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Antibacterial Activities of Epiroprim, a New Dihydrofolate Reductase Inhibitor, Alone and in Combination with Dapsone

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Epiroprim (EPM; Ro 11-8958) is a new selective inhibitor of microbial dihydrofolate reductase. EPM displayed excellent activity against staphylococci, enterococci, pneumococci, and streptococci which was considerably better than that of trimethoprim (TMP). EPM was also active against TMP-resistant strains, although the MICs were still relatively high. Its combination with dapsone (DDS) was synergistic and showed an in vitro activity superior to that of the TMP combination with sulfamethoxazole (SMZ). The EPM-DDS (ratio, 1:19) combination inhibited more than 90% of all important gram-positive pathogens at a concentration of 2 + 38 μg/ml. Only a few highly TMP-resistant staphylococci and enterococci were not inhibited. EPM was also more active than TMP against *Moraxella catarrhalis*, *Neisseria meningitidis*, and *Bacteroides* spp., but it was less active than TMP against all other gram-negative bacteria tested. Atypical mycobacteria were poorly susceptible to EPM, but the combination with DDS was synergistic and active at concentrations most probably achievable in biological fluids (MICs from 0.25 + 4.75 to 4 + 76 μg/ml). EPM and the EPM-DDS combination were also highly active against experimental staphylococcal infections in a mouse septicemia model. The combination EPM-DDS has previously been shown to exhibit activity in *Pneumocystis carinii* and *Toxoplasma* models and, as shown in the present study, also shows good activity against a broad range of bacteria including many strains resistant to TMP and TMP-SMZ.

Patients with human immunodeficiency virus disease suffer a multitude of infections, such as *Pneumocystis carinii* pneumonia, toxoplasmosis, and cytomegalovirus, fungal, and bacterial infections. This requires a number of different drugs either for therapy or for prophylaxis, and the total drug load is often the limiting factor. Broad-spectrum agents covering as many organisms as possible would have a considerable advantage for these patients. Co-trimoxazole (trimethoprim [TMP] and sulfamethoxazole [SMZ]) is frequently used for the prophylaxis and treatment of *P. carinii* pneumonia and has the advantage of excellent antibacterial activity and activity against *Toxoplasma* spp. (4). However, frequent adverse reactions occur at the high doses used in these patients.

We have recently described a new dihydrofolate reductase (DHFR) inhibitor, epiroprim (EPM; Ro 11-8958) (Fig. 1), which is considerably more active than TMP against the DHFR from *P. carinii* isolates but which is not active against the human DHFR (11, 12). The combination of EPM with dapsone (DDS), which is also used alone for the prophylaxis of *P. carinii* pneumonia (7), was highly effective against *P. carinii* isolates in rats (9, 14) and against toxoplasmosis in mice (3, 9) and in a model of mixed *Pneumocystis* and *Toxoplasma* infection (2). Since both EPM and DDS exhibit high levels of antibacterial activity, we investigated the activities of these compounds, alone and in combination, against a wide range of bacteria in comparison with those of TMP-SMZ and several other agents. The combination had excellent activity against gram-positive bacteria which was generally superior to that of TMP-SMZ.

(Part of the present study was presented at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy [11]).

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MATERIALS AND METHODS

Antibacterial agents. EPM was synthesized at the Pharmaceutical Research Laboratories, F. Hoffmann-La Roche Ltd., Basel, Switzerland. TMP, SMZ, and DDS were obtained from in-house sources. Concentrated stock solutions were prepared in dimethyl sulfoxide and were stored at −20°C. All other antibacterial agents were obtained from commercial sources, and stock solutions were prepared according to the instructions of the manufacturers.

Bacteria. The strains used in this study were from our own culture collection. They were obtained as single isolates from patients in different, mainly European hospitals. Most of the strains were not older than 5 years. The bacteria were identified by standard methods and were kept as stock cultures at −80°C. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included as quality control strains. Clinical mycobacterial isolates were obtained from G. Pfyffer, Swiss National Center for Mycobacteria, Zurich, Switzerland. *Mycobacterium smegmatis* ATCC 607 was obtained from the American Type Culture Collection, Rockville, Md.

