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Human islet isolation: status and future considerations*

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Abstract

Autologous human islet transplantation to mitigate or prevent surgically induced diabetes after total pancreatectomy for the relief of chronic pancreatitis-induced pain was pioneered in the late 1970s. Islet allotransplantation using islets from human donors to treat type 1 diabetes was first reported several years later. Over the last 40 years, methods for clinical islet transplantation have been methodically standardized to become the methods used today.

Human islets are also isolated from donors with pathologies with the aim to conduct a broad range of investigational studies. Human islet isolation undertaken for research can provide the opportunity to further optimize and develop methods that may be helpful in the clinic. The variability of conditions associated with donor and pancreas makes consistent success in isolating islets a challenge, thus, we should re-evaluate the effectiveness of our methods of islet isolation. In order to do this, we must first consider that the intended outcomes for clinical islet transplantation and experimental investigations involving islet isolation are, in fact, quite different and that these differences impact the status and future considerations of islet isolation procedures.

Abbreviations

CIT = Consortium for Islet Transplantation, CP = Chronic Pancreatitis, IBMIR = Instant Blood-Mediated Inflammatory Reaction, IEQ = Islet Equivalent, QOL = Quality of Life, T1D = Type 1 Diabetes, TPAIT = Total Pancreatectomy and Autologous Islet Transplantation.

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Introduction

Based on several studies we estimate a normal adult human pancreas to weigh approximately 90 grams and have a volume of 80 ml[1,2]. Estimates to the number of islets in a normal human pancreas range from 1 million[3], 3.2 million[4], to 3.6 and to 14.8 million[5] islets. Islet cells account for as much as 4.49% of the pancreas volume[4]. Korsgren et al[6] estimate that a normal 70 grams pancreas contains 500,000 Islet Equivalents (IEQ). One IEQ is the volume of a standard islet, i.e. a sphere with a diameter of 150 μm. Further, they state that most centers with an active clinical islet transplantation program report that they obtain between 300,000 to 600,000 IEQ/pancreas (between 4 and 10 ml islets by volume). Based on these calculations it seems likely that most of the native islets are successfully isolated from the donor pancreas. However, since islet total is normally highly variable, it may be difficult to isolate sufficient islets for transplantation even with proven methods.

Human islets for research

Several programs around the world focus on islet isolation and distribution of human islets for clinical and research purposes. The integrated Islet Distribution Program (IIDP) in the USA, the Alberta Islet Distribution Program (AIDP) in Canada, the European Consortium for Islet transplantation (ECIT), the Oxford Consortium for Islet Transplantation (OXCIT) in Europe, and the Clinical Islet Transplantation (CIT) Consortium International are currently active.

Our Allegheny Health Network (AHN) Islet Isolation Center works closely with researchers to provide islets from investigator-defined organ characteristics including donor age and type 1 diabetes (T1D) pathology. Within these and other protocols, flexibility is the key to success. Broad discretion is built into the protocols, which may

allow modifications to the key steps of pancreas digestion and islet purification. However, there are several general characteristics shared by most research isolations. Several thousand islets are often all that is needed for the various studies conducted by the investigators. Therefore, pre-isolation conditions that affect islet yield are not necessarily critical to the success or even the attempt to isolate islets. Conditions that would disqualify an organ donor from clinical islet allotransplantation such as surgical damage, cold ischemia time, and organ size, are minor factors to be considered. Protocols may be challenging to institute and not universally accepted; however, once found they can be effective in producing successful outcomes for the limited needs of research.

Islets for clinical transplantation

While clinical islet procedures for Total Pancreatectomy and Autologous Islet Transplantation (TPAIT) and allogeneic islet transplantation are similar, they are not identical because they are based on the expectation of different outcomes. Autologous islet isolation is a procedure in which islets are isolated from the excised pancreas of a patient suffering from chronic pancreatitis (CP) and returned to the patient in order to mitigate the effects of surgical diabetes. This type of transplantation is performed in the absence of immunosuppression. Clinical islet allotransplantation, in contrast, refers to the infusion of islets isolated from one or more deceased organ donors to replace endogenous insulin production in patients with T1D, typically after islet culture and in the presence of immunosuppression, with the goal to improve diabetes management, reduce hypoglycemic unawareness, and long-term complications. Although the primary goal to perform total pancreatectomy in severe CP is to treat pain, autologous islet transplantation may provide a sufficient islet mass that allows to achieve in many patients (approximately 30%) insulin independency, and to better control glycaemia in the majority of the recipients for several years[7]. Islets are isolated from the recipient's own pancreas, no immunosuppression is needed, and there is often less than 15 ml of tissue isolated which typically eliminates the need for purification. The challenge in isolating islets from pancreatic organs with CP is to process tissue with significant fibrotic infiltration, necrotic areas, blood remnants, and dilated pancreatic ducts. These conditions require adjusting the procedure protocols, increasing efficiency in breaking the extra cellular matrix, which allows to better release the islets. Higher islet numbers are associated with better outcome following intraportal infusion. In clinical islet allotransplantation, at least one, usually two and sometimes more deceased organ donors are needed to achieve success[8]. More stringent conditions (when compared to islet isolations for autologous islet transplantation) define success. Isolation fails to produce a useable batch of islets for allotransplantation approximately 50% of the time[9] most often due to the inability to provide an efficacious mass of islets consolidated into less than 10 ml of tissue required for patient safety. Approximately 44% of recipients have been reported to achieve insulin independence at one year[10], which falls to about 10% after 5 years, although, most recipients retain partial function - which provides some important benefits[11], including an abatement of the risk of hypoglycemia unawareness and diabetes complications. Islet isolation (independent of clinical outcome) is generally considered successful if 5,000-10,000 IEQ are isolated for allotransplantation and half that amount for autotransplantation. The CIT clinical trial CIT-07 must be considered the most complete attempt yet to standardize and optimize the production of a purified human islet product for allotransplantation in the USA. However, even this clinical trial was plagued by inconsistency in isolation and transplantation outcomes that cannot be totally laid at the door of post-transplant islet loss. To begin, only 52.5% of the lots produced for transplantation met the qualifications for release and ultimately only 75 were transplanted[9], one participating center reported only 24.3% of islet lots met release conditions. This inefficiency is not unusual although it is mainly exclusive to islet allotransplantation. The first step in the process of pancreas sourcing for islet isolation is donor selection, which is based on criteria designed to provide the best chance to isolate a large islet mass for transplantation. The inclusion criteria of the CIT-07 protocol are typical and includes (1) donor age between 15 and 65 years, (2) cause of death acceptable by transplant team, (3) maximum of 12-hour cold ischemia, (4) and an acceptable preservation fluid for transport[9]. TPAIT does not select donors and donors used for research are based on investigator-initi-

