

Failure to demonstrate an advantage in combining sulphamethoxazole with trimethoprim in an experimental model of urinary infection

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SYNOPSIS Co-trimoxazole was found to have a predominantly bacteriostatic effect upon 28 urinary isolates of *Enterobacteriaceae* in nutrient broth and was never bactericidal in artificially infected urine. The components of co-trimoxazole were tested individually and trimethoprim was found to be at least as effective as co-trimoxazole in nutrient broth and in urine. Trimethoprim alone produced some bactericidal effect in urine but this was antagonized by sulphamethoxazole.

Laboratory tests for evaluating these drugs may give a misleading impression of their activity *in vivo*. Further clinical comparisons should therefore be made between trimethoprim and co-trimoxazole to determine when trimethoprim should be used in preference to the combination.

Despite the absence of a full evaluation of trimethoprim itself, a combination with sulphamethoxazole (co-trimoxazole) was introduced into general clinical use in 1968 and has been widely used since. A Swiss clinical trial showed that there was very little overall difference between trimethoprim and the combination in the treatment of urinary tract infections (Hoigné, Müller, and Schneider, 1969). A later English investigation showed that trimethoprim was as effective as co-trimoxazole in domiciliary patients but the mixture appeared to be superior in hospital patients (Brumfitt and Pursell, 1972). The latter results may possibly be less conclusive than they appear because hospital infection may be due to epidemics of a few organisms or to transferable antibiotic resistance within a bacterial population. Co-trimoxazole has been shown to be more effective than its components in chronic urinary tract infections caused by *Enterobacteriaceae* (Gleckman, 1973). However, this last study was not representative of commonly occurring urinary infections since it involved a population with a mean age of 59 years consisting of about 48% males.

Some of the properties initially attributed to co-trimoxazole *in vitro* have been found to be unpredictable. It now appears that the combination is

more often bacteriostatic rather than cidal *in vitro* (Küchler and Koch, 1973; Lacey, Anderson, Lewis, and Gillespie, 1973; Lewis, Anderson, and Lacey, 1974) and that any cidal activity may be due to the trimethoprim component alone (Lewis *et al*, 1974).

In order to obtain a more realistic assessment of the activity of these drugs *in vitro* we have studied the antibacterial activity of co-trimoxazole and its components in artificial mixtures in urine and in urine from volunteers some of whom had taken therapeutic doses of these agents. The results indicate that trimethoprim alone may be expected to be as effective as co-trimoxazole in the treatment of urinary infections.

Methods

PATIENTS AND ORGANISMS

Twenty-eight isolates of *Enterobacteriaceae* were obtained from 12 outpatients and 16 inpatients who had significant urinary tract infections. Specimens were obtained from five hospitals within a 20 mile radius of Bristol over a period of several weeks in order to minimize the possibility of collecting the same organism or the same plasmid-mediated resistance determinant from different patients. The organisms were identified (Cowan and Steele, 1965) as *Escherichia coli*, 26; atypical *E. coli*, one; *Proteus morgani*, one. Nine of these organisms were resistant to sulphamethoxazole alone and one to both trimethoprim and sulphamethoxazole.

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DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICs) IN BROTH

Sulphamethoxazole and trimethoprim were incorporated in doubling dilutions, singly or in combination (usually in a ratio of 20:1 w/w) into plates containing Oxoid diagnostic sensitivity test agar with the addition of 5% (v/v) lysed horse blood. Further details have already been described (Lewis *et al.*, 1974). Resistance to sulphonamides was defined as an MIC of > 200 µg of sulphamethoxazole per ml; resistance to trimethoprim as an MIC of > 1.6 µg per ml.

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATIONS IN BROTH

As already described, using broth containing 5% (v/v) lysed horse blood (Lewis *et al.*, 1974). A bactericidal effect has been defined as a reduction of tenfold or more in viable count within a stated time.

ANTIMICROBIAL AGENTS

Sulphamethoxazole and trimethoprim lactate were obtained as powders for incorporation into various media from Burroughs Wellcome Ltd. Volunteers were given the following formulations: sulphamethoxazole tablets, 500 mg (Gantanol tablets, Roche Products); trimethoprim tablets, 80 mg (Burroughs Wellcome Ltd.); co-trimoxazole tablets, ie, sulphamethoxazole 400 mg with trimethoprim, 80 mg (Septrin tablets, Burroughs Wellcome Ltd).

