insulin, glucagons, and somatostatin with minimal inflammatory cell infiltration (Fig. 4). The secondary grafts from the original donor strain were rejected on days 15, 37, and 42, respectively, which was somewhat delayed when compared to controls (11.9 ± 0.8 days).

Microarray Gene Expression Profile After 3-Day Culture of MMC-Treated Islets

We compared the gene expression profile of MMC-treated and untreated islets cultured for 20 h and 3 days following MMC treatment. The genes expressed in MMC-treated islets followed by 20-h culture were compared to those of islets in culture alone. The fold change (FC) in gene expression of MMC-treated islets was quite similar to that of islets in culture alone when the values were plotted in relation to the expression value of the cultured alone islets (Fig. 5a). Upregulation (FC >2) and downregulation (FC <0.5) of gene expression was detected in 9 and 16 genes, while most of the remaining 1067 genes among 1091 were within the values of $0.5 < FC < 2.0$. On the other hand, prolongation of the culture period to 3 days induced various changes in gene expression, which included the upregulated expression of 442 genes and the downregulated expression of 158 genes with 490 genes within the value of $0.5 < FC < 2.0$ (Fig. 5b). Similarly, MMC treatment followed by 3-day culture resulted in upregulated expression of 236 genes and downregulated expression of 155 genes with 699

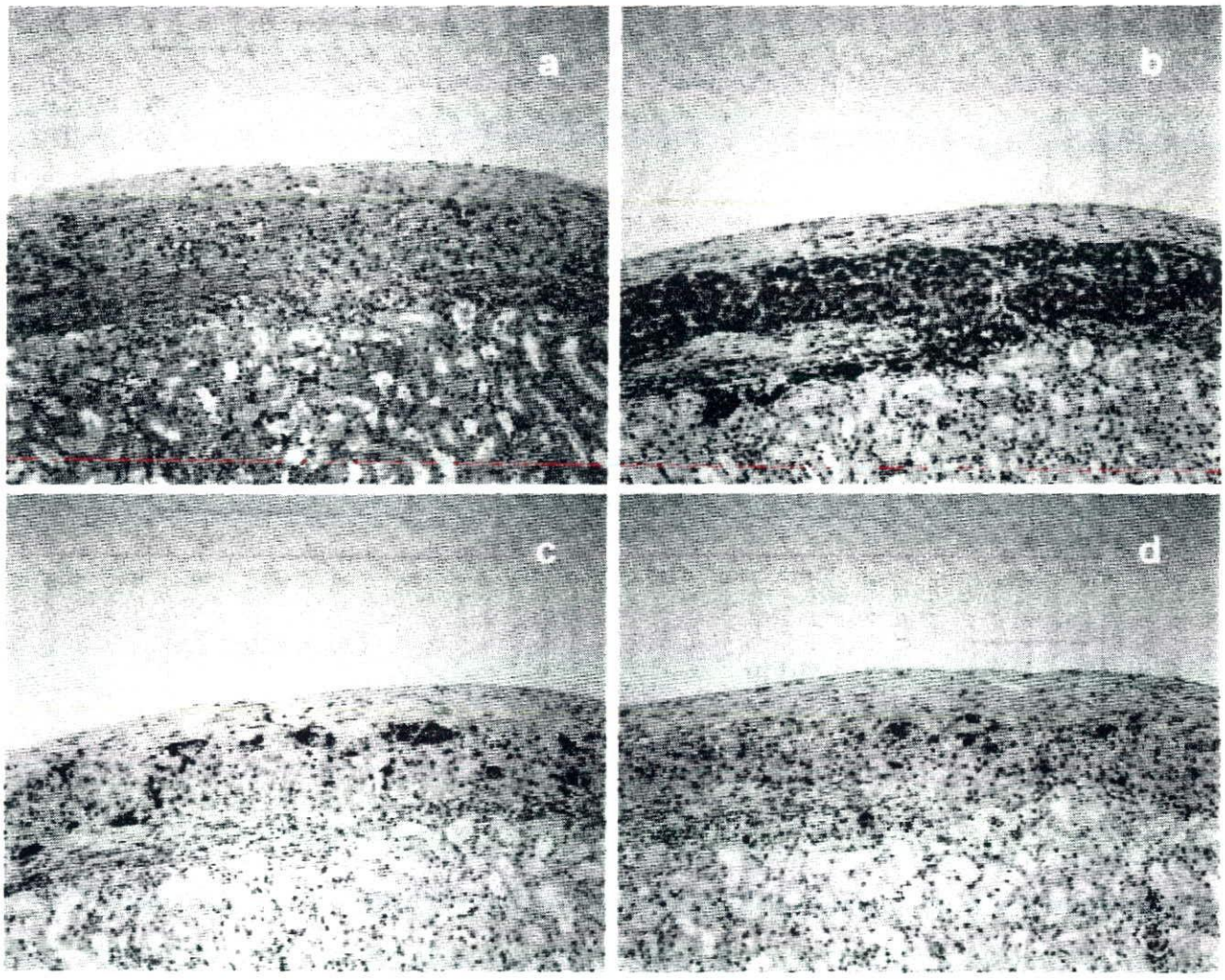

Figure 4. Immunohistological study of long-term functioning graft. One (No. 7557) of the long-term functioning xenografts was sacrificed on day 135 postgrafting and stained with H&E (a), anti-insulin (b), anti-glucagon (c), and anti-somatostatin (d) antibodies (original magnification ×40). The islet xenografts showed intact hormone-containing cells with minimal infiltration of inflammatory cells.

genes being within the value of $0.5 < FC < 2.0$ (Fig. 5c), suggesting that MMC treatment tended to downregulate the expression of many genes.

To determine the MMC-treatment associated gene expression profile rather than culture related effect, we compared the gene expression of MMC-treated and nontreated islets in culture for 3 days (Fig. 5d). Twenty-five upregulated genes (FC >5) were found in MMC-treated islets, with an expression value of >100 (Table 1). Furthermore, three downregulated (FC <0.2) genes were identified in cultured islets with an expression value of >100 (Table 1). Among upregulated genes, TGF-β, as well as type II activin receptor, which binds TGF-β superfamily, were both highly upregulated following MMC treatment compared to culture alone.

TGF-β Protein Expression of MMC-Treated and 3-Day Cultured Islets

To determine whether the strong upregulation of TGF-β mRNA expression detected in MMC-treated islets was associated with production of TGF-β protein within the islets, we analyzed TGF-β protein expression in MMC-treated islets (Fig. 6). Although vital staining of islets in both groups showed relatively compact shapes with some islets having PI-positive areas in the center, the process of fixing and embedding in paraffin affected the vulnerability of nontreated islets, compared to MMC-treated islets. TGF-β was expressed strongly in peripheral areas of MMC-treated islets (grade 4) compared with those of nontreated islets (grade 3).

