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Chapter 4

Clinical Use of Vancomycin in Cardiovascular Homograft Banking

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Abstract

Antibiotics are routinely used for the decontamination of cardiovascular homografts. This step of bioburden reduction is critical because manipulation during tissue recovery and processing as well as environmental factors may introduce micro-organisms to the homografts. As the consequences of implanting contaminated homografts are potentially life-threatening, stringent measures are taken to eliminate microbial transmission to recipients. This includes the application of aseptic techniques in tissue recovery and processing as well as antibiotic decontamination of homografts prior to long-term storage in liquid nitrogen vapour.

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Usually, to target a diverse spectrum of endemic micro-organisms isolated from tissues, a cocktail of different antibiotics are utilised. In 2011, a collation of heart valve processing practices from 24 international heart valve banks in North America, Europe, Australasia and South Africa revealed that vancomycin is one of the most commonly used antibiotics in cardiovascular homograft banking. 62.5% of the banks included vancomycin of concentration 50-500 μg/mL in their antibiotic cocktail. Antibiotic regimens were validated by individual banks prior to implementation to ensure that the antibiotic combination, incubation temperature and condition yielded optimal bactericidal effect. The test systems used to detect microbial contamination of the homografts were also validated to ensure the results were not compromised by the presence of residual antibiotics. At the author's tissue bank, vancomycin is preferred due to its broad spectrum and stability. This article briefly presents findings on the international banks' bioburden reduction practices and discusses the emerging importance of vancomycin in cardiovascular homograft banking.

Keywords: Heart valve banking; tissue banking; cardiovascular homograft banking; microbial contamination; antibiotic decontamination; vancomycin

Introduction

Effective eradication of microbial contamination of homografts is critical to ensure the provision of safe tissue products for implantation. However, procurement of aseptic homografts can be difficult to achieve. Microbial contamination of the homograft can originate from a number of sources, which include the donor, retrieval environment, or even the personnel conducting tissue recovery and processing procedures [1]. For instance, a significantly higher contamination rate of arterial tissues as compared to heart tissues had been reported, which was ascribed to the higher microbial load found in the abdominal compartment where arteries were recovered [2]. Elevated numbers of culture-positive results in homografts from multi-organ donors had also been described. This could occur especially when heart recovery was initiated after organ retrieval team had perforated the bowel or when the heart had been removed with instruments used to recover other tissues. An increase in incidence of contamination had also been reported for valves recovered in open mortuary areas due to reduced air quality of the mortuary environment [3].

Many homografts are recovered from multi-organ donors under conditions that make aseptic retrieval of the tissue difficult or impossible to achieve. Given the potentially life-threatening consequence of implanting a contaminated graft, stringent measures are taken to minimise microbial transmission to recipients as much as possible [4,5,6]. These include adherence to aseptic techniques during tissue retrieval and processing, prompt procurement of tissues after death, reduction in duration of tissue exposure to the retrieval environment, and maintaining separation of the thoracic cavity from the abdominal cavity where possible [3]. Although aseptic procedures are meticulously applied and sterile instruments and materials are used, contamination of recovered homografts cannot be completely eliminated [1].

To address this potential risk to the recipient, dissected homografts undergo a process of bioburden reduction. Although terminal sterilisation by gamma-irradiation can be used for tissues such as bone allografts, soft-tissues such as heart valves cannot be terminally sterilised. It is well recognised that terminal sterilisation of soft-tissues alters the homograft's biomechanical properties [7], inactivates cells within the graft and affects cell viability [8], significantly reducing graft durability and function *in situ*. For these reasons, the majority of heart valve banks worldwide now include a bioburden reduction step using a cocktail of antibiotics during processing to decontaminate homografts prior to long-term storage and implantation.

Bioburden Reduction of Homografts

Previously, decontamination of cardiovascular homografts was conducted using aggressive sterilisation methods, such as gamma-irradiation or chemical disinfection using formaldehyde, glutaraldehyde, beta-propriolactone and ethylene oxide. Although these techniques increased homograft availability, valve durability was adversely affected due to loss of cellular viability and subsequent structural deterioration of the valves [9]. Due to poor clinical outcomes among recipients of these terminally sterilised valves, their use in transplantation declined and remained low for several years. In 1968, Barratt-Boyes et al. developed high-concentration antibiotic decontamination procedures to disinfect heart valves for banking and transplantation [10,11]. These procedures have been refined over the years, with incubation in low-concentration antibiotic solution becoming the most common bioburden reduction method used for disinfecting cardiovascular homografts. Tissues processed using this method were found to maintain their structure,

biomechanical properties and cell viability, resulting in improved valve durability and function *in situ.* This outcome led to a revival in the clinical use of homografts for transplantation [3,4,12,13].

