

Personal Viewpoint

Islet Xenotransplantation: Are We Really Ready for Clinical Trials?

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Four clinical trials of porcine islet transplantation have been reported, and there are verbal reports that clinical trials on much larger scales are continuing in centers in China and Russia. The four reported trials are briefly reviewed and, in the light of the present status of experimental islet xenotransplantation, consideration is given to whether such trials are currently justified. The Ethics Committee of the International Xenotransplantation Association has (1) emphasized the need for encouraging studies in non-human primates before clinical trials should be undertaken, (2) mandatory monitoring for the transfer of porcine microorganisms, and (3) careful regulation and oversight by recognized bodies. Other aspects of the topic, such as the need for informed consent, are briefly discussed. We conclude that, at the present time, more data documenting convincing efficacy, focused on clinically applicable immunosuppressive regimens, are needed to justify the initiation of closely monitored clinical trials. A clinical trial may then be justified even though the potential risk to the patients, and possibly for society, will not be zero.

Key words: Clinical trials, pancreatic islets, xenotransplantation

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Introduction

Of all types of experimental xenotransplantation, islet transplantation is probably the closest to clinical application on a large scale. Over the past several years, progress has been made in the field of experimental islet xenotransplantation in pre-clinical non-human primate models (1), but significant questions remain as to whether progress has been sufficient to move toward clinical trials. Clinical trials of islet xenotransplantation were reported from the Soviet Union in the 1980s and 1990s [reviewed in (2)], and four more recent clinical studies using pig islets have been re-

ported (3–7); these trials are summarized in Table 1. In addition, however, there are verbal reports that clinical trials on much larger scales are continuing in centers in China and Russia.

Clinical Trials Reported to Date

In the early 1990s, Groth et al. (3) in Sweden were the first to report pig islet transplantation into immunosuppressed kidney allotransplant patients with diabetes ($n = 10$) (Table 1). Fetal pig islets were transplanted under the renal capsule ($n = 2$) or intraportally ($n = 8$). Diabetes was not reversed, although patients with intraportally transplanted islets excreted porcine C-peptide in their urine for varying periods of time, to a maximum of 460 days. It is questionable whether these patients benefited from the transplanted pig islets, since no reduction of insulin requirement was documented. However, the patients were not subjected to unnecessary immunosuppressive treatment, since this treatment was already being administered to protect their kidney allografts.

There have, however, been clinical studies that reported a decrease in exogenous insulin requirement after pig islet transplantation. In New Zealand, Elliott et al. (4) transplanted encapsulated neonatal porcine islets into the peritoneal cavity of two patients. One patient was non-immunosuppressed, and the other received immunosuppressive treatment for a prior kidney allotransplant (Table 1). A decrease of insulin requirement was observed and urinary porcine C-peptide excretion was detected in both patients for at least 14 months. Glycosylated hemoglobin (HbA1c) levels indicated better long-term control for up to 27 months in the non-immunosuppressed patient in whom, nine years after islet xenotransplantation, viable encapsulated islets were harvested by laparoscopy; these islets demonstrated insulin release after glucose stimulation *in vitro*. At this time, the patient claimed to continue to experience better glucose control (than pre-transplantation), although this claim was not supported by documented reduced exogenous insulin requirement; an improvement in the patient's HbA1c level was again documented (5).

More recently, Valdes-Gonzales and his colleagues (6) in Mexico reported transplantation of porcine islets, together with porcine Sertoli cells, into steel/Teflon stents placed

Table 1: Experience with clinical pig islet transplantation

First author (reference #)	Source pig islets	Recipients (n)	Site/number of islets (ICC/NPI) (when stated)	Immunosuppressive regimen	Outcome
Groth (3)	WT fetal	Group A: 2	A: Kidney capsule, 200,000 and 410,000	A and B: CyA (n = 10), prednisolone (n = 10)	A: No plasma C-pep. Mononuclear and eosinophilic infiltrates at day 21 (on kidney biopsy). B: Urine C-pep documented for up to 460 days (n = 4). Decreased exogenous insulin requirement (up to 34%), documented urinary C-pep production, and decreased glycosylated Hb for between 14–27 months.
Elliott (4,5)	WT neonatal	Group B: 8 2	B: Intraperitoneal, 330,000–1,020,000 1 million encapsulated in peritoneal cavity	ATG (n = 5) 15-deoxyspergualin (n = 5) Non-immunosuppressed (n = 1) CyA, AZA, prednisone (n = 1)	Nine years post Tx: viable islet cells identified in capsules (n = 1). Decreased exogenous insulin requirement for up to 4 years (n = 6). No serum C-pep. Glucose-stimulated serum porcine insulin (n = 3). A and B: Decreased exogenous insulin requirement (33–62%). C-pep for 1 year. C: No improvement.
Valdes-Gonzalez (6)	WT neonatal + Sertoli cells	12	14,000–21,000/kg subcutaneously, in collagen tubes in steel/teflon stents. Retransplants after 6 months (n = 11) and 3 years (n = 4)	Non-immunosuppressed	
Wang (7)	WT neonatal	Group A: 15 Group B: 3 Group C: 2	Groups A, B and C: Intra-hepatic artery, 5–7 million	A: CyA, MMF, prednisolone B: OKT-3, tacrolimus, sirolimus, prednisolone C: CyA, MMF	

