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Combined Activity of Sulfamethoxazole, Trimethoprim, and Polymyxin B Against Gram-Negative Bacilli

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The activity of the three two-drug combinations of sulfamethoxazole (SMX), trimethoprim (TMP), and polymyxin B (PB) against 52 clinical isolates of gram-negative bacilli was studied by a "checkerboard" agar dilution method. The organisms studied included strains of *Enterobacter* spp., *Klebsiella pneumoniae*, *Serratia marcescens*, *Providence*, *Proteus*, and *Pseudomonas aeruginosa*. The majority of these isolates were resistant to at least two of the three agents used in the combined studies and to the most commonly used antimicrobials. The TMP-PB combination demonstrated enhanced activity more frequently than the other two-drug combinations, showing synergism or addition in 85% of the combined studies; indifference or antagonism was also observed least frequently with TMP-PB. The great majority (83%) of *Enterobacter-Klebsiella-Serratia* isolates were susceptible to enhanced activity of all combinations. *Proteus-Providence* isolates were frequently susceptible (63%), but combined activity was indifferent or antagonistic against 60% of *P. aeruginosa*. Twelve isolates were selected for "killing-curve" assays in which an inoculum was incubated with SMX, TMP, and PB individually and in various two- and three-drug combinations. Surviving bacteria were counted at timed intervals over 24 h of incubation. The triple combination (SMX-TMP-PB) was synergistic against 9 of 12 isolates, whereas TMP-PB and SMX-PB showed synergism against 5 and 3 isolates, respectively. These data suggest that, although TMP-PB will often show enhanced activity against the gram-negative bacilli studied here, optimal antibacterial activity will be demonstrated when the three-drug combination is used.

A number of clinical and laboratory studies have indicated that combining the polymyxins with sulfonamides and trimethoprim (TMP) may well enhance antimicrobial activity by additive or synergistic mechanisms. The polymyxins and sulfonamides have been shown to exhibit synergistic activity against some strains of *Proteus*, *Pseudomonas*, and *Serratia* (9, 17, 20). In addition, the polymyxins and TMP are synergistic against some strains of *Serratia marcescens* and *Proteus* (9, 13). These combinations have been used on several occasions to successfully treat patients with serious infections due to these organisms (13, 20).

Greenfield and Feingold (9) demonstrated that sulfonamides and TMP both could sensitize *Serratia* to the lethal action of polymyxin B (PB) and suggested that the three-drug combination might be useful in the therapy of severe infections due to gram-negative bacilli. Simmons (21), using disk diffusion techniques, showed that the combination of colistin (polymyxin E), sulfamethoxazole (SMX), and TMP was more active than combinations of any two of these agents against 66 of 72 gram-negative bacillus strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella*, *Shigella*, and *Klebsiella aerogenes*). However, interpretation of results obtained by his technique for demonstrating synergism is difficult, and the organisms tested would be expected to be susceptible to a number of commonly used antimicrobials.

We decided to determine the antibacterial activity of SMX-TMP-PB combinations by using techniques for studying synergism with which there has been extensive experience. Selected as test organisms were those gram-negative bacilli resistant to multiple antimicrobials including, not infrequently, gentamicin and

carbenicillin. It would seem that any potential therapeutic application of the SMX-TMP-PB combination(s) would be against these resistant organisms.

MATERIALS AND METHODS

Chemicals and bacteria. PB (Aerosporin) and TMP were provided as standard laboratory powders by Burroughs Wellcome and Co., Inc. SMX (standard powder) was obtained from the Hoffmann-LaRoche Co., Inc. Stock solutions of each antimicrobial were prepared in concentrations 10 and 20 times the desired final concentration with sterile distilled water as the diluent. A few drops of 1 N NaOH were necessary to dissolve the SMX. Volumes of each antibiotic were stored frozen. Mueller-Hinton agar and broth and Trypticase soy agar were Difco products. Fresh, defibrinated horse blood was lysed by alternate freezing and thawing.

