Research and Development of Xenotransplantation using Animals

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Keywords: Xenotransplantation, Immune Rejection, Immunosuppression.

Abstract: Xenotransplantation is the transplantation of a tissue or organ from one species into another. It is expected to be used in the clinical treatment of end-organ failure in the future. In recent years, xenotransplantation is being widely studied because of its great potential. Because the homotransplantation donors are mainly derived from human origin and are in clinical shortage in quantity, while the xenotransplantation donors are from pigs, there is no apparent difference in organ quality between human and pig sources. xenotransplantation donors also have adequate quantity. This paper first introduces the general situation of xenotransplantation. Then, it discusses the technology and process of xenotransplantation in detail. What is more, this study also provides the prospects of xenotransplantation. It is hoped this article can provide new ideas to the research in xenotransplantation.

1 INTRODUCTION

Xenotransplantation is a new technique in the field of medicine. Xenotransplantation refers to the transplantation of tissue from one species into another. For example, from pigs to people. Different from homotransplantation, there are great differences between different species, which makes the possibility of successful transplantation very small. Animal organs are used as substitutes to reduce the shortage of organs. This technology is in great demand. Xenotransplantation is the only effective way to treat end-stage organs.

However, the shortage of organs continues to increase. The organs needed for transplantation mainly come from donors and criminals. Every year, more than 1 million people in the world need organ transplantation because of end-stage organ problems or accidents, but only 10% of them receive organ transplantation. Many people don't get treatment. Compared with human organ transplantation, xenotransplantation has enough donor sources and can be mass-produced, so its price should be lower than human organ transplantation (in some countries, the price of heart transplantation is about 450,000 yuan). The research of xenotransplantation began in 1667. In recent years, there are more and more researches on xenotransplantation. For example, 75% of heart valve surgery patients choose biological valves, i.e. pig valves or bovine pericardium, as tissue materials. The survival rate was over 90%. If the technology works. This will be great progress in the field of medicine, accelerating human civilization and prolonging human life.

There are abounding technology to achieve the goal that is applying xenotransplantation in daily life. The traditional technique implemented the transformation of heterologous organs in patients by a humanized modification for gene knockout of donor organs from transgenic animals and using immunosuppressants to promote transplantation. With the development of gene editing, CRISPR/Cas technology was also applied in xenotransplantation, which offers changes based on repairing broken DNA strands. Even though such technology is successfully used in xenotransplantation, some barriers also exist during application. For example, animals without

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Pang, K., Wan, T. and Yuan, X.
Research and Development of Xenotransplantation using Animals.
DOI: 10.5220/0011216500003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 461-466
ISBN: 978-989-758-595-1

correct gene editing cannot be converted into mature organ donors. Before implementing xenotransplantation, the heterologous organ required the inhibition of cellular immune responses, modifying the major histocompatibility complex, eliminating the cross-species transmission risk of virus from donor animals, and so on.

This article systematically introduces the general situation of xenotransplantation in this paper, including its development history, technical route, and the test results in recent years. Especially narrates the difficulties of current xenotransplantation technology. In addition, this paper also reasonably analyzes the relevant literature and predicts its bright future.

2 BASIC INTRODUCTION OF XENOTRANSPLANTATION

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2.1 Concept of Xenotransplantation

Xenotransplantation can provide an unlimited source of donors to humans through animals. Therefore, It is the dream of humans for many years. More and more people are researching xenotransplantation. In theory, it is the best choice to choose the closest primate (such as a monkey) as the source of graft. But the primate number is very rare. As a result, pigs, which are similar to human organs in size and function, become the best choice for researchers. In theory, for example, pig islets could be transplanted into humans. Because pig insulin works for humans. And pigs can reproduce rapidly, which makes it easy for them to be genetically modified.

The biggest problem of xenotransplantation is the rejection of transplanted organs. This is mainly due to the common rejection of cells and immune cells in organs. Lead to adverse reactions. It can even kill people. At the same time, there is a potential risk of xenotransplantation, that is, the risk of infection source transmission. For example, viruses in pigs spread from pigs to people. To solve these problems, scientists need further research.

2.2 The Status of Xenotransplantation

The effects of xenotransplantation on organs have been improved (Cowan 2017). It improves the research efficiency of xenotransplantation. Xenotransplantation is the best way to treat organ failure. Therefore, there is a huge demand for this technology. Many people worldwide are waiting for the success of xenotransplantation and integration into people's lives. However, there are many concerns about this technology. Scientists have proved that xenotransplantation is a feasible alternative to homotransplantation. However, the current situation of this technology is not mature.

However, with the continuous research of scientists, people have found a large number of obstacles to xenotransplantation, and designed many potential solutions. Scientists have made models of xenogeneic corneal transplantation. An anterior lamellar keratoplasty model of non-human primates in pigs was established. This model can predict the effect of transgenic pigs on xenogeneic corneal transplantation (Vabres 2020). At the same time, the experimental model of transplanting pig liver into baboon has also been completed (Navarro-Alvarez 2020). The experiment of islet transplantation from human to rodent has also been completed (Iuamoto 2017). These experiments and models have made a great step forward in human xenotransplantation.