DISCUSSION

We previously reported that crude digested islets pretreated with MMC prolonged graft survival time in a xenogeneic rat-to-mouse model when they were cultured at 37°C for 20 h (8). While the difference was significant, all xenografts eventually showed signs of rejection within 35 days. In this study, we extended the culture period up to 7 days. Marked prolongation of graft survival time was noted when MMC-treated islets were

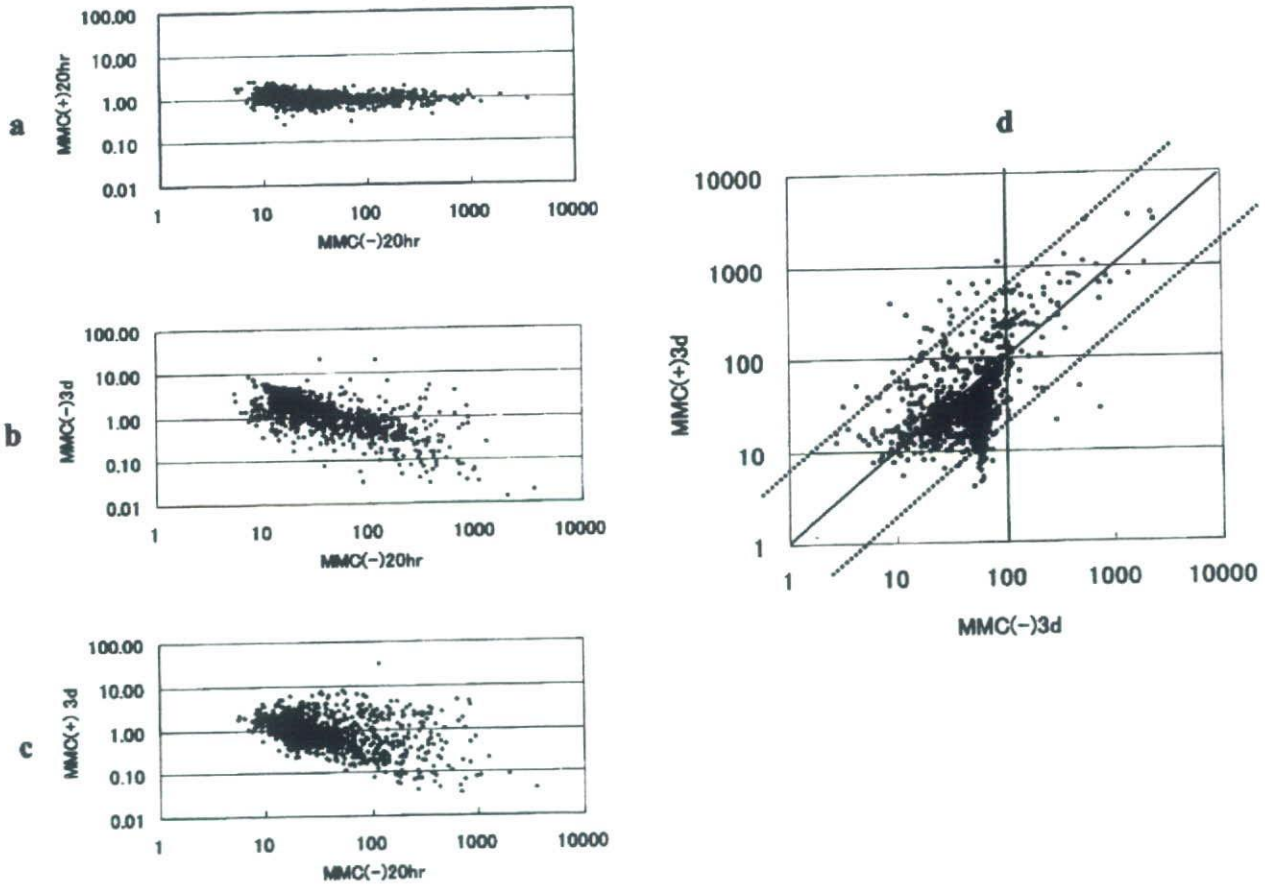

Figure 5. Gene expression of MMC-treated islets followed by 20-h or 3-day culture. Expression levels of various genes in MMC-treated islets cultured for 20 h (a) or 3 days (b) and those of untreated islets cultured for 3 days (c) were compared to those in untreated islets cultured for 20 h. Gene expression of MMC-treated and nontreated islets in culture for 3 days were compared between the two groups (d). Data are fold changes at 5, 1, or 0.2.

Table 1. Marked Up- or Downregulated Genes Following MMC Treatment and Culture for 3 Days Over Culture Alone

Affy No.	Genbank No.	Gene Title	Functions	Fold Change
Upregulated				
705	U03491	transforming growth factor-β3 (TGF-β3)	growth factors, cytokines, and chemokines	5.01
741	M32167	glioma-derived vascular endothelial cell growth factor	growth factors, cytokines, and chemokines	7.30
152	M35105	ros1 proto-oncogene	growth factor & chemokine receptors	5.09
534	X61479	macrophage colony-stimulating factor I receptor (CSF1R)	growth factor & chemokine receptors	6.57
536	U54791	LCR-1; G protein-coupled receptor	growth factor & chemokine receptors	5.23
559	S48190	type II activin receptor	growth factor & chemokine receptors	8.27
562	M84009	dopamine receptor D4 (D4 receptor; DRD4)	neurotransmitter receptors	24.01
1067	S47609	adenosine A2A receptor (ADORA2A)	other receptors (by ligands)	20.28
158	M86389	heat shock 27-kDa protein (HSP27)	heat shock proteins	6.15
162	X96394	multidrug resistance protein (MRP)	drug resistance proteins	8.83
750	U10156	growth hormone-releasing hormone (GHRH)	neuropeptides	11.36
896	L29090	guanine nucleotide-binding protein G(i)/G(s)/G(t) beta subunit 3	G-proteins	13.21
892	L19699	Ral B; GTP-binding protein	GTP/GDP exchangers and G-protein; GTPase activity modulators	7.17
1098	U57715	fibroblast growth factor receptor-activating protein 1 (FRAG1)	adaptors and receptor-associated proteins	10.65
453	M17086	cAMP-dependent protein kinase type I alpha regulatory subunit (PRKAR1A)	kinase activators and inhibitors	11.74
353	L20822	syntaxin 5 (STX5)	targeting	6.44
355	M95735	syntaxin 1B (STX1B)	targeting	7.19
350	D28512	synaptotagmin III (SYT3)	general trafficking	15.55
178	J02627	cytochrome P450 2E1 (CYP2E1)	simple lipid metabolism	9.06
373	M64797	testis fructose-6-phosphate 2-kinase/fructose 2,6-biphosphate (testis 6PF-2-K/fru-2,6-P2ase)	simple carbohydrate metabolism	10.51
376	AF019973	neuron-specific enolase (NSE)	energy metabolism	43.58
186	D83044	organic cation transporter 2 (OCT2)	xenobiotic transporters	5.28
184	AF008221 + AB004559	renal organic anion transporter (ROAT1) + multispecific organic anion transporter (OAT1)	xenobiotic transporters	11.51
253	M88751	voltage-gated dihydropyridine-sensitive L-type calcium channel beta 3 subunit (CCHB3)	voltage-gated ion channels	8.81
977	M16736	growth-accentuating protein 43 (GAP43)	functionally unclassified	8.85
Downregulated				
56	D26307	junD proto-oncogene	basic transcription factors	0.10
809	D31873	LIM domain kinase 1 (LIMK1)	nonreceptor protein kinases	0.07
983	L12382	ADP-ribosylation factor 3 (ARF3)	trafficking/targeting proteins	0.03

transplanted after 40 h or 3 days in culture following MMC treatment. Half of rat islet grafts survived indefinitely in B6 recipient mice with chemically induced diabetes. Although culture alone induced some prolongation of graft survival, the grafts were all eventually rejected.

Various modalities have been used to immunomodulate islet graft survival. These include islet culture (11,25), ultraviolet irradiation (UV) (15), specific monoclonal antibodies such as anti-Ia (4), anti-dendritic cells (5), and anti-ICAM-1 molecules (7). However, it has been difficult to produce indefinite survival of xenoislets without host immunosuppression except in one report by Hardy et al. (9), who showed that UV-irradiated Lew

islet xenografts could survive indefinitely in B10.BR recipient mice. However, this was not the case when the same islets were grafted into Balb/C mice, which eventually rejected them before 90 days postgrafting. In most of these experiments as well as those reported by Hardy et al., handpicked islets were used, which are less immunogenic compared to crude-digested islets that contain highly immunogenic contaminants. In the present study, we used crude-digested islets to test the feasibility of a treatment modality for preclinical islet transplantation.

