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β-Lactamase-Mediated Inactivation and Efficacy of Cefazolin and Cefmetazole in *Staphylococcus aureus* Abscesses

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Clinical reports and animal models have demonstrated that cefazolin may have decreased efficacy against some strains of *Staphylococcus aureus* because of type A β-lactamase-mediated hydrolysis. We sought to measure biologically active cefazolin concentrations within abscesses with high concentrations of *S. aureus* and compare the concentrations with those of cefmetazole, a β-lactamase-stable cephamycin. A type A β-lactamase-producing strain of *S. aureus* with a demonstrated inoculum effect against cefazolin (MIC at an inoculum of 5×10^5 CFU/ml, 1.0 μg/ml; MIC at an inoculum of 5×10^7 CFU/ml, 32.0 μg/ml) but not cefmetazole (MICs at inocula of 5×10^5 and 5×10^7 CFU/ml, 2.0 μg/ml) was used. Cefazolin or cefmetazole (100 mg/kg of body weight every 8 h for 8 days) was administered to rabbits with infected tissue cages. No differences in the concentrations of the two drugs in the uninfected tissue cages were observed. Higher concentrations of cefmetazole than cefazolin were found in infected tissue cages at day 3 (5.9 ± 0.7 versus 2.2 ± 0.3 μg/ml; $P < 0.01$), day 5 (9.1 ± 2.6 versus 3.6 ± 0.7 μg/ml; $P = 0.02$), and day 8 (9.4 ± 1.4 versus 4.8 ± 0.9 μg/ml; $P = 0.01$) after infection. Cefazolin and cefmetazole were equally effective in reducing the bacterial concentration in the abscess. In vitro experiments demonstrated greater cefazolin than cefmetazole degradation by *S. aureus*, but the differences were greater in serum than in abscess fluid supernatants. We conclude that abscess cefazolin concentrations are diminished by type A β-lactamase-producing *S. aureus*, but this did not affect drug efficacy in this model.

Some cephalosporins, especially cefazolin and cephaloridine, are relatively more susceptible in vitro to staphylococcal β-lactamases (20). Despite reports of cephalosporin failure in the treatment of *Staphylococcus aureus* infections in humans (3) and in animal models (5, 9), the relevance of these findings remains controversial, in part because of the presence of conflicting reports (4).

Some of the discrepancies reported may be due to differences in staphylococcal β-lactamases. Zygmunt et al. (27) demonstrated that type A β-lactamase more efficiently hydrolyzes cefazolin and cephaloridine than cefamandole and cefuroxime. In contrast, type B and type C β-lactamases more efficiently hydrolyze cefamandole than cefazolin. A small number of clinical trials also suggest that β-lactamase activity may be clinically important. Although conflicting data have been reported (6), some studies of cephalosporins used as prophylaxis in cardiac surgery have indicated that either cefamandole (13, 22) or cefuroxime (22) is more effective than cefazolin in prophylaxis of *S. aureus* wound infections. In addition, Kernodle et al. (14) demonstrated that staphylococcal type A β-lactamase production is associated with a greater number of clean wound infections with cefazolin prophylaxis than with cefamandole prophylaxis. No studies have measured the degree of staphylococcal β-lactamase inactivation at the infection site and correlated the degree of antibiotic deactivation with efficacy.

The goals of this investigation were (i) to use an *S. aureus* strain of known cephalosporinase activity (type A β-lactamase) to determine the degree of cefazolin inactivation at the infection site compared with that of a more stable cephamycin, (ii) to determine whether lower concentrations of an unstable cephalosporin in tissue adversely affect drug efficacy when the concentration remains greater than the MIC, and (iii) to compare antibiotic deactivation by β-lactamase in serum with deactivation at the site of a purulent infection. We chose to compare the results for cefazolin with those for cefmetazole because cefmetazole is known to be very stable to hydrolysis by the type A β-lactamase from strain PC-1 (which contains the plasmid on which the structural gene for the type A β-lactamase is located) (21, 27) and because it has pharmacokinetics similar to those of cefazolin in humans (12).

MATERIALS AND METHODS

Abscess model. New Zealand White rabbits were anesthetized, and three table tennis balls with 300 1.5-mm holes were implanted in the peritoneal cavities of the rabbits as described previously (7). The balls (capsules) became encased in connective tissue, developed a blood supply, and became filled with a sterile fluid that had the appearance of serum by 6 weeks after surgery. The capsules were then inoculated with 5×10^5 CFU of an *S. aureus* strain that was initially isolated from a bacteremic patient. Bacterial counts were performed by aspirating the capsule fluid, sonicating the sample at 90 W (Sonic dismembrator model 300; Fisher, Springfield, N.J.) for 15 s, serial diluting (10-fold), plating 100-μl volumes onto blood agar plates, and incubating the blood agar plates for 24 h. The lower limit of detection was 10^2 CFU/ml.

