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Daptomycin is more Bactericidal than Vancomycin and Vancomycin/Rifampin Combination in a Murine Model of Methicillin-Resistant *Staphylococcus aureus* Vascular Material Infection

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Abstract

Objective. Infection of vascular prosthesis is an emerging disease, mostly due to staphylococci, with limited data regarding efficacy of current antistaphylococcal agents. We aimed to assess the efficacy of Vancomycin and Daptomycin, with or without Rifampin in a murine model of meticillin-resistant *Staphylococcus aureus* (MRSA) vascular material infection. **Methods.** Squares of 1 cm^2 sterilized Dacron were incubated with murine serum at 37°C for 24 hours, and then implanted subcutaneously (SC) in the back of female Swiss mice. Forty-eight hours later the material was infected by percutaneous injection of 10^7 CFU MRSA suspension. Seventy-two hours later, mice were randomly assigned to 6 groups of 10 mice each: no treatment (C), Vancomycin 110 mg/kg bid SC (V), Daptomycin 50 mg/kg od SC (D), Rifampin 30 mg/kg bid intraperitoneally (R), Vancomycin + Rifampin (VR), and Daptomycin + Rifampin (DR). Forty-eight hours later, mice were euthanized and materials were explanted to determine the surviving bacterial count. Spleen from each mouse were removed and used for quantitative cultures. Mice with positive bacterial culture of spleen were considered to be bacteremic. **Results.** Control group showed stable infection during experimentation and 60% of animals were bacteriemic. Vancomycin demonstrated efficacy with significant decrease of bacterial count as compared to the Control group (- 1.9 log_{10}, P<0.05). Only 20% mice of this group were bacteriemic. Daptomycin was significantly more bactericidal than Vancomycin, with all the prosthesis sterilized after 48 hours of treatment and no bacteremic mouse (P<0.0001 vs Control and Vancomycin groups). The bactericidal activity of Vancomycin was significantly improved when combined with Rifampin (P<0.01), although the combination remained less bactericidal than Daptomycin.
Conclusion: In this murine model of MRSA vascular material infection, Daptomycin was superior to Vancomycin, whether or not combined with Rifampin.

Introduction

More than 400 000 vascular grafts are inserted annually in the United States. Graft insertion is complicated by infection in **0.5 - 4%** of cases[1]. **Vascular graft infections (VGI)** are associated with considerable mortality ranging from 10 to 25% within thirty days following the diagnosis. ***Staphylococcus aureus*** is the most commonly causative organism and methicillin resistant *S. aureus* (**MRSA**) account for almost 50% of *S. aureus* VGI[2].
Treatment of VGI is based on urgent surgical removal of the infected graft followed by prolonged antibiotherapy. Data on the best antibiotherapy to use are lacking since no well-designed trial to study antimicrobial treatment of VGI exists[3]. Moreover, there is very few if any experimental animal models dealing with the antimicrobial treatment of this kind of infection and more relevant data on VGI antibiotherapy are needed.
The aim of this study was to assess the efficacy of **Vancomycin**, D**aptomycin** and **Rifampin** alone or in combination with one of the two other drugs against MRSA in a **murine model of vascular material infection.**

Methods

Animals. Thirty 6 weeks-old female Swiss mice weighing approximately 25 g were maintained on a 12-hour light/dark cycle with free access to food and water. All experiments were in accordance with the Principles of Laboratory Animal Care and French regulations addressing animal experiments. The committee of animal ethics of the Pays de la Loire administrative region approved all animal experiments in this study. **Prosthetic devices.** Commercially available woven Dacron grafts were cut into 1 cm x 1 cm squares and sterilized. These Dacron sheets were then incubated under sterile conditions with serum of healthy Swiss mice during the 24 hours preceding their implantation at 37°C. **Surgical procedures.** Mice were anesthetized with isoflurane and ketamine (100 mg/kg, IP). A 10-mm horizontal incision in the center of the back was made to create a subcutaneous pocket. For each mouse, a Dacron sheet was implanted into this pocket. Skin was closed with sutures (Vicryl 5/0). **Bacterial inoculation.** BCB8 MRSA strains isolated from blood cultures were used for all experiments. Two days after Dacron implantation, a sterile saline solution (0.5 mL) containing 10^7 colony forming units (CFU) of MRSA was inoculated onto the graft surface with a tuberculin syringe. **Comparative groups.** Mice were randomized into six groups of ten each: (i) no treatment (Controls); (ii) Vancomycin group (subcutaneous injection, 110 mg/kg/12h); (iii) Daptomycin group (subcutaneous injection, 50 mg/kg/24h); Rifampin group (30 mg/kg/12 h, IP); Vancomycin + Rifampin group and Daptomycin + Rifampin group. Mice were treated during 48 hours and then euthanized following international guidelines. Dacron graft were removed, homogenized in 0.5 mL of saline buffer and used for quantitative bacterial cultures. Spleen were also weighed and homogenized in 1 mL of saline buffer for bacterial cultures. Animals with positive bacterial culture of spleen were considered to be bacteriemic. **Statistical analysis.** GraphPad prism software (La Jolla CA. United States) was used. P<0.05 was considered to be statistically significant.

