Heart Transplant from a Genetically-Modified Pig: A Paradigm Shift?

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Heart transplant remains the therapy of choice for patients with end-stage heart failure but is limited by chronic shortage of donated organs. Mechanical circulatory support (MCS) devices, with modern designs, have been used as destiny therapy, yielding better results that have positively impacted patient survival.[1] The indications for MCS have significantly increased and become part of the current context of potential candidates for transplant, be it as destiny therapy, bridge-to-transplant or bridge-to candidacy. However, there remains a considerable number of patients who would benefit from the transplant if the availability of donated organs was higher. According to the Brazilian Organ Transplant Association (ABTO), approximately 400 heart transplants are performed yearly in Brazil, but the demand for this procedure is 1,600 per year, *i.e.*, many patients die waiting for an organ.

A possible solution for this issue is xenotransplantation, the process of transplanting organs from other animals, which has gained increasing interest in the last years[2,3] for a combination of reasons. First, the efficacy of preclinical models has improved, with an increase in survival time of xenografts. Second, the rapid advances in genome editing, particularly the advent of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), which allowed the generation of donor pigs with multiple genetic protection modifications; what used to take years can now be done in months, with more accurate and comprehensive results. Third, the spectrum of the porcine endogenous retrovirus (PERV) significantly reduced. There is no evidence of PERV transmission in clinical trials of preclinical models, and novel treatment options and even elimination of these viral diseases are now available.[4] Due to its potential, the importance of xenotransplantation as a solution for the shortage of human organs and tissues remains a great hope for the transplant community, especially for the patients who face advanced disease and high mortality while waiting for a donated heart.

Keywords

Heart Transplantation; Transplantation, Heterologous; Allergy and Immunology; Genetically Modified Animals; Swine

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Many questions and ethical concerns have been raised about xenotransplantation. The risk of infection transmission from pigs may require a lifelong surveillance not only for transplant recipients but also for their family members. Another issue concerns the performance of animal experiments with a specific purpose of producing genetically engineered pigs for transplants and to save lives. Discussions among the community, regulatory agencies and animal protection agencies have played an important role in the advancement of research, that may become true in a near future.[5]

This year, with enthusiasm, we received the news of the first heart transplant from a pig to a male patient at the University of Maryland Medical Center. The regulatory agency of the U.S.A federal government (Food and Drug Administration) had approved xenotransplantation under "compassionate use" rules for emergency situations. The patient had refractory heart failure, severe sarcopenia, was in prolonged extracorporeal membrane oxygenation (ECMO), and considered not eligible for conventional heart transplant according to the medical staff.

The patient died approximately 50 days after the transplant, due to progressive cardiac hypertrophy and severe diastolic dysfunction. The patient had been transitioned back to ECMO support until the end. Biopsy and pathologic examination did not reveal any signs of humoral or cellular rejection. Many hypotheses have been proposed, that will probably be elucidated in the final publication of the data. One of the hypotheses is that the patient heart was affected by porcine cytomegalovirus, which may have contributed to refractory and irreversible dysfunction. Also, the physiological functioning and even gravitational aspects could have affected the graft function.

This pioneering experiment provided valuable indications for the possibility of the normal functioning of a genetically modified pig heart in a human person while the immune system is adequately suppressed. It is important that the valuable insights from this case guide future research and indications of the procedure.

The application of this groundbreaking research in a patient was only possible due to numerous previous work that helped to define the genetic engineering required to overcome the feared immunological and infectious barriers. This research started more than 30 years ago and constructed the basis for making this procedure feasible.

The reason for choosing pigs as donors was these animals have a shorter gestational period and time to maturity (around one year) and similar size for organs as compared with humans. Pigs have long been used in Human Medicine, for example for skin grafts and cardiac

valve implants. However, organ transplantations are far more complex than the use of highly processed tissues. The gene-edited pigs were produced by Revivicor (United Therapeutics Corporation, Virginia, USA), one of the several animal biotechnology companies at the frontiers of knowledge focused on producing organs for transplantation into humans. According to available information, 10 genes were supposedly manipulated – three knockout genes, one gene was inactivated to prevent hypertrophy and six human genes were inserted into its genome.

The next challenge was to neutralize the effect of pre-existing antibodies (AB) and manage potential incompatibility between the complement and the coagulation systems, and infection of the receptor by endogenous retroviruses.

The introduction of the CRISPR technology into xenotransplantation increased the speed of genetic manipulation in pigs. Thanks to this technology, researchers cannot only produce knockout and knock-in animas targeting multiple genes, but also exclude the expression that increases the risk of specific viral diseases. Genetically modified pigs using the CRIPSR technology have been used in several important studies involving AB and coagulation dysfunction. Today, there are more than 26 types of gene-edited pigs available for xenotransplantation research.[6]

Endothelial injury may occur within minutes due the activity of pre-existing AB against pig specificities. The AB-antigen binding triggers a hyperacute rejection after reperfusion of the xenograft. To prevent this complication, using genetic engineering, the three main genes responsible for the α-1,3-Gal, β4Gal and Neu5Gc proteins were inactivated by gene knockout, creating the triple-gene knockout pig.

The activation of the complement pathway and changes in the coagulation system may lead to the xenograft dysfunction within days or weeks, and consequent loss of the graft. At the human blood-porcine endothelial interface, porcine thromboregulatory molecules such as thrombomodulin, endothelial protein C receptor (EPCR), and thrombin activatable fibrinolysis inhibitor interact inappropriately with human coagulation pathway molecules. This can result in thrombotic microangiopathy in the xenograft and disseminated intravascular coagulation in the receptor.

To prevent the production of new AB or increase in pre-existing AB, the use of anti-CD40 monoclonal AB has been proposed, along with other components of a more comprehensive immunosuppressive regimen.[7]

The cardiac xenotransplantation, performed by Mohiuddin et al. evidenced the possibility of the long-term survival of cardiac grafts of genetically modified pigs.[8] Genetic modifications in pigs, combined with an intensive immunosuppressive therapy, based on a chimeric anti-CD40 monoclonal antibody, prevented humoral rejection and dysregulation of systemic coagulation pathway, promoting the cardiac xenograft survival, in addition to controlling inflammation and coagulation.[9]

Some lessons can be learnt from this first, groundbreaking case. Preoperative clinical conditions, such as sarcopenia, prolonged inactivity, and infections, made difficult the prompt recovery of the patient after transplantation. In this case the patient had pancytopenia, which prevented the use of the ideal immunosuppressive regimen. The severe interstitial edema, with myocardial necrosis and no cellular infiltrate, which led to ventricular dysfunction, will need to be better understood, including whether or not there was an influence of immunological components.[10]

We are truly experiencing an important paradigm shift in the field of transplantation. In the next years, we will witness great progress and research continuation towards feasible, safe and effective procedure in clinical practice. The University of São Paulo is planning the construction of a pig facility, focusing on research to make suitable clinical transplantation within the next five years. Again, research and science are playing their role in the progress of humanity. The future is happening now, right in front of our eyes.[11]

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