

(i) Title: Islet/stem cell transplantation – What’s new?

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Abstract:

Type 1 diabetes mellitus (T1DM) affects 345,000 children and adults in the UK, and a proportion of these patients develop hypoglycaemia unawareness. This often results in life-threatening episodes of severe hypoglycaemia. Transplantation of isolated islets of Langerhans is a minimally invasive procedure that can restore hypoglycaemia awareness in the majority of patients, and has huge potential for reversing T1DM. However, it is not currently available for treating children due to the requirement for life-long immunosuppression. This review will focus on the current practice of islet transplantation in the UK, as well as the challenges and recent developments in the field. These include optimising pancreas procurement and islet isolation, improving post-transplant islet engraftment and survival, and the use of renewable sources of islets such as stem cells. It will also discuss the novel strategies being developed to enable immunosuppression-free islet transplantation. The ultimate aim is to be able to offer this technology to young people soon after diagnosis, and thereby provide a safe and sustainable “cure” for T1DM.

Keywords: (5-10)

type 1 diabetes mellitus, islet cells, islet transplantation, stem cells, immunosuppression

Introduction

Type 1 diabetes mellitus (T1DM) affects 345,000 children and adults in the UK, and makes up about 5% of all diabetes cases. It is caused by a gradual destruction of the beta-cells of the pancreas via a presumed T-cell mediated autoimmune process, which eventually results in the patients becoming completely insulin deficient.

The mainstay of treatment of T1DM is exogenous insulin therapy, either in the form of subcutaneous injections (basal / bolus regimens) or continuous subcutaneous infusion via an insulin pump. This is combined with structured education programmes regarding diet and insulin dose adjustments, such as

DAFNE (Dose Adjustment For Normal Eating). The emphasis is on maintaining near normal glycaemic control in order to prevent macro- and micro-vascular complications, while avoiding episodes of severe hypoglycaemia (blood glucose <4.0 mmol/L).

Rationale for beta-cell replacement

Despite the development of new types of insulins over recent years, the pharmacokinetics of these molecules still do not fully emulate the responsiveness of endogenous insulin secretion from pancreatic beta cells, resulting in both periods of under-treatment (hyperglycaemia) and over-treatment (hypoglycaemia). In addition, insulin treatment only replaces one of the hormones previously produced by the destroyed islets of Langerhans. Hypoglycaemia is particularly dangerous in the short-term, as patients with type 1 diabetes also have blunted counter-regulatory mechanisms (such as glucagon secretion), and over time may lose their awareness of hypoglycaemia through a yet unknown mechanism. The results can be life-threatening episodes of severe hypoglycaemia, defined as requiring the assistance of another person to treat, with neuroglycopenic symptoms (such as agitation, loss of consciousness, seizures etc) occurring in the absence of warning symptoms of sympathetic activation (sweating, palpitations etc).

Specialised education programmes and technology (continuous glucose monitors and sensor-augmented insulin pumps) can help patients restore hypoglycaemia awareness by avoiding low blood glucose episodes. This seems to “re-train” higher centres to respond more appropriately to low glucose levels, and can significantly improve quality of life. However, there remains a proportion of patients who, despite attempts at optimising their medical treatment, education and trials of insulin pumps +/- continuous glucose monitoring, continue to experience recurrent life-threatening episodes of severe hypoglycaemia. It is in these patients that beta-cell replacement therapy, either in the form of a whole pancreas transplant or as transplantation of the isolated islets of Langerhans, currently has the greatest impact. The true goal, however, is to one day be able to reverse T1DM soon after diagnosis in both adults and children.

Whole Pancreas Transplantation

Allogenic pancreas transplantation was the first form of beta-cell replacement therapy used clinically in 1966, and after initial set-backs has gone on to become the gold standard for “reversing” T1DM. It is now mainly performed as a combination procedure in those patients with T1DM who require a kidney transplant (simultaneous pancreas and kidney, SPK) transplant, although pancreas transplant alone (PTA) is also considered in patients with T1DM who have poor glycaemic control and hypoglycaemia unawareness without significant nephropathy. Whole pancreas transplantation is currently associated with an 80-85% insulin-independence rate 1 year after transplantation, with over half of patients remaining insulin-independent for at least 5 years. However, whole pancreas transplantation remains a major procedure with a mortality rate of 4-7% and a re-operation rate of up to 30% in the first 6 months following the procedure. The main procedure-related complications are thrombosis and pancreatitis. Overall, whole pancreas transplantation is a highly successful procedure for adult patients, especially those who require a simultaneous kidney transplant anyway, but is unlikely to become the treatment of choice for children. The remainder of this overview therefore focuses solely on islet transplantation.

