Clinical Pharmacokinetics of 5-Methyltetrahydrohomofolate

Ti Li Loo, Liu Jiushi, Katherine Lu, and Niramol Savaraj

Department of Developmental Therapeutics, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

ABSTRACT

DL-N5-Methyltetrahydrohomofolate (MTHHF; NSC-139490; N5-[4-[(2-amino-3,4,5,6,7,8-hexahydro-4-oxo-5-methyl-6-pteridinyloxyethyl] amino] benzoyl]-L-glutamate), a new folate antagonist of relatively low toxicity, is active against experimental tumor systems resistant to methotrexate and consequently now in clinical trial. We investigated its clinical pharmacokinetics in six patients; four of them received by rapid i.v. infusion tracer doses of [N5-methyl-14C]MTHHF ranging from 13 to 16 mg/sq m, and 2 received 150 mg/sq m; the total radioactivity dose was 100 to 200 μCi/patient. MTHHF was assayed by radiochemical and chromatographic techniques. The elimination of MTHHF from the plasma followed a triexponential pattern, with a harmonic mean initial half-life of 20.1 min, an intermediary half-life of 4.5 hr, and a terminal half-life of 74.6 hr. The apparent volume of distribution was 1.6 liters/kg, suggesting rapid and extensive tissue binding. This, together with the low total clearance of 0.2 ml/kg/min, contributed to the long half-life of this agent. Although 68% of the administered dose was excreted in the urine on the first day, only an additional 1% was excreted on the ensuing 3 days. In the two patients who received the higher dose, very little MTHHF was found in the cerebrospinal fluid. In concentrations ranging from 25 to 500 μg/ml, the drug was about 50% bound to plasma protein. MTHHF was not metabolized in humans as also reported in animals. These results suggest that MTHHF is excreted in the bile to a certain extent. Moreover, since it tends to localize and persist in the body, to forestall cumulative toxicity, frequent administration of this agent should be undertaken only with caution.

INTRODUCTION

The continuing search for folate antagonists more efficacious than methotrexate as cancer chemotherapeutic agents has led to the synthesis of MTHHF3 (structure shown in Chart 1). Compared with its parent compound, tetrahydrohomofolate, not only is MTHHF less toxic but it is also more active against murine leukemia L1210. Above all, however, both are equally active against a variant of the L1210 tumor resistant to methotrexate (9). MTHHF is hence a second-generation folate antagonist of considerable promise. In preparation for its clinical trial, the pharmacological fate of MTHHF has been investigated in several animal species (2, 3). In this report, we describe our pharmacokinetic studies with very low doses of [5-methyl-14C]MTHHF in 6 volunteer patients with cancer to facilitate future clinical trial of this new agent.

MATERIALS AND METHODS

Drugs and Chemicals. MTHHF and [5-methyl-14C]MTHHF (7.3 mCi/mmol) were obtained from the Developmental Therapeutics Program of the Division of Cancer Treatment, National Cancer Institute. The labeled drug was 93% chemically pure and 95% radiochemically pure by radiochemical and chromatographic analyses. Glass-distilled chromatographic grade solvents were purchased from Burdick Jackson Laboratories, Muskegon, Mich. Other chemicals and reagents were supplied by regular commercial sources.

Patients. Volunteering for this study were 6 patients not responsive to conventional chemotherapy, 4 with metastatic breast carcinoma, one with Hodgkin’s disease, and one with angiolymphomatosus lymphadenopathy. Informed consents were obtained as required by institutional regulations. Initially, 4 patients received 13- to 16-mg/sq m doses, 250 μCi total, of the labeled drug. To exclude the possibility of dose-dependent pharmacokinetics, 2 additional patients with breast cancer were given a 150-mg/sq m dose of the drug containing the same dose of radioactivity; the highest nontoxic dose of MTHHF in beagle dogs by a single i.v. administration is 5000 mg/sq m (National Cancer Institute Investigational Drug-Pharmaceutical Data, March 1978). The drug was dissolved in 0.9% NaCl solution to concentrations of 1 to 5 mg/ml and filtered under sterile conditions through a Millipore Cathix bacterial filter with 0.45 μm pore size. The filtrate was tested for sterility and pyrogenicity. The sterile, pyrogen-free solution was administered i.v. to patients in 10 to 15 min. Blood samples of 10 ml each were drawn through a heparin lock from the arm opposite to the one used for drug administration. Urine was collected every 6 hr for 24 hr and then daily for 3 days. When possible, cerebrospinal fluid was sampled through an Ommaya reservoir. Table 1 summarizes patients’ diagnosis and drug dosages.