Susceptibility testing of bacteria other than mycobacteria. MICs were determined by the agar dilution method (1, 10). Nonfastidious organisms were grown on Iso-Sensitest agar (CM 471; Oxoid). Streptococci, pneumococci, and *Neisseria*, *Listeria*, and *Moraxella* spp. were grown on Iso-Sensitest agar supplemented with 5% sheep blood in the presence of 5% CO_2. Haemophili were tested on Iso-Sensitest agar supplemented with 5% chocolated sheep blood in the presence of 5% CO_2. Anaerobic bacteria (*Bacteroides* spp.) were tested in anaerobic jars

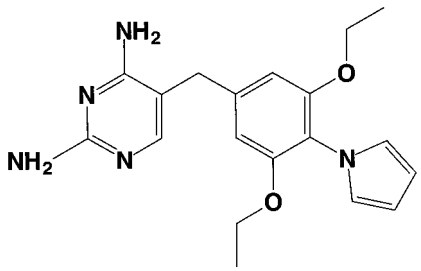

FIG. 1. Structure of EPM (Ro 11-8958).

TABLE 1. Comparative in vitro activities of EPM and its combination with DDS against gram-positive bacteria

Bacteria (no. of strains tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Staphylococcus aureus, methicillin susceptible, TMP susceptible (24)	EPM	0.06	0.125	0.03–0.125
	TMP	0.5	0.5	0.125–0.5
	TMP-SMZ[a]	0.06	0.06	0.03–0.125
	EPM-DDS[a]	0.03	0.06	0.015–0.06
	Ciprofloxacin	0.5	1	0.25–2
	Oxacillin	0.25	1	0.125–2
Staphylococcus aureus, methicillin resistant, TMP susceptible (34)	EPM	0.06	0.125	≤0.03–1
	TMP	0.5	1	0.125–4
	TMP-SMZ	0.06	0.25	0.03–1
	EPM-DDS	0.03	0.125	0.03–0.5
	Ciprofloxacin	0.5	32	0.125–128
	Oxacillin	128	>128	4–>128
Staphylococcus aureus, medium level of resistance to TMP (MIC, 8–128 μg/ml) (45)	EPM	8	32	1–128
	TMP	32	64	8–128
	TMP-SMZ	4	>8	0.5–>8
	EPM-DDS	2	4	0.25–8
	Ciprofloxacin	0.5	128	0.125–>128
	Oxacillin	32	>128	0.125–>128
Staphylococcus aureus, high level of resistance to TMP (MIC, ≥256 μg/ml) (11)	EPM	256	256	32–256
	TMP	512	>512	256–>512
	TMP-SMZ	>8	>8	0.25–>8
	EPM-DDS	>8	>8	0.125–>8
	Ciprofloxacin	0.25	2	0.25–8
	Oxacillin	0.5	4	0.25–>128
Coagulase-negative staphylococci,[b] TMP susceptible (36)	EPM	0.125	0.25	≤0.03–32
	TMP	0.25	4	0.25–4
	TMP-SMZ	0.125	0.5	0.03–4
	EPM-DDS	0.03	0.125	0.002–8
	Ciprofloxacin	0.25	1	0.125–64
	Oxacillin	0.5	16	0.06–>128
Coagulase-negative staphylococci,[c] TMP resistant (70)	EPM	32	64	0.25–128
	TMP	64	128	8–>128
	TMP-SMZ	8	>8	0.125–>8
	EPM-DDS	2	8	0.008–>8
	Ciprofloxacin	0.25	32	0.125–128
	Oxacillin	1	16	0.06–>128
Streptococcus pneumoniae, penicillin susceptible (19)	EPM	0.125	1	0.06–4
	TMP	4	64	2–128
	TMP-SMZ	0.25	1	0.25–8
	EPM-DDS	0.06	0.25	0.03–0.5
	Ciprofloxacin	2	4	1–8
	Oxacillin	0.06	0.125	0.06–2
	Penicillin G	≤0.06	≤0.06	≤0.06
Streptococcus pneumoniae, penicillin resistant (55)	EPM	16	32	0.125–64
	TMP	128	>128	8–>128
	TMP-SMZ	8	>8	0.5–>8
	EPM-DDS	2	2	0.06–4
	Ciprofloxacin	2	4	1–4
	Oxacillin	16	32	0.06–128
	Penicillin G	4	8	0.25–16
Streptococcus agalactiae (18)	EPM	2	64	1–128
	TMP	8	128	4–>128
	TMP-SMZ	0.25	4	0.25–8
	EPM-DDS	0.125	0.5	0.06–>8
	Ciprofloxacin	1	2	1–4
	Oxacillin	0.25	0.5	0.25–0.5

