17. Rickles FR, Edwards RL. Activation of blood coagulation in cancer: Trousseau's syndrome revisited. *Blood* 1983, **62**, 14–31.
18. Gabazza EC, Taguchi O, Yamakami T, *et al.* Coagulation-fibrinolysis system and collagen metabolism markers in lung cancer. *Cancer* 1992, **70**, 2631–2636.
19. Bick RL. Coagulation abnormalities in malignancy: a review. *Semin Thromb Haemost* 1992, **18**, 353–372.
20. Vogelzang NJ, Bosl GJ, Johnson K, Kennedy BJ. Raynaud's phenomenon: a common toxicity after combination chemotherapy for testicular cancer. *Ann Intern Med* 1981, **95**, 288–292.
21. Doll DC, List AF, Greco FA, Hainsworth JD, Hande KR, Johnson DH. Acute vascular ischemic events after cisplatin-based combination chemotherapy for germ-cell tumors of the testis. *Ann Intern Med* 1986, **10**, 48–51.
22. Licciardello JT, Moake JL, Rudy CK, Karp DD, Hong WK. Elevated von Willebrand factor levels and arterial occlussive complications associated with cisplatin-based chemotherapy. *Oncology* 1985, **42**, 296–300.
23. Schechter JP, Jones SE, Jackson RA. Myocardial infarction in a 27 year-old woman: possible complication of treatment with VP-16-213 (NSC-141540), mediastinal irradiation, or both. *Cancer Chemother Rep* 1975, **59**, 887–888.
24. Lazo JS. Endothelial injury caused by antineoplastic agents. *Biochem Pharmac* 1986, **35**, 1919–1923.
25. Gabazza EC, Taguchi O, Yamakami T, Machishi M, Ibata H, Suzuki S. Evaluating prethrombotic state in lung cancer using molecular markers. *Chest* 1993, **103**, 196–200.
26. Yen T, Walsh JD, Pejler G, Berndt MC, Geczy CL. Cisplatin-induced platelet activation requires mononuclear cell—a role of GMP-140 and modulation of procoagulant activity. *Br J Haematol* 1993, **83**, 259–269.
27. Juhan-Vague I, Valadier J, Alessi MC, *et al.* Deficient t-PA release and elevated PA inhibitor levels in patients with spontaneous or recurrent deep venous thrombosis. *Thromb Haemost* 1987, **57**, 67–72.
28. Adamson IYR, Bowden DH. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. *Am J Pathol* 1974, **77**, 185–198.
29. Nicholson GL, Custead SE. Effects of chemotherapeutic drugs on platelet and metastatic tumor cell-endothelial cell interactions as a model for assessing vascular endothelial integrity. *Cancer Res* 1985, **45**, 331–336.
30. Scates SM. Diagnosis and treatment of cancer-related thrombosis. *Semin Thromb Hemost* 1992, **18**, 373–379.

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

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The pharmacokinetic profiles of folinic 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 7OH-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 folinic acid at doses over 15 mg.

Key words: folinic acid, 5-methyltetrahydrofolate, metabolite, pharmacokinetic, methotrexate, rescue, repeated dosing

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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 folinic 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

is in turn converted to 5,10-methylene tetrahydrofolate, the biologically active coenzyme. D-CHO-THF is inactive.

The purpose of this study was to determine the optimal dose of leucovorin rescue after HD and intermediate dose (ID) MTX. The pharmacokinetic profiles of FA and its main metabolite were studied after oral administration of the drug in patients treated with MTX. High performance liquid chromatography (HPLC) permitted simultaneous monitoring of plasma levels of FA, $5CH_3$ THF, MTX and 7oHMTX [7] during 37 courses of MTX therapy in 25 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, $5CH_3FH4$ 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/m^2 in 6 h) and 16 courses of ID MTX (400 mg/m^2 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 05, T0+23 h 55, T0+29 h 55, T0+35 h 55, T0+41 h 55 and T0+47 h 55. 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 05, T0+35 h 55, T0+41 h 55, T0+47 h 55 and T0+53 h 55. 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 calculator-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 (C_{max}) and time of peak plasma concentration (T_{max}) of FA and $5CH_3FH_4$ were experimentally determined. Residual circulating levels (C_{min}) 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 multiexponential equation (two compartments model) using APIS software [8]. Total clearance (Cl) and half-life ($t_{1/2}$) 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 $P = 0.05$.

