ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1994, p. 1197–1199
0066-4804/94/$04.00+0

Vol. 38, No. 5

Levels of Dapsone and Pyrimethamine in Serum during Once-Weekly Dosing for Prophylaxis of *Pneumocystis carinii* Pneumonia and Toxoplasmic Encephalitis

MILOS OPRAVIL,* BEDA JOOS, AND RUEDI LÜTHY

Division of Infectious Diseases, University Hospital, Zurich, Switzerland

Received 27 September 1993/Returned for modification 7 February 1994/Accepted 7 March 1994

Concentrations of dapsone, monoacetyldapsone, and pyrimethamine were determined in 36 serum samples from human immunodeficiency virus-infected patients on prophylaxis with once-weekly administration of dapsone-pyrimethamine (200 mg of dapsone-75 mg of pyrimethamine). During day 1 after ingestion, median levels of 1,038 ng of dapsone per ml and 356 ng of pyrimethamine per ml were found. During days 6 to 7, the dapsone level fell to <20 ng/ml in five of nine serum samples, but the pyrimethamine level remained elevated (125 ng/ml). Concurrent, but separately ingested, didanosine administration did not seem to decrease the drug concentrations.

Dapsone and pyrimethamine are active against both *Pneumocystis carinii* and *Toxoplasma gondii* (3, 6, 8). We conducted a prospective trial evaluating once-weekly administration of 200 mg of dapsone and 75 mg of pyrimethamine for prophylaxis of *P. carinii* pneumonia and toxoplasmic encephalitis (16). Because no data regarding the achievable steady-state concentrations in human immunodeficiency virus-infected patients with this regimen are available, we examined serum samples from all compliant study participants in our center. We further sought to evaluate whether concurrent treatment with didanosine might decrease the concentrations of dapsone, as previously suggested (15).

The participants were human immunodeficiency virus-infected adults with symptomatic disease and/or <200 CD4 lymphocytes per μl. Simultaneous antiretroviral treatment was allowed. The study design was approved by the ethics committee, and all participants gave informed consent. Blood was collected randomly during the weekly dosing interval from patients who had regularly taken all of the last four doses as reported during the monthly follow-up visits.

Because both drugs were available only as 25-mg tablets in Switzerland, a combination capsule containing 200 mg of dapsone and 75 mg of pyrimethamine was produced for the study (G. Streuli & Co., Uznach, Switzerland). Bioavailability and pharmacokinetics after a single dose of a preliminary combination capsule containing 200 mg of dapsone and 100 mg of pyrimethamine were compared against the same single dose of commercially available tablets in three healthy volunteers before the study began. Both times, concentrations of dapsone and pyrimethamine in serum were determined every 2 h for the first 10 h and 24 and 72 h after ingestion.

Concentrations were determined by a slightly modified high-pressure liquid chromatographic procedure described originally by Edstein (9). Tests for linearity and recovery of the assay yielded correlation coefficients of >0.999 and extraction recoveries of >88%. The within-day coefficients of variation were 1.7% for dapsone and 1.1% for pyrimethamine, and the day-to-day coefficients were 4.4% for dapsone and 3.0% for pyrimethamine. Because 0.3 ml of serum was analyzed, the lower limits of detection were 20 ng/ml for dapsone and monoacetyldapsone and 30 ng/ml for pyrimethamine.

Individuals with a monoacetyldapsone/dapsone ratio of <0.35 were defined as slow acetylators; those with a monoacetyldapsone/dapsone ratio of >0.35 were defined as fast acetylators (19). The 95% confidence intervals of the median were calculated by the binomial-based method (4). The Mann-Whitney test was used for statistical comparisons.

In the preliminary evaluation of a combination capsule containing 200 mg of dapsone-100 mg of pyrimethamine, peak serum drug concentrations after a single dose in three healthy volunteers were 2,970 ± 495 ng/ml (mean ± standard deviation) for dapsone, 315 ± 29 ng/ml for monoacetyldapsone, and 859 ± 211 ng/ml for pyrimethamine. The areas under the curve (trapezoidal method) did not differ significantly between the combination capsule and the commercial tablets, with −8.2% for dapsone (0 to 24 h) and −3.6% for pyrimethamine (0 to 96 h postingestion), demonstrating normal bioavailability of the capsule used later in the trial.

Thirty-six serum samples were collected from 33 human immunodeficiency virus-infected patients on regular prophylaxis with once-weekly treatment with 200 mg of dapsone-75 mg of pyrimethamine. The study population consisted of 31 men and 2 women with a median age of 38 years (range, 29 to 68 years) and a median CD4 lymphocyte count of 160/μl (range, 10 to 450/μl) at study entry. The median duration of prophylaxis with dapsone-pyrimethamine before blood collection was 13 months (range, 1 to 24 months). At the time of blood collection, 14 patients had AIDS.

