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Review

Xenograft bioprosthetic heart valves: Past, present and future

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HIGHLIGHTS

- Glutaraldehyde fixation increased the success rate of heart valve transplantation.
- However calcification due to immune reactions causes valve deterioration.
- Identifying xenoantigens expressed on bioprosthetic valves will be important.
- Genetically-modified pigs will potentially provide better valves for implantation.

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ABSTRACT

The transplantation (implantation) of xenograft heart valves into humans has been carried out for >50 years. There has been considerable research into making this form of xenotransplantation successful, though it is not perfect yet. We review the understanding of the immune response to xenograft heart valves. Important steps in the history include understanding (i) the importance of glutaraldehyde in decreasing the immune response and (ii) the relationship between calcification (which is the main problem leading to xenograft failure) and the immune response. We subsequently discuss the importance of identifying xenoantigens that are important in leading to xenograft valve failure, and the potential of genetically-engineered pigs to allow the development of the 'ideal' heart valve for clinical valve replacement.

Contents

Abbreviations: Gal, galactose-α1,3-galactose; GBHV, glutaraldehyde-fixed bioprosthetic heart valve; GTKO, α1,3-galactosyltransferase gene-knockout; NeuGc, N-glycolylneuraminic acid.

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1. Introduction

Over 250,000 heart valves are replaced [1] worldwide each year in humans with valvular heart disease. Replacement of a heart valve can be accomplished using mechanical valves, or biological tissue, e.g., an autograft (from the patient's own tissues), a homograft (allograft, from a human cadaver), or a xenograft (from an animal source). A xenograft heart valve offers a number of advantages, and could provide an unlimited number of valves of various sizes. Realizing their potential for human heart valve replacement, a number of pioneer cardiac surgeons/scientists began to explore the possibility of using xenografts in the 1960s.

This review will outline some of the major developments in the use of xenografts for human valve surgery, and discuss future options, concentrating on the immune system's role in xenograft valve failure.

2. The early days – the past

The very first successful xenograft replacement of the aortic valve in a human was performed in September 1965 by Carpentier and his team in Paris [2]. By January 1968, Carpentier's group had implanted 61 porcine valves in 53 patients with a high failure rate. Only 60% of the valves were functioning well at 6 months and only ~45% at 1 year [3]. Although there were some technical/surgical reasons for some of the failures, there were histological features indicating an immune response to the xenograft tissue. Thus, studies were initiated to investigate the immune response to the valves and determine how to decrease this response.

The various studies resulted in a final protocol that eliminated soluble proteins by washing or electrolysis. Mucopolysaccharides and structural glycoproteins were denatured by oxidation using sodium periodate, then neutralized with ethylene glycol. Finally, the valves were placed in a glutaraldehyde-buffered solution which reacted to cross-link with other free amino groups of lysine or other amino acids. This process, especially the glutaraldehyde, greatly reduced the antigenicity of the valves, though the antigenic components (especially the structural glycoproteins) could not be totally eliminated.

This protocol increased the percentage of functioning valves at 1 year to 82% from the previous 45% [3]. At 5 years, the percentage of well-functioning xenograft heart valves was 77% in the mitral position, 89% in the aortic position, and 96% in the tricuspid position [4]. The use of glutaraldehyde led to the commercialization of these valves and many centers began implanting glutaraldehyde-fixed bioprosthetic heart valves (GBHVs) into patients, with a reasonably good success rate. However, with time, surgeons realized that many of these glutaradehyde-fixed valves were beginning to lose their function due to calcification.

3. The problem of calcification – the present

With the passage of time, GBHVs calcified and underwent structural deterioration, with narrowing of the valve orifice and tearing of the cusps, leading to valve leakage [5]. Considerable attention was paid to the chemical process [6] related to the glutaraldehyde crosslinking, and valve companies developed various proprietary methods in attempts to reverse the processes leading to calcification [7].

Minimal calcification was seen in valves implanted into elderly patients, but the incidence of calcification was much higher in younger patients [8]. Currently, in patients >65 years of age, <10% of GBHVs fail within 10 years, but there is a significantly higher rate of valve failure within 5 years in patients <35 years of age [1]. A possible explanation was that young patients had a more robust immune system and that there was an immune response to the GBHV [9]. Immune cells, such as lymphocytes and macrophages, secrete a number of cytokines (e.g., osteopontin), and these calcium-stimulating cytokines might be associated with early GBHV calcification and failure in young patients [10]. There is evidence in other disease states that calcification is associated with inflammation, and thus the same might be true for a GBHV [11–13]. However, there needed to be evidence that there is an immune response to glutaraldehyde-treated tissue, and, second, that this immune response correlated with the development of calcification.

Histological and ultrastructural studies of GBHVs removed from patients showed leukocytes destroying collagen fibers in the valve, with crystalline material present on their surfaces, suggesting it may have been acting as a nidus for calcification [14]. Using enzyme-linked immunosorbent assays and lymphocyte proliferation assays, Dahm et al. [15] showed that glutaraldehyde-treated bovine pericardial valves provoked cellular and humoral immune responses. Vincentelli et al. [16] and Grabenwoger et al. [17] provided evidence that it was not glutaraldehyde that led to the calcification, but it was the origin of the tissue (i.e., xenogeneic but not autologous). Manji et al. [18] carried out a study in a young animal discordant xenotransplant model (to try to mimic the human situation) that clearly established that glutaraldehyde-treated tissue induced both cellular and humoral immune response that could be decreased by immunosuppressive therapy with corticosteroids (Fig. 1). The extent of calcification correlated with the immune cell infiltrate.

4. Decreasing the immune response to GBHVs – the future

With the understanding that there is an immune response to a GBHV, a number of methods were explored in attempts to diminish the immune response and calcification. One approach was to try to identify important xenoantigens expressed on the GBHVs, with the intention that genetically-engineered pigs might provide the 'ideal' heart valve for clinical practice.

It has long been known that the most important antigen that stimulates xenograft rejection of tissues/organs from pigs/cows by non-human primates/humans is the galactose-α1,3-galactose (Gal) antigen (reviewed in Kobayashi 1999 [19]). Anti-Gal antibodies are present in humans, apes and Old World monkeys - anti-Gal IgM comprises 4–8% of total IgM and anti-Gal IgG about 1% of total IgG [19]. To overcome the Gal antigen–antibody immune response, α1,3-galactosyltransferase gene-knockout (GTKO) pigs (which do not express Gal) were produced in 2003 [20]. When transplanted into baboons, hearts from GTKO pigs do not usually undergo hyperacute rejection [21]. Studies were therefore initiated to determine the potential role of Gal antigens in the structural deterioration of GBHVs.

Gal antigens are present on commercially-available GBHVs [22,23], and receipts of these valves mount an immune response to the Gal antigen. Using enzyme-linked immunosorbent assays, Naso et al. [23] quantified the number of Gal epitopes expressed in valves from different companies, and reported that, among 7 different models of GBHVs, only the Epic™ valve completely shielded the Gal epitopes. Bloch et al. [24] documented that patients with a GBHV developed an increase in anti-Gal antibody post-valve implantation. Konakci et al. [25] found that fibrocytes interspersed in the connective tissue of porcine valves expressed Gal epitopes, and that patients receiving porcine GBHVs developed a significant increase in anti-Gal IgM compared to patients with a mechanical heart valve or those undergoing coronary artery bypass grafting. McGregor et al. [26] implanted either wild-type or GTKO pig valves into the mitral position in non-human primates. Over a period of 1 year,