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Comparative activity of cloxacillin and vancomycin against methicillin-susceptible *Staphylococcus aureus* experimental endocarditis

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***Objectives*: To compare the activity of cloxacillin and vancomycin against methicillin-susceptible *Staphylococcus aureus* and to determine how rapidly their bactericidal activity occurs in cardiac vegetations.**

***Methods*: *In vitro* and *in vivo* studies using an experimental model of endocarditis in rabbits. Animals were treated for 1, 2 or 3 days with cloxacillin 200 mg/kg intramuscularly three times a day or vancomycin 25 mg/kg intravenously twice a day.**

***Results*: Cloxacillin and vancomycin at concentrations 4- and 16-fold the MIC produced a modest decrease in the number of microorganisms at 4 h. After 24 h, cloxacillin produced a decrease in the counts of staphylococci from 2.19 to 4.84 $\log_{10}$ cfu/mL of inoculum. Only concentrations of vancomycin from 16- to 32-fold the MIC resulted in equivalent decreases. After 24 h of treatment, both antibiotics were equally effective in preventing mortality of rabbits. Cloxacillin produced a greater decrease in the number of staphylococci than vancomycin (3.50 ± 2.18 $\log_{10}$ cfu/g vegetation and 6.25 ± 1.28 $\log_{10}$ cfu/g vegetation, respectively; $P < 0.05$) and 41% of rabbits had sterile vegetations in comparison with none with vancomycin ($P = 0.035$). After 48 and 72 h of treatment, both antimicrobials exhibited equivalent activity.**

***Conclusions*: Vancomycin was less rapidly bactericidal than cloxacillin *in vivo*.**

Keywords: β-lactamase-resistant penicillins, glycopeptides, bactericidal activity, staphylococcal bacteraemia

Introduction

Vancomycin is the most widely used agent for the treatment of serious infections caused by Gram-positive pathogens. The common occurrence of methicillin-resistant *Staphylococcus aureus* infections in hospitals has led to an increasing use of vancomycin for the treatment of bacteraemia and other nosocomial infections.

Because of its favourable pharmacokinetics and reduced costs, vancomycin therapy is frequently continued even when isolated staphylococci are fully susceptible to oxacillin.[1] In addition, the widespread belief that vancomycin is as active as β-lactam antibiotics against *S. aureus* has been of paramount importance to reinforce the use of this antibiotic in infections where penicillins may be successfully used.[2] However, sustained bacteraemia has been reported among patients with staphylococcal endocarditis treated with vancomycin and relapses have also been described.[2,3] These findings raise the question of whether vancomycin may be equivalent to β-lactamase-resistant penicillins in the treatment of staphylococcal infections.

Because this controversial issue deserves further scrutiny, we investigated the activity of cloxacillin and vancomycin in an experimental model of endocarditis caused by methicillin-susceptible *S. aureus* (MSSA).

Materials and methods

In vitro studies

One strain of MSSA isolated from a patient with endocarditis was used. A microdilution method was used for susceptibility testing.[4] Concentrations of 5.5×10^5 cfu of staphylococci per mL were inoculated into Mueller–Hinton broth containing serial 2-fold dilutions of antibiotics. For cloxacillin testing, cation-supplemented Mueller–Hinton broth with 2% NaCl was used.

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Time–kill studies were performed in Mueller–Hinton broth with inocula of 10^8–10^9 cfu of staphylococci per mL.[4] The results are expressed as mean values.

Animal studies

This study was approved by the local institutional Committee for Animal Research and was conducted following the recommendations for ethical care of animals to be used under experimental conditions.

Aortic valve endocarditis was established in rabbits as reported previously.[5] Antimicrobial therapy was started 24 h after inoculation. Animals were placed into treatment groups as follows: (i) the control group received no treatment; (ii) the cloxacillin group consisted of animals treated intramuscularly with 200 mg of cloxacillin per kg three times a day; and (iii) the vancomycin group consisted of animals treated intravenously with 25 mg of vancomycin per kg twice a day.

Antimicrobial therapy was given for 1, 2 or 3 days. In order to determine the degree of endocardial infection, the entire valve and vegetations were weighed and homogenized in 0.9 mL of broth. The number of cfu of staphylococci per gram of vegetation was quantified by a pour plate method with trypticase soy broth. The results are expressed as $\log_{10}$ U, corresponding to 1 cfu/g.