We investigated the appropriate dose of MMC necessary to induce graft prolongation but not islet toxicity. We reported that MMC at doses of 10, 32, 50 and 100 μg/ml are effective for prolongation of graft survival, while glucose metabolism posttransplantation deteriorated when the dose exceeded 32 μg/ml in a rat-to-mouse combination (8). In a mouse model, a significant adverse effect was detected in both isografts and allografts when the dose of MMC was >32 μg/ml at 3 to 5 days postgrafting, whereas no adverse effect was found in mice bearing long-term functioning isografts regardless of the dose of MMC (3.2, 10, 32, 100 μg/ml) (17).

To consider the application of this treatment for human patients, it is necessary to evaluate the effect of MMC treatment on islets prior to transplantation. In this study, we examined islet viability by vital staining of islets and insulin secretory capacity in response to glucose and compared those data with previous in vivo findings. Vital staining using AO and PI demonstrated that MMC at doses <32 μg/ml appeared to be nontoxic without increasing the number of AO-positive cells. Insulin secretory capacity in response to glucose, which is a gold standard test for islet function in vitro, showed that the stimulation index was maintained when the MMC dose was <32 μg/ml. Both in vitro studies indicated that MMC treatment at a dose <32 μg/ml is nontoxic and preserves islet function at 20 h following MMC treatment. This finding correlates well with the

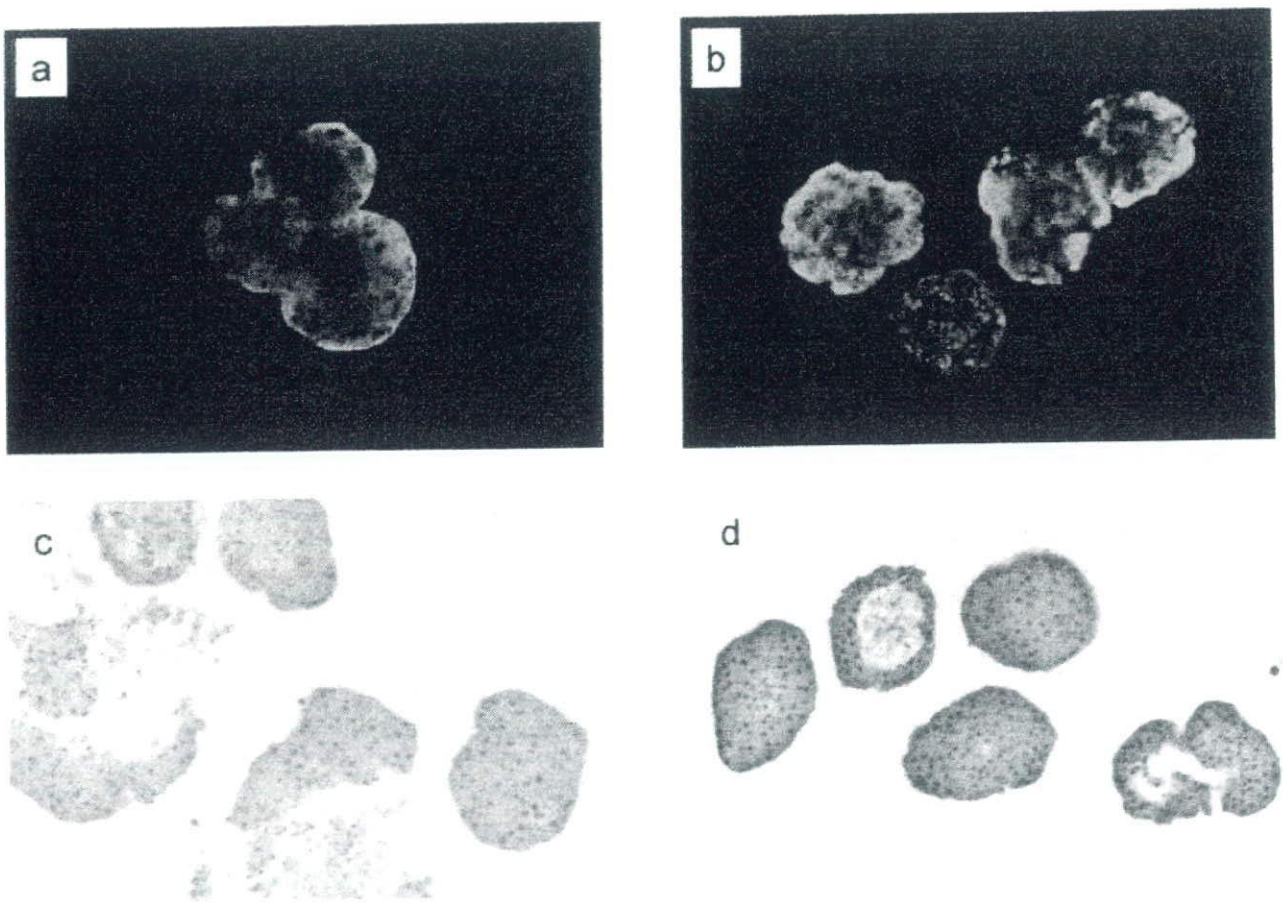

Figure 6. Vital staining and immunohistological study of MMC-treated and nontreated islets cultured for 3 days. MMC-treated (b, d) and nontreated islets (a, c) that were cultured for 3 days were stained by PI and AO (a, b), and were immunohisotologically studied using anti-TGF-β antibody (c, d). Vital staining of islets in both groups showed relatively compact shape with some islets having PI-positive areas in the center. TGF-β was expressed strongly in peripheral areas of MMC-treated islets as compared with those of nontreated islets.

previous results of an in vivo study (17), suggesting that these modalities could be applicable for testing viability after MMC treatment in human islet preparations.

Previous studies showed that culture alone did not induce indefinite survival of rat islets in mouse recipients even with a variety of modifications, including temperature (11), high oxygen concentration (14), and culture duration (25). This study also showed that prolongation of the culture period alone induced a significant, but only marginal, effect on protection of grafted islets from immune destruction. MMC treatment had a significant impact on graft survival time over culture for 20 and 40 h and 3 days, but not for 4 h or 7 days. Thus, there is a critical window in the post-MMC culture period that is necessary for significant graft survival. We previously showed that transient upregulation of TGF-β was responsible for prolongation of graft survival in a study using MMC-treated and 20-h cultured islets (10). Extending culture periods up to 40 h or 3 days induced further prolongation of graft survival time. Furthermore, some kind of unresponsiveness was induced in animals bearing long-term functioning grafts.

To determine the effect of MMC treatment on islets during the culture period, we examined the gene expression profiles of MMC-treated islets and found 25 relatively high-grade upregulated genes and 3 relatively high-grade downregulated genes. The highly upregulated genes (FC >20) included neuron-specific enolase, dopamine receptor D4, and adenosine A2A receptor, which were reported to be involved in the glycolytic pathway (18), neural–immune interactions (20), and signaling reactions (13), respectively. Interestingly, TGF-β, as well as type II activin receptor, which binds TGF-β superfamily (24), were both highly upregulated following MMC treatment compared to culture alone. TGF-β superfamily of ligands and receptors are known to stimulate cellular events in diverse processes ranging from cell fate specification during development to immune suppression (16). Data of microarray analysis were consistent with the immunohistological study of MMC-treated islets in which TGF-β upregulation was demonstrated after culture for 3 days. There have been some reports on various gene expressions of pancreatic islets during culture (3) or tolerated islets after transplantation (12); however, it is not yet determined which gene expression would be responsible for tolerance induction. In this study we were able to show that MMC treatment induced a variety of up- or downregulated genes. One of the responsible genes is upregulated TGF-β, and others hopefully will be identified in the future.

