The Journal of Medical Microbiology

Vol. 10 No. 3

SENSITIVITY OF *PSEUDOMONAS AERUGINOSA* TO SULPHONAMIDES AND TRIMETHOPRIM AND THE ACTIVITY OF THE COMBINATION TRIMETHOPRIM: SULPHAMETHOXAZOLE

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INFECTIONS with *Pseudomonas aeruginosa*, although relatively uncommon under normal conditions, may cause serious clinical problems because of the intrinsic antibiotic resistance of this species. Indeed, until the recent introduction of well-absorbed carbenicillin esters (indanylcarbenicillin and carfecillin), there was no specific oral therapy for urinary tract infections caused by *P. aeruginosa*. Whilst the majority of strains of this species are still sensitive to carbenicillin, there are local problems of resistance and there is clearly a need for an alternative form of oral therapy for this type of infection. Co-trimoxazole is one of the most successful orally administered preparations for the treatment of many urinary tract infections (Brumfitt and Pursell, 1972), but it has been stated that this combination should not be used to treat infections caused by *P. aeruginosa* (Fowle, 1968; Wellcome Medical Division, 1975). However, two observations suggested that co-trimoxazole might be more effective against these infections than has been realised. First, a high proportion of strains of *P. aeruginosa* isolated at this hospital were found to be sensitive to a 300- μ g disk of compound sulphonamide ("Sulphatriad"). Second, the minimal inhibitory concentrations (MICs) of trimethoprim (tm) and sulphamethoxazole (SMZ) for strains of *P. aeruginosa* were found to be approximately equal, in contrast to a ratio of about 1 : 20 found for other pathogens (Bushby, 1969). In the present paper we record the individual activities of various sulphonamides against strains of *P. aeruginosa* and we report synergy with the components of co-trimoxazole (tm and SMZ) against *P. aeruginosa*. A preliminary report of this work was presented at the 9th International Congress of Chemotherapy.

Received 3 Oct. 1975; revised version accepted 6 Aug. 1976.

MATERIALS AND METHODS

Antimicrobial agents. SMZ, tm lactate, and sulphadimidine (SDM) were generously given in powder form by, respectively, Roche Products, Welwyn, The Wellcome Foundation, London and Imperial Chemical Industries Ltd, Macclesfield. Sulphadiazine (SDZ) was obtained as a solution (Sulphadiazine Injection BP) containing 0.25 g per ml (May and Baker Ltd, Dagenham). SMZ and SDM were dissolved at a concentration of 15 mg per ml by suspending the solid in an appropriate volume of sterile water and adding sufficient 10M NaOH solution dropwise to obtain solution. Solutions of SMZ containing up to 150 mg per ml were similarly made with 40% (v/v) aqueous propylene glycol (British Drug Houses, Bournemouth) as solvent.

Sensitivity disks were obtained from Oxoid Ltd, London SE1. They contained SMZ 25 µg; or tm 2.5 µg; or compound sulphonamide (Sulphatriad = sulphathiazole 111 µg; SDZ 111 µg; sulphamerazine 78 µg), or co-trimoxazole (SMZ 23.75 µg; tm 1.25 µg).

Bacterial strains. Eighty-two strains of *P. aeruginosa* isolated from infected urines were tested. They were identified as described by Phillips (1969).

Microbiological techniques. The MICs of sulphonamides and tm were determined separately by an agar plate dilution method, in which both Sensitest agar (Oxoid) and the minimal salts agar (MSA) of Davis and Mingioli (1950) were used. As inoculum an overnight culture of the test organism in Hartley's digest broth (Southern Group Laboratories, London SE13), was diluted 10^3 in water; the strains were plated out in batches of 25 with a multiple inoculator delivering 3 µl of each diluted culture. Plates were read after overnight incubation at 37°C, the end point being taken as the minimum concentration that allowed the growth of three colonies or less, i.e., inhibition of at least 99.9% of the original inoculum.