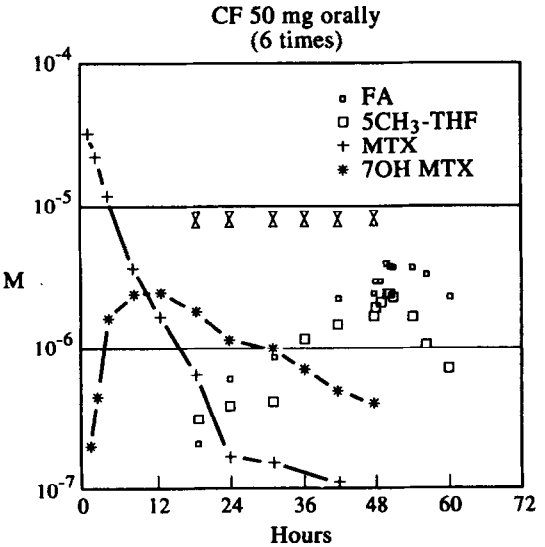

Figure 1. Pharmacokinetics profile of folinic acid (FA), 5-methyl tetrahydrofolate ($5CH_3$-THF), methotrexate (MTX) and 7-hydroxy methylmethotrexate (7OHMTX) after 50 mg (6 times) calcium folinate (CF) rescue following (18 h) administration of 630 mg MTX in 2-h infusion

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Table 1. Pharmacokinetic parameters of folinic acid (FA) and $5CH_3$ tetrahydrofolate ($5CH_3$ THF)

Groups (n)	Dose FA (mg)	C_{max}(μM) FA	C_{max}(μM) $5CH_3THF$	T_{max}(h) FA	T_{max}(h) $5CH_3THF$	C_{min}(μM) FA	C_{min}(μM) $5CH_3THF$	AUC (μM.h) FA	AUC (μM.h) $5CH_3THF$	$T_{1/2}$(h) FA	Cl (l/h) FA
I (15)	50	1.43	1.29	1.82	2.3	0.83	0.67	7.4	6.2	5.8	25.6
		(0.91)	(0.54)	(0.64)	(0.70)	(0.57)	(0.38)	(5.9)	(0.30)	(3.5)	(11.6)
II (7)	25	1.75	1.40	1.90	1.90	0.88	0.73	6.7	5.06	7.2	8.9
		(0.54)	(0.38)	(0.80)	(0.82)	(0.46)	(0.21)	(0.30)	(1.1)	(7.6)	(2.6)
III (10)	15	1.70	1.15	1.32	1.82	0.91	0.52	5.73	3.9	3.2	10
		(1.64)	(0.55)	(0.70)	(0.80)	(0.65)	(0.23)	(4.75)	(1.7)	(3.1)	(7.5)
IV (5)	7.5	0.98	0.83	1.56	1.65	0.72	0.30	6.47	3.6	5.8	3.78
		(0.52)	(0.27)	(1.08)	(0.75)	(0.35)	(0.17)	(2.54)	(0.78)	(2.6)	(2.18)
Total				1.65	2.02			6.75		5.2	16.6
				(0.75)	(0.78)			(4.5)		(4.2)	(13.4)