ATG = Anti-thymocyte globulin, AZA = azathioprine, C-pep = porcine C-peptide, CyA = cyclosporine, ICC = islet-like cell clusters, MMF = Mycophenolate mofetil, NPI = neonatal pig islet, WT = wild-type.

subcutaneously in non-immunosuppressed adolescent diabetic patients. For various reasons, this trial received considerable criticism in certain medical journals (8,9). Twelve patients received transplants, of whom 11 received a second transplant after 6–9 months and 4 were again retransplanted after 3 years. In half of the patients, a significant reduction in exogenous insulin requirement was documented for up to 4 years, including two patients who became temporarily insulin-independent (Table 1). Although promising data are available from rodent allotransplantation (10) and xenotransplantation (11) studies with regard to immunoprotection by Sertoli cells, the data available are not conclusive. Recently, it was reported that co-transplantation of neonatal porcine Sertoli cells with islets into diabetic rats did not contribute to graft survival compared to transplantation of islets alone (12). Moreover, the beneficial effect of co-transplantation of porcine Sertoli cells could not be confirmed in a non-human primate model of porcine islet transplantation; although in this study no subcutaneous stents were used, no long-term survival was found when neonatal islets, with or without co-cultured Sertoli cells, were transplanted into various sites of non-diabetic non-immunosuppressed macaques (13).

In 2005, Wang et al. (7) from China reported briefly on the transplantation of neonatal pig islets into the hepatic artery of 20 diabetic patients (Table 1). Various immunosuppressive regimens were administered. All patients who received a steroid-based regimen ($n = 18$) (some of them in combination with tacrolimus and sirolimus) showed a decrease of insulin requirement of 33–62% for up to 1 year, with the presence of porcine C-peptide and without changes in human C-peptide production. Furthermore, HbA1c levels were reported to be normal during this period. The two patients who were transplanted with a steroid-free protocol did not show any improvement in their diabetic status.

Clinical Trials in Relation to the Principles of the Ethics Committee of the International Xenotransplantation Association

Physicians and surgeons caring for diabetic patients, particularly those with unstable blood glucose levels who are at risk from sudden hypoglycemic attacks, are clearly stimulated by a desire to help their patients. With the limited supply of human islets, some physicians may wish to proceed with a clinical trial of xenotransplantation, and do not want to wait for confirmatory evidence of a regimen's efficacy from expensive and time-consuming studies of pig islet transplantation in non-human primates. However, several aspects of islet xenotransplantation need to be addressed to determine whether clinical trials are currently justified.

All four of the above trials may have been undertaken before the Ethics Committee of the International Xeno-

transplantation Association (IXA) published its principles on clinical trials of xenotransplantation. Without wishing to be critical of these trials, it is of interest to consider whether they would have met the recommendations of the Committee.

The Need for Preliminary Studies in Non-Human Primates

The principles set out by the Ethics Committee, published first in May, 2003 (14), and subsequently discussed (14,15), state the need for adequate pre-clinical data to justify a clinical trial, including promising data in a non-human primate model. The duration of survival of pig islet grafts in non-human primates necessary to justify a clinical trial was not defined by the Committee, as it believed this to be a determination that must take into account the strengths and limitations of the particular studies.

Are studies in non-human primates essential before embarking on a clinical trial? They are difficult, expensive and time-consuming. However, the relative ease with which success can be achieved on occasions in rodent models has not been found to be a good indicator of success in humans, whereas non-human primate studies better reflect the hurdles that need to be overcome to achieve clinical success. In view of the possibility of transferring a porcine microorganism to the patient, and perhaps to the community at large, a clinical trial should only be undertaken if there is clear evidence of success in a pre-clinical non-human primate model.