Islet Cell Transplantation

Pancreatic islet transplantation is a minimally-invasive procedure that involves only implanting the 2% of endocrine component of the pancreas (i.e. the part of the pancreas that has selectively been destroyed in T1DM). Like its whole pancreas counterpart however, one of its huge benefits over exogenous insulin treatment is that all pancreatic hormone producing cell-types are replaced. Although almost 500 clinical islet transplants were performed in the 1990s, it was not until the turn of the century that islet transplantation became routinely successful with the development of the 'Edmonton protocol'. This comprised a steroid-free immunosuppressive regimen, and a wide number of small modifications to the previous islet isolation and islet transplant protocols. In addition, recipients underwent 2-3 islet transplants in short succession to ensure a high islet mass was transplanted. As a result, in 2001 the Edmonton team published their seminal paper reporting 7 consecutive patients who had undergone successful islet transplantation for life-threatening hypoglycaemia unawareness, all of whom became insulin-independent. Since then, the islet transplant field has expanded rapidly and this procedure has now become an acceptable treatment for adults with severe hypoglycaemia unresponsive to optimised conventional treatment. Furthermore, as a cellular transplant there is huge potential to be able in due course to find alternative sources of islets and also to be able to manipulate / isolate the islet cells to potentially be able to avoid the need for immunosuppression in the future.

Islet Transplant Procedure

Islet transplantation involves two main steps, namely, human islet isolation and the islet infusion:

Islet Isolation

Islet isolation remains the more complex and variable step of islet transplantation. The challenge is to extract intact 'mini-organs' each comprising several thousand cells (the islets) from within an organ (the pancreas). Following retrieval (both donors after brain-death (DBDs) and donors after circulatory death (DCDs) are used) and transport of a donor pancreas to a clinical islet isolation centre, the pancreas is enzymatically and mechanically broken down. The resulting 'digest' is then purified using density-gradient centrifugation. The resulting 'pure' islets are incubated in culture medium for a minimum of 24 hours prior to transplantation. During this time, the islets can 'recover' from the islet processing and quantitative and qualitative assessments are performed. This period of incubation also allows the islet transplant to be planned as a semi-elective procedure rather than having to transfer patients to transplant centres as emergencies out-of-hours .

Islet Infusion

Although islet transplantation can be performed laparoscopically or through a mini-laparotomy, the majority are performed as a minimally-invasive percutaneous transhepatic procedure under local anaesthetic in the interventional radiology suite. Following administration of induction immunosuppression (currently alemtuzumab (Campath) in the UK), broad spectrum antibiotics, a tight variable-rate insulin infusion, and heparin, islets are infused into the portal vein. During the procedure, the portal pressure is monitored closely, but as the total packed volume of islets transplanted is rarely more than 5ml diluted in 500ml of transplant fluid, the risk of portal vein thrombosis is negligible. The islets then become trapped within the sinusoids of the liver and subsequently become revascularised and reinnervated from the surrounding liver parenchyma. Alternative implantation sites are currently being

explored, including subcutaneous tissue, intramuscular sites, and the omentum. However, although not ideal, the liver currently remains the most practical site.

Post-transplantation, patients remain on the tight variable-rate insulin infusion for about 48 hours in order to prevent endogenous insulin secretion during early islet engraftment. After this they are placed back onto their pre-transplant insulin doses and discharged home after about 72 hours. Peri-transplantation, maintenance immunosuppression is started (in the UK this comprises tacrolimus and mycophenolate). Following discharge, there is an intensive outpatient follow-up to titrate insulin requirements with islet function, and to ensure that blood levels of tacrolimus remain within a safe therapeutic range. Recipients are reviewed twice weekly for the first month and weekly for the next 2 months. Graft function is monitored by measuring stimulated c-peptide levels during a mixed meal tolerance test at 1 month, 3 months, 6 months, and yearly thereafter.

Results of Islet Transplantation

Although insulin-independence remains the ideal outcome measure for islet transplantation, many countries, including the National Institute for Health and Care Excellence (NICE) in the UK, have deemed that the primary outcome measures should currently be resolution of hypoglycaemia unawareness and stabilisation of HbA1C. Table 1 shows the outcome data from the UK and the USA, and demonstrates that hypoglycaemia awareness improved significantly with near resolution of severe hypoglycaemia episodes in both populations, despite differences in transplant practices (the majority of the USA group received 2 transplants, while the UK population received only 1 – as was standard practice at the time). Follow-up studies are ongoing to evaluate the long-term impact of islet transplantation.