Measurements of concentrations of antimicrobial agents in serum

Blood samples were taken from rabbits 60 min after administration of antibiotics and just before the next dose to measure their peak and trough levels. The concentration of vancomycin was measured by using TDX (Abbott Laboratories) and the concentration of cloxacillin was determined by a bioassay with *Bacillus subtilis* ATCC 6633.

Analysis of results

Differences in mean $\log_{10}$ cfu of staphylococci per g of vegetation were analysed by using the Kruskall–Wallis non-parametric test. In addition, the Mann–Whitney test was used when two groups were compared. For comparisons of two means, a P value of <0.05 was considered significant.

Results

In vitro studies

The MICs/MBCs of cloxacillin and vancomycin for the strain used in the experimental design were 0.25/0.5 and 1/2 mg/L, respectively.

The rates of decrease in the number of *S. aureus* per mL of broth in the time–kill studies with concentrations of antibiotics ranging from MIC values to 32-fold the MIC values are shown in Figure 1. With a high inoculum, cloxacillin and vancomycin at concentrations 4- and 16-fold the MIC produced only a modest decrease in the number of microorganisms at 4 h (reductions from 2.1 to 2.2 $\log_{10}$ cfu/mL and 1.69 to 2.08 $\log_{10}$ cfu/mL of inoculum, respectively). After 24 h, and depending on the concentration of antibiotic, cloxacillin produced a decrease in the mean counts of staphylococci from 2.19 to 4.84 $\log_{10}$ cfu/mL. Only concentrations of vancomycin from 16- to 32-fold the MIC resulted in decreases in the number of microorganisms at 24 h in the order of 4.79 $\log_{10}$ cfu/mL.

Therapeutic studies

Table 1 shows the results of treatment of endocarditis due to MSSA for 1, 2 and 3 days. The number of rabbits in each treatment group, the number of animals alive at each interval, the number with sterile vegetations and antibiotic concentrations are included.

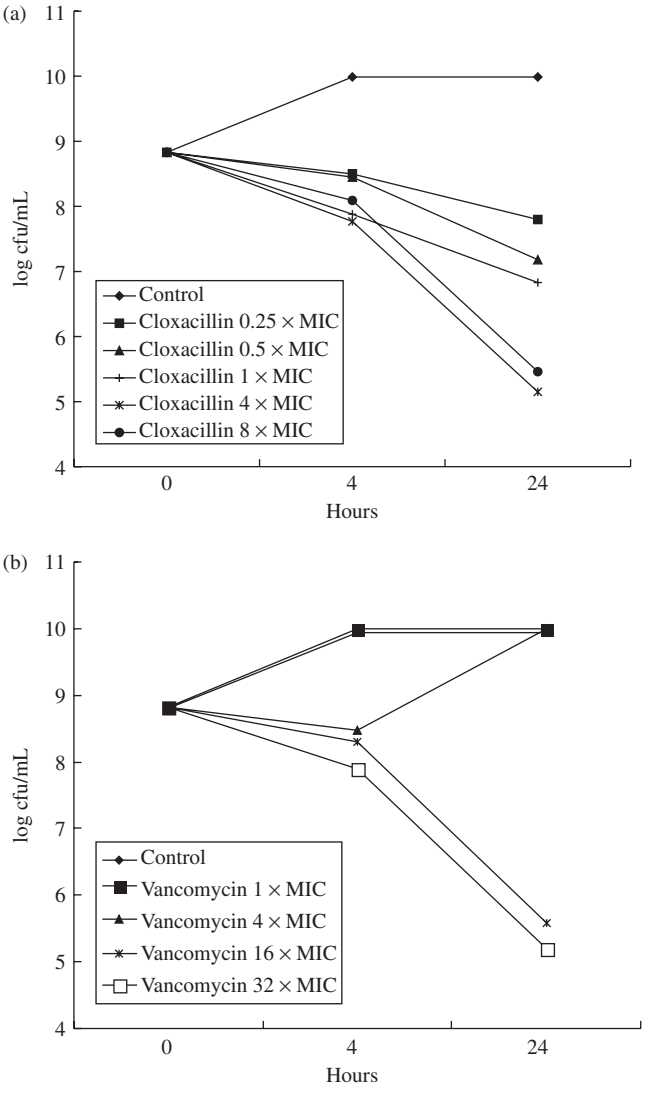

Figure 1. Time–kill studies of MSSA used in the experimental model of aortic endocarditis with different concentrations of cloxacillin (a) and vancomycin (b).

