Sulfamethoxazole-Trimethoprim Synergism for Neisseria gonorrhoeae

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Sulfamethoxazole and trimethoprim are synergistic against many bacteria in sulfamethoxazole/trimethoprim concentrations of 20:1, but single-dose therapy of gonorrhea with the combination is disappointing. We used agar dilution techniques to determine minimal inhibitory concentrations of sulfamethoxazole and trimethoprim for 168 gonococci isolated from men with acute urethritis in Atlanta, Ga. The geometric mean minimal inhibitory concentrations were 5.6 μg of sulfamethoxazole per ml and 24.3 μg of trimethoprim per ml, a ratio of 1:4. The concentration of sulfamethoxazole inhibiting 50% of gonococcal strains dropped only from 4.7 μg/ml to 2.9 μg/ml with the addition of a 1/20 dilution of trimethoprim. We studied synergism with various ratios of sulfamethoxazole to trimethoprim against 20 random strains. A ratio of 1:1 was always synergistic and was the most synergistic ratio for 15 strains, whereas the 19:1 ratio was never the most synergistic. The 19:1 ratio failed to show synergism against seven strains, but showed antagonism at this ratio with five of these seven. The sulfamethoxazole/trimethoprim ratio of 19:1 usually achieved in serum after oral administration is minimally synergistic and is sometimes antagonistic for gonococci.

The combination of sulfamethoxazole (SMX) and trimethoprim (TMP) is highly active against many bacterial species. The drugs are considered synergistic; that is, the effect of the combination is greater than the sum of the effects of each drug alone. Available as a tablet containing 400 mg of SMX and 80 mg of TMP, this fixed combination has been the basis of treatment for a variety of infections, including uncomplicated gonorrhea. A single-dose regimen is highly desirable, since it eliminates potential problems with patient compliance. Unfortunately, single-dose treatments of gonorrhea with SMX/TMP have usually been disappointing. Although studies among populations harboring relatively susceptible strains of gonococci seemed promising (4, 14, 21), recent trials in the United States and elsewhere have been unsatisfactory. Treatment of men for acute uncomplicated anogenital gonorrhea with single doses of eight or nine tablets yields cure rates ranging from 70 to 80% (8, 16, 19). These cure rates are significantly lower than those achieved with standard treatments. Treatment with a single dose of 12 tablets produced an 88% cure rate (7), but this dose has been associated with gastrointestinal (7) and other immediate side effects (9).

The combination of SMX and TMP in the tablets has been designed to yield serum ratios approximating 20:1 SMX/TMP. Single large doses yield serum ratios ranging from 12:1 to 30:1 SMX/TMP (9, 26). Many species of bacteria show significant synergism between the two components at these ratios. Previous work has suggested, however, that gonococci are somewhat unusual in that the ratio of SMX to TMP which gives maximum synergism in vitro may be quite different from that produced in serum. Austin and colleagues tested 54 fresh isolates at two ratios of SMX/TMP and found that 1:3 was more synergistic than 20:1 (1). Stolz et al. found that a 1:1 ratio and a 1:20 ratio of SMX/TMP were more synergistic than the 20:1 ratio for 5 gonococcal isolates (23). Phillips and colleagues tested 30 strains and found that ratios of SMX/TMP from 1:1 to 1:8 were generally more synergistic than ratios involving larger relative amounts of SMX (18). The ratio producing maximal synergy tended to be related to the ratio of the MICs of the individual drugs alone.

We thought that the relatively low efficacy of single-dose SMX/TMP therapy for acute gonorrhea might result in part from a lack of synergism between the drugs. We determined the susceptibility to SMX and TMP of freshly isolated strains of N. gonorrhoeae and determined the synergistic effect of various combinations of the two drugs.
MATERIALS AND METHODS

Gonococci were isolated from 168 men with acute gonococcal urethritis attending a venereal disease clinic in Atlanta, Ga. Gonococci were identified by growth on modified Thayer-Martin medium as typical oxidase-positive colonies of gram-negative diplococci with the ability to metabolize glucose but not maltose or sucrose. Antimicrobial susceptibilities were determined by agar dilution on Oxoid Diagnostic Sensitivity Test agar containing 5% lysed horse blood (11). Organisms were suspended in broth at $10^7$ colony-forming units per ml as determined by light scatter. The suspensions were diluted so that a Steers replicator delivered inocula of approximately $5 \times 10^8$ colony-forming units. After the inocula had dried, the plates were incubated for 24 h under 10% carbon dioxide at 35°C. The minimal inhibitory concentration (MIC) was the lowest concentration of antimicrobial agent that prevented growth of the organism. A faint haze caused by the inoculum was ignored.

Beta-lactamase production was assayed by using the chromogenic cephalosporin (17).

A random sample of 20 isolates was tested for susceptibility to the combination of SMX/TMP at 19:1, 2:1, 1:1, 1:2, and 1:4. The fractional inhibitory concentration (FIC) was calculated as the MIC of the drug in combination divided by the MIC of the drug alone. The FIC index was defined as the sum of the FICs for SMX and TMP in each combination (6). An FIC index greater than one indicates antagonism between the two drugs. An FIC index less than one suggests synergism, the effect of the combination being greater than the sum of the effects of the individual drugs alone. The smaller the FIC index, the greater the synergistic effect.

