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Review Article

Xenotransplantation from Transgenic Animals: A Critical Analysis

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ABSTRACT: The current shortage in human organs has made xenotransplantation a potential source of organ transplant in humans. The use of primates could not provide the needed results in humans as many attempted organ transplants involving primate-to-humans were not so successful. Coupled with this, is their limited number and the fact that they are endangered species. Consequent upon this, transgenic pigs became the candidate of choice with the potential of solving this issue of organ shortage. Apart from having organ identical with humans, they have high reproductive rate and can be efficiently manipulated genetically to obtain desired results in humans. However, there are potential problems which exist with xenotransplantation using transgenic pigs as an alternative source. Currently, most of these problems are being overcome. This will not only reduce the problems of organ shortage but will open a new chapter in xenotransplantation from transgenic animals in the near future. This article discusses the potential benefits of using transgenic animals as a practical solution to human organ transplant. It further points out some of the potential problems of using these animals and how these problems are currently being overcome.

KEYWORDS: Xenotransplantation; Allotransplantion; Transgenic animals; Xenogenic; Zoonosis; Xenograft

Introduction

Xenotransplantation has become a promising area in recent times as a result of the shortage of allogenic organs (Bach, 1997). 1950s, allotransplantation (organ transplant between related species) was a major alternative for the treatment of end-stage disease of the liver, kidney, heart and lungs (Dorling *et al.,* 1997). This is no longer so today, as the current shortage of human solid organs for transplantation has led to a sudden drift from allotransplantation to xenotransplantation (organ transplant between distantly related species). The number of patients awaiting organ transplant has increased over the years while the number of available donors has greatly reduced. As a result of this crisis in organ shortage, many people are left at the mercy of the availability of organs for their survival.

Xenotransplantation has a long history. In 1910, Unger carried out the first attempt in xenotransplantation which involved renal transplant from chimpanzees to humans. In this case, it was unsuccessful and the patient died due to blood clotting (Jonathan *et al.,* 1995). Reemstma and colleagues also carried out a similar work with the intention of obtaining a positive result. However, there was a success of a nine month graft survival in one of the patients who later died. Another report was that of Starzl and co-workers in the same year. Here, they used a baboon-to-human transplantation but had a poorer graft survival. The patients in both cases were reported to have died as a result of infectious complications and nonspecific doses of high level of immunosuppressive agents that were used (Jonathan *et al.,* 1995). Consequent upon these failures was the introduction of cyclosporine A in 1983 (Petersen *et al.,* 2008), an immunosuppressive drug with the intention of overcoming the problem of rejection of xenogenic organs. Further to this, cyclosporine was administered to “baby Fae” after transplantation with a baboon heart. She was reported dead twenty days later (Bailey *et al.,* 1985).

Several attempts of xenotransplantation from primates had not been so successful. In addition to this was the ethical issues raised in regards to their use, particularly the issue of self. Primates were considered endangered species and their number was equally at a decreasing rate. This might not be able to meet the current increase in the demand for organ transplant (Petersen *et al.,* 2008). This has led scientists into the search for an alternative and better animal donor. Pigs were then considered as potential candidates of choice because ethical concern for pig is less and besides, they produce large size match organs with human and they have very high reproductive capacity with large litters (Turk *et al.,* 2004). To be able to demonstrate this success in humans, pig-to-primate xenotransplantation was performed and some considerable successes were achieved.

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However, the use of pig organs in human xenotransplantation has also faced some challenges. There are ethical concerns like: the risk of disease infection (Porcine endogenous retrovirus) and the issue of interfering with nature (Hughes, 1998). The major problem is that of Hyperacute rejection (HAR), Delayed Xenograft Rejection (DXR) and T-cell mediated rejections, which are all immunologic problems (Bach, 1998). Thus, transgenesis has tried to overcome some of these problems as seen in this article.

The need for Xenotransplantation from Transgenic Animals

Xenotransplantation from transgenic animals has reached a new level as a result of the shortage in human organs. It has almost become the only alternative to human organ transplant, particularly the use of transgenic pig which has a wider ethical acceptance than non-human primates that were used before.

Based on a report, about 45,000 people in the USA with most of them younger than 65 years needed heart transplant. On the other hand, only 2000 human hearts are transplanted annually. These number keeps on increasing as new patients are being added to the waiting list. In this kind of situation, a lot of people would die because there is no guarantee that those on the waiting list would have a heart transplant not talk more of those being added to the waiting list.

The emergence of transgenic pig as a source of organ for xenotransplantation has the capacity of providing a solution to this lingering crisis. The porcine (pig) has a very high reproductive rate which means an inexhaustible supply of organs to the teaming population of patients on the waiting list for organ transplant.

The possibility of using xenograft from transgenic pig as a likely option for preventing some notorious and recurrent primary diseases is currently receiving attention. As a result of this fact, species difference between pig and humans in susceptibility to diseases may lead to transplant in a particular group of people (D'Apice *et al*., 1997). This xenograft could be immune against some diseases like HIV and hepatitis B (Dorling *et al.*, 1997). This means that there is a possibility of xenotransplantation even in patients harbouring this form of diseases.

There is a potential of extending therapeutic possibilities of some diseases when transgenic pigs are used. As reported by Kovarik and Mandel (1999), there is only one amino acid difference between pig and human insulin and a similar case has equally been used in the treatment of diabetes. This implies that manipulating this insulin genetically can lead to the treatment of diabetes mellitus – a disease that has no cure at present.