Despite the widespread use of antibiotics and its significant impact on successful decontamination of homografts, standardisation of the bioburden reduction step has not been achieved [2,10,13,14,15]. Indeed, a recent survey of 24 heart valve banks identified extensive differences in the composition and concentrations of antibiotics used and in the incubation conditions (durations and temperatures) applied to achieve bioburden reduction [15]. Lack of standardisation of this step is not surprising for a number of reasons. Firstly, banks in different parts of the world contend with different endemic micro-organisms of various antibiotic sensitivities. In addition, some banks have patented the bioburden procedures for their purposes, while others have established protocols that meet their requirements based on experience and outcomes. In all cases, the procedures used had been validated and met the banks' final product requirements.

Emerging Importance of Vancomycin in Tissue Banking

There is a general trend of increasing antibiotic resistance among micro-organisms. For this reason, it is imperative to understand antimicrobial susceptibility patterns of micro-organisms commonly isolated from homografts when choosing an antibiotic combination for the bioburden reduction step. It is well established that the types of microbial contaminants vary with geographical location, and that selection pressures exerted by antibiotic use alters antimicrobial susceptibility patterns. Even so, it has been reported that the susceptibility patterns displayed by micro-organisms isolated from homografts are similar to those found in other clinical circumstances within the same geographical region [6]. This finding provides a rational basis for developing an antibiotic cocktail that will be effective in decontaminating homografts retrieved in the same region.

Vancomycin is a unique glycopeptide structurally unrelated to any currently available antibiotic. Its primary mode of action is achieved by inhibiting bacterial cell wall synthesis of susceptible micro-organisms.

Table 1. Antibiotic regimens of heart valve banks which use vancomycin as one of the antibiotics for decontamination of cardiovascular homografts

Bank	Concentration of vancomycin used	Other antibiotics used	Incubation conditions
Europe: a total of 11 banks surveyed			
E1	500 µg/mL	Gentamicin: 50 µg/mL Piperacillin: 500 µg/mL Nystatin: 2500 U/mL	Room temperature (21°C), 24 hours in the dark
E2	50 µg/mL	Cefoxitin: 240 µg/mL Lincomycin: 120 µg/mL Colimycin: 100 µg/mL	4°C, 24 hours
E3	50 µg/mL	Gentamicin: 4000 µg/mL Imipenem: 200 µg/mL Nystatin: 2500 U/mL Polymixin B: 200 µg/mL	2 - 8°C, 18 - 24 hours
E4	50 µg/mL	Gentamicin: 4000 µg/mL Ciprofloxacin: 200 µg/mL Amphotericin B: 50 µg/mL	Room temperature (21ºC), 24 hours
E5	50 µg/mL	Metronidazol: 50 µg/mL Amikacin: 50 µg/mL Amphotericin B: 5 µg/mL	4ºC, 24 hours
E6	50 µg/mL	Tobramycin: 50 µg/mL Cotrimoxazole: 50 µg/mL	4ºC, 6 - 24 hours
E7	Information not provided	Lincomycin, Polymyxin B sulphate	4°C, 48 hours
E8	500 µg/mL	Amphotericin B: 250 µg/mL Fungoral: 100 µg/mL Colistin: 200 µg/mL Gentamicin: 530 µg/mL	4 - 8°C, 24 hours

Table 1. (Continued)

Bank	Concentration of vancomycin used	Other antibiotics used	Incubation conditions
E9	500 μg/mL	Cefuroxime: 250 μg/mL Gentamicin: 80 μg/mL Ciproflaxacin: 200 μg/mL Colistin: 1000 IU/mL Amphotericin B: 20 μg/mL	37°C, 18 - 24 hours
North America: a total of 6 banks surveyed			
N1	50 μg/mL	Gentamicin: 80 μg/mL Cefoxitin: 240 μg/mL	33 - 38°C, 18 - 26 hours
N2	Information not provided	Cefoxitin, Colymycim-M, Lincomycin	4°C, 24 hours
N3	50 μg/mL	Cefoxitin: 240 μg/mL Polymyxin B: 100 mg/mL Lincomycin: 120 μg/mL	1 - 10°C, 22 - 26 hours
N4	50 μg/mL	Colymycin M: 75 mg/mL Cefoxitin: 100mg/mL Lincomycin: 300 mg/mL	4°C, 24 ± 2 hours
Australasia and South Africa: a total of 7 banks surveyed			
A1	50 μg/mL	Cefoxitin: 240 μg/mL Lincomycin: 120 μg/mL Polymyxin B: 100 μg/mL Amphotericin B: 25 μg/mL	First soak: 4ºC, 24 hours; 2nd soak: 4ºC, 24 hours; Transfer to Hank's Balanced Salt Solution at 4ºC until frozen
A2	50 μg/mL	Amikacin: 100 μg/mL	4°C, 24 - 28 hours

Studies have shown that vancomycin can also alter the permeability of cell membrane and may selectively inhibit ribonucleic acid synthesis [16].