2.3 Results of Xenotransplantation and Its Effect on People

In recent years, there is a growing interest in xenotransplantation. The effect of xenotransplantation on many organs has been significantly improved. It improves the research efficiency of xenotransplantation. The PERV virus has subsided. However, the prevention of xenograft rejection is still a great challenge (Cowan 2017).

In 2016, Swiss researchers made a breakthrough in this field. With the administrative and security parts of clinical preliminaries entering the program and using polygenic pigs. The endurance pace of pig xenotransplantation in non-human primate models has been altogether improved (Yung 2017). To make xenotransplantation a clinical reality, it is necessary to work on the resistant procedure. The most

interesting possibility soon is the utilization of homozygotes α-Organs of galactose quality knockout pigs.

Variety of organic pathways in the all-out range of xenograft dismissal (Hoerbelt 2004). With the continuous advancement of human progress and the efforts of scientists, there will be more and more breakthroughs in xenotransplantation. Then the solution and the technology are officially recognized and put into use. So that the choice of patients whose organs have reached the end of their life is no longer limited to allogeneic transplantation. Xenotransplantation will become the best choice for them.

3 BARRIERS OF XENOTRANSPLANTATION AND THE TECHNOLOGY TO BREAKTHROUGH

3.1 Obstacles and Strategies to Overcome in Xenotransplantation

When human organ donor is in a shortage, it is well known that the application of xenotransplantation can acquire more benefit than homotransplantation technology. However, before a huge amount of organ supplementation from animals, some obstacles need to be overcome, for example, the immunological rejection of heterologous organ in the human body prevents the goal to utilize animals as an organ donor (figure 1).

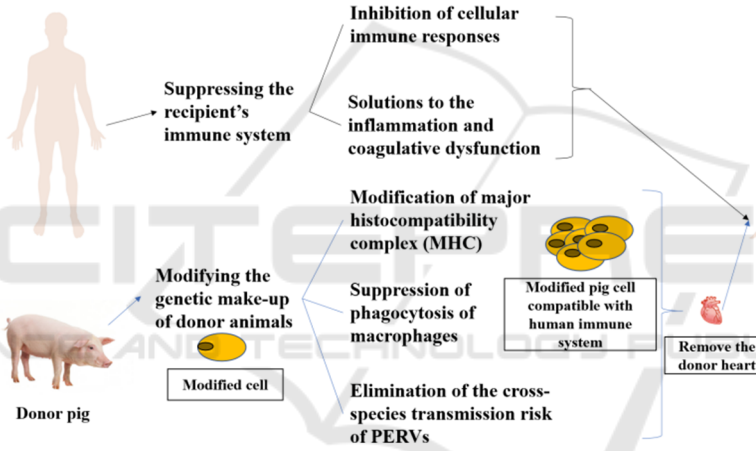

Figure 1: The main obstacle in xenotransplantation lies in how to eliminate the immune rejection after the operation. For patients, immunosuppressant adjuvant therapy is needed to reduce cellular immune response and optimize treatment for transplant-induced inflammation and coagulopathy. For donor pigs, editing and modification at the gene level should be carried out in advance, which mainly includes the modification of MHC, the inhibition of macrophage function, and the elimination of endogenous viruses that can be transmitted across species.

In recent years, the development of genetic technology and research of immunosuppression medications had prepared the basics for the effective regulation of these flaws. As an example, the porcine gene had been optimized to enable human immunoreaction decline, also, it decreased liveness rates of porcine endogenous retroviruses (PERVs), prevented coagulation disorders and other reactions. Meanwhile, researchers need to ensure the organ is functioning normally and living for a long period after xenotransplantation, for instance, operate corresponding immunosuppression strategy to transfer the pancreas from transgenic pig to human and make certain the organ working, as usual, to return blood sugar and insulin level to normal. Deeper research is necessary, applying the immunosuppression strategy still has a limitation for the health of the patient. Moreover, another strategy, immunotherapy, can build a mouse model (gene and tissue of mice are substituted by human genes and cells), simulating the growing environment of xenograft organs and tissue under the human immune system. This model is one of the latest advances in immunosuppression strategies.

3.2 Suppressive Regulation of Immune Response

Many experiments had been done in the study of xenotransplantation, especially in the control of the immune response. For example, the application of TEVMP with good mechanical and physiological characteristics as a biological artificial artery in pigs to simulate the physiological blood flow system of the human body, and the study of xenograft rejection in blood vessels (Kim 2021). In addition, many transplantation experiments have been performed to challenge xenoimmunological rejection, such as the implantation of human cancer cells in humanized mice (which have a human immune system). The mice were also tested for tumor treatment results which have replaced the original human patients (Jin 2021).

