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In Vitro and In Vivo Efficacy of the Combination Trimethoprim-Sulfamethoxazole against Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*

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The in vitro susceptibilities of 16 independent, geographically distinct clinical isolates of methicillin-resistant *Staphylococcus aureus* to trimethoprim (TMP) in combination with sulfamethoxazole (SMX) were evaluated. Although methicillin-resistant *S. aureus* strains appear to be universally resistant to SMX, the combination TMP-SMX was found to be synergistic in vitro (in combination, the MICs of both drugs decreased 6- to 25-fold) as well as in vivo (5- to 6-fold reduction in TMP at 50% effective doses).

Since 1974, methicillin-resistant *Staphylococcus aureus* has emerged as both a nosocomial and a community-acquired pathogen in the United States. These strains have become established hospital flora, with infections caused by methicillin-resistant *S. aureus* becoming increasingly prevalent (13, 16). Vancomycin (VM) hydrochloride is generally effective in the treatment of infections involving methicillin-resistant *S. aureus*, although poor clinical responses have been observed, perhaps caused by the tolerance of *S. aureus* to VM (5, 7, 15). The combination of trimethoprim (TMP)-sulfamethoxazole (SMX) has been reported to be active in vitro against these resistant strains, although only a limited number of isolates have been examined. In addition, several anecdotal reports suggest that TMP-SMX is valuable in the treatment of *S. aureus* infections unresponsive to seemingly adequate antibiotic treatment (2, 4, 12, 15). This study compares the in vitro activities of VM, TMP, SMX, and the combination TMP-SMX against a heterogeneous collection of clinical strains of methicillin-resistant *S. aureus* and investigates whether the combination of TMP and SMX is synergistic in vitro and in vivo.

TMP-lactate and SMX were provided as standard powders by Burroughs Wellcome Co., Research Triangle Park, N.C. VM hydrochloride was obtained from Sigma Chemical Co., St. Louis, Mo.

We studied 16 strains of methicillin-resistant *S. aureus* (MICs, 25 to >100 μg/ml). Disk diffusion susceptibility tests with methicillin were performed in our laboratory to confirm the resistance of these strains (11). The clinical isolates were obtained from the Centers for Disease Control, Atlanta, Ga., and represent independent, geographically distinct strains (Linda McDougal, personal communication). Identification was confirmed biochemically by the use of standard Gram stain, catalase, and coagulase tests.

MICs and MBCs were determined by a microtiter broth dilution technique similar to that described by Gavan and Barry (6). Briefly, fresh bacterial suspensions were made in Wellcotest broth (Burroughs Wellcome Co.) supplemented with NaCl (2%) from overnight cultures and adjusted to a 0.5 McFarland turbidity standard to contain 10^8 CFU/ml. Wellcotest broth is a thymidine-free, all-purpose bacteriological growth medium. Twofold dilutions of antimicrobial agents, performed with a semiautomated microtiter system by using Wellcotest broth supplemented with NaCl (2%) (Dynatech Laboratories, Inc., Alexandria, Va.), were inoculated with 0.010 ml of a 1:16 dilution of the bacterial suspension for a final concentration of $>5 \times 10^5$ CFU/ml. All plates were incubated at 30°C for 48 h. The MIC was defined as the lowest concentration of the antibiotic that prevented visible growth. MBC determinations were performed by subculturing 0.010-ml samples from wells showing no visible growth and incubating them for 48 h at 30°C. The MBC was defined as the lowest antibiotic concentration showing ≥99.9% killing of the initial bacterial inoculum at 48 h (1). Parallel tests were performed with a lower-salt medium (0.1% NaCl) and a higher incubation temperature (37°C) as independent variables.

The MICs and MBCs of VM, TMP, SMX, and TMP-SMX (1:20 fixed ratio) for the 16 *S. aureus* strains examined in this study are shown in Table 1. VM demonstrated good in vitro activity against all strains of methicillin-resistant *S. aureus*, as did TMP. In contrast, SMX showed relatively poor in vitro activity against these isolates (MICs = 50 μg/ml for 13 of 16 strains). These data support those of Seligman (14), who found that all of the 47 strains of methicillin-resistant *S. aureus* that he examined were resistant to >64 μg of SMX per ml. In addition, VM, TMP, and TMP-SMX showed excellent bactericidal activity against these isolates. For all organisms tested, the MBC was no more than twofold greater than the MIC (Table 1). Duplicate tests, using a lower NaCl concentration (0.1%) in the growth medium and a higher incubation temperature (37°C), showed virtually identical MIC and MBC results (data not shown).

The definition of synergy is dependent on methodology and to some degree is quite arbitrary (10; L. S. Young, Clin. Microbiol. Newsl. 2:1–3, 1980). Synergy was defined for this study as a fourfold or greater decrease in the MIC when agents were combined. Clearly, the combination of TMP-SMX is synergistic in vitro; TMP and SMX demonstrated 6- to 12.5-fold and 12.5- to 25-fold decreases, respectively (MIC_{90}s; Table 1), despite the fact that the strains were relatively resistant to SMX as a single agent.

