Clinical trials in cardiac xenotransplantation: are we ready to handle ethical issues?

Short Title: Cardiac xenotransplantation and ethical issues.

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Abstract

Heart allotransplantation has become one of the methods of choice in the treatment of severe heart failure. In the face of its difficulties, such as the unmet balance between organ supply and demand, the use of xenotransplantation might be an attractive option in the near future, even more with the ongoing progress achieved regarding the avoidance of hyperacute rejection and primary organ disfunction, maintenance of xenograft function and control of xenograft growth.

To make possible this translational challenge, some points must be taken into account indeed, and they are the equipoise of human benefit and animal suffering, the risk of unknown infections, a well prepared informed consent, ethical and religious beliefs, and the role of cardiac xenotransplantation in a ventricular assistance device era.

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Introduction

In recent years, heart allotransplantation has become one of the methods of choice in the treatment of severe heart failure, in selected cases. However, difficulties with the small number of donors remains a challenge and limit its use. Therefore, the use of xenotransplantation, which is the name given to organ transplantation between different species, becomes an attractive option in this clinical scenario. Hardy and cols. performed the first cardiac xenotransplantation, using a chimpanzee heart, in 1964. After 20 years, Dr. Bailey and cols. performed the first

baboon-to-human cardiac xenotransplantation in a neonate, who was a victim of hypoplasic left heart syndrome (HLHS). That landmark procedure, better known as the "Baby Fae case", raised several ethical questions and concerns about the clinical use of cardiac xenograft. Currently, genetically modified porcine models have transformed the clinical tests in nonhuman primates by decreasing the risk of cardiac xenograft rejection[1]. Even in a mechanical ventricular assist device (VAD) era, permanent implantation of a pig heart can be a future and feasible treatment option for selected patients with advanced heart failure. Therefore, is not too early to discuss the ethical challenges in the likely future clinical trials involving that promising therapy.

For this purpose, this article highlights the following key ethical issues: the use of animal organs and its implications, the risk of unknown serious infections, the role of the informed consent in this scenario, and the significance of the cardiac xenotransplantation in face of the new VAD age.

A brief history of cardiac xenotransplantation in human beings – The "Baby Fae" case

The first-ever cardiac xenotransplantation was performed in 1964. Hardy and cols. transplanted a chimpanzee heart in a 64-year-old man with severe ischemic cardiomyopathy. Unfortunately, two hours after the procedure, a possible acute vascular rejection occurred and the patient died[2]. Cooley, Ross, Marion, and Barnard also reported other unsuccessful attempts in adults[3-5]. In 1984, Stephanie Fae Beauclair was the first infant subject of cardiac xenotransplant procedure. Baby Fae was a victim of a very aggressive type of congenital cardiovascular malformation, which is HLHS[6]. The incidence of HLHS is low in neonatal patients, however, it is responsible for approximately 23% of neonatal deaths[7]. Before proceeding the baboon-to-human transplant, a long and detailed approval process was traversed.

In August 1983, Dr. Bailey submitted the research protocol and informed consent to the Loma Linda University Medical Center (LLU) institutional review board (IRB), which was approved two months after the first submission[8]. Also, members of the National Institutes of Health (NIH) were invited to examine the LLU IRB approval process. They concluded that the procedure had been performed according to the ethical guideline, including the appropriate explanation about the informed consent to the infant's parents[8, 9]. Nevertheless, informed consent failed to clearly explain why the possibility of searching for a human heart has not been made[8,9].

Czaplicki and and cols published, in 1992, a known case report of pig-to-human transplant. Despite the death of the patient 23 hours after the procedure, this surgery was particularly important because they removed the pre-formed natural antibodies from the pig heart and described the lack of xenograft rejection in the failed heart[10]. One unpublished case was performed in 1996 in India[11].

Where do we stand in 2021?

A brief clinical review of the current status of cardiac xenotransplantation is necessary, at this point, to provide a better understanding of our posterior ethical discussion. The primary barrier for the success of the heterotopic and orthotopic cardiac xenotransplantation is the immunological challenge[4, 11]. Usually, hearts from lower mammals are rapidly destroyed after human transplantation due to an aggressive hyperacute rejection, which is mainly mediated by the expression of the α (1,3) galactose disaccharide (Gal) carbohydrate on the vascular endothelium of the heart xenograft. Human anti-Gal natural antibodies bind to the new heart causing platelet aggregation, endothelial cell dysfunction, and vascular thrombosis[11]. To eliminate the α Gal antigen, Phelps, and cols. developed pigs genetically modified, mutating the α-galactosyltransferase gene (GGTA-1). This gene encodes the enzymatic function to produce α

Gal antigen. The new genetic procedure marginalized any role of anti-Gal antibody in the rejection of pigs donor organs[11,12].

