Trimethoprim—Sulphamethoxazole: Comparative Study in Urinary Infection in Hospital

D. S. REEVES,* M.B., B.S.; M. C. FAIERS,† M.B., B.S., D.OBST.R.C.O.G.; R. E. PURSELL,‡ A.I.M.L.T.
W. BRUMFITT,§ M.D., PH.D., M.R.C.P., M.C.PATH.

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Summary: Trimethoprim is inhibitory for a wide range of bacteria, and when used in combination with a sulphonamide marked synergy has been reported.

In order to test its value in the treatment of urinary infections 154 hospital patients with infections of varying severity and due to a wide range of organisms were treated with combinations of sulphamethoxazole and trimethoprim. Combinations of these substances in two different ratios (2:1 and 10:1) were used in 113 patients, and one week after the end of treatment about three-quarters were cured by both combinations. In a second study 106 patients were treated with a sulphamethoxazole-trimethoprim combination (5:1), ampicillin, or sulphadimidine. The cure rate with the 5:1 combination was higher than that found with ampicillin or sulphadimidine both one week after finishing treatment (sulphamethoxazole-trimethoprim 85%, ampicillin 70%, sulphadimidine 40%) and at the fourth- to fifth-week follow-up (sulphamethoxazole-trimethoprim 67%, ampicillin 52%, sulphadimidine 15%).

The results obtained with the various sulphamethoxazole-trimethoprim combinations did not indicate that a particular ratio was superior for treating urinary infections in general or for those caused by any particular species of organism.

Laboratory studies showed that many bacteria causing urinary infections in hospital were sensitive to trimethoprim, and the therapeutic results could have been largely predicted from a knowledge of the in-vitro sensitivity tests to trimethoprim alone. For example, sulphamethoxazole-trimethoprim in the treatment of *Proteus mirabilis* infections was less successful than in those due to *Escherichia coli*, and this finding was clearly reflected in the higher minimal inhibitory concentrations for trimethoprim of *Pr. mirabilis*.

The sulphamethoxazole-trimethoprim combination was simple to administer, free from side-effects, and gave satisfactory results in the treatment of urinary infections that occurred in hospital patients.

Introduction

Trimethoprim (2,4-diamino-5-(3, 4, 5-trimethoxybenzyl) pyrimidine) was fully described by Roth, Falco, Hitchings, and Bushby (1962), though previous work had shown synergy with sulphonamides (Hitchings, 1961). Synergy may be due to the sequential blocking of folic acid synthesis by the two compounds so that synthesis of bacterial deoxyribonucleic acid is inhibited. Acting by substrate competition sulphonamides prevent incorporation of *p*-aminobenzoic acid into the bacterial

folate metabolic pathway, while trimethoprim blocks dihydrofolate reductase—the enzyme that converts dihydrofolate to tetrahydrofolate.

In man sulphonamides and trimethoprim are of low toxicity, since *p*-aminobenzoic acid is not involved in human folate metabolism and the affinity of trimethoprim for human dihydrofolate reductase is negligible. Thus the combination has a selective inhibitory action on bacterial metabolism and is suitable for antibacterial therapy in man.

Darrell, Garrod, and Waterworth (1968) carried out detailed in-vitro studies on the antibacterial spectrum of trimethoprim both alone and in combination with sulphafurazole. Trimethoprim was found to be bacteriostatic when tested alone, but with sulphonamides synergy was noted and the combination was bactericidal. The enhanced effect against Gram-negative bacilli indicated that a sulphonamide-trimethoprim combination would be valuable in the treatment of urinary infection in hospital, where success is often prejudiced by antibiotic resistance and organic and functional abnormalities of the urinary tract.

We describe here two clinical trials using sulphamethoxazole-trimethoprim combinations for the treatment of urinary infections that occurred in hospital. In the first trial we studied only sulphamethoxazole-trimethoprim, but used two combinations (10:1 and 2:1). The encouraging results led us to carry out a second study in which we compared a 5:1 combination of sulphamethoxazole and trimethoprim with sulphadimidine and ampicillin.

Methods

Criterion of Infection.—Diagnosis based on a single specimen is known to be unreliable and we have found the error to be much accentuated in specimens collected from patients on admission to hospital (unpublished data). Therefore the sole criterion of infection was a bacterial count exceeding 10^5 bacteria per ml. in two consecutive urine specimens showing the same bacterial species on both occasions.