Vancomycin is known to be active against a large number of gram-positive aerobes and anaerobes, such as *Staphylococcus aureus* (including methicillin-resistant strains), *Staphylococcus epidermidis* (including multiple-resistant strains), viridan streptococci, enterococci, and clostridia, amongst others [16,17]. However, it exhibits no significant activity against most gram-negative bacteria, such as *Pseudomonas* species and *Esherichia coli*, which are commonly found in homografts worldwide [17]. Therefore, to cover a wider microbial spectrum, vancomycin must be combined with other antibiotics to effectively reduce microbial load of the homografts.

In 2011, a survey was conducted by co-author Linda Manning to compile information on heart valve processing techniques, including antibiotic regimens, utilised by 24 international heart valve banks from Europe, North America, Australasia and South Africa [15]. As presented in Table 1, results revealed that 62.5% of the banks used vancomycin as one of the antibiotics to decontaminate their homografts. Among the banks that used vancomycin, 60% of them added an aminoglycoside, such as gentamicin, amikacin or tobramycin to the antibiotic mix. Aminoglycosides are preferred because they are bactericidal and active *in-vitro* against a wide spectrum of aerobic and facultative gram-negative bacilli [18]. In addition, the bactericidal activity of vancomycin in combination with an aminoglycoside was found to be synergistic, especially against *Staphylococcus aureus*, enterococci and viridan streptococci [16]. Currently, gentamicin is the aminoglycoside of choice by 66.7% of the banks surveyed using this vancomycin-aminoglycoside combination. This is probably because the combination of vancomycin and gentamicin reportedly yields the most predictable synergistic activity against most sensitive strains of enterococci, viridan streptococci, methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, as well as against one-third to one-half of *Staphylococcus epidermidis* strains [17].

In addition to its effectiveness against a broad spectrum of micro-organisms, vancomycin is known to be stable at 4^{o}C and 37^{o}C, which are the two temperatures most commonly used for bioburden reduction step. Its potency remains unaffected after 6 hours of exposure at 37^{o}C and 24 hours at 4^{o}C [13]. One bank had validated vancomycin stability for up to 48 hours at 4^{o}C. 73.3% of the banks conducted bioburden reduction in the cold (1-10^{o}C), with the majority incubating at 4^{o}C for 18-24 hours. Only 13.3% of the banks performed bioburden reduction step at room temperature (21^{o}C) and the other 13.3% conducted this step at physiological temperatures (33-38^{o}C). Regardless

of the antibiotic combinations or incubation conditions used, in each case, the procedure had been validated to meet the banks' final product requirements. For these reasons, many banks are hesitant to change their protocols, especially as procedural changes would require additional validation, which can be both costly and time-consuming.

Although the current antibiotic regimens utilised by international heart valve banks is referred to as 'low dose', the concentrations of antibiotics used exceeds the minimum inhibitory concentration (MIC) of most micro-organisms [4,19]. For instance, the MIC of vancomycin is approximately 0.5-16 μg/mL against *Staphylococcus aureus* [6,17,20], 4 μg/mL against Group B streptococci (*Streptococcus agalactiae*) [17], 1 μg/mL against *Enterococcus facecalis* [6], and 0.25 μg/mL against *Bacillus subtilis* [20]. From the survey, it was revealed that 76.9% of the banks decontaminated homografts with 50 μg/mL vancomycin and 23.1% used 500 μg/mL, both of which significantly exceeds the MIC of these susceptible pathogens.

Benefits and Concerns of Residual Antibiotics

It is known that antibiotic-decontaminated homografts show increased resistance to infection [21]. There are diverse opinions as to the actual cause of this phenomenon. However, the most widely accepted view is that residual antibiotics present in the implanted homograft act prophylactically in the recipient [3,4,19]. This is particularly important when homograft replacement is used as a treatment for infective endocarditis or infection of bioprosthetic or mechanical valves [19].

