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PANCREATIC TRANSPLANTATION*

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Despite intensive medical management by diet, exercise and exogenous insulin therapy complied with by the patient, a proportion of patients with diabetes mellitus, largely but not exclusively of type 1, continue to progress to end-stage disease, with debilitating retinopathy, central and peripheral vasculopathy, nephropathy, neuropathy and enteropathy. These patients develop blindness and renal failure, often at a young age.

Diabetes mellitus afflicts an estimated 80 million people worldwide; 5 to 10% are classified as Type I and are dependent upon exogenous insulin for life. Approximately 2% of the UK population is affected by diabetes: one million people in England and Wales.[1] It is the commonest cause of blindness under the age of 60 in Britain.[2] Clinical renal disease develops in about one fifth of the insulin-dependent group, although incipient nephropathy, reflected by microalbuminuria, is much more common.[3,4] Diabetes accounts for about 10% of the end stage renal failure population. Neuropathy, both somatic and autonomic, also occurs, with particular effects on the legs and feet: reduction in sensation can lead to ulceration and in combination with peripheral vascular disease, to gangrene and the need for amputation.[1] The risk of lower limb amputation in the British diabetic patient is 40 times that for the general population. Macrovascular disease, in the form of ischaemic heart disease, stroke and peripheral vascular disease is two to five times more common in diabetics than in the general population, and is the main cause of premature death in diabetes.[1,5] Diabetic ketoacidosis is an important cause of death in diabetics aged under 50. Hypoglycaemia is also common:[6] the average rate of hospital admissions for hypoglycaemia alone is 0.1 admissions per patient per year.[7] Even mild chronic hypoglycaemia may cause neurological damage and some young patients die from nocturnal hypoglycaemia.[8] The economic cost of diabetes is estimated to be 5% of total NHS expenditure, and this excludes the costs of families helping to look after their diabetic relatives and the costs to the country from lost productivity.[9] A large proportion of this expenditure is required for the treatment of the debilitating complications of this disease.

Whole organ pancreas transplantation

The goals of pancreas transplantation are to eliminate the morbidity associated with labile blood glucose levels, to stabilise or improve secondary diabetic complications, and to improve the quality of life of patients with diabetes mellitus by restoring normal glucose metabolism. With improvements both in surgical techniques and in immunosuppression, the results of combined simultaneous 'whole organ pancreas and kidney' (SPK) transplantation have improved significantly during the past decade and now approach those for other solid organ allografts.[10,11] SPK transplantation has not been popular in the UK because of anxieties about the management of serious

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postoperative complications, particularly graft pancreatitis, wound infection, bladder leaks and profound sepsis, and scepticism about the potential benefits. Improvements in surgical techniques, particularly in the USA, have now reduced the postoperative morbidity to acceptable levels and SPK transplantation is no longer regarded as an experimental procedure in that country.[11,12] A definite answer to whether this results in a reduction of long-term diabetic complications will not be known for several years, but recent results are encouraging.[13-16] The recent study by the American Diabetes Association has shown that improvements in glucose control will reduce the development of complications.[17]

The stabilisation of diabetic control, the avoidance of exogenous insulin and the ability to return to a normal diet for the first time since childhood are indisputable benefits of this procedure. In our own experience of sixteen SPK patients transplanted during the past four years, 81% have survived with 75% and 69% kidney and pancreas graft survivals respectively (average 24 months follow-up): their post-transplant glucose metabolism has been excellent, and five patients are now back in full-time employment for the first time for several years. This degree of improvement in the quality of life indicates that long-term national savings will be made in terms of previous expenditure on community support staff and government paid benefits, and in terms of taxation of future SPK recipient incomes.

The surgical techniques involved continue to evolve. At Leeds we have concentrated on the combined retrieval of both liver and pancreas from the donor by use of a donor iliac artery conduit to the splenic and superior mesenteric arteries (preserving the coeliac axis for the liver). The aim has been for as short a period of organ preservation as possible, with the recipient admitted just prior to organ retrieval, and a cross-match performed using donor blood at that time. The recipient operative approach has been through a short lower midline incision, with an extraperitoneal approach to the right external iliac vessels for kidney implantation, followed by intraperitoneal transplantation of the pancreas using the left common iliac vessels, with anastomosis of the donor duodenum to the bladder. Urinary pH and amylase have been monitored daily but more emphasis has been placed on the detection of kidney allograft rejection by biochemical analysis and regular biopsies.

