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β-Lactam–β-Lactamase-Inhibitor Combinations Are Active in Experimental Endocarditis Caused by β-Lactamase-Producing Oxacillin-Resistant Staphylococci

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Optimal therapeutic strategies for serious infections caused by borderline and heterotypic oxacillin-resistant *Staphylococcus aureus* (BORSA and ORSA) strains have not been fully characterized. Recent evidence suggests that the dominant penicillin-binding protein of ORSA strains (PBP 2a) shows good affinity for ampicillin and that these strains commonly produce β-lactamase. Therefore, we compared the in vivo efficacy of the combination of ampicillin plus sulbactam with that of vancomycin against ORSA strains. Also, the moderate resistance of BORSA strains appears to be attributable mainly to the hyperproduction of β-lactamase. Therefore, we also studied the in vivo efficacy of ampicillin plus sulbactam against such organisms. Experimental aortic endocarditis was induced in rabbits by the following three strains: β-lactamase-producing BORSA strain VP-986, β-lactamase-producing ORSA strain 67-0, and its β-lactamase-negative clone. In animals with BORSA endocarditis, ampicillin plus sulbactam and oxacillin were highly effective in reducing mean intravegetation bacterial densities, with each being significantly better than either ampicillin alone or no therapy. In animals with endocarditis caused by the β-lactamase-producing ORSA strain, ampicillin plus sulbactam was significantly better at reducing mean vegetation bacterial densities than the other regimens. For endocarditis caused by the β-lactamase-negative ORSA clone, ampicillin was better than vancomycin in reducing mean intravegetation bacterial densities. These data show that infections caused by β-lactamase-producing BORSA strains respond therapeutically in a manner similar to that of infections caused by oxacillin-susceptible strains, with both oxacillin and ampicillin plus sulbactam being highly efficacious. Moreover, high-dose ampicillin treatment strategies were effective in the therapy of ORSA endocarditis; this efficacy is presumably related to the relatively high affinity profile of this compound (compared with that of oxacillin) for the functionally dominant ORSA PBP 2a.

Clinical infections of both community and nosocomial origins caused by heterotypic oxacillin-resistant *Staphylococcus aureus* (ORSA) strains continue to be major therapeutic and infection control challenges worldwide (9, 11, 20, 23). Recently, a variety of infectious syndromes caused by *S. aureus* strains exhibiting low-level, or borderline, resistance (MICs of 1 to 4 μg/ml) to oxacillin (BORSA strains) have been documented (13). The vast majority of ORSA and BORSA strains have been shown to produce β-lactamases which hydrolyze many penicillins (e.g., penicillin G and ampicillin) and narrow-spectrum cephalosporins (e.g., cefazolin) and which are irreversibly inhibited by sulbactam and clavulanate (13–15, 18). Optimal treatment for deep-seated infections (such as endocarditis) due to BORSA strains has not been fully delineated to date. Moreover, although vancomycin has been generally thought to be the agent of choice for invasive ORSA infections (20), recent studies have questioned the overall success of this agent in cases of staphylococcal endocarditis (5, 19). In addition, in vitro studies have suggested that certain β-lactam agents, such as ampicillin, have relatively high binding affinities for the functionally dominant penicillin-binding protein (PBP) of heterotypic ORSA strains, PBP 2a (6, 7). The current study was designed to investigate the role of combinations of β-lactams and β-lactamase inhibitors in the therapy of experimental endocarditis caused by β-lactamase-producing BORSA and ORSA strains in comparison with standard treatment regimens with oxacillin and vancomycin, respectively.

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MATERIALS AND METHODS

Organisms. The *S. aureus* strains used in this investigation were kindly provided by Henry F. Chambers, San Francisco, Calif., and have been characterized in detail elsewhere (4). They were BORSA strain VP-986 (a β-lactamase-producing clinical isolate) and both β-lactamase-producing and β-lactamase-negative clones of ORSA strain 67-0, which expresses heterotypic oxacillin resistance.

Antibiotics. Vancomycin was supplied by Eli Lilly Research (Indianapolis, Ind.), oxacillin was supplied by Beecham Laboratories (Bristol, Tenn.), and ampicillin and sulbactam were supplied by Roerig-Pfizer (New York, N.Y.). Stock solutions of each agent (1,000 μg/ml) were kept at −70°C until thawed on the day of in vitro studies. For use in animal treatments, antibiotics were reconstituted just prior to administration.