As far as we are aware, no specific pre-clinical studies in non-human primates were carried out, or reported in the peer-reviewed literature, by the above four groups before embarking on clinical trials in the aforementioned human studies. Before the study by Elliott's group, however, Sun et al. had reported normoglycemia after transplantation of encapsulated pig islets into non-immunosuppressed spontaneously-diabetic monkeys (16). Non-human primate models of xenotransplantation are not widely available, and the expense and effort involved in these models may preclude this type of research in many centers. Nevertheless, if encouraging data have been obtained from rodent studies, there are centers with the facilities, expertise and even the financial support, where such research can be carried out on a collaborative basis. Although difficult, it is no longer a persuasive argument that this type of research was not available to the group considering embarking on a clinical trial.

Monitoring for Porcine Microorganisms

The IXA Ethics Committee addressed the need for monitoring for transfer of porcine infectious agents after

transplantation. The issue of the potential transmission of an infectious agent from pig to human continues to be raised, not only with regard to porcine endogenous retrovirus (PERV) but also to other porcine viruses such as the herpes viruses, e.g. cytomegalovirus and lymphotropic herpes virus (17,18). To reduce these risks, the Committee recommended that source animals should be obtained from closed colonies from which known and potential pathogens have been excluded. PERV are expressed in porcine islets, though expression does not necessarily mean that there will subsequently be release of virus (19). Cytomegalovirus, but not lymphotropic herpes virus, can readily be eliminated from a pig herd by early weaning (17).

Furthermore, the possibility of transmission of microorganisms that have not yet been identified, and that could possibly mutate and develop increased virulence, has caused concern, and requires careful consideration whenever a clinical trial is proposed. Monitoring for novel organisms, as far as is conceivably possible, needs to be built into the trial.

In the above trials, islets were not always harvested from pigs in closed 'high-health' status herds (and the definition of 'high-health' varies from center to center). In the Swedish trial, which was undertaken before concern was raised on the potential risks of PERV, the islets were harvested from non-high-health Swedish Landrace pig fetuses. In the New Zealand trial, the islet-source pigs were from a herd of high-health status, although PERV were present. The Mexican trial also used piglets that were bred in New Zealand in a specific pathogen-free (but not PERV-free) environment. No details on pig source in the Chinese trial were given in the presented abstract.

Furthermore, close monitoring of recipients of any pig xenograft was strongly recommended by the IXA Ethics Committee. Monitoring for transmission of infectious disease has been carried out in the patients in all four trials, if on occasion this has been retrospectively. No adverse infectious event has resulted to date from any of the trials. Follow-up of the patients who have received transplants of porcine islets demonstrated no evidence that they have become infected with PERV (4,6,7,20,21). However, in some cases, there was little or no evidence of long-term survival of the islets, and it is likely that the PERVs were destroyed with the islets. Although the absence of recipient infection with a porcine microorganism is very encouraging, the data remain inconclusive. Our understanding of expert opinion is that, although more investigation is necessary before pig tissue can be declared completely safe, the risk of an adverse effect from PERV is now considered to be low and, indeed, possibly acceptable (22,23).

However, as PERVs provide only one of many potential infectious risks, every effort must be taken to ensure this risk is minimized before clinical trials should be consid-

ered justified. In view of the potential infectious risk to the community, even from microorganisms hitherto unidentified, the perceived risk/benefit ratio must be very carefully considered, more so than with other medical or surgical innovations.

Regulation/Oversight

The IXA Ethics Committee addressed the need to control infectious disease risks through oversight by recognized national bodies [e.g. the Food and Drug Administration (FDA) in the United States]. As none of the four trials outlined above was carried out in the United States, it cannot be expected that any of them were monitored by the FDA, but some form of oversight with regard to infectious organisms would have been highly advisable. Furthermore, the Committee recommended that any future trial should be conducted with detailed oversight by an institutional committee, i.e. be supervised by a recognized official committee at the medical center where the trial is taking place (e.g. an Institutional Review Board in the United States).

In this respect, those carrying out the Swedish trial obtained approval by their institutional human ethics committee, although there appeared to be no 'national' oversight, probably because no such authority was in existence at that time. The trial by Elliott et al. was carried out with approval of ethical and statutory bodies, although it is not clear at what level these bodies operated. Those carrying out the Mexican trial obtained consent and oversight from institutional and national bodies within Mexico, although no specific 'xenotransplantation authority' exists in that country. Details on the recently reported trial from China are not yet available.