PREPARATION OF URINE FOR USE AS A BACTERIAL MEDIUM

Urine was collected on the third day of a course of treatment with each antimicrobial agent. Healthy volunteers were given either co-trimoxazole, two tablets twice daily; or trimethoprim, 80 mg, two tablets twice daily, or sulphamethoxazole, 500 mg, two tablets twice daily. The urine was cooled to 4° after voiding, sterilized by filtration, and then pooled. The possibility that filtration altered the antibacterial properties of the urine was excluded. Some specimens of urine were stored at -20° before use; freezing led to the precipitation of solid matter but did not affect the antimicrobial properties.

For certain experiments 24-hr collections of mid-stream urine from healthy individuals were treated as described above. Sulphamethoxazole, or trimethoprim, or the combination (ratio 20:1 w/w) were then added to the urine as already described (Lewis *et al.*, 1974).

BEHAVIOUR OF ORGANISMS IN URINE

Overnight broth cultures (10 ml) of organisms were washed twice by centrifugation and then resuspended in saline to remove substances accumulating

in the broth which might inhibit trimethoprim or sulphonamides. Prewarmed urine was inoculated with between 10⁵ and 10⁶ organisms per ml and immediately sampled for viable bacteria (Miles, Misra, and Irwin, 1938) on MacConkey agar plates. The viable count was determined again after incubation of the flasks on a shaker at 37° for two, four, and six hours.

Results

ANTIBACTERIAL EFFECT OF CO-TRIMOXAZOLE AND ITS COMPONENTS IN NUTRIENT BROTH MEDIUM

Co-trimoxazole and its components when tested individually were usually bacteriostatic against most organisms in nutrient broth containing 5% (v/v) lysed horse blood (table I).

Antibacterial Agent Added (32 × MIC in each case)	No. of Cultures Killed at Time Shown ¹		
	Two Hr	Four Hr	Six Hr
Sulphamethoxazole	0	0	0
Trimethoprim	0	2	5
Co-trimoxazole	0	1	3

Table I Effect of added antibacterial agents upon 28 urinary isolates of Enterobacteriaceae in nutrient broth containing 5% (v/v) lysed horse blood

¹Cidal effect defined in methods section.

ANTIBACTERIAL PROPERTIES OF CO-TRIMOXAZOLE AND ITS SEPARATE COMPONENTS IN URINE

In order to obtain optimal activity of sulphonamides and trimethoprim *in vitro* certain precautions have to be taken to neutralize naturally occurring antagonists in the media. The nature and extent of such antagonists in the body are unknown so that any attempt to correlate antibacterial activity *in vitro* with projected activity in clinical practice is at best only approximate. The activity of these agents was therefore determined in urine where it is found that sulphamethoxazole, or trimethoprim, or a 20:1 (w/w) was seldom bactericidal (table II). Trimethoprim was significantly superior (Student paired t test) to co-trimoxazole at two hr ($P = 0.02$) but the two agents were not significantly different at four hr ($P = 0.45$) or six hr ($P = 0.55$). Co-trimoxazole was superior to sulphamethoxazole at six hr ($P = 0.05$) but not at two hr ($P = 0.65$) or four hr ($P = 0.75$). Sulphamethoxazole antagonized the cidal effect of trimethoprim on the occasions when this

Test System	Time of Incubation		
	Two Hr	Four Hr	Six Hr
Urine control	0 2.8 (0.8→6.3)	0 100 (1.3→340)	0 1010 (13→4700)
Urine containing 1 mg per ml sulphamethoxazole	0 0.97 (0.3→3.4)	0 1.0 (0.2→3.1)	0 1.8 (0.09→7)
Urine containing 50 µm per ml trimethoprim	0 0.84 (0.2→2.5)	5 0.34 (0.02→8.0)	8 1.4 (0.008→20)
Urine containing 1 mg per ml sulphamethoxazole and 50 µg per ml trimethoprim	0 1.0 (0.5→2.7)	0 1.1 (0.5→6.9)	0 1.1 (0.01→6.3)

Table II Effect of added antibacterial agents upon 28 urinary isolates of Enterobacteriaceae in urine¹

¹For each observation the top figure shows the number of organisms giving a bactericidal response. The middle figure is the mean value of the ratio: $\frac{\text{viable count at time shown}}{\text{original viable count}}$. The lower bracketed figure is the observed range of these ratios.

occurred. Strains found to be sulphonamide resistant by the disk test failed to grow in urine containing sulphamethoxazole, and there was in fact no significant difference in the degree of inhibition of sulphonamide 'resistant' or 'sensitive' strains. Likewise there was no significant difference in the response of sulphonamide 'resistant' or 'sensitive' strains to either trimethoprim or co-trimoxazole (Student t test of means).

