Acta Bioethica 2022; 28 (1): 149-156

ARE WE READY FOR PIG-TO-HUMAN CLINICAL XENOTRANSPLANTATION TRIALS?

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Abstract: The invention and widely use of organ allotransplantation provides effective treatment of some originally fetal diseases such as liver/kidney failure and has saved million of lives around the globe. However, the scarcity of human organs has caused many patients, who could have been treated, to die while waiting for suitable organs around the world. Pig-to human xenotransplantation provides a potential solution to solve this tough problem. Pig organs have been considered as major sources of xenotransplantation because of the sufficient number of donors, the sizes of organs, and physiologically structural similarities. However, xenotransplantation also has some problems, such as the possibility of spreading animal diseases to human, the interspecies immunological barrier, organs of animal origin challenging human nature, and potential informed consent issues. This article will discuss these potential issues and to see whether it is the suitable time to conduct clinical xenotransplantation trials in humans.

Key words: pig-to-human, xenotransplantation, zoonotic diseases, immunological barriers, psychological issues, informed consent

¿Estamos listos para ensayos clínicos de xenotrasplante del cerdo al humano?

Resumen: La invención y el amplio uso de trasplantes alógenos proporciona tratamiento efectivo de algunas enfermedades de origen fetal, como la insuficiencia renal y hepática, y ha salvado a millones de pacientes en el mundo. Sin embargo, la escasez de órganos humanos ha causado que muchos pacientes en el mundo, que podrían haber sido tratados, murieran por esperar un órgano adecuado. El xenotrasplante del cerdo al humano proporciona una solución potencial para resolver este difícil problema. Los órganos de cerdo han sido considerados como fuentes mayores para xenotrasplantes debido al suficiente número de donantes, el tamaño de los órganos y estructuras fisiológicas similares. No obstante, el xenotrasplante también tiene algunos problemas, como la posibilidad de expandir enfermedades animales a humanos, la barrera inmunológica entre especies, el desafío para la naturaleza humana de tener órganos de origen animal y problemas potenciales de consentimiento informado. Este artículo discute estos temas potenciales y plantea si estamos en un momento apropiado para realizar ensayos clínicos de xenotrasplantes en humanos.

Palabras clave: xenotrasplante de cerdo a humano, enfermedades zoonóticas, barreras inmunológicas, temas psicológicos, consentimiento informado

Estamos prontos para ensaios clínicos de xenotransplante porco-para-humanos?

Resumo: A invenção e amplo uso de alotransplante de órgãos propicia tratamento efetivo para algumas doenças originalmente fetais tais como falência hepática/renal e tem salvo milhões de vidas em todo o globo. Entretanto, a escassez de órgãos humanos tem causado a morte de muitos pacientes - que poderiam ter sido tratados – aguardando por órgãos apropriados em todo o globo. Xenotransplante porco-para-humanos propicia uma solução potencial para resolver este difícil problema. Órgãos de porco tem sido considerados como as principais fontes de xenotransplante por causa do número suficiente de doadores, do tamanho dos órgãos e de similaridades estruturais fisiológicas. Entretanto, xenotransplante também tem alguns problemas, tais como a possibilidade de disseminar doenças animais aos humanos, a barreira imunológica entre espécies, órgão de origem animal desafiando a natureza humana e aspectos potenciais de consentimento informado. Esse artigo discutirá esses aspectos potenciais e verificará se é o momento adequado para conduzir ensaios clínicos de xenotransplante em humanos.

Palavras chave: porco-para-humano, xenotransplante, doenças zoonóticas, barreiras imunológicas, aspectos psicológicos, consentimento informado

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Introduction

In ancient Greek mythology, the typical image of Chimera has a head like a lion, a tail like a snake, and a goat's head on its back. The medical term "Chimera" in biological transplantation, which means chimera, was derived from this.

Transplantation has been proved to be a very effective way to treat end-stage organ failure such as liver and kidney failure. Allotransplantation is the transplantation of cells, tissues, or organs from one individual to another individual within the same species, whereas xenotransplantation refers to any cross-species transplantation (e.g., pig to human). The increasing life expectancy of humans has resulted in increasing number of patients who in need of organ transplants. Insufficient human organ resources have caused many patients to die while waiting for suitable donor organs. In 2018, there were 113,000 patients in the United States who need an organ transplant, but only 36,528 patients received a transplant*(1)*. Similarly, there are more than 300,000 patients in China who need organ transplants, but only about 16,000 organs are available each year*(1)*. Therefore, it is urgent to find other resources to meet the demands. Xenotransplantation is a possible solution to this problem.