Bacteriological Techniques.—Organisms were identified by standard methods. O antisera against *Escherichia coli* and *Proteus mirabilis* were used to distinguish different serotypes of these species.

Criterion of Cure.—Examination of a urine specimen from patients was carried out at 72 hours, two weeks, and six weeks after starting treatment, and eradication of the original organism was taken to be a cure. Reinfection by an organism which was different from the one originally causing infection was not regarded as a treatment failure but another episode of infection.

Sensitivity Testing.—Initial testing was carried out by the impregnated disc technique, the sensitivity to sulphamethoxazole-trimethoprim being assessed by a single disc containing 47.6 µg. of sulphamethoxazole and 2.4 µg. of trimethoprim.

Subsequently the minimum inhibitory concentration (M.I.C.) of sulphadimidine, ampicillin, and trimethoprim was measured against all infecting organisms. M.I.C.s of sulphadimidine and trimethoprim were carried out by the plate dilution technique

* Senior Registrar.

† Senior House Officer.

‡ Research Assistant.

§ Clinical Pathologist.

Edgware General Hospital, Edgware, Middlesex.

with the use of lysed horse blood agar plates (Darrell *et al.*, 1968). For ampicillin the tube dilution technique was used.

Patients

All patients were treated in hospital and the majority were known to have acquired their infection after admission. They were of all ages from 15 years upwards and included post-partum, gynaecological, genitourinary, and medical patients (Table I). All were seen by one of us, who made a clinical assessment, initiated treatment, and saw the patient at follow-up (often as an outpatient).

TABLE I

	Study 1				Study 2					
	Sulphamethoxazole-trimethoprim 2:1		Sulphamethoxazole-trimethoprim 10:1		Sulphamethoxazole-trimethoprim 5:1		Ampicillin		Sulphadimidine	
	No.	%	No.	%	No.	%	No.	%	No.	%
Patients treated	65	—	48	—	41	—	30	—	35	—
Females	47	72	39	81	29	71	24	80	27	77
Age:										
15-40 years	9	14	12	25	10	24	6	20	9	26
41-60 years	7	11	5	10	9	22	7	23	7	20
> 60 years	49	75	31	65	22	54	17	57	19	54
Mean (years)	(66)		(61)		(58)		(60)		(56)	
Abnormalities of urinary tract or instrumentation	30	46	23	48	21	51	17	57	15	43
Symptoms	44	68	29	60	30	73	24	80	29	83
Pyuria	27	41	20	42	31	76	24	80	21	60
Blood urea:										
40-80 mg.	21/42	50	12/31	39	12	31	12	40	5	14
> 80 mg.	1/42	2	2/31	6	1	2	2	7	3	9

No particular cross-infecting organism which accounted for a high proportion of infections was found at any time during the study.

Design of Trials

The first trial (study 1) was designed to test the effectiveness of 2:1 and 10:1 combinations of sulphamethoxazole and trimethoprim, and 113 patients were treated and followed up. The results were encouraging, and therefore a second trial (study 2) was carried out in order to compare sulphamethoxazole-trimethoprim with ampicillin and with sulphadimidine. At this stage we learnt that the commercial preparation of sulphamethoxazole-trimethoprim would be a 5:1 ratio (Seprin) and it was decided to use this combination in the trial. Patients were allocated to treatment groups by random selection. The result of sensitivity testing was not taken into consideration in allotting treatment in either study 1 or study 2. In study 2 if the infection failed to respond to the primary treatment within three days one of the remaining two substances was given on a random basis; 142 courses of treatment were given to 106 patients.

Dosage.—Sulphamethoxazole-trimethoprim dosage was two tablets night and morning. The actual quantity of each compound in the tablet was 2:1, sulphamethoxazole 250 mg. and trimethoprim 125 mg.; 5:1, sulphamethoxazole 400 mg. and trimethoprim 80 mg.; 10:1 sulphamethoxazole 500 mg. and trimethoprim 50 mg. The dose of ampicillin was 500 mg. eight-hourly and of sulphadimidine 1 g. six-hourly. All treatment was given by mouth for a period of seven days.

