Cell Transplantation, Vol. 16, pp. 707–715, 2007
Printed in the USA.

0963-6897/07 $90.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

Effect of Pretransplant Preconditioning by Whole Body Hyperthermia on Islet Graft Survival

H. Brandhorst,* M. Olbrich,† A. Neumann,‡ H. Jahr,‡ and D. Brandhorst*

*Department of Oncology, Radiology & Clinical Immunology, University Hospital, 75185 Uppsala, Sweden
†Department of Internal Medicine, University Hospital Eilbeck, 22081 Hamburg, Germany
‡Third Medical Department, University Hospital, 35385 Giessen, Germany

Previous observations in heat-shocked pig islets revealed the ambivalent character of the stress response simultaneously inducing processes of protection and apoptosis. To clarify whether the proapoptotic character of the stress response is reduced in heat-exposed islets still embedded in their native environment, hyperthermia was performed in the present study either as whole body hyperthermia (WBH) prior to pancreas resection or as in vitro heat shock (HS) after isolation. HS (42°C/45 min) was induced in donors 12 h before isolation (WBH, $n = 32$) or in freshly isolated islets prior to 12 h of culture at 37°C (in vitro HS, $n = 25$). Islets continuously incubated at 37°C served as controls ($n = 34$). Proinflammatory treatment was performed with H_2O_2, DETA-NO, or a combination of IL-1β, TNF-α, and IFN-γ. Quality assessment included islet yield, viability staining, static glucose incubation, and nude mouse transplantation. WBH was significantly less effective than in vitro HS to induce HSP70 overexpression and to increase islet resistance against inflammatory mediators. Although characterized by an unaltered Bax to Bcl-2 ratio, islets subjected to WBH partially failed to restore sustained normoglycemia in diabetic nude mice. The inflammatory response observed in the pancreas of WBH-treated rats was associated with significantly reduced viability that seems to have a higher predictive value for posttransplant outcome compared to islet in vitro function or mitochondrial activity. In contrast, in vitro HS significantly decreased transcript levels of Bcl-2, but did not affect posttransplant function compared to sham-treated islets. These findings suggest that WBH is primarily associated with increased necrosis as a secondary tissue type-specific effect of pancreas damage while in vitro HS mainly induces apoptosis.

Key words: Rats; Islet isolation; Heat shock; Inflammation; Apoptosis; Whole body hyperthermia

INTRODUCTION

The production of isolated islets is associated with numerous variables that determine the critical islet mass to induce long-term insulin independence in type 1 diabetic recipients (25). Detrimental factors such as brain death, surgical trauma, or enzymatic pancreas digestion are characterized by the activation of proinflammatory and proapoptotic mechanisms affecting islet yield and viability (1,13,24,28). After intraportal transplantation islets are exposed to immediate nonspecific inflammatory reactions, reducing islet graft function (5,9).

One important strategy of cells to survive under otherwise lethal conditions is the universal heat shock response (29). The formation of heat shock proteins (HSPs) is of considerable relevance for islet survival in an inflammatory environment (3), because islets are characterized by an extremely decreased level of antioxidant enzymes in comparison with other organs (18). However, previous observations in isolated pig islets demonstrated that the induction of HSPs by hyperthermic stress simultaneously induces two distinct processes with protective or apoptotic characteristics, which can finally deteriorate posttransplant function (10). As a consequence, reduction of hyperthermia not only prevented a significant upregulation of proapoptotic proteins such as Bax, Fas, or DNA fragmentation factor, but minimized overexpression of HSP27, HSP70, or HSP90 as well (7,10). In this context it should be considered that isolated islets are exposed to a nonphysiological ambient environment, which represents a proapoptotic stimulus for these microorgans (21). On the other hand, heat shock performed in donor rats as whole body hyperthermia (WBH) significantly increased islet resistance against warm ischemia (22) and improved morphology of pancreatic isografts after prolonged cold ischemia (27).

Received November 9, 2006; final acceptance March 12, 2007.
Address correspondence to Daniel Brandhorst, Department of Oncology, Radiology & Clinical Immunology, University Hospital Uppsala, Dag Hammarskjölds väg 20, 75185 Uppsala, Sweden. Tel: +46-70-4250636; Fax: +46-18-611-0222; E-mail: Daniel.Brandhorst@klinimm.uu.se

These findings suggest that islet cells still embedded in their native pancreatic matrix can compensate proapoptotic stimuli to a greater extent than isolated islets (8,24). To clarify the effect of the ambient environment of heat-exposed rat islets on yield, viability, and resistance against inflammation, heat shock (HS) was induced in the present study either as WBH or in vitro HS performed prior to or after isolation, respectively.

MATERIALS AND METHODS

Islet Isolation

Male Lewis rats (Harlan, Hanover, Germany) weighing 300–350 g were used as pancreas donors. Anesthesia of fasting animals was performed utilizing 160 mg/kg of pentobarbital (Narcoren, Merial, Hallbergmoos, Germany). Prior to resection pancreases were intraductally distended with 20 mg collagenase (Serva, Heidelberg, Germany) dissolved in 12 ml of cold Hank's balanced salt solution (HBSS, Biochrom, Berlin, Germany). Stationary pancreas digestion and islet purification were carried out as previously described in detail (14). Purified islets were suspended in bicarbonate-free tissue culture medium (TCM) 199 supplemented with 5% fetal calf serum, 20 mmol/L HEPES, 2 mmol/L *N*-acetyl-L-alanyl-L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Biochrom).

Hyperthermic Preconditioning

WBH was performed 12 h before pancreas resection ($n = 32$) as described previously (19). After IP administration of 55 mg/kg pentobarbital and 4 ml saline, anesthetized and hydrated rats were immersed in a water bath at 43°C for approximately 15 min until rectally measured body temperature raised to 42°C. This temperature was maintained for an additional 30 min. Subsequently, heat-exposed rats were placed in a water bath adjusted to 30°C to return body temperature to basal levels. After drying, pretreated rats were allowed to recover for 12 h with free access to food and water.