(Table 1)

Islet Programme in the UK (NHS)

Although 12 islet transplants were performed in the UK in the 1990s (9 in Oxford; 3 in Leicester), none of these patients became insulin independent. However, between 2005 and 2008, 12 Edmonton-type islet transplants were funded by Diabetes UK (performed in Oxford, King's College, and Royal Free). The encouraging outcomes led to NHS Commissioning Islet Transplantation as a clinical service in 6 centres in England and Wales in 2008 (Oxford, King's College, Royal Free, Newcastle, Manchester, and Bristol), shortly followed by a Scottish funded centre in Edinburgh. Two clinical islet isolation centres were commissioned in England and Wales (Oxford and King's College) in a 'hub and spoke' model. This means that the UK is one of only a few countries (which include Canada but not the USA) in which islet transplantation is reimbursed by the health system. Interestingly, a recent economic evaluation of allogenic islet transplantation found that it is cost-effective compared to whole pancreas transplantation in England and Wales, and there is currently much attention on whether the procedure will be reimbursed in the USA following publication earlier this year of the findings from a large multicentre trial in North America (CIT-07).

The UK is also unique in that in 2010, a joint pancreas allocation system was established for whole pancreas transplantation and islet transplantation. This is a major advance, as in most countries whole pancreas still has priority for organ allocation, and as such islet isolation centres often receive highly marginal organs for attempted islet extraction.

The challenge of making islet transplantation applicable to a wider group of recipients including children

Although islet transplantation has been a highly successful treatment for a select group of adult patients with severe T1DM, there are a number of challenges to overcome before this important treatment can be used for a wider group of patients, and before it can ultimately be applicable to children. These include the need to: 1) optimise pancreas procurement and islet isolation; 2) improve post-transplant islet engraftment and islet survival; 3) overcome pancreas shortage, including finding an alternative renewable source of islets; 4) develop strategies that avoid the need for life-long immunosuppression.

1) Optimisation of Pancreas Procurement and Islet Isolation

Human islet isolation remains a highly variable procedure with less than 50% of processed pancreases resulting in a transplantable islet yield. The problem is that the enzymatic methods used for pancreas digestion are fairly crude and have not been developed based on a detailed understanding of the pancreatic matrix. The current challenge is to try to understand the molecular structure of the islet-exocrine interface for the full range of different donor variables so that targeted enzyme blends can be developed that can routinely ensure optimal islet liberation from the surrounding tissue. Furthermore, the shortage of donor pancreases means that most groups are increasingly accepting marginal donors for their transplant programmes. This includes an increased use of organs from DCD donors, in addition to the more optimal DBD donors. DCDs are subject to a greater degree of warm ischaemia, something that is particularly deleterious to islets. A number of approaches are being investigated to optimise pancreas preservation after retrieval, and also to see if ischaemic damage can be reversed. Hypothermic perfusion is a process in which the retrieved organ is connected to a machine via its arterial and venous supply that pumps a preservation fluid (usually a proprietary solution called UW) in pulses at a temperature of between 4 – 10°C. The technique was originally used in the late 1970s, though it has made a come-back over the past decade. The exact mechanism of action is still unclear, however it is thought to preserve the vascular endothelium and clear toxic metabolites from the microcirculation. Most experience comes from the field of kidney transplantation, and there is emerging evidence of its role in pancreas transplant both whole organ and islet.

An alternative technique being explored is ex-vivo normothermic perfusion. This involves connecting the donor organ to a circuit similar to extra-corporeal membrane oxygenation and using a red-cell based perfusate. Again the mechanism of action is poorly understood, but recent reports for a number of organs (including kidney and liver) and for DCD pancreases have been encouraging.

2) Improving post-transplant islet engraftment and islet survival

It is thought that about 40-50% of transplanted islets are destroyed within minutes to hours of infusion into the liver by a mechanism called “instant blood mediated inflammatory reaction” (IBMIR). This involves platelet aggregation and activation of the complement and coagulation systems. Strategies to prevent this occurring involve adding heparin to the bag of islets prior to infusion, with emphasis also shifting to suppressing the acute inflammatory injury of islets. There is now evidence that incorporating an anti-TNF- α agent in the induction regimen improves islet transplant outcomes, and other targets such as IL-1 receptor are being investigated. In addition, groups are exploring coating islets with different compounds, including heparin, in order to prevent IBMIR islet destruction.

