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EDITORIAL

Xenotransplantation

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Xenotransplantation (xenoTx), or interspecies transplantation, is defined as transplantation of organs, tissues or cells across different species and represents one of the most interesting proposals to address donor shortage. The prospect of extracting tissues and organs from immunologically modulated animal donors, with little to no chance of rejection in humans, makes xenoTx attractive for clinical purposes[1-6]. Being able to provide good quality organs at any moment, this propose would improve clinical outcomes and reduce the waiting time and mortality of patients in the transplantation waiting list.

By the early 1980s, there were several attempts at xenoTx using great apes, pigs, and sheep[6]. At that time, the concept of brain death was not yet well established causing important shortages of suitable organs for transplantation[6]. Several articles were published describing a total of 33 kidney xenoTxs in humans. Particularly notable is a series of 13 cases with donor chimpanzees who had a maximum survival of nine months, described in 1964 by Reemtsma et al.[7]. Starzl et al.[8] performed six kidney transplantations using donor baboons, with a maximum survival of about two months. Twelve liver xenoTxs were also performed, such as in the series by Starzl et al. who carried out four transplantations between 1966 and 1974 using chimpanzees graft, with a maximum survival of 14 days[6]. Starzl's group also performed two more transplantations with baboon donors between 1992 and 1993, with a maximum survival of 70 days[9]. Hardy et al. performed in 1964 the first heart xenotransplantation in man with only a day survival[6]. After that, six more attempts were made without significant success also; however, Bailey et al. in 1984 performed the longest survival of heart xenotransplant utilizing cyclosporin therapy and the heart functioned for 20 days[6].

However, complications such as hyperacute rejection (HAR) which rapidly destroys the graft, the advent of encephalic death establishing cadaveric donors and the possibility of interspecies infections transmission currently impede clinical applications of xenoTx, thereby justifying research on the subject[1-9].

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XenoTx is classically divided into two types: concordant and discordant. Discordant xenoTx is carried out across very different species, such as between pigs and humans. Concordant xenoTx involves individuals of similar species, such as rats and mice, or great apes and humans. The degree of difference between the species involved determines the intensity of xenoTx's humoral immune response involving preformed interspecies antigens/antibody reaction. Thus, discordant xenoTx usually causes HAR, with total graft destruction within few hours. In concordant xenoTx, there is usually acute vascular rejection with graft destruction in a few days[1-3].

Indeed, the biggest limitation of xenotransplantation is the HAR, an immune reaction that rapidly destroys the graft. Considered an immunological catastrophe, its pathophysiology in xenoTx is not yet fully understood. It is known to be mainly humoral in nature, mediated by the activation of mainly IgM and IgG class antibodies known as xenoreactive antibodies. In HAR there is a strong deposition of antibodies in the vascular endothelium of the graft, increasing the migration, adherence, and activation of leukocyte membrane receptors[1-10].

This activation leads to the release of anaphylatoxins, histamine and serotonin, which activate other mediators such as platelet-activating factor (PAF), and inflammatory cytokines. Changes in the vascular endothelium expose the basement membrane, thus promoting the activation of platelets and complement factors C2a and C5a. There is also inhibition of natural anticoagulants such as heparin sulfate and eco-adenosine diphosphate, with the formation of microthrombi and fibrin deposits[1-5]. The final consequence of this reaction is the presence of microvascular thrombosis and interstitial hemorrhage, which quickly destroys the graft. If the mechanisms of HAR can be inhibited, the grafts usually evolve into acute vascular rejection. Macroscopically, grafts with HAR initially show discoloration and later a purple color, vascular congestion, and parenchymal hemorrhage[1-3].

Several aspects of the pathophysiology of RHA are still unknown, mainly concerning its treatment. Among the mechanisms involved in the immune response of RHA that still need better clarification, we highlight the participation of cell-mediated immunity, the coagulopathy that occurs in this reaction, the involvement of inflammatory and regulatory cytokines in addition to their superfamily, the chemokines[9]. The difficulty in understanding HAR is likely because there is a lack of appropriate models for its study.