Organs from non-human primates (NHPs) were tried in early clinical xenotransplantation because they are phylogenetically closer to humans than other species*(2)*. However, researchers found that NHPs were not ideal organ sources because of ethical concerns, the high risk of cross-species transmission of zoonotic diseases, difficulties in breeding*(3)*.

Scientists have tried to use pigs as sources of organs for xenotransplantation since the 1990s,

Pigs have several advantages over NHPs*(4)*. Pigs have higher reproductive abilities and relative shorter maturation period. The size and physiological structure of pig organs are more similar to that in humans. Lower risk of spreading infectious diseases between pigs and humans. Pork is one of the most commonly consumed meats in the world, so for many people, transplanting pig organs seems morally justified. A relatively cheaper and more accessible procedure than existing remedies for organ failure. However, pigs have greater genetic distance to humans than NHPs. Therefore, it is more likely to cause immunological rejection. The progress in the field of genetic engineering and the development of new generations of immunosuppressive drugs in recent years have effectively attenuated immune rejection and dramatically increased the survival time of xenotransplants in preclinical trials*(5-7)*.

In the following sections, we will discuss the progress and problems related to pig-to-human xenotransplantation and to evaluate whether it is the suitable time to perform clinical trials in humans.

The immunological barriers to xenotransplantation

Although pig organs offer great prospects for human transplantation, genetic incompatibilities between species result in immunological barriers that need to be overcome to achieve successful xenotransplantation.

When a wild-type pig organ is transplanted into a human, the graft is inevitably rapidly and completely destroyed (within minutes to hours) by the host immune system, which is called hyperacute rejection (HAR)*(8)*. HAR is mediated by pre-existing natural antibodies in recipients against a donor graft antigen, galactose-α1,3-galactose (α-Gal), which is a carbohydrate that expresses on a variety of pig cells in all breeds of pigs*(9)*. In contrast, α-Gal epitope is absent in humans and anti-α-Gal antibodies are naturally induced in all healthy humans during neonatal life*(10)*. Consequently, pre-existing human anti-α-Gal antibodies can destroy implanted pig organs in a very short period of time.

Another type of rejection is cellular xenograft rejection, which happens within days to weeks following transplantation. It involves both innate and adaptive immune responses. Immune cells participating in this process includes T cell, B cell, macrophages, natural killer cells and so on.

In recent years, the construction of new genetically modified pigs and the use of novel and more

potent immunosuppressive drugs have greatly extended the survival time of xenotransplants in preclinical NHP models. 5-year graft survival rates in the United States for primary kidney/liver/lung transplants from living donors were 85%/78.1%/53.3%, respectively*(11-13)*. 5-year heart graft survival rates were 75.6% in combined heart-liver transplantation in the United States*(14)*. But compared with allotransplantation in humans, the survival time of xenotransplantation is still relatively short. The longest survival of a life-sustaining pig kidney xenotransplantation was 499 days in a rhesus macaque*(6)*. The life-supporting function of pig hearts was extended to 195 days in baboons*(15)*. The longest survival time for life-supporting pig liver and lung xenografts were only 29 and 14 days in NHPs until 2019*(7,16)*. Therefore, the current protocols for xenotransplantation need to be further optimized to achieve similar survival time as allotransplantation in humans.

Zoonotic diseases

Human endogenous retroviruses (HERVs), which are mostly defective and replication-incompetent, Porcine endogenous retroviruses(PERVs), in contrast, are able to actively replicate and produce infectious viral particles in normal pig cells*(17)*.

A major risk for xenotransplantation is the cross-species transmission of zoonotic diseases to humans. One important reason for not using primate organs as sources of xenotransplantation is the potential high risk of cross-species transmission of primate diseases. Compared with pathogens carried by other animals, microorganisms from NHPs are relatively easy to evolve to cross the interspecies barrier and infect humans because humans are genetically closer to NHPs than to other species*(18)*. Theoretically, infectious materials that circulate within pigs are not easy to spread to people. In addition, most pathogens can be eliminated by using specific pathogen-free (SPF) animals and by the implementation of strict biosecurity measures*(19)*. However, there is an exception. Porcine endogenous retroviruses cannot be eliminated using above strategies because they are integrated into porcine genomes among different pig breeds and organs. Although, there is no direct evidence that PERVs can infect NHPs or human, the risk cannot be completely ruled out. Once adapted to humans, it would be another potential disaster just like HIV/AIDS pandemic. Once latent infections are established, it would be impossible to eradicate by current knowledge.