Results

Treatment

Study 1.—Table I shows an analysis of some clinical and laboratory findings. It can be seen that the two groups of patients are similar. Analysis of the infecting organisms (Table II) also shows the two groups to be similar except for

a predominance of *Klebsiella* spp. in patients treated with the 2:1 combination. The results of treatment are shown in Table III. It can be seen that these were the same in the two groups at one week and similar at four to five weeks. The results of treatment with the 10:1 and 2:1 combinations were analysed separately in relation to the two most common infecting organisms *E. coli* and *Pr. mirabilis* (Table IV and Fig. 2). The cure rates for *E. coli* were similar with both combinations, as were those for *Pr. mirabilis*. It is apparent, however, that *Pr. mirabilis* infections were more difficult to eradicate (Table IV).

TABLE II.—Infecting Organisms

	Study 1				Study 2					
	Sulphamethoxazole-trimethoprim 2:1		Sulphamethoxazole-trimethoprim 10:1		Sulphamethoxazole-trimethoprim 5:1		Ampicillin		Sulphadimidine	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>E. coli</i> * (including atypical coli)	39	60	30	63	22	54	17	57	30	86
<i>Pr. mirabilis</i>	15	23	10	21	4	10	2	7	3	9
<i>Klebsiella</i> spp.	9	14	1	2	7	17	2	7	1	3
<i>Str. faecalis</i>	1	2	4	8	4	10	6	20	0	0
<i>Staph. pyogenes</i>	1	2	2	4	3	7	1	3	1	3
<i>Staph. albus</i>	0	0	0	0	1	2	0	0	0	0
<i>Ps. aeruginosa</i>	0	0	0	0	0	0	1	3	0	0
Mixed:										
<i>Pr. mirabilis</i> and <i>Str. faecalis</i>	0	0	0	0	0	0	1	3	0	0
<i>Str. faecalis</i> and <i>Staph. pyogenes</i>	0	0	1	2	0	0	0	0	0	0

* Common *E. coli* serotypes: study 1, 40%; study 2, 48%.

TABLE III.—Treatment with Sulphamethoxazole-trimethoprim in Combinations of 2:1 and 10:1 Study 1

Treatment	No. of Patients Treated	Cure			
		1 week after End of Treatment		4 weeks after End of Treatment	
		No.	%	No.	%
2:1	65	50	77	56	86
10:1	48	37	77	42	87

TABLE IV.—Results for *E. coli* and *Pr. mirabilis* in Study 1

Treatment	No. of Patients Treated	<i>E. coli</i>		<i>Pr. mirabilis</i>	
		No. of Patients Treated	Cure after 4-5 weeks	No. of Patients Treated	Cure after 4-5 weeks
2:1	33	24 (73%)	13	7 (54%)	
10:1	24	19 (79%)	10	4 (40%)	
Total	57	43 (76%)	23	11 (48%)	

Study 2.—As in study 1, Tables I and II show that the groups of patients receiving treatment with the three substances were similar and that the organisms causing infection were also evenly distributed, except that there were no *Streptococcus faecalis* infections in the patients given sulphadimidine. Table V shows the results of primary treatment in the three groups. At both one week and four to five weeks the sulphamethoxazole-trimethoprim combination gave the highest cure rate. Ampicillin also gave good results. The results with sulphadimidine were poor. With all three substances a remarkable number of patients who had apparently been cured at one week relapsed at four to five weeks. This may be related to the high proportion of patients with urinary tract abnormalities in this study. Further treatment of patients who failed primary treatment with one of the other two compounds showed a good cure rate after one week with sulphamethoxazole-trimethoprim (89%) and a reasonably good cure rate with ampicillin (67%) (Table VI). However, follow-up over a longer period of time (Table VI) showed that these patients were even more prone to relapse than those who had only the primary treatment (Table V).

TABLE V.—Results of Primary Treatment in Study 2

Treatment	No. of Patients Treated	Cure			
		1 week after End of Treatment		4-5 weeks after End of Treatment	
		No.	%	No.	%
Sulphamethoxazole-trimethoprim 5:1 ..	41	35	85*	27	67
Ampicillin ..	30	21	70*	14	52
Sulphadimidine ..	35	14	40	4	15

* $P=0.102$ for the difference between the results at one week for sulphamethoxazole-trimethoprim and ampicillin.

TABLE VI.—Results of Secondary Treatment (Patients Who Had Already Failed One Drug) in Study 2

Treatment	No. of Patients Treated	Cure			
		1 week after End of Treatment		4-5 weeks after End of Treatment	
		No.	%	No.	%
Sulphamethoxazole-trimethoprim 5:1 ..	18	16	89	6	46
Ampicillin ..	12	8	67	1	17
Sulphadimidine ..	6	0	0	0	0

Toxicity and Side-effects.—Assessment of gastrointestinal side-effects in ill patients in hospital is difficult, but no instance of severe nausea, vomiting, or diarrhoea was noted to coincide with any of the three treatments. Minor skin rashes developed in one patient after completing the course of treatment with sulphadimidine and in one after treatment with sulphamethoxazole-trimethoprim. Two patients receiving sulphameth-

oxazole-trimethoprim and one receiving ampicillin developed skin rashes during treatment. The drugs were therefore stopped and the patients were excluded from the study.

