Cancer Chemother Pharmacol (1987) 20: 78–80

Cancer Chemotherapy and Pharmacology

Short communication

Methotrexate (MTX) concentration in tumors following low-dose MTX*

Naomi J. Winick, Barton A. Kamen, Anita Streckfuss, Johnny Craig, Fred McGuirt, Robert L. Capizzi, Frederick Sklar, and Dale Coln

Departments of Pediatrics and Surgery, The University of Texas Health Science Center at Dallas TX and Oncology Research Center, Bowman Gray School of Medicine, Winston-Salem, NC, USA

Summary. Methotrexate (MTX) is a folate analog competitive with reduced folates for cellular transport and metabolism. Since the normal plasma folate concentration is only 10^{-8} *M*, we tested the possibility that there may be a saturable uptake of MTX by proliferating tumor tissue at plasma MTX concentrations of only 10^{-7} to 10^{-6} *M*. Patients with advanced malignancies, refractory to accepted therapy, were given low-dose oral MTX (30–60 mg/m^2 total dose in four to eight divided doses). Tumor tissue was biopsied 18–24 h after the last oral dose of MTX. The concentrations of MTX and its polyglutamated derivatives were measured in these samples. Forty-eight percent of the drug in the tumor samples was present as a polyglutamated derivative.

Introduction

Methotrexate (MTX), an antifolate, has been used as an anticancer agent for 30 years. During this time, administered doses have increased from 0.5 mg to >30 g in attempts to overcome resistance and improve the therapeutic response. A detailed knowledge of MTX biochemistry and the existence of a rescue agent (5-formyltetrahydrofolic acid) have allowed for the use of high-dose MTX [5, 6]. Randomized clinical trials, however, have yet to establish a therapeutic advantage of the use of high-dose versus low- or moderate-dose MTX [3, 12, 16]. In addition, few publications have documented the concentration of MTX and polyglutamates in tumor tissue following high-dose therapy [15].

Recently, studies documenting folate (and MTX) transport at nanomolar concentrations in vitro [1, 8] have suggested that transport may not limit tissue MTX concentrations at plasma concentrations $\geq 5 \times 10^{-8}$ *M*. We tested this hypothesis by measuring tumor MTX concentrations in patients who had received low-dose oral MTX. All patients had malignant disease, refractory to accepted therapy.

* Partially supported by American Cancer Society grant ACS CH-228 (BAK) and American Society of Clinical Oncology grant (NJW) BAK is a Scholar of the Leukemia Society of America

Offprint requests to: Naomi J. Winick, Department of Pediatrics, 5323 Harry Hines Blvd., Dallas, TX 75235-9063, USA

Materials and methods

Patients. The six patients included in this study had the following malignancies: osteogenic sarcoma metastatic to lung (two patients), recurrent fibrosarcoma of the left chest (one patient), choroid plexus carcinoma (one patient), recurrent medulloblastoma (one patient), and squamous cell carcinoma of the head and neck (one patient). Informed consent was obtained prior to therapy, with all patients having been scheduled for a diagnostic or excisional biopsy prior to consideration for this study.

Treatment. Five patients received oral MTX at 7.5 mg/m^2/dose every 6 h for four to eight doses; one patient received 1.25 mg/m^2/dose every 8 h for three doses. The last dose was always taken 18–24 h prior to surgery to assure a negligible serum MTX concentration, thus avoiding any difficulty in the interpretation of tumor MTX concentrations.

Tissue preparation. Tissues were homogenized in 50 m*M* Tris-HCl, pH 8.3, containing 10 m*M* EDTA, 150 m*M* 2mercaptoethanol and processed to prevent hydrolysis of the MTX polyglutamates [9, 10, 13]. Prior to separation by reverse-phase high-pressure liquid chromatography (HPLC) samples were further purified on a DEAE cellulose column [9].

MTX assay. MTX concentrations were measured by a sequential radioligand binding assay using chicken liver dihydrofolate reductase as the binder [10]. MTX and its polyglutamates are bound equally well. Accordingly, pretreatment of the samples with γ-glutamyl carboxypeptidase was not necessary. The absolute sensitivity of this assay is approximately 0.1 pmol MTX.