Rats that served as islet donors for controls and in vitro HS were treated in the same manner with regard to timing and dosage of anesthesia. For in vitro HS ($n = 25$) quantified aliquots of freshly purified islets were suspended in supplemented TCM 199 and incubated for 45 min in a water bath at 42°C prior to 12-h culture at 37°C in humidified atmosphere (10). This protocol was determined in preliminary experiments performed to obtain maximum HSP70 expression (data not shown). Freshly isolated islets continuously incubated at 37°C served as controls (37°C, $n = 34$).

Islet Characterization

Isolated islets were quantified as islet equivalents (IEQ) immediately after isolation and in vitro HS (23). Islet viability was simultaneously examined by the trypan blue exclusion assay (14) and additionally assessed after overnight culture at 37°C.

After overnight culture duplicate samples of five hand-selected islets with an average diameter of 150–200 μm were subjected to static incubation for 120 min in CMRL 1066 supplemented with 2.8 or 20 mmol/L glucose. After incubation islets were recovered and sonified in acid ethanol for subsequent determination of intracellular insulin content (16). Insulin content and release were measured utilizing an enzyme immunoassay specific for rat insulin (Mercodia, Uppsala, Sweden).

To assess mitochondrial alterations induced by thermal stress (30), quantified aliquots of preconditioned or untreated islets were placed in 24-well plates (Costar, Bodenheim, Germany) and incubated in duplicate for overnight culture. The mitochondrial activity was determined by conversion of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS, Promega, Mannheim, Germany) into formazan. After addition of 20% (v/v) MTS and subsequent 3-h incubation at 37°C in a humidified atmosphere, the optical density of the supernatant of each well was measured in duplicate in a 96-well plate reader at 490 nm and normalized to islet protein content as determined by a detergent-compatible microprotein assay (Bio-Rad, München, Germany). Blanks were calculated from empty medium treated in the same manner.

Immunohistology

Pancreata of preconditioned ($n = 2$) and untreated rats ($n = 2$) were fixed for 60 min in Zamboni's solution, washed, embedded in OTC compounds (Sakura Finetek Europe, Zoeterwoude, Netherlands), frozen in liquid nitrogen, and cryosliced (8 μm). Tissue slices were incubated overnight at 4°C with guinea pig anti-insulin serum (final dilution 1:400; Dako Cytomation, Hamburg, Germany) mixed with a monoclonal mouse anti-rat macrophage antibody (final dilution 1:100; anti-CD163, clone ED2, Serotec, Düsseldorf, Germany). Binding of primary antibodies was then visualized by a 1-h reaction at room temperature with a mixture of donkey anti-goat-RhodRedX and donkey anti-mouse-FITC (Jackson ImmunoResearch, West Groove, PA, USA) diluted 1:400 with phosphate-buffered saline (PBS) containing 5% rat serum (Jackson ImmunoResearch). Nuclei were counterstained (blue fluorescence) with Höchst 33342 (Calbiochem, Schwalbach, Germany) for 5 min at room temperature. After washing, the slices were embedded into Slow Fade Light Antifade (Molecular Probes, Eugene, OR, USA). Blanks were performed by using an equivalent concentration of nonspecific mouse IgG1. Fluorescence was assessed with a Leica DM-LB microscope

(Wetzlar, Germany) equipped with a Leica DC-200 camera, Leica IM 1000 software, and filters for excitation of blue light (505 nm), green light (580 nm), and UV light (400 nm). From each sample sections from 10 different locations were analyzed.

Western Blot Analysis

Samples of heat-exposed and untreated islets were washed three times in cold HBSS and homogenized in cold PBS supplemented with 1% sodium dodecyl sulfate (Bio-Rad), 5% sodium deoxycholate, 10% Igepal (Sigma, Deisenhofen, Germany), and 15% protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Subsequent to centrifugation at $3000 \times g$ supernatants were snap frozen in liquid nitrogen and stored at −80°C until further use. HSP70 was detected as previously described (11) utilizing a monoclonal anti-rat HSP70 antibody (Becton-Dickinson, Heidelberg, Germany) and a peroxidase-conjugated anti-mouse secondary antibody (Dako, Hamburg, Germany). The level of protein expression was quantified by band densitometry and calculated as arbitrary units (AU) utilizing the software analySIS (SIS, Stuttgart, Germany). HSP70 expression was normalized to sample protein content.

RT-PCR Analysis

RT-PCR analysis was utilized to investigate the effect of different hyperthermic treatments on the expression of antiapoptotic Bcl-2 and proapoptotic Bax due to the low sensitivity of Western blot analysis for these proteins. After preliminary experiments total RNA was extracted from preconditioned or control islets 12 h after heat exposure by means of the Perfect RNA mini kit (Eppendorf, Hamburg, Germany) followed by cDNA synthesis utilizing the Cloned AMV first-strand cDNA synthesis kit (Invitrogen, Inchinnan, UK). PCR was performed by means of the Hotmaster Mix (Eppendorf) using the following oligonucleotide primers: rat β-actin (forward, 5′-CGT AAA GAC CTC TAT GCC AA-3′ and reverse, 5′-AGC CAT GCC AAA TGT CTC AT-3′), rat Bax (forward, 5′-AGA GGC AGC GGC AGT GAT-3′ and reverse, 5′-AGA CAC AGT CCA AGG CAG-3′), and rat Bcl-2 (forward, 5′-GCG AAG TGC TAT TGG TAC CTG-3′ and reverse, 5′-ATA TTT GTT TGG GGC AGG TCT-3′). mRNA expression was analyzed by semiquantitative PCR analysis and expressed as AU normalized to β-actin (6).

