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Evaluation of Bacterial Interference and β-Lactamase Production in Management of Experimental Infection with Group A Beta-Hemolytic Streptococci

ITZHAK BROOK* AND JAMES D. GILMORE
Naval Medical Research Institute, Bethesda, Maryland 20814-5055

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The in vivo effects of penicillin and cefprozil therapy on the interaction between organisms commonly recovered from inflamed tonsils were studied by using a subcutaneous abscess model in mice. These organisms were group A beta-hemolytic streptococci (GABHS), *Streptococcus salivarius* (which is capable of interfering with GABHS), and *Staphylococcus aureus*. In mice infected with GABHS and *S. salivarius* alone or in combination, penicillin eliminated both organisms and cefprozil eliminated GABHS and *S. aureus* but not *S. salivarius*. Penicillin did not, however, reduce the number of GABHS or *S. salivarius* in the presence of *S. aureus*. The present study demonstrated the ability of β-lactamase-producing *S. aureus* to protect GABHS from penicillin. However, no such protection was present following the administration of cefprozil. Furthermore, the preservation of *S. salivarius* that interferes with GABHS growth may provide protection from reinfection with GABHS. This study supports and provides an explanation for the increased efficacies of cephalosporins administered orally over that of penicillin when treating patients with acute GABHS pharyngitis or tonsillitis.

The failure of penicillin to eradicate group A beta-hemolytic streptococci (GABHS) from the throats of an appreciable proportion of patients with streptococcal pharyngitis or tonsillitis is of clinical concern (8). Various theories have been offered as explanations for this phenomenon. One explanation is that β-lactamase-producing bacteria (BLPB) can protect GABHS by inactivating penicillin (1, 22). An alternative explanation is that preservation of the alpha-hemolytic streptococci (AHS) as part of the normal oral flora is an important element in the eradication of GABHS. Some of these bacteria have been shown to compete and thus interfere with the growth of GABHS (7, 9). Since AHS are very susceptible to penicillin, eradication of AHS by penicillin may create a microbiological "vacuum," allowing reinfection or the persistence of GABHS.

The higher eradication rates consistently seen with cephalosporins administered orally over that seen with penicillin in the therapy of pharyngitis or tonsillitis caused by GABHS (18) has largely been unexplained. One advantage of the orally administered cephalosporins is their activity against BLPB, such as *Staphylococcus aureus* (16). In contrast, orally administered cephalosporins are generally less effective than penicillin against AHS, while they maintain good activity against GABHS (12). We theorized that the improved clinical efficacy of the orally administered cephalosporins in treating pharyngitis or tonsillitis caused by GABHS resides in their ability to eradicate *S. aureus* and preserve AHS.

The present study was designed to investigate the effects of penicillin and cefprozil therapy on the in vivo interaction between organisms commonly recovered from inflamed tonsils by using a subcutaneous abscess model in mice. These organisms were GABHS, AHS (*Streptococcus salivarius*, which is capable of interfering with GABHS), and a β-lactamase-producing *S. aureus* isolate.

* Corresponding author.

MATERIALS AND METHODS

Antimicrobial agents. The drugs penicillin V (150 mg/kg of body weight per day) and cefprozil (30 mg/kg/day) were used. They were dissolved in 0.1-ml volumes of sterile water and were given by oral gavage at intervals of 12 h starting 24 h after inoculation with the organisms.

Organisms. The organisms used in the experiments described here were recent clinical isolates collected from patients with acute tonsillopharyngitis. These included one isolate each of GABHS, *S. salivarius*, and *S. aureus*. The GABHS was a mucoid encapsulated isolate that was determined to be type 18 with T-agglutination. The bacteria were kept frozen in skim milk at −70°C. The organisms were identified by conventional methods (14). The *S. aureus* strain was a β-lactamase producer, as determined by the chromogenic cephalosporin method (17). The *S. salivarius* isolate tested was found to possess an inhibiting substance toward GABHS, as determined by the method of Grahn et al. (10).

Animals. All experiments were performed with 10- to 12-week-old $B_6D_2F_1$ female mice (weight, 20 to 25 g) raised under conventional conditions. Mice were obtained from Jackson Laboratories, Bar Harbor, Maine.

Susceptibility tests. Isolates were tested for their susceptibilities to antimicrobial agents by the microdilution technique (14) in Schaedler's broth enriched with vitamin K_1 and hemin (England Laboratories, Beltsville, Md.).

Inoculum preparation. The bacteria were grown on blood agar with a brain heart infusion base (Difco). The mice were inoculated subcutaneously in the right groin with 0.1 ml of the appropriate bacterial suspension at a concentration of 10^9 organisms per ml. The inoculated bacteria were harvested from bacteria at the logarithmic phase of growth.