Unfortunately, some urinary problems have been experienced with this approach (chemical cystitis and urethritis) and 40% have required enteric conversion i.e. disconnection of the donor duodenum from the bladder and anastomosis to the small bowel. In addition, urine pH and amylase have proved to be notoriously unreliable indicators of rejection. Therefore recently this protocol has been changed to primary enteric anastomosis.

In addition within this unit in Leeds one pancreas-only graft (successful at three years) was carried out, and four multivisceral grafts (liver-pancreas-small bowel) with two successes: one was lost to CMV, one to rejection. Immunosupression has been with cyclosporin, steroids and azathioprine. A 30% OKT3 usage compares to 5% in the renal allograft population. Multivisceral grafts have received FK506 and it is planned to use mycophenolate mofetil, a new immunosuppressive with effects on both B and T lymphocytes, in the near future.

Although pancreas transplantation is a relatively new method of therapy in the UK, the gravity of the surgery and the need for long-term immunosuppression means that whole-organ grafting must be reserved for patients with end-stage disease. A much more sensible and innovative approach would be to attempt to prevent the

development of the debilitating complications of diabetes by improving blood glucose metabolism at an early stage.

Insulin infusion pumps

The most sophisticated method of insulin delivery currently available for clinical use is continuous insulin infusion by a pump (which may be implanted subcutaneously) but even with this, it is not possible to achieve perfect metabolic control.[18] This is probably because of the absence of closed-loop regulation of the insulin delivery by the concomitant blood glucose level; tight control of the blood glucose increases the risk of hypoglycaemia and the patients tend to set their glucose homeostasis at a level which protects them from hypoglycaemia.[19] In addition, patients can only try to imitate glucose homeostasis by reacting to self-monitored glucose levels in blood and urine. Animal models using implanted pumps giving a low background insulin secretion, augmented at meal times by squeezing the chamber or by electric currents, have been relatively successful for periods as long as 10 months.[20,21] The development of a closed-loop insulin delivery system would be a major breakthrough for diabetic therapy, and some recent work has concentrated on the development of biosensors designed to react to glucose levels.[22,23]

Islet transplantation

Pancreatic islet cell transplantation has been studied extensively as an alternative to whole-organ pancreas grafting, and has a number of theoretical and practical advantages. There are about one million islets of Langerhans in a human pancreas and each has a diameter of about 150 microns with 60% of the islet mass made up of beta cells that secrete insulin. About 10%, or 100,000 islets - a volume of less than 1 ml, should be enough to provide normoglycaemia in a 70 kg human.[24] Islets can be isolated from pancreatic tissue by using collagenase digestion, followed by purification by hand-picking the islets from the tissue suspension or by density gradient separation using ficoll (a high density sugar) or albumin.[25] Some difficulties in large animal models and in humans are still encountered particularly with the purification stage. The islets can be transplanted into the liver via the portal vein or by a subcapsular injection into the kidney or spleen. Rejection of the islets remains a problem and the success rates of this type of transplantation have been poor in the clinical setting.[26]

Recent work has concentrated on the development of a bio-artificial or hybrid artificial pancreas and this approach is based on the concept of immuno-isolation. Living pancreatic islet cells are isolated from the immune system by an artificial coat or membrane with semipermeable properties. The membrane pore size is defined such that small molecules such as glucose, insulin, and nutrients can pass freely through it, but antibodies and white blood cells are excluded. Immune recognition and the rejection response cannot then occur. Several attempts at the development of an hybrid artificial pancreas have been made, with varying degrees of success. Each islet can be surrounded by a thin capsule, or a number of islets can be placed inside a larger macrocapsule or hollow fibre. They can be classified as extravascular or intravascular.

Microencapsulation of islets in a spherical calcium alginate hydrophilic bead was introduced by Lim and Sun in 1980.[27] This work demonstrated that islets micro-encapsulated in alginate/poly-L-ysine/polyethyleneimine remained morphologically and functionally intact for as long as 4 months in culture and they

were able to establish normoglycaemia for 2 weeks when implanted into diabetic rats.[27] A variety of polymers are being explored, including agarose, polyacrylamide-coated agarose, polyvinyl alcohol cross-linked with a styrylpyridinium group, cellulose sulphate, polycationic polymers, and a water-insoluble polyacrylate.[24,28-34] Unfortunately, fibrosis around the membrane, eventually limiting the diffusive exchange, has been a significant problem. With alterations in the characteristics of the capsule, advances are being made and some of the more recent results have been more encouraging.[35]

