protein Assay kit I; Bio-Rad, Hercules, CA).

**Islet isolation, culture, and transplantation.** After 2, 4, and 6 h of mechanical ventilation with and without CNS injury, a midline abdominal incision was made; the bile duct was cannulated and injected with 5 ml of cold M199 medium containing 1.5 mg/ml rodent Liberase (Roche Diagnostics, Indianapolis, IN). Islets were isolated as described (16). An aliquot of islets was cultured as islet engraftment (IEQ) under a humidified atmosphere of 95% air and 5% CO2 using diaphenylthiocarbazone (Sigma) staining. One IEQ was the islet tissue mass equivalent to a spherical islet of 150 μm in diameter. Islets were handpicked individually under the microscope to ensure pure islet preparations. Islets were cultured in RPMI 1640 medium (11 mmol/l glucose), supplemented with 10% FCS (Gibco, Grand Island, NY), 100 units/ml penicillin, and 0.1 mg/ml streptomycin (Gibco), and 25 mmol/l HEPES (Gibco) at 37°C in a humidified atmosphere of 95% air and 5% CO2. Syngeneic rats (males, 280–300 g) were made diabetic by intraperitoneal injection of streptozotocin (STZ; 85 mg/kg body wt; Sigma) freshly dissolved in citrate buffer (pH 4.5). Blood glucose levels were obtained after tail snipping using a portable glucose meter (Roche Accu Check III). Diabetes was defined as nonfasting glucose levels >250 mg/dl for at least 3 consecutive days. Twenty-four hours after isolation, 3,000 IEQ were reperfused into the portal vein via 25-G needle connected to PE50 tubing as described (16).

**Islet viability.** Islet viability was assessed immediately after islet isolation in single islet cells after islet incubation at 37°C for 17 min with 0.25% EDTA-trypsin (Sigma) followed by syringe injection through progressively narrower gauge needles sized from 16 to 22 as previously described (17). Then, islets cells were exposed to acridine orange (100 μg/ml) and ethidium bromide (100 μg/ml) and subjected to two-color fluorescence microscopy. Viable cells were identified with green fluorescence; nonviable cells were identified with red fluorescence. Approximately 2.5–106 cells were counted per islet isolation. Islet cell viability in culture was determined by a colorimetric assay that detects the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (18).

**Terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick-end assay.** Six hours after brain death, pancreata were removed, fixed in Bouin’s solution, embedded in paraffin, sectioned (4 μm thick), and immunostained after deparaffinization and rehydration. Islet cell death was detected by enzymatic in situ detection kit (Roche Molecular Biochemicals) according to the manufacturer’s instructions. Staining was achieved using 3,3-diaminobenzidinetetrahydrochloride (Sigma). Following to terminal deoxynucleotidyltransferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL), sections were stained with polyclonal anti-insulin antibody (diluted 1:150; DAKO, Carpinteria, CA), developed with biotinylated secondary antibody (diluted 1:100; Vector Laboratories, Burlingame, CA). Staining was then revealed with streptavidin–biotinylated horseradish peroxidase (diluted 1:100; Sigma) followed by 3,3-diaminobenzidinetetrahydrochloride (Sigma). Following procedures previously described (15). The aorta and portal vein were cannulated with 22-G angiocatheters (Johnson & Johnson, Arlington, TX). Both renal arteries, the hepatic artery, and the suprarenal aorta were ligated. The pancreas was covered with a piece of sterile plastic to maintain adequate hydration and temperature. Oxygenated Krebs-Ringer bicarbonate buffer (KRB) containing 0.25% BSA and glucose (2 or 26 mmol/l), at 37°C and a constant flow of 2 ml/min, was used as perfusate. Before sample collection (portal vein cannula), the pancreas was perfused with KRB supplemented with 2 mmol/l glucose for a 15-min equilibration period. The pancreas was then perfused with KRB/26 mmol/l glucose for 20 min. Finally, 20 mmol/l arginine (Sigma) was perfused, in the continued presence of 26 mmol/l glucose, for an additional 20 min. Insulin concentrations were measured in effluent perfusate at 1 and 5 min and every fifth minute thereafter with an ELISA kit (Rat Insulin ELISA Kit; Crystal Chem, Chicago, IL). This assay has an intra-assay coefficient of variation of 3.5% and interassay precision of 6.3%.