3) Overcoming the challenge of pancreas shortage including finding a renewable islet source

At present, there is a worldwide mismatch between the number of available donor pancreases and the number of potential recipients with T1DM who would benefit from islet transplantation. Various ways of addressing this shortage include the introduction of 'opt-in' rather than 'opt out' systems for organ donation, which is used in some European countries and has been shown to increase donor numbers, and the use of more marginal donors, as described above. However, whilst these different approaches have their impact, ultimately what is required is an alternative renewable islet source. The two main approaches being explored are the use of islet stem cells, and the use of xenogeneic (animal) islets:

Islet Stem-Cells and Islet Regeneration

The potential for stem cells to revolutionise the treatment of chronic conditions has been reported for a number of years, however there are few tangible examples of this technology transitioning into clinical application. This has partly been due to ethical concerns regarding the use of human embryonic stem cells (derived from the blastocyst of *in vitro* fertilised embryos which are not being implanted), but also the risks of uncontrolled growth and teratoma formation. Adult fully differentiated cells (such as fibroblasts and adipocytes) can also be converted into pluripotent cells (termed inducible pluripotent stem cells) by using viral vectors to introduce or activate specific genes.

Other than the risk of tumour formation, the main obstacle has been manipulating these stem cells down specific differentiation pathways to generate the cells of interest. In the diabetes field, the best current techniques involve stepwise induction protocols which result in "beta-like" cells, meaning that they are able to secrete insulin in response to glucose but not as quickly nor to the same degree as fully mature human islets. Nevertheless, animal studies have shown that these cells are able to restore normoglycaemia in diabetic models.

An alternative approach to using controversial human embryonic tissue could be cells taken from the placenta after birth. Amniotic epithelial (AE) cells can be isolated from the new-born's placenta, which would usually be discarded, and are able to differentiate down all three germ-layer paths. Evidence is emerging of their ability to differentiate into pancreatic endocrine cells (a multi-hormonal stage which precedes the "beta-like" cells), with some success in improving glycaemic control in rodent models of diabetes. AE cells also have certain characteristics which set them apart from the other stem-cell types: they carry a lower risk of graft-versus-host disease (as the placenta is an immune-privileged site) and appear to have no risk of teratoma formation. What's more, the worldwide availability of this tissue and the relative ease of isolation means that research into this cell source, though still at an early stage, has the potential for much wider application in the future.

The ideal situation, which would overcome the problems of all the above techniques, would be to regenerate beta-cells. Whether or not there exists new beta-cell formation in adult humans is a hot topic of debate in the field of developmental biology. Evidence in favour of this comes partly from findings that patients who have had T1DM for over 50 years still have beta-cells in their pancreas at autopsy, but whether these cells are new or "immune-resistant" is unclear. Research from the field of type 2 diabetes into the mechanisms of beta-cell-mass expansion and beta-cell death indicates a degree of trans-differentiation between alpha- and beta-cells, and indeed between cells lining the pancreatic duct and alpha-cells. Novel compounds are under development which reportedly "guide" regenerating cells down the path to beta-cells, and pilot clinical trials are underway in patients with type 2 diabetes. Whether these

same compounds could have a role in T1DM treatment is unknown, but regenerating endogenous immune-resistant beta-cells would be the epitome of diabetes cures.

Xenotransplantation

Although a number of different xenogeneic sources of islets have been explored for use in transplantation, porcine islets have generated the most interest. Pig insulin is very similar to human insulin (only 1 amino acid difference out of 51) and was of course used extensively in the past to treat T1DM. Research over the past 10 years has focused on pig-to-nonhuman primate islet transplantation models, with excellent outcomes for 6 months post transplantation. In addition, there is now preliminary safety data from small clinical trials in humans. While it is potentially an abundant source of islet cells, there are specific issues that need to be overcome before pig islets can be routinely used in humans. Firstly, IBMIR is more pronounced in xenogeneic islet transplantation leading to much higher rates of destruction. Secondly, questions remain about the possible transmission of pathogenic organisms – though there have been no documented cases. There are also concerns about the development of xeno-antibodies and the appropriate immunosuppression regimens. Transgenic technology has allowed the development of a number of pig strains which are less immunogenic and some research groups have even developed porcine strains in which islets secrete immunosuppression into the surrounding tissues. Finally, and probably most importantly, xenotransplantation would have to be proven to be cost-effective in order for it to gain widespread support from commissioning bodies.

4) Developing Strategies to avoid life-long immunosuppression

Although the challenges described above are preventing more patients from receiving a successful islet transplant, the primary obstacle preventing islet transplantation from being made available to children is the need for life-long immunosuppression. Whilst the relative risks of immunosuppression (tumour formation, nephrotoxicity, and increased susceptibility to infection etc.) can be justified if these are outweighed by the benefits of an islet transplant (e.g. for life-threatening hypoglycaemia etc.), it is hard to justify these risks in patients who have not yet developed the life-threatening or life-shortening complications of T1DM. However, many groups are currently researching novel approaches to enabling islet transplantation to be performed without immunosuppression. Achieving this goal would be the real “game changer” for the field. In broad terms there are 2 approaches, namely altering the recipient’s immune system to create immune tolerance of the islet graft, or isolating the islets from the immune system within an immunoprotective device (encapsulation).

