International Journal of Cardiology 198 (2015) 201–205

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Selective propensity of bovine jugular vein material to bacterial adhesions: An in-vitro study☆

Zakaria Jalal [a], Louise Galmiche [b], David Lebeaux [c,d], Olivier Villemain [a], Georgia Brugada [a], Mehul Patel [a], Jean-Marc Ghigo [b], Christophe Beloin [b,1], Younes Boudjemline [a,d,1,*]

[a] Centre de Référence Malformations Cardiaques Congénitales Complexes — M3C, Necker Hospital for Sick Children, Assistance Publique des Hôpitaux de Paris, Pediatric Cardiology, Paris, France
[b] Institut Pasteur, Unité de Génétique des Biofilms, Département de Microbiologie, Paris, France
[c] Hôpital Necker Enfants Malades, Laboratoire d'Anatomopathologie, Paris, France
[d] Université Paris Descartes, Sorbonne Paris Cité, Paris, France

ARTICLE INFO

Article history:
Received 12 May 2015
Accepted 1 July 2015
Available online 6 July 2015

Keywords:
Endocarditis
Melody valve
Fallot
Transcatheter pulmonary valve

ABSTRACT

Background: Percutaneous pulmonary valve implantation (PPVI) using Melody valve made of bovine jugular vein is safe and effective. However, infective endocarditis has been reported for unclear reasons. We sought to assess the impact of valvular substrates on selective bacterial adhesion.
Methods: Three valved stents (Melody valve, homemade stents with bovine and porcine pericardium) were tested in-vitro for bacterial adhesion using *Staphylococcus aureus* and *Streptococcus sanguinis* strains.
Results: Bacterial adhesion was higher on bovine jugular venous wall for *S. aureus* and on Melody valvular leaflets for *S. sanguinis* in control groups and significantly increased in traumatized Melody valvular leaflets with both bacteria (traumatized vs non traumatized: $p = 0.05$). Bacterial adhesion was lower on bovine pericardial leaflets.
Conclusion: Selective adhesion of *S. aureus* and *S. sanguinis* pathogenic strains to Melody valve tissue was noted on healthy tissue and increased after implantation procedural steps.

1. Introduction

Since the first reported case in 2000, percutaneous pulmonary valve implantation (PPVI) using Melody® valve (Medtronic, Inc., Minneapolis, MN, USA), has provided an effective alternative to surgical pulmonary valve replacement in selected patients with right ventricular outflow tract dysfunction [1]. Several cases of infective endocarditis (IE) have been described [2]. McElhinney et al. reported an annualized rate of IE at 2.4% per patient-year [3]. Interestingly reports about IE involving surgically placed Contegra® conduits (Medtronic, Inc., Minneapolis, MN, USA) made with bovine jugular vein (BJV) as the Melody® valves are scarce [4]. Albanesi et al. reported that Contegra® infection affected 11.3% of cases after a median time of 4.4 years. Our team reported recently that IE was more frequent after PPVI than surgical pulmonary valve replacement. IE was more frequent in patients with BJV valves, whether surgically or percutaneously implanted compared with other valvular substrates suggesting a potential propensity of endocarditis [5]. This was recently confirmed by Van Dijck et al. who showed that the Melody valve and the Contegra conduit have a significantly higher incidence of IE than homografts [6]. Cases of IE are also published with Edwards Pulmonic Sapiens® (Edwards Lifesciences, Irvine, CA) valve made with bovine pericardium and Corevalve® (Medtronic, Inc., Minneapolis, MN, USA) made with porcine pericardium [7]. One of the main differences between surgery and PPVI is that percutaneous valves undergo several traumatic manipulations before, during crimping and during implantation whereas surgical prostheses are placed with minimal handling. Traumatic injury to biological valve leaflets has been reported during valved stent preparation [8,9]. In this in-vitro study we aimed to evaluate the impact of PPVI procedural steps on bacterial adhesion of common pathogenic gram positive bacteria to the Melody® valve substrate, and compare these results with other tissues used for valved stent fabrication (i.e. bovine and porcine pericardium).

2. Methods

2.1. Valvular substrates

Experimental study was performed using 3 types of valved stents:

1) The Melody® valve was obtained from Medtronic and stored in its commercial packaging (glutaraldehyde-based solution).