Currently, there is a limited number of pig-to-primate orthotopic cardiac transplants, and the maximum survival reported was 195 days[1]. Besides the hyperacute rejection problem, the primary organ dysfunction, better known as perioperative cardiac xenograft dysfunction (PCXD) due to problems in thromboregulation and ischemia-reperfusion injury after cardiopulmonary bypass, are some of the most important targets to researchers in this field[10].

Recently, new studies have shown some important advances towards those targets. Langin and cols concluded that consumption coagulopathy was prevented in the baboons by expressing the human thrombomodulin protein in the grafts, which reduces coagulation levels. The immune response, in turn, was reduced with the expression of the human protein CD46 in grafts from genetically modified pigs[1, 12].

Another important point that has been taken into account is the maintenance of graft function[13]. Iwase et al[14] demonstrated that xenotransplantation recipients had a rapid decline of free triiodothyronine after cardiac xenotransplantation and this information was then used to improve the graft perfusate[15] by adding hormones to the solution used to protect the graft. Besides improving the myocardial protection, the strategy also involved a continuous 8C perfusion in a non-static preserved graft[15].

The control of post-transplantation xenograft growth was also demonstrated to ensure long-term orthotopic function in baboons[1]. During maintenance therapy methylprednisolone was reduced gradually, the baboons received antihypertensive treatment to mimic the lower porcine

levels and the prodrug temsirolimus, a new mTOR inhibitor[16], which may prevent thrombotic microangiopathic lesions[17] and promotes myocardial hypertrophic attenuation[18].

Clearly, with the advances in the orthotopic cardiac xenotransplantation, we are closer[19] to start a clinical trial in human beings since we have better control of the perioperative problems related to the procedure and a prolonged survival expectation to justify this therapy in humans at this point. This is affirmed by a statement of the International Society of Heart and Lung Transplantation since preclinical efficacy is supported when a majority (60%) of xenotransplants of porcine to nonhuman primates (NHP) models´ life-sustaining survival is greater than three months[8,20].

The equipoise of human benefit and animal suffering

We will start with the non-controversial point of view regarding the use of animals like xenograft donors. All sectors of our society agree that animals used for research or clinical xenotransplantation purposes must be treated humanely and respectfully. Animal donor safety and welfare must always be the purpose[20] so they should be subjected to as little pain as possible and the best conditions, including food, water, and shelter. Prior institutional approval may be obtained and the minimum necessary number of animals should be used[21,22] from closed herds rigidly monitored[20]. Since the genetic modifications do not change the character of the species, the laboratory creation of genetically modified pigs is considered socially acceptable.

Of course, that would be ideal if the genetically modified animals could be alive after organ donation. Also, it is not feasible yet producing animals without brains or central nervous systems, which would be a way to avoid pain and suffering. For many societies, the use of pigs can be considered less controversial than nonhuman primate use. Pigs are historically created in

captivity and used for foods in several regions around the world. Therefore, the use of pigs as a source for xenograft can be better accepted for the public opinion and researcher community[21,22]. Regarding the nonhuman primate, for many members of society, their complex social behaviors, the financial and practical problems involving breeding plenty of human-size animals, and the concern about viral transmission are crucial barriers to the use of that kind of animal.

Various animal rights lawyers argue breeding and using animals for human purposes is a flagitious action. However, all the new drugs and devices that were responsible to improve the survival and quality of life of humans and animals came from animal research. In this way, without animal experimentation, plenty of current scientific advances would not be possible. Besides that, xenotransplantation products can be applied only when traditional medical options available have been failed and for highly selected patients[20, 23].

The risk of unknown infection disease

In a clinical trial, the potential risk of an unknown infection should be balanced against the benefits of the procedure being studied. Every clinical trial and every new experiment as well can be faced with some sort of unknown discovery or serious adverse event though[24–26]. Any time infection and xenotransplantation are discussed, the deadly filoviruses infection by Marburg and Ebola virus are evocated[27]. First of all, it is important to remember that both serious diseases were not caused by monkeys in the experimental atmosphere. Secondly, the probability of the modern biotechnology producing primates and pigs free of infection cells is high[24–28].

In pigs, the porcine endogenous retroviruses (PERV) are always present[29, 30] and is the most worrying transmissible pathogen. Although they appear to do the pig no harm, is concern that PERV can be pathogenic in human cells and their zoonotic potential is unknown[28].