HPLC. The HPLC method used to separate the MTX polyglutamates is a modification of that described by Jolivet and Schilsky [7] and detailed elsewhere [9]. A 20- to 100-μl aliquot containing 10–40 pmol of total MTX is injected onto a μ-Bondapak C18 column equilibrated with 5 m*M* tetrabutyl ammonium phosphate (PIC A, Waters Associates) in water. The use of a convex gradient from 20%–40% acetonitrile in 5 m*M* tetrabutyl ammonium phosphate allows for the early elution of MTX followed by metabolites with larger polyglutamate chain lengths. As the MTX $(Glu)_n$ present in the biopsy samples is below

Table 1. Methotrexate (MTX) and MTX polyglutamates in tumor samples

Tumor	Total MTX dose (mg)	Total tumor MTX (nmol/g wet weight)	% of total MTX				
			Glu_1	Glu_2	Glu_3	Glu_4	Glu_5
Squamous cell (head and neck)	12.5	0.08	60	0	8	24	7
Medulloblastoma	60	0.36	41	50	7	1.3	0
Osteogenic sarcoma lung metastasis	10	0.07	60	26	11	2	0
Osteogenic sarcoma lung metastasis	10	0.15	47	40	10	2	0.5

Samples were obtained 18–24 h following the last oral dose of MTX
By convention, MTX = MTX $(Glu)_1$
Polyglutamate analysis was done with HPLC (see text)

that needed for spectral detections, 80 250 µl (0.25 min) fractions were collected during each 20-min run and fractions assayed for MTX using the radiobinder assay. Recoveries of $MTX(Glu)_n$ ranged from 80% to 110%.

Results

The mean tumor concentration of MTX following low-dose drug therapy was 0.39 nmol/g wet weight (range 0.03–1.3 nmol/g wet weight). The concomitant plasma MTX concentration was 0.001 µ*M*, precluding any significant contribution of plasma drug concentration to the MTX concentration measured in the tumor samples. Normal lung tissue obtained from one patient had 0.018 nmol MTX/g wet weight. This represents 12% of the MTX concentration found in adjacent tumor tissue.

Adequate sample size allowed for an analysis of MTX polyglutamates in four tumor specimens (Table 1). The mean proportion of the total MTX present as a polyglutamate metabolite [$\geq MTX(Glu)_2$] was 48%±9.6%.

Discussion

The efficacy of cancer chemotherapy depends upon both tumor cell kill and relative host toxicity. Recently, a specific receptor-mediated pathway for folate (and MTX) accumulation in proliferating cells has been demonstrated at physiologic plasma concentrations of folate [1, 8]. This implies that the transport of vitamin (and drug) at plasma concentrations of $\geq 5 \times 10^{-8}$ *M* may not limit the final intracellular drug concentration. Specifically, once above a threshold concentration, the time of exposure to the drug, rather than the peak concentration, may be the important variable in determining cell kill. This has been supported by clinical experience with prolonged infusions of low doses of MTX [4] as well as by laboratory studies in vivo and in vitro [11, 18].

Samuels et al. [15] measured total MTX and MTX polyglutamate concentrations in biopsy samples from patients with osteogenic sarcoma following therapy with high doses of intravenous MTX (8–12 g/m^2). They found a mean tumor MTX concentration of 3.6 nmol/g wet weight as compared to the value of 0.39 nmol/g wet weight documented here. One notes, however, that the mean concomitant plasma MTX concentration following high-dose therapy was 1.9 µ*M*. The plasma MTX concentration following low-dose therapy was less than 0.001 µ*M*. This value precludes any contribution of plasma MTX to the total MTX concentration measured in the tumor samples. In addition, the mean proportion of the total MTX present as a polyglutamated metabolite was 48%±9.6% following low-dose therapy and only 13.6%±11% in the six samples analyzed following high-dose MTX [15]. The increase in the dose of MTX from approximately 50 mg/m^2 to >10000 mg/m^2 was associated with a change in the total $MTX(Glu)_n$ content from 0.19 to 0.49 nmol.

The tumor MTX concentrations documented here suggest that the frequent oral administration of low doses of MTX may allow for a significant concentration of MTX in malignant tissue. Given the variation in the rate and extent of MTX polyglutamation in normal and neoplastic tissues [2, 14, 17], more data are needed in order to be able to define the effects of tumor type and leucovorin administration on MTX concentrations and polyglutamate formation. Clinical trials should be designed to explore the efficacy of low-dose repetitive MTX so as to compare this relatively safe and cost-efficient method of drug delivery to the use of high single-bolus doses of parenteral MTX.