☆ Acknowledgment for grant support: Z.J. was supported by a grant from the Fédération Française de Cardiologie. C.B. and J-M.G. are supported by the Fondation pour la "Recherche Médicale" grant (Equipe FRM DEQ20140329508) and the French Government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant no. ANR-10-LABX-62-IBEID). D. L. was supported by a grant from the AXA Research Fund.
* Corresponding author at: Hôpital Necker Enfants Malades, Cardiologie Pédiatrique, 149 rue de Sèvres, 75015 Paris Cedex, France.
E-mail address: younes.boudjemline@nck.aphp.fr (Y. Boudjemline).
[1] CB and YB contributed equally to this work.

http://dx.doi.org/10.1016/j.ijcard.2015.07.004
0167-5273/

2) Bovine pericardium: valvular leaflets were sampled from a bovine pericardial patch (10 × 15 cm — Edwards Lifesciences, Irvine, USA), cut into a 21-mm homemade 3 leaflet valvular mold and sutured onto inner side of a platinum and iridium stent (CP8Z34, Numed Inc., Canada). Valved stents were stored in 0.625% glutaraldehyde until use.
3) Porcine pericardium: valvular leaflets were sampled from a porcine pericardial patch (8 × 6 cm — Vascutek Terumo Ltd., Swillington, Leeds). Porcine pericardial valved stents were then prepared and stored in 0.625% glutaraldehyde until use.

2.2. In-vitro manipulations

For each valved stent, we compared 2 experimental conditions reproducing the procedural steps leading to a conventional PPVI (Fig. 1). Before manipulation, valved stents were rinsed twice for 2 min each in two 500-ml saline baths to remove glutaraldehyde.

2.2.1. Condition I: control group

Valved stents were not manipulated (non-traumatized group, Fig. 1A).

2.2.2. Final condition

Valved stents were manually crimped on sterile syringes (5 and 2.5-ml) and then onto 22-mm balloon of the 22-French Ensemble® delivery system. The sheath was advanced to cover the balloon-mounted valved stent during 5 min. This duration was chosen arbitrarily and aims to reproduce the crimping duration during a conventional PPVI. The compressed prostheses were regularly flushed with a saline solution. The sheath was then drawn back and valved stents were deployed in a 20-mm Goretex conduit by inflation of the inner and outer balloons of the delivery system. The balloons were then deflated and the delivery system removed. After valve deployment, a post-dilatation using a 22-mm high-pressure balloon (Atlas Gold, Bard Peripheral Vascular, Inc., Tempe, AZ, USA) inflated at 20 atm for 5 s was performed.

For each Melody® valved stent in each condition, 3 samples of the BJV wall adjacent to the leaflets within sinuses were taken using an 8-mm diameter (i.e. 0.5 cm^2) trepan for bacteriological tests. After sampling, valvular leaflets (and BJV wall fragments for Melody® valved stent) were stored in 0.625% glutaraldehyde until bacteriological processing (within 24 h).

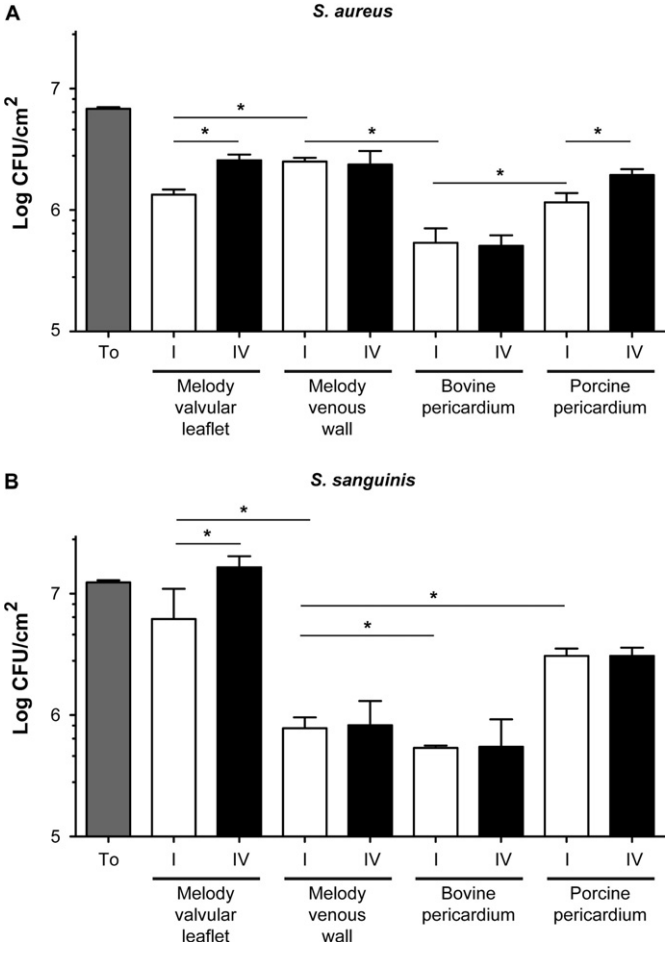

Fig. 1. Adhesion of *Staphylococcus aureus* (A) and *Streptococcus sanguinis* (B) in control group (I) and after manipulation (IV) according to substrate type. To corresponds to the initial inocula that were incubated during 1 h in absence of tissues. CFU = colony forming units, mean and standard deviation are shown; $^*p < 0.05$.