Antibiotic susceptibility testing. The MICs of individual antibiotics ampicillin, oxacillin, sulbactam, and vancomycin against the various staphylococcal strains were determined by the microdilution method in cation-supplemented Mueller-Hinton broth plus 2% NaCl at a final inoculum of ~5 × 10^5 CFU/ml. MICs were read after 24 h of incubation at 35°C

as the highest antibiotic concentration yielding no visible growth. Borderline oxacillin resistance was defined as an MIC of 1 to 4 μg/ml, heterotypic oxacillin resistance was defined as an MIC of ≥8 μg/ml, and ampicillin resistance was defined as an MIC of ≥16 μg/ml (16). Results of MIC testing were utilized to design studies of enhanced bactericidal effect of β-lactam–β-lactamase-inhibitor combinations described below.

The ability of sulbactam to enhance the inhibitory activities of ampicillin or oxacillin against β-lactamase-producing BORSA and ORSA strains was also studied in the microtiter system at a final inoculum of $\sim 5 \times 10^5$ CFU/ml. The range of final ampicillin and oxacillin concentrations was 0.125 to 128 μg/ml, while that for sulbactam was 0.062 to 64 μg/ml; the combination of the β-lactam plus sulbactam was tested at a 2:1 ratio across this concentration range (from 0.125 μg of ampicillin per ml plus 0.062 μg of sulbactam per ml to 128 μg of ampicillin per ml plus 64 μg of sulbactam per ml) to parallel the clinically available formulations (e.g., Unasyn). An enhanced inhibitory effect was considered to occur if the MIC of the β-lactam-plus-sulbactam combination was at least fourfold less than those of both single agents.

To confirm that an enhanced bactericidal effect was achieved with the oxacillin-plus-sulbactam or the ampicillin-plus-sulbactam combination, the timed kill curve technique was used. A final inoculum of $\sim 10^6$ logarithmic-phase staphylococcal cells per ml was incorporated into either antibiotic-free or antibiotic-containing cation-supplemented Mueller-Hinton broth with 2% NaCl. The final β-lactam or sulbactam concentration used was equivalent to either twice or eight times the respective drug's in vitro MIC for the respective strains, as determined in the microtiter study above. At time zero and at 4, 6, and 24 h of incubation, 100 μl from each growth tube was quantitatively cultured in cation-containing Mueller-Hinton agar with 2% NaCl and incubated for an additional 48 h before surviving CFU per milliliter were counted. For subculture tubes containing ampicillin, penicillinase (PenAse, 1,000 U/ml; Difco, Detroit, Mich.) was incorporated into the subculture media to minimize ampicillin carryover effects. A fall in CFU of ≥2 $\log_{10}$ units per ml below that effected by the β-lactam or sulbactam alone was defined as evidence of an enhanced bactericidal effect of the drug combination. The magnitudes of bactericidal effects of ampicillin or oxacillin in combination with sulbactam were compared with those rendered by the single agents ampicillin, oxacillin, and vancomycin, each tested at twice and eight times their respective MICs for BORSA and ORSA strains.

Experimental endocarditis. The experimental rabbit model was used to assess the therapeutic efficacies of the various regimens in this study against BORSA and ORSA endocarditis. New Zealand White rabbits underwent transcarotid-transaortic catheterization as previously described (17) and then 24 h later were challenged intravenously (i.v.) with $\sim 10^7$ CFU of the BORSA or ORSA strain to be evaluated. Twenty-four hours after i.v. bacterial challenge, blood cultures were performed to document induction of endocarditis, and then bacteremic animals were randomly assigned to receive either no therapy or their first antibiotic treatment. Animals with BORSA endocarditis received either no therapy, ampicillin (100 mg/kg of body weight intramuscularly [i.m.] every 6 h), ampicillin plus sulbactam (20 mg/kg i.m. every 6 h), or oxacillin (100 mg/kg i.m. every 6 h); antibiotic therapy was given for either 3 or 5 days. These regimens were based on prior pharmacokinetic studies in this laboratory to achieve either supra-MIC levels in serum (for the single agents) or levels in serum exceeding those necessary for an enhanced in vitro bactericidal effect (for the drug combinations).