Drug Assay. Blood samples were centrifuged at 10,000 x g for 10 min to separate the plasma from the cells; the plasma was kept frozen until use. For radioactivity determination, 0.2 ml of the plasma or other physiological fluids was admixed with 11 ml of PCS (a toluene-based phase-confirmed liquid scintillation counting fluid supplied by Amer sham/Searle Corp.) and counted in a Packard Model 2650 liquid scintillation spectrometer; quenching was automatically corrected with an external standard; counting efficiency for 14C was above 90%. To assay for unchanged MTHHF, the plasma was deproteinized with one- tenth of its volume of 20% sulfosalicylic acid and applied to an ITLC-SA sheet (Gelman Instrument Co.). The chromatogram was developed in 1-butanol-acetic acid-water (20:3:7, v/v/v) by ascending flow. Upon completion of development, the ITLC-SA sheet was visualized under UV illumination and cut into 1-cm squares, and each square was placed in a counting vial. The radioactivity therein was determined after the addition of 11 ml of PCS as before. The Rf of authentic MTHHF was 0.60. No other radioactive spot was detected in any of the physiological fluids. To ensure the absence of degradation and metabolism of MTHHF, a HPLC analysis was additionally used. This consisted of a Waters Model ALC-204 liquid chromatograph equipped with a Model 600A pump and a U6K injector. The following varieties of Varian equipment were also used: a Vari-chrom detector; a CDS-111 integrator; and a Model 9176 recorder. Separation was achieved with a 3.9-mm x 30-cm C18Bondapak reverse-phase column, eluted with 85%...
RESULTS

Clinical Pharmacokinetics of MTHHF. Chart 2 shows the elimination of unchanged MTHHF from the plasma of 2 patients; one receiving the drug at 16 mg/sq m and the other being given 150 mg/sq m. The cumulative urinary excretion of MTHHF was 68% of the administered dose at 24 hr and 69% at 96 hr (Chart 3). In other words, the excretion was virtually complete 24 hr after drug administration. In Table 1, we also present the pharmacokinetic parameters of MTHHF in these 6 patients.

MTHHF disappeared from the plasma of patients who received the drug in a triexponential fashion, with an initial half-life of 20.1 min, an intermediate half-life of 4.5 hr, and a terminal half-life of 74.6 hr. The mean total clearance was 0.2 ml/kg/min, and the apparent volume of distribution was 1.6 liters/kg. These parameters in the 2 patients who received the higher dose of 150 mg/sq m, did not deviate significantly from those of the 4 patients who were given the lower doses. Evidently, dose-dependent pharmacokinetics was not apparent.

Cerebrospinal fluid sampled from the Ommaya reservoir was periodically analyzed in the 2 patients who received the high dose of MTHHF. In one, the drug was detectable 18 min after administration and remained so for 6 hr; however, the maximal concentration of 0.2 μg/ml was sustained during 1 and 2 hr. In the other, MTHHF was detectable for 48 hr; the 6- and 24-hr samples showed the highest concentration of 0.3 μg/ml.

MTHHF was about 51 ± 2% (S.D.) bound to human plasma protein in concentrations of 25 to 500 μg/ml. There was no evidence of MTHHF metabolism in humans.

Table 1

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose (mg/sq m)</th>
<th>Initial (min)</th>
<th>Intermediary (hr)</th>
<th>Terminal (hr)</th>
<th>VD (liters/kg)</th>
<th>Clearance (ml/kg/min)</th>
<th>Urinary excretion (% in 96 hr)</th>
<th>Diagnosis</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>11.1</td>
<td>3.3</td>
<td>116</td>
<td>3.0</td>
<td>0.3</td>
<td>59 Lymphoma</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>13.2</td>
<td>3.4</td>
<td>58</td>
<td>1.0</td>
<td>0.2</td>
<td>62 Lymphoma</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>54.2</td>
<td>14.4</td>
<td>58</td>
<td>1.5</td>
<td>0.3</td>
<td>59 Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>34.5</td>
<td>4.4</td>
<td>116</td>
<td>2.0</td>
<td>0.2</td>
<td>88 Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>22.6</td>
<td>3.6</td>
<td>50</td>
<td>1.3</td>
<td>0.3</td>
<td>69 Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>24.7</td>
<td>6.1</td>
<td>115</td>
<td>1.0</td>
<td>0.1</td>
<td>75 Breast carcinoma</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>20.1 ± 7.1</td>
<td>4.5 ± 1.9</td>
<td>74.6 ± 14.4</td>
<td>1.6 ± 0.3</td>
<td>0.2</td>
<td>69 ± 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Harmonic mean.
As previously reported in experimental animals (2, 3), in all of the plasma and urine specimens, the radioactivity was detectable exclusively in the MTHHF fraction by ITLC and HPLC analysis.

