Pharmacokinetics of Folinic Acid and 5-Methyltetrahydrofolic Metabolite After Repeated Oral Administration of Calcium Folate Following Methotrexate Treatment


The pharmacokinetic profiles of folic acid (FA) and its active metabolite, 5-methyltetrahydrofolic acid, were studied after oral administration of decreasing doses of calcium folinate during 37 courses of high and intermediate dose methotrexate treatment in 25 lymphoma patients. FA was administered at a dose of 6 × 50 mg in 15 courses, 6 × 25 mg in seven courses, 6 × 15 mg in 10 courses and 6 × 7.5 mg in 5 courses. FA, 5-methyltetrahydrofolic acid, methotrexate and 70H-methotrexate were assayed simultaneously by high performance liquid chromatography. When FA was administered at doses between 50 and 15 mg, maximum concentrations of both the drug and its metabolite were always obtained after 1 to 2 h and remained stable. The same was true for the equilibrium concentration of the two products at doses over 15 mg. These findings suggest saturation of absorption and metabolism of folic acid at doses over 15 mg.

Key words: folic acid, 5-methyltetrahydrofolic acid, metabolite, pharmacokinetic, methotrexate, rescue, repeated dosing


INTRODUCTION

The clinical use of high dose (HD) methotrexate (MTX) treatment is feasible in association with the subsequent administration of 5-formyl tetrahydrofolate (THF), commonly known as folic acid (FA) [1, 2]. Rescue has been successfully achieved by both the oral and intravenous routes [3, 4]. However, many questions concerning the optimal dose, mode and frequency of administration of FA remain unresolved.

Commercially available FA is a racemic mixture of L-CHO-THF and D-CHO-THF. Absorption of L-CHO-THF is 4 times greater than D-CHO-THF: 80 versus 20% [5]. L-CHO-THF is rapidly converted to 5-methyl tetrahydrofolic acid [6], which
Patients

Drugs

LYMPHOMA ranging in age from 26 to 62 years (mean 43) entered the study. They underwent 21 courses of HD MTX (1500 mg/m² in 6 h) and 16 courses of ID MTX (400 mg/m² in 2 h). Conventional hydration methods were used before and after treatment. Kidney function tests performed before treatment were always normal.

Lymphoma patients. The criteria of surveillance were clinical status, white blood cell (WBC) count, platelet count, hepatic enzyme level and serum creatinine level.

MATERIALS AND METHODS

Drugs

MTX and aminopterin (AMT) were supplied by Lederle Laboratories (Oullins, France). Standard FA, 5CH₃FH₄ and 2-mercaptoethanol were purchased from Sigma (St Louis, Missouri, U.S.A.). 7-OH-MTX was purified on DEAE cellulose [7]. Solutions of standard folates were stored in the dark in 0.2 M 2-mercaptoethanol at -20°C to prevent oxidation. All other reagents used for HPLC assay were of analytical grade.

Patients

25 patients (17 males and 8 females) with non-Hodgkin’s lymphoma ranging in age from 26 to 62 years (mean 43) entered the study. They underwent 21 courses of HD MTX (1500 mg/m² in 6 h) and 16 courses of ID MTX (400 mg/m² in 2 h). Conventional hydration methods were used before and after both protocols. MTX treatment was administered as part of a multidrug regimen, but it was injected alone at an interval of at least 1 week from the previous course of treatment. Kidney and liver function tests performed before treatment were always normal.

FA rescue was administered by the oral route 16 h after the beginning of ID MTX and 18 h after the beginning of HD MTX. Six doses of calcium folinate (prepared by dissolving Lederfoline® in 10 ml of 5% dextrose) were administered every 6 h at 50 mg in group I (15 courses), 25 mg in group II (7 courses), 15 mg in group III (10 courses), and 7.5 mg in group IV (five courses). 8 patients received two to three different FA dosages. Toxicity was evaluated according to WHO criteria. Laboratory tests showed no evidence of normal kidney or liver toxicity. No difference was noted between the four groups with regard to haematological or mucosal toxicity.