Prior pharmacokinetic studies in our laboratory (1) had shown that a 100-mg/kg dose of ampicillin would not achieve levels in serum above 15 μg/ml for a substantial part of the dosing interval; this is the ampicillin concentration required for 50% saturation of PBP 2a of ORSA strains in vitro (6). For this reason, the ampicillin dose was increased to 200 mg/kg for the treatment of ORSA endocarditis. Thus, animals with ORSA endocarditis caused by the β-lactamase-producing clone received 5 days of either no therapy, ampicillin (200 mg/kg i.m. every 6 h), ampicillin plus sulbactam (20 mg/kg i.m. every 6 h), or vancomycin (15 mg/kg i.v. twice daily). Animals with ORSA endocarditis caused by the β-lactamase-negative clone received 5 days of either no therapy, ampicillin (200 mg/kg i.m. every 6 h), or vancomycin (15 mg/kg i.v. twice daily).

Groups treated with sulbactam alone were not included in this study because the β-lactamase inhibitor exhibited no intrinsic antibacterial activity against the BORSA or ORSA strains in vitro.

For assessment of therapeutic efficacy, all animals were sacrificed by i.v. sodium pentobarbital overdose at least 24 h after the last drug dose to minimize antibiotic carryover effects. At the time of sacrifice, the heart was removed, and the chambers of the left side were examined for confirmation of both catheter position and macroscopic vegetative endocarditis on the aortic valve and in the left ventricle. All vegetations from a single animal were removed, homogenized, and quantitatively cultured as described by Carrizosa and Kaye (3); for calculation of the mean bacterial densities per gram of vegetation, culture-negative vegetations were considered to contain 2 $\log_{10}$ CFU/g (3). Prior to quantitative culturing, penicillinase (1,000 U/ml) was added to all homogenates from animals which received ampicillin regimens, in order to further minimize antibiotic carryover.

Antibiotic pharmacokinetics in serum. The pharmacokinetics of semisynthetic penicillins such as oxacillin and nafcillin, as well as those of vancomycin and sulbactam, have been well described in the recent literature for the rabbit model of endocarditis (1, 7). We did, however, study the pharmacokinetics of ampicillin at a 200-mg/kg dose to ensure that ampicillin levels in serum exceeded 15 μg/ml for a substantial proportion of the dosing interval as noted above. Blood for determination of ampicillin levels was drawn at 30, 60, 120, 180, and 240 min after a 200-mg/kg i.m. dose. Ampicillin levels were determined by high-performance liquid chromatography (courtesy of Roger Bawden, Houston, Tex.).

Statistical analysis. The Fisher's exact test (with Bonferroni's correction factor) was used for comparing proportional data, while the Kruskal-Wallis test with correction for multiple treatment groups (10) was used for comparing differences between $\log_{10}$ CFU per gram of vegetation.

RESULTS

Antibiotic susceptibility testing. The microtiter MICs (micrograms per milliliter) of the drugs for the BORSA strain were as follows: oxacillin, 2; ampicillin, 16; and sulbactam, >64. The combination of ampicillin plus sulbactam lowered the MICs for this strain to 4 μg/ml (ampicillin) and 2 μg/ml (sulbactam). In contrast, the addition of sulbactam to oxacillin did not lower the oxacillin MIC. These data were paralleled in the time kill curves; the addition of sulbactam to

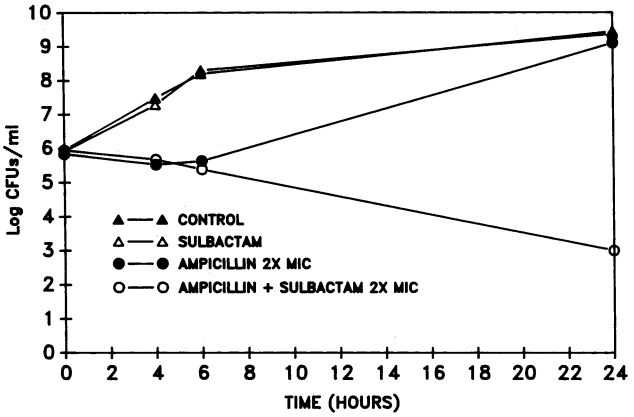

FIG. 1. Timed kill curve of β-lactamase-producing BORSA strain VP986. Each antibiotic agent was tested at twice its in vitro MIC (ampicillin alone, 32 μg/ml; sulbactam alone, 16 μg/ml; ampicillin plus sulbactam in combination, 8 and 4 μg/ml, respectively). Data are the means of two separate experimental runs.