Old world monkeys (baboon, gorilla, chimpanzee, and orangutan) are the animals with the potential for xenoTx, as they share more than 95% of genetic similarity with humans. Those animals, as well as humans, do not express galactose-α-1,3-galactose (Gal-α-1,3-Gal), an oligosaccharide present in most pathogenic bacteria for these species. Through immunological evolution, these species extinguished this molecule from their genome and developed preformed antibodies against it, making them more resistant to serious infections. In contrast, other mammal species such as swine did not show this evolutionary development and express Gal-α-1,3-Gal. Thus, swine organs transplanted in humans are rapidly destroyed by preformed primate antibodies against porcine antigens, mainly Gal-α-1,3-Gal[5]. However, primates have many limitations for use in research or as donors, as they are animals that easily transmit zoonosis, do not mate properly in captivity due depression, are in danger of becoming extinct, cause great social repudiation, above all[10].

Swine are animals with great potential for use in experimental and clinical xenoTx. Despite promoting a discordant reaction in humans with consequent HAR, their organs, especially the liver, have physiological and morphological similarities with those of humans. Other advantages using swine include low maintenance and mating costs, they can mate in captivity without problems, their organs size is suitable for both small and large patients, lower social restriction and the contemporary possibility of producing transgenic pigs free of pathogens and immunocompatible with humans to avoid rejection[10-14].

Swine have also been studied as organ donors in temporary liver assistance during fulminant hepatitis. Temporary liver assistance aims to keep these patients clinically stable until the appearance of a compatible donor[10-11]. This procedure showed biochemical and neurological improvement in patients with fulminant hepatitis[12,13]. Recently, transgenic pigs have been developed by various methods of genetic modification, aiming to make them immunologically similar to humans. The use of these animals in preclinical xenoTx research showed promising results[13,14].

The development of genetic engineering technology for the production of transgenic pigs holds great promise for xenoTx. The products of gene edition can product swine with α1,3-galactosyltransferase gene knockout- (GTKO) and / or other adaptations of gene expression (such as the inclusion of human genes), for example, of regulatory proteins of the complement factor, or those related to the CD47 complex of signal regulatory protein α (SIRPα), and / or genes regulating thrombosis in humans. Some authors believe that these genetic manipulations promoted by a methodology called CRISPR / Cas9, which induces genetic manipulation producing immunological tolerance and combating complications such as thrombocytopenia, combined with new immunosuppressive regimes should control HAR of the xenograft and improve the survival of swine grafts[3,15,16]. This technology may produce swine that could be an inexhaustible source of organs for transplantation.

Tools for gene editing in pigs are improving rapidly, such that precise cuts in DNA have to be generated to successfully exclude genes. The development of means to replace pig genes with human genes with precision is very desirable for the future development of pig donors for xenotransplantation. Recently Dos Santos et al.[17], in a collaboration between LIM 37 with the Indiana University School of Medicine, used a CRISPR / Cas9 to product a thrombomodulin (pTHBD) gene knockout swine and replace it with a plasmid containing an antibiotic selection marker without a promoter and the exon for human thrombomodulin[17]. The PhiC31 recombinase was used to remove the antibiotic selection marker to create porcine aorta endothelial cells that express human thrombomodulin instead of pTHBD, driven by the endogenous pig promoter. The selection cassette without a promoter allowed efficient enrichment of cells containing the correctly inserted transgene allowing expression of the human transgene by the endogenous pTHBD promoter. Gene regulation was maintained after gene replacement because the endogenous pig promoter was kept intact in the correct position. Therefore, these authors concluded that Cas9 technology and recombinase make the human to pig orthotopic exchange viable and pave the way for the creation of swine with human genes that can be expressed in the appropriate tissues that preserve gene regulation. These gene therapy improvements may allow the use of genetic modified swine as safe donors for organ transplantation in a near future[17].

The *Laboratório de Investigação Médica 37* (Medical Investigation Laboratory 37) develops an unprecedented line of research on multivisceral xenotransplantation. This model was idealized due to the great lack of organs for this type of transplantation, which is currently indicated for several serious diseases of the digestive system such as congenital diseases, intestinal failure with complications of prolonged parenteral nutrition, some abdominal tumors restricted to the abdomen, abdominal catastrophes, among others[18-23].

