Efficacy of Sulfamethoxypyridazine in a Murine Model of Pneumocystis carinii Pneumonia

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Sulfamethoxazole is the component of co-trimoxazole responsible for its efficacy against Pneumocystis carinii pneumonia, but this drug is associated with frequent adverse effects. Sulfamethoxypyridazine is significantly more effective than sulfamethoxazole against a murine model of P. carinii and might be a candidate for testing in infected patients.

Prophylaxis against Pneumocystis carinii pneumonia (PCP) has made an important contribution to extending the life expectancy of patients with human immunodeficiency virus infection. It has been estimated that PCP prophylaxis delays the onset of the first AIDS-related illness by 6 to 12 months (9). The common use of PCP prophylaxis may have led to a dramatic drop in the incidence of PCP. In 1987, for example, PCP represented 50% of the AIDS-defining illnesses in the Multicenter AIDS Cohort Study, while in 1991, it accounted for only 25% (9). The most widely used and effective prophylactic agent is co-trimoxazole (trimethoprim-sulfamethoxazole [TMP-SMX]) (13). Several laboratories have now demonstrated that SMX accounts almost entirely for the antipneumocystis activity of TMP-SMX, with TMP contributing little (11, 12, 17). Dapsone, a sulfone related to the sulfa drugs, is an important second-line agent (16).

Unfortunately, up to 40% of patients on TMP-SMX must discontinue use of the drug due to adverse effects (16). Alternative regimens, including desensitization regimens, have now been found to reduce the incidence of adverse effects but not to eliminate them (4). Thus, a better prophylactic agent would be very useful.

Only a handful of the 15,000 available sulfa drugs have ever been tested against P. carinii. Accordingly, we screened 44 sulfa drugs and found 2, sulfamethoxypyridazine (SMPR) and sulfisoxazole, that appeared to be equal to or better than SMX as inhibitors of recombinant P. carinii dihydropteroate synthase in vitro (8). In a preliminary study of P. carinii-infected rats, sulfisoxazole was found to be only modestly effective, while SMPR was found to be highly effective (7). SMPR was also found to be as effective as SMX in P. carinii-infected rats at 1 mg/kg of body weight/day (10). In the present study, we attempted to compare the efficacy of this agent with that of SMX in a mouse model.

SMPR and SMX were obtained from Rhone-Poulenc-Rorer (Centre de Recherche de Vitry-Alfortville, Vitry-Alfortville, France) and Hoffmann-La Roche (Nutley, N.J.), respectively. Both drugs were administered via drinking water to transtracheally infected BALB/c mice by our previously published protocol (2). Drugs were administered daily to groups of 10 mice either for 6 weeks beginning 1 day after infection (prophylactic protocol) or for 3 weeks beginning 3 weeks after infection (therapeutic protocol). Mice were sacrificed at the cessation of therapy and assessed for intensity of infection as previously described (2). Briefly, Giemsa- and silver-stained slides (stained for trophozoites and cysts, respectively) were scored from 0 (no organisms in 50,000× microscopic fields) to 5 (>100 organisms in 1,000× field). Scores were determined by two microscopists in a blinded manner and then averaged. Data from four experiments were pooled and analyzed by linear regression analysis with SPSS for Macintosh, version 6.1. Each experiment had a control group of 10 mice; the pooled Giemsa and silver stain scores from all control groups averaged 4.38 and 3.45, respectively (Table 1) and were used as the controls for all calculations. The effective drug doses which could reduce these Giemsa and silver stain scores by 50% (ED50s and ED90s, respectively) were calculated with the equations calculated by SPSS for Macintosh, version 6.1. Two-tailed t tests were performed with Microsoft Excel, version 4.0.

Both SMPR and SMX were administered to mice by the therapeutic protocol in four different experimental cohorts (Table 1). Good correlations between the infection scores and the logarithms of the doses were obtained for SMPR by both silver and Giemsa staining for the therapeutic and prophylactic protocols; adjusted R2 values ranged from 0.75 to 0.84. In contrast, the dose-response relationship for SMX could be fitted only poorly, with adjusted R2 values of only 0.19 and 0.14 for the Giemsa and silver stain scores, respectively. However, rough estimates of the ED50 and ED90 for both drugs were obtained and were much lower for SMPR than SMX. The calculated therapeutic ED50 for SMPR were 0.06 and 0.08 mg/kg/day as determined by the Giemsa and silver stain scores, respectively. In contrast, the calculated ED50 were 0.48 and 0.49 mg/kg/day for SMX. The ED90 difference was even greater with a range of 0.17 to 0.19 mg/kg/day for SMPR versus a range of 2.4 to 2.8 mg/kg/day for SMX.

All dosages were calculated at the end of each experiment by taking into account the amount of drinking water ingested by each mouse. Because of variability in mouse behavior, SMPR and SMX were not ingested at the same dosages. However, when Giemsa stain scores for each group of 10 animals were compared by t tests, SMPR at either 0.1 or 0.3 mg/kg/day was found to be significantly more effective than SMX at 0.18, 0.25, or 0.47 mg/kg/day (P < 0.01). Thus, despite some variability in the experimental results, SMPR was statistically significantly more effective than SMX.

SMPR was only slightly more effective when given by the prophylactic protocol than when given by the therapeutic pro-
protocol (Table 1). The ED_{50} and ED_{90} were 0.03 and 0.11 to 0.12, respectively. The difference between protocols was statistically significant only at the 0.03-mg/kg/day dosage (P < 0.01).

The difference in efficacy observed in this study between SMPR and SMX may be due to differences in drug half-lives. The half-lives of SMPR in humans and rats are 37 and 13 h, respectively. In contrast, SMX half-lives are much shorter, only 11 and 5.2 h in humans and rats, respectively (1, 6). The half-lives of these drugs in mice have not been measured, but one can presume that SMPR also has a substantially longer half-life than SMX in mice. Thus, the increased efficacy of SMPR compared to SMX could be due to the fact that at equivalent doses, SMPR accumulates to higher levels in blood than SMX. However, it is also possible that other factors, such as distribution into the lung and a higher affinity for the target enzyme, might account for the difference in efficacy.

There are several reasons why SMPR might be considered as an alternative to TMP-SMX for the prophylaxis and treatment of PCP. First, SMPR is likely to be safe. Sulfapral, which is a combination of sulfamethizole and SMPR, had the lowest fatality rate and the second-lowest serious reaction rate of nine sulfa drugs analyzed (3). Yet the daily dose of Sulfapral was approximately 6 mg/kg (for a 70-kg man) (3), which is substantially higher than the effective daily doses of SMPR determined here (0.1 and 0.3 mg/kg). Second, since SMPR has a longer half-life, patients might be able to take the drug at less frequent intervals. Third, by using a sulfa drug without TMP, adverse effects due to TMP can be avoided (5).

On the other hand, there are several reasons why it might not be appropriate to proceed with clinical trials for SMPR. First, SMPR and SMX are structurally related, so it is unlikely that patients allergic to SMX will be able to tolerate SMPR. Second, SMPR can cause severe adverse effects, such as Stevens-Johnson syndrome (14, 15), although there is no evidence that such adverse effects are more common for SMPR than SMX (3). Third, evidence for the appearance of sulfa-resistant strains of P. carinii has recently been presented (13), implying that new classes of antipneumocystis agents will be needed.

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### REFERENCES