**Determination of insulin content.** Insulin was extracted from pancreata obtained from additional animals (n = 4) with and without brain death (6 h) following procedures previously described (15). Briefly, the whole pancreas was centrifuged at 10,000 g for 10 min at 4°C, and the supernatant was stored at −20°C for future insulin determination. After neutralization, the insulin content in the extracts was measured by ELISA as described above. Insulin concentration was expressed as islet total protein (Bio-Rad Protein Assay kit I; Bio-Rad, Hercules, CA).

**Brain death induction.** Brain death was produced by balloon inflation of a Fogarty catheter introduced into the subarachnoid space through an occipital burr hole as previously described (10). Briefly, anesthesia was induced with diethyl ether in all animals and maintained by intraperitoneal administration of pentobarbital (30 mg/kg Nembutil Sodium Solution; Abbott Laboratories, Chicago, IL). All animals were tracheotomized and intubated with a blunt-tipped 4F Feeding Tube (Davol, Cranston, RI) and mechanically ventilated with intermittent positive pressure for 6 h (respiratory rate 105/min, tidal volume 4 ml; Rodent ventilator, model 683, Harvard Instruments, South Natick, MA). The arterial blood pressure was monitored continuously via a PE 50 catheter (Scientific Commodities, Lake Havasu City, AZ) placed into the femoral artery and attached to a transducer and recorder (HP 78534C, Hewlett-Packard). For avoiding ischemic effects, rats with mean arterial pressure (MAP) <75 mmHg for >5 min were excluded. All animals received 0.5 ml of lactated Ringer solution intravenously for 30 min during the 6-h period. A 1-mm hole was drilled through the skull 0.3 cm lateral to the sagittal suture. A No. 3 Fogarty catheter (Fogarty Arterial Embolotomy Catheter, 3F; Baxter Healthcare, Irvine, CA) was then inserted through the burr hole. For raising the intracranial pressure gradually, the balloon was inflated with 40 μl/min saline until respiration ceased. Using this protocol, previous studies demonstrated brain death by electroencephalogram and magnetic resonance imaging (10). The absence of reflexes, apnea, and maximally dilated and fixed pupils confirmed the condition. The average balloon volume that consistently abolished all electroencephalographic activity of the cerebral cortex is 200 ± 30 μl. The balloon was kept inflated during the entire 6-h follow-up. In instances in which animals exhibited hypotension, balloon volume was reduced by 10 μl/min until the animal became normotensive (MAP >75 mmHg). In sham-operated control rats, a burr hole was drilled, the catheter was inserted, but the balloon was not inflated. Body temperature, measured with a rectal probe, was maintained at 37°C by means of a dorsal thermopad.

Urine production was monitored via a PE 50 catheter placed directly into the urinary bladder. The average urinary output of the rats with central injury was 1.5 ± 0.1 ml/h. No significant difference was observed between the groups (17). The average MAP was found in brain death animals (79.3 ± 4.1 mmHg) versus mechanically ventilated animals without central nervous system (CNS) injury (77.8 ± 1.8). After these protocols, the majority of animals (72%) were considered satisfactory as pancreas donors after 6 h of brain death.

**Serum TNF-α, IL-1β, and IL-6 levels.** A total of 0.5 ml of whole blood was collected from the femoral vein catheter at 2, 4, and 6 h after central injury and placed in microcentrifuge tubes (Microtainer; Becton Dickinson, Rutherford, NJ). The tubes were then centrifuged at 15,000 g for 10 min at 4°C. Serum was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored at −80°C until analysis. Rat TNF-α (Bioresource International, Camarillo, CA) and IL-1β and IL-6 (Quantikine M; R & D Systems, Minneapolis, MN) were assessed by enzyme-linked immunosorbent assay (ELISA) following the manufacturer’s recommendations.