Acknowledgement. The authors acknowledge the secretarial assistance of Mrs. Dawn Miller.

References

1. Antony A, Kane M, Portillo R, Elwood P, Kolhouse F (1985) Studies of the role of a particulate folate-binding protein in the uptake of 5-methyltetrahydrofolate by cultured human KB cells. J Biol Chem 26: 14911
2. Curt GA, Jolivet J, Carney DN, Bailey BD, Drake JC, Clendeninn NJ, Chabner BA (1985) Determinants of the sensitivity of human small-cell lung cancer cell lines to methotrexate. J Clin Invest 76: 1323
3. DeConti RC, Schoenfeld D (1981) A randomized prospective comparison of intermittent methotrexate, methotrexate with leucovorin, and a methotrexate combination in head and neck cancer. Cancer 48: 1961
4. Djerassi I, Farber S, Abir E, Neikirk W (1967) Continuous infusion of methotrexate in children with acute leukemia. Cancer 20: 233
5. Djerassi I, Rominger CJ, Kim JS, Turchi J, Suvansri U, Hughes D (1972) Phase I study of high doses of methotrexate with citrovorum factor in patients with lung cancer. Cancer 30: 22
6. Goldin A, Mantel N, Greenhouse SW, Venditti JM, Humphreys SR (1954) Effect of delayed administration of citrovorum factor on the antileukemic effectiveness of amethopterin in mice. Cancer Res 14: 43
7. Jolivet J, Schilsky RL (1981) High-pressure liquid chromatography analysis of methotrexate polyglutamates in cultured human breast cancer cells. Biochem Pharmacol 30: 1387
8. Kamen BA, Capdevilla A (1986) Receptor mediated folate and methotrexate transport is regulated by the intracellular folate pool. Proc Natl Acad Sci USA 83: 5983

9. Kamen BA, Winick NJ (1986) Analysis of methotrexate polyglutamates in vivo. Meth Enzymol 122: 339
10. Kamen BA, Takach PL, Vatev R, Caston JD (1976) A rapid, radiochemical-ligand binding assay for methotrexate. Anal Biochem 70: 54
11. Keefe DA, Capizzi RL, Rudnick SA (1982) Methotrexate cytotoxicity for L5178Y/asn lymphoblasts: relationship of dose and duration of exposure to tumor cell viability. Canc Res 42: 1641
12. Kirkwood JM, Canellos GP, Ervin TJ, Pitman SW, Weichselbaum R, Miller D (1981) Increased therapeutic index using moderate dose methotrexate and leucovorin twice weekly vs. weekly high dose methotrexate-leucovorin in patients with advanced squamous cell carcinoma of the head and neck: a safe new effective regimen. Cancer 47: 2414
13. Krakower GR, Kamen BA (1983) In situ methotrexate polyglutamate formation in rat tissues. J Pharmacol Exp Ther 227: 633
14. Poser RG, Sirotnak FM, Chello PL (1981) Differential synthesis of methotrexate polyglutamates in normal proliferative and neoplastic mouse tissues in vivo. Cancer Res 41: 4441
15. Samuels LL, Feinberg A, Moccio DM, Sirotnak FM, Rosen G (1984) Detection by high-performance liquid chromatography of methotrexate and its metabolites in tumor tissue from osteosarcoma patients treated with high-dose methotrexate/leucovorin rescue. Biochem Pharmacol 33: 2711
16. Taylor SG IV, McGuire WP, Hauck WW, Showel JL, Lad TE (1984) A randomized comparison of high-dose infusion methotrexate versus standard-dose weekly therapy in head and neck squamous cancer. J Clin Oncol 2: 1006
17. Whitehead VM, Perrault MM, Stelcner S (1975) Tissue-specific synthesis of methotrexate polyglutamates in the rat. Cancer Res 35: 2985
18. Winick NJ, Krakower G, Kamen BA (1985) Metabolism of MTX to polyglutamyl derivatives and relationship to folate pools in vivo. In: Proceedings of Second Workshop on Folyl and Anti-Folyl Polyglutamates. Praeger, New York, p 297

Received November 10, 1986/Accepted March 25, 1987