Results

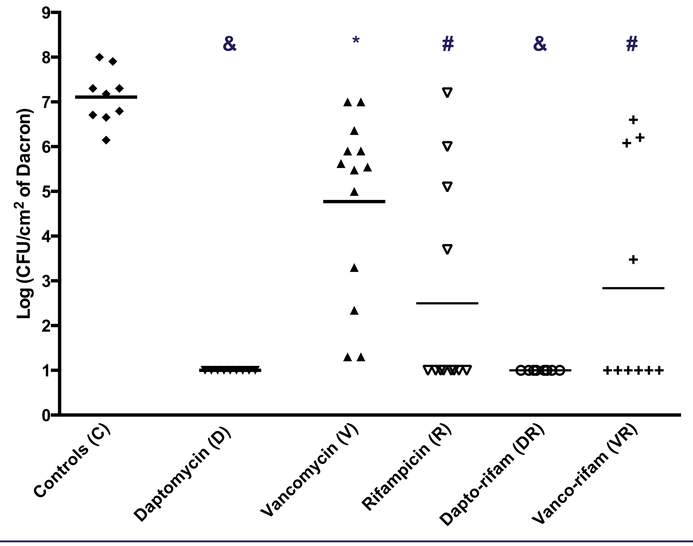


Figure 1: Results of graft culture after two days of treatment

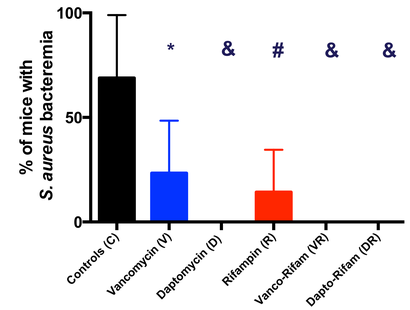
&: P < 0.0001 D and DR vs C, V, R and VR

*: P < 0.05 V vs C

#: P < 0.01 R and VR vs C

Bacterial culture of the Dacron graft. Control group showed stable infection during experimentation. Vancomycin demonstrated efficacy with significant decrease of bacterial count as compared to the control group: - 1.9 log_{10}, P < 0.05. Daptomycin was dramatically more efficient than Vancomycin and Rifampin in this experimental model since all materials were sterile after 2 days of treatment (P < 0.0001). The bactericidal activity of Vancomycin was significantly improved when combined with Rifampin (P<0.01), although the combination remained less bactericidal than Daptomycin. All these results are exposed in the figure 1. No antibiotics resistance emerged after treatment.

Spleen cultures (figure 2). 60 % of controls animals were bacteriemic. Only 20% of mice were bacteriemic in the Vancomycin group (P<0.05) and 15% in the Rifampin group (P<0.01). Once again, Daptomycin demonstrated higher efficacy than Vancomycin with no mouse with positive spleen culture (P < 0.0001) .



&: P< 0.0001 D, DR and VR vs C, V, R

*: P< 0.05 V vs C; #:P<0.01 R vs C

Figure 2: Results of spleen culture after two days of treatment

Conclusion

Evaluation of efficacy of antibiotics can be achieved with this model of murine vascular graft infection.
In this model, Daptomycin demonstrated much more efficacy than Vancomcyin against MRSA, whether or not combined with Rifampin.
New experiments are needed to confirm these results and to test other antibiotics or associations.

References

1. Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med 2004;350:1422-9
2. Jansen K, Girgenti D, Scully I, Anderson A. Staphylococcus aureus vaccine: problems and prospects. Vaccine 2013;31:2723-30
3. Fitzgerald S, Kelly S, Humphreys H. Diagnosis and treatment of prosthetic aortic graft infections: confusion and inconsistency in the absence of evidence and consensus. J Antimicrob Chemotherapy 2005;56:996-9

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