Abscess fluid supernatants. At 14 days after bacterial inoculation, a supernatant pool of abscess fluid was prepared from animals not used in the treatment studies. The capsules were aspirated, centrifuged at 200 × *g* for 20 min, filter sterilized (pore size, 0.45 μm; Acrodisc; Gelman, Ann Arbor, Mich.), pooled, and stored at −70°C for later in vitro analysis.

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Treatment studies. Additional rabbits with capsules that had been infected for 14 days were given cefazolin (100 mg/kg of body weight) or cefmetazole (100 mg/kg) intramuscularly every 8 h for 8 days or were given no antibiotic. Capsule sampling for bacterial and antibiotic quantification was repeated 2 h after the antibiotic dose after days 3, 5, and 8 of antibiotic administration. At day 5, blood was obtained at 45 min, 105 min, and 8 h after antibiotic administration for determination of antimicrobial concentrations in serum. The results of the efficacy experiments for cefmetazole were compared with the previously reported results for cefazolin and the controls that received no antibiotic (2).

Antibiotic assays. Antibiotics were measured by bioassay (1) by using *Bacillus subtilis* ATCC 6633 as the test organism for cefazolin and *Micrococcus luteus* ATCC 9341 as the test organism for cefmetazole. Antibiotic levels in serum or abscess fluid were based on standard curves made by using known antibiotic dilutions prepared with both normal rabbit serum and pooled abscess fluid supernatants. These curves, however, were superimposable. The lower limit of sensitivity for both antimicrobial agents was 1.2 μg/ml. For cefazolin, the mean r value for the standard curves was 0.9983 ± 0.0005 (n = 5); for cefmetazole the r value was 0.9926 ± 0.0021 (n = 7). The coefficient of variation between assay runs for cefazolin at a concentration of 250 μg/ml was 4.77%, and at 5 μg/ml it was 5.04%; for cefmetazole it was 5.51% at 250 μg/ml and 17.6% at 5 μg/ml.

In vitro antimicrobial tests. The MIC and MBC for *S. aureus* were determined by the method of Taylor et al. (25) (inoculum, 2.5 × 10^5 CFU/ml). In addition, MIC testing at inocula of 5.0 × 10^5 and 5 × 10^7 CFU/ml (9) was performed. In vitro antibiotic deactivation assays were performed by adding 200 μl of 3.8 × 10^8 or 3.8 × 10^7 CFU of *S. aureus* per ml to 1.8 ml of either sterile filtered abscess fluid or rabbit serum containing 32 μg of either cefazolin or cefmetazole per ml. Tubes were incubated at 35°C for 24 h. At 0, 3, and 24 h, 400 μl was removed, sterile filtered, and frozen at −70°C for antibiotic quantification.

β-Lactamase typing and quantification. The *S. aureus* β-lactamase was typed by using ratios of substrate hydrolysis and was quantified by D. S. Kernodle, Vanderbilt University, Nashville, Tenn., by previously described methods (15).

Statistics. Results are expressed as means ± standard errors of the means. The results of the antibiotic concentrations were compared by a Student's t test. In the treatment studies in which means were calculated, a $\log_{10}$ CFU per milliliter value of less than 2 was averaged as 2. Results of the efficacy experiments were compared statistically by the Kruskal-Wallis H test, and if a difference was found, a comparison between two groups was done by the Mann-Whitney U test.

RESULTS

The MICs and MBCs for the test *S. aureus* strain at an inoculum of 2.5 × 10^5 CFU/ml were 0.5 and 1.0 μg/ml, respectively, for cefazolin and 2.0 and 8.0 μg/ml, respectively, for cefmetazole. At an inoculum of 5 × 10^5 CFU/ml, the cefazolin and cefmetazole MICs were 1.0 and 2.0 μg/ml, respectively. At an inoculum of 5 × 10^7 CFU/ml, the MICs were 32.0 and 2.0 μg/ml, respectively. The *S. aureus* strain used was typed as a type A β-lactamase producer. Following induction of β-lactamase production by growth on agar containing subinhibitory concentrations of methicillin for 14 h (15), the strain degraded cefazolin at a rate of 0.23 μg/min/10^8 CFU.

