SPECIAL ARTICLE

Xenotransplantation Today

ABSTRACT

The "mainstream" research in xenotransplantation concerns the pig organ/tissue engraftment to non human primates. During the last decade, a breakthrough in xenotransplantation research was made with transgenic pigs, resolving the problem of hyper-rejection. But, these pigs should undergo further genetic manipulation to prevent expression of other antigens towards which at least some humans have naturally occurring antibodies. The most remarkable problem remains coagulation dysfunction between the recipient and donor. The life span of engrafted organs is still not good enough to get a long term therapeutic effect. Xenogenic transplantation is also concerned with the problem of transmissive biological agents and serious ethical issues. The long-term basic and pre-clinical studies are necessary to solve multilevel problems before the xenogenic organ transplantation comes to the clinical level, from non human primate to humans.

KEY WORDS

genetic manipulation, transgenic pigs, coagulation dysfunction, ethical issues

DOI: 10.7251/SMD1201008I *(Scr Med 2012;43:8-10)*

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Submitted: April 26, 2012
Accepted: May 02, 2012

Transplantation of animal organs and tissues to humans is a very old idea. Some xenografts, as pig heart valves and tissues for orthopedic and general surgical procedures are used in medicine. In fact, these "grafts" represent only structural tissues from which pig cells have been removed to be, after transplantation, repopulated with human recipient cells. However, the transplantation that should provide viable animal organs that will continue to function in human organism is a completely different case and not yet a clinical reality.

Several animal species have been historically considered as organ "donors" due to various limitations both of biological and ethical order. The pig is considered to be the most appropriate animal for this purpose with respect to the size of its organs and the fact that it is philogenically distant enough to avoid ethical and moral problems appearing when non human primates are considered. Most pre-clinical experiments were performed by grafting pig organs, cells and tissues to non human primates. With that respect, the distinction should be made between transplants of solid organs and groups of cells such the pancreatic islets (which come without blood vessels). Typically, a xenotransplantation should overcome following barriers:[1]

1) Immunological barrier - Hyperacute rejection i.e. antibody-mediated complement activation initiated by naturally occurring (T-cell independent) antibodies directed against Gal (alpha 1,3 Gal) and possibly non Gal antigens.

- Acute humoral xenograft rejection i.e. antibody-mediated but probably independent of complement, initiated by natural and/or elicited (T-cell dependant) antibodies; possible role for macrophages, natural killer cells and lectins.
- Acute cellular xenograft rejection – T-cell mediated cellular response
- Probably graft vasculopathy (chronic rejection) - uncertain mechanism.

2) Microbioligical and safety issues – potential for transfer of micro-organisms from donor pig organ to human recipient as bacteria, exogenous viruses (e.g. porcine megalovirus, porcine endogenous retroviruses; potential for transfer of porcine micro-organisms from human organ recipient to his/her human contact.

3) Physiological barrier – incompatibilities in coagulation factors between pig and human being could result in pro-coagulant state with risk in progression to graft thrombosis or disseminated intra-vascular coagulation.

An important advance in matter of xenogeneic transplantation was performed by knocking-out the gene for alpha

1,3-galactosil-transferase in donor pigs.[1] That gene encodes the enzyme that is responsible for production of gal oligosaccharide (GT-KO pigs). The first homozygous GT-KO pigs were successfully bred in 2002,[2] and some of the problems were solved; the organs originating from GT-KO pigs became resistant to acute hyper-rejection. But, these pigs should undergo further genetic manipulation to prevent expression of other antigens towards which at least some humans have naturally occurring antibodies. The most remarkable problem remains a coagulation dysfunction between recipient and donor. For example, when a pig heart is transplanted, such dysfunction takes the form of thrombotic microangiopathy that causes ischemic injuries to the myocardium and can ultimately result in consumptive coagulopathy. It is more pronounced with a pig kidney transplant (consumptive coagulopathy developed at early stage).[3] It seems that the pig vasculo-endothelial cells are sufficient to activate primate platelets and peripheral blood mononuclear cells even in absence of antibodies or complement. In addition, presence of pig organ in the primates also triggers a systemic inflammatory response involving innate immune cells, platelet and leukocyte and complement cascade activation.

The best results of organ rate survival obtained with pig hearts transplanted to baboons combined with an intensive immunosuppressive treatment varies from 76 days to 6 months.[4] With all these improvements, including the genetic modifications and an optimized immunosuppressive regimen, the pig organ graft survival in non human primates remains relatively short:[4] for lung only one to two days, for liver couple to seven days, and for the heart and kidney about three months. Much better results were obtained for cornea (400 days) and cellular/tissue preparations: pancreatic islet 400 days, neuronal cells 500 days. This life span of engrafted organs is certainly better now (GT-KO pigs) than those before genetic interventions on donor pigs, but they are not good enough to get a long term therapeutic effect. The goal is instead to get a "bridge" to allo-transplantation, i.e. to maintain a patient alive while waiting the human organ. This approach might be specially applied in future, for heart and liver transplantation.

Apart phase 1/2 clinical studies (human patients) based on pig pancreatic islets (encapsulated or not), the other procedures are still at the level of pre-clinical studies on non human primates and lots of problems should be solved before switching to clinical trials. But at the level of these pre-clinical studies on non human primates, in addition to numerous problems to increase the organ graft survival, the researchers also face the ethical ones as the "animal rights issue" - questioning experimentation on non human primates.

The transfer of these procedures to clinical trials would certainly demand a long time. Some of developing applications of pig cells tissues and organs could be simply abandoned due to successful development of human and cell tissue engineering. For example, an industrial *ex vivo* production of red blood cells from "induced pluripotent stem cells"[5] could be much better solution than the transfusion of pig red blood cells; this principle has not been solved even at fundamental level.

Although some recent studies report a long term absence of porcine endogenous retrovirus effect in chronically immunosuppressed patients after treatment with porcine-based bio-artificial liver,[6] a number of other microbiological factors including the emerging zoonose agents could potentially complicate the situation. If we compare a potential pig-human xenotransplantation (cellular, organ and tissue level) with allogeneic and even autologous cell and tissue therapy, we could realize how complex the problem is: any new clinical protocol based on human cell and tissues is submitted to draconian verification before authorization of a clinical trial.[7] Since the 90's, the precaution principle was introduced as an absolute requirement for development of new cell- and tissue-based therapies. For example, all *ex vivo* transformation procedures should be performed without any contact with molecules of animal origin. If it is impossible, the animal molecules ("clinical lots") should be derived from "certified animals" (which are "clean" for several generations for transmissive viruses, prions, and similar agents). Furthermore, even if the molecule was not injected in patient's bodies (or it is present in traces) during the therapeutic procedure, the risk of its immunogenicity and other biological interferences should be evaluated. This rigorous approach, related to health safety, should not be applied only to cell therapy, but also to other transplantation-based therapies, including the xenotransplantation.

With respect to all these factors, it seems to be evident that the perspective of xenotransplantation the same for cell and tissue-based therapies and organ transplantation. In first case, the long phase 3 clinical trials are necessary, to fully evaluate cell/tissue-based xenotransplants (for example, pancreatic islets) before introducing these procedures in routine medical practice. When the xenogeneic organ transplantation is in question, the long-term basic and pre-clinical studies are necessary in order to solve multilevel problems before switching to a clinical level (from non human primate to humans). But even after solving all biological problems, the issues as graft safety, logistics, regulatory and ethical aspects will be in front of xenotransplantation clinical trials.

Although xenogeneic transplantation is an interesting issue, and a field in which major breakthrough were performed recently (transgenic pigs), it is too early, in my opinion, to claim "the next medical revolution".

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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