Reliably high concentrations of dapsone were found only on days 1 to 3 and fell below the limit of detection in five of nine patients whose serum samples were collected beyond day 5. Monoacetyldapsone was detectable in most patients only during the first 4 days. In contrast, pyrimethamine concentrations remained relatively stable during the entire weekly dosing interval (Table 1).

At the time of blood collection, 10 patients were receiving didanosine (13 serum samples), 16 were receiving zidovudine (2 together with zalcitabine), and 7 had no antiretroviral treatment. No differences in the drug concentrations between patients receiving didanosine and those without it are visible (Fig. 1).

* Corresponding author. Mailing address: Division of Infectious Diseases, University Hospital, 8091 Zurich, Switzerland. Phone: 41 1255 3322. Fax: 41 1255 3291.

TABLE 1. Concentrations of dapsone, *N*-monoacetyldapsone, and pyrimethamine during the weekly dosing interval[a]

Day(s) of serum collection	No. of serum samples	Median time after intake (h)	Dapsone concn (ng/ml)	*N*-Monoacetyldapsone concn (ng/ml)	Pyrimethamine concn (ng/ml)
1	7	18 (7–24)	1,038 (24–1,290)	193 (<20–667)	356 (144–659)
2–3	6	49 (32–71)	258 (195–421)	59 (48–153)	281 (189–434)
4–5	14	97 (80–117)	44 (<20–127)	25 (<20–42)	195 (161–355)
6–7	9	145 (122–163)	<20 (<20–49)	<20 (<20–<20)	125 (<30–250)

[a] Median values are shown, with 95% confidence intervals given in parentheses. The lower detection levels were 20 ng/ml for both dapsone and *N*-monoacetyldapsone and 30 ng/ml for pyrimethamine. The results are representative of 36 serum samples from 33 participants in the prophylaxis study who regularly took all doses within the last 4 weeks before blood collection.

The acetylator type could be determined in 18 patients. Nine patients (50%) were slow acetylators. Dapsone levels did not differ between slow and fast acetylators; the median concentrations were 224 ng/ml (95% confidence interval, 134 to 1,198 ng/ml) and 267 ng/ml (95% confidence interval, 45 to 1,038 ng/ml), respectively, when all evaluable values were compared ($P = 0.48$). The times since the last dose received did not differ between these two groups.

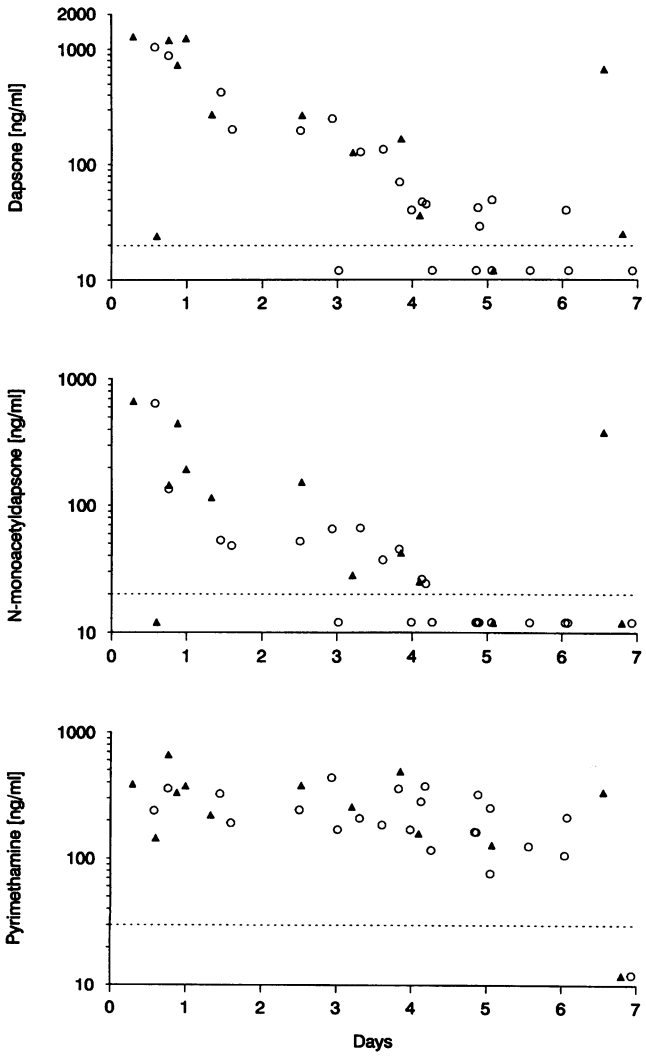

FIG. 1. Serum drug concentrations of dapsone, monoacetyldapsone, and pyrimethamine in relation to time since last dose. The dashed lines delineate the lower detection limits; values below them represent undetectable concentrations. ▲, patients with concurrent didanosine therapy; ○, patients without concurrent didanosine therapy.