In this research, we compared HAR in three combinations of species used in multivisceral xenotransplantation. Multivisceral grafts (esophagus, stomach, small intestine, colon, liver, pancreas, spleen, and kidneys) were removed and implanted heterotopically in the donor-recipient combinations dog-pig (n = 5); pig-dog (n = 5) and rabbit-pig (n = 15). Multivisceral allotransplantation [pig-pig (n = 5), dog-dog (n = 4) and rabbit-rabbit (n = 5)] comprised the control group. Three hours after reperfusion, graft samples were collected for histopathology and immunohistochemistry. Models using dogs were interrupted due to a ban on the use of this animal for research by federal law. The HAR was visually observed in all xenografts about 15

minutes after reperfusion. The autopsy revealed HAR in all organs of the multivisceral xenograft; however, we observed a HAR less severe in the liver compared to the esophagus, stomach, small intestine, colon, pancreas, spleen, and kidneys. IgG fixation by immunohistochemistry was strong in xenografts and absent in allografts. HAR was absent in all allografts[18-23]. Therefore, we can verify that the three different models for multivisceral xenotransplantation in this experiment are relevant to the study of HAR and underwent a similar evolution. IgG expression by immunofluorescence was strong at the sites of HAR and we showed for the first time that the liver is more tolerant of HAR than other abdominal organs after multivisceral transplantation[18-23]. The results of these surveys had a major impact international and were the reason for the publication of an article by a group from the University of Pittsburgh which highlighted the relevance of the findings of the forementioned research[24].

In conclusion, xenotransplantation is a potential solution for organ shortage; however, HAR and the possibility of interspecies infections transmission currently hamper this procedure. Advances in xenotransplantation research and CRISPR / Cas9 biotechnology may product transgenic swine imunnocompatible with humans and free of pathogens to serve as organ donors in a near future.

REFERENCES

1. Yang YG, Sykes M. Xenotransplantation: current status and a perspective on the future. Nat Rev Immunol.2007;7:519-31. doi: 10.1038/nri2099.
2. Cooper DK. Clinical xenotransplantion--how close are we? Lancet. 2003;362:557. doi:10.1016/S0140-6736(03)14118-9
3. Ekser B, Ezzelarab M, RHAa H, van der Windt DJ, Wijkstrom M, Bottino R, Trucco M, Cooper DK. Clinical xenotransplantation: the next medical revolution? Lancet. 2012;379:672-83. doi:10.1016/S0140-6736(11)61091-X.
4. Denner J, Tönjes RR. Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. Clin Microbiol Rev. 2012;25:318-43. doi: 10.1128/CMR.05011-11.
5. Zhong R. Gal knockout and beyond. Am J Transplant. 2007;7:5-11. doi:10.1111/j.1600-6143.2006.01615.x.
6. Taniguchi S, Cooper DK Clinical xenotransplantation: past, present and future. Ann R Coll Surg Eng.1997;79:13-9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2502626/.
7. Reemtsma K, Mccracken BH, Schlegel JU, Pearl MA, Pearce CW, Dewitt CW, Smith PE, Hewitt RL, Flinner RL, Creech O Jr. Renal heterotransplantation in man. Ann Surg. 1964;160(3):384-408. doi: 10.1097/00000658-196409000-00006
8. Starzl TE, Marchior TL, Peters GN, Kirkpatrick CH, Wilson WEC, Porter D. Rifkind KA, Ogden DA, Hitchcock CR, Waddell WR. Renal heterotransplantation from baboon to man: experience with 6 cases. Transplantation. 1964;2:752-76. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2972727/.
9. Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M. Baboon-to-human liver transplantation. Lancet. 1993;341:65-71. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2962592/.
10. Dehoux JP, Gianello P. The importance of large animal models in transplantation. Front Biosci. 2007;12:4864-80. doi: 10.2741/2434.
11. Ibrahim Z, Busch J, Awwad M, Wagner R, Wells K, Cooper DK. Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. Xenotransplantation. 2006;13(6):488-99. Doi:10.1111/j.1399-3089.2006.00346.x.
12. Chari RS, Collins BH, Magee JC, DiMaio JM, Kirk AD, RHAland RC, McCann RL, Platt JL, Meyers WC.Treatment of hepatic failure with ex vivo pig-liver perfusion followed by liver transplantation. N Engl J Med.1994;331:234-37. doi: 10.1056/NEJM199407283310404.