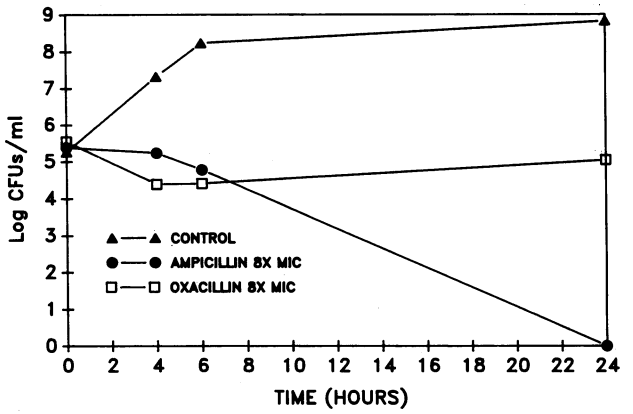

FIG. 3. Timed kill curve of β-lactamase-negative clone of ORSA strain 67-0. Each antibiotic agent was tested at eight times its in vitro MIC (ampicillin concentration, 64 μg/ml; oxacillin concentration, 512 μg/ml). Data are the means of two separate experimental runs.

ampicillin (each at 2× MIC) significantly enhanced the killing of the BORSA strain, compared with the single agents (Fig. 1); similar findings were noted with both agents at 8× MIC (data not shown). In contrast, sulbactam plus oxacillin had no effect on in vitro killing of the BORSA strain (data not shown).

The microtiter MICs (micrograms per milliliter) of the drugs for the β-lactamase-producing ORSA strain were as follows: oxacillin, 64; ampicillin, >64; vancomycin, 2; and sulbactam, >64. The microtiter MICs for the β-lactamase-negative ORSA clone were very similar to those for the β-lactamase-producing strain, except for a lower ampicillin MIC (8 μg/ml) observed for the former strain. The combination of ampicillin plus sulbactam reduced the MICs of each single agent for the β-lactamase-producing ORSA strain to 8 μg/ml for ampicillin and to 4 μg/ml for sulbactam. In contrast, the combination of sulbactam plus oxacillin did not reduce the MICs for either single agent against this strain. Timed kill curves documented that the combination of ampicillin plus sulbactam (each at 2× MIC) substantially enhanced the bactericidal effect in comparison with either single agent alone against the β-lactamase-producing ORSA strain (Fig. 2); similar kill curves were generated with each agent at 8× MIC (data not shown). In contrast, the combination of sulbactam plus oxacillin had no similar synergistic effects (data not shown). Ampicillin alone at either twice or eight times its MIC (Fig. 3) had a significant in vitro bactericidal effect against the β-lactamase-negative ORSA clone, paralleling that seen with vancomycin at similar multiples of the MIC (data not shown).

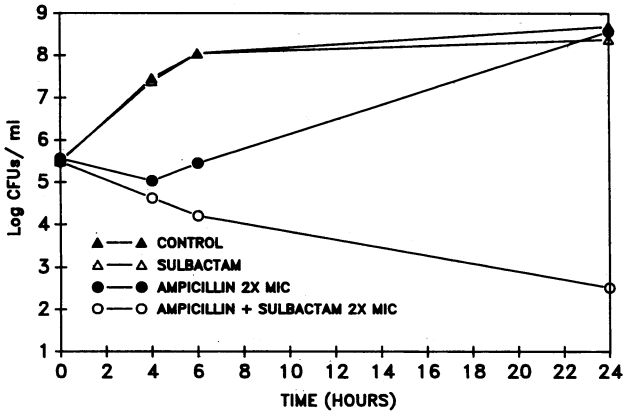

FIG. 2. Timed kill curve of β-lactamase-producing ORSA strain 67-0. Each antibiotic agent was tested at twice its in vitro MIC (ampicillin alone, 128 μg/ml; sulbactam alone, 128 μg/ml; ampicillin plus sulbactam in combination, 16 and 8 μg/ml, respectively). Data are the means of two separate experimental runs.

Experimental endocarditis. (i) BORSA. After the third day of treatment, vegetations from animals receiving either oxacillin or ampicillin plus sulbactam contained significantly lower bacterial densities than untreated controls or animals treated with ampicillin alone (Table 1). After the fifth day of therapy, all animals receiving either ampicillin alone or no therapy had either been previously sacrificed or died; in contrast, those animals receiving either oxacillin or ampicillin plus sulbactam continued to have low residual bacterial densities in vegetations, significantly lower than densities in vegetations observed with either untreated controls or animals treated with 3 days of ampicillin alone. Therapy with ampicillin plus sulbactam rendered more vegetations culture negative over the 5-day treatment period (11 of 13) than did oxacillin therapy (6 of 13), although this difference was not statistically significant.