Cytotoxicity Assay

Quantified aliquots of preconditioned or untreated islets were placed in 24-well plates (Costar) and incubated in duplicate for 24 h with 0.05 mmol/L hydrogen peroxide (H_2O_2) or 1.0 mmol/L of the nitric oxide (NO) donor DETA-NO (Alexis, Grüningen, Germany) generating the double molar amount of NO. Cytokine incubation was performed for 48 h with a combination of recombinant human IL-1β (10 U/ml), TNF-α (112 U/ml), and IFN-γ (112 U/ml) (Cell Concepts, Umkirch, Germany) (15). Mitochondrial activity of islets treated with inflammatory mediators was expressed as the percentage of formazan production in corresponding islets cultured without addition of noxious agents.

Islet Transplantation

In vivo function of islets was assessed in NMRI nude mice (Harlan) rendered diabetic by a single IV injection of 240 mg/kg streptozotocin (Sigma) 3 days prior to transplantation of 700 IEQ beneath the kidney capsule. Islets were transplanted in a donor-to-recipient ratio of 1:1. Blood samples taken from the tail vein were processed in a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA, USA) to determine nonfasting serum glucose levels. Pretransplant serum glucose levels of all recipients exceeded 350 mg/dl. After transplantation nonfasting serum glucose levels <200 mg/dl were defined as normoglycemic and considered as graft function. Nephrectomy (Nx) of graft-bearing kidneys was performed 32 days posttransplant to demonstrate immediate return of hyperglycemia.

Data Analysis

All values are expressed as means ± SE. Statistical analysis of data was performed by the Wilcoxon test for paired-matched samples and by the Mann-Whitney test for independent samples. Graft function (time of normoglycemia) was analyzed utilizing the Log-rank test. Significance was expressed as p-value and considered for $p < 0.05$. Values of $p > 0.05$ were termed nonsignificant (NS).

RESULTS

Islet Characterization

The results of islet characterization are presented in Table 1. Viability of freshly purified islets, either untreated, exposed to in vitro HS, or isolated from donors subjected to WBH, consistently exceeded 90%. Islet yield was slightly lower (NS) in rats subjected to WBH (978 ± 76 IEQ, NS) compared with untreated donors providing control islets (1104 ± 92 IEQ) or islets heat shocked in vitro (1285 ± 151 IEQ). In comparison with the purity of untreated islets (88 ± 3%) or islets exposed to in vitro HS (87 ± 3%), islet preparations isolated after WBH were contaminated with increased quantities of damaged exocrine tissue resulting in a significantly reduced purity (68 ± 10%, $p < 0.01$). After visual inspection exocrine contaminations were thoroughly removed for subsequent experiments. During overnight culture, viability decreased significantly in each experimental

Table 1. Characterization of Heat-Exposed Rat Islets

Hyperthermia	*n*	Yield (IEQ)	Purity (%)	Viability (%) Day 0	Viability (%) Day 1	Formazan (OD/mg)	Insulin Content (μU/IEQ)
37°C	34	1104 ± 92	88 ± 3	95.0 ± 0.4#	85.8 ± 2.8	3.4 ± 0.3§	734 ± 81
WBH	32	978 ± 76	68 ± 10*§	94.6 ± 1.1#	72.0 ± 4.0†‡	2.9 ± 0.2‡	1100 ± 55*§
In vitro HS	25	1285 ± 151	87 ± 3	94.6 ± 0.2¶	84.1 ± 5.1	2.2 ± 0.2	754 ± 98

WBH, whole-body hyperthermia; HS, heat shock; IEQ, islet equivalent.
*$p < 0.01$, †$p < 0.001$ versus 37°C.
‡$p < 0.05$, §$p < 0.01$ versus in vitro HS by Mann-Whitney test.
¶$p < 0.05$, #$p < 0.001$ versus day 1 by Wilcoxon test.

group. This reduction was strongest after WBH (72.0 ± 4.0%) compared with control conditions (85.8 ± 2.8%, $p < 0.001$) or in vitro HS (84.1 ± 5.1%, $p < 0.05$).

Static glucose incubation revealed a significant decline in the stimulated insulin discharge in heat-pretreated islets in comparison to islets isolated from untreated rats ($p < 0.05$) (Fig. 1). The decrease in basal insulin release reached significance only in islets subjected to in vitro HS ($p < 0.05$ vs. WBH). The corresponding intracellular insulin contents are shown in Table 1. WBH-treated islets were characterized by a significantly greater intracellular insulin content (1100 ± 55 μU/IEQ, $p < 0.01$) than control islets (734 ± 81 μU/IEQ) or islets exposed to in vitro HS (754 ± 98 μU/IEQ).

With regard to mitochondrial activity, a significant reduction was observed only in islets subjected to in vitro HS (2.2 ± 0.2 OD/mg, $p < 0.05$ vs. WBH; $p < 0.01$ vs. 37°C) but not after WBH (2.9 ± 0.2 OD/mg, NS) when compared to untreated islets (3.4 ± 0.3 OD/mg).