Bacterial strains were isolated from clinical material and identified by the Bacteriology Laboratory of the Wadsworth Veterans Administration Hospital Center. Isolates were recovered from a variety of clinical specimens, although the majority were from urines. A total of 52 clinical isolates was studied (12 *K. pneumoniae*, 9 *Enterobacter* spp., 9 indole-positive *Proteus* species, 8 *S. marcescens*, 6 *Providence*, and 8 *P. aeruginosa*). A standard inoculum was prepared from 6- or 24-h Mueller-Hinton broth cultures of each organism by adjusting the turbidity to an opacity equivalent to a 0.5 McFarland nephelometer density standard. A 1:10 dilution of this was used as the final inoculum. Saline was the diluent in the agar plate dilution method, and Mueller-Hinton broth was used for the "killing curves."

MIC of individual antimicrobials. The individual minimal inhibitory concentration (MIC) of PB, SMX, and TMP for 52 isolates of gram-negative bacilli was determined by an agar plate dilution method using a Steers replicator (23). Agar plates containing appropriate dilutions of each antimicrobial were prepared by mixing 17 ml of melted (and cooled to 48 to 50 C) Mueller-Hinton agar with 1.0 ml of lysed horse blood and 2.0 ml of the antimicrobial (at 10 times the final concentration). This mixture was then poured into a petri dish. Control plates were prepared by substituting sterile, distilled water for the antimicrobial. Each well of the replicator block was filled with the standard inoculum, and the mechanical inoculator was used to inoculate each plate in triplicate. Control plates were inoculated before and after each set of antimicrobial plates, and all plates were incubated at 37 C for 18 to 24 h.

The MIC was recorded as the lowest concentration of antimicrobial inhibiting at least 80% of growth. In almost all instances this corresponded to total inhibition of growth.

Studies of combined antimicrobial activity. So-called checkerboard MIC studies with two-drug combinations were by the method of Sabath (18). The following combinations were studied: PB-SMX, PB-TMP, and SMX-TMP. An agar plate dilution method with a Steers replicator was used as described above, except that 1.0 ml of each of the two antimicrobials (at 20 times final concentration) was added to the melted-agar tube before the plate was poured. The checkerboard was achieved by combining in separate plates each of the four concentrations of one agent (e.g., TMP: 12.5, 6.25, 3.12, and 1.56 μg/ml) with each of the four concentrations of the second agent (e.g., SMX: 200, 100, 50, and 25 μg/ml). Thus, there would be separate plates containing 12.5 μg of TMP and 200 μg of SMX per ml, 12.5 μg of TMP and 100 μg of SMX per ml, 12.5 μg of TMP and 50 μg of SMX per ml, etc. MICs were determined for each antimicrobial in the combination, and isobolograms (Sabath) were plotted.

Killing-curve assays of combined antimicrobial activity were performed against 12 bacterial isolates. These isolates were expected to show some degree of susceptibility to combined antimicrobial activity on the basis of results from the checkerboard studies. A technique similar to that of Bulger and Kirby (3) was used. Basically, killing of bacteria after incubation in tubes containing individual and various two- and three-drug combinations of SMX, TMP, and PB was measured by counting colonies of survivors at various times. The concentrations of antimicrobials used were based on demonstrated clinically achievable blood levels after high-dose therapy, i.e., 135-mg PB, 1.0-g TMP, and 6.0-g SMX daily doses. They also approximated the 1:20 optimum for TMP-SMX synergism. The concentrations were: PB, 1.0 to 3.0 μg/ml; TMP, 5 to 10 μg/ml (14); and SMX, 100 μg/ml. Specific concentrations used in each assay were subinhibitory for the particular organism being studied. For each assay, a series of eight tubes was set up, one tube as the control, three tubes containing a single antimicrobial, three tubes with each two-drug combination, and one tube containing all three antimicrobials. The single antimicrobial tube contained 9.4 ml of inoculum, 0.5 ml of lysed defibrinated horse blood, and 0.1 ml of antimicrobial. The two-agent combination tubes contained 9.3 ml of inoculum, 0.5 ml of lysed defibrinated horse blood, 0.1 ml of antimicrobial no. 1, and 0.1 ml of antimicrobial no. 2. The three-drug combination tube contained 9.2 ml of inoculum, 0.5 ml of lysed defibrinated horse blood, and 0.1 ml of each antimicrobial. The control tube contained no antimicrobial. Tubes were incubated at 37 C. At the start of incubation and after 2, 4, 6, and 24 h, portions were obtained from each tube for making pour plates. Several dilutions were made in Mueller-Hinton broth, mixed in tubes of melted Trypticase soy agar, poured immediately into petri dishes, and incubated at 37 C for 24 h. Colony counts were performed, and killing curves were plotted for the assay with each of the 12 organisms studied.