Sample collection

Five-millilitre peripheral blood samples were collected in tubes containing lithium heparin, with approximately 20 mg of sodium ascorbate. During ID MTX treatment, samples were taken before MTX treatment (T₀) and then at T₀+1 h, T₀+2 h, T₀+6 h, T₀+12 h, T₀+18 h, T₀+23 h, T₀+29 h, T₀+35 h, T₀+41 h, T₀+47 h. During HD MTX treatment, samples were taken before MTX treatment (T₀) and then at T₀+2 h, T₀+4 h, T₀+6 h, T₀+12 h, T₀+18 h, T₀+24 h, T₀+35 h, T₀+41 h, T₀+47 h and T₀+53 h. After administration of the last dose of FA, blood samples were again taken at 15 min, 30 min, 1 h, 2 h 30, 3 h, 6 h, 12 h in both protocols. Immediately after collection, the plasma was removed and stored at −20°C in 0.2 M 2-mercaptoethanol.

HPLC analysis

HPLC analysis was performed with a Hewlett-Packard HP 1090 equipped with a Rheodyne fixed-loop injector of 100 μl, a detector-integrator HP 3390A, and a filter photometric detector at 280 nm. Immediately before analysis, plasma samples were deproteinised with trichloracetic acid (5%). AMT (5 μM) was used as an internal standard. After centrifugation at 15 000 g for 5 min, the supernatant was injected directly into a Waters spherical C18 (150 × 4.6 mm; particle size 5 μm) column protected by a Rheodyne inlet filter (pore size 2 μm). The temperature of the column was adjusted to 40°C. Elution was carried out at a flow rate of 0.8 ml/min with 0.1 M sodium acetate buffer (pH 5.5) as solvent B. The sensitivity of the method was 5.10−8 M. Reproducibility was lower than 10% for each compound [7].

Pharmacokinetic analysis

The peak plasma concentration (Cmax) and time of peak plasma concentration (Tmax) of FA and 5CH₃FH₄ were experimentally determined. Residual circulating levels (Cmin) was the mean of three measurements made at steady state, i.e. after the third FA administration. Kinetic parameters for FA and MTX are fitted with a multieponential equation (two compartments model) using APIS software [8]. Total clearance (Cl) and half-life (t½) of elimination were estimated.

Statistical methods

All results are expressed as means (standard deviation). Pharmacokinetic parameters were compared using a two-way variance analysis (ANOVA) using Statview software. The level of significance was P = 0.05.

Figure 1. Pharmacokinetics profile of folic acid (FA), 5-methyl tetrahydrofolate (5CH₃-THF), methotrexate (MTX) and 7-hydroxy methotrexate (7OHMTX) after 50 mg (6 times) calcium folinate (CF) rescue following (18 h) administration of 630 mg MTX in 2-h infusion.

[Diagram of pharmacokinetics profile of folate acid (FA), 5-methyl tetrahydrofolate (5CH₃-THF), methotrexate (MTX) and 7-hydroxy methotrexate (7OHMTX) after 50 mg (6 times) calcium folinate (CF) rescue following (18 h) administration of 630 mg MTX in 2-h infusion.]

Correspondence to S. Monjanel-Mouterde.
N. Tubiana-Mathieu, C. Lejeune and Y. Carcassonne are at the Service d’Oncoologie et de Rationotherapy, and S. Monjanel-Mouterde, B. Payet, J. Catalan and J.P. Cano are at the Laboratoire de Pharmacocinétique et Toxicocinétique, CHU Timone, Marseilles, France.
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<th>Groups (n)</th>
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<th>T_{max} (h)</th>
<th>C_{min} (μM)</th>
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RESULTS

Pharmacokinetic parameters of FA and 5CH3-THF

Figure 1 shows the FA and 5CH3-THF profiles observed after repeated oral administration of FA (50 mg) in a patient treated with 400 mg/m2 MTX infusion. FA is rapidly metabolised to 5CH3-THF. The Cmax of FA and 5CH3-THF were obtained after the third administration. After the last administration, FA and 5CH3-THF increased for 2 h, reaching a Cmax of 3.81 µM and 2.35 µM, respectively. The FA level decreased with a half life value of 2.20 h.

