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ORIGINAL ARTICLE

Microbial Contamination of Clinical Islet Transplant Preparations Is Associated with Very Low Risk of Infection

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Abstract

Background: Several published studies have analyzed microbial contamination rates of islet products, ranging from 0% to 16%. However, few studies make reference to potential clinical consequences for transplant recipients and possible impact on islet survival.
Materials and Methods: The current study defines rates of microbiological contamination of islet products under current good manufacturing practice conditions in 164 patients receiving 343 transplants at a single institution.
Results: Nineteen (5.5%) islet preparations showed positive microbial growth with a majority (79.4%) due to Gram-positive organisms. The most frequently identified microorganism was coagulase-negative *Staphylococcus* (nine of 19 [47.3%]), followed by polymicrobial organisms (eight of 19 [42.1%]). No patient developed signs of clinical infection, and there were no hepatic abscesses evident on imaging by ultrasound or magnetic resonance imaging (none of 19 [0%]), despite the use of potent T-depletional induction. Finally, we could not demonstrate any negative impact of microbiological contamination on long-term islet graft survival.
Conclusions: Microbiological contamination of the final islet preparation appears to have little or no effect on patients or on islet survival when appropriate antibiotics are given. However, preparation sterility should be guaranteed at all cost in order maximize patient safety and avoid potential complications in immunosuppressed patients.

Introduction

Clinical islet transplantation is an accepted treatment modality to stabilize frequent hypoglycemia or severe glycemic lability in highly selected subjects with type 1 diabetes and poor glycemic control that cannot be stabilized by other means.[1,2] Established final islet product release criteria must be met prior to clinical transplantation and must include adequate islet yield, purity, tissue volume, viability, negative Gram stain, and post hoc confirmation of microbiological sterility, an important consideration in the setting of immunodepletion and immunosuppression for transplant recipients.[3]

Several studies have reported microbial contamination rates of islet products, ranging from 0% to 16% during pancreatic retrieval, in transport media, in islet isolation, and during islet culture.[4–8] It is generally believed that the major source of bacterial contamination arises from the retrieved duodenal segment of small bowel attached to the pancreas. However, few studies have explored the potential clinical consequences for transplant recipients or the potential impact on islet survival. The objective of the study was to monitor the rate of microbiological contamination of islet products under current good manufacturing practice (cGMP) conditions at a large-volume transplant center and the clinical consequences for patients, in terms of both infectious complications and graft function.

Subjects and Methods

Patients

Between March 1999 and July 2012, the clinical islet transplant program in Edmonton, AB, Canada, has carried out 358 islet transplants procedures in 171 subjects with type 1 diabetes mellitus under a series of evolving induction and maintenance immunosuppressive protocols. Patients received a median of two procedures (range, one to four). Seven

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subjects participating in a National Institutes of Health trial (CIT-04) using belatacept (Nulojix®; Bristol-Myer Squibb, Devens, MA) induction were excluded from the current analysis. Thus, the study population consisted of 164 subjects receiving 343 islet transplantation procedures, with a female:male ratio of 88:76 and a mean age of 46.3 years. All subjects underwent complete pretransplant evaluation. Informed consent was obtained, and ethical approval for this study was covered under protocol 1120, approved by the Health Research Ethics Board at the University of Alberta and by Health Canada.

Transplant procedures

Islets were prepared as previously described, using a modified Ricordi protocol.[9–12] In brief, human cadaveric pancreata were recovered from deceased donors and transported to the cGMP-grade clinical islet isolation laboratory. Upon arrival, the pancreatic duct was cannulated, and collagenase blend enzyme solution was perfused transductally (Serva collagenase NB1; Crescent Pharmaceuticals, Islandia, NY) with Liberase HI or, more recently, mammalian tissue-free enzyme (Roche Diagnostics Corp., Indianapolis, IN).[13] The pancreas was enzymatically and mechanically dissociated in a Ricordi chamber and then purified on a refrigerated centrifuge (model Cobe 2991; Cobe BCT, Lakewood, CO) with continuous density gradient separation with Ficoll™ (Sigma-Aldrich, St. Louis, MO) or, more recently, Biocoll™ (Biochrom AG, Berlin, Germany) separating solution (Cedarlane®, Burlington, ON, Canada).[14] The majority of the islet preparations were placed in culture (median, 13.0 h; range, 6.4–23.0 h) before infusion to facilitate timing of islet infusion or as part of the immunosuppressive protocol. Subjects then underwent percutaneous transhepatic portal access in the Radiology Department under local anesthesia and with fluoroscopic and ultrasonic guidance, and islets were infused under gravity pressure from a 100-mL medium-containing intravenous islet bag.[15] Portal pressure was monitored during and after infusion, and afterward the catheter tract was ablated to minimize the risk of bleeding.