In conclusion, MMC pretreatment of rat islets and culture for 3 days at 37°C induced marked prolongation of graft survival in nonimmunosuppressed recipient mice, with half of the grafts surviving indefinitely. This effect was obtained within a specific culture period and was supported by microarray gene profile analysis. The results of these two manipulations were reproducible and may offer a strategy for the preclinical application of this protocol in human islet transplantation.

ACKNOWLEDGMENTS: This work was supported in part by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology and in part by a Grant-in-Aid for Research on Human Genome, Tissue Engineering Food Biotechnology, Health Sciences Research Grants, Ministry of Health, Labor and Welfare of Japan.

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REPUBLISHED PAPER

Pancreas preservation for pancreas and islet transplantation

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Current Opinion in Organ Transplantation 2008, 13:445–451

Purpose of review

To summarize advances and limitations in pancreas procurement and preservation for pancreas and islet transplantation, and review advances in islet protection and preservation.

Recent findings

Pancreases procured after cardiac death, with in-situ regional organ cooling, have been successfully used for islet transplantation. Colloid-free Celsior and histidine-tryptophan-ketoglutarate preservation solutions are comparable to University of Wisconsin solution when used for cold storage before pancreas transplantation. Colloid-free preservation solutions are inferior to University of Wisconsin solution for pancreas preservation prior to islet isolation and transplantation. Clinical reports on pancreas and islet transplants suggest that the two-layer method may not offer significant benefits over cold storage with the University of Wisconsin solution: improved oxygenation may depend on the graft size; benefits in experimental models may not translate to human organs. Improvements in islet yield and quality occurred from pancreases treated with inhibitors of stress-induced apoptosis during procurement, storage, isolation or culture. Pancreas perfusion may be desirable before islet isolation and transplantation and may improve islet yields and quality. Methods for real-time, noninvasive assessment of pancreas quality during preservation have been implemented and objective islet potency assays have been developed and validated. These innovations should contribute to objective evaluation and establishment of improved pancreas preservation and islet isolation strategies.

Summary

Cold storage may be adequate for preservation before pancreas transplants, but insufficient when pancreases are processed for islets or when expanded donors are used. Supplementation of cold storage solutions with cytoprotective agents and perfusion may improve pancreas and islet transplant outcomes.

Keywords

islet isolation, islet transplants, pancreas preservation, pancreas procurement, pancreas transplants

Curr Opin Organ Transplant 13:445–451
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1087-2418

Introduction

Pancreas and islet transplants are two options for patients who, because of serious diabetic conditions, will benefit from β-cell replacement. The numbers of clinical pancreas transplants have increased exponentially and outcomes have improved markedly, especially during the past two decades [1]. The improvements in outcomes are attributed to better recipient care with respect to surgical techniques and immunosuppressive regimens and to better organ procurement and preservation protocols. In this review, we summarize the recent literature regarding the current state-of-the-art in pancreas procurement and preservation. Our focus is on pancreas preservation before islet isolation, which appears to have more stringent requirements than pancreas preservation for whole-pancreas transplants, due to the added stresses experienced by the islets during the isolation and purification process. In addition, we provide a brief review of current advances in islet protection and preservation during isolation and culture.

Pancreas procurement

There are several well established techniques for multi-organ procurement. The two main techniques for pancreas are en-bloc procurement, and in-vivo dissection [2].

In a recent report, Brockmann *et al.* [3•] performed a meta-analysis of the literature on organ procurement for transplants and favored rapid en-bloc removal of the abdominal organs with separation on the back table. Dalle Valle *et al.* [2] reported that pancreas grafts procured by in-vivo dissection and in-situ separation

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maintained excellent quality. Dalle Valle noted that successful in-vivo dissection was influenced by the experience and ability of the operating surgeons, more so than when the back-table technique was used, suggesting that the back-table technique might be preferred in the absence of a dedicated team of experienced surgeons.

The techniques described above are most likely sufficient for organ procurement from brain-dead donors, but they may be inadequate for procurement of organs from donors after cardiac death. The increasing demand for, and limited availability of, organs for transplants have prompted the use of pancreases procured from donors after cardiac death [4]. Such pancreases have also recently been successfully processed for islets and used for clinical islet transplants in Japan [5,6••,7,8•]. The in-situ regional organ cooling system originally developed for kidney procurement [9] was modified for pancreas procurement [8•]. After confirmation of brain death, a double-balloon catheter is inserted into the aorta through the femoral artery; the tip of the double-balloon catheter is placed above the level of the celiac axis [9]. Ultrasound is used to confirm balloon placement. In order to prevent warm ischemic damage, regional in-situ cooling of the pancreas and kidney is achieved by infusion of a hypothermic preservation solution. The flush is initiated immediately after the donor's cardiac arrest and continued until organ procurement is complete. A venous catheter is also placed in the inferior vena cava via the femoral vein to vent the perfusate.

Pancreas preservation for pancreas transplantation

The two main methods used for experimental and clinical organ preservation are static cold storage, which we will simply refer to as cold storage, and machine perfusion [10–20,21•,22•,23–34,35•,36,37•,38–44]. The hypothermic pulsatile machine perfusion technique originally developed by Carrel and Lindbergh in 1935 [45–47] has been widely used for clinical kidney transplants [48] but not for clinical pancreas preservation.

Early experiments with canine segmental pancreas grafts, reported by Florack *et al.* [16], demonstrated that failure rates with machine perfusion were 30% at 24 h and 40% at 48 h. There were no failures at 24 and 48 h with cold storage. These results, along with the complexities associated with machine perfusion of the pancreas, have made cold storage the preferred and most widely used method for pancreas preservation [44].

Pancreases preserved with cold storage and transplanted immediately as vascularized grafts nearly always restore insulin independence in the recipient. When pancreases are preserved for islet transplantation, two to three donor pancreases may be required per recipient to achieve insulin independence [49], in some but not all centers [50].

The need for more than one donor pancreas per recipient for islet transplants may be attributed to at least three problems: first, to the lower quality of pancreases selected and offered for islet transplants compared with those organs offered for whole-pancreas transplantation; second, to exposure of islets to a series of damaging physicochemical stresses during isolation that may amplify the damage caused during cold storage; and third, to the further damage of islets during purification and culture [38].

University of Wisconsin solution (UWS) has been the standard preservation solution for pancreas transplantation for almost 20 years [36]. Recently, multiple reports have suggested that other preservation solutions may be effective alternatives to UWS [18–20,22•].

In 2006, Englesbe *et al.* [21•] reported the results of a multicenter study using histidine-tryptophan-ketoglutarate (HTK). The study population consisted of 77 consecutive pancreas recipients: 41 were in the UWS group and 36 were in the HTK group. Pancreas graft function, at 90 days posttransplant, the technical graft loss rates, and the pancreatic leak rates were similar between the groups, with no significant differences in postoperative amylase and lipase levels. Similarly, in 2007, Becker *et al.* [22•] reported no significant differences in patient survival or graft survival between UWS ($n=47$) and HTK ($n=48$) groups. Furthermore, peak lipase on postoperative day 1, serum amylase and C-reactive protein were not significantly different between the two groups. Some reports, including the one by Becker *et al.* [22•], have indicated that HTK-flushed pancreata appeared more edematous [18–20,21•,22•]. However, this edema did not appear to impair early graft function [22•].