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TABLE 1—*Continued*

Bacteria (no. of strains tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Streptococcus pyogenes (20)	EPM	1	128	0.25–128
	TMP	8	256	2–256
	TMP-SMZ	2	>8	0.25–>8
	EPM-DDS	0.5	>8	0.125–>8
	Ciprofloxacin	0.5	1	0.5–2
	Oxacillin	0.06	0.06	≤0.03–0.06
Viridans group streptococci[d] (15)	EPM	1	64	0.06–64
	TMP	8	128	1–256
	TMP-SMZ	0.5	4	0.125–4
	EPM-DDS	0.25	1	0.015–2
	Ciprofloxacin	2	8	2–16
	Oxacillin	0.5	0.5	0.06–64
Enterococcus faecalis (24)	EPM	≤0.03	16	≤0.03–128
	TMP	0.25	64	0.125–256
	TMP-SMZ	0.06	0.25	0.03–>8
	EPM-DDS	0.008	0.125	≤0.008–4
	Ciprofloxacin	2	32	0.5–>128
	Oxacillin	16	32	8–64
	Vancomycin	2	>16	1–>16
Enterococcus faecium (16)	EPM	≤0.03	64	≤0.03–64
	TMP	0.5	256	0.125–256
	TMP-SMZ	0.5	>8	0.125–>8
	EPM-DDS	0.03	>8	0.008–>8
	Ciprofloxacin	8	>128	1–>128
	Oxacillin	>128	>128	16–>128
	Vancomycin	2	>16	0.5–>16
Listeria monocytogenes (10)	EPM	≤0.03	0.06	≤0.03–0.06
	TMP	0.25	0.25	0.125–0.25
	TMP-SMZ	0.06	0.06	0.03–0.06
	EPM-DDS	0.06	0.06	0.03–0.06
	Ciprofloxacin	2	2	2–4
	Oxacillin	4	4	2–4

[a] The MICs of TMP-SMZ and EPM-DDS are expressed as the values for the TMP and the EPM components, respectively. Ratio, 1:19.
[b] Includes *S. epidermidis* ($n = 26$), *S. cohnii* ($n = 1$), *S. capitis* ($n = 1$), *S. haemolyticus* ($n = 1$), *S. hominis* ($n = 2$), *S. saprophyticus* ($n = 2$), *S. simulans* ($n = 1$), and *S. warneri* ($n = 2$).
[c] Includes *S. epidermidis* ($n = 58$), *S. haemolyticus* ($n = 5$), *S. simulans* ($n = 2$), *S. hominis* ($n = 3$), *S. auricularis* ($n = 1$), and *S. lentus* ($n = 1$).
[d] Includes *S. bovis* ($n = 3$), *S. mitis* ($n = 3$), *S. mutans* ($n = 3$), *S. salivarius* ($n = 3$), and *S. sanguis* ($n = 3$).

