Enhanced In Vitro Activity of Dihydrofolate Reductase and Dihydropteroase Synthase Inhibitors in Combination against *Nocardia* spp.

Common treatments for nocardial infections include the sulfonamides used alone or in combination, e.g., trimethoprim-sulfamethoxazole, with amikacin, imipenem, or ceftriaxone. Treatment is especially difficult in cases involving the central nervous system or disseminated disease or with drug-resistant species such as *Nocardia farcinica* and *Nocardia otitidiscaviarum* (5).

Sulfamethoxazole and dapsone inhibit dihydropteroate synthase and hence prevent the conversion of bacterial para-aminobenzoic acid to dihydrofolic acid. The active putative metabolite of PS-22 is a dihydrotriazine, a sister compound of WR99210 (1). WR99210 is a dihydrofolate reductase inhibitor that has demonstrated in vitro activity against *Nocardia* species (3). Theoretically, the combination of a dihydrofolate reductase inhibitor with a dihydropteroate synthase inhibitor should lead to enhanced activity, as both these enzymes lie on the folate biosynthesis pathway. This was evaluated in vitro.

The active putative metabolite of PS-22 and dapsone was

provided by Jacobus Pharmaceutical Co., Princeton, N.J.; sulfamethoxazole was purchased from Sigma Chemical Co., St. Louis, Mo. Twenty strains of *Nocardia* spp. from the American Type Culture Collection and clinical isolates provided by the Clinical Microbiology Laboratory, Upstate Medical University (Betty Ann Forbes), Syracuse, N.Y., were used in this study. Experimental in vitro procedures were modeled after those listed in the paper of Meyer et al. (4). Varying concentrations of the active putative metabolite of PS-22 were tested against fixed concentrations of sulfamethoxazole and dapsone. The concentration of sulfamethoxazole at 10 µg/ml was physiologically achievable. The concentration of dapsone was fixed at 1 µg/ml, as following the administration of 100 mg of the drug orally serum drug concentrations range from 0.4 to 1.2 µg/ml (2).

Table 1 shows the results of the drug combinations against various *Nocardia* species. The in vitro activity of the active putative metabolite of PS-22 was generally enhanced by combination with dapsone or sulfamethoxazole. These results agree with the findings of McNeil et al. (3) and demonstrate that the rational drug combination of a dihydrofolate reductase inhibitor combined with a dihydropteroate synthase inhibitor provides good targets for the evaluation of agents that work at these sites.

TABLE 1. MICs (µg/ml) of a WR99210 analogue alone and in combination with dapsone or sulfamethoxazole against various strains of *Nocardia* spp.

Sp. and strain	PS-22 metabolite	PS-22 metabolite-dapsone ^a	PS-22 metabolite-sulfamethoxazole ^b
<i>Nocardia asteroides</i>			
651	0.5	0.125	0.008
1170	0.25	0.03	≤0.015
1260	0.5	0.03	≤0.004
1964	>16	>16	4
2039	0.5	0.03	0.008
720	0.5	0.03	0.008
F71743	0.5	0.125	0.06
N9	>16	>16	>16
N22	2	0.25	0.06
S3840	0.5	0.5	0.008
W34408	0.25	0.06	≤0.004
<i>Nocardia caviae</i>			
243	>16	>16	2
2497	>16	>16	>16
<i>Nocardia farcinica</i>			
ATCC 3318	>16	16	2
<i>Nocardia nova</i>			
1276	0.5	0.125	0.015
N5	0.5	0.125	0.06
<i>Nocardia otitidiscaviarum</i>			
ATCC 14629	>16	>16	>16
S49536	>16	>16	2
<i>Nocardia species</i>			
1247	2	0.5	0.008
N8	8	4	1

^a 1 µg/ml.

^b 10 µg/ml.

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