RESULTS

MIC. Table 1 summarizes the MIC data for PB, SMX, and TMP individually against clinical isolates of gram-negative bacilli. The two categories of MICs chosen for each agent are those expected to be below and above readily achievable serum levels with conventional

TABLE 1. *MIC data*

Organism	Total no. of strains	No. of strains in each MIC category[a]					
		PB[b]		SMX		TMP	
		≤1.56	≥12.5	≤50	≥100	≤1.56	≥6.25
Enterobacter spp.	9	3	6	0	9	6	3
Klebsiella pneumoniae	12	12	0	0	12	12	0
Proteus (indole +)	9	0	9	0	9	1	8
Providence	6	0	6	0	6	0	6
Pseudomonas aeruginosa	8	8	0	1	7	0	8
Serratia marcescens	8	1	7	0	8	2	6

[a] Number of strains of each organism susceptible (listed under lower MIC) or resistant (listed under higher MIC) to each antimicrobial.
[b] Drug.

doses. The nine strains of indole-positive *Proteus* sp. consisted of three strains of *P. morganii*, five strains of *P. rettgeri*, and one strain of *P. vulgaris*. *Enterobacter* strains were not speciated. These data show that 24 of 52 strains were inhibited by ≤1.56 μg of PB per ml (susceptible), and the remainder (28 of 52) were inhibited by concentrations of PB ≥12.5 μg/ml (resistant). *K. pneumoniae* and *P. aeruginosa* strains were all susceptible, whereas 22 of 23 *Proteus*, *Providence*, and *S. marcescens* strains were resistant. Six of nine *Enterobacter* spp. strains were resistant.

All except one of the 52 strains were resistant to SMX, being inhibited only by ≥100 μg/ml. A single strain of *P. aeruginosa* was inhibited by 50 μg or less of SMX per ml and would be considered susceptible.

All 12 strains of *K. pneumoniae* and 6 of 9 *Enterobacter* spp. strains were susceptible to TMP (MIC ≤1.56 μg/ml). Twenty-eight of the remaining 31 strains (includes *Proteus*, *Providence*, *P. aeruginosa*, and *S. marcescens*) were resistant (MIC ≥6.25 μg of TMP per ml). Only one of nine and two of eight *Proteus* and *S. marcescens* strains, respectively, were susceptible.

These data demonstrate that the majority of all strains were resistant to at least two of the three antimicrobial agents studied. In addition, most strains of *Proteus*, *Providence*, and *S. marcescens* were resistant to all three antimicrobials. This pattern of general resistance serves as a basis for demonstrating enhanced activity against these microorganisms by various combinations of PB, SMX, and TMP.

Checkerboard studies. Figure 1 illustrates four examples of isobolograms constructed from the checkerboard MIC data obtained with the bacterial strains in this study. A more or less straight line is seen in isobol 1A when PB and SMX are combined against a strain of *S. marcescens*, denoting additive activity. Isobol 1B is concave or bowed inwardly, demonstrating synergism between TMP and SMX against a *Providence* strain. The horizontal and vertical limbs of isobol 1C meet at approximately a right angle, representing indifferent activity between PB and SMX against a strain of *P. morganii* (lack of any effect of one agent on the activity of the other). Isobol 1D is convex or bows outwardly away from the individual MIC values