Examination of abscesses. Animals were killed by cervical dislocation on the seventh day after inoculation, and the abscess material was removed aseptically. The site and the histology of the abscesses were confirmed by hematoxylin and eosin staining. Abscesses were homogenized inside a glove box in 1.0 ml of sterile saline in a ground glass tissue

TABLE 1. Effects of therapy on number of organisms in abscesses in mice[a]

Inoculum	Therapy	Mean ± SD log no. of bacteria/abscess			Mean ± SD peak level of antibiotic in abscess (μg/g)
		GABHS	*S. salivarius*	*S. aureus*	
Single-organism infection					
GABHS	None	9.1 ± 0.6			0
	Penicillin	NG[b]			38.2 ± 4.0
	Cefprozil	NG			10.6 ± 1.2
S. aureus	None			8.7 ± 0.8	0
	Penicillin			7.5 ± 0.4	5.0 ± 0.6
	Cefprozil			2.4 ± 0.4	11.6 ± 1.2
Mixed-organism infection					
GABHS, *S. salivarius*	None	7.4 ± 0.6	7.0 ± 0.4		0
	Penicillin	NG	NG		36.0 ± 3.5
	Cefprozil	NG	5.8 ± 0.4		10.8 ± 1.0
GABHS, *S. aureus*	None	9.9 ± 0.7		9.2 ± 0.6	0
	Penicillin	8.1 ± 0.6		8.6 ± 0.7	28.6 ± 0.4
	Cefprozil	NG		NG	
GABHS, *S. salivarius*, *S. aureus*	None	8.1 ± 0.8	6.8 ± 0.7	9.8 ± 0.8	0
	Penicillin	7.8 ± 0.6	5.1 ± 0.4	8.5 ± 0.6	6.2 ± 0.5
	Cefprozil	NG	4.7 ± 0.7	2.0 ± 0.4	11.4 ± 0.9

[a] There were 10 mice in each group.
[b] NG, no growth.

homogenizer. Tenfold serial dilutions of the homogenates were made with sterile saline, and 0.1 ml of each dilution was spread in triplicate onto enriched brain heart infusion and blood agar plates. Colonies were counted after the plates were incubated for 48 h at 37°C in a CO_2 incubator (14). Characteristic colonies of all organisms were identified by Gram staining and biochemical tests (14).

Experimental designs. Each experimental group included 30 mice. One group each was inoculated with a single organism: GABHS, *S. aureus*, or *S. salivarius*. One group each was inoculated with a combination of two or three organisms: GABHS with *S. salivarius*, GABHS with *S. aureus*, and GABHS with *S. salivarius* and *S. aureus*. Each group was further divided into two subgroups that received different antimicrobial therapies and a control subgroup of 10 mice each. These subgroups received either penicillin V, cefprozil, or sterile saline. Each experiment was repeated twice.

Measurement of levels of antimicrobial agents. The levels of antimicrobial agents in the abscesses were measured in specimens collected on the fifth day of therapy at 1 h after drug administration (expected peak). Because of the small amount of pus, the specimens from five mice in each group were pooled. The antibiotic levels in the abscess specimens from the mice were determined by a modified well diffusion assay technique (14) with *Micrococcus luteus* ATCC 9341 (American Type Culture Collection, Rockville, Md.).

Statistical analyses. Statistical analyses were performed by Student's *t* test of independent means.

RESULTS

Abscess induced by a single organism. A subcutaneous abscess was induced in 96% of the mice inoculated with GABHS, 100% of the mice inoculated with *S. aureus*, and none of the mice inoculated with *S. salivarius* alone. Without therapy, the abscesses reached a maximum size of 5 to 9 mm in diameter within 5 to 7 days. Up to 90% of the abscesses drained spontaneously at 9 to 12 days and healed within 18 to 24 days. There was no mortality or extension of the infection to other sites.

Abscess induced by mixtures of two or three organisms. GABHS was inoculated in combination with either *S. salivarius* or *S. aureus* or was inoculated with both organisms (Table 1). Following injection of 0.2 to 0.3 ml of saline containing 10^8 of each organism, an abscess developed within 24 to 48 h without therapy. The abscesses reached a maximum size of 10 to 16 mm within 5 to 7 days and drained spontaneously at 8 to 12 days. Of interest is the fact that the number of GABHS was significantly less when GABHS were mixed with *S. salivarius* than when they were used alone.

Histological examination of the abscess. Histological studies of two abscesses selected from each group (Table 1) were shown to have a central area of necrosis, fibrin, and bacteria surrounded by a band of leukocytes and a distinct collagen capsule.

Effect of therapy on bacterial counts per abscess. In the groups of mice inoculated with GABHS alone, no viable organisms were recovered from the site of inoculation following treatment with penicillin or cefprozil. The mean number of *S. aureus* was not significantly reduced by penicillin, but it was lowered by cefprozil ($P < 0.001$). In mice infected with a combination of GABHS and *S. salivarius*, penicillin eliminated both bacteria. In contrast, cefprozil eradicated GABHS but preserved *S. salivarius*. In mice infected with GABHS and *S. aureus*, penicillin was ineffective in reducing the number of either organism; however, cefprozil eliminated both organisms. In mice infected with GABHS, *S. salivarius*, and *S. aureus*, penicillin did not significantly reduce the number of any organism. In contrast, cefprozil eliminated GABHS, significantly reduced the number of *S. aureus* ($P < 0.05$), and did not alter the number of *S. salivarius*.

