Efficacy of Oxacillin and Ampicillin-Sulbactam Combination in Experimental Endocarditis Caused by β-Lactamase-Hyperproducing Staphylococcus aureus

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Optimal therapy of infections caused by borderline oxacillin-susceptible, β-lactamase-hyperproducing Staphylococcus aureus has not been established. We used a rat model of aortic valve endocarditis to examine efficacies of antibiotic regimens against a borderline oxacillin-susceptible strain as compared with a fully susceptible S. aureus strain. Animals were treated with oxacillin alone or in combination with sulbactam or with ampicillin-sulbactam combinations at two dose levels. Infections caused by the borderline susceptible and fully susceptible strains responded equally well to oxacillin alone, with residual bacterial titers in vegetations falling to 4.8 ± 1.6 and 4.4 ± 1.7 (mean ± standard deviation) log_{10} CFU/g, respectively. Addition of sulbactam to oxacillin (1:2) did not enhance the efficacy of oxacillin against either strain in the animal model. A high-dose regimen of ampicillin-sulbactam (2:1) yielding mean (± standard deviation) levels in serum of 16.8 ± 7.4 and 9.5 ± 1.1 µg/ml, respectively, proved equally effective against both strains (bacterial titers, 6.6 log_{10} CFU/g). However, at lower doses (8.3 ± 2.6 and 5.9 ± 2.4 µg/ml), the combination showed greater efficacy against the fully susceptible strain, with residual titers of 7.1 ± 2.0 versus 9.0 ± 1.6 log_{10} CFU/g (P < 0.05). In vitro studies revealed that the β-lactamase inhibitor sulbactam was also a potent inducer of staphylococcal β-lactamase at clinically relevant concentrations. Based on this short-term in vivo therapy study, oxacillin would be predicted to be clinically effective in the therapy of infections caused by borderline oxacillin-susceptible strains of S. aureus, while the combination of ampicillin with sulbactam appears to be inferior to oxacillin alone against such infections.

In 1986, McDougal and Thornsberry (8) described isolates of Staphylococcus aureus displaying borderline susceptibility to antistaphylococcal penicillins. These strains were inhibited by methicillin and oxacillin at concentrations of 4 and 2 µg/ml, respectively, and were characterized by the production of large amounts of β-lactamase. Addition of a β-lactamase inhibitor such as clavulanic acid or sulbactam restored activities of the penicillins to levels which inhibit fully methicillin-susceptible S. aureus strains. Such strains appear to be distinct from those demonstrating chromosomally mediated intrinsic β-lactamase resistance, which has been associated with the presence of a low-affinity penicillin-binding protein, termed PBP 2a or 2' (5). To differentiate borderline susceptible strains from the latter group of intrinsically resistant organisms further, they have also been called acquired-resistant strains (7, 8). Whether infections caused by borderline susceptible staphylococcal isolates would respond to β-lactams as well as infections caused by fully susceptible strains, or whether these isolates should be considered the same as intrinsically resistant strains for clinical and therapeutic purposes, is unclear and remains to be determined.

In this study, we established an aortic valve endocarditis model in rats, using a fully oxacillin-susceptible strain of S. aureus or a borderline susceptible clinical isolate of S. aureus, to evaluate the role of β-lactamase production in the therapeutic efficacies of oxacillin alone, oxacillin combined with sulbactam, and ampicillin-sulbactam in combination.

(This work was presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy [C. Thauvin-Eliopoulos, L. B. Rice, G. M. Eliopoulos, and R. C. Moellering, Jr., Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 367, 1988].)

MATERIALS AND METHODS

Bacterial strains. The two strains of S. aureus used throughout this study were clinical blood culture isolates recovered in Boston, Mass. Susceptibilities were determined by microdilution broth titration in cation-supplemented Mueller-Hinton broth (CSMHB; BBL Microbiology Systems, Cockeysville, Md.) with 2% NaCl (9, 16). Strain 1 was borderline oxacillin susceptible (BOSSA) with methicillin and oxacillin MICs of 4 and 1 µg/ml, respectively. Addition of sulbactam, 16 µg/ml (8), reduced MICs of these agents to 2 and 0.25 µg/ml, respectively. Strain 2 was a fully oxacillin-susceptible S. aureus (OSSA) strain with methicillin and oxacillin MICs of 2 and 0.25 µg/ml, respectively. Sulbactam alone inhibited both strains at 250 µg/ml. Antimicrobial susceptibilities were also determined in CSMHB plus 2% NaCl supplemented with 50% rat serum, using inocula of 5 x 10^4 to 1 x 10^5 CFU/ml. Inhibitory endpoints were read at 24 and 48 h of incubation at 35°C. At 48 h, 0.01-ml samples were transferred to antibiotic-free plates for determination of MBCs (11).