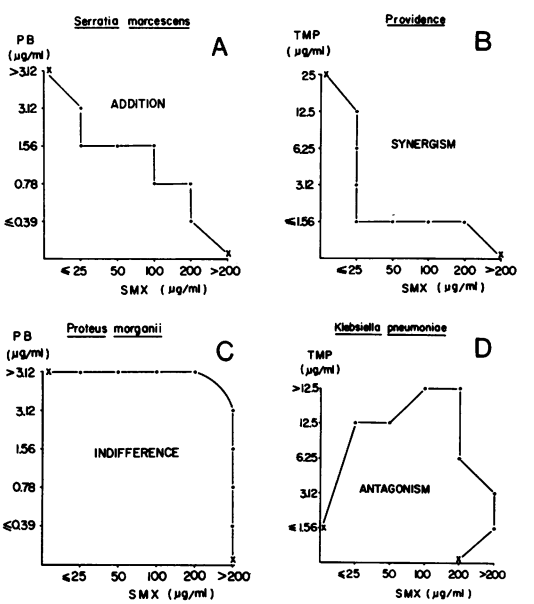

FIG. 1. *Isobolograms constructed from checkerboard MIC data showing additive combinations of PB, SMX, and TMP against gram-negative bacilli. Concentration (A), synergistic (B), indifferent (C), or antagonistic (D) activity of value marked by "X" denotes MIC of that antimicrobial for the specific organism.*

(marked by the X's on the coordinates) for TMP and SMX against this strain of *K. pneumoniae*. This pattern indicates antagonism.

Table 2 summarizes the data derived from all the isobolograms prepared from the results of the checkerboard studies. Three isobols (one for each two-drug combination) were prepared for each bacterial strain (total of 156 isobols). However, 51 isobols had to be discarded because MIC values for at least one of the antimicrobials were too low to permit determination of combined activity. Four strains (one *Enterobacter* spp., two *K. pneumoniae*, and one *P. aeruginosa*) were entirely eliminated because MIC values were too low on all three isobols. Enhanced activity (instances of synergism or additive activity in bracketed figures) occurred 31 of 46 times (67%) with the SMX-PB combination and 23 of 27 times (85%) with TMP-PB (Table 2). For these two combinations, additive activity occurred more frequently than synergism. Enhanced activity occurred somewhat less frequently (17 of 32 times or 53%) when TMP and SMX were combined, although synergism was demonstrated more often than addition (11 versus 6 times). This combination showed indifferent activity more often than the other two (13 of 32 times or 41%). Antagonism occurred most frequently (8 of 46 times or 17%) when SMX and PB were combined. The TMP-PB combination, then, demonstrated enhanced activity more frequently and indifference or antagonism less frequently than the other two-drug combinations against all the organisms considered here.

TABLE 2. *Activity[a] of antimicrobial combinations*

Antimicrobial combination	Synergism[b]	Addition	Enhanced activity[c]	Indifference	Antagonism	Total[d] isobols
SMX-PB	9	22	31	7	8	46
TMP-PB	9	14	23	2	2	27
TMP-SMS	11	6	17	13	2	32

[a] Type of activity determined from isobolograms constructed from checkerboard MIC studies against gram-negative bacilli.

[b] Indicates number of isobols in each category; i.e., 9 isobols showed synergism between PB and SMX, whereas 22 showed additive activity.

[c] Total isobols showing enhanced (synergism or addition) activity between each antimicrobial combination.

[d] Total isobols showing combined activity of each combination. Unequal totals resulted because 51 of 156 isobols were discarded due to excessively low MIC values for at least one of the antimicrobials.

In Table 3 the different types of combined activity are tabulated according to the frequency with which each was demonstrated against three different groups of gram-negative bacilli. The numbers represent the total number of combined tests (or isobols), including all three two-drug combinations. The great majority (37 of 44 or 83%) of *Enterobacter-Klebsiella-Serratia* isolates were susceptible to enhanced activity; synergism and addition occurred with about the same frequency. Enhanced activity was also demonstrated frequently (26 of 41 times or 63%) against *Proteus-Providence* isolates.

On the other hand, the data show indifferent or antagonistic activity against 12 of 20 (60%) *P. aeruginosa* isolates. Clearly, these organisms will infrequently be susceptible to the activity of any of these two-drug combinations, whereas the *Enterobacter-Klebsiella-Serratia* group will be susceptible most of the time and *Proteus-Providence* will often be susceptible.