Immunohistological Pancreas Characterization

Immunocytochemical pancreas characterization revealed infiltration of CD163-positive macrophages in the pancreas of WBH-treated rats (Fig. 2A), whereas T-lymphocytes were only rarely detectable (not shown). The green-stained macrophages were mainly present at the periphery of pancreatic lobuli. A low number of macrophages were found in the parenchyma but were

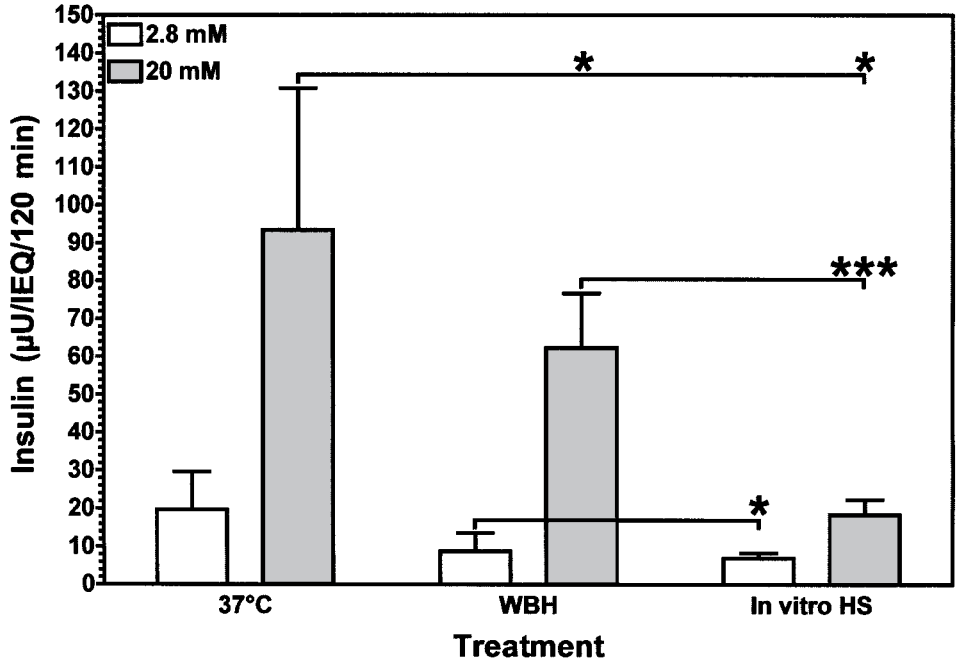

Figure 1. Insulin secretion capacity (μU/IEQ/120 min) measured during static incubation in 2.8 mM (white columns) or 20 mM glucose (gray columns). Statistical analysis by Mann-Whitney test revealed *$p < 0.05$ and ***$p < 0.001$ as indicated.

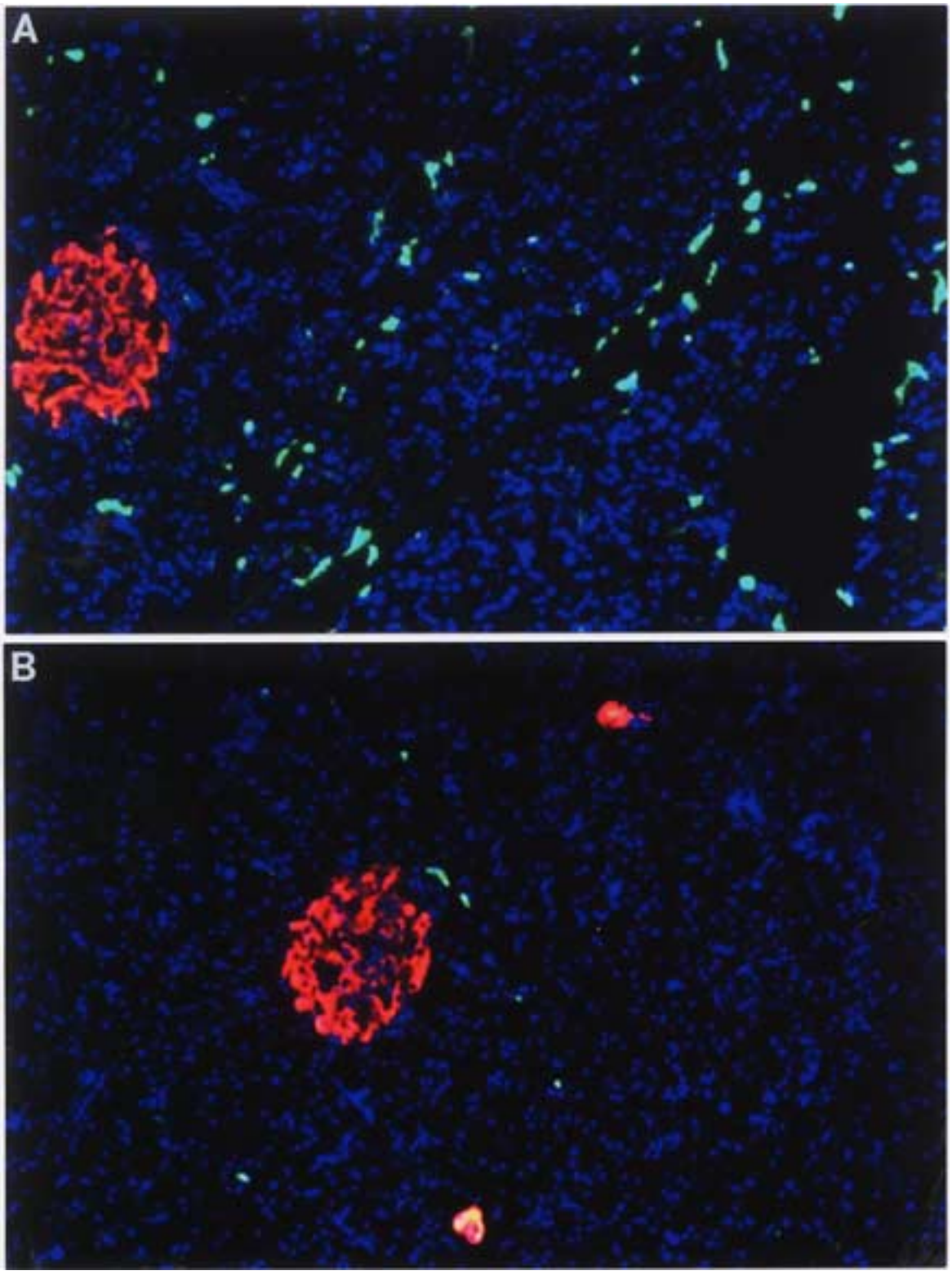

Figure 2. Double immunocytochemical staining for CD163-positive macrophages (green) and insulin-positive cells (red) in the pancreas resected from a rat subjected to either (A) whole body hyperthermia or (B) control conditions. Original magnification 100×. The pictures are representative for two animals that were investigated in each experimental group.

not localized in close vicinity to red-stained islets. Because macrophages could not be detected in untreated rats (Fig. 2B), these findings suggest that WBH, as applied in the present study, induces a significant inflammatory response in pancreatic exocrine tissue.

