Resistance to infection of long-term cryopreserved human aortic valve allografts

Viola Steffen, MD,[a,b] Georg Marsch, MD,[a] Karin Burgwitz,[a,b] Christian Kuehn, MD, PhD,[a,b] and Omke E. Teebken, MD, PhD[a]

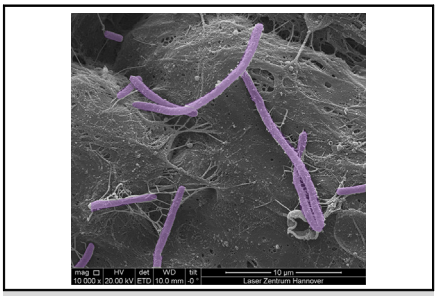
ABSTRACT

Objective: To analyze the in vitro antimicrobial activity of 3 antibiotic regimens (group A, gentamicin-piperacillin-vancomycin-metronidazole-amphotericin B; group B, gentamicin-piperacillin-flucloxacillin-metronidazole-amphotericin B; and group C, meropenem-vancomycin-tobramycin-colistin-amphotericin B) used in the processing of cryopreserved human ascending aortic tissue and aortic valves against *Staphylococcus epidermidis* and *Staphylococcus aureus.* The results were additionally compared with the infection resistance of cryopreserved ascending aortic tissue against *Escherichia coli* and *Pseudomonas aeruginosa*.

Materials: Each of 10 cryopreserved human allografts (CHAs) was divided into 25 pieces (separating aortic wall and valve). Eighteen segments were microbiologically tested, and 7 pieces underwent scanning electron microscopy. A bacterial solution (4 mL; optical density, 0.20 ± 0.02) was used for contamination. After incubation, the optical density of the solution was measured. CHAs underwent sonication to release viable adherent bacteria. The number of attached bacteria was quantified by the colony forming units per square centimeter of CHA surface.

Results: Antibiotic regimen groups B and C were more efficient than group A in eradicating gram-positive organisms adherent to the aortic wall ($P < .001$). Group C showed enhanced resistance against *E coli* compared with group A or B ($P < .001$), whereas group B appeared to be more effective against *P aeruginosa* ($P < .001$). With reference to each antibiotic regimen, ascending aortic tissue showed significantly less bacterial contamination with staphylococcal bacteria than valve grafts ($P \leq .01$).

Conclusions: CHAs possess antibacterial activity despite long-term storage over 5 years. Antibiotic combinations applied during CHA processing have a significant influence on their infection resistance. Ascending aortic tissue shows a significantly enhanced bacterial resistance against staphylococcal bacteria compared with aortic valves. (J Thorac Cardiovasc Surg 2016;151:1251-9)



Elongated *Escherichia coli* on cryopreserved aortic wall allograft.

Central Message

The infection resistance of CHAs depends on antibiotic pretreatment and is superior in the aortic wall compared with valve tissue.

Perspective

Cryopreserved human allografts (CHAs) can be recommended to patients with destructive infections or high risk of (re)infection. Our in vitro study offers novel insight into the principles of antibacterial activity of CHAs. Enhanced retention of antibiotic agents may improve the infection resistance of CHAs. The risk of reinfection can be reduced by adapting the CHA decontamination protocol to the spectrum of infection-causing bacteria.

See Article page 1239.

See Editorial Commentary page 1249.

See Editorial page 1230.

Prosthetic graft infections are responsible for major morbidity and drastically increased mortality rates (20%-80%) and therefore remain among the most challenging complications in cardiothoracic surgery.[1-3]

The incidence of prosthetic valve endocarditis ranges from 0.3% to 1.2% per patient year, with a cumulative risk of 5% at 10 years.[1,2] Furthermore, 0.9% to 1.9% of patients develop a prosthetic graft infection following surgery on the thoracic aorta.[3]

Most graft infections are caused by gram-positive bacteria, namely *Staphylococcus aureus* and *Staphylococcus epidermidis*.[2,4,5] The increased incidence of highly

From the [a]Department of Cardiothoracic, Transplantation, and Vascular Surgery, and [b]CrossBIT Research Center for Biocompatibility and Immunology of Medical Implants, Hannover Medical School, Hannover, Germany.

Funding sources: Internal funds.

Received for publication July 12, 2015; revisions received Oct 18, 2015; accepted for publication Nov 18, 2015; available ahead of print Dec 23, 2015.

Address for reprints: Omke E. Teebken, MD, PhD, Department of Cardiothoracic, Transplantation, and Vascular Surgery, Hannover Medical School, Carl-Neuberg-Str 1, OE 6210, 30625 Hannover, Germany (E-mail: Teebken.Omke@MH-Hannover.de).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2015.11.029

Scanning this QR code will take you to the article title page.