**Determination of IL-6, IL-1β, and TNF-α mRNA.** Pancreatic tissue from donors were collected from the femoral vein catheter at 2, 4, and 6 h after central injury and placed in microcentrifuge tubes (Microtainer; Becton Dickinson, Rutherford, NJ). The tubes were then centrifuged at 15,000 g for 10 min at 4°C. Serum was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored at −80°C until analysis. Total mRNA was prepared using the RNAeasy 96 Total RNA Isolation Kit (Qiagen) according to the manufacturer’s protocol. RNA concentration was estimated from absorbance at 260 nm. The expression level of specific mRNAs was quantified using an ELISA-based assay (Quantikine Rat mRNA; R&D Systems) following the manufacturer’s instructions. One attomole of IL-6 mRNA is ~361 femtometres, 1 attomole of IL-1β mRNA is ~452 femtometres, and 1 attomole of TNF-α mRNA is ~257 femtometres. The results were expressed as femtomoles per islet.
In vitro glucose-stimulated insulin release. Glucose-stimulated insulin secretion was determined in triplicate for each glucose concentration tested by static incubation as described (15). After 60 min of incubation, buffer was removed and frozen at −20°C until insulin measurement as described above. Protein was measured after neutralization with HCl using a commercial kit (Bio-Rad Protein Assay kit I) following the manufacturer’s instructions. Determination of nuclear NF-κB, c-jun, ATF-2, and CREB-1 activities. Immediately after islet isolation, nuclear extracts were prepared from handpicked isolated islets using a nuclear extraction kit (Transfactor Extraction Kit; BD Biosciences Clontech Laboratories, Palo Alto, CA) following the manufacturer’s instructions. Briefly, DNA binding of NF-κB p50, c-jun, ATF-2, and CREB-1 was assessed in nuclear extracts using an ELISA-based format (BD Mercury NF-κB p50, c-jun, ATF-2, CREB-1 Transfactor Kits; BD Biosciences Clontech Laboratories), following the manufacturer’s instructions. Intraportaline glucose tolerance test. Ten days after islet transplantation, glucose disposal rate (Kg) was analyzed in 16-h fast recipients by intraperitoneal glucose tolerance testing (1 g of glucose/kg body wt i.p.) as previously reported (19). Statistical analysis. All data are expressed as mean ± SE. Comparison between groups was performed by t test using SPSS 10.0 for windows (ACITIS, University of Texas at Austin, Austin, TX). Statistical significance was established at P < 0.05.

RESULTS

Activation of PICs by donor brain death. Shortly after brain death induction, a significant increase in serum TNF-α, IL-6, and IL-1β was demonstrated in a time-dependent manner (Fig. 1). For excluding the possibility that hypotension or ischemia-reperfusion injury produced these changes, serum from mechanically ventilated rats without CNS injury was examined. No increase in serum PIC was observed at any experimental time points, emphasizing the effects of central injury. There is little available information on the expression of PIC in the pancreas after massive central injury. As demonstrated in Fig. 1D, we have noted upregulation of TNF-α, IL-6, and IL-1β mRNA by RT-PCR analysis; no such activity was apparent in non–brain death donors. Comparable upregulation of adhesion molecules such as P- and E-selectin were also demonstrated (data not shown).

Suppression of islet function by brain death. Because PICs have a profound impact on β-cell function and death (13), we examined insulin secretory responses before islet isolation by in situ perfusion of the pancreas 6 h after CNS injury. The relationship between glucose and insulin secretion from the perfused pancreas is shown in Fig. 2. As the glucose concentration in the perfusate was increased from 2 to 26 mmol/l, insulin secretory responses were significantly higher in non–brain death donors (7,890.56 ± 286.22 pmol/l; P < 0.0001) compared with brain death donors (3,244.34 ± 47.3 pmol/l; P < 0.05). No difference in insulin concentrations were observed in control normal rats (no mechanical ventilation) versus rats that were mechanically ventilated for 6 h without CNS injury (Fig. 2A). The mean insulin response to 20 mmol/l arginine (Fig. 2B) in the presence of 26 mmol/l glucose was also significantly lower in brain death donors (3,244.34 ± 334.4 pmol/l) compared with non–brain death animals (7,890.56 ± 314.35 pmol/l; P < 0.0001).