Islet Immunoalteration (Immune Tolerance)

As lymphocytes mature, they develop tolerance to native tissue while maintaining their responsiveness to foreign pathogens. This process is disrupted in auto-immune conditions (such as T1DM), but is also the basis of immune rejection in transplantation. By manipulating the system into a state of immune tolerance it could be possible to protect the transplanted tissue while not increasing the risk of infections. There is growing interest in targeting regulatory T-cells and the use of anti-CD3 antibodies (such as oteelixizumab) in both the transplant world and in preventing the onset of T1DM in high risk individuals.

Islet Immunoisolation (Encapsulation)

There are 2 broad types of encapsulation: microencapsulation, in which individual islets are coated with alginate materials, and macroencapsulation, in which the whole islet graft is placed within an implantable device. In both cases the principles are the same, namely that the capsule consists of a semipermeable membrane which forms a physical barrier between the cells and the recipient, allowing glucose and oxygen to exchange freely but preventing contact with the immune system. This allows transplantation to occur without requiring immunosuppression, but also allows the islets to be contained and easily removed, something that is vital for islets from either a stem cell or a xenogeneic source.

Such is the interest generated by macroencapsulation devices that there are already pilot clinical trials underway using this technology together with xenogeneic islets (BetaO2) or human stem-cell derived cells (ViaCyte and TheraCyte). However, there are a number of technical aspects that are still under development. Firstly, low oxygen levels within the core of the device can lead to cell necrosis. The BetaO2 has addressed this issue, but requires the user to connect to an oxygen exchange pump every day via an injection into a subcutaneous port. Secondly, the inefficiency of current beta-like cells means that large numbers are likely to be required for good glycaemic control in humans. This would probably mean multiple devices being inserted into the subcutaneous tissue. Thirdly, there is the potential for breakdown of the membrane or fibrosis around the capsule material which could go undetected. Finally, it is important to point out that a number of these innovations are being driven by private companies and as such there is an element of secrecy surrounding their products (e.g. the technique for deriving the pancreatic progenitor cells by ViaCyte has not been disclosed). Progress in this field needs to be evidence-based, and this will likely require careful collaboration between industry and academic institutions.

Conclusion

Islet transplantation has huge potential for reversing T1DM. It is minimally invasive, safe, and over the past 10 years has achieved excellent results in selected adult pancreases. However, its full potential is yet to be realised. Whilst a number of challenges remain to further improve the outcomes of islet transplantation, the primary challenge preventing this important treatment being translated from adults to children is the need for life-long immunosuppression. As such, currently the procedure is justifiable if the complications of T1DM outweigh the potential risks of immunosuppression. Novel strategies are being developed that should enable people with T1DM to be transplanted without the need for or with minimal immunosuppression. Once this goal has been reached, islet transplantation really could become a 'cure' for young people soon after diagnosis.

Practice Points

- Type 1 diabetes affects 345,000 children and adults in the UK
- Islet transplantation is a viable treatment for patients with hypoglycaemia unawareness with life-threatening hypoglycaemia episodes
- It is currently only used in adults due to the need for life-long immunosuppression
- Research is focusing on finding alternative sources of islet cells, and on developing immunosuppression-free transplantation methods
- This would allow the treatment to be offered to children soon after diagnosis

Islet transplant outcomes						
	UK (Brooks et al., 2013)			USA (Hering et al., 2016; CIT-07)		
	Pre-transplant	1 year	2 years	Pre-transplant	1 year	2 years
Graft function			80%		94%	
Target HbA1c < 7.0% (53mmol/mol)			70%		87.5%	71%
Hypoglycaemia awareness (reduction indicates benefit)	Gold score:			Clarke score:		
	6		3	6	<1	<1
Insulin independence		45%	15%		52%	
Severe hypoglycaemia episodes	episodes/patient-years			At least one severe hypoglycaemia episode/year		
	20	0	0.3*	100%	4%**	
* 0.7 in those who had lost graft function						
** these patients were documented as taking too much insulin against medical advice						

Table 1: Islet transplant outcomes at 2 years in the UK and a sub-population in the USA currently in the CIT-07 trial.

Conflict of interest statement

The authors declare no conflict of interest.

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