Celsior, an extracellular, low-viscosity preservation solution originally designed for heart transplantation has also been used for experimental pancreas [23–25] and other organ preservation [26–32]. For whole-pancreas transplantation, the use of Celsior has been controversial [23–25]. Baldan *et al.* [24] demonstrated that Celsior was an effective alternative to UWS for pancreas procurement in a pig autotransplant model, whereas Uhlmann *et al.* [25] reported, using the same model, that the use of Celsior was associated with increased ischemia-reperfusion injury, when compared with UWS. Recently, in a pig allotransplant model, Garcia-Gil *et al.* [33] demonstrated that lipid peroxidation after reperfusion of pancreases preserved in Celsior and UWS was similar.

The first prospective, randomized study comparing UWS ($n=50$) with Celsior ($n=50$) for clinical pancreas

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transplants was recently reported by Boggi *et al.* [34]. The authors demonstrated that Celsior and UWS had similar safety profiles for pancreas preservation. Another study reported by Manrique *et al.* [35•] comparing Celsior ($n = 28$) with UWS ($n = 44$) for pancreas preservation demonstrated that 2-year recipient survival rates, 2-year graft survival rates, pancreas leakage rates, and clinical graft pancreatitis rates were similar in both groups.

Pancreas preservation for Islet transplantation

UWS has been used since the 1980s as the pancreas preservation solution [36] for clinical islet transplants. Salehi *et al.* [37•] recently reported that islet yields from human pancreases preserved in HTK or UWS were equivalent. A recent study by Hubert *et al.* [51•], demonstrated that the islet isolation yields, as measured in islet equivalents per gram pancreas, from pancreases preserved with Celsior solution were 2.1-fold lower than those obtained when UWS was used ($P < 0.05$). Based on these results, Hubert *et al.* [51•] suggested that colloid-free preservation solutions might be suboptimal for pancreas perfusion and cold storage prior to islet isolation and transplantation [38].

Two French groups recently demonstrated the possibility of clinical application of solution de conservation d'organes et de tissus (SCOT), which has been shown to have some immunoprotective effects on islet cells. SCOT, an extracellular solution containing polyethylene glycol (PEG), is an oncotic agent, which may induce immunocamouflage of the graft's surface antigens [52]. In addition, Hubert *et al.* [51•] demonstrated that cell swelling and pancreas edema were not significant following 12 h of cold storage with SCOT, compared with UWS – a finding that may be related to the presence of PEG. Giraud *et al.* [53] demonstrated that SCOT could improve islet yield when used during isolation and could prolong islet allograft survival without immunosuppression when used for culture, as compared with control solutions.

There is consensus among the major islet transplantation centers that islet yields and quality can be improved with better pancreas procurement techniques and by the use of cold-preservation techniques that are not necessarily needed for whole-pancreas transplants. The two-layer method (TLM) for pancreas preservation is an example of a coordinated effort to improve islet yield and quality by improving pancreas oxygenation during preservation. In 2002, the University of Minnesota [38], University of Miami [39], and University of Alberta [40,41] reported that the TLM improved islet yield and increased islet transplant opportunities. Since then, the TLM has been widely used by islet transplant centers worldwide.

The mechanisms by which the TLM improves human islet yield and quality are not fully understood. A standard explanation is the improved oxygenation of the pancreas during cold storage with the TLM. It has been suggested that during islet isolation and transplantation, apoptosis is initiated and executed mainly through the mitochondrial and mitogen-activated protein kinase (MAPK) pathways [54–56]. Matsuda *et al.*, using a rat model [57], and Ramachandra *et al.*, using human pancreases [58••], demonstrated improved islet yield from pancreata preserved with the TLM. These results may be attributed to inhibition of apoptosis mediated by the mitochondrial pathway.

Noguchi *et al.* [59•], using a pig model, reported that the islet yield from pancreases preserved with the TLM using a modified ET-Kyoto solution (Otsuka Pharmaceutical Factory, Inc., Tokyo, Japan) was significantly higher when compared with the TLM using UWS. Interestingly, Noguchi *et al.* found no significant difference in islet viability, in-vitro or in-vivo function between the two preservation methods [59•]. They also recently demonstrated improvements in islet isolation using M-Kyoto solution [60•] instead of UWS [61] for ductal injection. They hypothesized that M-Kyoto solution is less likely to inhibit collagenase activity than UWS [62].

Brandhorst *et al.* [63], demonstrated that a simpler preservation method, the one-layer method (OLM), using oxygenated perfluorocarbon, could be used as an alternative to the TLM. They suggested that short-term storage in oxygenated perfluorocarbon improved the in-vitro but not the in-vivo function of pig islets that were damaged by warm ischemia. Three hours of additional pancreas 'oxygenation' with the OLM significantly increased ATP content in pig islets exposed to 30 min of warm ischemia, resulting in recovery of in-vitro function but not in significant improvements in posttransplant function [64•]. These observations are quite interesting as the perfluorocarbon does not contain any substrate for ATP regeneration, does not contain any additives with antioxidant properties, and does not include any of the compounds that are expected to prevent cellular edema during cold storage.

In contrast to observations by Brandhorst *et al.* in the porcine model, Kuroda's group [65], using a rat transplant model, demonstrated that pancreata damaged by 30 min of warm ischemia were restored after 3 h of TLM preservation [63]. These discrepancies may be explained by species-dependent differences in pancreas size and texture [66•]. Porcine and human pancreata are much thicker, and are often covered by significant amounts of fat, as compared with canine pancreases. Papas *et al.* [67], using a porcine model, demonstrated that only 15% of the total pancreatic volume was oxygenated during TLM

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preservation with oxygen-saturated perfluorocarbon, suggesting the need for substantial improvement in pancreas oxygenation and preservation, even when the TLM is used.

In a recent large-scale clinical study, the Edmonton group [68•] demonstrated no beneficial effect of the TLM with preoxygenated perfluorocarbon on islet isolation and islet transplant outcomes ($n = 75$). In addition, the Uppsala group [69••] recently reported results from 200 islet isolations, and found that the TLM did not improve islet isolation or clinical outcome posttransplant.

The 2007 Annual Report of the Collaborative Islet Transplant Registry (CITR) [70] provided information on islet characteristics with regard to pancreases preserved by the TLM or UWS. The data were collected from the 31 active islet transplant programs in North America from 1999 to 2006. According to the data, islet yields did not significantly differ between pancreata preserved with the TLM ($366{,}467 \pm 11{,}418$, $n = 161$) or UWS ($390{,}532 \pm 7{,}440$, $n = 330$). Additional information on islet viability did not reveal any substantial differences between the two groups. The membrane integrity tests, however, utilized for generating the reported viability data are known to be insensitive [71]. In addition, the retrospective analysis reported by the CITR did not indicate the exact methods of perfluorocarbon oxygenation in the TLM, (saturated compared with continuous oxygen supply), the duration of cold ischemia in the two groups, or any relationship or overlap between the pancreas procurement teams and the processing and infusion teams.

The different perfluorocarbon oxygenation methods (presaturated without replenishment compared with continuous oxygen supply) are unlikely to explain the controversial findings on the benefits of the TLM in pancreas preservation. Even in the best-case scenario for oxygenation (perfluorocarbon continuously bubbled and fully saturated with oxygen), the oxygen penetration depth cannot be expected to exceed 1 mm, leaving a large portion of human and porcine pancreata oxygen-limited [66•,67].

It is hypothesized that more sophisticated preservation protocols may be necessary and should replace cold storage, with or without TLM, in order to improve islet yields and quality. This is supported by recent data [73•] indicating that machine perfusion of porcine pancreases for 24 h improved isolation yields by approximately three-fold compared with cold storage and by approximately two-fold compared with fresh procurement. The mechanisms behind these improvements are unclear; it has been suggested that edema caused by perfusion may facilitate more efficient islet isolation [73•]. In addition, intermittent capillary perfusion at low flow rates may facilitate pancreas preservation by maintaining better functional capillary density, compared with cold storage [74]. Capillary perfusion may be important for pancreas transplants, but may be less of an issue for islet transplants.