After 24 h, both treatments were equally effective in preventing early mortality of rabbits. Cloxacillin produced a decrease in the number of staphylococci that was significantly greater than that produced by vancomycin. Forty one per cent of rabbits treated with cloxacillin had sterile vegetations in comparison with none in the group treated with vancomycin ($P = 0.035$).

After 48 h of treatment, both antimicrobials exhibited an equivalent activity in reducing the number of organisms in vegetations and 70% of the animals treated with vancomycin had sterile vegetations. After 72 h of antimicrobial therapy, 60% of those treated with cloxacillin and 80% of those treated with vancomycin had sterile vegetations. At this point, both antibiotics still showed an equivalent activity in reducing the number of staphylococci in cardiac vegetations. The efficacy of vancomycin to reduce the number of staphylococci in vegetations increased

Table 1. Results of treatment of experimental endocarditis due to MSSA during 24, 48 and 72 h

Duration of treatment and antimicrobial regimen	No. of animals alive/ no. of animals used/no. with sterile vegetations	Antibiotic levels peak/trough (μg/mL) (mean ± SD)	Log_{10} cfu/g vegetation (mean ± SD)
24 h			
none (control)	0/5/0		9.86 ± 0.81
cloxacillin	12/12/5	14.0 ± 3.1/0.28 ± 0.1	3.50 ± 2.18*,**
vancomycin	13/14/0	49.1 ± 10.8/5.7 ± 1.3	6.25 ± 1.28*
48 h			
none (control)	0/6/0		9.66 ± 0.81
cloxacillin	9/9/3	20.3 ± 13.7/0.5 ± 0.1	3.72 ± 2.23*
vancomycin	10/10/7	53.0 ± 7.02/5.4 ± 1.7	2.63 ± 2.16*,***
72 h			
none (control)	0/8/0		9.74 ± 0.81
cloxacillin	10/10/6	15.5 ± 6.92/0.7 ± 02	2.39 ± 1.81*
vancomycin	10/10/8	44.5 ± 7.7/2.8 ± 1.3	2.54 ± 2.21*,***

*$P < 0.05$ for all comparisons of cloxacillin and vancomycin versus control.
**$P < 0.05$ for the comparison of cloxacillin versus vancomycin.
***P non-significant for the comparisons of cloxacillin versus vancomycin.

from day 1 to day 2 of treatment ($P = 0.006$) although not from day 2 to day 3 ($P = 0.93$).

Discussion

Reliance on vancomycin for the treatment of infections caused by MSSA rests on the assumption that it is as effective as β-lactam antibiotics, an assumption that remains open to debate.

Some reports have shown slow clinical response and failures in patients with endocarditis caused by *S. aureus* treated with vancomycin and others have found that nafcillin was superior to vancomycin in preventing bacteriological failure for MSSA bacteraemia.[2,3,6] However, these observations must be treated cautiously due to their retrospective nature and the inclusion of bias such as unremoved foreign bodies, haemodialysis dependence and deep-tissue infection.

Overall, differences in the killing rate of *S. aureus* produced by oxacillin or vancomycin *in vitro* are not found. However, Small and Chambers[3] using 10 clinical isolates of MSSA found that at four times the MIC, nafcillin resulted in a significantly greater reduction in bacterial counts than vancomycin after 24 h of incubation.

On the experimental evidence, the results of treatment of *S. aureus* endocarditis in animals have shown a similar activity of glycopeptide antibiotics in comparison with penicillin, cloxacillin or nafcillin.[5,7,8] These studies assessed the efficacy of the drugs after 3 days of treatment but did not measure the speed at which each antibiotic produced the bactericidal effect on vegetations. In addition, some of these experiments were conducted with bacterial counts well below the microbial concentration found during the disease in humans.[8] For these reasons and in order to determine how rapidly cloxacillin and vancomycin produce their bactericidal activity in cardiac vegetations, we treated animals for 1, 2 and 3 days under the most stringent conditions of high-bacterial-count endocarditis.

Although both cloxacillin and vancomycin treatments seemed equally effective in avoiding early mortality of rabbits, cloxacillin showed a faster bactericidal activity, produced a greater decrease in the number of staphylococci in vegetations and resulted in a higher number of animals with sterile vegetations after 24 h of treatment. These differences subsided after 48 h of antimicrobial therapy. While the activity of cloxacillin did not increase in parallel with the duration of therapy, the efficacy of vancomycin increased from day 1 to day 2.