Scientists have attempted to eradicate PERVs. Some breakthroughs have been made in recent years. In 2015, Yang et al. demonstrated that the inactivation of all copies of the PERVs genomes in a porcine cell line using CRISPR-Cas9 can achieve more than 1000-fold reduction in PERV transmission to human cells*(20)*. In 2017, PERV-inactivated pigs were generated from PERVs-inactivated primary porcine cell line through somatic cell nuclear transfer by the same group*(21)*. There is no doubt that this innovation will push forward the progress of xenotransplantation.

The risk of transmission of pig infectious diseases to healthy humans is generally very low because of the species barrier. However, xenotransplant recipients may be more susceptible to animal infectious agents since they will have to take immunosuppressive drugs to overcome host immune-mediated rejection, which will consequently undermine their immune systems. Besides, animal immune systems cannot be effectively reconstituted in implanted animal organs within the human hosts, which may further increase potential risks of animal diseases. Animal pathogens may be able to establish productive infections in implanted animal organs or tissues inside the human body since those tissues are their natural hosts. Then, animal pathogens may gradually mutate and adapt to cross the species barrier and infect human tissues within the host. Those animal-derived pathogens may eventually be able to spread among other members of the community after accumulating sufficient mutations.

Compared with other organs, some organs are more likely to become the targets of animal pathogens. For example, lung is an important target organ for many infectious diseases such as influenza, SARS and COVID-19. Human and avian influenza viruses can establish infections in

pig's lung at the same time and more virulent and transmissible recombinant influenza viruses containing genetic materials from both human and avian influenza viruses can be generated. Besides, Lungs are directly connected to the environment through the respiratory tract, so it is easier to be exposed to the source of infectious pathogens. Therefore, should we also consider these risks when choosing animal organs for transplant?

Psychological issues

Allotransplantation has been widely conducted in clinical and save many lives every year. However, there are also some negative impacts related to it. Recipients may experience psychological problems after transplant. A recipient must successfully incorporate the alien organ into his or her sense of identity. Failing to finish this process often results in recipients' psychological problems such as symptoms of depression, emotional distress, anxiety, and other forms of mental issues. It is generally easier for invisible organ such as liver, lung, and kidney, but relatively difficult for externally visible organ such as facial and hand transplants. Clint Hallam was the first patient received a hand allograft*(22)*. He could not bear the strange hand and stopped taking immunosuppressive drugs. The transplanted limb was amputated in the end. Facial transplant recipients often wonder if they are themselves when seeing their transplanted faces.

Heart is often viewed as the seat of the soul, and source of love and emotions. Therefore, people may believe that their hearts are more closely linked with their personal identity. This could explain why fewer people are willing to donate their hearts compared to other organs such as kidneys and livers. Recipients of hearts are also more likely to experience a disruption to their own identity and bodily integrity following transplants*(21)*.

The situations would be even worse as regarding xenotransplantation. When people say an individual harboring animal organ, it is often to insult someone. For example, the Chinese idiom "a man with a wolf's heart and a dog's lungs" is used to describe a cold-hearted person, who have a lack of empathy or sympathy for others. Participants may start to wonder if they are a complete human after transplant since they have animal organs within their bodies. They may feel interconnected with animals and treat the xenotransplant as an intruder or stranger. They may even believe that they harbor animals' feelings and character. Therefore, it may be harmful for their personal identities and bodily integrity. Only internally invisible organs of animals should be used for xenotransplantation because externally visible organs will always remind the recipients that they have animal organs and even have some characteristics of animals. In addition, it will make it easy for others to know that they have transplanted animal organs.

Compared with allotransplantation, the psychological stresses placed on xenotransplant recipients can also come from others. Social stigma and discrimination are potential serious problems which may hinder xenotransplantation. Humans are superior to animals. If people know that someone around them transplanted with animal organ, it is hard to imagine that people would not discriminate against this person. If xenotransplant recipients feel discriminated against because of their conditions, they are very likely to experience psychological problems such as depression, anxiety disorder, suicidal ideation. Although HIV-1/AIDS cannot be cured based on current knowledge, it has become a manageable chronic disease due to the discovery of combination antiretroviral therapy. However, social stigma and discrimination against HIV/AIDS remain a major unsolved problem. It can negatively impact the mental health of patients, making them feel shame, isolated, depressed, and anxiety. Some individuals deliberately or maliciously spread HIV to intentionally harm others via condomless sex, sharing syringes or needles containing infected blood. These actions have seriously threatened social stability and put everyone's health at risk. Therefore, social stigma and discrimination are not easy to solve. Effective measures to solve this issue need to be proposed before proceeding with clinical xenotransplantation trials.