Cytotoxicity Assay

Twenty-four-hour incubation with H_2O_2 or DETA-NO substantially reduced the mitochondrial activity of rat islets either untreated ($p < 0.001$) or subjected to WBH ($p < 0.001$) or in vitro HS ($p < 0.01$) compared with corresponding islets cultured without proinflammatory stimuli. In relation to the other experimental groups, islets pretreated by in vitro HS were characterized by the highest resistance against H_2O_2 ($73.2 \pm 6.5\%$, $p < 0.01$) or DETA-NO ($85.4 \pm 4.6\%$, $p < 0.01$ vs. 37°C; $p < 0.05$ vs. WBH) (Table 2). As shown in Table 2, WBH had no protective effect against H_2O_2 or DETA-NO-mediated toxicity compared with control conditions. Cytokines, as used in the present study, did not reduce mitochondrial activity.

HSP70 Protein Expression and mRNA Expression of Bax and Bcl-2

Western blot analysis revealed that the HSP70 expression in islets subjected to WBH (386.2 ± 50.0 AU/μg, $n = 33$) was only slightly higher compared with control islets (281.9 ± 34.6 AU/μg, $n = 26$, NS) but significantly lower in comparison with islets exposed to in vitro HS (517.6 ± 49.3 AU/μg, $n = 15$, $p < 0.05$ vs. WBH; $p < 0.001$ vs. 37°C). These findings correlated with the observation that islets heat shocked in vitro were characterized by the highest resistance against H_2O_2 and DETA-NO in relation to the other experimental groups (Table 2).

In order to evaluate whether proapoptotic pathways were stimulated by different procedures applied for hyperthermia, transcripts levels of Bax and Bcl-2 were determined. In comparison to control conditions, proapoptotic Bax mRNA expression was not significantly altered in islets by any hyperthermic treatment (Table 3). Likewise, no effect on Bcl-2 mRNA expression was observed in relation to untreated islets (0.53 ± 0.10 AU), if islets were subjected to WBH (0.48 ± 0.06 AU, NS). In contrast, in vitro HS reduced mRNA expression of antiapoptotic Bcl-2 by 50% (0.20 ± 0.03 AU, $p < 0.01$), finally resulting in a significantly increased Bax-to-Bcl-2 ratio ($p < 0.01$).

Table 2. Mitochondrial Activity (%) of Heat-Exposed Rat Islets After Cytotoxic Treatment

Hyperthermia	*n*	H_2O_2 (%)	DETA-NO (%)	Cytokines (%)
37°C	34	50.7 ± 3.6*	63.4 ± 3.7*	103.9 ± 6.6
WBH	32	53.4 ± 4.1*	72.3 ± 5.0†	118.7 ± 11.7
In vitro HS	25	73.2 ± 6.5	85.4 ± 4.6	125.9 ± 14.3

WBH, whole body hyperthermia; HS, heat shock.
*$p < 0.01$, †$p < 0.05$ versus in vitro HS by Mann-Whitney test.

Table 3. mRNA Expression (AU) of Bax and Bcl-2 in Heat-Exposed Rat Islets

Hyperthermia	*n*	Bax	Bcl-2	Bax/Bcl-2
37°C	5	0.90 ± 0.11	0.53 ± 0.10*	1.94 ± 0.41*
WBH	6	0.71 ± 0.05	0.48 ± 0.06*	1.63 ± 0.23*
In vitro HS	5	0.85 ± 0.08	0.20 ± 0.03	4.65 ± 0.65

WBH, whole body hyperthermia; HS, heat shock.
*$p < 0.01$ versus in vitro HS by Mann-Whitney test.

Islet Function in Diabetic Nude Mice

As demonstrated in Figure 3, diabetic nude mice transplantation of islets either untreated or heat shocked in vitro resulted in immediate and sustained reversal of hyperglycemia. In contrast, graft function of WBH-treated islets was only transient and characterized by partial dysfunction that deteriorated over time ($p < 0.05$ vs. 37°C by Log-rank test). Nephrectomy of graft-bearing kidneys was performed at day 32 and induced immediate return of hyperglycemia in all normoglycemic recipients and a further increase of serum glucose in hyperglycemic mice.

DISCUSSION

Previous studies in isolated pig islets revealed the dual character of the heat shock response, which can simultaneously trigger mechanisms for protection and apoptosis (10). After isolation islets are exposed to a nonphysiological ambient environment, representing a proapoptotic stimulus for this endocrine tissue (21). The aim of the present study was to clarify whether the proapoptotic character of the stress response can be reduced when islets are still embedded in their native pancreatic matrix (8,24).

