Bioavailability of Oral Trimetrexate in Patients with Acquired Immunodeficiency Syndrome

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The combination of the lipophilic antifolate trimetrexate and the rescue agent leucovorin has shown promise in the treatment of Pneumocystis carinii pneumonia in patients with acquired immunodeficiency syndrome. The pharmacokinetic behavior of trimetrexate administered either by intravenous bolus or orally was studied in six patients with acquired immunodeficiency syndrome with a reversed-phase high-pressure liquid chromatography assay. The mean clearance following bolus injection was 38 ml/min per m², with a range of 15 to 55 ml/min per m². The postdistributive half-life ranged from 6 to 16 h. With oral administration, the mean bioavailability was 44% (range, 19 to 67%). An oral dose of 60 mg/m² (162 μmol/m²) resulted in concentrations in plasma that approximated those achieved with a 30-mg/m² (81-μmol/m²) intravenous dose. The toxicity of this combination regimen was minimal. It appears that the oral route is a practical route of administration for trimetrexate in patients with acquired immunodeficiency syndrome requiring long-term outpatient treatment or prophylaxis for P. carinii pneumonia.

Pneumocystis carinii pneumonia is the most common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS). The attack rate is at least 65% in this patient population, and this infection accounts for 25% of all deaths (6, 14). Conventional therapy with the combination of trimethoprim and sulfamethoxazole or pentamidine isethionate is associated with a 20 to 40% failure rate and a 60 to 100% incidence of adverse reactions (12). For these reasons new antiprotozoal drugs are being evaluated.

A novel approach that has shown promise for the treatment of P. carinii pneumonia in patients with AIDS is the use of a regimen combining trimetrexate (2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline [TMTX]) and leucovorin (5-formyl-tetrahydrofolate) (1). TMTX is a lipid soluble antifolate that readily crosses both mammalian and protozoan cell membranes independently of the folate membrane transport system (7, 11). Like methotrexate, the classical folate antagonist, TMTX is a potent inhibitor of the enzyme dihydrofolate reductase, which converts dihydrofolate to the active, chemically reduced tetrahydrofolate form (5). Recent studies have shown TMTX to be a much more potent inhibitor of protozoal dihydrofolate reductase than the conventionally used antifolates trimethoprim and pyrimethamine. Leucovorin, which is transported across the mammalian cell membrane by a specific transport system that is not present in the pneumocystis cell wall, can selectively rescue host cells from the toxic effects of TMTX.

The pharmacokinetics of TMTX administered by intravenous (i.v.) bolus has been described in the initial phase I trials performed in cancer patients refractory to conventional treatment (3, 10, 13). The drug is cleared primarily by biotransformation and has a postdistributive half-life of 12 to 24 h (3, 13). However, the bioavailability of TMTX following oral administration in humans has not been reported. The oral route is clearly more convenient for the treatment or prophylaxis of protozoal disease in patients with AIDS. In this report we present results of a pharmacokinetic study of TMTX in patients with AIDS who received the drug by both the i.v. and oral routes.

MATERIALS AND METHODS

Drug formulation and administration. TMTX was supplied by the Investigational Drug Branch of the National Cancer Institute as a sterile lyophilized powder in 6-ml vials containing 50 mg of the drug as the glucuronate salt. The drug was reconstituted with 1.9 ml of sterile water (25 mg/ml) and injected by i.v. bolus. Because of its insolubility in chloride-containing solutions, TMTX could be injected only into i.v. lines containing 5% glucose in water. The i.v. preparation, mixed in the same fashion, was also used for oral administration.

Patients. Six patients with AIDS (ages 27 to 45 years) who were being treated for P. carinii pneumonia (four patients) or toxoplasmosis (two patients) with daily i.v. TMTX (30 mg/m² or 81 μmol/m²) administered as a bolus injection and leucovorin (20 mg/m² every 6 h, i.v. or peroral) by an approved National Cancer Institute protocol participated in this study. Further details of the drug doses and schedules and the results of the trial have been reported separately (1). None of these patients were receiving sulfamethoxazole at the time of study. Liver function and renal function were assessed in all patients prior to pharmacokinetic studies and found to be within the normal range. Informed consent was obtained from all patients prior to participation in this study.

All patients had completed at least 3 weeks of treatment with i.v. TMTX and leucovorin. Prior to discontinuation of i.v. TMTX, a dose was monitored by drawing heparinized blood samples prior to the dose and at 5 and 30 min and 1, 2, 4, 6, 8, and 24 h after the dose. Plasma was separated by sedimentation and stored frozen until assayed. In the patients with P. carinii pneumonia, oral TMTX (60 mg/m² or
162 μmol/m²) and leucovorin administration was started after a 3-week hiatus during which no TMTX was administered. The two patients with toxoplasmosis were switched to chronic oral therapy with TMTX and leucovorin. On day 3 of oral therapy, an oral dose of TMTX was monitored in the same fashion as the i.v. dose. All patients fasted prior to the oral dose. Patients were monitored for toxicity with biweekly blood counts and a weekly chemistry profile.

