In Vitro Activities of Trimethoprim and Sulfamethoxazole Against *Listeria monocyto genes*

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The in vitro activities of trimethoprim and sulfamethoxazole against clinical isolates of *Listeria monocyto genes* were examined separately and in combination with a microtiter broth dilution system. Sulfamethoxazole demonstrated variable activity and was generally bacteriostatic. Trimethoprim alone was bactericidal against 96% of isolates at $\leq 0.5 \mu g/ml$. The bactericidal action of trimethoprim against *L. monocyto genes* was generally potentiated by sulfamethoxazole even when isolates were relatively resistant to sulfamethoxazole alone.

*Listeria monocyto genes* is an important cause of bacteremia and meningitis in immunocompromised patients (6, 12, 19). Standard therapy with ampicillin or penicillin G may result in relapse (19, 22, 28, 30) This may be related to the fact that many strains of *Listeria*, although inhibited by relatively low concentrations of penicillin, require much higher levels for bactericidal effect (14, 20). Alternative drugs include tetracyclines, erythromycin, and chloramphenicol, which are also bacteriostatic in activity against *Listeria* (15, 32). Although the addition of aminoglycosides to a penicillin results in enhanced in vitro killing (14, 20) and improved survival in some animal models of listeric infection (9, 26), the clinical superiority of combination therapy has not been established. There is no bactericidal antibiotic for use in penicillin-allergic patients.

In this study, we present data concerning the in vitro activities of trimethoprim (TMP) and sulfamethoxazole (SMZ) separately and in combination (TMP-SMZ) against 26 clinical isolates of *L. monocyto genes*.

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**MATERIALS AND METHODS**

**Bacterial strains and media.** All strains tested were clinical isolates recovered from blood or cerebrospinal fluid of patients from New England, Mid-Atlantic, South Atlantic, and South Central states. A variety of serotypes were tested. The medium used for broth dilution studies was tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) supplemented with thymidine phosphorylase (0.1 IU/ml; Burroughs-Wellcome Research Laboratories, Research Triangle Park, N.C.). This medium was chosen since preliminary experiments confirmed that the bacteria grew well in this medium and endpoints were much easier to determine than with Mueller-Hinton broth. This has been confirmed by others (32).

**Antimicrobial agents.** TMP and SMZ (supplied by Hoffmann-LaRoche, Inc., Nutley, N.J.) were initially dissolved to make stock solutions containing 1,000 and 19,000 $\mu g/ml$, respectively. All stock solutions were filter sterilized (Nalge, Inc., Rochester, N.Y.) and stored at $-70^\circ C$ until use. Dilutions of antimicrobial agents were prepared in sterile test tubes (18 by 150 mm).

**Susceptibility testing and antimicrobial interaction.** Isolates were tested for susceptibility to SMZ and TMP separately and in combination, using a fixed ratio of 19 parts SMZ to 1 part TMP. Serial twofold dilutions were made, and from these tubes, standard 96-well U-bottom microtiter plates were filled, using a 96-channel semiautomatic dispenser (MIC-2000; Dynatech Laboratories, Inc., Alexandria, Va.) to dispense 100 $\mu l$ per well (34). An overnight growth of bacteria in broth was further diluted in TSB, and the turbidity was adjusted to a MacFarland 1 standard, approximately $3.5 \times 10^8$ colony-forming units per ml. Four milliliters of standardized inoculum was added to 36 ml of sterile water. Using a replicator inoculator (Dynatech Laboratories), we added 0.0015 ml of inoculum, as prepared above, to each well, resulting in a final inoculum size of approximately $5 \times 10^4$ colony-forming units per ml. Plates were sealed with transparent plastic and incubated 18 to 20 h at 35°C. Minimal inhibitory concentrations (MICs) were determined by visual inspection. *Streptococcus faecalis* (ATCC 29212) was used as a control organism (21). A 0.001-ml calibrated loop was inserted into the bottom of each well not showing visible growth and agitated. This material was subcultured to antibiotic-free sheep blood agar plates and incubated overnight twice in a CO$_2$ incubator at 35°C. Minimum bactericidal concentrations (MBC; approximately $\geq 99.8\%$ killing) were defined as the concentration of the antibiotic yielding

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no more than one colony on subculture. Each strain was tested three to six times. If a discrepancy between trials was noted, the median value was used. Representative strains were also examined by time-kill curves with TMP and SMZ separately and in combination by previously described techniques (20).

Synergy was defined in terms of MBCs, using the fractional lethal concentration index (FLC) (3). This was derived by the formula \([\text{MBC of SMZ in presence of TMP}/\text{MBC of SMZ alone}] + [\text{MBC of TMP in presence of SMZ}/\text{MBC of TMP alone}]\). Synergy was present when the FLC was \(\leq 0.5\); an additive (partially synergistic) effect was said to be present when the FLC was \(>0.5\) and \(<1\). Indifference was present when the MBC of TMP was unchanged (FLC = 1), and antagonism was said to be present when the MBC was greater after the addition of SMZ (FLC > 1).