The present finding, that the heat-induced HSP70 overexpression in rat islets is associated with an enhanced in vitro resistance against H_2O_2 and NO, confirmed previous studies in rat islet single cells (4). The extent of islet resistance against inflammatory mediators correlated with the quantity of HSP70 expression and was significantly higher after in vitro HS compared with WBH. Because the temperature measured in the colon reflects accurately the thermal profile of different organs including the pancreas (2), we suppose that the intensity of thermal stress during WBH was identical with in vitro HS. The decreased HSP70 expression in preconditioned

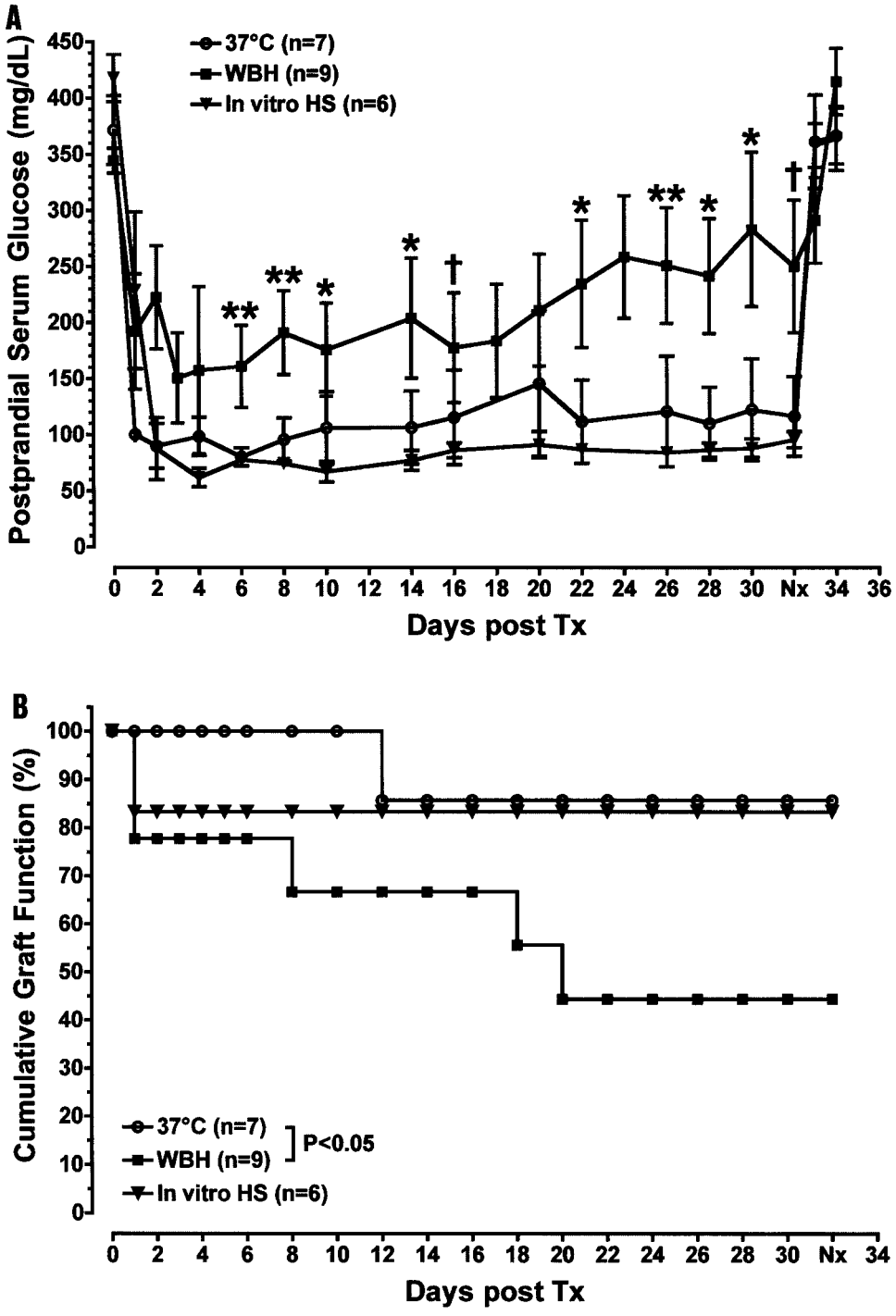

Figure 3. Function of isolated rat islets transplanted beneath the kidney capsule of diabetic NMRI nude mice. Posttransplant function is expressed as either (A) postprandial serum glucose or (B) cumulative graft function. Islets were either incubated under control conditions at 37°C (open circles, $n = 7$) or subjected to heat shock performed in vitro (in vitro HS, filled triangles, $n = 6$) or as whole body hyperthermia (WBH, filled squares, $n = 9$). Graft removal through nephrectomy (Nx) was performed as indicated at day 32 posttransplant. Analysis of serum glucose levels by Mann-Whitney test revealed $*p < 0.05$ and $**p < 0.01$ for WBH vs. control conditions and in vitro HS, or $\dagger p < 0.05$ versus control conditions only. Statistical analysis by Log-rank test revealed $*p < 0.05$ for WBH-treated islets versus control islets as indicated.

donors may also be related to a faster degradation of stress proteins, reflecting cellular recovery from hyperthermia (17). The delay of cellular recovery in cultured islets can be related to the observation that standard culture conditions stimulate the stress response in islets, not only increasing the expression of proapoptotic proteins but also the formation of HSP70 (21,26). Therefore, we hypothesize that the time needed for complete recovery of heat-exposed islet cells seems to be shorter in the living donor than in isolated islets maintained in an artificial microenvironment.

Western blot analysis in isolated pig islets demonstrated that the heat-induced HSP70 overexpression correlate with an increased expression of Bax (10). In the present study it was found, that rat islets expressed a constant level of Bax mRNA and a marked downregulation of antiapoptotic Bcl-2 mRNA after in vitro HS in comparison with untreated and WBH-exposed islets. Thus, in vitro HS resulted in a twofold increase of the Bax over Bcl-2 ratio, clearly suggesting induction of proapoptotic processes (20).