Abbreviations and Acronyms

CHA = cryopreserved human allograft
OD_{600nm} = optical density
PBS = phosphate-buffered saline
SEM = scanning electron microscopy

virulent and antibiotic-resistant microorganisms such as *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) additionally hinders a successful eradication of infection, and consequently, investigations into this problem are currently ongoing.[2,6]

Various therapies for prosthetic graft infections involving the aortic valve and the ascending aorta have been described; for example, adaptive antibiotic treatment, antiseptic irrigation, and perhaps most importantly, surgical debridement.[2,7] Because extra-anatomical rerouting is not possible at the site of the aortic root and the arch, surgical options involve either salvaging the existing graft by aggressive debridement, irrigation, and coverage with healthy tissue[8] or graft removal and renewed in situ replacement with synthetic grafts or biological tissue; for example, cryopreserved human allografts (CHAs).[7,9-12]

CHAs represent effective alternatives for the treatment of infections, with extremely low reinfection rates.[13] Native and prosthetic valve endocarditis,[12,14,15] as well as vascular prosthetic infections,[6] show promising outcomes after CHA replacement. Furthermore, CHAs appear to be effective even in complex destructive infections involving the aortic root or ascending aorta.[5,16] CHAs are not recommended for routine aortic valve or ascending aortic surgery, but can be considered for patients with destructive infections or high risk of (re)infection.[17]

The reason why CHAs exhibit this clinically observable bacterial resistance remains unclear, but may be related to their viability, which allows an improved transfer of antibiotic agents and immunocompetent cells through the wall into the perigraft space.[18] Moreover, their antimicrobial activity might be attributed to processing and, in particular, to the storage of CHAs in antibiotics.[19] However, this topic remains poorly investigated to date.

The aim of this in vitro study was to evaluate the antimicrobial activity of CHAs, taking a closer look at 3 clinically approved antibiotic regimens used in the processing of human ascending aortic tissue and aortic valves with respect to *S epidermidis* and *S aureus*, as gram-positive pathogens. Additionally, we compared these data with the infection resistance of cryopreserved ascending aortic tissue against gram-negative pathogens; for example, *E coli* and *P aeruginosa.* We hypothesized that the choice of antibiotics has significant influence on infection resistance, and that valve and aortic wall tissue behave in a different manner.

MATERIALS AND METHODS

Bacterial Strains

The strains were 20044DSMZ for *S epidermidis*, 20231DSMZ for *S aureus*, 1103DSMZ for *E coli,* and 0411208MDPA01 for *P aeruginosa*. Isolates were subcultured overnight in tryptic soy broth (Oxoid, Hampshire, United Kingdom). The bacterial concentration measured by optical density (OD_{600nm}), as used for graft contamination, amounted to 0.20 ± 0.02. This OD_{600nm} corresponded to 6×10^7 CFU/mL for *S epidermidis*, 8×10^7 CFU/mL for *S aureus*, 3×10^6 CFU/mL for *E coli,* and 3×10^6 CFU/mL for *P aeruginosa*.

CHAs

Ten aortic valve allografts, including a segment of the ascending aorta, were obtained from the Deutsche Gesellschaft für Gewebetransplantation or its predecessor DSO-G. These allografts were rejected for transplantation because their storage time had exceeded 5 years. CHAs were subjected to the standard procedure of processing, consisting of harvesting from multiorgan donors, preparation, antibiotic treatment, cryopreservation, and thawing as described previously.[6] The CHAs were assigned to three groups according to their antibiotic treatment (Table 1): group A (gentamicin, piperacillin, vancomycin, metronidazole, and amphotericin B), group B (gentamicin, piperacillin, flucloxacillin, metronidazole, and amphotericin B), and group C (meropenem, vancomycin, tobramycin, colistin, and amphotericin B).

Experimental Protocol

Each CHA was divided into 25 pieces under sterile conditions as illustrated in Figure 1. The valves were dissected into 7 samples (0.75 cm^2 or approximately 0.4 cm^2 for controls). The wall specimens (1 cm^2) were contaminated with both gram-positive and gram-negative microorganisms, whereas the cusps were incubated with the bacteria most frequently responsible for prosthetic valve endocarditis: *S epidermidis* and *S aureus*.[4] In detail, the piece of CHA (aortic wall or cusp) was placed into 4 mL subcultured bacterial suspension (OD_{600nm}, 0.20 ± 0.02) and incubated at 37°C and 5% carbon dioxide for 24 hours ($n = 21$). Two specimens of the aortic wall and 2 of the valve were incubated in sterile tryptic soy broth and served as controls.

Microbiologic tests. Following incubation, all grafts were washed 3 times in 50 mL phosphate-buffered saline (PBS) (Dulbecco; Biochrom, Berlin, Germany) to eliminate unattached bacteria. The OD_{600nm} of the bacterial suspension was measured to evaluate bacterial growth in the surroundings of the specimens. The samples were transferred into sterile Falcon tubes containing 5 mL PBS, and the remaining viable adherent bacteria were released by sonication at low power (37°C, 100%) for 20 minutes.[20] 100 μL of this PBS-solution containing the dislodged bacteria was plated onto tryptic soy broth-agar plates. All plates were incubated (37°C; 5% carbon dioxide), and colonies were visually identified and counted with a Molecular Imager (Gel-Doc XR; Bio-Rad Laboratories, Munich, Germany). The results were used to determine the number of attached bacteria per square centimeter of CHA surface.

Scanning electron microscopy. After incubation, each sample was washed three times in PBS to remove unattached microorganisms. Samples were subsequently fixed in a 2.5% glutaraldehyde and sodium-cacodylate buffer (0.1 M; pH 7.3), after which they were passed through an increasing concentration of acetone before dehydration in a critical point dryer (CPD-030; Bal-Tec GmbH, Balzers, Liechtenstein), sputtered with gold, and observed by scanning electron microscopy (SEM) (Quanta-400F; FEI Company, Hillsboro, Ore), at 20 kV and a resolution of 1 nm.

Statistical Analysis

All data are expressed as mean ± standard error of the mean. The Mann-Whitney test was chosen to compare the different groups. SPSS version 21.0 (IBM-SPSS Inc, Armonk, NY) and GraphPad Prism version