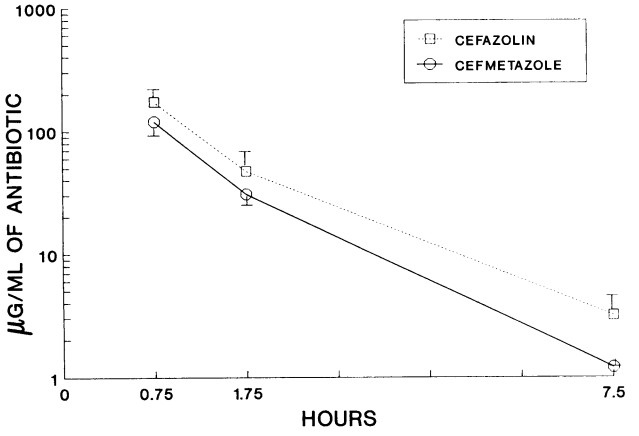

FIG. 1. Concentrations of cefazolin and cefmetazole in serum after administration of 100 mg/kg intramuscularly on day 5 of antimicrobial treatment. Bars represent standard errors.

The mean antibiotic concentration measured in serum at 0.75 h was higher for cefazolin than cefmetazole (173 ± 14.4 versus 120 ± 15.7 μg/ml; P = 0.024; n = 9) (Fig. 1). The cefazolin and cefmetazole half-lives in serum were 31.7 and 30.3 min, respectively. In only one animal was the concentration of either drug detectable in serum when it was measured at 7.5 h. It received cefazolin, and the concentration in serum was 9 μg/ml.

Five capsules in animals treated with cefazolin and six capsules in animals treated with cefmetazole cleared the infection ($\log_{10}$ CFU/ml, <2). Antibiotic concentrations in these capsules after the infection cleared were similar: 17.0 ± 6.0 μg/ml for cefazolin and 18.0 ± 6.9 μg/ml for cefmetazole. However, in infected capsules, cefmetazole concentrations were significantly higher than the concentrations of cefazolin on each day on which they were measured (Fig. 2).

The efficacy results are shown in Fig. 3. Combining all treatment groups and the controls, there was a mean of 7.25 ± 0.10 CFU/ml at day 0, with no significant differences

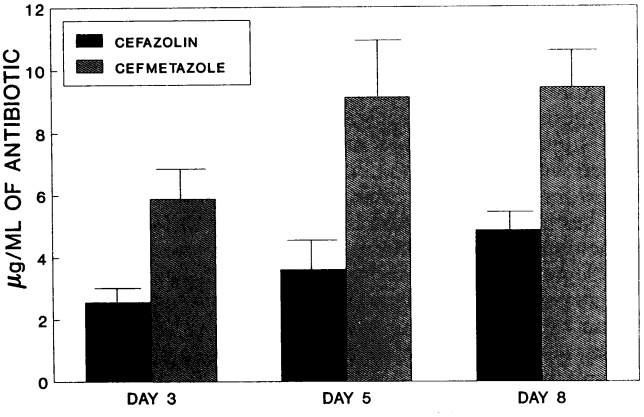

FIG. 2. Concentrations of cefazolin and cefmetazole in infected capsules at day 3 (P < 0.01 between cefazolin [n = 18] and cefmetazole [n = 10]), day 5 (P = 0.02 between cefazolin [n = 17] and cefmetazole [n = 17]), and day 8 (P = 0.01 between cefazolin [n = 18] and cefmetazole [n = 11]). Bars represent standard errors.

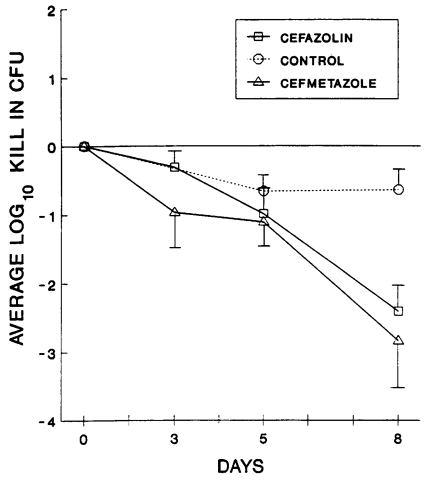

FIG. 3. Antimicrobial efficacy in the abscess model. At day 8, P was <0.01 between each treatment group and the controls (n = 7), and P was >0.10 between the cefmetazole (n = 16) and cefazolin (n = 18) treatment groups. Bars represent standard errors.

between the groups. There were no differences in efficacy between cefazolin and cefmetazole on day 3, 5, or 8.