RESULTS

Pharmacokinetic parameters of FA and $5CH_3$-THF

Figure 1 shows the FA and $5CH_3$THF profiles observed after repeated oral administration of FA (50 mg) in a patient treated with 400 mg/m^2 MTX infusion. FA is rapidly metabolised to $5CH_3$-THF. The C_{min} of FA and $5CH_3$THF were obtained after the third administration. After the last administration, FA and $5CH_3$THF increased for 2 h, reaching a C_{max} 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 $5CH_3$THF measured during ID and HDMTX regimens. There is no significant difference between the four groups for T_{max} for FA and $5CH_3$THF (mean values were 1.65 ± 0.75 and 2.02 ±0.78 h, respectivcly). The C_{max} of FA and $5CH_3$THF determined after the last administration of FA were 1.43±0.91 μM and 1.29±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 C_{min} of FA and $5CH_3$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 $5CH_3$THF values were statistically lower in group IV than in group II (P=0.025). Interindividual 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±4.2 h). Similarly, mean total plasma clearance of FA in the four groups was 16.8±13.4 l/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 $5CH_3$THF 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 $5CH_3$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, C_{max} FA remained constant to 15 mg and decreased at 7.5 mg, while C_{max} $5CH_3$THF remained constant to 7.5 mg. Conversely, C_{min} FA remained constant to 7.5 mg, while C_{min} $5CH_3$THF 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 $5CH_3$THF can also be explained by saturation in the metabolic process for FA doses between 50 to 15 mg. Saturation did not seemed 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 pharmacokinetics 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.

1 Frei E, Jaffe N, Tattersall MHN, Pittman S, Parker L. New approaches to cancer chemotherapy with methotrexate. *N Engl J Med* 1975, **292**, 846–851.

2. Frei E, Blum RH, Pitman SW, *et al.* High dose methotrexate with leucovorin rescue. Rationale and spectrum of antitumour activity. *Am J Med* 1980, **68**, 370–376.

3. Stoller RG, Kaplan HG, Cummings J, Calabresi P. A clinical and pharmacological study of high-dose methotrexate with minimal leucovorin rescue. *Cancer Res* 1979, **39**, 908–912.

4. Rosen G, Caparros B, Huvos AG, *et al.* Preoperative chemotherapy for osteogenic sarcoma. Selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. *Cancer* 1982, **49**, 1221–1230.

5. Straw AJ, Szapary D, Wynn WT. Pharmacokinetics of the diestereoisomers of leucovorin after intravenous andoral administration to normal subjects. *Cancer Res* 1984, **44**, 3114–3119.

6. Nixon PF, Bertino JR. Effective absorption and utilisation of oral formyltetrahydrofolate in man. *N Engl J Med* 1972, **286**, 175–179.

7. Payet B, Fabre G, Tubiana N, Cano J-P. Plama kinetic study of folinic acid and 5-methyltetrahydrofolate in healthy volunteers and cancer patients by high-performance liquid chromatography. *Cancer Chem Pharm* 1987, **19**, 319–325.

8. Iliadis A. Apis: a computer program for clinical pharmacokinetics. *J Pharm Clin* 1985, **4**, 573–577.

9. Schilsky RL, Choi KE, Vokes EE, *et al.* Clinical pharmacology of the stereoisomers of leucovorin during repeated oral dosing. *Cancer* 1989, **63**, 1018–1021.

10. Bertrand R, Jolivet J. The natural and unnatural diestereoisomers of leucovorin. Aspects of their cellular pharmacology. In Rustum Y, Mac Guire JJ, eds. *The Expanding Role of Folates and Fluoropyrimidines in Cancer Chemotherapy: Advances in Experimental Medicine and Biology*. New York, Plenum 1988, **244**, 15–32.

11. Taguchi H. The metabolism of folinic acid (leucovorin) following oral and parenteral administration. *J Nutr Sci Vitaminol* 1981, **39**, 908–912.

12. Mac Guire BW, Sia LL, Haynes JD, Kisicki JC, Gutierrez ML, Stokstad ELR. Absorption kinetics of orally administered leucovorin calcium. *NCI Monogr* 1987, **5**, 47–56.

13. Greiner PO, Zittoun J, Marquet J, Cheron J-M. Pharmacokinetics of (L-) folinic acid after oral and intravenous administration of the racemate. *Br J Clin Pharmacol* 1989, **28**, 289–295.

14. Hines JD, Zakem MD, Adelstein DJ. Bioavailability of high-dose oral leucovorin. *NCI Monogr* 1987, **5**, 57–60.

15. Patel R, Newman EM, Villacorte DG. Pharmacology and phase I trial of high-dose oral leucovorin plus 5-fluorouracil in children with refractory cancer: a report from Children's Cancer Study Group. *Cancer Res* 1991, **51**, 4871–4875.