(BBL) on Wilkins-Chalgren agar containing menadione (0.5 mg/ml), hemin chloride (5 mg/l), and thymidine phosphorylase (0.2 U/ml; Sigma). Agar plates containing serial twofold dilutions of antibiotics were inoculated with the help of a multipoint inoculator (Denley A400) to yield about 1×10^4 to 5×10^4 CFU per spot. Plates were evaluated after incubation at 35°C for 18 to 20 h. MICs were defined as the lowest concentration of antibiotic that prevented clearly visible growth. A barely visible haze and the growth of up to five colonies per spot were disregarded.

Susceptibility testing of mycobacteria. MICs for mycobacteria were determined by a microdilution method (13). Strains were routinely cultivated in Middlebrook 7H9 broth (pH 6.8; lot no. 21113; Difco) or on Middlebrook 7H10 agar (Difco). Both media were supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment (lot no. 751344; Difco). For testing of the antifolates, the medium was supplemented with 0.2 U of thymidine phosphorylase (Sigma) per ml. The wells of the microtiter tray containing test compounds in 50 μl of 7H9 broth were inoculated with 10^5 CFU/ml. After inoculation, the plates were covered, sealed in plastic bags, and incubated at 37°C without CO_2; *Mycobacterium marinum*, however, was incubated at 30°C. When the organisms had reached good visible growth in control wells, the MICs were read in indirect light by using a Dynatech reading stand. The optimal incubation times were found to be 7 days for slowly growing mycobacteria (*Mycobacterium avium* complex [MAC], *Mycobacterium marinum*, and *Mycobacterium kansasii*) and 3 days for rapidly growing mycobacteria. The MIC was defined as the lowest concentration of a compound which inhibited visible growth, neglecting a barely visible haze of growth.

Synergy testing. The synergistic activity of the EPM-DDS combination was tested by checkerboard analysis (5) in microtiter trays. Middlebrook 7H9 broth was used for mycobacteria, and Iso-Sensitest broth (Oxoid) was used for staphylococci and enterococci. The inoculum size was approximately 5×10^4 CFU/ml. The fractional inhibitory concentration index (ΣFIC) was calculated by the following formula:

$$\Sigma\text{FIC} = \frac{\text{MIC of EPM in combination}}{\text{MIC of EPM alone}} + \frac{\text{MIC of DDS in combination}}{\text{MIC of DDS alone}}$$

Synergy is defined as a ΣFIC ≤ 0.5, additive is a ΣFIC of 1.0, and antagonism is a ΣFIC of >2.0.

Experimental septicemia in mice. Septicemia was induced in outbred Swiss albino mice (Jbm MoRo [specific pathogen free]; weight, 16 to 20 g) (1). The mice were infected by intraperitoneal injection of diluted overnight cultures of the test organisms. Bacterial challenge doses were four times the number of organisms required to kill 50% of the untreated animals within 72 h. Test compounds were administered orally 1 and 3 h after the bacterial challenge. Control and treatment groups at each dose consisted of five mice each. The 50% effective dose (in milligrams per kilogram of body weight) was calculated by probit analysis as described by Finney (6) from the survival rates on day 4 after infection.

RESULTS AND DISCUSSION

The results obtained with EPM and the comparison agents against 397 gram-positive bacteria are summarized in Table 1. EPM was generally two- to eightfold more active than TMP

TABLE 2. Comparative in vitro activities of EPM and its combination with DDS against gram-negative bacteria