Microbiological testing

The majority of clinical islet preparations were placed in culture containing ciprofloxacin (Cipro®; Bayer AG, Toronto, ON, Canada) at a concentration of 20 mg/mL. Samples for Gram stain and microbiological culture were taken immediately before transferring islets to the final container for transplant and evaluated at the Provincial Laboratory of Public Health at the University of Alberta Hospital. Both a negative Gram stain and an endotoxin content of <5 endotoxin units (EU)/kg of recipient's body weight are mandatory requirements prior to islet release for transplantation. Results of samples undergoing microbiological culture for aerobic and anaerobic bacterial, fungal, mycoplasmal, and mycobacterial contamination were made available from 2 to 7 weeks post-transplant and did not constitute product release testing.

Antibiotic coverage

All patients undergoing islet transplantation in Edmonton routinely receive prophylactic antibiotic treatment consisting of a single dose of cefazolin (Baxter Co., Mississauga, ON, Canada), 1 g intravenously preprocedure, or clindamycin (Sandoz Canada Inc., Boucherville, QC, Canada), 600 mg intravenously, if there was a known allergy to cephalosporins or severe penicillin allergy.

In the rare case of contaminated islet preparation discovered after transplantation, consultations from transplant infectious diseases were made to design treatment strategy. Imaging studies (ultrasound, computed tomography, and magnetic resonance imaging), as clinically indicated, was used to rule out development of intrahepatic abscess, and directed antibiotic therapy was given according to microbiological culture results. In the absence of symptoms, normal liver imaging, and absence of abscess, patients received a minimum of 7 days of broad-spectrum (culture-sensitive) antibiotic or antifungal treatment by the peroral route, if appropriate, or intravenously, where the peroral route was judged to be inadequate.

Immunosuppression protocols

Induction and maintenance immunosuppressive protocols have evolved in our program over time. Initially our practice was to induce with an interleukin-2 receptor monoclonal antibody (daclizumab [Zenapax®; Hoffmann-La Roche Ltd., Mississauga], 2 mg/kg intravenously at transplant and at 5 days posttransplant), combined with tacrolimus (Prograf®; Astellas Pharma Canada Inc., Markham, ON, Canada) for a target trough level of 3–6 ng/mL and sirolimus (Rapamune®; Pfizer Canada Inc., Kirkland, QC, Canada) for target trough levels of 12–15 ng/mL for the first 90 days and 8–10 ng/mL thereafter (the "Edmonton Protocol").[2,12] Subsequently, basiliximab (Simulect®; Novartis Pharmaceuticals Canada Inc., Dorval, QC, Canada) (20 mg intravenously on Days 0 and 4) has been used in place of daclizumab, with the combination of tacrolimus (target trough level of 8–10 ng/mL) and mycophenolate mofetil (Cell Cept®; Hoffmann-La Roche Ltd.) (up to 2 g daily in divided dose as tolerated). Before 2003, daclizumab was given at a dose of 1 mg/kg every 2 weeks for five doses.[10]

Other protocols included the use of infliximab (10 mg/kg) given at the time of transplant, combined with daclizumab; alternative use of basiliximab (two doses of 20 mg) or etanercept (Embrel®; Amgen Canada Inc., Mississauga) (50 mg weekly); or, most recently, potent lymphocyte depletion protocols based on alemtuzumab (Mabcampath®; Genzyme Canada, Mississauga) or anti-thymocyte globulin (rabbit) (Thymoglobulin®; Genzyme Canada).