RESULTS

No isolate elaborated a beta-lactamase. Figure 1 shows the cumulative percentage of strains inhibited at various concentrations of TMP alone, SMX alone, and SMX to which had been added a 1/20 dilution of TMP. Gonococci were more susceptible to SMX than to TMP. The concentration of SMX inhibiting the growth of 50% of strains was 4 µg/ml, whereas the concentration of TMP inhibiting 50% of strains was 16 µg/ml. At a 20:1 ratio of SMX to TMP, the concentration of SMX inhibiting 50% of strains only dropped from 4 to 2.7 µg/ml. The concentration of SMX which, alone, inhibited 50% of strains would inhibit but 65% of strains in combination. Thus the ratio of 20:1 SMX/TMP is only slightly more active against gonococci than is SMX alone.

Figure 2 shows the distribution of the ratios of MICs of SMX alone and TMP alone for the 168 isolates. Of the strains, 94% had ratios showing equal or greater resistance to TMP than to SMX.

Table 1 shows the mean FIC index for each ratio of SMX/TMP against the 20 random strains tested. The 1:1 ratio is significantly more synergistic than any of the others (analysis of variance, $P < 0.01$). The 1:1 ratio was most synergistic for 15 of the 20 strains tested, and the 19:1 ratio was never the most synergistic ($P = 0.0003$). The 1:1 ratio was synergistic against all strains tested, but the 19:1 ratio was synergistic against only 13 strains ($P = 0.02$). The 19:1 ratio was, frankly, antagonistic against 5 of the 20 strains, whereas the 1:1 ratio was never antagonistic ($P = 0.07$).

DISCUSSION

The gonococci isolated in this study showed the same degree of susceptibility to SMX and TMP alone as had been found with isolates from Seattle and Indianapolis in 1973 (1), but they were more susceptible to both drugs than were

![Figure 1. Cumulative percentage of 168 strains of N. gonorrhoeae inhibited by TMP alone, SMX alone, and SMX to which had been added, a 1/20 dilution of TMP.](http://aac.asm.org/)

**FIG. 1.** Cumulative percentage of 168 strains of *N. gonorrhoeae* inhibited by TMP alone, SMX alone, and SMX to which had been added, a 1/20 dilution of TMP.

![Figure 2. Ratios of MICs of SMX alone and TMP alone for 168 strains of *N. gonorrhoeae*.](http://aac.asm.org/)

**FIG. 2.** Ratios of MICs of SMX alone and TMP alone for 168 strains of *N. gonorrhoeae*.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>FIC index*</th>
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<tbody>
<tr>
<td>19:1</td>
<td>0.719 ± 0.395</td>
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<tr>
<td>2:1</td>
<td>0.506 ± 0.283</td>
</tr>
<tr>
<td>1:1</td>
<td>0.396 ± 0.171</td>
</tr>
<tr>
<td>1:2</td>
<td>0.620 ± 0.243</td>
</tr>
<tr>
<td>1:4</td>
<td>0.419 ± 0.142</td>
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* Each value represents mean ± standard deviation.
isolates from Boston, Mass., during the same year (2). Both groups found, as did we, that adding TMP at 1/16 to 1/20 the concentration of SMX had little effect on in vitro activity against the isolated strains.

The definition of in vitro antimicrobial synergism is somewhat controversial. A killing curve, a graph of the rate at which a bacterial inoculum is reduced over time by antibiotics alone or in combination, is often used to determine synergism (24). Killing curve studies are difficult to perform with *N. gonorrhoeae* because the bacteria tend to undergo autolysis in broth cultures at physiological pH. Static methods such as determination of the FIC index eliminates this problem. Comparative studies have shown excellent correlation between these two classes of approaches (25), and they seem equally reliable as predictors of clinical effectiveness (20).

Garrod and Waterworth suggested that the maximally effective ratio of the two drugs in combination should relate to the ratio of the activities of the drugs alone against gonococci (10). Phillips et al. found this relationship to be roughly true (18). Almost 95% of our isolates had ratios of the MICs of SMX/TMP ranging from 1:1 to 1:64, with the majority between 1:1 and 1:8. Thus we might have expected that the optimal ratios for SMX and TMP would be 1:2 to 1:4.

Indeed, the addition of a 1/20 concentration of TMP to SMX did not significantly increase the efficacy of the SMX. The concentration of SMX inhibiting 50% of gonococci inhibited only 65% in the 19:1 ratio with TMP.

The combination of equal parts of SMX and TMP seems to be most efficient for inhibiting *N. gonorrhoeae*. The 19:1 serum ratio usually achieved after a 5:1 therapeutic dose is considerably less effective. Ratios of SMX/TMP in other tissues and fluids are different from the serum ratios, however, and may more closely approximate ratios yielding better synergism (6). SMX/TMP ratios in urine have ranged from 1:4 to 3.5:1 after single and repeated doses (3, 13, 22). TMP is probably concentrated by the prostate (15), and prostatic ratios in men may be very close to 1:1. Which tissue levels are specifically important for the treatment of acute gonorrhea is unclear, although cure rates have been related to serum levels of penicillin (12).

The relative excess, from the standpoint of synergism, of SMX in serum should not interfere with the efficacy of the combination (5). However, the single-dose treatment of acute gonorrhea is limited by gastrointestinal tolerance. It may therefore be possible to construct a somewhat more effective but still tolerable single-dose regimen by administering SMX and TMP in doses yielding a more favorably synergistic ratio in serum. It may be possible to determine such an effective combination when TMP becomes available as a single drug.

**LITERATURE CITED**