ated criteria rather than the need to optimize islet yield so may disregard any or all of these. One of the primary considerations of clinical islet allotransplantation must be the volume of tissue to be transplanted. The favored route of islet delivery is via the portal vein, for islets to engraft into the liver; however, this method is subject to portal vein thrombosis and other potential adverse events and so care is given not to transplant too much tissue to the detriment of the recipient. This requires a purification step to be added to consolidate as many islets for allotransplantation as possible into the allowed volume. TPAIT often produces less tissue so that a consolidation is not required. AHN data shows that adult subjects did not need purification 62% of the time and this rose to 83% for pediatrics (unpublished data). In research isolation, purification may be beneficial to the investigator but is not strictly required, as large yields of islets are not needed.

Islets lost along the way

Ricordi et al[9] report that the CIT-07 post-digestion median of isolated islets was 708,470 IEQ (382,000 IQR). This number fells to 582,370 IEQ (267,931 IQR) after purification, a loss of 18% of the original amount. A further reduction to 490,174 IEQ (226,835 IQR) after 1-3 days of culture, a loss of 16% from the post-purification total and 31% lost from the original total. This is in-line with published estimates of islet loss at 13% after 1 day of culture that increases to 35% by day 3 due to overexpression of inflammatory mediators and contamination from exocrine tissue[12]. We are left with approximately 69% of the original total of islets and if we factor in a slight inefficiency in organ digestion, our total may be closer to only having 50% of native donor islets available for allotransplantation. The percentage of islets available for transplant after TPAIT would probably be higher considering the lack of culturing and likelihood of no purification step, however, the baseline number of islets in the CP organ would be significantly less than in the donor organs selected for allotransplantation of islets. For non-clinical research purposes, the percentage of islets unrecovered is usually unimportant. Islet loss continues and is even more severe after transplantation. The Instant Blood Mediated Inflammatory Reaction (IBMIR) occurs within seconds of contact between the donor islets and the recipient blood stream. 25% of islets were determined to be lost in the first 19 min after infusion began based on radioactivity given off by labeled islets as part of clinical islet allotransplantation[13]. *In vitro* studies modeling IBMIR with human islets and syngeneic mouse islet transplantation suggest that the loss may be closer to 50-60% in just a few days[14-16]. Estimates of islet loss range up to 70%[17] or even 90%[11] one-month post-transplant. 10% islet survival after transplantation would roughly correspond to 7% of the originally isolated islets surviving to potentially engraft. Obviously, any improvement in islet isolation efficiency would be welcome, more especially towards rendering islets more resistant to the events that characterize early engraftment.

Looking forward

What does the future of islet isolation hold? The effectiveness of the individual procedure is directly related to the expected outcome. Islets isolated for research and as part of TPAIT can provide successful outcomes on a consistent basis. The standardized methods of isolation for islet allotransplantation are effective to a large extent, however, having the most severe measure of success, many preparations cannot be utilized. Some costs must be borne, and incentives made available to encourage testing new approaches including organ provision and transport. Perhaps the biggest boost for allotransplantation would be a method of islet infusion that allows a greater volume of tissue to be transplanted. This would help to eliminate much of the need for islet purification and reduce failed isolation attempts. Alternatively, novel methods for islet purification, with reduced islet loss would be advantageous. New digestive cocktails and culture conditions may also provide some much-needed consistency. Several experimental purification steps that are less damaging to islets have been tested in animal models; however, nothing has yet been able to replace the density gradient method despite its well-known detrimental effect on islets[18,19]. Methods to optimize digestive enzyme have been instituted with good results in several clinical allotransplantation centers but they are not widely utilized elsewhere[20,21]. Considering that any improvements are likely to be small, efforts might be better used to preserve islets after transplantation rather than to find more to transplant considering that 2/3 of all islet loss occurs within the first month post-transplant.

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Conflict of Interest:
No author declares a conflict of interest.

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