Bacteria (no. of strains tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Haemophilus influenzae (24)	EPM	2	8	0.25–32
	TMP	0.25	1	≤0.06–2
	TMP-SMZ[a]	0.125	0.5	0.03–1
	EPM-DDS[a]	1	4	0.125–8
	Ciprofloxacin	≤0.03	≤0.03	≤0.03–0.06
Haemophilus parainfluenzae (13)	EPM	8	16	1–32
	TMP	0.5	0.5	0.125–1
	TMP-SMZ	0.125	0.25	0.06–0.5
	EPM-DDS	4	8	0.5–8
	Ciprofloxacin	≤0.03	0.06	≤0.03–0.06
Moraxella catarrhalis (17)	EPM	8	8	8–16
	TMP	64	64	32–128
	TMP-SMZ	0.5	0.5	0.25–0.5
	EPM-DDS	0.5	0.5	0.25–0.5
	Ciprofloxacin	0.125	0.125	0.06–0.125
Acinetobacter spp.[b] (17)	EPM	64	128	1–128
	TMP	4	16	0.5–128
	TMP-SMZ	0.25	8	0.125–>8
	EPM-DDS	0.5	>8	0.125–>8
	Ciprofloxacin	0.5	2	≤0.03–8
Neisseria meningitidis (10)	EPM	16	16	8–128
	TMP	64	128	64–>256
	TMP-SMZ	0.25	2	0.06–4
	EPM-DDS	1	4	0.5–4
	Ciprofloxacin	≤0.03	≤0.03	≤0.03
Escherichia coli (19)	EPM	4	>128	2–>128
	TMP	0.5	>128	0.25–>128
	TMP-SMZ	0.06	>8	0.06–>8
	EPM-DDS	2	>8	1–>8
	Ciprofloxacin	≤0.03	≤0.03	≤0.03
Shigella flexneri (6)	EPM	>128	>128	>128
	TMP	>256	>256	>256
	TMP-SMZ	>8	>8	>8
	EPM-DDS	>8	>8	>8
	Ciprofloxacin	≤0.03	≤0.03	≤0.03
Salmonella spp.[c] (13)	EPM	1	>128	0.5–>128
	TMP	0.125	>256	0.125–>256
	TMP-SMZ	0.06	>8	0.06–>8
	EPM-DDS	1	>8	1–>8
	Ciprofloxacin	≤0.03	≤0.03	≤0.03
Klebsiella pneumoniae (19)	EPM	8	16	4–>128
	TMP	0.5	1	0.25–>256
	TMP-SMZ	0.25	0.5	0.06–8
	EPM-DDS	4	>8	1–>8
	Ciprofloxacin	≤0.03	0.5	≤0.03–1
Proteus vulgaris (19)	EPM	32	128	4–>128
	TMP	2	8	0.5–32
	TMP-SMZ	0.5	1	0.125–4
	EPM-DDS	2	8	1–>8
	Ciprofloxacin	0.06	0.25	≤0.03–1
Proteus mirabilis (19)	EPM	32	>128	8–>128
	TMP	2	>256	1–>256
	TMP-SMZ	0.5	>8	0.125–>8
	EPM-DDS	4	>8	2–>8
	Ciprofloxacin	0.125	0.125	0.06–4

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TABLE 2—*Continued*

Bacteria (no. of strains tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Citrobacter freundii (18)	EPM	4	>128	1–>128
	TMP	0.25	>256	≤0.06–>256
	TMP-SMZ	0.125	>8	0.03–>8
	EPM-DDS	1	>8	0.015–>8
	Ciprofloxacin	≤0.03	0.25	≤0.03–8
Morganella morganii (19)	EPM	16	>128	2–>128
	TMP	2	>256	0.5–>256
	TMP-SMZ	0.125	>8	0.06–>8
	EPM-DDS	4	>8	1–>8
	Ciprofloxacin	≤0.03	≤0.03	≤0.03
Enterobacter cloacae (20)	EPM	4	8	0.5–>128
	TMP	0.5	1	≤0.06–>256
	TMP-SMZ	0.125	0.5	0.03–>8
	EPM-DDS	1	>8	0.25–>8
	Ciprofloxacin	≤0.03	≤0.03	≤0.03–0.125
Serratia marcescens (18)	EPM	>128	>128	4–>128
	TMP	4	>256	1–>256
	TMP-SMZ	0.5	>8	0.125–>8
	EPM-DDS	>8	>8	4–>8
	Ciprofloxacin	0.125	2	0.06–2
Pseudomonas aeruginosa (19)	EPM	>128	>128	>128
	TMP	128	>256	16–>256
	TMP-SMZ	4	>8	2–>8
	EPM-DDS	>8	>8	>8
	Ciprofloxacin	0.125	4	0.06–32
Bacteroides spp. (12)	EPM	4	4	0.25–8
	TMP	16	32	8–>32
	Ciprofloxacin	8	16	2–>32
	Metronidazole	0.5	1	0.25–8

