Surgical treatment of diabetes mellitus by islet cell and pancreas transplantation

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The incidence and progression of chronic diabetic complications can be reduced by achieving normoglycaemia (box 1). Unfortunately the recent Diabetes Control and Complications Trial has shown that intensive, subcutaneous insulin regimens that improve blood glucose control puts the patient at three times the risk of developing severe hypoglycaemia.¹ Intensive subcutaneous insulin regimens can never mimic the physiological fluctuations of *in vivo* insulin secretion. An alternative option to achieve near normoglycaemia is by transplantation of the whole pancreas (vascularised pancreas transplantation). Some would argue that this is perhaps a cumbersome approach when only the islet cells are needed to restore physiological levels of blood glucose, but perhaps more importantly pancreas transplantation (box 2) has an appreciable high rate of morbidity and mortality compared with kidney transplantation alone.² With these factors in mind investigators have tried to isolate and transplant individual islet of Langerhans cells.

Box 1: Chronic diabetic complications

- Nephropathy.
- Neuropathy.
- Retinopathy.
- Peripheral vascular disease.
- Coronary artery disease.
- Cerebrovascular disease.

Box 2: Pancreas transplantation

- Major surgical procedure.
- Higher rate of morbidity and mortality.
- Need for immunosuppression.
- Improves quality of life.
- Reverses some diabetic complications.
- 82% insulin independent at one year.

Box 3: Islet transplantation

- Minor radiological procedure.
- Low morbidity and mortality.
- Intrahepatic implantation.
- Potential for no immunosuppression.
- Potential for use in young newly diagnosed diabetic patients.
- 14% insulin independent at one year.

The advantages of islet cell transplantation (box 3) are that it requires only local anaesthesia and is a minor radiological procedure having minimal risk to the patient. Unfortunately the merits of both pancreas and islet cell transplantation have to be weighed against the need for immunosuppression and for those patients having a pancreas transplant the risk of the surgical procedure.³ For patients who survive a pancreas transplant some of these risks are offset by the improved quality of life.⁴ In the majority of patients pancreas and islet cell transplantation have been reserved for type 1 diabetic patients who already have or are about to receive a renal transplant. These patients will therefore have a need for the necessary immunosuppression as a renal transplant is life saving. Ideally islet cell transplantation needs to be performed before diabetic complications have become established, in young newly diagnosed diabetic patients. In these patients it is difficult to justify the immunosuppression because not all patients will develop severe diabetic complications.

Over the last decade many investigators have been able to isolate a significant number of purified islets from the human pancreas. Recent evidence indicates that to consistently achieve insulin independence islets will have to be prepared from more than one human donor pancreas. Another problem thwarting its current progress has been graft rejection.⁵ At present there is no early marker for islet allograft rejection and by the time hyperglycaemia has ensued restoration of normoglycaemia is impossible and the graft is lost. One can therefore conclude that while the safety of islet cell transplantation has been proved, its efficacy is still far from ideal and pancreas transplantation is currently the more successful option.⁵

Pancreas transplantation

The International Pancreas Transplant Registry has reported well over 10000 cases world wide, most in the USA.⁶ In the UK only five centres (Cardiff, Liverpool, Leeds, and both St Mary's and King's College in London) regularly perform the procedure. A total of 130 pancreas transplants have been performed in the UK during the last 14 years.⁷

A pancreas transplant can be performed simultaneous with a kidney (SPK; 90%), or after a kidney transplant (4%) and in specialised centres in non-uraemic patients, pancreas transplant alone (6%). Survival rates are better for SPK because acute rejection can be treated earlier coinciding with the simultaneous rise in serum creatinine that is indicative of acute rejection of the kidney. Nevertheless, patients

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have higher rates of infection, surgical complications, increased rates of acute rejection, and more hospital admissions when compared with kidney transplants alone.^{8,9}

Performing a pancreas transplant in a non-uraemic patient is one of the most contentious areas of pancreas transplantation. These patients have "brittle" (labile) diabetes or hypoglycaemic unawareness that is regarded as potentially more harmful than the combined risk of the immunosuppression and surgical risks. The centre in Minneapolis has the largest experience (n=225), showing acceptable rates of graft survival (80% at one year) and patient survival (90% at one year).¹⁰