Table 4 breaks down the susceptibility of

TABLE 3. *Combined activity[a] against groups of gram-negative bacilli*

Bacilli	Synergism (29)[b]	Addition (42)	Enhanced activity[c]	Indifference (22)	Antagonism (12)	Enhanced activity	Total isobols[d] (105)
Enterobacter-Klebsiella-Serratia	19	18	37	2	5	7	44
Proteus-Providence	9	17	26	15	0		41
Pseudomonas	1	7	8	5	7	12	20

[a] Type of combined activity (synergism, etc.) determined from isobolograms constructed from "checkerboard" MIC studies of each two-drug combination; i.e., SMX + PB, TMP + PB or TMP + SMX.

[b] Indicates number of isobols (or tests of combined activity) demonstrating each type of activity against each group of gram-negative bacilli; i.e., of 44 isobols constructed for the *Enterobacter-Klebsiella-Serratia* group, 19 showed synergistic activity of the various two-drug combinations against these organisms.

[c] Total isobols showing enhanced (synergism or addition) or lack of enhanced (indifference or antagonism) activity between the two-drug combinations against each group of organisms.

[d] Total isobols showing all types of combined activity of each combination. Unequal totals resulted because 51 of 156 isobols were discarded because of excessively low MIC values for at least one of the antimicrobials.

TABLE 4. *Combined activity[a] of each two-drug combination against groups of gram-negative bacilli*

Antimicrobial combination	Synergism[b]	Addition	Enhanced activity[c]	Indifference	Antagonism	Total isobols[d]
Enterobacter-Klebsiella-Serratia						
SMX-PB	9	12	21	2	3	26
TMP-PB	2	5	7	0	0	7
TMP-SMX	8	1	9	0	2	11
Proteus-Providence						
SMX-PB	0	9	9	5	0	14
TMP-PB	7	7	14	0	0	14
TMP-SMX	2	1	3	10	0	13
Pseudomonas						
SMX-PB	0	1	1	0	5	6
TMP-PB	0	2	2	2	2	6
TMP-SMX	1	4	5	3	0	8

[a] Type of combined activity (synergism, etc.) determined from isobolograms constructed from checkerboard MIC studies.

[b] Indicates number of isobols (or tests of combined activity) demonstrating each type of activity against each group of gram-negative bacilli; i.e., of 26 isobols constructed for the SMX-PB combination against the *Enterobacter-Klebsiella-Serratia* group, 9 showed synergism.

[c] Total isobols showing enhanced (synergism or addition) activity between the two-drug combinations against each group of organisms.

[d] Total isobols showing all types of combined activity of each combination against each group of organisms. Totals are unequal because some isobols were discarded because of excessively low MIC values for at least one of the antimicrobials.

each group of gram-negative bacilli according to each two-drug combination and the type of combined activity demonstrated. These data extend that of Table 3 and indicate that TMP-PB always showed enhanced activity against the *Enterobacter-Klebsiella-Serratia* and *Proteus-Providence* groups. SMX-PB and TMP-SMX frequently showed enhanced activity only against the *Enterobacter-Klebsiella-Serratia* isolates. None of these two-drug combinations can be expected to consistently demonstrate synergism or addition against *P. aeruginosa*.

Killing-curve studies. Figure 2 illustrates five separate killing curves constructed from the colony counts determined during incubation of the gram-negative bacilli with the three antimicrobials, individually and in various combinations. Synergism was defined as at least a 3.5- to 4-log decrease in colony count with a given antimicrobial combination as compared to the effect of the most active single agent. Determinations were generally made on the basis of the 24-h colony counts. However, on two occasions the 6-h reading for the TMP-PB combination against isolates of *Providence* and *S. marcescens* indicated synergism, whereas significant regrowth had occurred by 24 h (Fig. 2D). In these two instances, the 6-h reading was chosen and synergism was considered present. This judgment was based on the fact that Bulger and Kirby (3) considered the rate of killing during the first 4 to 6 h the most critical part of this test.