Difficulty in swallowing the large sulphamethoxazole-trimethoprim tablets was an occasional complaint, especially in older patients.

Microbiology

Study 1.—M.I.C.s were estimated for all organisms against sulphadimidine (Fig. 1) and trimethoprim (Fig. 2). In Fig. 1 the sensitivities are given for all organisms isolated, those for *E. coli* and *Pr. mirabilis* are also given separately. As previously found at this hospital (Brumfitt and Percival, 1967) a large proportion of organisms were resistant to sulphonamides. Fig. 2 shows the M.I.C.s of trimethoprim for all organisms treated with the two sulphamethoxazole-trimethoprim combinations. It can be seen that failure of treatment is associated with a higher resistance to trimethoprim than the successes. Taken as a whole the M.I.C.s for *Pr. mirabilis* are higher than those for *E. coli*, and this is reflected in the relatively poor results found when treating proteus infections (Table IV). It is also seen that this difference occurred in spite of the similar distribution of M.I.C.s for these two organisms of sulphadimidine (Fig. 1).

Study 2.—Fig. 3 relates the M.I.C.s for the infecting bacteria to the results of the treatment achieved by the respective antimicrobial substances. The M.I.C.s of trimethoprim alone are given for the group treated with sulphamethoxazole-trimethoprim. Again, as in study 1, the sensitivity to the antimicrobial agent and the results of treatment are closely related. For all three antibacterial substances used there was a level of drug concentration (M.I.C.) above which the proportion of treatment failures rose steeply (Fig. 3, Table VII). It is noteworthy that in the urine this level was greatly exceeded by all three drugs (at the dose given).

FIG. 1.—M.I.C.s of sulphadimidine for all organisms isolated from patients in study 1. In addition, the M.I.C.s for *E. coli* and *Pr. mirabilis* are shown separately. FIG. 2.—M.I.C.s of trimethoprim for individual organisms isolated in study 1. These patients were treated with a combination of sulphamethoxazole and trimethoprim. Treatment failures (dark column) and successes (light columns) are shown separately.

FIG. 3.—M.I.C.s of trimethoprim, sulphadimidine, and ampicillin for the individual organisms isolated from patients in study 2. Treatment failures (dark columns) and successes (light columns) are shown separately.

Serology.—The *E. coli* strains treated were serotyped in order to see whether they showed the expected distribution for hospital patients and whether resistance was predominant in certain serotypes. Common urinary serotypes accounted for 44% of all *E. coli* strains, a figure very similar to that found in this hospital previously (Grüneberg, Leigh, and Brumfitt, 1968). No particular serotype was found to be excessively common or to be associated with a greater resistance to the three drugs used than the other serotypes. Thus there was no evidence for significant cross-infection with a highly resistant organism.

TABLE VII.—*Relationship of Cure at Two Weeks to M.I.C. for Primary Treatment in Study 2*

	M.I.C. (μ g./ml.)					
	Ampicillin		Sulphadimidine		Trimethoprim*	
	≤ 25	> 25	≤ 50	> 50	≤ 1.0	> 1.0
No. of infections studied	19	11	18	17	39	2
No. and percentage cured	15 (79%)	5 (45%)	11 (61%)	4 (24%)	35 (90%)	0

* These patients received sulphamethoxazole-trimethoprim (5 : 1).

Emergence of Resistance.—The results of study 2 were analysed to see whether increased resistance of the infecting organism to the drug used developed during therapy. Resistance was recorded only when increase in resistance was four-fold and when the two organisms isolated were otherwise identical. Failure of treatment with sulphadimidine was associated with the development of resistance in four patients, with sulphamethoxazole-trimethoprim in one patient, and no resistance developed during ampicillin therapy.