Synergy was sought by carrying out MIC determinations with serial concentrations of tm in the presence of different concentrations of SMZ. Isobolograms were constructed from the results, and drug interaction was expressed in terms of fractional inhibitory concentrations (FIC; Elion, Singer and Hitchings, 1954); $FIC = MIC \text{ of drug in combination} / MIC \text{ of drug acting alone}$. For two interacting drugs A and B, the sum of the FICs ($\Sigma FIC = FIC_A + FIC_B$) expresses the extent of interaction. For example, if the MIC of each drug is reduced four-fold in combination, $FIC_A = FIC_B = 0.25$. Hence, $\Sigma FIC = 0.5$. If the effect is purely additive, $\Sigma FIC = 1$, and if the drugs are antagonistic $\Sigma FIC > 1$. To allow for inter-experimental variations in these studies, both with tm/SMZ and other combinations (Kerry, Hamilton-Miller and Brumfitt, 1975), any value of $\Sigma FIC < 0.7$ has been taken to indicate synergy.

Disk sensitivity testing was done with Sensitest agar plates inoculated with aqueous 10^3 dilutions of 6-h cultures and read after overnight incubation.

RESULTS

Disk sensitivity tests

All 82 strains of *P. aeruginosa* were resistant to tm and SMZ when these agents were tested separately and together as co-trimoxazole. Sixty-nine (84%) of the strains were sensitive to Sulphatriad.

MICs of tm and of sulphonamides

The MICs of three sulphonamides and of tm for 71 strains were determined by plate dilution on Sensitest agar; 12 of the strains tested were resistant and 59 sensitive to Sulphatriad in the disk test. It is clear (table I) that there was good correlation between qualitative resistance to Sulphatriad and high level resistance to SDZ, SMZ and SDM. This correlation was best for SMZ, of

TABLE I

Activity of three sulphonamides and of trimethoprim against Pseudomonas aeruginosa

Antimicrobial agent	Number of sensitive (S) and resistant (R) strains* tested	Number of strains with MIC (μ g per ml) value of						
		31	62.5	125	250	500	1000	>1000
Sulphadiazine	59 (S)	6	17	19	14	2	1	0
	12 (R)	...	...	1	0	1	2	8
Sulphamethoxazole	59 (S)	4	7	18	16	5	9	0
	12 (R)	...	...	...	...	...	...	12
Sulphadimidine	59 (S)	...	...	...	...	1	1	57
	12 (R)	...	...	...	...	...	...	12
Trimethoprim	59 (S)	...	14	13	17	10	3	2†
	12 (R)	...	...	1	4	3	2	2†

* As determined by disk test with 300 μ g compound sulphonamide (Sulphatriad).† MIC of these 4 strains was 2000 μ g per ml.

TABLE II

Activity of trimethoprim and sulphamethoxazole and their therapeutically attained levels

Antimicrobial agent	MIC (μ g per ml)*	Therapeutically attained concentration (μ g per ml)
Trimethoprim	108	} tm/SMZ 96.5/80.2† 53/159‡
Sulphamethoxazole	227	
Combination (tm/SMZ)	11.4/16.4	

* Mean values for 22 *P. aeruginosa* strains, all sensitive to 300- μ g sulphonamide disk; † Schwartz and Rieder (1970); ‡ Kaplan *et al.* (1973).

which the MIC for all the Sulphatriad-resistant strains was >1 mg per ml. These strains are referred to subsequently as "highly resistant" to sulphonamides, whilst the majority of the strains, all sensitive to Sulphatriad and with MICs of SMZ ≤ 1 mg per ml, are designated "moderately resistant". The same classification has been used before for *Klebsiella* spp. (Hamilton-Miller and Grey, 1975).

For the highly resistant strains, the mean MIC of SMZ and of tm were 16.4 and 0.44 mg per ml, respectively. It was impossible to determine the MIC of SDZ and SDM for these strains because these compounds did not remain in solution in agar at concentrations above about 10 mg per ml.