Figure 2A shows that only the three-drug combination shows synergism against a strain of *S. marcescens*. Both the three-drug and the TMP-PB combinations demonstrate synergism against the strain of *P. morganii* seen in Fig. 2B. In Fig. 2C, the triple combination has virtually killed all *P. vulgaris* organisms by 6 h, showing marked synergism. By 24 h the killing effect of SMX-PB also qualifies as synergism, although this effect is much less dramatic. TMP-PB and the triple combination are both synergistic against a strain of *Providence*, although the TMP-PB effect is seen only at 6 h (Fig. 2D). Only the three-drug combination has a synergistic effect against the strain of *P. aeruginosa* shown in Fig. 2E.

Table 5 summarizes the data on synergism of the various antimicrobial combinations against those 12 strains of gram-negative bacilli studied by killing-curve assays. The triple combination (SMX-TMP-PB) was synergistic against nine of the isolates. TMP-PB showed synergism against five isolates, and SMX-PB showed synergism against three isolates. In this assay system with these bacteria, the TMP-SMX combination was not synergistic. Although this

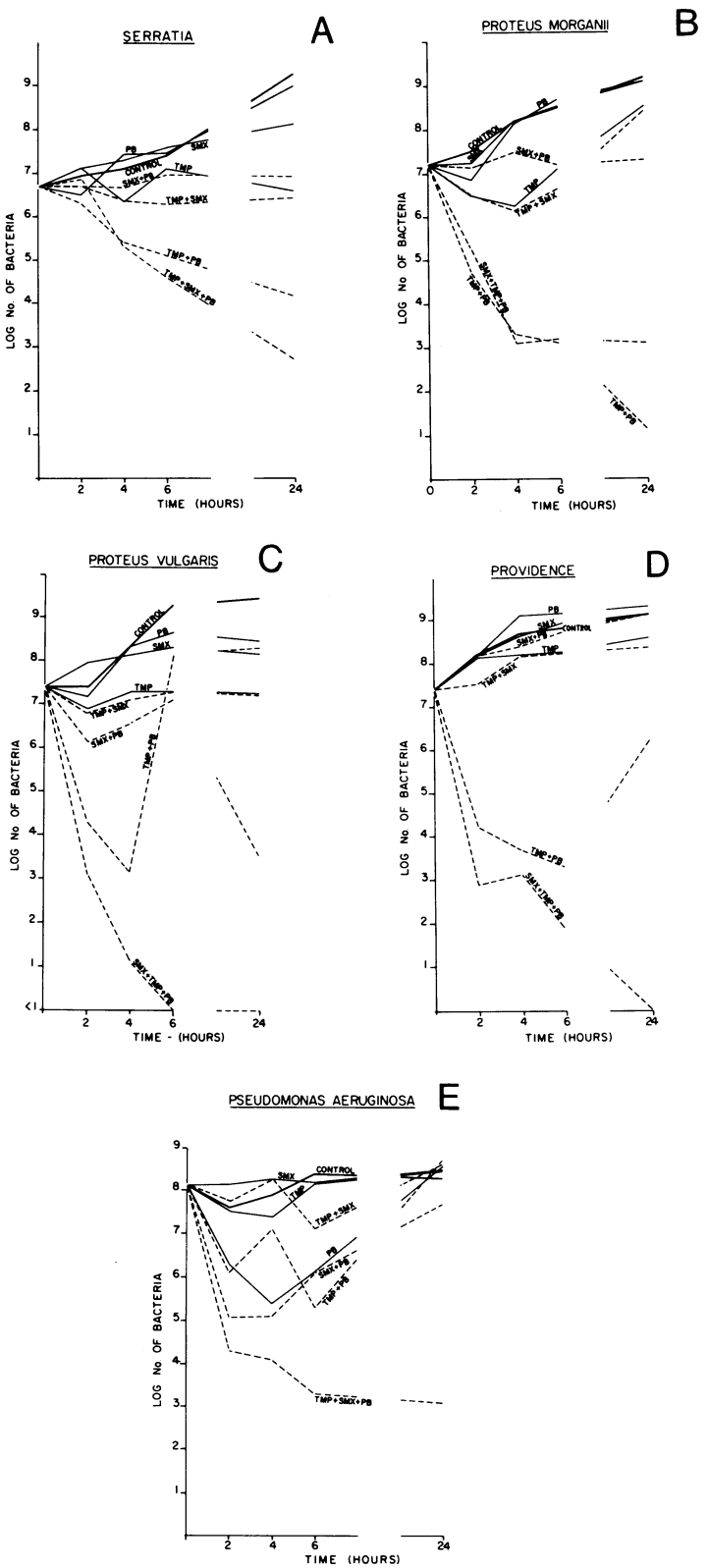

FIG. 2. *Killing curves constructed from colony counts performed after incubation of gram-negative bacilli in PB, SMX, and TMP, individually and in various combinations. The three-drug combination is most active and has good killing power against each of these organisms. Activity against P. aeruginosa was less than that against the other gram-negative bacilli.*

TABLE 5. *Killing curve studies demonstrating synergistic combined activity*[a]

Organism	No. of isolates	Synergism[b]			
		STP[c]	TMP + SMX	SMX + PB	TMP + PB
Enterobacter spp.	1	1	0	1	1
Klebsiella pneumoniae	1	1	0	0	1
Proteus (4)-*Providence* (1)	5	4	0	2	2
Pseudomonas aeruginosa	2	1	0	0	0
Serratia marcescens	3	2	0	0	1

[a] Instances of synergism (a 3.5- to 4-log decrease in survival) demonstrated by each two-drug and the three-drug combination against 12 isolates of gram-negative bacilli. Killing curves constructed from colony counts of surviving bacteria over 24 h.