The alternative, and currently more promising, approach is macro-encapsulation, in which the membrane is formed in a hollow tube. During 1991-2, I worked with Professor A. P. Monaco's group at Harvard Medical School, in association with Dr W. L. Chick's team at Biohybrid Technologies Inc., Shrewsbury, MA, and Dr B. Solomon's team at W. R. Grace and Co., Lexington, MA. These teams have been investigating two types of micro-encapsulation devices: an intravascular device where blood flows through the tubes of a membrane and the islets are placed outside of it, and an extravascular device where the islets are inside tubes of membrane and the tubes are then distributed within the peritoneal cavity.[36-41]

The vascularised immuno-isolation devices have been tested in a canine diabetic model and have a clear clinical potential for both allograft and xenograft islet transplantation. The hybrid pancreas devices used in these animals consist of a single coiled hollow fibre ultrafiltration membrane fabricated from an acrylic copolymer and contained within a disk-shaped acrylic housing. The membrane was developed by W. R. Grace and Co. and has a nominal molecular weight cut-off between 50,000 and 80,000 Da (insulin has a molecular weight of 6,000 Da; IgG antibody's molecular weight is about 150,000 DA) and a wall thickness of 120-140 microns, much of which is support structure. The membrane is formed into a tube with an internal diameter of 5.7 mm and a length of approximately 33 cm, providing 60 to 65 cm of membrane surface area. It is surrounded by an annular cavity of approximately 5 ml which defines the islet chamber, and access to the chamber is achieved through two syringe ports capped with silicone. Each end of the membrane is connected to a polytetrafluoroethylene (PTFE) vascular graft with a matched internal diameter. The PTFE grafts are anastomosed end-to-side to the external iliac artery and common iliac vein in the dog model but could be anastomosed to a number of different sites in a human.

Vascular patency of unseeded devices has now been demonstrated, with several devices patent and *in situ* after more than three years. Pancreatectomised dogs received seeded devices. The pancreatic islets, once isolated, have been embedded in a matrix of nutrient media and injected into the device via the seeding ports: 200,000 to 400,000 islets per animal. The results from the first 23 experiments indicated that a single device was unable to consistently maintain normal fasting blood glucose levels in this severe model of diabetes. In double-device studies i.e. each animal receiving two devices, 17 of 25 dogs receiving canine islets functioned successfully for between 1 and 9 months, and a further 2 animals functioned for 1 year, with reversal to the diabetic state following device removal.[36,37,39,41] No other group has achieved this level of long-term islet viability and function without immunosuppression.

Recent work from this group has concentrated on xenograft islets: bovine and porcine islets tested in the dog model. No evidence of any cellular rejection process has been noted. An approximately 40% success has been achieved with porcine islets

to date, and most of the problems have related to islet isolation rather than device failure.[42,43] The pig has ethical attractions as a source of xenograft islets as a pancreas can be successfully retrieved at the slaughterhouse and the rest of the animal then processed for consumption.

This group's work on extravascular devices has involved the use of multiple tubular (2 to 4 cm x 5mm) membrane diffusion chambers ('straws') seeded with islet allografts and widely distributed throughout the peritoneal cavity. Following successful rodent work, the devices have been tested in several dogs.[40] A 50% success rate was achieved at 3 months, but there have been problems with membrane breakage. The initial intravenous glucose tolerance curves are much more impressive with the intraperitoneal devices suggesting that this approach is more efficient. The development of a protective carousel should solve the breakage problem.

Current work is aimed at improvements in porcine islet isolation, device design improvements, and islet replacement protocols for failing devices. Some success has been achieved with a single larger vascularised device in the canine model. F.D.A. approval has been achieved for early clinical testing of the vascularised devices but more work needs to be done on improvements for the intraperitoneal devices before clinical application can be considered. It is envisaged that a mini-laparotomy will be necessary for intraperitoneal placement, but laparoscopic implantation is also being considered.

CONCLUSIONS

Currently, allograft transplantation remains the only long-term successful replacement therapy for the patient with a major failing organ. The difficulties of donor organ shortage, the requirement for major surgery, and the side-effects of chronic immunosuppression provide a substantial impetus for work on artificial organ systems. At present, the hybrid approach, making use of a combination of living cells and biomaterials, shows the most promise. One major advantage of the immunoisolation approach is that xenograft cells can be used. The retrieval of tissue from food-source animals gets around many of the ethical arguments against the current search for xenograft whole-organ transplantation using primates or bioengineering methodology.

The future of this vital interface and partnership between basic science, clinical medicine and device manufacture will depend on a partnership of scientific institutions, hospitals and industry with basic scientists, clinicians and engineers working together.

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