It can be seen that the sulphonamides differed in their activity against the moderately resistant strains that formed the majority of those tested. SDM was very poorly active, while SDZ (with a mean MIC of 125 μ g per ml) was significantly more active than SMZ (mean MIC = 202 μ g per ml) ($P < 0.01$). The antibacterial activity of tm resembled that of SMZ, tm having a mean MIC of 205 μ g per ml. For the individual strains in this group there was a good correlation ($r = 0.506$, $P < 0.001$) between the MIC of tm and of SMZ.

TABLE III

Synergy between trimethoprim and sulphamethoxazole against 14 strains of Pseudomonas aeruginosa

Medium	Mean MIC (μg per ml) for each agent tested			
	alone		in combination	Mean ΣFIC^*
	tm	SMZ	tm/SMZ	
Minimal salts agar	124.5	134.6	8.8/ 8.1	0.13
Sensitest	100	232	10.3/18.6	0.2

* Values of $\Sigma\text{FIC} < 0.7$ (see *Methods*) indicate synergism.

Synergy between SMZ and tm

Eight highly resistant strains, with MICs of SMZ in excess of 2.5 mg per ml, were tested on Sensitest agar against combinations of tm and SMZ. Synergy was not demonstrated (mean $\Sigma\text{FIC} = 0.97$).

Conversely, when moderately resistant strains, with MICs of SMZ ranging from 100 to 400 μg per ml, were tested on Sensitest agar, marked synergy between tm and SMZ was noted. The results from these tests were averaged and are presented in table II. It can be seen that the fall in the MIC as the result of combining the two compounds was approximately 10-fold for tm and 15-fold for SMZ. The data in table II include concentrations of tm and SMX that have been found in the urine of subjects receiving a therapeutic dose of co-trimoxazole (160 mg tm + 800 mg SMZ) (Schwartz and Rieder, 1970; Kaplan *et al.*, 1973). Comparison of these figures with the inhibitory concentrations shows clearly that, whereas the levels in urine of the components separately would not be inhibitory, there is a considerable therapeutic margin when tm and SMZ are combined; the degree of synergy should result in complete inhibition of all the strains tested.

When 14 of the above 22 strains were tested in parallel on Sensitest agar and MSA (table III) the activity of tm was virtually unaffected by the change in medium, but SMZ was almost twice as active in the defined medium, an effect noted also by Amyes (1974). The amount of synergy was slightly greater in MSA.

The mean MIC of tm for the 22 strains tested for synergy was only about half that for all the moderately resistant group (table I). This was not because of deliberate selection of strains with greater sensitivity to tm, but because, for technical reasons, it was more economical to test batches of strains that had the same, or similar, MIC values for the two substances.

DISCUSSION

There have been only a few previous studies on synergy between tm and SMZ against *P. aeruginosa*. Bushby and Barnett (1967) stated that there was

no synergy for 10 strains at a ratio of 1 : 20, but it is not clear from their text how the testing was done. On the basis of these findings, Fowle (1968) suggested that co-trimoxazole was not indicated for the treatment of any infections caused by *P. aeruginosa*. However, subsequently both Simmons (1970) and Ritzerfeld and Hasch (1972) have reported synergy. The former used strains very sensitive to sulphonamide (MIC of SMZ < 12.5 µg per ml) and a diffusion technique that did not allow quantitation, and the latter used the conventional 1 : 20 ratio only.