In vitro susceptibilities. Penicillin was effective against GABHS and *S. salivarius* (MIC, 0.06 mg/liter), but it was inactive against *S. aureus* (MIC, 128 mg/liter). Cefprozil was

active against GABHS (MIC, 0.12 mg/liter) and *S. aureus* (MIC, 1 mg/liter), but not *S. salivarius* (MIC, 32 mg/liter).

Antibiotic concentrations. There were no significant differences in the concentrations of cefprozil in the abscess contents among animals who were inoculated with a single bacterium or multiple bacteria (Table 1). The penicillin concentrations in abscesses in which *S. aureus* was present (alone or in combination) were lower than those found in abscesses induced by GABHS ($P < 0.05$).

DISCUSSION

The present study demonstrated the ability of β-lactamase-producing strains of *S. aureus* to protect GABHS from penicillin in vivo. There was no such protection when the animals were treated with cefprozil, which is stable to the β-lactamase produced by *S. aureus* (12).

In vitro and in vivo studies have demonstrated the protection phenomenon. A 200-fold increase in the resistance of GABHS to penicillin was observed when it was inoculated with *S. aureus* (22). An increase in resistance was also noted when GABHS were grown with *Haemophilus parainfluenzae* (21). When GABHS were mixed with cultures of *Bacteroides fragilis*, the resistance of GABHS to penicillin increased 8,500-fold (4).

Several studies in animals demonstrated the ability of the enzyme β-lactamase to influence polymicrobial infections. Hackman and Wilkins (11) showed that penicillin-resistant strains of *B. fragilis*, *Prevotella melaninogenica*, and *Prevotella oralis* protected a penicillin-susceptible *Fusobacterium necrophorum* isolate from penicillin therapy in mice. Brook and colleagues (3), utilizing a subcutaneous abscess model in mice, demonstrated the protection of GABHS from penicillin by *B. fragilis* and *P. melaninogenica*.

Recurrent pharyngitis or tonsillitis caused by GABHS is still a serious clinical problem. Failure to eradicate GABHS from patients treated with penicillin can occasionally lead to rheumatic fever and, rarely, to glomerulonephritis. As a last resort, many physicians refer their patients for elective tonsillectomy.

One explanation for the failures of penicillin described above is that repeated penicillin administration may result in a shift of the oral microbial flora, resulting in selection of BLPB (1) that, by hydrolyzing penicillin in the area of the infection, can protect not only themselves but also penicillin-susceptible pathogens. The recovery of aerobic and anaerobic BLPB in over three-fourths of the patients with recurrent GABHS tonsillitis (6, 19, 23), the ability to measure β-lactamase activity in the core of their tonsils (5), and their responses to antimicrobial agents that are effective against BLPB (2, 13) support the role of these organisms in the inability of penicillin to eradicate GABHS from patients with recurrent tonsillitis.

The presence of AHS that inhibit the growth of GABHS was first described by Crowe and colleagues (7). They found that increased bacteriocin production by AHS resulted following GABHS colonization. This led to the hypothesis that bacteriocin production may result from a selective pressure exerted by GABHS and also that these substances might actually inhibit colonization of the upper respiratory tract and/or aid in the eradication of GABHS. Roos and colleagues (20) demonstrated that both production of β-lactamase by the normal oropharyngeal flora and the lack of colonization of the pharynx with inhibiting AHS correlated with the failure of penicillin to cure GABHS tonsillitis.

The results of the present study help to explain why a cephalosporin may be more effective than penicillin in eradicating susceptible isolates of GABHS in patients with pharyngitis or tonsillitis. This involves the combination of the two phenomena: the effect of BLPB and the presence of interfering AHS. When all three organisms (GABHS, *S. aureus*, and *S. salivarius*) were present, penicillin failed to eradicate GABHS because it was inactivated by the β-lactamase produced by *S. aureus*. In contrast, cefprozil, which is stable to the β-lactamase produced by *S. aureus*, eliminated GABHS and reduced the number of *S. aureus* but did not reduce the number of *S. salivarius*. This outcome is a desirable one, since not only is GABHS eliminated but the presence of *S. salivarius*, which can interfere with the regrowth of GABHS and thus decrease the potential for relapse, is also preserved. This phenomenon may also occur in the clinical setting, where GABHS is eliminated from the pharynx or tonsils but both are still colonized with the interfering AHS, which may prevent reinfection with GABHS.

Although only a single strain of each organism was used in the present study, the isolates that we used represent typical isolates of each bacterial species. The GABHS used were the most common ones that cause tonsillitis (15), and the susceptibilities of AHS to cephalosporin were similar to those of 8 of 10 *S. salivarius* isolates that we studied (1a). The present study supports and provides an explanation for the increased efficacy of orally administered cephalosporins over those of penicillins in eradicating GABHS from patients with acute pharyngitis or tonsillitis (18). However, further clinical studies are needed to verify these findings in patients.

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