Nevertheless, there is no current evidence that retrovirus might be dangerous for human beings[26]. They are part of the genome and therefore cannot be eliminated by specified pathogen-free breeding[28,31], nevertheless, some strategies may be tested such as the use of antiviral drugs, which have still not been used in pigs to treat infections, selection of pigs from closed herds with low copy number and low expression of PERV proviruses at the RNA and vaccination of the donor pigs[28].

Even in human heart allotransplantation one or more infectious agents can be transmitted by the donor to the organ receptor (e.g. cytomegalovirus, Epstein-Barr virus, and hepatitis B or C). Some of them are also present in pigs, like cytomegalovirus and herpes virus, but except for PERV, all potentially zoonotic viruses can be eliminated by specified designated pathogen-free breeding[28]. Providing exogenous infection-free organs, tissues and cells is a major advantage of xenotransplantation over allotransplantation[32] even more with the methods described above, but we must take into account the challenges of translational science[33] since we might face some problems with theoretical unknown pathogens and how known agents will behave in an immunosuppressive environment.

Informed Consent – A critical issue for organ receptors, their families and medical staff

We already mentioned informed consent during the "Baby Fae" case description. In that case, the proposed informed consent was accepted by the LLU IRB, albeit some criticism about its content, can be done[6]. First of all, in a patient with advanced heart failure, the informed consent should be obtained as soon as possible, because the risk of clinical deteriorating is very high.

To fully understand a long text about an inclusion process in a clinical trial involving cardiac xenograft is not an easy task for hospitalized patients in a Cardiac Care Unit, even more under several intravenous medications, dialysis, or sedation[21]. Secondly, due to the risk of unknown infections, the enrolled patient can be aware that he or she needs to be monitoring for the rest of his or her life, which means that the patient should be on the clinical trial forever. It seems like a feasible and fair situation due to the global risk to be avoided, however, that practice denies the right to withdraw from the study at any time, a fundamental right warranted by the Declaration of Helsinki[34]. Remaining in the infection risk matter, the patient may be aware if in case of serious and unknown infection, he or she can be isolated from the social life for quarantine, including avoiding intimate relationships or pregnancy.

Should the close contacts of patients and medical personnel sign the informed consent? Since the xenotransplantation clinical trials will directly affect the life of close people around the receptor, the informed consent may be secured from relatives and medical staff[35].

Lastly, religious beliefs, risk of suicide, and grating of consent to necropsy at the time of death may also be appropriately accessed in the informed consent.

The role of cardiac xenotransplantation in the ventricular assistance device (VAD)

HeartMate II (Thoratec, Pleasanton, CA, USA) and HeartWare (HeartWare International, Framingham, MA, USA) were the two first FDA approved continuous-flow mechanical circulatory support devices for bridge-to-transplant and destiny therapy[36]. For instance, the REMATCH trial showed a one year- survival of 68% in ineligible for heart transplantation patients that received the HeartMate II device[37]. The SynCardia Total Artificial Heart (SynCardia Systems Inc, Tucson, Arizona), on the other hand, reported the longest-term survivor

alive after 16.4 years post-implantation[38]. Given these results, how can we think about an experimental procedure that offers to us, until now, 195-days survival in a baboon orthotopic heart receptor?

Despite the positive survival outcomes, we have several adverse events in VAD therapy as well. Bleeding and thromboembolic events, device-related infections, device malfunction, and neurological complications[39] are among the most relevant serious adverse events. Aortic insufficiency and right ventricular failure are other complications of chronic support with continuous-flow pumps[36]. Besides the adverse events, a reasonable number of patients have some sort of contraindication for VAD therapy. Based on these barriers, Cooper, and cols. have described some requirements to the cardiac xenotransplantation attain the necessary equipoise for the first clinical trials as mentioned above[25] and among them, the most significant was a heart graft survival for at least three months with some primates surviving more than 6 months after an orthotopic pig transplant[40].

Besides that, patients requiring mechanical circulatory support are part of a heterogeneous group like the devices designated for acute or chronic heart failure. The available literature provides insufficient data for precise recommendations regarding patient and device selection and the timing of intervention[41]. Another point is the effect of VAD therapy in the financial support of health systems since it is a very expensive therapy and heart failure is a major cause of death worldwide.

Benefits already acquired from VAD therapies must act together with possible advantages of porcine xenografts which will lead to improvements in the conduction of advanced heart failure in the future.

Conclusion

In conclusion, with the ongoing progress in the cardiac xenotransplantation scenario in a pig-to-nonhuman primate model, we are closer than ever to start a clinical trial. With the unmet balance between organ supply and demand, heart xenotransplantation might become an alternative for advanced disease even in a VAD era. The equipoise of human benefit and animal suffering, the risk of unknown infections, a well-prepared informed consent, ethical and religious beliefs must be taken into account to make this great translational challenge happen though.

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