Individuals receiving 100 mg of dapsone and 12.5 mg of pyrimethamine once weekly for malaria prophylaxis had peak serum drug levels of 1,134 to 1,420 ng/ml for dapsone and 111 to 116 ng/ml for pyrimethamine (10, 11); the 24-h values were 374 to 803 ng/ml for dapsone and 91 to 121 ng/ml for pyrimethamine (11, 13). Five days after ingestion, concentrations of dapsone fell well below 50 ng/ml in these studies (10, 13). According to the elimination half-lives of 23 to 25 h for dapsone, 22 h for monoacetyldapsone, and 80 to 105 h for pyrimethamine (10, 11), the decrease in the concentrations of dapsone and its principal metabolite monoacetyldapsone throughout the dosing interval, as well as the maintenance of pyrimethamine levels above 100 ng/ml in most of our patients, is to be expected. After extrapolation for the different dosages, serum drug concentrations measured in our study, both after a single dose in healthy volunteers and after multiple doses in human immunodeficiency virus-infected individuals, were thus consistent with the reported values.

On the basis of tissue culture experiments, the 50% inhibitory concentrations of dapsone were 550 to 600 ng/ml against *T. gondii* and <100 ng/ml against *P. carinii* (1, 6, 8). Pyrimethamine displays a strong inhibitory effect against *T. gondii* at concentrations of 40 to 200 ng/ml (1, 5, 7) but is only a weak inhibitor of *P. carinii* dihydrofolate reductase at concentrations of 700 to 945 ng/ml (2, 3).

Peak pyrimethamine concentrations of >750 ng/ml were estimated to be necessary to successfully treat toxoplasmic encephalitis (18), and peak dapsone concentrations of 900 to 2,300 ng/ml were observed in patients successfully treated for *P. carinii* pneumonia (12). Comparison with our data suggests that the serum drug levels achieved during prophylaxis were not sufficient to completely suppress the parasites during the entire weekly dosing interval. However, synergism between dapsone and pyrimethamine, as demonstrated against *T. gondii* (8), might contribute to prophylactic efficacy despite low serum drug levels. Moreover, constant maintenance of high serum drug levels may be less important for prophylaxis than for treatment, as shown by the experience with intermittent trimethoprim-sulfamethoxazole dosing for prevention of *P. carinii* pneumonia. In a study using thrice-weekly trimethoprim-sulfamethoxazole dosages, the trough levels were undetectable in the majority of patients despite prophylactic efficacy, illustrating that the dosing interval may exceed the half-lives of the individual compounds severalfold (17).

To avoid potential loss of dapsone efficacy (15), our patients on concurrent didanosine therapy were instructed to take dapsone-pyrimethamine separately (at least 2 h before or 6 h after didanosine). By following this recommendation, our

measured concentrations seem to exclude a gross interaction caused by didanosine (Fig. 1). However, until more detailed pharmacokinetic evaluations rule out any possible interaction between didanosine and dapsone (as well as other drugs), it appears prudent to keep recommending separate ingestion of didanosine.

The acetylator phenotype was equally divided between the slow and fast acetylators. The acetylation ratio remains constant over several days (13, 14) and could therefore be determined independently of the time since ingestion of the drugs. In concordance with data indicating that the acetylation ratio does not change the rate of dapsone elimination (14), the serum drug concentrations of dapsone did not differ between slow and fast acetylators in our study. Because neither the therapeutic response nor the rate of side effects seems to depend on the acetylator phenotype (19), concentrations of monoacetyldapsone are of less relevance in evaluating clinical events.

In conclusion, the data of this study warrant further investigation of the effects of once-weekly dapsone-pyrimethamine dosing for prophylactic purposes.

This study was supported in part by the Swiss National AIDS Research Program (grants 90-7051 and 91-7059).

We are indebted to Karola Hodapp for help with the data collection, Markus Flepp for support with the study of the volunteers, and Sandra Opravil for editorial assistance.