13. Levy MF, Crippin J, Sutton S, Netto G, McCormack J, Curriel T, Goldstein RM, Newman JT, Gonwa TA, Banchereau J, Diamond LE, Byrne G, Logan J, Klintmalm BG. Liver transplantation after extracorporeal hepatic support with trangenic (hCD55/hCD59) porcine livers. Transplantation. 2000;69:272-80. doi:10.1097/00007890-200001270-00013.

14. Gock H, Nottle M, Lew AM, d'Apice AJ, Cowan P. Genetic modification of pigs for solid organ xenotransplantation. Transplant Rev. 2011;25:9-20. doi: 10.1016/j.trre.2010.10.001.

15. Ekser B, Klein E, He J, Stolz DB, Echeverri GJ, Long C, Lin CC, Ezzelarab M, Hara H, Veroux M, Ayares D, Cooper DKC, Gridelli B. Genetically-engineered pig-to-baboon liver xenotransplantation: histopathology of xenografts and native organs. PLoS One. 2012;7:e29720. doi:10.1371/journal.pone.0029720.

16. Waghmare SK, Estrada J, Reyes L, Li P, Ivary B, Sidner RA, Burlak C, Tector AJ. Gene targeting and cloning in pigs using fetal liver derived cells. J Surg Res. 2011;171:e223-9. doi: 10.1016/j.jss.2011.07.051.

17. Nunes Dos Santos RM, Carneiro D'Albuquerque LA, Reyes LM, Estrada JL, Wang ZY, Tector M, Tector AJ. J CRISPR/Cas and recombinase-based human-to-pig orthotopic gene exchange for xenotransplantation. Surg Res.2018;229:28-40. doi: 10.1016/j.jss.2018.03.051.

18. Galvao FH, Pompeu E, de Mello ES, da Costa Lino Costa A, Mory E, Dos Santos RM, Santos VR, Machado MC, Bacchella T. Experimental multivisceral xenotransplantation. Xenotransplantation. 2008;15:184-90. doi:10.1111/j.1399-3089.2008.00470.x.

19. Galvao FH, Farias AQ, Pompeu E, Waisberg DR, Teixeira AR, de Mello ES, Costa AC, Galvao R de C, Santos VR, Chaib E, Carrilho FJ, D'Albuquerque LA. Endoscopic features in a model of multivisceral xenotransplantation. Xenotransplantation. 2010;17:423-8. doi: 10.1111/j.1399-3089.2010.00609.x.

20. Galvao FH, Soler W, Pompeu E, Waisberg DR, de Mello ES, Costa AC, Teodoro W, Velosa A, Capelozzi V, Antonangelo L, Chaib E, D'Albuquerque LA. IgG profile in hyperacute rejection after multivisceral xenotransplantation. Xenotransplantation. 2012;19:298-304. doi: 10.1111/xen.12002.

21. Galvao FHF. Modelo experimental de xenotransplante multivisceral [Tese Livre Docência].São Paulo: Faculdade de Medicina da Universidade de São Paulo; 2012.

22. Soler WV, Waisberg DR, Pompeu E, Costa A, Capelozzi VL, Chaib E, D'Albuquerque LA, Galvao FH. Multivisceral Xenotransplantation: the effect of different species combination. Transplantation. 2018;102:S735. doi: 10.1097/01.tp.0000543721.67531.64.

23. Galvao FH, Soler WV, Lee AD, Waisberg DR, Pompeu E, Costa A, D'Albuquerque LA. Evidence of liver tolerance to severe hyperacute rejection in a pioneer model of multivisceral xenotransplantation. Transplantation. 2019;103:S137. doi: 10.1097/01.tp.0000576252.23264.c8.

24. Kumar G, Ekser B, Abu-Elmagd KM Cooper DK. Multivisceral xenotransplantation. Does it have a future? Xenotransplantation. 2010;17:418-22. doi:10.1111/j.1399-3089.2010.00613.x.