The implementation of recently developed tools for pancreas and islet quality assessment [71,72•] is expected to contribute to more thorough and sensitive evaluation of existing, as well as much-needed new, pancreas preservation strategies, and perhaps help answer some of the long-standing questions in the field.

Islet protection and preservation

Goto *et al.* recently exploited the ability of perfluorocarbon to store high amounts of oxygen to better oxygenate islets during the isolation process [75•]. They demonstrated that the use of oxygenated perfluorocarbon during islet isolation resulted in improved islet yield and quality, which suggests that hypoxia during islet digestion may damage islets [75•].

Ichii *et al.* [76••] demonstrated that the addition of nicotinamide to the processing medium significantly improved islet yield and increased the success rate of isolation. Nicotinamide, a cytoprotective compound, may ameliorate injuries caused by oxidative stresses and various cytokines. In addition, nicotinamide supplementation of the processing medium reduced the islet production of tissue factor, which may trigger thrombotic reactions after portal islet infusion. Nicotinamide also reduced the islet production of macrophage chemoattractant protein (MCP-1), which has a potent chemotactic activity for monocytes. Korsgren's group [77] demonstrated that nicotinamide supplementation of culture medium was effective in reducing both tissue factor and MCP-1 production by islets, which led to the inhibition of the instant blood-mediated inflammatory reaction when islets came into contact with blood.

Islet isolation activates the c-Jun NH2-terminal kinase (JNK), a member of the stress-activated group of MAPKs [55,56]. Noguchi *et al.* investigated the efficacy of a JNK inhibitory peptide (JNKI) to inhibit cell apoptosis in islets isolated from pancreata preserved with the TLM [78,79]. They demonstrated that JNKI prevented islet cell apoptosis induced immediately after isolation and that JNKI improved islet yield and islet graft function after 1 day of culture [78,79]. Similar findings were recently reported by Ito *et al.* using an inhibitor of p38 MAPK during islet preservation [80].

Conclusion

The management of donor pancreas preservation affects clinical outcomes in pancreas, and especially islet,

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transplant recipients. In-situ regional organ cooling may protect the pancreas from warm ischemic injury when managing donors after cardiac death. Colloid-free preservation solutions such as Celsior and HTK, have been demonstrated to perform comparably to UWS when used for pancreas preservation for subsequent pancreas transplants. Colloid-free preservation solutions were reported to be inferior to UWS for preservation of pancreases intended to be used for islet isolation. Pancreas preservation for islet isolation and transplants may have different requirements: cold storage with or without the TLM may be insufficient. Recent studies with large numbers of pancreases did not demonstrate significant differences in islet yields and in clinical islet allotransplant outcomes with pancreases stored with TLM when compared with cold storage. Pancreas size and texture variations and methods of perfluorocarbon oxygenation may influence the oxygenated volume fraction and the quality of the preserved pancreas. Agents that block stress-signaling pathways and that may interfere with apoptosis, such as JNK and p38 inhibitors, may improve islet yield and quality. Nicotinamide supplementation of the medium has also been reported to be effective for preserving islets. The lack of real-time, quantitative objective tools for assessing the quality of pancreases during and after cold storage and the quality of islets after isolation has hindered progress. The recent development and implementation of such tools [71,72•] is expected to contribute significantly, in the near future, to needed advances in preservation of pancreases to be used for islet isolation and transplantation.

Acknowledgements

This work was partially supported by a grant from the National Center for Research Resources (U42 RR016598) National Institutes of Health (NIH), and a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (R43 DK070400) NIH.

We acknowledge the contributions of Dr Bernhard Hering for critical review, and Dr Raja Kandaswamy for consultation, as well as Mary Knatterud and Lauren Rogers for preparation of the manuscript.

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レクチャー　1型糖尿病診療の update

6 糖尿病に対する外科的治療法
—膵臓移植と膵島移植—

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●Key Words● 膵臓移植，膵島移植，インスリン離脱

Summary

●膵臓移植は全身麻酔下の手術が必要で侵襲度は大きいが，1度の移植で高いインスリン離脱率を維持可能である．膵島移植はインスリン離脱に複数回の移植が必要で，インスリン離脱したとしてもそれを長期間維持するのは難しい．しかし，高い安全性と血糖値の不安定性に対する治療効果は確認されている．

●膵臓移植と膵島移植の選択については，両者のメリット・デメリットおよび患者の病期を考慮して選択されるべきである．また，医学的に耐術性がない，あるいは膵臓移植の適応から外れる症例でも，膵島移植なら適応となる場合がある．

はじめに

膵臓移植と膵島移植はどちらも1型糖尿病等のインスリン依存状態糖尿病に対する外科的治療法で，膵β細胞を補充し適正なインスリン分泌を再開することによって糖代謝を正常化させることを目的としている．糖代謝の正常化により血糖値は安定化しquality of life（QOL）は劇的に向上する．また，糖尿病性合併症の発症・進展を予防することにもなる．膵臓移植は臓器移植であるのに対して，膵島移植は膵臓から膵島（＝ランゲルハンス島，ラ氏島）のみを分離して移植する細胞（組織）移植である．

本稿では，膵臓移植と膵島移植の現況と，それらの選択について述べる．

膵臓移植

膵臓移植は1966年米国ミネソタ大学において世界で初めて行われた．1度の移植で高いインスリン離脱率を維持可能で，特に膵腎同時移植ではQOLの改善のみでなく救命・延命効果も認められ，糖尿病性腎不全に対する根治療法として定着している．

膵臓移植の分類

膵臓移植は，ドナーによって，①脳死ドナー膵臓移植，②心停止ドナー膵臓移植，③生体ドナー膵臓移植に分類できる．大多数が脳死ドナー膵臓移植であるが，心停止ドナー膵臓移植も少数の報告例がある．生体ドナー膵臓移植は健常なドナーの膵体尾部を用いるものであるが，死

体ドナー数が極端に少ない日本では最近その数が増えてきている．

次に術式によって，①膵腎同時移植（Simultaneous Pancreas Kidney Transplant：SPK），②腎移植後膵移植（Pancreas Transplant after Kidney：PAK），③膵単独移植（Pancreas Transplant Alone：PTA）に分類できる．膵腎同時移植（SPK）は糖尿病性腎不全患者に対して膵臓と腎臓を同時に移植するものである．腎移植後膵移植（PAK）は糖尿病性腎不全で予め腎移植を受けた患者に対して膵臓移植を追加するものである．膵単独移植（PTA）は非腎不全糖尿病患者に対して膵臓のみを単独で移植するものである．

膵液のドレナージ法には膀胱ドレナージ法（Bladder Drainage：BD）と腸管ドレナージ法（Enteric Drainage：ED）がある．膀胱ドレナージ法（BD）は膵頭部に付いている十二指腸をレシピエントの膀胱に吻合して膵液を膀胱内に誘導する方法で，尿中のアミラーゼ値をモニターリングすることで，拒絶反応の補助診断が可能という長所がある．しかし，感染などの尿路系合併症が多くなる．一方，腸管ドレナージ法（ED）は，十二指腸をレシピエントの小腸に吻合して膵液を腸管内に誘導する生理的な方法である．近年，免疫抑制法が進歩したこと，またBD施行後に尿路系合併症によるEDへの変更手術（enteric conversion）が必要となる症例がしばしばあることから，EDが多くなっている．

静脈血の還流法には，グラフトの静脈をレシピエントの腸骨静脈に吻合する大循環系ドレナージ法（Systemic Drainage）と上腸間膜静脈に吻合して門脈系に環流させる門脈系ドレナージ法（Portal Drainage）がある．