Management of patients receiving contaminated islet preparation

When a positive culture was obtained from the islet product, a personalized management was designed based on the patient's characteristics, the immunosuppression regimen, the transplant interval, and the identity of the organism(s) isolated in culture. Routine abdominal ultrasound and abdominal computed tomography or magnetic resonance imaging were also performed to rule out liver abscess when a positive culture was received, and complementary antimicrobial treatment was ordered depending on the particular microorganism growing in the culture medium and its sensitivity profile. Further measures were also available upon clinical evidence of infection.

Table 1. Microbial Species Cultured from 19 Contaminated Human Islet Preparations

Pathogen	*Frequency*	*Percentage*
Staphylococcus sp.	9/19	47.3
Enterococcus sp.	4/19	21
C. albicans	4/19	21
Streptococcus sp.	3/19	15.8
Aerobic spore-bearing bacilli	3/19	15.8
Micrococcus sp.	1/19	5.26
Diphtheroid sp.	1/19	5.26
Rothia sp.	1/19	5.26
Mycoplasma sp.	1/19	5.26
Propionibacterium sp.	1/19	5.26
Ureaplasma urealyticum	1/19	5.26
Polymicrobial contamination	8/19	42.1

Graft function

In addition to standing graft function determination based on insulin requirement, glycemic control, hemoglobin A1C, protection from hypoglycemia, and fasting C-peptide testing, more definitive stimulated C-peptide levels were obtained at the time of mixed meal tolerance testing scheduled at intervals post-transplant (3 monthly for 1 year and then every 6 months thereafter). Loss of C-peptide production was defined by stimulated C-peptide levels below 0.2 nmol/L after mixed meal tolerance testing or in the presence of a fasting glucose level of >15 mmol/L.

Statistical analysis

Results are expressed as mean ± SE values or the median (25^{th}–75^{th} percentile) as appropriate. Comparisons were made with a two-tailed Student's *t* test, paired or unpaired as appropriate. Graft survival analysis was performed using Kaplan–Meier analysis with the log-rank test to compare differences between groups. All statistical analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, IL). Significance was considered when $P < 0.05$.

Results

After more than 358 procedures, no single islet preparation showed a positive Gram stain or endotoxin level greater than 5 EU/kg. Of those 343 islet transplants included in this study, 19 (5.5%) showed positive microbial growth in the final islet preparation. This prevalence is well within the incidence previously reported by centers worldwide.[4,5,8,16] Thus two groups of patients are reported: those with contamination ($n = 18$) (one patient received two contaminated islet preparations) and those without ($n = 146$). Among the contaminated islet preparations, the most common isolates were Gram-positive organisms (79.4%), followed by *Candida* sp. (11.7%) and Gram-negative organisms (8.8%) (Table 1). The most frequently identified microorganisms were coagulase-negative *Staphylococcus* (nine of 19 [47.3%]), followed by *Enterococcus* and *Candida albicans* (four of 19 [21%] for each). Eight islet preparations had polymicrobial contamination (42.1%). Other

Table 2. Summary of 18 Patients Receiving an Islet Transplant with Microbial Contamination in the Final Preparation