Prior to the identification of transgenic pigs as a potential source of human organ donor, transplantation involving allograft requires that donor organ is hurriedly transplanted unto the recipient (Dorling *et al.*, 1997). The advent of transgenic pig with increased rate of reproduction will result in the availability of organs awaiting transplant. Therefore, this will allow for planned and unhurried operation contrary to the era of allotransplantation.

Potential Problems of Xenotransplantation and their Solutions

Ethical issues

Ethical concerns have been raised concerning the use of pigs in xenotransplantation. Some based it on the fact that it is unnatural to remove the organs of animals, which they believe interferes with nature (Hughes, 1998). But when we look at this from another angle, it is obvious that issues concerning human health have been a matter of clinical trials using animals. Since pigs are consumed by humans as food, there should be no problem in sacrificing them for the benefit of mankind. If some consider this act unnatural, then no animal should even be killed in the first place for human consumption. On the other hand, since the number of human organ donor is reducing as reported earlier, if transgenic pigs are not used, this could lead to more death tolls as a lot of people awaiting organ transplant would not even have any. There should be no problem if animal organs can provide the solution to the shortage of human organs; after all, it is for the gain of humanity. There is equally the fear that this animal will suffer in isolation and also be genetically manipulated as the aforementioned case. However, if some of these genetic manipulations are not done, human beings could be faced with the puzzle of not knowing what to do to save the human race. Regulations can even be put in place to ensure that the animals are treated humanely.

Risks of disease infection

This is one problem that has perturbed public concern on the use of transgenic pigs as an alternative source to human organ transplant. People have been bothered on the possibility of cross-species infection (zoonosis) from the pigs to humans. This case has been aggravated due to an earlier controversy on the origin of HIV (human immunodeficiency virus) which many claimed was a cross-species infection from the Chimpanzee's SIV (simian immunodeficiency virus) to humans. Other cases of animal diseases like the flu epidemic and SARS (severe acute respiratory syndrome) whose origin was traced to animals were also highlighted.

As a result of the above, there is fear that a possibility of the porcine endogenous retrovirus (PERV) crossing over to the human population and evoking another era of epidemic is inevitable. Thus, it has been reported and well documented by Paradis *et al*., (1999) that twenty one pig-to-human transplants carried out; no cases of porcine retroviral transmission were documented. Further to this, since cases of retroviral transmission have not been reported and of which we hope would not occur between pigs and humans, transgenic pigs may serve a better option for xenotransplantation.

Graft rejection

Graft rejection seems to be the major problem confronting the progress of xenotransplantation involving pigs. The main one is hyperacute rejection, described as the first and most destructive, occurring within minutes (Petersen *et al.*, 2008). It is an immunologic response by the recipient against the xenograft. This has been described as the reaction of the natural antibody IgM (immunoglobin M) isotype (xenoreactive antibody) to the xenograft endothelium and leading to the formation of membrane attack complements (Gambiez *et al.,* 1992). The after effects of the reaction includes; endothelial cell activation, cell damage, aggregation of platelets, platelet adhesion and intravascular graft thrombosis which results in the loss of graft function (Platt *et al.,* 1991).

It has been found that the gal epitope is expressed in non-primate mammals, promisians and New World monkeys but not expressed in humans, Old World monkeys and Apes (Macher and Galili, 2008). The enzyme responsible for the synthesis of the gal epitope (alpha 1, 3 galactose linkage) is the alpha 1, 3 galactosyl transferase (gene symbol GGTA1). Therefore, when there is a pig-to-human xenotransplantation, as a result of the gal epitope and enzyme in human, complement cascade is activated and a subsequent hyperacute rejection is initiated (Petersen *et al.*, 2008). This happens because xenoreactive antibodies found in human blood attack the epitope, causing graft loss.

As a result of this problem, a lot of strategies have been developed and tested in pigs and mice (Cowan *et al.,* 1998; Costa *et al*., 1999; Mckinzie *et al*., 2000) of which transgenic modification of the donor animal (pig) is the most promising (Petersen *et al.,* 2008). Gene knock out can now be applied in pigs to generate alpha 1, 3 galactosyltransferase knock out pigs by homologous recombination in primary fibroblast cultures as well as the use of differentiated primary cells with somatic cell nuclear transfer (Dai *et al.,* 2002; Harrison *et al.,* 2002; Phelps *et al.,* 2003). With this promising technology, the case of hyperacute rejection will soon be a thing of the past as transgenic pigs are already being generated even though other genes may be involved.

Furthermore, another immunologic issue is delayed xenograft rejection which occurs some days later due to procoagulation and infiltration of natural killer (NK) cells and monocytes in the host organs (Bach, 1998). Transgenic approaches that would block upregulation of inflammatory genes in the endothelial cells or using transgenic donors (pigs) expressing DAF (decay accelerating factor) would prevent delayed xenograft rejection (Dorling *et al.,* 1997).

Conclusion

The increase in the number of people seeking organ transplant has been increasing enormously. Due to lack of success in allotransplantation caused by shortage in human organs, non-human primates were thought to be a solution. However, ethical concerns and the reason that they are a group of endangered species coupled with their limiting number have failed to yield considerable results.

Consequent upon this was the identification of pigs as potential organ donor for human organ transplant. Xenotransplantation involving transgenic pigs has equally faced serious controversy of which the gains outweigh the potential effects. If these potential effects are taken care of, which are currently being handled by genetic engineering; the use of transgenic animals would be an inevitable source of organs for xenotransplantation in the near future.

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