ANTIBACTERIAL PROPERTIES OF URINE FROM INDIVIDUALS RECEIVING THERAPEUTIC DOSES OF CO-TRIMOXAZOLE OR ITS COMPONENTS

The finding that co-trimoxazole was not bactericidal in urine was confirmed when the same organisms were incubated in urine from either of two individuals who had received on separate occasions, sulphamethoxazole, trimethoprim, or the combina-

tion (ratio 5:1 w/w) as co-trimoxazole (table III). Trimethoprim was significantly superior to co-trimoxazole at four hr ($P = 0.003$) but the agents were not significantly different at two hr ($P = 0.75$) or at six hr ($P = 0.60$). Co-trimoxazole was significantly superior to sulphamethoxazole at all times. It was notable that sulphamethoxazole again antagonized the cidal effect of trimethoprim and that no significant correlation was found between apparent sensitivity to sulphonamides by the disk test and response to co-trimoxazole or its components in urine.

Co-trimoxazole or its individual components had a bacteriostatic effect in urine upon the single isolate of *E. coli* which appeared to be resistant to these agents by the antibiotic disk test. The clinical significance of this finding is difficult to assess.

Confirmation that the effect of co-trimoxazole was bacteriostatic was provided when similar results were obtained when a selection of 18 of the

Drug Regime	Time of Incubation		
	Two Hr	Four Hr	Six Hr
Sulphamethoxazole 1 g twice daily	0 1.9 (0.6→10)	0 12 (0.6→88)	0 27 (0.3→162)
Trimethoprim 160 mg twice daily	0 0.75 (0.1→4.6)	6 0.31 (0.01→1.3)	9 0.46 (0.01→4.4)
Co-trimoxazole (sulphamethoxazole 0.8 g with trimethoprim 160 mg, twice daily)	0 0.69 (0.3→1.3)	0 0.60 (0.1→1.7)	0 0.55 (0.1→1.1)

Table III Effect of urine from individuals receiving various antibacterial agents upon 28 urinary isolates of Enterobacteriaceae¹

¹See footnote to table II.

test organisms was incubated with urine from a third individual who had received therapeutic doses of this agent.

Discussion

Although the study of organisms in urine described here may not relate necessarily to the natural environment, since no attempt was made to study these agents at concentrations found in kidney tissue, it was probably closer to the conditions found in urinary infections than experiments performed in nutrient broth. The main conclusion was that co-trimoxazole or its components do not produce a reliable destruction of bacteria in urine and that trimethoprim alone would appear to be as effective, and possibly more so, than the mixture. It is puzzling that trimethoprim has not been subjected to a more extensive clinical evaluation in its own right.

Failure to show any difference between the response of sulphonamide-sensitive or -resistant organisms to co-trimoxazole in urine is supported by parallel findings in a clinical study of urinary tract infections (Acar, Goldstein, and Chabbert, 1973).

Variations in the response of organisms to these various antibacterials after different periods of incubation suggest that bacterial response may be a function of the stage of growth.

The clinical significance of laboratory tests for sensitivity to these agents remains in doubt and one cannot at present improve on the observation that therapeutic results with co-trimoxazole in urinary tract infections may be largely predicted from a knowledge of sensitivity tests *in vitro* to trimethoprim alone (Reeves, Faiers, Pursell, and Brumfitt, 1969).

The use of trimethoprim instead of co-trimoxazole is known to cause fewer side effects (Brumfitt and Pursell, 1972). The possible increased chance of selecting antibiotic resistant populations by a single agent may now be largely discounted in clinical practice (Lacey *et al.*, 1973; Lewis *et al.*, 1974), although the use of the combination for this reason did seem to be quite reasonable at the time when co-trimoxazole was introduced. The components of

co-trimoxazole are incompatible in parenteral preparations. If our observations are found to apply in clinical practice, trimethoprim alone may well prove to be a useful drug for parenteral use. Whilst we agree with Brumfitt and Percival that it would be unwise at present to recommend the substitution of trimethoprim for co-trimoxazole there is obviously an urgent need for further clinical comparisons of these two agents in order to identify conditions for which the trimethoprim component should be given alone.

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