Patient number	*Pathogen(s)*	*Clinical manifestations*	*Treatment*	*Most recent C-peptide*	*Imaging*	*Outcome*
1	*Staphylococcus* sp.	None	Cephalosporin course	Negative	Negative US, CT	No effect
2	Aerobic Gram-negative bacilli/*C. albicans*	None	Cephalosporin/ antifungal course	Positive	Negative US, CT	No effect
3	*Staphylococcus* sp.	None	Cephalosporin course	Positive	Negative US	No effect
4	*Bacillus* sp./*Streptococcus viridans*	None	Cephalosporin course	Positive	Negative US	No effect
5	*Staphylococcus* sp.	None	Cephalosporin course	Positive	Negative US	No effect
6	*Enterococcus* sp.	None	Vancomycin course	Negative	Negative US, CT	No effect
7	*Streptococcus*/*Micrococcus* sp.	None	Vancomycin course	Positive	Negative US, MR	No effect
8[a]	*Staphylococcus* sp. Aerobic spore-bearing bacilli	None	Cephalosporin course Negative	Negative Positive	Negative US Negative US, CT	No effect
9	*Rothia* sp./streptococci	None	Cephalosporin course	Positive	Negative US	No effect
10	*Mycoplasma*	None	Quinolone course	Negative	Negative US	No effect
11	*Staphylococcus* sp./ *Propionibacterium*	None	Cephalosporin course	Positive	Negative US	No effect
12	*C. albicans*/diphtheroids	None	Quinolone and antifungal course	Positive	Negative US, CT	No effect
13	*Enterococcus* sp./*C. albicans*	None	Linezolid and antifungal course	Positive	Negative US, CT	No effect
14	*C. albicans*	None	Fluconazole course	Positive	Negative US, CT	No effect
15	*Staphylococcus* sp.	None	Cephalosporin course	Negative	Negative US	No effect
16	*Staphylococcus* sp./ *Enterococcus* sp.	None	Linezolid course	Positive	Negative US, CT	No effect
17	Aerobic spore-bearing bacilli	None	Cephalosporin course	Positive	Negative US	No effect
18	*C. albicans*	None	Fluconazole course	Positive	Negative US, CT	No effect

[a]Patient 8 received contaminated preparations on two occasions.
CT, computed tomography; MR, magnetic resonance; US, ultrasound.

microorganisms also cultured in lesser frequency were *Pseudomonas* sp., *Streptococcus* sp., aerobic spore-bearing bacilli, and *Mycoplasma* sp.

All patients in whom islet cultures were positive received appropriate antimicrobial prophylaxis, usually of 1–2 weeks in duration. No patient in our series experienced clinical features of infection related to the procedure regardless of the induction immunosuppression used, and blood cultures sent for those patients resulted in no growth (Table 2).

We also analyzed the possible impact of contaminated preparations on short- and long-term islet graft function assessed by various post-transplant tests over a mean follow-up of 134.6 months. The analysis did not demonstrate any significant difference between the two groups in terms of measurable or stimulated C-peptide peak values at 1 month post-transplant and later. Insulin-independence rates were similar among both study groups, and, finally, the mixed meal tolerance test (Ensure® [Abbott Nutrition Canada, Saint-Laurent, QC, Canada] test) and other metabolic monitoring tools (intravenous glucose tolerance test, oral glucose tolerance test, and arginine stimulation test) also failed to demonstrate any differences in graft function after receiving noncontaminated/contaminated islet preparations. Moreover, we could not demonstrate any negative impact of microbiological contamination upon long-term islet graft survival, with loss of stimulated C-peptide over time (Fig. 1). Mean C-peptide survival was 112.4 months in subjects with no contamination ($n=146$) versus 112.7 months in those receiving contaminated preparations ($n=18$) (log-rank $P=0.39$).