The reasons for the different behaviour of cloxacillin and vancomycin in this experimental model are not known. Generally speaking, high-inoculum *S. aureus* infections produce a significant impact on the activities of both β-lactams and vancomycin. In an *in vitro* pharmacodynamic model with simulated endocardial vegetations using an inoculum of 5.5 log_{10} cfu/g, nafcillin and vancomycin demonstrated equivalent bactericidal activity. However, at a high inoculum in the order of 9.5 log_{10} cfu/g neither nafcillin nor vancomycin achieved bactericidal activity.[9]

As shown previously by others using oxacillin,[3] our *in vitro* studies also showed a greater ability of cloxacillin to reduce the number of microorganisms after 24 h of incubation, and lower concentrations of this antibiotic produced greater reductions in bacterial counts than vancomycin. Only concentrations of vancomycin from 16- to 32-fold the MIC resulted in equivalent decreases in the number of microorganisms at 24 h. These findings suggest a greater intrinsic activity of cloxacillin against staphylococci.

The inoculum effect on vancomycin activity may be related to a reduction of concentrations of vancomycin in cultures due to binding of free vancomycin to cell wall material. Reduction of vancomycin concentrations *in vitro* is related to the size of the bacterial population and so very dense cultures will bind more drug.[10] These findings suggest that the dosing of vancomycin is of particular importance in infections such as endocarditis with dense inocula.

These studies indicate that vancomycin may be less rapidly bactericidal than cloxacillin *in vivo* and might explain the longer duration of fever and bacteraemia seen in some patients with staphylococcal endocarditis treated with vancomycin. Our findings give experimental support to such clinical observations and

provide *in vivo* evidence of the faster bactericidal activity of cloxacillin in cardiac vegetations. Since rapid sterilization of the vegetations and bloodstream may be important in preventing valvular damage and metastatic complications, cloxacillin should be preferred to vancomycin for the treatment of MSSA endocarditis.

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Transparency declarations

We do not have commercial or any other associations that may pose a conflict of interest.

References

1. Roghmann MC, Perdue BD, Polish L. Vancomycin use in a hospital with vancomycin restriction. *Infect Control Hosp Epidemiol* 1999; **20**: 60–3.

2. Levine DP, Fromm BS, Reddy BR. Slow response to vancomycin or vancomycin plus rifampin in methicillin-resistant *Staphylococcus aureus* endocarditis. *Ann Intern Med* 1991; **115**: 674–80.

3. Small PM, Chambers HF. Vancomycin for *Staphylococcus aureus* endocarditis in intravenous drug users. *Antimicrob Agents Chemother* 1990; **34**: 1227–31.

4. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. *Manual of Clinical Microbiology*, *8th edn*. Washington, DC: American Society for Microbiology, ASM Press, 2003; 1108–27.

5. Chambers HF, Sande MA. Teicoplanin versus nafcillin and vancomycin in the treatment of experimental endocarditis caused by methicillin-susceptible or -resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1984; **26**: 61–4.

6. Chang FY, Peacock JE Jr, Musher DM *et al*. *Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. *Medicine (Baltimore)* 2003; **82**: 333–9.

7. Cantoni L, Glauser MP, Bille J. Comparative efficacy of daptomycin, vancomycin, and cloxacillin for the treatment of *Staphylococcus aureus* endocarditis in rats and role of test conditions in this determination. *Antimicrob Agents Chemother* 1990; **34**: 2348–53.

8. Cantoni L, Wenger A, Glauser MP *et al*. Comparative efficacy of amoxicillin-clavulanate, cloxacillin, and vancomycin against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* endocarditis in rats. *J Infect Dis* 1989; **159**: 989–93.

9. LaPlante KL, Rybak MJ. Impact of high-inoculum *Staphylococcus aureus* on the activities of nafcillin, vancomycin, linezolid, and daptomycin, alone and in combination with gentamicin, in an *in vitro* pharmacodynamic model. *Antimicrob Agents Chemother* 2004; **48**: 4665–72.

10. Ekdahl C, Hanberger H, Hällgren A *et al*. Rapid decrease of free vancomycin in dense staphylococcal cultures. *Eur J Clin Microbiol Infect Dis* 2005; **24**: 596–602.