Antimicrobial agents. Ampicillin and sulbactam standard reference powders and the 2:1 combination for in vivo studies were generously provided by Roerig-Pfizer, Inc., New York, N.Y. Oxacillin was obtained from Bristol Lab...
oratories, Evansville, Ind. Methicillin was obtained from Sigma Chemical Co., St. Louis, Mo.

**Evaluation of β-lactamase activity.** β-Lactamase activity of cell lysates was determined by a spectrophotometric method (10). Bacteria were grown in dextrose phosphate broth (GIBCO Diagnostics, Madison, Wis.) to log phase at 35°C and harvested when cell densities reached 10^6 CFU/ml. Cell pellets were lysed by incubation with lysostaphin (50 μg/ml) (Sigma) and Triton X-100 (0.05% final concentration) (BioRad Laboratories, Richmond, Calif.). For studies of β-lactamase induction, cells were grown in the presence of ampicillin or sulbactam at concentrations of 0.1× the MIC of each drug.

**Production of bacterial endocarditis.** Bacterial endocarditis was established by the technique of Santoro and Levison (12), with minor modifications as described previously (14, 15). Nonbacterial thrombotic endocarditis was created in male Sprague-Dawley rats (180 to 250 g) by insertion of a polyethylene catheter (PE 10 Intramedic tubing) across the aortic valve via the right carotid artery. At 20 min after catheterization, 10^6 CFU of the infecting strain in saline were injected through the catheter, which was ligated and left in place throughout the experiment. Establishment of bacterial endocarditis was ascertained by positive blood cultures drawn just before onset of therapy. Only animals with both initial blood cultures positive for the infecting strain and correct placement of the catheter across the aortic valve (as established at autopsy) were included in the study. With both strains, colony counts in cardiac vegetations reached 8.0 to 8.5 log_{10} CFU/g by 12 h after infection.

**Antimicrobial therapy.** Treatment was started 12 h after bacterial challenge and continued for 3.5 days. All antimicrobial agents were delivered by continuous intravenous infusion through an indwelling central venous catheter inserted through the external jugular vein into the superior vena cava, as described previously (14). Antimicrobial agents were carefully delivered with syringe pumps (Orion Research, Inc., Cambridge, Mass.). Animals were randomly assigned to one of the following five groups: (i) oxacillin alone (600 mg/kg per day), (ii) oxacillin (600 mg/kg per day) plus sulbactam (300 mg/kg per day), (iii) ampicillin (450 mg/kg per day) plus sulbactam (225 mg/kg per day), (iv) ampicillin (1,000 mg/kg per day) plus sulbactam (500 mg/kg per day), and (v) an untreated control group.

**Monitoring of therapy and outcome.** On day 3 of therapy, antibiotic levels in serum were measured by using an agar well diffusion technique (1), with *Bacillus subtilis* as the test organism for ampicillin and oxacillin levels and with a β-lactamase-producing strain of *Pasteurella haemolytica* as the test organism for sulbactam levels. Animals surviving 3.5 days of treatment were sacrificed 2 h after discontinuation of therapy. Cardiac vegetations were aseptically removed, weighed, homogenized, and diluted in saline for bacterial titer determination. Colony counts were performed on mannitol-salt agar plates with and without penicillinase to control for potential antibiotic carry-over. The lower limit of detection by this method was 2.3 log_{10} CFU/g of vegetation.

**Statistical evaluation.** The chi-square test with Yates correction was used for evaluation of nominal data. Differences in bacterial titers within vegetations among treatment groups were assessed by analysis of variance followed by the Bonferroni method for multiple comparisons (4).

**RESULTS**

**In vitro susceptibility studies.** Activities of antimicrobial agents against the test strains determined in CSMB plus 2% NaCl supplemented with 50% rat serum are shown in Table 1. MICs of oxacillin against the two strains were identical in this medium, and these were not affected in the presence of sulbactam. The MIC of ampicillin-sulbactam (2:1, expressed in terms of the ampicillin component) against the BOSSA strain was fourfold higher than that against the OSSA strain. In the absence of rat serum, MICs (micrograms per milliliter) against the BOSSA and OSSA strains in CSMB plus 2% NaCl were as follows: ampicillin-sulbactam, 4 and 2; oxacillin, 1.0 and 0.25; oxacillin-sulbactam, 0.25 and 0.125. Strain 1 (BOSSA) demonstrated greater tolerance to the bactericidal effects of oxacillin (MIC/MIC ratio, 32) and ampicillin-sulbactam (MIC/MIC ratio, 8) than did strain 2 (OSSA) (MIC/MIC ratios, 2 for both drugs).