Despite the potential benefits, there are concerns with regards to the presence of antibiotic residues. Firstly, there are concerns that the presence of residual antibiotics may cause an allergic or hypersensitive reaction in the recipient. Skin rashes and medication-associated fever have been reported in 1-8% of patients receiving vancomycin [17]. However, given the concentration of vancomycin used in homograft decontamination is very low as compared to therapeutic dosage, the risk to recipients of vancomycin-treated homografts is probably minimal [17,19]. In addition, vancomycin only comes into temporary contact with homografts, and most of it is removed during processing and thawing. Tissue banks accredited by the American Association of Tissue Banks (AATB) practise the rinsing of homografts in two

phases - (1) after incubation in antibiotics prior to storage, and (2) after thawing of homograft prior to implantation into the recipient. Studies had demonstrated that antibiotics, such as vancomycin, are diluted to a negligible level (≤0.05%) after rinsing the homograft prior to implantation [19]. Routine follow-ups on 51 recipients from the authors' tissue bank had found no cases of allergic reactions as a result of residual antibiotics or vancomycin.

Another concern is that the presence of antibiotics on post-antibiotic incubation tissue and solution samples has the potential to compromise the reliability of microbiological test methods performed to validate the tissue decontamination process [4,13,22,23]. To counteract this potential bacteriostatic/ fungastatic effect (BF effect) and ensure validity of test results, the detection system employed must either (1) contain an effective neutralising agent (for instance, blood culture bottles), and/or (2) allow sufficient rinsing with a suitable rinsing solution (for instance, in membrane filtration) to effectively remove inhibitory substances.

Microbial contamination detection systems using defined nutrient broths have shown to be effective in detecting low-level contamination in tissue and solution samples collected at various stages during homograft processing. For example, the BacTec bottle system had been validated against a range of micro-organisms listed in the British Pharmacopoeia Appendix XVI E. Microbiological Control of Cellular Products [24] and it was found to be suitable for the detection of low-level microbial contamination (<10 colony-forming units) in homografts and rinse solutions in terms of specificity and sensitivity. This type of test method contains a neutralising agent that effectively eliminates the masking of microbial contamination by residual antibiotics. The disadvantage of this type of detection system is that only a limited volume (~10 ml) of sample can be tested per bottle.

It was recommended that to counteract the BF effect and ensure a valid sterility test, 50-2000ml of solution per tissue should be tested, depending on tissue size [22]. To achieve this, the membrane filtration method is capable of testing a larger volume of solution. The filter disc can also be placed onto nutrient plates to quantitate microbial contamination levels on the tissue if required. For these reasons, membrane filtration is considered by many banks to be the gold standard for microbiological assessment of solutions [1]. In this method, an appropriate amount of test solution (10% or more of the total volume) is flushed through a size exclusion membrane capable of retaining micro-organisms. It is then transferred into a nutrient broth or placed onto a nutrient plate. The United States Pharmacopeia <71> guideline states that the filter disc's pore size should be no greater than 0.45-micron for effective

microbial retention [25]. The benefits of this technique are (1) a large volume of solution can be tested, (2) should the solution contain substances that cause BF effect, such as antibiotics, rinsing the filter disc with a suitable rinse agent can eliminate most, if not all, of these inhibitory substances, and (3) bioburden levels can be quantitated, if required. However, there were few drawbacks to this method too. Firstly, it is more time-consuming than the blood bottle test system. Secondly, it is more expensive [1]. However, due to the importance of microbial contamination detection in ensuring the safety of clinical homografts, and the perception that filtration is a more robust test method than direct inoculation into culture broths, many tissue banks are adopting this method to detect microbiological contamination in homograft samples.

Conclusion

Evaluation of microbiological culture results is critical in tissue banking. The discard of only the culture-positive tissue or all tissues retrieved from the same donor is dependent on several factors. These include the species of micro-organism identified, the diversity of micro-organisms detected, the number of pre-processed and post-processed tissue and solution samples contaminated, and microbial contamination results of other tissues recovered from the same donor. All the banks have a list of "exclusion micro-organisms", consisting of highly virulent and/or spore-forming microbes [26], the presence of which in any test sample will result in tissue discard.

One of the most critical factors in processing soft-tissue products for implantation is utilising a bioburden reduction step that effectively eliminates microbial contamination on the homograft [26]. For cardiovascular homografts, incubation in a low-concentration antibiotics solution is now the most common bioburden reduction method used for decontamination. Given the importance of this step in ensuring product safety, it is essential that the procedure is validated and that the antibiotic cocktail used is effective against the microflora commonly isolated from homografts. As vancomycin has a broad antimicrobial spectrum of activity, especially in combination with other antibiotics such as the aminoglycosides, and is stable over a wide range of temperatures, an increasing number of heart valve banks are including vancomycin for this critical step of bioburden reduction. This would lead to an increase in the availability of safe, contaminant-free homografts for transplantation.

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