Drug assays. TMTX concentration in plasma was measured by a specific reversed-phase high-pressure liquid chromatography assay which has been previously described (2). This assay is specific for TMTX since it separates the parent drug from its more polar metabolites. Trimethoprim (Sigma Chemical Co., St. Louis, Mo.) was added to plasma samples prior to extraction as an internal standard. TMTX was extracted and concentrated from plasma using C₁₈ SEP-PAK cartridges (Waters Associates, Inc., Milford, Mass.) and injected onto a 5-μm C₁₈ Nova-PAK radial column (Waters Associates). The mobile phase contained 0.02 M KH₂PO₄ (pH 4.5) and 25% acetonitrile and was pumped at a rate of 2 ml/min. In the presence of sulfamethoxazole, the mobile phase had to be modified to 0.05 M KH₂PO₄ (pH 4.5) and 18% acetonitrile, because of the sulfamethoxazole coeluted with TMTX. The eluent was monitored with a UV detector at a wavelength of 240 nm. Retention times for trimethoprim and TMTX were 2.8 and 5.0 min (3.6 and 10.9 min with modified mobile phase), respectively. The lower limit of sensitivity for the concentrated samples was 0.05 μmol/liter.

Pharmacokinetic calculations. Pharmacokinetic parameters were determined by model-independent methods. Area under the plasma concentration-time curve (AUC) from 0 to 24 h was derived by the trapezoidal method (8). Total clearance was calculated from the intravenous data by dividing the dose by the AUC. The terminal half-life was determined by regression analysis. Volume of distribution at steady state was derived from the area under the moment curve (15). The bioavailability of the oral dose (F) was calculated from the following equation (8): 

\[ F = \frac{\text{dose}^{\text{oral}} \cdot \text{AUC}^{\text{oral}}}{\text{dose}^{\text{i.v.}} \cdot \text{AUC}^{\text{i.v.}}} \]

where p.o. means peroral.

RESULTS

The pharmacokinetic parameters for TMTX following both i.v. and oral administration are listed in Table 1. With i.v. administration there was some variability in the rate of drug elimination, with a four- to fivefold range in total clearance (15 to 55 ml/min per m²) and a two- to threefold range in the postdistributive half-life (6 to 16 h).

Followiing oral administration, the rate of absorption was variable, with peak concentrations occurring from 0.5 to 4 h after the dose. An average of 44% of the dose was absorbed, with a range from 19 to 67%. Patient 4, who had the lowest fraction absorbed, actually achieved a total TMTX exposure equivalent to that of the other patients as measured by both peak concentration and AUC (Table 1). Concentrations in plasma with the oral dose were equivalent to those following an i.v. dose in the postdistributive phase when twice the i.v. dose was administered orally (Fig. 1).

In these six patients, the only side effect of the oral TMTX and leucovorin was the bitter taste of the TMTX solution.

DISCUSSION

The bioavailability of oral TMTX in patients with AIDS who were infected with either P. carinii or toxoplasmosis

![FIG. 1. Plasma disappearance curves of TMTX after an i.v. bolus dose of 30 mg/m² (○) and an oral dose of 60 mg/m² (■) in six patients with AIDS. Points and error bars represent the means and standard deviations.](image-url)
was 44%. With a single daily oral dose of 60 mg/m² (162 μmol/m², which was twice the effective intravenous dose), concentrations in plasma approximated those achieved with the i.v. dose and were maintained above or near 1 μmol/liter, a concentration that inhibited P. carinii in vitro for 24 h. However, the variability in the rate of drug clearance noted in this study and previous pharmacokinetic studies with cancer patients suggests that adjustments in the dosing schedule based on therapeutic drug monitoring may be required in patients with either very rapid or delayed clearance.

The incomplete bioavailability of this drug may be due either to incomplete absorption or to presystemic metabolism of the drug. Although no data were generated in this study to define the mechanism of the limited bioavailability, in previous pharmacokinetic studies in monkeys, higher circulating concentrations of dihydrofolate reductase-inhibiting metabolites of TMTX were detected after oral administration than after i.v. administration, suggesting some presystemic metabolism of the drug (2).

The oral absorption of the classical antifolate methotrexate has been well studied and appears to be a saturable, carrier-dependent process. As the dose of methotrexate is increased, the absorption becomes prolonged and incomplete (4, 9). Further studies with rat jejunum have demonstrated that methotrexate shares a carrier-mediated transport system with the naturally occurring folates (16). It is unlikely that TMTX is dependent on this carrier for absorption, since cellular uptake is independent of the folate carrier. Up to 60% of a 60-mg/m² (162-μmol/m²) dose of TMTX is absorbed, in contrast to the small fraction of a comparable 80-mg/m² (176-μmol/m²) dose of methotrexate that reaches the systemic circulation (9).

The primary toxicities of TMTX in patients with refractory cancer who did not receive concurrent leucovorin include myelosuppression, mucositis, a maculopapular rash, and elevations in hepatic transaminases (3, 13). On a daily schedule for 5 days, the tolerable dose in this patient population is 8 mg/m² (22 μmol/m²), well below the 30-mg/m² (81-μmol/m²) dose administered daily and indefinitely with leucovorin in patients with AIDS who experience minimal toxicity. Thus it appears that leucovorin decreases the frequency of adverse reactions.

In conclusion, it appears that the oral route will be practical for patients with P. carinii pneumonia who can be managed as outpatients or for patients who require long-term prophylaxis. Further trials to evaluate the efficacy of an oral regimen of TMTX and leucovorin in these situations are needed. General use must also await the development of a formulation of the drug specifically for oral administration.

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LITERATURE CITED