[b] Number of times each drug combination showed synergism; i.e., STP was synergistic against the one isolate of *Enterobacter* spp. and against four of the five isolates of *Proteus* and *Providence* studied.

[c] STP, Sulfamethoxazole-trimethoprim-polymyxin B.

sample is small, these killing-curve results suggest that the triple combination (SMX-TMP-PB) will frequently show marked synergism with dramatic killing of gram-negative bacilli, whereas the effect of the two-drug combinations is, in comparison, less impressive.

DISCUSSION

This report indicates that significant antimicrobial activity is demonstrated against gram-negative bacilli by combining PB, SMX, and TMP in three- and two-drug combinations. Two different methods were used to study combined activity. Checkerboard dilution MIC studies using each of the two-drug combinations were carried out with 52 different isolates of gram-negative bacilli. The more time-consuming killing curves, showing the activity of the individual agents as well as the two- and three-drug combinations, were performed with only 12 isolates. The results obtained with the two different methods are not entirely comparable, i.e., TMP-SMX showed no synergism against the 12 isolates by the killing-curve assay and yet was deemed synergistic by checkerboard MICs against 8 of 11 *Enterobacter-Klebsiella-Serratia* isolates. The differences are undoubtedly methodological and subject to varied interpretations. However, certain conclusions can be drawn from both sets of data. The checkerboard MIC studies demonstrate that TMP-PB is the two-drug combination that will show enhanced antimicrobial activity against the great majority of *Enterobacter-Klebsiella-Serratia* and *Proteus-Providence* groups of bacteria most consistently. Furthermore, when SMX, TMP, and PB were combined in the killing-curve assay in concentrations similar to those achievable in the blood with high-dose therapy, the three-drug combination showed significantly greater killing than any single agent or two-drug combination. Optimal antibacterial activity against gram-negative bacilli, then, can be expected when SMX, TMP, and PB are utilized as a three-drug combination.