Discussion

Symptomatic patients in domiciliary practice usually have infections due to *E. coli* which are sensitive to the commonly used chemotherapeutic agents, and a cure rate of 80 to 90% can be expected in such patients. This is not the case in hospitals, where, though *E. coli* still predominates (Table II), resistance is greater and a wider range of bacteria are concerned (Figs. 1, 2, and 3). The greater resistance in hospital patients is particularly apparent with sulphonamides. Using sulphadimidine we obtained a cure rate of only 15% at a four-week follow-up and this result could have been predicted from the M.I.C.s for sulphonamides.

Trimethoprim is highly active against organisms causing urinary infection in hospital, and, as judged by the M.I.C.s (Figs. 2 and 3), it would be effective when used alone. However, in-vitro studies have shown that its use in combination with a sulphonamide was synergistic, reduced the emergence of resistant strains, and enhanced the bactericidal effect (Darrell *et al.*, 1968; Bushby and Hitchings, 1968). In the treatment of gonorrhoea the synergistic effect of the combination seems certain, for the results with the combination were greatly superior to those obtained when either trimethoprim or sulphonamides were used alone (Csonka and Knight, 1967). A similar comparison between trimethoprim alone and in combination with a sulphonamide in the treatment of urinary infections would seem worth while, but in the meantime it is probably best to use trimethoprim in combination with a sulphonamide.

Sulphamethoxazole was chosen for use in the combination because its serum half-life is similar to that of trimethoprim. However, it is uncertain that the 5:1 ratio will always prove to be the best mixture. Darrell *et al.* (1968) showed that for particular organisms the optimal ratio for maximum synergy varies. It is possible, therefore, that for individual serious infections specially chosen combinations of a sulphonamide and trimethoprim may be needed.

In our present studies we have made no attempt to demonstrate in vitro synergy, and when we used three different combinations of sulphamethoxazole and trimethoprim no significantly increased cure rate was found with any of these combinations against a particular organism. Although the patients treated with the 5:1 combination of sulphamethoxazole and trimethoprim were not studied simultaneously with those given 10:1 and 2:1, examination of Tables I and II shows the patients and infecting organisms to be similar enough to justify comparison of the results of treatment. The results presented therefore provide no evidence to support or refute synergistic action in vivo.

Ampicillin is established as a valuable drug for the treatment of urinary infections, but the cure rate with sulphamethoxazole and trimethoprim at one and four weeks after treatment was greater (Table V—ampicillin, 70 and 52%; sulphamethoxazole-trimethoprim, 85 and 67%). However, trimethoprim had not previously been used in this hospital and it remains to be seen whether this advantage will be lost if its more widespread use leads to a change in the present favourable ratio of sensitive to resistant strains (Figs. 2 and 3). Nevertheless, the sulphamethoxazole-trimethoprim mixtures are a valuable addition to the drugs available for the treatment of urinary infection in hospital. Good results with simple therapy (two tablets twice daily) were obtained overall and we also obtained highly satisfactory results in individual cases. For example, a patient with severe acute pyelonephritis who was subsequently shown to have a bacteraemia rapidly became asymptomatic and was bacteriologically cured.

For routine sensitivity testing a combined disc containing sulphamethoxazole and trimethoprim is adequate, but when sulphamethoxazole-trimethoprim is being considered for use in serious infections a test with three discs should be carried out. Two of the discs contain the individual compounds and the third the combination, thus making it possible to distinguish organisms which are sensitive to only one of the drugs from those which are sensitive to both. When accurate assessment of individual sensitivity and synergy is important for choosing treatment, tests are needed with the two substances incorporated into a suitable solid medium.

In domiciliary patients follow-up four to six weeks after therapy shows that a small but significant proportion of those free from infection one week after treatment have relapsed (Williams, Brumfitt, Leigh, and Percival, 1965). However, in the present study the tendency to relapse at the later follow-up is much greater (Table V). In the particular patients studied this may be due to abnormalities of the urinary tract which allowed small numbers of organisms to persist in tissues and reinfect the urine subsequently. We have pointed out that adequate urine levels alone did not ensure eradication—a finding which suggests the presence of tissue involvement. In addition, further analysis of the results showed that the tendency to relapse was much greater when the patient remained in hospital, even though there was no indwelling catheter. This may be associated with abnormal function of the urinary tract due to the underlying illness and resulting immobilization.

It is apparent, therefore, that in spite of the continuous introduction of new antimicrobial agents the prevalence of urinary infection in hospital remains unchanged and will continue to pose therapeutic problems, especially in chronically ill patients.

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Requests for reprints should be addressed to Dr. W. Brumfitt, Edgware General Hospital, Edgware, Middlesex.

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