Strains of *P. aeruginosa* make up about 2% of the bacterial strains isolated from samples of infected urine at this hospital, and all appear to be resistant to co-trimoxazole by the conventional disk test. Our results suggest that, for most of these strains, this resistance is an artefact created by the use of a disk containing an inappropriate ratio of tm : SMZ of 1 : 20. The MIC values of SMZ for the strains tested were of two kinds, namely (1) values of > 1000 µg per ml for the minority (16%) that were resistant to a 300-µg Sulphatriad disk, and (2) values with a mean of about 200 µg per ml for the majority (84%) that were sensitive to a Sulphatriad disk. The minority population would clearly be resistant to co-trimoxazole under any circumstance; not only were the MICs of tm and SMZ grossly in excess of levels attainable in the urine, but synergy could not be demonstrated. Concentrations of the individual components of co-trimoxazole similar to those found in the urine, when acting alone, did not inhibit the larger group of strains that were sensitive to a Sulphatriad disk; however, due to the high degree of synergy demonstrated, these organisms should be susceptible to the combination of drugs at the levels likely to be found in the urine.

These results raise doubts as to the validity of carrying out sensitivity tests on bacteria isolated from urine with a disk containing tm and SMZ in a ratio of 1 : 20. This disk represents the ratio of the two drugs achieved in blood. In urine, it is clear that the ratio achieved is often very different, being between 1 : 3 (Kaplan *et al.*, 1973) and 1 : 1 (Schwartz and Rieder, 1970). Such a ratio does not favour synergic action against the common urinary tract pathogens such as *Escherichia coli* and *Proteus mirabilis*, but these species will often be inhibited anyway by the high concentrations of the individual components present in urine. On the other hand, the levels of the individual components present in the urine are almost ideal for synergy against *P. aeruginosa*, and by virtue of the degree of synergy most strains of this species should be sensitive to co-trimoxazole in the urinary tract. Thus, *P. aeruginosa* may be grouped as regards co-trimoxazole sensitivity with gonococci (Phillips *et al.*, 1970) and *Bacteroides fragilis* (Phillips and Warren, 1974), all three species being relatively resistant to tm, but sensitive to co-trimoxazole because of the degree of synergy.

There is, therefore, a strong case for using a disk containing the more appropriate ratio of tm to SMZ of 1 : 2 (e.g., tm 20 µg + SMZ 40 µg) when testing strains isolated from urine for sensitivity to co-trimoxazole. Use of the conventional disk is illogical and may give misleading results. It is, of course, accepted practice with other antimicrobial agents (e.g., ampicillin) to

test pathogens from the urinary tract with disks of considerably greater potency than those used for testing strains recovered from other sites in the body.

A search of the literature to determine how often co-trimoxazole has been used in the treatment of urinary tract infections due to *P. aeruginosa* has revealed only 19 well-documented cases, and in nine the infection was eradicated (Grüneberg and Kolbe, 1969; Coppi, 1970; Hofstetter, 1970; van Camp, 1970; Fourrier and Vallet, 1971; and Stratford and Dixon, 1971). It is clear that a further clinical trial is justified.

SUMMARY

The activities of three sulphonamides and trimethoprim against strains of *Pseudomonas aeruginosa* have been studied. Sulphadiazine had most activity, sulphadimidine had little, and the activity of sulphamethoxazole was intermediate. According to their sensitivity to sulphamethoxazole, strains were divided into two groups: "highly resistant" (16%, MIC > 1000 µg per ml) and "moderately resistant" (84%, MIC ≤ 1000 µg per ml). The former were resistant on disk testing to Sulphatriad 300 µg. Sulphamethoxazole and trimethoprim did not act in synergy against them.

The moderately resistant strains were sensitive to Sulphatriad; trimethoprim and sulphamethoxazole showed marked synergy against them in agar-plate dilution tests. The concentrations of trimethoprim and sulphamethoxazole necessary for synergy lay for each drug within the range of concentrations at which they have been found in urine, and the ratio of their MICs when acting in synergy was similar to the ratio of their concentrations in urine.

It is suggested that a disk containing trimethoprim and sulphamethoxazole in a ratio of 1 : 2 rather than 1 : 20 would be more appropriate when testing strains from urine for their sensitivity to co-trimoxazole.

D. G. is a Junior Research Fellow of the Royal Free Hospital Medical School.

We thank Professor W. Brumfitt for constant advice and encouragement, and Ms Wendy Atkin of The Wellcome Foundation for a literature search.

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