In the in vitro antibiotic deactivation assay, cefazolin was degraded more than cefmetazole (Table 1). Cefazolin inactivation was greater in serum than in the abscess fluid supernatant, and inactivation of cefazolin demonstrated a dose-response effect, since more inactivation was observed with a higher concentration of bacteria.

DISCUSSION

The results of the study indicate that after administration of identical doses, cefazolin was present at lower concentrations than cefmetazole within an *S. aureus* abscess. Since peak cefazolin concentrations in serum were higher than those of cefmetazole and concentrations in uninfected tissue cages were similar for cefazolin and cefmetazole, the likely explanation for this finding is cefazolin degradation by the type A staphylococcal β-lactamase. Although use of a radiolabeled drug would have been helpful in proving drug deactivation, our study is supported by similar work of O'Keefe et al. (19) and Joiner et al. (11), who found that *Bacteroides fragilis* β-lactamase production is a major determinant of antibiotic concentrations in abscesses for drugs susceptible to hydrolysis by the β-lactamase.

We measured drug concentrations in the tissue cage 2 h after the antibiotic dose. Previous studies with this model have demonstrated very slow elimination of cephalosporins from the capsule fluid (7, 17), so it is unlikely that measuring the concentration in the capsule fluid at other times would have affected our results. Previous studies have demonstrated that cefazolin is 61.2 to 95% protein bound in rabbit serum at concentrations of 82 to 100 μg/ml (6, 17), while cefmetazole is 53 to 59% protein bound at concentrations of 30 to 100 μg/ml (16, 18). Although we did not measure protein binding in our studies, the slightly higher level of protein binding of cefazolin measured previously would have favored higher steady-state cefazolin concentrations in the infected tissue site, since it is a protein-containing extravascular site (7, 8, 13).

Despite the decreased concentration of cefazolin compared with that of cefmetazole, the drugs were equally efficacious in reducing the bacterial concentration within the abscess, perhaps because the mean concentration of cefazolin remained greater than the MIC on all days tested. We tested only one staphylococcal strain known to produce type A β-lactamase and demonstrated to have an in vitro inoculum effect. Had we used a strain that produced more β-lactamase, it is possible that the concentration of cefazolin in the abscess would have fallen below the MIC, which could have affected our efficacy results. In a neutropenic mouse infection model, the duration of time when the cefazolin concentration exceeds the MIC is most predictive of efficacy (26). Therefore, some degree of drug deactivation may not affect efficacy as long as the concentration exceeds the MIC. The issue remains controversial, however, since in rabbit meningitis models in which neutrophils are present, β-lactam efficacy is enhanced as the drug concentration increases up to 100 times the MBC (24). Of note is that in our model, although the concentration of cefazolin in the abscess was lower than the concentration of cefmetazole, the concentration-to-MIC ratios were similar since cefmetazole had a slightly higher MIC when measured at inocula of 2.5×10^5 or 5.0×10^5 CFU/ml.

The results of the in vitro antibiotic deactivation assay presented in Table 1 demonstrate that cefazolin deactivation is greater in serum than in abscess fluid supernatant. The human (3) and animal model studies (5, 9, 23) that have demonstrated that cefazolin is less effective in the treatment of *S. aureus* infections were all either endocarditis studies or studies which produced rapidly fatal high-grade bacteremia. To our knowledge, no study has demonstrated a decreased efficacy of cefazolin in the treatment of more localized purulent infections.

Cefazolin use for the treatment of staphylococcal infections has recently been termed "a drug for your mother-in-law, not for your mother" (10). Our study has demonstrated for the first time in an animal model that an organism that produces only a moderate amount (14) of type A β-lactamase can result in lower antibiotic concentrations at the site of infection compared with the concentrations of a more stable cephamycin. The concentration remained greater than the MIC, however, and efficacy was not affected, perhaps because cefazolin is degraded to a lesser degree in an abscess supernatant than it is in serum. Further work should be done in an attempt to correlate the degree of β-lactamase production in vitro with the degree of drug deactivation and efficacy in vivo in order to understand the role of cefazolin in the treatment of localized staphylococcal infections.

TABLE 1. Cefazolin and cefmetazole inactivation in vitro after 24 h of incubation with *S. aureus*

Medium	% Inactivation[a]			
	High inoculum		Low inoculum	
	Cefazolin	Cefmetazole	Cefazolin	Cefmetazole
Abscess fluid	48.4	26.8	35.8	26.8
Serum	80.3	41.2	48.4	32.1

[a] High-inoculum tubes contained 3.8×10^7 CFU/ml; low-inoculum tubes contained 3.8×10^6 CFU/ml. All tubes contained 32 μg of the antimicrobial agent per ml at time zero.

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