TABLE 1. Treatment of BORSA endocarditis

Therapy group	Mean vegetation bacterial density on therapy day[a]:		
	0	3[b]	5
Control	8.07 ± 1.45 (6)	7.60 ± 1.1 (3)	—[c]
Ampicillin	ND[d]	7.66 ± 2.9 (8)	—
Oxacillin	ND	3.47 ± 1.6 (5)	2.2 ± 0.3 (8)
Ampicillin + sulbactam	ND	3.07 ± 1.6 (5)	2.05 ± 1.3 (8)

[a] Values are mean $\log_{10}$ CFU per gram of vegetation ± standard deviation. Values in parentheses are numbers of animals sacrificed.

[b] Day 3 comparisons: oxacillin versus control, $P < 0.01$; ampicillin plus sulbactam versus control, $P < 0.05$; oxacillin versus ampicillin, $P < 0.05$; ampicillin plus sulbactam versus ampicillin, $P < 0.05$.

[c] —, All animals had either died or been sacrificed.

[d] ND, Not done.

TABLE 2. Treatment of endocarditis due to ORSA β-lactamase-producing strain

Therapy group	Mean vegetation density on therapy day[a]:	
	0	5[b]
Control	9.59 ± 0.58 (9)	8.41 ± 1.6 (5)
Ampicillin	ND[c]	9.53 ± 1.3 (9)
Ampicillin + sulbactam	ND	2.33 ± 0.63 (9)
Vancomycin	ND	6.65 ± 3.1 (9)

[a] Values are mean $\log_{10}$ CFU per gram of vegetation, ± standard deviation. Values in parentheses are numbers of animals sacrificed.
[b] Day 5 comparisons: ampicillin plus sulbactam versus ampicillin, $P < 0.0005$; ampicillin plus sulbactam versus vancomycin, $P < 0.005$; vancomycin versus ampicillin, $P < 0.05$.
[c] ND, Not done.

(ii) ORSA. For endocarditis caused by the β-lactamase-producing ORSA strain (Table 2), 5 days of vancomycin treatment reduced bacterial densities in vegetations below those of untreated controls sacrificed at therapy day 0 or after the fifth day of therapy, although these differences were not statistically different. In contrast, animals treated with ampicillin plus sulbactam for 5 days had bacterial densities in vegetations significantly below those of untreated controls as well as those of animals receiving ampicillin alone or vancomycin. Although vegetations were rendered culture negative in more animals given ampicillin plus sulbactam for 5 days (five of nine) than in their counterparts given either ampicillin alone (zero of nine) or vancomycin (one of nine), these values were not significantly different.

To confirm the in vivo efficacy of ampicillin against ORSA strains, this agent was then compared with vancomycin in treatment of endocarditis caused by the β-lactamase-negative clone (Table 3). There was a significant difference between mean bacterial densities in vegetations from untreated controls sacrificed at day 0 versus those from animals receiving either ampicillin or vancomycin therapy for 5 days. Bacterial densities in vegetations from animals receiving ampicillin for 5 days were lower than those from their vancomycin-treated counterparts, although this difference did not reach statistical significance.

Antibiotic pharmacokinetics. Because of the salutary treatment outcome of animals with ORSA endocarditis receiving ampicillin-based regimens, we performed a pharmacokinetic analysis of ampicillin in this model. As noted in Fig. 4, the high-dose ampicillin treatment (200 mg/kg) resulted in peak levels above 200 μg/ml; also, ampicillin levels exceeded the in vitro ampicillin MIC for ORSA strains for ~2 h postdose (8 μg/ml for the β-lactamase-negative clone and 8 μg/ml for the β-lactamase-positive clone in the presence of 4 μg of sulbactam per ml). The area under the time-concentration curve for ampicillin was 376 μg · h/ml; the terminal half-life was 0.25 h. For comparison, recent studies from our laboratory defined the half-lives and the areas under the time-concentration curves for sulbactam and vancomycin to be 0.34 h and 27.5 μg · h/ml and 1.10 h and 180.9 μg · h/ml, respectively, in the aortic endocarditis model in rabbits (1). Chambers et al. have recently reported a mean 1-h concentration in serum and a half-life for the semisynthetic penicillin nafcillin to be 28 ± 11 μg/ml and 1.7 h, respectively, in the aortic endocarditis model in rabbits (7).