HOW TO PERFORM A PANCREAS TRANSPLANT

Over 40 different modifications of pancreas transplantation have been described but most are of historical interest.¹¹ The graft is placed within the peritoneal cavity and revascularised to the external iliac artery and vein. One of the non-physiological consequences of this technique is that insulin is secreted into the systemic venous circulation. Portal venous drainage has been described¹² and is favoured by some in order to avoid the potential atherogenic effects of peripheral hyperinsulinaemia.¹³ It is also more physiological in that it delivers insulin directly to its main target organ, the liver. However, this is technically more difficult and there is an appreciable risk of portal venous thrombosis.

Exocrine secretions are essentially managed in three different ways: either drainage into the bladder or small intestine, and although not commonly used outside France, duct obliteration by injection with a sclerosant. The advantages of bladder drainage are that a decline in urinary amylase allows the earlier detection of acute graft rejection.¹⁴ Another important consideration is that up to 25% of all pancreas transplants performed with bladder drainage will need enteric conversion at a later date.^{15,16} The reasons for this are metabolic acidosis, recurrent urinary tract infections, bladder mucosal dysplasia, and reflux pancreatitis and consequently enteric drainage is rapidly regaining popularity.

EFFECT OF PANCREAS TRANSPLANTATION ON DIABETIC COMPLICATIONS

Numerous uncontrolled studies have demonstrated the effect of pancreas transplantation on the quality of life.⁴ Some patients have complications that are perhaps too far advanced for a pancreas transplant to have any significant advantage.⁵ Also, direct comparisons between recipients of a pancreas and kidney transplant with patients having only a kidney transplant alone are often difficult to interpret because recipients having the simultaneous procedure are often younger and fitter. However approximately 90% of patients report improvements in their quality of life.^{4,17}

A number of studies indicate a reduction in incidence and prevention of glomerular hypertrophy and mesangial proliferation in kidney allografts when combined with a pancreas transplant.¹⁸ More recent evidence now indicates reversal of established lesions of diabetic

nephropathy in native kidneys after at least five years of normoglycaemia.¹⁹ It must also be appreciated that renal function after a pancreas transplant can deteriorate three times more rapidly than diabetic patients with overt nephropathy treated with insulin injections. This is because of the nephrotoxic nature of cyclosporin²⁰ but more recently this has perhaps been curtailed by the use of tacrolimus.

In the majority of patients, once the proliferative changes of diabetic retinopathy have taken place, a successful pancreas transplant is unlikely to reverse them. This has been shown by a number of different comparisons, including kidney/pancreas recipients versus patients whose grafts have rejected and kidney/pancreas recipients versus those having a kidney transplant alone.²¹ Indeed despite physiological control of blood glucose for up to 12 months, retinopathy can deteriorate.^{21,22} One must also keep in mind that more conservative treatments such as photocoagulation are known to stabilise diabetic retinopathy and therefore vascularised pancreas transplantation cannot be justified as an appropriate substitute.

In cases of advanced neuropathy a pancreas transplant will have no benefit. In less severe cases, a pancreas transplant can improve peripheral and autonomic neuropathy.^{23,24} Finally if combined pancreas and kidney transplantation is to have a major impact for uraemic diabetic patients it still needs to be proved against the development of macrovascular angiopathy. To date there are no trials demonstrating good evidence in favour of reducing macrovascular complications.

MORBIDITY AND MORTALITY

Pancreas transplantation has a high rate of morbidity and mortality when compared with islet transplantation. Manske *et al* report three year patient survival after simultaneous pancreas and kidney transplantation to be 68%, compared with over 90% for those patients who receive only a kidney transplant.² More recently, however, Tyden *et al* have demonstrated that in well matched groups patients receiving a SPK, in comparison to those receiving a kidney transplant alone, have a distinct long term survival advantage if they can survive the first year after transplantation (80% *v* 20% mortality at 10 years).²⁵ During the first 12 months of transplantation approximately 10% of patients may die in contrast to less than 1% receiving only a kidney transplant. Stratta reported that 12% of patients will die within the first 18 months of surgery.²⁶ Infection, myocardial infarction, and sudden death were the most common causes of mortality.²⁶ Nevertheless rates of graft survival are continually improving. Graft survival is now 94% and insulin independence 82% (actuarial five year survival 65%).⁶ On balance pancreas transplantation is perhaps a more expensive treatment than subcutaneous insulin injections in the short term, but to a young uraemic diabetic patient the risk of death and early expense are perhaps worth taking in preference for the improved quality of life and the prospect of better long term survival.