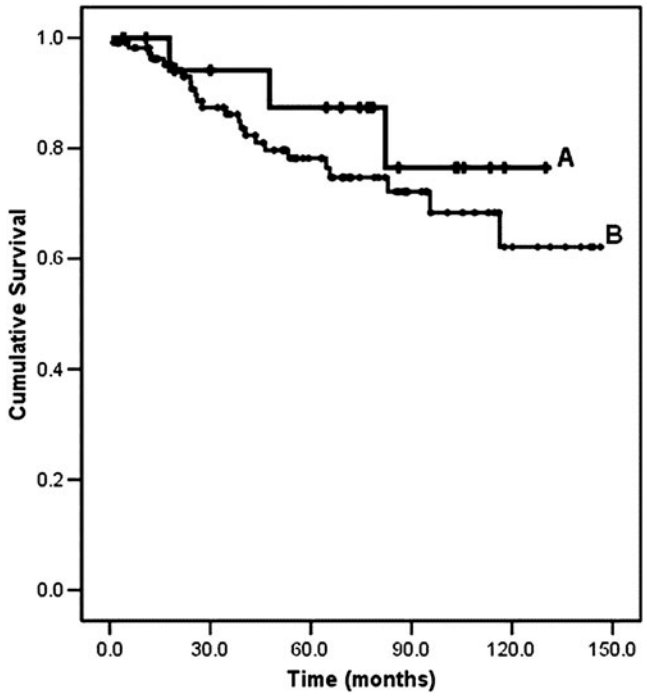

FIG. 1. Kaplan–Meier graft survival curves comparing the maintenance of stimulated C-peptide levels in subjects who (curve A) did or (curve B) did not receive contaminated islet preparations. Mean C-peptide survival was (curve B) 112.4 months in subjects with no contamination ($n=115$) versus (curve A) 112.7 months in those receiving contaminated preparations ($n=18$) (log-rank $P=0.39$).

Discussion

We herein report on the largest single-center series of intraportal islet transplantation, with respect to risk of transplantation of microbiologically contaminated islet preparations in immunocompromised recipients. It is important that, despite a 5.5% risk of transplantation of a contaminated product, there were no clinical sequelae and no negative impact on islet function in any of the 18 recipients. Clearly, despite the introduction of rigorous cGMP manufacturing conditions and strict protocols for handling of biological tissues, there is still a potential risk for transmission of microbiological contaminants.[3–5,8,16] Because all of the materials, reagents, and media are strictly quarantined and monitored and because full aseptic technique used is throughout the processing, it seems most likely that the greatest source of microbiological contamination originates from the donor pancreas and in the retrieved segment of donor duodenum. Previous studies addressing contamination of the pancreas preservation solution (University of Wisconsin solution or histidine–tryptophan–ketoglutarate) both in whole pancreas and in islet isolation suggest that the donor duodenal segment provides the greatest source of contamination.[4,8]

Our study reports a contamination rate of 5.5% with absolutely no influence of harvesting techniques or preservation solutions as previously reported by our center.[8] In fact, previous studies on microbial contamination during islet isolation have suggested that a majority of microorganisms are washed, diluted, or eliminated during pancreas processing. However, de novo contamination during the last stages of the process is still reported in various centers.[4,8]

Previous reports on this topic make minimal reference to the consequences of patients receiving these contaminated islets in the presence of potent T-directed immunodepletion and immunosuppression regimes (121 of 358 [33.8%]). To our knowledge, previous reports have not addressed clinical risk, impact, and potential negative effects on islet engraftment and survival. In the current study, we looked for possible infectious complications in 18 subjects receiving positive-culture islet preparations. Our current series expands on previous findings and clearly demonstrates the exceedingly low risk of clinical sequelae provided the initial microbiological load is low (negative Gram stain and low endotoxin content) and appropriate antimicrobial prophylaxis is used. Furthermore, the intrahepatic site for islet delivery may provide an especially protective environment for a potentially contaminated islet product, because of the excellent intrahepatic blood flow, exposure to prophylactic, and treatment-directed antibiotics where indicated, and most likely because of the presence of large numbers of phagocytic Kupffer cells.[17]

When looking into the possible long-term effects of this contamination, we could not find any significant differences in islet survival between the two groups. This finding is similar to another study recently published by our group when analyzing the influence of cytomegalovirus infection in clinical islet transplanted patients.[18] In that analysis we reported an association between the use of T-depletion immunosuppressant and the occurrence of cytomegalovirus transmission.

The sterile technique during islet isolation continues to be of paramount importance in line with the continual improvement in islet transplantation safety. The use of cGMP

conditions remains standard and an integral part of the safety of clinical-grade islet manufacture. Despite these standards, microbiological contamination of islet products may still occur on rare occasions, but this condition is only identified after the transplant procedure. Fortunately, it appears to have little or no effect on patient health or on islet survival. Nonetheless, utilization of sterile preparations should remain a priority in order to maximize patient safety and avoid potential complications in immunosuppressed patients. Microbial surveillance remains an important element in clinical islet transplantation, and, where it is found, the risks can be negligible provided appropriate directed antimicrobial therapies are given.[4]

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Author Disclosure Statement

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