The wholeness of the body is not only somethings attached to the body and/or inside the body, but a matter of contributing to the body

as a functional whole*(23)*. Hence, the removal of malfunctioning organs does not cause much damage to bodily integrity. Violation of bodily integrity is generally regarded as acceptable because of the potential benefits and restoration of bodily functions that can be attained by the xenograft-recipient human dignity.

Genetic engineering

Enormous progress has been made in gene editing technologies over the past decade due to the discovery and wide use of the CRISPR/Cas9 gene editing system. The CRISPR/Cas9 platform is a promising technology for targeted genome editing due to its easiness, flexibility, specificity, high efficiency, and low cost. For example, genes that related to family inherited diseases can be eliminated to avoid the occurrence of related fatal diseases.

As discussed above, potential animal-derived diseases and interspecies immune barriers greatly hinder the progress of xenotransplantation. CRISPR/Cas9-based gene editing brings unprecedented opportunities and potential for the field of xenotransplantation. In recent years, scientists have tried to eliminate the risks of cross-species transmission of zoonotic diseases and immunological barrier using genetical modifications*(19-21,24)*. These modifications greatly prolong the survival time of the graft in the allogeneic body and basically eliminate the risk of PERV transmission.

However, it has only been about ten years since this technology was discovered to be widely used. People are not fully aware of the potential risks of this technology. A large number of long-term preclinical experiments are required to prove its safety. Several groups have reported that Cas9 attaches to unintended genomic locations, named off-target phenomena*(25-27)*. Some studies have indicated that CRISPR/Cas9 technique may unintentionally enhance the risk of cancer*(28,29)*. CCR5-edited babies may be more resistant to HIV infection*(30)*. However, rashly performing clinical trial has caused the twin sisters to be in fear for the rest of their lives.

In addition, some genes that need to be knocked out may have important functions. Although they currently seem to have very few functions or even non-functional, it may be due to our insufficient understanding of them. Knockout of these genes can have serious consequences. Some genes that seem to be unimportant now may have potentially important functions that have not yet been discovered. CCR5 seems not important right now, but we cannot rule out that it may have important functions, especially in the long term. Therefore, every small step should be very cautious to avoid serious consequences.

Informed consent

The informed consent of the recipient

More preclinical experiments should be conducted before translating into human clinical studies. Patients should be provided with complete information of the advantages and disadvantages of this new technology, potential risks, and possible adverse consequences. In clinical trials, researchers must not conceal defects to attract patients to attend their programs. This will not only harm the interests of patients, influence public trust in science. A lack of real data will also affect the development of this technology in the long run.

Currently, we have very little knowledge about the safety and feasibility of xenotransplantation in humans. We will get more knowledge only after many clinical trials have been conducted. Xenotransplant recipients may transmit animal-derived diseases to their sexual partners, family, friends and eventually to the public. Since this risk in xenotransplantation organs cannot be completely ruled out, necessary measures must be taken to prevent potential transmission of zoonotic diseases. This problem is extremely complicated and need to be discussed among experts from different fields. It involves the privacy, confidentiality, and personal freedoms of patients. While taking necessary measures, it is also important to ensure the basic privacy and basic human rights of patients.

The information of xenotransplant patients must be highly confidential and cannot be leaked. It will cause great harm to patients and their fami-

lies if this kind of information is revealed. The patients have already suffered tremendous pain from the diseases. At the same time, they also need to bear their psychological issues regarding animal tissues.

Global consent

Xenotransplantation undoubtedly affects the interests of all people around the globe since the spreading of zoonotic diseases cannot be completely ruled out. Therefore, everyone in the world has the right to be correctly informed. All clinical trials should be conducted under the premise of ensuring public safety. However, we cannot hinder the development of related technologies because of potential risks. In the future, more and more people will need organ transplantation to treat their diseases and extend their lifespan. In the case of ensuring safety, we should promote the development of this technology step by step.

Conclusion

Pig-to-human xenotransplantation provides a huge potential for solving the scarcity of human organs and saving terminally ill patients. In recent years, the widespread application of gene editing due to the discovery of the CRISPR/Cas9 gene editing system has provided technical operability for solving immunological rejection and preventing the spread of animal diseases such as PERV. However, it is irresponsible to conduct pig-to-human clinical xenotransplantation trials at this moment due to the remaining risk of transmitting animal diseases, relatively short graft survival time, psychological issues, potential side effects of gene editing, and issues related to informed consent.

Author contributions

Laichun Zhang conceived and wrote the manuscript, Lijun Ling provided technical support.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Received: January 3, 2022
Accepted: January 13, 2022