膵臓移植後合併症

移植後早期の合併症として，移植した膵臓の静脈（門脈）または動脈内に血栓ができる場合があり，移植膵臓の摘出が必要となることがある．他に移植膵膵炎，感染がある．感染が進んで移植膵臓の摘出が必要になる場合もある．レシピエントが死に至るといった重篤なものもある．

BDにおいては膵液がそのまま尿として排泄されるので，脱水や膵液中の重炭酸の喪失による代謝性アシドーシスを来す．高度な場合は，Enteric Conversionが必要なことがある．また，尿路感染と排尿障害が起こることがある．

世界の膵臓移植の現況

国際膵臓移植登録（International Pancreas Transplant Registry：IPTR）によると最近では世界で年間1,700件前後実施され，2006年12月の時点で累積症例数は26,000例を越している．膵臓移植のカテゴリー別の成績（2000年1月から2004年6月）は，患者1年生存率はSPK 95%，PAK 95%，PTA 98%，グラフト1年生着（インスリン離脱）率はSPK 85%，PAK 78%，PTA 76%である．5年生着（インスリン離脱）率はそれぞれSPK 69%，PAK 58%，PTA 58%である[1]．各術式数をみると，SPKが71%，PAKが21%，PTAが8%を占めていて，多くの場合SPKが行われている．

日本の膵臓移植の現況

日本では，臓器移植法施行前に1984～1994年までに15例の膵臓移植が行われた．1997年

の臓器移植法施行以降は 2007 年 12 月末までに 58 例が行われた．内訳は脳死ドナー移植 42 例，心停止ドナー移植 2 例，生体ドナー移植 14 例である．カテゴリー別でみると，SPK46 例，PAK6 例，PTA6 例である．日本の膵臓移植はコンディションが良くないドナー（marginal donor）の膵臓を使用することが多いにもかかわらず，欧米と遜色ない移植成績である．

2006 年 4 月 1 日より脳死および心停止ドナーを用いた膵臓移植は保険適応となっている．

膵島移植

膵島移植は，膵島（ランゲルハンス島）を膵臓から分離し，レシピエントに移植する細胞（組織）移植である．大がかりな手術を必要とせず，通常は局所麻酔によって経皮経肝的に門脈内に留置したカテーテルから膵島を注入することによって肝臓内に移植する．

膵島移植の分類

膵島移植もドナーによって，①脳死ドナー膵島移植，②心停止ドナー膵島移植，③生体ドナー膵島移植に分類できる．海外では脳死ドナーを用いているが，日本では世界でも珍しい心停止ドナー膵島移植を行っている．生体ドナー膵島移植は 2005 年 1 月に京都大学で実施され世界で初めて成功した[2]．

次に術式によって，①膵島単独移植（Islet Transplant Alone：ITA），②腎移植後膵島移植（Islet Transplant after Kidney：IAK），③膵島腎同時移植（Simultaneous Islet Kidney Transplant：SIK）に分類できる．通常 ITA が行われているが，最近 IAK による移植腎の保護効果が注目されている．SIK は現在ほとんど行われていない．

膵島移植後合併症

分離膵島を経門脈的に肝臓に移植するため，門脈閉塞の可能性がある．実際には，移植組織量を 10 mL 以下とし，さらにヘパリンを同時に投与することで，そのリスクを減らしている．他に肝臓の穿刺部位からの出血の可能性がある．総じて膵島移植は侵襲の少ない手技で行えるため，移植に関連した重篤な合併症はない．

世界の膵島移植の現況

膵島移植は 1970 年代から実験的に行われていたが，当初はうまくいかなかった．ところが，2000 年にカナダのアルバータ大学からいわゆるエドモントンプロトコールが発表されて移植成績が飛躍的に向上し世界に普及した．このプロトコールが現在の臨床膵島移植の標準となっている．その主な特徴は，①免疫抑制剤にステロイドを使わずに，シロリムスと少量のタクロリムスを使用すること，②一人の患者に複数回の移植を行うこと，③腎機能が保たれている患者を対象とすること，である[3]．現在では 70 以上の施設で 700 人以上の患者が既に受けているが，膵島移植に関連した死亡症例は 1 例もなく，高い安全性が確認されている．

2005 年にアルバータ大学における膵島移植後の長期成績が発表され，インスリン離脱率は移植後 5 年で約 10%と膵臓移植と比べてかなり低い結果であった．しかしながら，移植膵島の生着率（C-ペプチド陽性で判定）は 5 年で 80%と高く，再びインスリン注射が必要となった場合でも内因性のインスリン分泌がある限りは血糖

値の安定化を得ることができると報告された[4]．即ち，ほとんどの患者において移植した膵島は徐々にその数は減少するが，少なくとも移植後5年間は血糖の調節に貢献することが分かった．

この発表から，現在では膵島移植は血糖値の安定化を得ることができる安全性の高い治療法であると認識されている．しかしながら，インスリン離脱を目標とした場合には，現在の技術では複数回の移植が必要であること，長期間の維持が困難であるなどの限界がある．

日本の膵島移植の現況

日本では膵島移植は組織移植の範疇に入るため，脳死ドナーを用いることができず，日本組織移植学会が定めたガイドラインに基づいて心停止ドナー膵島移植を行っている．実施に際しては，膵・膵島移植研究会で定められた規則の下で行っている．2004年4月に京都大学で第1例目が実施され，2007年3月までに全国で17人に33回の移植が行われた．

京都大学ではこれまで10名の患者に計19回の移植を行った（うち1例は生体ドナー移植）．移植後は全例で必要インスリン量は減少し，特に複数回の移植を受けた7名中3名がインスリン治療から離脱することができた．また，1回の移植ではインスリン治療から離脱することができなくても，インスリンの基礎分泌補充量が減少し，血糖値の不安定性の指標であるM値とMAGEは著明に低下し，重症低血糖は消失することが示された[5]．

当初日本では，エドモントンプロトコールに則って腎機能が保たれている患者を対象にITAのみを実施してきた．2006年9月に膵・膵島移植研究会でIAKの実施が承認されてからは，3名の腎移植後患者に4回のIAKが行われている．

表．膵臓移植と膵島移植の比較

	膵臓移植	膵島移植
麻酔	全身麻酔	局所麻酔
侵襲度	大	小
移植回数	1回	2～3回
費用	保険診療	自費または研究費
拒絶診断	可	不可
移植成績		
インスリン離脱（5年）	60～70%	約10%

膵臓移植と膵島移植の選択について

膵臓移植と膵島移植のどちらを選ぶかについては，病期によるところが大きく，膵臓移植は糖尿病性腎不全（透析）患者に対して腎臓移植と同時に行われ（SPK），膵島移植はまだ腎機能が保たれている患者が対象（ITA）となるのが一般的である．しかしながら，腎機能が保たれている患者に膵臓移植（ITA）も行われている．糖尿病性腎不全であらかじめ腎移植を受けた患者に対しては膵臓移植（PAK）も膵島移植（IAK）も行われている．膵臓移植は全身麻酔下での手術を要し，局所麻酔下でカテーテルを用いて行える膵島移植に比べて侵襲度が大きい．しかしながら，1回の移植でインスリン離脱でき，移植後5年のインスリン離脱率は60～70%と膵島移植の約10%に比べて成績が格段に良い．膵島移植ではインスリン離脱するのに複数回の移植を必要とし，またインスリン離脱を維持するのが難しいが，血糖値の安定化は高率に得ることができる．費用については，膵臓移植の方が日本でも保険適応になっている点で有利である（表）．これらの膵臓移植と膵島移植のメリットとデメリットを患者に十分説明し，個々の事情に応じて選択してもらうことが重要である．ただし，重度の心疾患などがあり耐術性のない症