Figure 1 The automated method for islet isolation.

Islet transplantation

For successful islet isolation the donor pancreas has to be retrieved with great care. Young donors are preferred (20–50 years) with minimal warm ischaemia. Prolonged cold ischaemia is also damaging to the pancreas (for example, greater than 12 hours).

ISLET ISOLATION

The pancreas is digested using an enzyme called collagenase (Human Liberase).²⁷ This is required to separate islets from surrounding acinar tissue by digesting the pancreatic extracellular matrix. Collagenase is derived from cultures of *Clostridium histolyticum* bacteria²⁷ but can have extreme batch to batch variation. The pancreas digestion phase is still the most critical and highly variable stage of islet isolation. There are many reasons for this other than the collagenase itself; these include organ donor variables (for example, donor age, body mass index), warm and cold ischaemia during the procurement process, and the inconsistencies of islet purification. More recent advances have included the development of endotoxin

free agents for islet isolation, as endotoxin is damaging to islets.²⁸

One of the milestones in islet isolation was the introduction of Camillo Ricordi's method of automated pancreas dispersion (fig 1).²⁹ Collagenase is administered within the pancreatic duct. The pancreas is placed within a stainless steel chamber containing steel spheres. The chamber is gently shaken and the pancreas gradually becomes dispersed. This process takes about 45 minutes. Islets totally devoid of surrounding acinar ("cleaved") are desirable to allow density gradient purification (fig 2). Approximately 15–40 ml of pancreatic digest is usually collected containing cleaved islets, uncleaved islets, acinar tissue, ductal and lymphoid remnants. This tissue is then purified.

Islets and acinar tissue have different buoyant densities allowing large scale separation on a density gradient layered onto a COBE 2991 processor.^{30–31} Purification optimises islet yield by reducing the load of unwanted contaminating acinar tissue making transplantation a much safer procedure. Purification may also improve islet revascularisation. Furthermore large volumes of unpurified pancreatic digest infused into the portal vein or spleen have led to portal hypertension, hepatic or splenic infarction, disseminated intravascular coagulation, splenectomy, and death.³²

TRANSPLANTATION SITE

Many transplant sites have been attempted experimentally, including the renal subcapsular space, the spleen, and intraperitoneal space. The intraportal site is the most common. By using the liver islets can be implanted percutaneously under radiological guidance with a local anaesthetic.³³

ISLET CELL ALLOGRAFT REJECTION

Graft rejection is one of the major problems curtailing successful islet cell transplantation. Many experimental studies highlight the diabetogenic effects of cyclosporin and tacrolimus.

Figure 2 Isolated human islets stained red with dithizone.

Peak concentrations of immunosuppressive drugs are also high in portal venous blood and are likely to be too toxic for islet engraftment.³⁴ With the introduction of newer non-diabetogenic immunosuppressive regimens (for example, rapamycin (Sirolimus), deoxyspergualin, mycophenolate mofetil (MMF), humanised interleukin-2 receptor antagonists (Daclizumab) and humanised anti-CD 154 antibodies³⁵) it is hoped that islet cell allograft loss through allograft rejection will be minimised. Preliminary reports from the Edmonton group,³⁶ using a non-diabetogenic steroid free protocol (Sirolimus, Dacluzimab, and low dose tacrolimus) can dramatically improve the rates of insulin independence at one year (100% n=7) but only when islets are retrieved from multiple organ donors. These patients were also non-uraemic type 1 diabetic patients. The rationale for this approach was that the patient's extremely labile diabetes and hypoglycaemic unawareness were considered to be potentially more life threatening than the side effects of the immunosuppression.

