

## Trimethoprim-Sulfamethoxazole in Murine Toxoplasmosis

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The combination trimethoprim-sulfamethoxazole had no greater activity in a murine model of toxoplasmosis than did sulfamethoxazole alone.

Although trimethoprim alone is essentially inactive against *Toxoplasma gondii* in experimental models in vitro (8) and in vivo (4, 8, 9), a number of studies have purportedly shown effectiveness of the combination trimethoprim-sulfamethoxazole against murine (9) and human toxoplasmosis (2, 6, 7). Whether the combination is more active than sulfamethoxazole alone has not been shown. The studies in human cases were uncontrolled and in patients with self-limited forms of toxoplasmosis (e.g., lymphadenopathy). The question is not solely of academic interest as this combination is being used in and recommended for human toxoplasmosis.

### MATERIALS AND METHODS

**Mice.** Swiss-Webster mice, purchased from Simonsen Laboratories, Inc., Gilroy, Calif., and weighing 18 to 20 g, were employed in all experiments.

**Preparation and administration of trimethoprim and sulfamethoxazole.** Trimethoprim (lot no. 56538) and sulfamethoxazole (lot no. 734094) were supplied in the powder form by E. Grunberg, Hoffmann-La Roche, Inc., Nutley, N.J. The amount of drug in the diet was given in milligram amounts per 4 g of food since mice consumed approximately 4 g of the diet each day (3). The drugs were mixed individually or together with the powdered form of normal mouse chow (S/L maintenance diet, Simonsen Laboratories, Inc., Gilroy, Calif.). Initial experiments, performed to determine the palatability of the drug-chow mixtures, revealed that, over a period of 14 days, uninfected mice consumed essentially the same amount of each of the drug-chow diets as they did of the diet without drug. The drug-chow diet was begun immediately after infection and continued for 14 days.

**Toxoplasma inoculum.** Mice were inoculated intraperitoneally with 0.2 ml of saline containing  $5 \times 10^4$  trophozoites of the RH strain harvested from the peritoneal fluid of infected mice, as previously described (5). Control mice were inoculated with saline alone. Animals were followed for time to death. In two experiments, portions of liver, spleen, and brain of survivors were inoculated intraperi-

toneally into normal mice to test for residual infection.

### RESULTS

Five separate experiments were performed. In each experiment there were 10 mice per group. The results were essentially the same in each. In Table 1 are representative results obtained in one of the experiments. As previously found (8), trimethoprim had no demonstrable activity in the doses used. Sulfamethoxazole, however, was active; in three experiments, the mortality at 156 mg/kg was 0, 30, and 90%. In all experiments, 312 mg/kg resulted in 100% survival and 78 mg/kg resulted in 100% mortality. The organs of mice treated with 625 mg/kg were essentially free from *T. gondii*, as mice inoculated with their organs did not die of the

TABLE 1. Effect of trimethoprim and sulfamethoxazole alone and in combination on murine toxoplasmosis

Treatment	g of drug/ 100 g of diet	Approx dose (mg/kg)	Mor- tality (%) <sup>a</sup>	Time to death (days)
Trimethoprim	0.1	250	100	7
	0.05	125	100	8
	0.025	62.5	100	7
Sulfamethoxazole	1.0	2,500	0 <sup>b</sup>	
	0.5	1,250	0 <sup>b</sup>	
	0.25	625	0 <sup>b</sup>	
	0.125	312	0 <sup>b</sup>	
	0.062	156	90	10
	0.031	78	100	8
Trimethoprim (T) plus sulfamethoxazole (S)	0.1 (T) + 0.062 (S)	39	100	7
	0.1 (T) + 0.031 (S)		100	8
Saline			100	7
			100	7

<sup>a</sup> There were 10 mice per group.

<sup>b</sup> Mice were treated for 14 days and thereafter observed for 60 days. No deaths in these groups occurred during the follow-up period.

infection (less than 10 organisms of the RH strain will cause death in 100% of inoculated mice). No synergistic effect was noted when trimethoprim (250 mg/kg) was added to the sulfamethoxazole.

### DISCUSSION

The results obtained in this study did not reveal any synergistic effect of trimethoprim and sulfamethoxazole against murine toxoplasmosis. In the one other study in which the effectiveness of sulfamethoxazole alone and in combination with trimethoprim was examined, Sander and Midtvedt (9) found that trimethoprim had a significant sulfonamide potentiating effect in experimental toxoplasmosis in mice. After 20 days of treatment, survival was greater ( $p < 0.005$ ) in mice receiving trimethoprim (100 mg/kg) plus sulfamethoxazole alone (50 mg/kg). However, mortality, after the 20 days of treatment was concluded, was higher in the groups that received both drugs (59%) than in those treated with the sulfonamide alone (20%).

The lack of effectiveness of trimethoprim alone when compared with the remarkable effectiveness of pyrimethamine and the absence of demonstrated efficacy of the former drug when combined with sulfamethoxazole (the combination presently marketed worldwide) suggest that the combination trimethoprim-sulfamethoxazole should not be depended upon in cases of human toxoplasmosis.

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