**DISCUSSION**

The elimination of MTHHF from the plasma of patients who received the drug i.v. was slow, with a prolonged average half-life in excess of 3 days (Table 1). This long terminal half-life is attributable to the relatively large apparent volume of distribution coupled with the low total clearance of the agent (4). Although all 6 patients had cancer, none showed any evidence of renal or hepatic dysfunction. Consequently, these observations strongly suggest that MTHHF was extensively bound to tissues, a contention also supported by the excretion studies. Since urinary excretion of MTHHF was 68% of the dose in 24 hr (Chart 3) and thereafter no more than an additional 1% in the next 3 days, and since the drug was not metabolized, the remaining 31% of the dose must have been either excreted in the bile or bound to tissues and thereby retained in the body, or both. Bile samples were not available from these patients; unfortunately, our attempts to collect the feces were also not successful. However, considerable amounts of the drug was found in the feces of rats, dogs, and monkeys (2, 3), undoubtedly from the bile. In our experience, anticancer drugs excreted in the bile of animals are inevitably excreted also in the bile of humans, although not necessarily to the same extent. In that event, the biliary excretion of MTHHF coupled with enterohepatic recirculation additionally could contribute to the long plasma half-life of this agent. In the animal species mentioned above, MTHHF binding and retention by the liver, kidneys, and intestines have been demonstrated (2, 3). Probably in humans, MTHHF is similarly bound extensively to various tissues and only gradually released. Accordingly, to prevent serious cumulative toxicity, caution must be exercised when using this agent frequently.

Species differences in the pharmacological disposition of this drug are remarkably few. Only in rodents was the elimination of MTHHF from the plasma biphasic with a comparatively short terminal half-life of about 1 hr (2, 3). In monkeys and dogs as well as in humans, the plasma disappearance of MTHHF was triphasic with much longer terminal half-lives ranging from less than 15 hr in monkeys, to 27 to 45 hr in dogs (2), to more than 3 days in our patients. The drug was about 50% bound to human plasma protein and only somewhat more extensively (65 to 80%) to dog and monkey plasma proteins. In all the species studied, 50 to 80% of the administered dose was excreted in the urine; the remaining dose was in the feces. Hepatobiliary excretion of MTHHF was not determined in our patients, but in experimental animals it was considerable. No MTHHF metabolite was ever detected in the blood and urine of humans or animals. However, using \([N\text{-methyl}^{14}C]\)MTHHF in the monkey, a trace (0.3%) of the administered radioactivity was found in the expired air as radioactive carbon dioxide. Evidently, metabolism of MTHHF, oxidative N-demethylation in particular was minimal.

From the studies of El Dareer et al. (2), we estimated that after an i.v. dose of 150 mg/sq m, the apparent volume of distribution of MTHHF in liters/kg was 2.8 in the dog but 1.1 in the monkey, whereas the total clearances in ml/kg/min were 1.7 and 1.1, respectively, in the 2 species. When the dose was increased 10-fold to 1.5 g/sq m, the apparent volume of distribution was 2.7 in the dog and 1.3 in the monkey, whereas the corresponding values of total clearance were 2.2 and 1.8, respectively. Further, as in our human studies, at these dose levels MTHHF pharmacokinetics showed no dose dependency. However, in contrast with our clinical results, the total clearance of MTHHF in the dog and the monkey was significantly greater. Since the apparent volumes of distribution of this drug were comparable in the 3 species, the small total clearance alone would be responsible for the very long plasma half-life of the drug in humans.

Structurally, MTHHF is a homologue of MTHF (Chart 1), a naturally occurring folate derivative in human serum and a metabolite of citrovorum factor, the “rescue” agent commonly used in high-dose methotrexate therapy. Pharmacokinetic studies of MTHF derived from i.v. (6) and p.o. (8) citrovorum factor have revealed that its plasma terminal half-life in humans was about 2 hr, in distinct contrast to 3 days with MTHHF. Based on the results of Mehta et al. (8), we estimated the apparent volume of distribution of MTHF to be 640 ml/kg and the total clearance to be about 3 ml/kg/min. These parameters differ strikingly in magnitude from those of MTHHF. Apparently the insertion of an additional methylene group between C9 and C10 of \(N\text{-methyltetrahydrofolate}\) has greatly modified its lipophilicity and drastically altered its pharmacokinetics. Because it is considerably protein bound and most probably highly ionized at physiological pH, MTHHF shows little propensity to cross the blood-brain barrier. As a consequence, it would probably not be effective for the treatment of cancers of the central nervous system.

The mechanism of antitumor action of MTHHF is obscure. Although the L-diastereoisomer of this agent is a substrate for the cobalamin methyltransferase of *Escherichia coli* and cells of certain mammalian species (11), the cytotoxicity of L-MTHHF is not related to its demethylation to L-tetrahydrohomofolate and the subsequent inhibition of thymidylate synthesis. In any
event, in vivo demethylation and metabolism of MTHHF has never been detected in humans or experimental animals (2, 3). MTHHF shares a carrier transport mechanism with methotrexate and reduced folate cofactor, but the relationship between this observation and the antitumor activity of MTHHF remains to be elucidated.

REFERENCES


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