[a] The MICs of TMP-SMZ and EPM-DDS are expressed as the values for the TMP and the EPM components, respectively. Ratio, 1:19.
[b] Includes *A. anitratus* (n = 10), *A. baumanii* (n = 6), and *A. lwoffi* (n = 1).
[c] Includes *S. typhi* (n = 4) and other *Salmonella* species (n = 9).

against TMP-susceptible strains. It was also more active against TMP-resistant strains, but the resulting MICs were still relatively high. The combination EPM-DDS (1:19) was generally two- to fourfold more active than TMP-SMZ, and the MICs compared favorably with those of the comparison drugs, especially against methicillin-resistant staphylococci. Staphylococci have been subdivided into several categories according to their resistances to methicillin and/or TMP in Table 1. EPM was generally eightfold more active than TMP against susceptible *S. aureus* strains but was mostly only 1 dilution step more active against coagulase-negative staphylococci. High levels of activity of a combination of TMP with DDS against oxacillin-resistant strains of *S. aureus* were reported by Lambertus et al. (8); in our study, EPM-DDS was distinctly more active. Against penicillin-susceptible pneumococci, EPM was as much as 32- to 64-fold more active than TMP. There was a close association between penicillin and TMP resistance: 99% of the penicillin-resistant pneumococci tested were also TMP resistant. EPM was distinctly more active than TMP against these strains, but the MICs were up to 100-fold higher than those for TMP-susceptible strains. The combination EPM-DDS exhibited high levels of activity against TMP-resistant pneumococci, with MICs at which 90% of isolates are inhibited (MIC_{90}s) of 2 + 38 μg/ml. Most enterococci were highly susceptible to EPM and the combination EPM-DDS, with *Enterococcus faecium* being

TABLE 3. Comparative in vitro activities of EPM and EPM-DDS against nontuberculous mycobacteria

Mycobacterial organism	MIC (μg/ml)[a]					
	EPM	EPM-DDS (1:19)	TMP	TMP-SMZ (1:19)	CLM	RMP
M. fortuitum ZH 5	32	0.25	>128	0.06	16	>32
M. chelonae ZH 9	>128	8	>128	>16	4	>32
M. smegmatis 607	2	≤0.06	4	0.125	0.5	16
M. marinum ZH 11	8	≤0.03	16	≤0.03	0.25	0.125
M. kansasii ZH 1	4	≤0.03	64	≤0.03	0.25	0.125
M. kansasii ZH 4	8	≤0.03	64	≤0.03	0.25	0.125
MAC 158-0	64	1	>128	1	0.5	0.25
MAC 3530-0	64	0.5	>128	0.25	4	1
MAC ZH 12	128	4	>128	2	2	1
MAC ZH 13	64	1	>128	0.5	4	32
MAC ZH 14	64	1	>128	1	4	2
MAC ZH 15	64	0.5	>128	0.5	4	4

[a] The MICs of TMP-SMZ and EPM-DDS are expressed as the values for the TMP and the EPM components, respectively. Abbreviations: RMP, rifampin; CLM, clarithromycin.