REFERENCES

1. **Allegra, C. J., D. Boarman, J. A. Kovacs, P. Morrison, J. Beaver, B. A. Chabner, and H. Masur.** 1990. Interaction of sulfonamide and sulfone compounds with *Toxoplasma gondii* dihydropteroate synthase. J. Clin. Invest. **85**:371–379.
2. **Allegra, C. J., J. A. Kovacs, J. C. Drake, J. C. Swan, B. A. Chabner, and H. Masur.** 1987. Activity of antifolates against *Pneumocystis carinii* dihydrofolate reductase and identification of a potent new agent. J. Exp. Med. **165**:926–931.
3. **Broughton, M. C., and S. F. Queener.** 1991. *Pneumocystis carinii* dihydrofolate reductase used to screen potential antipneumocystis drugs. Antimicrob. Agents Chemother. **35**:1348–1355.
4. **Campbell, M. J., and M. J. Gardner.** 1989. Calculating confidence intervals for some non-parametric analyses, p. 71–79. *In* M. J. Gardner and D. G. Altman (ed.), Statistics with confidence. British Medical Journal, London.
5. **Chamberland, S., H. A. Kirst, and W. L. Current.** 1991. Comparative activity of macrolides against *Toxoplasma gondii* demonstrating utility of an in vitro microassay. Antimicrob. Agents Chemother. **35**:903–909.
6. **Cushion, M. T., D. Stanforth, M. J. Linke, and P. D. Walzer.** 1985. Method of testing the susceptibility of *Pneumocystis carinii* to antimicrobial agents in vitro. Antimicrob. Agents Chemother. **28**:796–801.
7. **Derouin, F., and C. Chastang.** 1989. In vitro effects of folate inhibitors on *Toxoplasma gondii*. Antimicrob. Agents Chemother. **33**:1753–1759.
8. **Derouin, F., C. Piketty, C. Chastang, F. Chau, B. Rouveix, and J. J. Pocidalo.** 1991. Anti-*Toxoplasma* effects of dapsone alone and combined with pyrimethamine. Antimicrob. Agents Chemother. **35**:252–255.
9. **Edstein, M.** 1984. Quantification of antimalarial drugs. II. Simultaneous measurement of dapsone, monoacetyldapsone and pyrimethamine in human plasma. J. Chromatogr. **307**:426–431.
10. **Edstein, M. D., K. H. Rieckmann, and J. R. Veenendaal.** 1990. Multiple-dose pharmacokinetics and in vitro antimalarial activity of dapsone plus pyrimethamine (Maloprim) in man. Br. J. Clin. Pharmacol. **30**:259–265.
11. **Jones, C. R., and S. M. Ovenell.** 1979. Determination of plasma concentrations of dapsone, monoacetyl dapsone and pyrimethamine in human subjects dosed with maloprim. J. Chromatogr. **163**:179–185.
12. **Lee, B. L., I. Medina, N. L. Benowitz, P. Jacob, C. B. Wofsy, and J. Mills.** 1989. Dapsone, trimethoprim, and sulfamethoxazole plasma levels during treatment of *Pneumocystis* pneumonia in patients with the acquired immunodeficiency syndrome (AIDS). Evidence of drug interactions. Ann. Intern. Med. **110**:606–611.
13. **Lee, H. S., T. Y. Ti, P. S. Lee, and C. L. Yap.** 1985. Simultaneous estimation of serum concentrations of dapsone, monoacetyldapsone, and pyrimethamine in Chinese men on maloprim for malaria prophylaxis using reversed-phase high performance liquid chromatography. Ther. Drug Monit. **7**:415–420.
14. **May, D. G., J. A. Porter, J. P. Uetrecht, G. R. Wilkinson, and R. A. Branch.** 1990. The contribution of *N*-hydroxylation and acetylation to dapsone pharmacokinetics in normal subjects. Clin. Pharmacol. Ther. **48**:619–627.
15. **Metroka, C. E., M. F. McMechan, R. Andrada, L. J. Laubenstein, and D. P. Jacobus.** 1991. Failure of prophylaxis with dapsone in patients taking dideoxyinosine. N. Engl. J. Med. **325**:737.
16. **Opravil, M., A. Heald, A. Lazzarin, B. Hirschel, R. Lüthy, and the Swiss Group for Clinical Studies on AIDS.** 1993. Dapsone-pyrimethamine vs. aerosolized pentamidine for combined prophylaxis of PCP and toxoplasmic encephalitis, abstr. PO-B10-1429. IX Int. Conf. AIDS, Berlin, Germany.
17. **Stein, D. S., R. C. Stevens, D. Terry, S. C. Laizure, S. Palte, D. J. Lancaster, J. J. Weems, and C. L. Williams.** 1991. Use of low-dose trimethoprim-sulfamethoxazole thrice weekly for primary and secondary prophylaxis of *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. **35**:1705–1709.
18. **Weiss, L. M., C. Harris, M. Berger, H. B. Tanowitz, and M. Wittner.** 1988. Pyrimethamine concentrations in serum and cerebrospinal fluid during treatment of acute *Toxoplasma* encephalitis in patients with AIDS. J. Infect. Dis. **157**:580–583.
19. **Zuidema, J., E. S. Hilbers Modderman, and F. W. Merkus.** 1986. Clinical pharmacokinetics of dapsone. Clin. Pharmacokinet. **11**: 299–315.