Nevertheless, neither expression of proapoptotic proteins nor in vitro function or mitochondrial activity could predict posttransplant function of islets heat exposed in any way. Although the secretory capacity of islets was reduced to the greatest extent after in vitro HS, transplanted islets of that group were characterized by a metabolic capacity comparable to that of control islets. Moreover, the highest intracellular insulin content was found in WBH-treated islets that yielded the lowest outcome after transplantation. Because no valid data are available yet, we can only speculate whether islets treated by hyperthermia prior to pancreas donation are more resistant to insulin loss induced by the stressful isolation and purification procedure (12).

In agreement with previous studies demonstrating that the heat-induced deterioration of stimulated insulin release is reversible (31), we found that in vitro HS does not affect sustained reversal of hyperglycemia after islet transplantation into diabetic nude mice. In contrast, although characterized by an unaltered mitochondrial activity, transplantation of WBH-treated islets revealed partial islet dysfunction. In view of the finding that islet yield, purity, and viability after overnight culture were deteriorated in WBH-treated rats, it may be of particular interest that a proinflammatory response was observed in the pancreas of preconditioned rats. Although macrophage infiltration was restricted to exocrine tissue, islet damage as a secondary effect of uncontrolled enzyme discharge from injured acinar tissue should be considered. However, this observation is in conflict to the observation that the highest intracellular insulin content was found in WBH-treated islets that were characterized by the lowest posttransplant function. In terms of islet graft function, evaluation of membrane integrity by the trypan blue exclusion assay seems to have a higher predictive value than glucose-stimulated insulin release or mitochondrial activity.

The contradictory observation that WBH improves function of syngenically transplanted rat livers (19) seems to reflect the complexity and variability of this procedure. Comparative investigations in preconditioned rats clearly demonstrated that the kinetic and the quantity of the stress response is tissue type specific, resulting in an extreme range of the HSP70 accumulation measured in different organs (2).

In summary, the present study demonstrates that WBH is less effective than in vitro HS to induce persistent HSP70 overexpression in rat islets needed for increased islet resistance against inflammatory mediators. Although characterized by an unaltered Bax to Bcl-2 ratio, islets subjected to WBH failed to restore completely normoglycemia in diabetic nude mice. The inflammatory response observed in the pancreas of WBH-treated rats was associated with significantly reduced viability that seems to have a higher predictive value for posttransplant outcome compared to islet in vitro function or mitochondrial activity. In contrast, in vitro HS reduced antiapoptotic Bcl-2 but did not deteriorate posttransplant function. These findings indicate that the dimension of necrotic cell death found after WBH is larger compared to the extent of apoptosis observed after in vitro HS.

REFERENCES

1. Abdelli, S.; Ansite, J.; Roduit, R.; Borsello, T.; Matsumoto, I.; Sawada, T.; Allaman-Pillet, N.; Henry, H.; Beckmann, J. S.; Hering, B. J.; Bonny, C. Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure. Diabetes 53(11):2815–2823; 2004.
2. Beck, S. C.; Paidas, C. N.; Tan, H.; Yang, J.; De Maio, A. Depressed expression of the inducible form of HSP 70 (HSP 72) in brain and heart after in vivo heat shock. Am. J. Physiol. 269(3 Pt. 2):R608–613; 1995.
3. Bellmann, K.; Hui, L.; Radons, J.; Burkart, V.; Kolb, H. Low stress response enhances vulnerability of islet cells in diabetes-prone BB rats. Diabetes 46(2):232–236; 1997.
4. Bellmann, K.; Wenz, A.; Radons, J.; Burkart, V.; Kleemann, R.; Kolb, H. Heat shock induces resistance in rat pancreatic islet cells against nitric oxide, oxygen radicals and streptozotocin toxicity in vitro. J. Clin. Invest. 95(6): 2840–2845; 1995.
5. Bennet, W.; Groth, C. G.; 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. 105(2):125–133; 2000.
6. Berney, T.; Molano, R. D.; Cattan, P.; Pileggi, A.; Vizzardelli, C.; Oliver, R.; Ricordi, C.; Inverardi, L. Endotoxin-mediated delayed islet graft function is associated with increased intra-islet cytokine production and islet cell apoptosis. Transplantation 71(1):125–132; 2001.