TABLE 4. Activities of the EPM-DDS combination against selected bacteria tested by the checkerboard methodology

Organism	MIC (μg/ml)			ΣFIC[a]
	EPM	DDS	EPM-DDS[a]	
S. aureus ATCC 25923	0.06	8	0.007/1	0.25
S. aureus 151/4559	0.125	8	0.015/0.5	0.19
S. aureus 743	>512	>512	8/64	<0.14
S. epidermidis ATCC 14990	0.03	>64	0.0035/8	<0.25
S. epidermidis HAL9	64	512	8/64	0.25
E. faecalis ATCC 29212	0.03	>256	0.007/0.5	<0.25
E. faecalis 10	0.5	>256	0.06/0.5	<0.13
E. faecium 10	0.03	256	0.007/256	1.25
MAC ZH13	64	32	0.5/4.5	0.15
MAC ZH14	64	32	1/9	0.30

[a] Data for the combination resulting in the lowest ΣFIC are provided.

less susceptible than *Enterococcus faecalis*. The synergistic action of the combination EPM-DDS, defined as a ΣFIC of ≤0.5, was observed in strains of *S. aureus*, *Staphylococcus epidermidis*, and *E. faecalis*, even in strains resistant to both TMP and DDS (see Table 4). In contrast, no synergistic response was obtained in *E. faecium*.

Among the 282 gram-negative strains (Table 2), only *Moraxella catarrhalis*, *Neisseria meningitidis* and *Bacteroides* spp. were more susceptible to EPM than to TMP. The activity of TMP-SMZ was always better than that of EPM-DDS against gram-negative organisms. Nontuberculous mycobacteria were poorly inhibited by TMP alone, with EPM having distinctly lower, but still high MICs for these organisms (Table 3). The combinations with DDS were nearly equivalent in activity. *M. kansasii* and *M. marinum* were extremely susceptible (MICs, ≤0.03 + 0.6 μg/ml for EPM + DDS). Both EPM-DDS and TMP-SMZ had considerable activity against MAC, with MICs ranging from 0.25 + 4.75 μg/ml to a maximum of 4 + 76 μg/ml (Table 3). A synergistic response was obtained in strains of MAC (Table 4). *Clostridium difficile* was resistant to both DHFR inhibitors (MICs, >32 μg/ml) (data not shown).

The in vitro activity of EPM translated well to in vivo models: EPM, applied as a single agent, was active in mouse septicemia models of *S. aureus* infection (Table 5). The EPM-DDS combination was highly active in these models, and the drugs showed synergistic activity in vivo.

TABLE 5. In vivo efficacy of EPM alone and combined with DDS against experimental septicemia in mice

Organism[a]	Compound[b]	ED_{50} (mg/kg) (95% confidence limit)[c]
S. aureus Schoch	TMP	7.2 (3.0–17.0)
	EPM	3.5 (2.2–5.7)
	DDS	14.2 (8.8–22.8)
	EPM-DDS (1 + 4)	0.77 + 3.08 (0.46 + 1.84–1.26 + 5.04)
S. aureus Smith	TMP	16.8 (12.1–24.4)
	EPM	11.7 (7.4–18.4)
	DDS	>25
	EPM-DDS (1 + 4)	<0.35 + 1.4

[a] The infective doses were 1×10^7 CFU per mouse for the Schoch strain and 2×10^4 CFU per mouse for the Smith strain.
[b] Compounds were administered orally 1 and 3 h after bacterial challenge.
[c] ED_{50}, 50% effective dose.

The combination of EPM with DDS, which has already been demonstrated to have good in vivo activity against opportunistic infections caused by *P. carinii* (9, 14) and *Toxoplasma gondii* (3, 9), also shows activity against a broad range of bacteria causing respiratory tract infections. It is also likely that most atypical mycobacteria, which cause systemic infections once the CD4-cell count drops to <200/mm^3, would be inhibited at concentrations achievable in biological fluids. The combination EPM-DDS would therefore offer a distinct step forward toward the goal of maximal protection with a low overall drug load. This and other such synergistic combinations would be worthy of further investigations in clinical studies.

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