Brain death reduced β-cell survival and insulin content. Pancreatic sections co-stained for insulin and TUNEL displayed numerous TUNEL-positive islet cells in animals with brain death (6 h) compared with animals without CNS injury (Fig. 3). Quantification of these TUNEL-positive β-cells yielded an index of insulin-producing cells death five- to sixfold higher (P < 0.001) in brain...
Death donors as compared with controls (Fig. 3C). Fur-
thermore, total pancreatic insulin levels were reduced 6 h
after induction of brain death (controls 0.22 ± 0.01 vs.
brain death 0.10 ± 0.03 μg/mg tissue; *P < 0.05; +P < 0.001).

**Effects of brain death in islet recovery.** As shown in
Fig. 4, islet recovery was significantly reduced in brain
death donors (at 6 h 602.3 ± 233.4 IEQ/pancreas for prepurifica-
tion yields) compared with mechanically ventilated ani-
mals without CNS injury (1,792.5 ± 325.4 IEQ; *P < 0.05). There was no significant difference in mean pro-
cessed pancreas weight between groups (data not shown).
Animals with mechanical ventilation without CNS injury
presented a slight reduction in islet recovery compared
with controls that did not reaches statistical significance
(mechanical ventilation for 6 h 1,792.5 ± 325.4 IEQ/pancreas vs. normal animals 2,396.3 ± 487.3 IEQ/pancreas).

**Islet viability after isolation from brain death versus
non–brain death donors.** Islet viability, assessed imme-
diately after isolation in both dissociated islet cells and
intact cultured islets, is shown in Fig. 5. As previously
demonstrated in pancreas sections, islet viability after

**FIG. 5.** Islet viability after isolation from brain death versus
non–brain death donors. Islet viability, assessed imme-
diately after isolation in both dissociated islet cells and
intact cultured islets, is shown in Fig. 5. As previously
demonstrated in pancreas sections, islet viability after

**FIG. 3.** Effects of brain death on β-cell survival. Paraffin-embedded
4-μm pancreatic sections obtained 6 h after brain death (A) or me-
chanical ventilation without CNS injury (B) were co-stained for insulin and
TUNEL-positive cells as described in RESEARCH DESIGN AND METHODS.
Twenty-five islets were examined per animal (n = 5), and an apoptotic
index (percentage of TUNEL-positive cells per islet) was determined.
Results are expressed as mean ± SE (n = 6); +P < 0.001.
Isolation was significantly reduced in brain death donors at 2, 4, and 6 h after CNS injury compared with controls (Fig. 5A). Islet viability determined in culture by MTT reduction showed similar results (Fig. 5B). Islet viability was significantly reduced at 1, 2, and 3 days in cultured islets recovered from brain death donors compared with controls \( (P < 0.05) \). Apoptosis is likely the principal form of \( \beta \)-cell death in diabetes and early stages of islet transplantation (13). In this regard, higher incidence of apoptosis was observed in isolated islets from brain death donors (enrichment factor at 6 h 6.1 \( \pm \) 1.3) versus non–brain death donors (1.08 \( \pm \) 0.39; \( P < 0.001 \)).

**Effects of brain death on nuclear activities of proapoptotic and inflammation-related transcription factors.** Studies using high-density oligonucleotide arrays identified NF-κB as a central transcription factor in the process of PIC-induced \( \beta \)-cell gene expression and death (13,20). As demonstrated in Fig. 6, islets isolated from brain death donors presented significantly higher NF-κB \( p50 \) activity compared with non–brain death donors \( (P < 0.001) \). Recent studies have pointed toward activation of c-Jun NH2-terminal kinase as the signal transducer for islet isolation processing, oxidative stress, and PIC toxicity to \( \beta \)-cells (21–23). Transcription factors c-Jun and ATF-2 are regulated by c-Jun NH2-terminal kinase and have been implicated with onset of apoptosis (24). In this regard, c-Jun and ATF-2 nuclear activities were increased in islets obtained from brain death donors compared with controls. To characterize further the effects of brain death, we assessed the activity of transcription factor CREB-1, which is associated with survival in insulin-producing cells (25). It is interesting that in correlation with activation of proapoptotic pathways after the induction of brain death, we observed a significant decrease in CREB-1 activity in isolation was significantly reduced in brain death donors at 2, 4, and 6 h after CNS injury compared with controls (Fig. 5A). Islet viability determined in culture by MTT reduction showed similar results (Fig. 5B). Islet viability was significantly reduced at 1, 2, and 3 days in cultured islets recovered from brain death donors compared with controls \( (P < 0.05) \). Apoptosis is likely the principal form of \( \beta \)-cell death in diabetes and early stages of islet transplantation (13). In this regard, higher incidence of apoptosis was observed in isolated islets from brain death donors (enrichment factor at 6 h 6.1 \( \pm \) 1.3) versus non–brain death donors (1.08 \( \pm \) 0.39; \( P < 0.001 \)).