To extend these in vitro observations, the capacity for TMP and SMX to potentiate each other in vivo against strains of methicillin-resistant *S. aureus* was examined in an animal model. Based on preliminary mouse virulence studies, two isolates (*S. aureus* 48-676-71 and 48-126-72) were selected, and three replicate studies were performed with

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TABLE 1. In vitro activity of VM, TMP, SMX, and TMP-SMX (1:20 ratio) against 16 strains of methicillin-resistant *S. aureus*[a]

Antimicrobial agent	MIC (MBC) in μg/ml		
	50%[b]	90%[c]	Range
VM	1.6 (1.6)	1.6 (1.6)	1.6–3.1 (1.6–3.1)
TMP	1.2 (1.2)	2.5 (2.5)	0.3–2.5 (0.3–2.5)
SMX	50.0 (100.0)	50.0 (100.0)	25.0–50.0 (50.0–100.0)
TMP-SMX	0.2/4.0 (0.2/4.0)	0.2/4.0 (0.2/4.0)	0.05/1.0–0.4/8.0 (0.1/2.0–0.8/16.0)

[a] Test conditions: incubation temperature, 30°C; test medium, Wellcotest broth supplemented with NaCl (2%).
[b] MIC (MBC) for 50% of strains tested.
[c] MIC (MBC) for 90% of strains tested.

TABLE 2. Therapeutic efficacy of TMP, SMX, and the combination TMP-SMX against methicillin-resistant *S. aureus* infections in mice

Methicillin-resistant *S. aureus* strain	Compound	MIC (μg/ml)	ED_{50}[a] (mg/kg)	95% Confidence limit
48-676-71	TMP-SMX	0.2-4.0[b]	9.6[c]	8.1–11.0
	TMP	0.8	58.1	31.0–85.2
	SMX	50.0	333.0	133.7–532.3
48-126-72	TMP-SMX	0.2-4.0[b]	6.4[c]	2.9–9.4
	TMP	0.8	32.5	24.4–40.6
	SMX	50.0	194.2	121.7–266.7

[a] Mean values from three separate studies; see the text.
[b] MIC at 1:20 ratio TMP-SMX.
[c] ED_{50} of the TMP component; this value is one-fifth the SMX ED_{50}. For both *S. aureus* strains, the ED_{50}s of TMP in combination with SMX were significantly lower ($P < 0.05$) than the ED_{50}s of TMP alone.

each isolate. Cultures were grown overnight at 30°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with NaCl (2%). Female CD-1 strain mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were infected by an intraperitoneal injection of 0.5 ml of diluted overnight cultures in 3% mucin (type 1701-W; Wilson Laboratories, Pharmaceutical Div., Wilson and Co., Chicago, Ill.) and randomized to treatment groups. Inocula were standardized to produce 90 to 100% mortality in vehicle-treated mice in 72 h. Serial twofold dilutions of TMP, SMX, and the combination of TMP-SMX (at the 1:5 ratio used clinically) were administered orally to groups of 10 mice each at 1, 3, and 5 h postinfection. Individual 50% effective dose (ED_{50}) values were calculated from the number of mice surviving for 4 days by probit analysis using Litchfield's correction for groups with 100 or 0% survival (9); subsequent analyses were performed on sets of three such values obtained from the replicate studies.

The results of these studies are presented in Table 2. Consistent with the in vitro results, TMP alone was more active against the methicillin-resistant *S. aureus* isolates tested than SMX alone was. In addition, treatment with the TMP-SMX combination resulted in significantly improved therapeutic activity as evidenced by a five- to sixfold reduction in TMP ED_{50}s. The magnitude of this improved activity suggests that the combination of TMP-SMX is synergistic in vivo.

This study confirmed and extended a previous observation (14) that TMP-SMX is an effective combination against methicillin-resistant *S. aureus* in vitro. Importantly, the 16 strains of methicillin-resistant *S. aureus* examined here represented independent, geographically distinct isolates rather than a single isolate possibly spread by cross infection in a single institution. TMP-SMX proved to be an effective combination in vitro against these strains, showing bactericidal activity at the MIC (or twofold higher). It should be noted, however, that Ellison et al. (3) were unable to permanently eradicate the methicillin-resistant *S. aureus* carrier state by using the combination TMP-SMX in colonized patients; however, many therapeutically effective drugs do not eradicate the carrier state. All methicillin-resistant *S. aureus* clinical isolates we have examined to date were resistant to SMX, with MICs ranging from 25 to >100 μg/ml. The precise nature of this apparent linkage of SMX and methicillin-resistance is unclear. Despite this SMX resistance, the combination of TMP-SMX proved to be synergistic in vitro as well as in vivo.

It should be noted that attainable levels of TMP-SMX in human plasma exceed the MICs and MBCs of the combination for the methicillin-resistant *S. aureus* isolates reported in this study (Table 1). For example, a single oral administration of the equivalent of 160 mg of TMP-800 mg of SMX resulted in mean peak blood levels (1 to 4 h after administration) of 1.2 to 1.9 μg of TMP and 25 to 60 μg of non-protein-bound SMX per ml in healthy adult volunteers (8). Thus, these pharmacokinetic data, as well as the experimental data presented in this study and preliminary clinical results (N. Markowitz, L. Saravolatz, D. Pohlod, C. Cendrowski, E. Quinn, M. Somervile, R. Del Busto, J. Cardenas, and E. Fisher, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 903, 1985), suggest that the combination of TMP-SMX may be a valuable alternative agent for the treatment of infection caused by methicillin-resistant *S. aureus*.

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