TABLE 3. Treatment of endocarditis due to ORSA β-lactamase-negative strain

Therapy group	Mean vegetation density on therapy day[a]:	
	0	5[b]
Control	9.29 ± 0.48 (5)	—
Ampicillin	ND[c]	3.67 ± 2.08 (12)
Vancomycin	ND	5.75 ± 2.60 (12)

[a] Values are mean $\log_{10}$ CFU per gram of vegetation, ± standard deviation. Values in parentheses are numbers of animals sacrificed.
[b] Day 5 comparisons: ampicillin versus day 0 controls, $P < 0.00005$; vancomycin versus day 0 controls, $P < 0.05$; ampicillin versus vancomycin, $P = 0.10$. —, All control animals had either been previously sacrificed or died.
[c] ND, Not done.

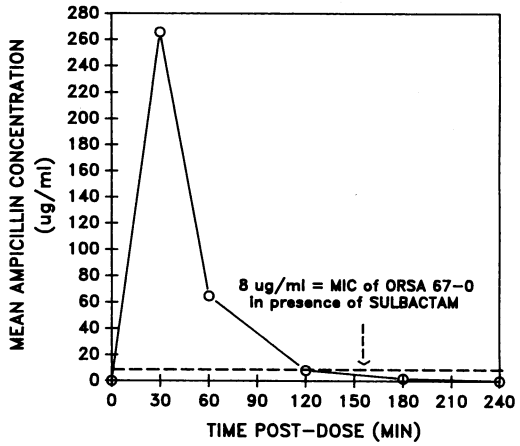

FIG. 4. Time-concentration pharmacokinetic curve following a 200 mg/kg i.m. dose of ampicillin to animals with experimental ORSA endocarditis. Each point on the curve represents the mean of at least four data sets.

DISCUSSION

Clinical strains of *S. aureus* that are resistant to the semisynthetic penicillins such as methicillin, nafcillin, and oxacillin (ORSA strains) generally share the following characteristics (11, 12): (i) heterotypic resistance, with only a small fraction of the cell population (<1%) manifesting the drug resistance phenotype; (ii) β-lactamase production; (iii) chromosomally-mediated expression of the dominant penicillin-binding protein (PBP 2a) with very low affinity for methicillin, nafcillin, or oxacillin; and (iv) uniform susceptibility to vancomycin. Of interest, the abnormal PBP 2a of ORSA strains has a relatively high affinity for penicillin G and ampicillin, with 50% saturability observed at <20-μg/ml concentrations in vitro (6, 7). Recently, a number of investigators have reported the isolation of clinical *S. aureus* strains that exhibit a borderline, or low-level, resistance to oxacillin (4, 13–15, 18) (BORSA strains; MICs between 1 and 4 μg/ml). BORSA strains generally hyperproduce extracellular β-lactamase (13); however, in contrast to typical ORSA strains, BORSA strains tend to have a normal repertoire of PBPs, with expression of PBP 2a conspicuously absent (4). The mechanism(s) of low-level oxacillin resistance in these strains has been thought to be at least in part related to β-lactamase hyperproduction (14). However, recent work by Tomasz et al. (22) has shown that, although quantitatively normal, the major PBP components of certain BORSA strains (particularly PBPs 1 and 2) may manifest relatively low affinities for methicillin. Such strains have been termed MODSA (for modified PBPs) to distinguish them from

BORSA strains containing PBPs of normal structure and function.

The current study was designed to examine in vivo the potential role of β-lactam–β-lactamase-inhibitor combinations in the therapy of experimental endocarditis caused by β-lactamase-producing ORSA and BORSA strains as well as to examine the potential in vivo activity of ampicillin in the therapy of ORSA infections. Several interesting findings came from this investigation. It appears that BORSA strains act more like oxacillin-susceptible strains than true ORSA strains both in vitro and in vivo. In vitro, β-lactamase-producing BORSA strains were readily killed by oxacillin, with little added bactericidal benefit accruing from the addition of the β-lactamase inhibitor sulbactam. In contrast, whereas ampicillin had poor in vitro activity against BORSA strains, presumably because of hyperproduction of β-lactamase, the addition of sulbactam to ampicillin substantially enhanced the inhibitory and bactericidal activities of the β-lactam. These findings were mirrored in vivo in experimental endocarditis due to the BORSA strain. Ampicillin alone was ineffective in this model, while ampicillin plus sulbactam and oxacillin were each highly efficacious in reducing intravegetation BORSA densities. Our data parallel the recent findings of Thauvin-Eliopoulos et al. with the rat model of BORSA endocarditis in which β-lactam–β-lactamase-inhibitor combinations proved salutary (21). It thus appears from our data and others that infections caused by BORSA strains respond therapeutically more like those caused by oxacillin-susceptible *S. aureus* than ORSA strains and would not require vancomycin for cure.