As a deeper study, CD47 is a ligand for macrophage inhibitory receptors, if introducing human inhibitory regulators of macrophage into the pig, it could effectively inhibit the rejection reaction of macrophage in the human body after xenotransplantation. By the technique of chromatin transfer, researchers successfully obtained ideal hCD47 Knock-in Pigs, after two rounds of clone. Moreover, to test the results' effectiveness, the researchers transplanted porcine progenitor cells into mice and found that several alleles in the mice were able to bind with hCD47. This result showed that the expression of hCD47 had a significant protective effect on the transplantation and persistence of porcine cells in this model, possibly by regulating phagocytosis of macrophages (Tena 2014).

3.3 Second Sectionxenotransplantation Experiment Practice

Interestingly, there have also been practices involving xenografts, for instance, using pig islet grafts as substitutes for allografts. Researchers evaluated clinically available drug immunosuppression regimens, such as belimumab for maintenance and adalimumab for control of inflammatory responses. The experimental subjects were non-human primates. After transplantation of pig islets, blood glucose was normal, and there were no organ failures and no serious adverse reactions. The survival days of the transplanted organs in all three recipients were more than 100 days (Kim 2021).

To ensure long-term insulin independence, in another paper, researchers used several strategies. The implantation of embryonic porcine pancreas tissue into diabetic animals is a key aspect of xenotransplantation, to be more precise, the location of the islet implantation is also critical. In previous experiments, it was placed in the liver, where low oxygen and inflammatory reactions could hinder the survival of islets. However, when implanted into the renal capsule, the islet was well protected, but the site was prone to ischemic injury. Finally, when the islet was transplanted into the gastric submucosal-space, its direct contact with the blood flow is delayed, but the arterial blood can continue to maintain. As a result, transporting oxygen and nutrients can enable the graft to survive. Another advantage is that drainage of venous blood allows insulin to work directly in the liver (Marigliano 2011).

4 REDUCE IMMUNE REJECTION AND INFLAMMATION

There are still many problems to be overcome in xenogeneic heart transplantation. Such problems mainly focus on postoperative immune rejection, inflammatory reaction, and infection. At the same time, there are also some ethical and religious issues (Garcia 2021). At present, various research teams have proved several possible methods to reduce immune rejection and inflammation.

4.1 Gene Editing Pig

First of all, it is necessary to cultivate donor pigs with gene editing in routine xenotransplantation experiments, knock out or modify some genes that can cause immune rejection, and porcine cell virus genes, to reduce the potential risk of immune rejection (Tomasi 2021). Due to the different genes of patients, there will be slight differences between gene editing pigs and different recipients. These differences may cause a serious inflammatory reaction after transplantation (Reichart 2021). Li et al. designed a new method to quickly detect the xenogeneic immune response of humans to pigs, which can express special endothelial cell markers, verify the stimulation strength of inflammatory cytokines, and detect xenogeneic immune response by the culture and transformation of immortalized primary porcine living derived cell (Li 2021).

In addition, how to improve the production efficiency of gene editing pigs is also one of the hot issues. Cho et al. introduce the human endothelial protein C receptor (hEPCR) and human thrombomodulin (hTM) genes into porcine neonatal

ear fibroblasts. It can be used as donor cells for reclining to increase production efficiency. The cloned fetal kidney cells also have the same function (Cho 2022).

The survival time of organs after the operation can be improved, and immune rejection can be reduced by improving the preservation method of the donor's heart before operation (Goerlich 2021). Corbin et al. Found that compared with traditional preservation methods, low-temperature standing preservation was carried out on the ice, and used XVIVO© Heart solution (XHS) based cardioplegia can improve the survival rate and function of grafts.

4.2 Proinflammatory Factor and Cardiomyocyte

There are two additional ways to reduce the occurrence of immune rejection and inflammation after surgery. One is to continuously apply anti-CD40 antibodies and other immunosuppressants and control the expression of pro-inflammatory factor hTNF (Hara 2021, Mohiuddin 2016). When human blood contacts pig hearts, it induces the activation and proliferation of cytotoxic T cells and NK cells. hTNF is one of the important factors in the process of activation and proliferation.

The other is by inhibiting the harmful proliferation of donor heart cells (Längin 2018). Research shows excessive proliferation and hypertrophy of cardiomyocytes will lead to multifocal myocardial necroses and thrombosis and cause secondary liver failure. Matthias et al. tested with baboons. They weaned the recipient baboons at an early stage, applied hypertension treatment, and took additional tacrolimus, which finally reduced the excessive growth of the heart.

5 CONCLUSIONS

In summary, this paper analyzes the basic introduction of xenotransplantation, the current situation of xenotransplantation, the results of xenotransplantation, and its impact on human beings. Some effective strategies for suppressing the immune system can enhance the survival rate and durability of transplanted organs. However, some methods still need a deeper study that researchers have not eliminated the threat of inflammatory reaction and virus infection carried by allogeneic organs completely. The next research will focus on Gene editing pigs. Those donor pigs in use today can survive as long as six months with just a few genes knocked out, and it is not hard to imagine that these xenoorgans could survive even longer when more genes are modified. Researchers can also find more suitable immunosuppressants with better effects and fewer side effects. In the next study, they can gradually start clinical research under international recognition and supervision.

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