**RESULTS**

The MICs and MBCs of SMZ and TMP separately and in combination for *Listeria* isolates are shown in Fig. 1. SMZ inhibited 90% of strains at \(\leq 9.5\) \(\mu\)g/ml; however, only 50% of the strains were killed by 152 \(\mu\)g/ml, and all strains had MBCs of \(\geq 19\) \(\mu\)g/ml (Fig. 1). TMP alone inhibited all isolates at \(\leq 0.06\) \(\mu\)g/ml, and 96% were killed by \(\leq 0.5\) \(\mu\)g/ml. A single isolate (serotype 4b, from Charity Hospital, New Orleans, La.), although inhibited by 0.03 \(\mu\)g/ml, yielded growth on subculture despite 500 \(\mu\)g of TMP per ml when used as a single agent and 304 \(\mu\)g of SMZ per ml with 16 \(\mu\)g of TMP per ml in combination. This "tolerance" of the lethal effect of TMP was present in both stationary and log-phase cultures and was present at 48 as well as 24 h.

Using an FLC of \(\leq 0.5\) as the criterion for synergy, synergy could be demonstrated with 16 of the 26 strains, and an additive effect was demonstrated with 9 of the 26. Although the MIC of the single TMP-tolerant strain was reduced from 0.03 to 0.015 \(\mu\)g/ml in the presence of 0.3 \(\mu\)g of SMZ per ml, an effect of the MBC could not be determined. Antagonism was not encountered.

Four strains were also tested by time-kill curves with an inoculum size of \(10^6\) colony-forming units per ml, and a typical strain which was synergistically killed by the combination of SMZ and TMP is shown in Fig. 2. Concentrations of agents were chosen so that the concentration of SMZ was less than the bacterium's MIC, and TMP exceeded the MIC but was less than the MBC. SMZ at a concentration of 0.6 \(\mu\)g/ml yielded a growth curve identical to growth in antibiotic-free broth. An approximately 10-fold reduction of bacterial counts was observed after exposure to TMP at its MIC at 24 h, but

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**FIG. 1.** Activities of SMZ and TMP separately and in combination.
concentrations of bacteria approached that seen in the control by 48 h. For the strains examined by killing curves, a 100-fold increase in killing by the addition of SMZ to TMP was seen when the concentration of TMP was less than its MBC. The increased rate of killing by the 20:1 combination of SMZ and TMP was not apparent until 24 h of incubation, at which time >99.9% of the original inoculum had been killed. The bactericidal effect persisted at 48 h.

**DISCUSSION**

In general, the use of bactericidal antimicrobial agents appears to be desirable (and often necessary) in the therapy of bacterial meningitis (24). In meningitis, despite the presence of abundant phagocytic cells in the cerebrospinal fluid, opsonization is impaired due to the relatively low concentration of complement present (7, 11, 31). In addition, listerias may escape killing by their ability to survive intracellularly (17).

The fixed combination of TMP and SMZ is often bactericidal against susceptible species (1, 5). TMP-SMZ has been used with success in many infections due to intracellular pathogens, including *Pneumocystis carinii*, *Chlamydia trachomatis*, *Salmonella typhi*, *Nocardia asteroides*, *Brucella sp.*, *Pseudomonas pseudomallei*, *Mycobacterium marinum*, and *Plasmodium falciparum* (35). Both TMP and SMZ penetrate well into the central nervous system (2, 23, 29, 33). This combination shows promise in the therapy of meningitis due to a variety of pathogens (8, 10, 13, 25), and at least two patients with meningitis due to *L. monocytogenes* have been treated successfully with TMP-SMZ (16, 18). It is of historical interest that many patients (most before 1960) with listeriosis were treated successfully with sulfonamides alone, in doses ranging from 0.5 to 10 g daily (27). Despite this, there are few data in the literature on the in vitro susceptibility of *Listeria* to TMP-SMZ, although Boisivon recently has independently demonstrated the bactericidal activity of TMP-SMZ in fixed combination against *Listeria* (4).

In this study, we found that SMZ as a single agent displayed variable activity against *Listeria*, with some isolates being highly susceptible and even killed by ≤19 μg/ml, whereas others were not even inhibited by as much as 304 μg/ml. In contrast, TMP alone was bactericidal at ≤0.5 μg/ml against 25 of 26 isolates, and the bactericidal effect of TMP was often potentiated by SMZ even when isolates were relatively resistant to SMZ as a single agent. The striking bactericidal activity of TMP against *Listeria* prompted us to look at a number of other TMP-containing combinations in vitro. We have recently shown that the bactericidal action of TMP is antagonized by the addition of ampicillin,
doxycycline, and rifampin (D. L. Winslow and G. A. Pankey, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, A3, p. 1). These latter antibiotics are bacteriostatic against Listeria, and combinations containing these agents do not appear to have any advantage in vitro over ampicillin or penicillin. However, we believe TMP alone or in combination with SMZ merits in vivo study in the treatment of listeric infections.

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