Theoretically islet cell transplantation has the potential to be performed without immunosuppression by achieving immunological tolerance. This is a process where the recipient becomes immunologically tolerant to donor tissue and the recipient does not reject the islet cell graft. One technique that has been extensively investigated, in the hope of achieving immunological tolerance, is immunomodulation.

Immunomodulation either by gamma or ultraviolet irradiation, culturing islets with monoclonal antibodies or at low temperature (22°C) and cryopreservation have proved to be successful experimentally but not in the clinical situation.⁵ The principals of immunomodulation are based on the concept that some cell types called passenger leucocytes (for example, dendritic cells) within an allograft are more likely to stimulate rejection because of expressing major histocompatibility complex class II antigens. Pretreatment of islet cell allografts by immunomodulation may delete passenger leucocytes making graft rejection less likely. The mechanisms of immunological tolerance are still incompletely understood but it is hoped that in the future a combination of immunosuppressive agents, immunomodulation, and the application of monoclonal antibodies will eventually reach this goal.

Other strategies to prevent islet rejection have included islet encapsulation where islets are protected by an artificial membrane but most encapsulated islet grafts still fail because of capsule fibrosis and bioincompatibility.³⁷

ISLET CELL AUTOIMMUNITY

Another problem with islet cell transplantation is that diabetes is an autoimmune disease. Isolated cases of autoimmune recurrence have been described after pancreas transplantation.³⁸ Even therapeutic immunosuppression (for example, cyclosporin, steroids, and azathioprine) has previously not been able to prevent autoimmune recurrence. For example, a twin

recipient receiving a segmental pancreas allograft has developed recurrence of autoimmunity. Autoimmune recurrence undoubtedly plays a role in the failure of islet allografts. Roep *et al* have investigated autoimmune responses after islet transplantation.³⁹ Patients with minor alloimmune and autoimmune responses against islet allografts maintained function for more than a year. Where there was strong alloreactivity grafts rapidly failed with varying degrees of autoimmune reactivity. Lastly, hyperautoreactivity can occur in the absence of alloreactivity with progressive loss of the islet graft.³⁹

RESULTS OF ISLET CELL TRANSPLANTATION

Over 300 islet allografts have been performed world wide. The majority have been performed in America (for example, Minnesota, Miami). In Europe, Geissen and Milan have performed the majority.⁴⁰ Insulin independence for more than one week has been documented in 33 of the 305 recipients performed during 1974–96. Only 4% remain insulin independent at one year (n=13). The Geissen group have reported the most recent success.⁴¹ At one year 45% of those with islet after kidney (IAK) transplantation (n=20) and 74% of those with simultaneous islet/kidney (SIK) transplantation (n=31) have demonstrated significant islet function with normalised glycated haemoglobin. Daily insulin requirements have been reduced to 24 IU from 40 IU after SIK transplantation and 12 IU from 45 IU after IAK transplantation. Insulin independence at one year is equivalent: for IAK transplantation 20% (4/20) and SIK transplantation 19% (6/31). This improvement was deemed to be as a result of better immunosuppression (cyclosporin with MMF or azathioprine) and peritransplant protocols (for example, pentoxifyline, antioxidants, and nicotinamide).

Many other factors can partly explain the failure of islet cell allografts such as transplantation of insufficient numbers of islets. As few as 2500 islets/kg body weight can lead to insulin independence after islet autotransplantation, as allograft rejection does not occur.³² For type 1 diabetic patients receiving an allograft who have developed insulin independence the mean number of islets transplanted has been approximately 8500 international islet equivalents/kg.^{36, 40} The average 70 g pancreas contains anything from 305 000 to 1.5 million islets (4% of total pancreas volume) and therefore implies that current islet isolation protocols are inefficient. Investigators have therefore cryopreserved islets from multiple donors to increase islet mass but islet losses are still a big problem and pooling islets from multiple donors may also increase the risk of rejection but it now seems to be the only way of consistently achieving insulin independence.³⁶

Conclusion

There is no doubt that islet cell transplantation has far less morbidity and mortality than whole pancreas transplantation and theoretically could be suitable for more of the diabetic population but significant advances will still have to be

made if it is to achieve its full potential and match the results of pancreas transplantation.

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