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islets obtained from brain death donors compared with controls.

**In vitro functionality of islets recovered from brain death versus non–brain death donors.** Islet glucose responsiveness 6 h after CNS injury was assessed by static incubation (Fig. 7). The insulin secretory response after a glucose challenge for islets recovered from mechanically ventilated animals without CNS injury was similar to islets from normal animals (data not shown). In contrast, glucose-stimulated insulin secretion was markedly blunted in islets from brain death donors. Because a significant percentage of islet cells obtained from brain death donors are not viable, we examined whether increasing the number of IEQ/well would reduce the difference in insulin release observed between groups. Doubling the brain death islet mass did not reach the insulin levels obtained from non–brain death islets (188.4 ± 78 pg insulin/mg protein vs. 358.8 ± 103, respectively; data not shown; P < 0.05).

**Effects of brain death on islet functionality after transplantation.** We compared the performance of islets obtained from brain death donors versus non–brain death donors using an optimal mass (3,000 IEQ/recipient) in STZ-induced syngeneic recipients. One hundred percent of the recipients that received islets from control or non–brain death donors were euglycemic (nonfasting glucose <11 mmol/l) at 7 and 30 days posttransplantation. In contrast, only 20% of the animals that were treated with islets that were recovered from brain death donors were found euglycemic 7 days posttransplantation and none at 30 days (P < 0.001) (Fig. 8A). For further defining transplanted islet function, glucose disposal rate was calculated from intraperitoneal glucose tolerance testing 10 days posttransplantation (Fig. 8B). Glucose disposal rate was markedly reduced in brain death islet recipients compared with controls.

**DISCUSSION**

The studies of Shapiro and colleagues (1,2) demonstrated the efficacy of human islet transplantation for type 1 diabetes. However, human pancreatic tissue is severely limited, a situation worsened by the fact that the Edmonton protocol requires the utilization of islets from 1–3 donor pancreata to achieve exogenous insulin independence. Presently, brain death donors are the sole source of pancreatic tissue for islet transplantation. Organ donor characteristics such as medical history, age, length of hospitalization, and cause of death have a significant impact on islet recovery after isolation (26,27). Moreover, hypothermic preservation and the islet isolation process trigger further damage to pancreatic islets. Because current pancreas preservation and islet isolation techniques
recover <50% of the available human islets, new strategies to improve islet yields will be critical to enhance the therapeutic efficacy of islet transplantation. To this end, a new protocol for pancreas preservation (double layer [perfluorochemical/University of Wisconsin]) has demonstrated promising results (28–30).