例，重篤な血管合併症のため膵臓移植適応から外れる症例，腹腔内感染が原因で移植膵臓を摘出した既往があり膵臓再移植では感染の再燃が予想される症例などは，膵島移植が良い適応となる．

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Points

◆膵臓移植は手術侵襲が大きいが，高いインスリン離脱率を維持可能である．

◆膵島移植は安全性が高いが，インスリン離脱を長期間維持するのは困難である．しかし，血糖値の安定化を得ることはできる．

心停止ドナー膵島移植

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Islet Transplantation using Donation after Cardiac Death Donor

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1．はじめに

膵島移植は，膵臓から膵島を分離し移植するという細胞移植である。1型糖尿病等のインスリン依存状態糖尿病に対して，膵β細胞を補充し適正なインスリン分泌を再開することによって糖代謝を正常化させることを目的としている。

海外では脳死ドナーからの膵臓を用いているが，膵島移植は組織移植の範疇に入るため，日本では制度上脳死ドナーを用いることができない。そこで，心停止ドナー膵島移植を行なっている。膵島分離は脳死ドナー膵を用いても十分な膵島収量を得ることが一般的に困難であるが，いわゆる“マージナルドナー”である心停止ドナーの膵臓から膵島を分離することはさらに困難である。

本稿では，心停止ドナーを用いた本邦の膵島移植の現況と当院における取り組みについて述べる。

【キーワード】
膵島移植，膵島分離，マージナルドナー，心停止ドナー，インスリン依存状態糖尿病

2．世界の膵島移植の状況

膵島移植は1970年代に実験医療として開始されたが，当初は，インスイリン注射からの離脱症例はなかった。膵島分離が難しく，十分な膵島収量を得られないことがその大きな原因であった。1988年にDr. Ricordiが大量の膵島を分離する方法（Ricordi法）を開発し[1)]，翌年の1989年に初のインスリン離脱症例を出した。しかしながら移植成績は芳しくなく，しばらくは一部の施設でのみ行われる実験的治療法であった。膵島移植が世界中に広まったのは，2000年にカナダのアルバータ大学からエドモントンプロトコール[2)]が発表されて移植成績が向上してからのことである。このプロトコールが現在の臨床膵島移植の標準となり，これまでに世界で60以上の移植施設で700人以上の患者が移植を受けている。

膵島移植の成績については，2005年にアルバータ大学から移植後5年間の成績が発表された。インスリン離脱率は移植後2年で約40%，5年で約10%であった（図1）。膵島の生着率（C-ペプチド0.3ng/ml以上で判定）については移植後5年でも80%あり，これらのインスリン基礎分泌がある人は，再びインスリン注射が必要となっても

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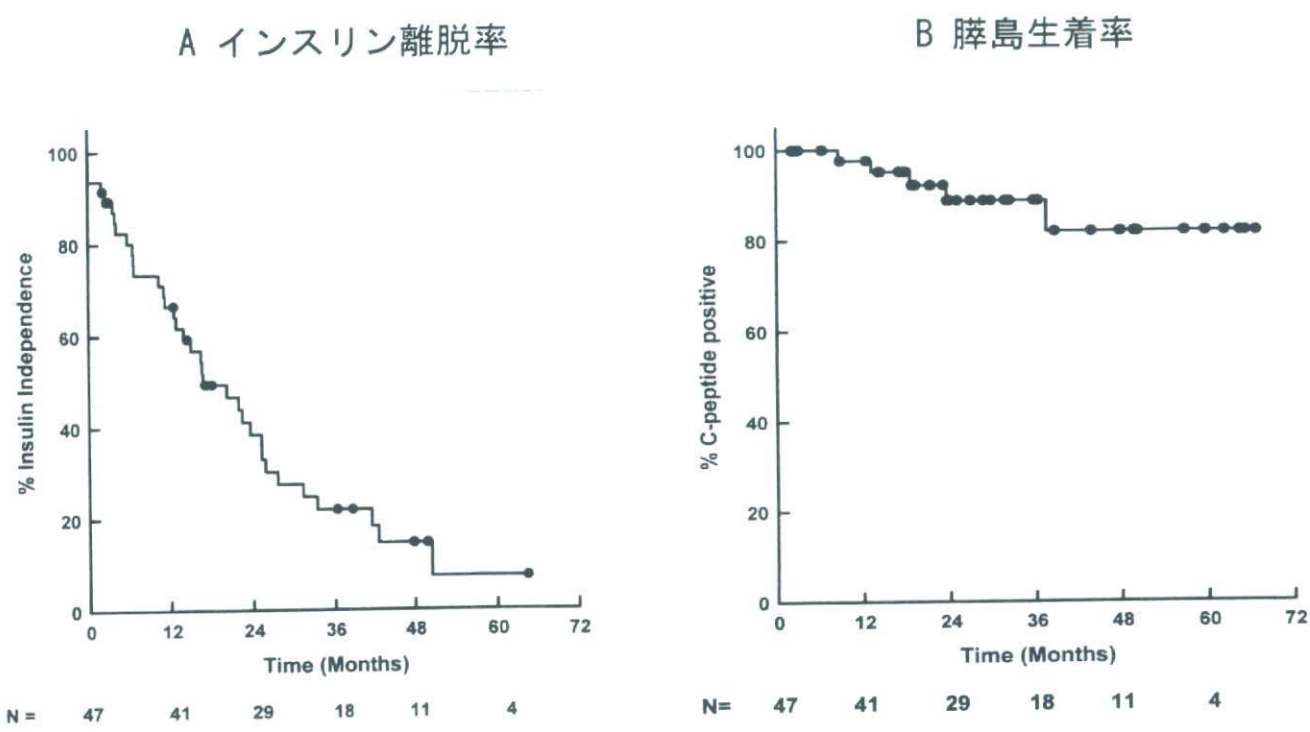

（文献(3)より改変引用）

図1. 膵島移植の成績（アルバータ大学）

HbA 1 c：６％台と血糖値の安定化を維持できていた[3]。

マージナルドナーの定義ついて，ピッツバーグ大学の基準を引用すると，①年齢は45歳以上，②循環動態が不安定で，昇圧剤が単剤でDopamine>10 μg/kg/minまたは２剤以上使用されている，③心停止ドナーのいずれかに該当するものとしている[4]。海外でのマージナルドナーを用いた膵島移植については，報告例が少ない。マイアミ大学では膵臓保存に二層法を使うことで50才以上のマージナルドナー膵を用いても膵島分離で十分な収量を得て移植を行なうことができた[5]。心停止ドナー膵島移植については，ペンシルバニア大学からの１例報告があるのみである[6]。

3．日本の膵島移植の状況

膵島移植は組織移植の範疇に入るため，日本では原則として心停止ドナーを用いている。ドナーの適応基準は，日本組織移植学会の「ヒト組織を利用する医療行為に関するガイドライン」[7]を遵守して感染症，悪性疾患などを除外し，さらに，膵・膵島移植研究会膵島移植班が定めた膵島移植実施マニュアル[8]に従って，①年齢は70歳以下，②温阻血時間は原則として30分以内が望ましい，③膵臓保存法はUW液による単純浸漬保存または二層法を用いることが望ましい，と定められている（表１）。

膵臓から分離した膵島を移植に供するか否かの判断基準“新鮮膵島移植の基準”についても表２の通り膵島移植実施マニュアル[8]で定められている。すなわち，レシピエント体重あたり5,000 IEQ/kg 以上の膵島収量があり，純度30% 以上，組織量10 ml 未満，viability 70% 以上，エンドトキシン５EU/kg 未満（患者体重），グラム染色陰性などの基準を満たした場合に新鮮膵島移植を行うこととされている。

膵島移植は一人につき３回まで行うことが可能で，2004年４月に本邦初症例が実施されてから