Of particular interest, ampicillin demonstrated potent in vitro and in vivo activity against ORSA strains in our study. In vitro, the addition of sulbactam to ampicillin substantially enhanced the inhibitory and bactericidal activities of the β-lactam against a β-lactamase-producing ORSA strain, rendering the β-lactam now as active in vitro as vancomycin. The ampicillin concentration utilized for timed kill curve analyses (16 μg/ml) mirrored that reported by Chambers and Sachdeva (6) to show high-affinity binding to PBP 2a of the ORSA strain used in our study. These investigators reported that at 15 and 17 μg of ampicillin and penicillin G per ml, respectively, ~50% saturation of PBP 2a was observed by radiofluorometric densitometry; of note, these 50% PBP saturation levels for ampicillin and penicillin G corresponded closely to the in vitro MIC for the resistant subpopulation of this heterogeneous ORSA strain (6). In the current study, ampicillin-based regimens were superior to vancomycin regimens in the therapy for ORSA endocarditis. For the β-lactamase-producing ORSA strain, the ability to lower intravegetation bacterial densities was substantially better with ampicillin plus sulbactam than with vancomycin; similar findings were seen with ampicillin treatment alone and vancomycin therapy for ORSA endocarditis caused by the β-lactamase-negative clone. A similar efficacy of β-lactam–β-lactamase inhibitor agents in the treatment of ORSA endocarditis was recently reported by Chambers et al. (ticarcillin-clavulanate, ampicillin-sulbactam [7]); however, in that study, only a minority of vegetations were sterilized because of the persistence of a resistant subpopulation of ORSA cells. The somewhat better outcome in the current study, vis-à-vis lower mean intravegetation bacterial densities, is undoubtedly related to the higher-ampicillin-dosage strategy used in our study (200 mg/kg administered four times daily versus 100 mg/kg administered thrice daily [7]). Cantoni et al. (2) recently used a high dosage of amoxicillin (150 mg/kg every 5 h) in combination with clavulanate (30 mg/kg every 5 h) in the rat model of aortic ORSA endocarditis and achieved in vivo efficacies similar to that observed with ampicillin-sulbactam in the current investigation.

The relatively inferior outcome with vancomycin treatment in our investigation is probably multifactorial and may be related to the short treatment course (5 days) or the relatively low dosage (30 mg/kg/day) of vancomycin used; Chambers et al. (7) and others have reported more salutary outcomes with higher-dosage vancomycin therapy (50 mg/kg/day) of experimental ORSA endocarditis. However, it has also recently been stressed by Small and Chambers (19) that intrinsic kill rates of *S. aureus* strains may be substantially slower with vancomycin than with β-lactams. Moreover, Cremieux et al. (8) have recently shown that the complex glycopeptides, such as teicoplanin and vancomycin, tend to penetrate infected experimental cardiac vegetations to a lesser degree than either β-lactam or aminoglycoside agents.

The high-dose ampicillin treatment strategies for ORSA endocarditis in the current study may be criticized for being well above the recommended doses for humans. However, one of the goals of our study was to attempt to confirm the in vivo biological significance of prior in vitro findings of relatively avid PBP 2a binding of ampicillin in ORSA strains. In order to do this, such high-ampicillin-dose regimens were required to reliably approach or exceed the 50% PBP 2a saturation level (15 μg/ml) for a significant part of the dosing interval (2 h). Although it is unlikely that such dose regimens could be utilized clinically for human ORSA infections, our findings and those of Cantoni et al. (2) and Chambers et al. (7) should give credence to the potential role of high-avidity PBP 2a-binding β-lactams in treating ORSA infections. Also, these studies will, we hope, provide impetus for further development of novel β-lactam compounds with high-level PBP 2a binding affinities, making future clinical dosage strategies for serious ORSA infections feasible.

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