**β-Lactamase activity.** As expected, β-lactamase activity of cell lysates from strain 1 was substantially greater than that of strain 2 (Fig. 1). Both ampicillin and sulbactam induced production of β-lactamase, but in each case the resulting hydrolytic activity was greater with BOSSA lysates. This effect was particularly striking when BOSSA cells were induced by incubation with sulbactam. The resulting lysate achieved complete hydrolysis of substrate within 5 min.

**Effects of preincubation with sulbactam or clavulanate on**
β-lactamase activity were also studied in five additional BOSSA isolates. In four of these, exposure to sulbactam induced β-lactamase activity, resulting in ≥90% nitrocefin hydrolysis within 2 to 4 min, with the fifth strain hydrolyzing this amount of drug by 60 min. In each case, preincubation with clavulanic acid retarded nitrocefin hydrolysis by the resulting cell lysate. Sulbactam induced β-lactamase activity in two strains of OSSA which produced β-lactamase, but, as expected, did not affect one penicillin-susceptible, non-β-lactamase-producing isolate.

Experimental endocarditis. Results of therapy are shown in Table 1. Antibiotic levels in serum achieved with both oxacillin and ampicillin-sulbactam are clinically attainable in humans and exceeded MICs against the test organisms. Survival of animals in each treatment group exceeded that of control animals (P ≤ 0.02), but did not differ among the groups of treated animals. Oxacillin was equally effective against BOSSA and OSSA infections, with residual titers within vegetations of 4.8 ± 1.6 and 4.4 ± 1.7 log_{10} CFU/g, respectively. Addition of sulbactam to oxacillin did not enhance activity of the latter against either strain. Residual bacterial titers were higher among animals treated with this combination than among those treated with oxacillin alone, but these differences were not statistically significant. Likewise, the high-dose ampicillin-sulbactam regimen was equally effective against BOSSA and OSSA infections. This regimen was less effective than oxacillin alone against both strains (P = 0.01 for BOSSA and P = 0.002 for OSSA), but was comparable to the oxacillin-sulbactam regimen. Statistically significant differences in response of animals infected with BOSSA or OSSA strains were evident only among those treated with low-dose ampicillin-sulbactam. With this regimen, significantly greater reductions in vegetation bacterial titers were observed with the OSSA than with the BOSSA strain (P < 0.05).

**DISCUSSION**

Since the recognition that strains for which oxacillin MICs are ≥1 μg/ml constitute a small but significant proportion of recent isolates of *S. aureus* at some centers (7, 18), there has been considerable concern about appropriate therapy of infections due to such strains. Specifically, would a β-lactam alone prove successful or would such isolates more closely resemble methicillin-resistant *S. aureus* and fail to respond predictably? It has become amply clear, however, that mechanisms of resistance to penicillinase-resistant penicillins cannot always be accurately predicted solely from levels of susceptibility. Strains of *S. aureus* for which oxacillin MICs are between 1 and 2 μg/ml appear to represent a heterogeneous group, some of which hyperproduce β-lactamase, as defined by McDougall and Thornsberry (8), and others of which may attribute oxacillin resistance to alterations in penicillin-binding proteins (3, 13). In the present study, we ascertained that the BOSSA strain studied was a hyperproducer of β-lactamase as compared with a clinical isolate demonstrating full susceptibility to oxacillin.
This study showed oxacillin to be equally effective against the BOSSA and OSSA strains, with residual bacterial titers of 4.4 to 4.8 log_{10} CFU/g. This observation is consistent with results of other animal studies which have compared response of fully susceptible and intermediate susceptible strains of *Staphylococcus aureus* to therapy with antistaphylococcal penicillins. Cantoni et al. (2), using a rat endocarditis model, found similar responses to cloxacin of two strains, with oxacillin MICs of 0.25 and 2 μg/ml. Chambers et al. (3), using a rabbit model of endocarditis, noted identical responses to nafcillin of a fully susceptible strain (nafcillin MIC, 0.5 μg/ml) and one characterized by a nafcillin MIC of 4 μg/ml. Penicillin-binding proteins of the latter strain were examined and found to be similar to the pattern of methicillin-susceptible *S. aureus* strains. However, in neither of these studies was the level of β-lactamase production in intermittently resistant isolates formally tested. These data are also consistent with results of a prospective analysis of clinical infections due to *S. aureus* strains with different levels of resistance to oxacillin (7). Treatment with a penicillinase-resistant penicillin was successful in 88.6% of infections caused by fully susceptible strains and 7 of 10 (70%) infections caused by acquired oxacillin-resistant isolates (oxacillin resistant by disk diffusion; oxacillin MIC of ≤2 μg/ml by broth dilution). These results are difficult to interpret, however, in view of the fact that four of five (80%) infections caused by oxacillin-resistant isolates (MIC, >8 μg/ml) also responded to treatment with a penicillinase-resistant penicillin.