The significance of these in vitro data is difficult to determine at the present time. The combination of TMP-SMX is commercially available in the form of a fixed-ratio tablet containing 80 mg of TMP and 400 mg of SMX. This combination is recommended in the United States for the treatment of chronic urinary tract infections due to susceptible organisms (primarily gram-negative bacilli). The studies reported here indicate that TMP-PB is superior to the other two-drug combinations and nearly as active as the three-drug combination. However, since TMP is not available as a single agent, any combined therapeutic effort at the present time requires the use of PB and the combination TMP-SMX tablet. Practical problems arise in attempting to therapeutically achieve enhanced antibacterial activity similar to that demonstrated in the laboratory. The TMP-SMX tablets are given orally at 6-h intervals. Alternating peak and valley blood levels are produced during these dosage intervals, and absorption from the gastrointestinal tract may be irregular in any given individual. PB, in contrast, is administered parenterally at much longer intervals (8 or 12 h). Thus, blood levels of all three agents optimal for synergistic or additive activity may be present for only short periods of time during a course of treatment. Furthermore, whereas TMP and SMX seem to be rather freely diffusable (5), the tissue diffusion of PB is markedly limited. Kunin and Bugg (11) have shown that much of the PB injected in rabbits becomes bound to phospholipids of tissue cell membranes. Such bound drug has no antibacterial activity. Levels of free (active) PB in tissues were much lower, and repeated injections resulted in accumulation of tissue-bound PB with no increase in free drug.

Another question concerning the in vivo therapeutic efficacy of PB relates to the work of Chen and Feingold (4) which demonstrated that divalent cations (magnesium and calcium) antagonize the bactericidal effect of PB on *P.*

aeruginosa and *E. coli* cells. They concluded that in infections with some gram-negative bacilli, physiological concentrations of divalent cations might limit the efficacy of PB.

In spite of the above problems, the potential therapeutic use of the synergistic combination SMX-TMP-PB should be of interest, especially in the case of gram-negative bacteremias. Here, serum levels of antimicrobials may be more important than diffusion of free drug into tissues. Bacteremias (and other infections) due to nosocomial multiple antibiotic resistant gram-negative bacilli are currently one of the most serious problems in hospitalized patients. Many of these organisms (species of *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Providence*, and *Pseudomonas*) are susceptible only to gentamicin and/or carbenicillin, resulting in widespread use of these agents. This excessive use may well result in increasing resistance of gram-negative bacilli to these agents. Indeed, there have been recent reports of such resistance (7, 8, 22). New aminoglycoside antimicrobials (tobramycin, sisomicin, and BBK8) active against these organisms (6, 15) have been developed, but some cross-resistance with gentamicin does occur (2), and one would expect that widespread use of these newer agents would eventually lead to increasing bacterial resistance to them. In contrast, there has been no significant development of resistance to polymyxin in the past 20 years (1). Moreover, one would expect that combined use of three antimicrobials acting by different mechanisms (PB by damage to cytoplasmic membrane and SMX-TMP by interference with folate metabolism) would decrease the likelihood of development of resistance.

There has been some limited clinical experience with combinations of PB, SMX, and TMP in instances where infecting bacteria have become resistant to all other available agents. Rosenblatt et al. (16) have reported two cases of bacterial endocarditis in narcotic addicts, one due to *S. marcescens* and a second due to *P. aeruginosa*, treated with the three-drug combination. There was temporary clearing of bacteremia, although subsequent cardiac valve surgery was required for a cure. There have been several recent reports of treatment of *P. cepacia* endocarditis with combinations of SMX, TMP, and PB. Hamilton et al. (10), Seligman et al. (19), and Neu et al. (12) treated a total of four patients with SMX-TMP only and achieved sterilization of the blood. However, relapse occurred in two patients after discontinuation of therapy, and cardiac valve surgery was required in three patients. Rahal et al. (14) also treated four patients with *P. cepacia* endocarditis with the three-drug combination and cardiac valve surgery. Three of the patients were cured. These authors also studied 11 strains of *P. cepacia* by a tube-dilution method and showed that addition of therapeutic concentrations of SMX and TMP significantly lowered the MIC of PB for all the strains to the very susceptible range (0.09 to 0.78 μg/ml). They concluded that a combination of all three agents appears to provide optimal activity in vitro against *P. cepacia*.

In conclusion, the combination SMX-TMP-PB appears to demonstrate synergistic activity against gram-negative bacilli frequently resistant to multiple antimicrobials. Clinical trials of the three-drug combination are probably warranted in patients with serious infections due to these organisms, who have failed to respond clinically to other antimicrobials and whose infecting organisms are resistant in vitro. Furthermore, susceptibility to the synergistic effect of SMX-TMP-PB should also be demonstrated in the laboratory.

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