Xenotransplantation: on the way to Clinical Application?

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INTRODUCTION

Heart transplantation is the accepted therapeutic option that improves the life expectancy and quality of life of patients with end-stage heart failure. One of its major limitations is donor shortage that leads to a high mortality rate in the waiting list. Public and Transplantation Societies policies and campaigns did not significantly improve this scenario. Several strategies to increase the heart donor pool have been proposed and results have been reported and discussed in consensus conferences.

These strategies include the use of "high-risk donors", or the so-called "marginal donors", *e.g.* elderly people or donors with a minor or correctable cardiopathy. Nevertheless, they also had no significant impact in the donor pool.

The use of animals' hearts or xenotransplantation is the only possibility of overcome this problem.

The first human cardiac transplantation was a xenotransplantation performed by Dr. James Hardy[1]. After, Bernard's first interhuman transplant, xenotransplantation was performed by Dr. Cooley (1968) [2,3], Dr. Ross (1968)[4], Dr. Barnard (two cases, 1977)[5], and Dr. Bailey (1984)[6], using hearts from sheep, pig, and baboon. Except for Dr. Bailey's case (20 days), the patients survived only hours. It was clear that hyperacute rejection was an outstanding barrier.

Some advantages of xenotransplantation are: 1 – unlimited availability of organs; 2 – planned operation; and 3 – repeated elective transplant. Potential disadvantages are: 1 – possibility of an animal infectious disease; 2 – great immunological differences; and 3 – physiological differences[7].

EXPERIMENTAL BACKGROUND

It was soon clear that the major problem of xenotransplantation was the immunological barrier, leading to acute humoral xenograft rejection or delayed rejection (acute vascular rejection).

The increased interest in this kind of transplantation led to the choice of the pig as donor, instead of a nonhuman primate. The reasons for this choice were: the number of piglets, the fact that the pigs' hearts are easier to manipulate and that their hearts are bigger than the nonhuman primates' hearts, and the less chance of zoonosis.

When we look at the evolution of the species, humans came from a separation of other mammals 75-80 million years ago. The monkeys of the New World separated from the branch of Old World monkeys and humans 30-40 million years ago. At this particular period, it was deleted the gene that produces the galactosyl-α-1,3 galactose, or simply GAL, a carbohydrate that is the most important antigen in the xenotransplantation. GAL is expressed in most of the cells of all mammals, including pigs, except for Old World monkeys and humans. Unfortunately, GAL is present, for instance, in intestinal bacteria, and humans have preformed antibodies (Ab) against GAL[8].

The first strategies to avoid hyperacute rejection were depletion of Ab by plasmapheresis and immunoadsorption and depletion or inhibition of complements, among many others. Nevertheless, Ab return with graft loss[9,10].

Initial genetically engineered pigs were animals that expressed one or more human complement regulatory proteins (CRP). This is of paramount importance because when the Ab antiGAL binds to the antigen, it triggers the complement cascade with coagulation of the vessels[11].

Later on, transgenic pigs' development, besides the manipulation to express human CRP, included the most important manipulation, which is deletion of the gene that express α-1,3galactosyl transferase, so these pigs do not have GAL in their cell surface. This kind of gene knockout (GETKO) pig was the major achievement in this field.

As thrombotic microangiopathy was still a problem through several mechanisms, including other xenotransplant non-GAL

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antigens, other genetic manipulations were included in GETKO/CRP pigs that consisted in the inclusion of human antigens and anticoagulant genes, specially the one called thrombomodulin.

Besides the abovementioned strategies for decreasing humoral acute rejection and its consequences, considering the relationship between inflammation and rejection as well as the evidence of inflammatory response in xenotransplantation, several agents to inhibit cytokine activity and the initial use of transgenic pigs to express anti-inflammatory genes were investigated.

In regard to cellular rejection, there is no evidence of histologic lymphocytic infiltration in xenotransplantation organs. In baboons with long survival, there is no evidence of graft vascular disease and this probably is due to the multiple immunosuppressive strategies.

The most important groups in the United States of America and Europe are working in an experimental model of pig to baboon xenotransplantation. The donors are GTO and CRP transgene pigs, with or without thrombomodulin expression[11].

Leading research laboratories use these engineered pigs and other multiple approaches to decrease cellular immune response. Besides, antithymocyte globulin, cyclosporine/tacrolimus, and mycophenolate mofetil strategies included B-cell depletion with anti-CD20 Ab and costimulation blockade with anti-CD40.

These research groups have used transgenic pigs with two- or three-gene manipulations and have associated these to B-cell depletion and length of use of costimulation blockade with different dose duration.

Some groups compared the survival rates of these different protocols. The number of recipient baboons are usually small, but some survived more than 100 days, some more than 500 days, and one reached 945 days[12].

So, long-term survival is a reality in a model of pig to baboon xenotransplantation. It is matter of concern that due to the high cost of the experiments, the number of animals is small, and it is difficult to establish definitely the necessity of each intervention and possible complications, specially infections.

The physiological differences are not always considered. For instance, the fact that pigs are quadruped and their body temperature is 38ºC in contrast with the fact that primates and humans are biped and their body temperature is 37ºC may play a role.

Another issue is the monitoring of the xenograft. Endomyocardial biopsy probably will not be useful and one field of investigation is the use of circulating xenograft specific micro-ribonucleic acid as a biomarker of organ survival and immune rejection[13-15].

Even with the possibility of graft loss, it remains the possibility of repeated transplantation and xenotransplantation as a bridge to allotransplantation.

CLINICAL CONSIDERATIONS

With the ongoing advances in cardiac xenotransplantation, an initial discussion regarding the potential clinical applications has now begun. An invited overview by members of an U19-funded xenotransplantation research group has recently begun addressing which patient population would be the best to start initial trials in organ xenotransplantation, including the heart. Potentially, it could be those patients who are currently disproportionately affected by the ongoing shortage of available allografts, including retransplant candidates, those who are highly sensitized to or with significant allosensitization to preexisting Ab against human leukocyte antigen, and individuals who have contraindication to mechanical circulatory device support. Additionally, circumstances precluding well-tolerated and secure implantations of a ventricular assist device, including friable tissue, amyloidosis, or anatomical conditions secondary to complex congenital heart diseases.

CONCLUSION

The unique potential for xenotransplantation to address the current issues of scarce donor resources and limitations of the present technology has immense implications for the future of heart failure management. Although still experimental, recent scientific advances in xenotransplantation have boosted the prospect of clinical applications. Although barriers in immunomodulation, coagulation dysfunction, and transplant physiology are resolved, other interventions, specific implementation protocols, and indications for xenotransplant therapy will need to be addressed. Before expanded criteria are considered acceptable, xenografts will likely initially be used in cases where allotransplantation and device therapy is contraindicated or unavailable.

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