7. Boreham, D. R.; Dolling, J. A.; Maves, S. R.; Miller, S.; Morrison, D. P.; Mitchel, R. E. Heat-induced thermal tolerance and radiation resistance to apoptosis in human lymphocytes. Biochem. Cell Biol. 75(4):393–397; 1997.
8. Bottino, R.; Balamurugan, A. N.; Tse, H.; Thirunavukkarasu, C.; Ge, X.; Profozich, J.; Milton, M.; Ziegenfuss, A.; Trucco, M.; Piganelli, J. D. Response of human islets to isolation stress and the effect of antioxidant treatment. Diabetes 53(10):2559–2568; 2004.
9. Bottino, R.; Fernandez, L. A.; Ricordi, C.; Lehmann, R.; Tsan, M. F.; Oliver, R.; Inverardi, L. Transplantation of allogeneic islets of Langerhans in the rat liver: Effects of macrophage depletion on graft survival and microenvironment activation. Diabetes 47(3):316–323; 1998.
10. Brandhorst, D.; Brandhorst, H.; Kumarasamy, V.; Maataoui, A.; Alt, A.; Brendel, M. D.; Bretzel, R. G. Hyperthermic preconditioning protects pig islet grafts from early inflammation but enhances rejection in immunocompetent mice. Cell Transplant. 12(8):859–865; 2003.
11. Brandhorst, D.; Hammes, H. P.; Brandhorst, H.; Zwolinski, A.; Nahidi, F.; Alt, A.; Bretzel, R. G. Pretransplant induction of HSP-70 in isolated adult pig islets decreases early islet xenograft survival. Cell Transplant. 9(3):423–430; 2000.
12. Brandhorst, H.; Brandhorst, D.; Brendel, M. D.; Hering, B. J.; Bretzel, R. G. Assessment of intracellular insulin content during all steps of human islet isolation procedure. Cell Transplant. 7(5):489–495; 1998.
13. Contreras, J. L.; Eckstein, C.; Smyth, C. A.; Sellers, M. T.; Vilatoba, M.; Bilbao, G.; Rahemtulla, F. G.; Young, C. J.; Thompson, J. A.; Chaudry, I. H.; Eckhoff, D. E. Brain death significantly reduces isolated pancreatic islet yields and functionality in vitro and in vivo after transplantation in rats. Diabetes 52(12):2935–2942; 2003.
14. Jahr, H.; Hussmann, B.; Eckhardt, T.; Bretzel, R. G. Successful single donor islet allotransplantation in the streptozotocin diabetes rat model. Cell Transplant. 11(6):513–518; 2002.
15. Kawahara, D. J.; Kenney, J. S. Species differences in human and rat islet sensitivity to human cytokines. Monoclonal anti-interleukin-1 (IL-1) influences on direct and indirect IL-1-mediated islet effects. Cytokine 3(2):117–124; 1991.
16. Korbutt, G. S.; Elliott, J. F.; Ao, Z.; Smith, D. K.; Warnock, G. L.; Rajotte, R. V. Large scale isolation, growth, and function of porcine neonatal islet cells. J. Clin. Invest. 97(9):2119–2129; 1996.
17. Landry, J.; Bernier, D.; Chretien, P.; Nicole, L. M.; Tanguay, R. M.; Marceau, N. Synthesis and degradation of heat shock proteins during development and decay of thermotolerance. Cancer Res. 42(6):2457–2461; 1982.
18. Lenzen, S.; Drinkgern, J.; Tiedge, M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic. Biol. Med. 20(3): 463–466; 1996.
19. Matsumoto, K.; Honda, K.; Kobayashi, N. Protective effect of heat preconditioning of rat liver graft resulting in improved transplant survival. Transplantation 71(7):862–868; 2001.
20. Mizuno, N.; Yoshitomi, H.; Ishida, H.; Kuromi, H.; Kawaki, J.; Seino, Y.; Seino, S. Altered bcl-2 and bax expression and intracellular Ca2+ signaling in apoptosis of pancreatic cells and the impairment of glucose-induced insulin secretion. Endocrinology 139(3):1429–1439; 1998.
21. Paraskevas, S.; Maysinger, D.; Wang, R.; Duguid, T. P.; Rosenberg, L. Cell loss in isolated human islets occurs by apoptosis. Pancreas 20(3):270–276; 2000.
22. Perdrizet, G. A.; Rewinski, M. J.; Schweizer, R. T.; Scharp, D. W. Heat shock and recovery protects pancreatic islets from warm ischemic injury. Transplant. Proc. 26(6):3477–3478; 1994.
23. Ricordi, C.; Gray, D. W. R.; Hering, B. J.; Kaufman, D. B.; Warnock, G. L.; Kneteman, N. M.; Lake, S. P.; London, N. J. M.; Socci, C.; Alejandro, R.; Zeng, Y. G.; Scharp, D. W.; Viviani, G.; Falqui, L.; Tzakis, A.; Bretzel, R. G.; Federlin, K.; Pozza, G.; James, R. F. L.; Rajotte, R. V.; DiCarlo, V.; Morris, P. J.; Sutherland, D. E. R.; Starzl, T. E.; Mintz, D. H.; Lacy, P. E. Islet isolation assessment in man and large animals. Acta Diabetol. Lat. 27:185–195; 1990.
24. Rosenberg, L.; Wang, R.; Paraskevas, S.; Maysinger, D. Structural and functional changes resulting from islet isolation lead to islet cell death. Surgery 126(2):393–398; 1999.
25. Ryan, E. A.; Lakey, J. R.; Paty, B. W.; Imes, S.; Korbutt, G. S.; Kneteman, N. M.; Bigam, D.; Rajotte, R. V.; Shapiro, A. M. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. Diabetes 51(7):2148–2157; 2002.
26. Sandberg, J. O.; Margulis, B.; Jansson, L.; Karlsten, R.; Korsgren, O. Transplantation of fetal porcine pancreas to diabetic or normoglycemic nude mice. Evidence of a rapid engraftment process demonstrated by blood flow and heat shock protein 70 measurements. Transplantation 59(12): 1665–1669; 1995.
27. Shindo, Y.; Asanuma, Y.; Furuya, T.; Shibata, S.; Sato, T.; Itoh, H.; Koyama, K. Pretransplant heat loading on pancreatic graft reduces posttransplant ischemia-reperfusion injury. Transplant. Proc. 34(4):1329–1334; 2002.
28. Ulrichs, K.; Meyer, T.; Klock, G.; Chodnewska, I.; Czub, S.; Hamelmann, W.; Beutner, U.; Thiede, A. Monitoring of enzymatic digestions on porcine pancreatic tissue using a simple histological assay. Transplant. Proc. 30(2):355; 1998.
29. Welch, W. J. Mammalian stress response: Cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol. Rev. 72(4):1063–1081; 1992.
30. Welch, W. J.; Suhan, J. P. Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. J. Cell Biol. 101(4): 1198–1211; 1985.
31. Welsh, M.; Eizirik, D. L.; Strandell, E. Heat-shock treatment of mouse pancreatic islets results in a partial loss of islet cells but no remaining functional impairment among the surviving beta cells. J. Mol. Endocrinol. 1(1):27–31; 1988.