The IXA Ethics Committee also recommended that efforts should be made to work toward international guidelines to ensure monitoring of patients who have received a xenotransplant. To date, no mechanism is in place to monitor (or prevent) a patient with a xenotransplant performed in one country from traveling freely to other countries. (This is important, not only with regard to the xenotransplantation of islets and organs, but also to the xenotransplantation of numerous other cell types, such as fetal sheep cells, that are used as 'therapy' for diverse conditions.) Nonetheless, the recent adoption of resolution WHA57.18 by the 192 countries represented by the World Health Assembly represents a step toward the development of international recommendations and guidelines for well-monitored global application of xenotransplantation practices (24). FDA guidelines on xenotransplantation can be found through their website (www.fda.gov/cber/xap/xap.htm), which also contains links to guidelines from the Council of Europe, EMEA (European Agency for the Evaluation of Medicinal products), and World Health Organization.

Other Considerations

There are several other aspects of clinical trials of xenotransplantation that we have not touched on in this brief commentary, but which must be considered by those planning a clinical trial. Adequate facilities for archiving of tissues and/or blood from both the organ-source pig and the recipient must be available. The potential recipient must have been made aware of the need for life-long monitoring after the transplant, even if the graft fails to function. There are those who believe that procreation of recipients of xenografts should be avoided until the safety of these procedures is assured; this topic must be fully addressed with the potential recipient. In view of these considerations, at this stage in its development, a strong case could be made for xenotransplantation to be performed only in patients in whom the xenograft would be life-saving. With the availability of insulin and islet allotransplantation, patients with diabetes do not generally fall into this category. Although the ideal of immunological tolerance to porcine islets cannot yet be achieved, the immunosuppressive regimen should, at the least, be modest rather than intensive, thus reducing the risks of long-term therapy to the patients.

Finally, the question of whether minors (children and adolescents) should be included in initial clinical trials remains highly controversial. Although their inclusion may well be justified if the procedure will be life-saving, it would be much less justified if this is not the case. In view of the potential risks and possible restrictions on their lifestyles following receiving a xenograft, is it fair to impose these burdens on minors if they are not in a position themselves to give fully informed legal consent? Space precludes a detailed discussion of informed consent for xenotransplantation, but this important topic has been fully considered by the U.S. Secretary's Advisory Committee on Xenotransplantation (25,26).

Conclusions

Although valuable data may be obtained from clinical trials of islet xenotransplantation, and although efforts toward clinical trials should not be unduly impeded, we have to question whether there is sufficient encouraging experimental data in non-human primate models to warrant further clinical studies at the present time. Over the past years, some data have become available from pig-to-non-human primate studies (1) but, except for a preliminary report by the Minneapolis group on successful survival of pig islets in cynomolgus monkeys for periods of several months using an immunosuppressive regimen that is very unlikely to be clinically applicable (27), there are no studies that support a conclusion that clinical trials are likely to be successful (1).

Although some patients have required less, or even no, insulin (at least temporarily) after pig islet transplantation,

and thus the patients have individually benefited from the trial, it remains uncertain whether this was related to improved medical management, e.g. meticulous attention to diet, regular monitoring of blood glucose, excellent medical advice and management, etc., rather than to the function of the transplanted islets. Diabetic patients are likely to be more carefully monitored and more attentive to maintaining good control of their own condition when participating in a well-organized clinical trial.

The future may host more clinical islet xenotransplantation studies. If so, in the opinion of the IXA Ethics Committee, it would be mandatory that convincing experimental data in non-human primate models as to the efficacy of the approach are available to indicate the likelihood of a successful outcome and to justify exposure to potential risks. Careful monitoring (in collaboration with a recognized national authority) for potential transmission of infectious microorganisms is also surely mandatory. All aspects of the trial should be under the supervision of an institutional (and possibly a national) committee or authority. Such oversight and monitoring will surely not only safeguard the individual patient and the community, but will also increase the likelihood of obtaining valuable data from the trial, even if it is not fully successful in achieving its goals.

At this point in time, more experimental data from non-human primate models documenting convincing efficacy, focused on clinically-applicable immunosuppressive regimens, are needed to justify the initiation of clinical trials. These studies should also be designed to provide further data on the safety of the procedure, particularly with regard to the transfer of porcine viruses (including, but not limited to, PERV) to the non-human primate recipient of the porcine islets. A carefully monitored clinical trial may then be justified even though the potential risk to the patients, and possibly for society, would not be zero.

What results are required in a non-human primate model of porcine islet transplantation to justify progress to a clinical trial remain uncertain. The criteria for a clinical trial are particularly difficult to determine for patients with diabetes, since the disease is not as rapidly fatal as many other conditions for which xenotransplantation offers hope. A strong case could be made for a consensus meeting, and/or the setting up of an expert advisory committee [as convened by the International Society of Heart and Lung Transplantation in 2000 (28)] to determine these criteria.

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