2.3. Bacterial adhesion

2.3.1. Materials

We used relevant clinical strains of *S. aureus* and *S. sanguinis* isolated from blood cultures of 2 patients who were diagnosed with infective endocarditis involving a Melody® valve.

2.3.2. Preparation

Samples were rinsed twice for 2 min each in 500-mL saline baths to remove glutaraldehyde before bacterial adhesion tests. Bacteria were cultured in Trypticase™ Soy Broth + 0.25% glucose (*S. aureus*) or Brain–Heart Infusion (*S. sanguinis*) at 37 °C + 5% CO_2 and diluted in Phosphate Buffered Saline solution to create bacterial suspensions of 7.10^6 colony forming units/mL (CFU/mL) of *S. aureus* and 1.2×10^7 CFU/mL of *S. sanguinis* (CFU)/mL. We chose these inocula after preliminary experiments in order to obtain adhesion levels low enough to observe potential increased adhesion without saturation of the tissue but higher than the limit of detection. Adhesion protocol was inspired from previous studies on *Streptococci* [10]. Two milliliters of the bacterial suspension were dropped on sterile valvular samples (8-mm diameter, 0.5-cm^2 surface) previously transferred in 6 well plates and incubated for 60-min at 37 °C + 5% CO_2. Unbound bacteria were removed by Phosphate Buffered Saline washing.

2.3.3. Analysis

We compared adhesion between non-traumatized control and traumatized tissue. For each bacterium, 2 samples of each control or traumatized tissue were used for scanning electron microscopy to assess location of bacterial adhesion. Other samples to measure bacterial adhesion were transferred into tubes containing 1-mL of Phosphate Buffered Saline, enzymatically treated by 25-μg/mL of collagenase type-II (Sigma Aldrich Co. St. Louis, USA) during 15-min at 37 °C and bacteria were released by mild sonication and mechanical grinding of the samples. The resulting suspensions were serially diluted with Phosphate Buffered Saline and cultured at 37 °C + 5% CO_2 for 24–48 h. The CFUs were counted to determine the number of viable adherent bacteria, and the bacterial density (CFU/mL and CFU/cm^2) was calculated. Non-traumatized supplementary sample was incubated with a sterile solution and served as sterility control for each test.

2.3.4. Scanning electron microscopy

Samples were fixed in a 0.6% glutaraldehyde, cacodylate buffer and ruthenium red and processed for scanning electron microscopy.

2.4. Statistical analysis

Results were expressed as mean (standard deviation) or median (range) for continuous variables or as a number (percent) for categorical variables. The data from these experiments were analyzed with nonparametric Mann–Whitney or Kruskal–Wallis tests. The value of statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Bacterial adhesion

3.1.1. Quantitative analysis of adhesion

We first analyzed bacterial adhesion quantitatively (Fig. 1). In control groups (non-traumatized), bacterial adhesion was significantly different regarding tissular substrate and bacterial strain. *S. aureus* adhered the highest on bovine Melody® venous wall and *S. sanguinis* on Melody® valvular leaflets. The lowest adhesion was obtained on bovine pericardial leaflets for both bacteria. Bacterial adhesion increases moderately but significantly in traumatized-group compared with control group for Melody® valvular leaflets with both bacteria (non-traumatized vs traumatized groups: $p = 0.05$ for *S. aureus* and *S. sanguinis*). Moreover, adhesion significantly increased between two conditions for porcine pericardial leaflets with *S. aureus* ($p = 0.046$).

3.1.2. Scanning electron microscopy

The observations made by scanning electron microscopy on valvular leaflets or bovine jugular venous wall, traumatized or not, and contaminated by *S. aureus* or *S. sanguinis*, are presented in Figs. 2 and 3. Qualitatively profiles of adhesion were identical on the different types of tissue. In control groups, microscopic observations revealed that, regardless of the studied substrate, bacteria adhered over the entire sample surface with patchy distribution. In traumatized samples, although we observed adhesion of bacteria within microcavities and defects,