In attempting to reconcile the above results with the fourfold difference in broth dilution oxacillin MICs and the significant difference in β-lactamase activity generated by the two strains used in our study (Fig. 1), it is noteworthy that susceptibility testing performed in the presence of 50% rat serum yielded identical oxacillin MICs against the two strains. Continuous administration of oxacillin (or repetitive antibiotic dosing in the studies cited), resulting in drug concentrations in serum 10-fold higher than the MIC, may have partially negated any differences in β-lactamase production apparent in the static environment of standard susceptibility testing. While it is theoretically possible that our results might also be explained by lower virulence of the BOSSA strain, this seems highly unlikely in view of the fact that bacterial titers in vegetations of untreated animals inoculated with either strain were identical both 12 h after infection (8.0 to 8.5 log_{10} CFU/g) and at sacrifice on day 4 (10.4 to 10.5 log_{10} CFU/g). Tolerance to the bactericidal effects of oxacillin which we observed with strain 1 (MBC/MIC ratio, 32) appears to be common among BOSSA isolates (18), but is of uncertain relevance in vivo. Woods and Yam (18) have reported that tolerance to oxacillin among β-lactamase-hyperproducing strains of *S. aureus* determined by MBC testing did not predict poor bactericidal activity of the drug by time-kill techniques.

The combination of ampicillin plus sulfactam administered in the high-dose regimen, also demonstrated identical efficacy against BOSSA and OSSA strains despite a 4-fold difference in MICs and a 16-fold difference in MBCs against the two isolates (Table 1). At the lower dose level, however, the combination was significantly less effective against the BOSSA strain. In the latter case, levels of ampicillin in serum exceeded the MIC (of ampicillin in combination with sulfactam) by only twofold. In addition, sulfactam levels were likely to have been inadequate in view of the linear relationship between sulfactam concentration and ampicillin MBC (in combination) against penicillinase-producing *S. aureus* (6).

In this study, oxacillin was more effective than ampicillin-sulfactam (high dose) against both isolates tested. While it might be possible to attribute this result to a superior antibiotic level in serum/MIC ratio (10:1 for oxacillin, 4:1 for ampicillin-sulfactam) for the BOSSA isolates, alternative explanations would clearly have to be sought in the case of the OSSA isolates (10:1 for oxacillin, 16:1 for ampicillin-sulfactam). Examination of attained drug levels in serum in relation to bactericidal endpoints yields similar conclusions. Our results are also in contrast to those of other studies which found ampicillin-sulfactam comparable to nafcillin against methicillin-susceptible *S. aureus* (17) and amoxicillin-clavulanate comparable to cloxacillin against both a fully oxacillin-susceptible strain and one for which the oxacillin MIC was 2 μg/ml (2).

We believe that these results may be explained by the observation that sulfactam displayed a striking ability to induce production of β-lactamase in each of the two strains (Fig. 1). This phenomenon was probably reflected in the diminished (although not statistically significant) efficacy of oxacillin against both strains when the drug was combined with sulfactam. This hypothesis provides an explanation for the discordance of our results with those of the aforementioned studies with amoxicillin plus clavulanic acid (2), which does not appear to be a potent inducer of the β-lactamase (data not shown), and with ampicillin plus sulfactam (17), which was dosed to achieve peak levels in serum substantially higher than the steady-state mean levels in serum achieved in the present study. Attainment of such high drug concentrations in serum may be important because the capacity of sulfactam to induce β-lactamase may well reach a maximum level at modest drug concentrations, while the β-lactamase-inhibitory activity of the agent appears to increase proportionally with drug concentration (6).

We conclude that, against the representative strains studied here, both oxacillin and ampicillin-sulfactam (high dose) were as effective against a β-lactamase-hyperproducing BOSSA as against an OSSA strain. At the doses used, ampicillin-sulfactam was less effective than oxacillin. Higher drug concentrations of ampicillin-sulfactam in serum would probably have enhanced the efficacy of this combination. We have shown that sulfactam could induce production of β-lactamase in these strains. Combination of sulfactam with oxacillin, while not affecting survival, appeared to diminish efficacy in terms of final bacterial titers within vegetations, although this effect did not reach statistical significance.

**LITERATURE CITED**