Table 1 shows the plasma pharmacokinetic parameters of FA and 5CH3-THF measured during ID and HDRMTX regimens. There is no significant difference between the four groups for T1/2 for FA and 5CH3-THF (mean values were 1.65 ± 0.75 and 2.02 ± 0.78 h, respectively). The Cmax of FA and 5CH3-THF determined after the last administration of FA were 1.43 ± 0.91 µM and 1.79 ± 0.54 µM, respectively, in group I, 1.75 ± 0.54 µM and 1.40 ± 0.38 µM in group II, 1.70 ± 1.64 µM and 1.15 ± 0.55 µM in group III, and 0.98 ± 0.25 µM and 0.83 ± 0.27 µM in group IV. No statistical difference was noted between the first three groups. Only FA values in group IV were significantly lower than in group II.

The Cmin of FA and 5CH3-THF were 0.83 ± 0.57 µM and 0.67 ± 0.39 µM, respectively, in group I, 0.88 ± 0.46 µM and 0.73 ± 0.21 µM in group II, 0.91 ± 0.65 µM and 0.52 ± 0.24 µM in group III, and 0.72 ± 0.35 µM and 0.30 ± 0.15 µM in group IV. No statistical difference was noted in the first three groups. Only 5CH3THF values were statistically lower in group IV than in group II (P = 0.025). Intergroup variations were noted within each group of patients.

As shown in Table 1, there was no significant difference between the groups with regard to half-life of FA (mean value was 5.2 ± 4.7 h). Similarly, mean total plasma clearance of FA in the four groups was 16.8 ± 13.4 h, but it should be noted that non-significantly lower values were observed in group IV. This finding was consistent with the stability of AUC in the four groups with a mean value of 6.75 ± 4.5 µM.h. AUC stability was less evident for 5CH3THF compound with a progressive but not statistically significant decrease being observed from group I to group IV (6.2 ± 3.0 µM.h to 3.6 ± 0.78 µM.h).

DISCUSSION

Until now the optimal dosage for leucovorin rescue after HD or ID MTX was not known. Correlation of the pharmacokinetic parameters of FA and its active methylated derivative with those of MTX and 7OHMTX provides a foundation for such optimisation.

Whatever the route chosen for FA administration, i.e. intramuscular, intravenous or oral, the active isomer L-CHO-THF is intensively and quickly converted to 5CH3-THF. While D-CHO-THF is not metabolised [8-13], the advantage of the oral route is that mainly the L form is absorbed [5]. Another advantage of the oral route is to reduce the amount of unnatural isomer in the plasma [14]. As previously reported, modifying the dosages of FA had no effect on the pharmacokinetics of MTX.

Our results have shown that when leucovorin was administered at doses from 50 to 7.5 mg every 6 h per day, Cmax FA remained constant to 15 mg and decreased at 7.5 mg, while Cmax 5CH3THF remained constant to 7.5 mg. Conversely, Cmin FA remained constant to 7.5 mg, while Cmin 5CH3THF was constant to 15 mg and decreased at 7.5 mg. These results were in agreement with those of MacGuire [12] and Patel [15].

Clearance of FA decreased at lower dose. These results confirm the hypothesis of saturation of FA intestinal absorption, in spite of an interindividual variability. This saturation prevents accumulation of FA and the competition of inactive and active form of FA.

The results concerning 5CH3THF can also be explained by saturation in the metabolic process for FA doses between 50 to 15 mg. Saturation did not seem to occur at 7.5 mg.

Although the concentration of folates necessary for optimal effectiveness is not known, the plasma concentration of total folates obtained in our study seemed to be sufficient for Clinical rescue in HD MTX and ID MTX treatment.

With this mode of administration, clinical toxicity was the same with all four dosages.

Most pharmacokinetic data concerning the use of oral leucovorin had been obtained from normal volunteers [5, 9, 14]. The originality of this study is that it was performed in MTX-treated patients and provides clinical guidelines for leucovorin rescue.