Although brain death has been well defined neurologically, knowledge of systemic changes that occur after this event remains limited. It is recognized that brain death has defined effects on hemodynamic stability, hormone regulation, and inflammatory reactivity that predispose for additional organ injury during hypothermic preservation and reperfusion after transplantation (7). In this regard, brain death is associated with structural myocardial damage (31), intense nonimmune inflammation in kidney isografts (8), and higher incidence of acute and chronic allograft rejection (12,32). Isografts (8), and higher incidence of acute and chronic allograft rejection (12,32–34). This study represents the first examination of brain death’s impact on islet recovery and functionality in vitro and in vivo after transplantation. As previously reported, we found an association between brain death and a proinflammatory condition characterized by high levels of PICs. High levels of IL-6, IL-1β, and TNF-α mRNA were demonstrated in the pancreas subsequent to brain death induction. It has been postulated that islet exposure to PICs induces significant islet dysfunction and death, mainly by apoptosis. We found a significant reduction in insulin secretory response during in situ pancreas perfusion in brain death animals compared with mechanically ventilated controls. The extent of this defect was greater after arginine stimulation of insulin release. Moreover, we observed more apoptotic islet cells in pancreatic sections obtained from brain death animals compared with controls. Taken together, these results suggest that brain death significantly reduces β-cell mass and functionality before organ preservation and islet isolation. Other factors, such as hypotension, oxidative stress, and hyperglycemia, also could be important in the detrimental effects of brain death on pancreatic islets, and they are currently under investigation in our laboratory.

To examine the direct effects of brain death on islet transplantation, we first analyzed islet yields from brain death versus mechanically ventilated rats without CNS injury. We found a time-dependent decrease in islet yields in brain death animals compared with controls. Furthermore, islet viability after isolation and culture was reduced in islet preparations obtained from brain death donors. In contrast with our results, better islet viability is routinely obtained in human islet isolation from brain death donors. These differences could be explained by different resistance of β-cells to the toxic effects of PICs and oxidative stress between species (35,36). Furthermore, islet viability assessed by fluorescein diacetate/propidium iodide staining, used routinely in human islet preparation quality control, may overestimate the viability count. Finally, the high-pressure brain death induced by the Fogarty catheter may represent a much more severe model of brain death than that encountered in most multiorgan donors in the clinical setting.

Brain death was also associated with impairment in isolated islet functionality assessed in vitro in static incubations. In this regard, islets recovered from brain death donors presented lower insulin release after a glucose challenge compared with control islets. Although we increased the β-cell mass in islet preparations from brain death donors, we failed to demonstrate equivalent insulin release after glucose stimulation compared with controls. These results demonstrate (independent of β-cell mass reduction) an association between brain death and impairment in islet functionality after islet isolation.

At the molecular level, we showed that islet preparations obtained from brain death donors presented higher nuclear activity of NF-κB p50, c-Jun, and ATF-2 compared with controls. It is interesting that the activation of the “protective” transcription factor CREB-1 (25) was found to be significantly reduced in islet preparations obtained from brain death donors. Overall, these results suggest that islets recovered from brain death donors presented higher activity of proapoptotic and proinflammatory signaling pathways.

To examine the consequences of brain death on islet transplantation, we evaluated the performance of islet preparations obtained from brain death versus non–brain death donors in a syngeneic model of islet transplantation using STZ-induced diabetic rats. We found significant reduction in islet functionality, as evaluated by nonfasting glucose levels and glucose disposal rate after transplantation, in animals that received islets from brain death donors. Because brain death increases immunogenicity of the organs (i.e., increases expression of adhesion molecules, chemokines, major histocompatibility complex classes I and II, and B7) (11), further reductions in islet functionality are expected in an allogeneic system. Moreover, the detrimental effects of brain death on islet recovery and functionality could be further exacerbated by the common use of high inotropic support before organ procurement, leading to increased ischemia of islets.

The results presented herein need to be considered in the design of experimental islet transplantation. In this regard, in virtually all experimental studies of islet transplantation, young, healthy, living animals are used as donors. Moreover, the experimental system described in the present study provides a potentially clinically relevant model in which to study therapeutic strategies to prevent or reduce the effects of brain death to pancreatic islets.

In summary, our studies demonstrated profound physiological and structural derangements in pancreatic islets subsequent to brain death induction. In this regard, brain death is associated with significant reduction in islet yields and functionality in vitro and in vivo after transplantation. Because virtually all experimental studies in islet transplantation involve the use of healthy, normal donors, these results need to be considered in the design of future experimental transplant studies. It is conceivable to postulate that prevention or reduction of deleterious effects of brain death would mitigate islet loss and improve islet engraftment and survival after transplantation. Moreover, development of such strategies could improve the quality of organs from “marginal” donors, broadening the criteria for donor acceptance for islet isolation and transplantation.

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