

Rescue From High-Dose Methotrexate With 5-Methyltetrahydrofolate^{1,2}

Avner Reggev* and Isaac Djerassi³

5-Formyl-tetrahydrofolate [citrovorum factor (CF)] is commonly used for preventing or reversing toxicity due to treatment with high-dose methotrexate (HDMTX). In vivo, CF is converted to 5-methyltetrahydrofolate (MTHF) and then to the 5,10-methylene tetrahydrofolate, which is the active coenzyme involved in thymidine synthesis. In this study, MTHF was used for protection from toxicity following larger doses of MTX than previously studied, doses which could not be tolerated without effective "rescue." Fifteen patients were given 18 courses of MTX in escalating doses (1–24 g) followed by MTHF. The toxic effects observed included: leukopenia, thrombocytopenia, mucositis, nausea, vomiting, and elevation of serum creatinine and serum transaminase. The side effects were reversible and all patients recovered fully. "Matched pairs" comparison of CF and MTHF rescue in five patients showed no difference in rescue efficacy between the two agents. Since ten of the treatments consisted of 12 and 24 g of MTX, doses potentially fatal without rescue, MTHF is an effective agent for prevention of MTX toxicity. [Cancer Treat Rep 70:251–253, 1986]

The clinical use of high-dose methotrexate (HDMTX) is made possible by the subsequent administration of 5-formyl-tetrahydrofolate, commonly known as citrovorum factor (CF). In vivo, CF is rapidly converted to 5-methyltetrahydrofolate (MTHF) and then to 5,10-methylene tetrahydrofolate, the biologically active coenzyme. MTHF could, therefore, be used as an alternative agent for rescue from MTX toxicity. Because it is closer metabolically to the active coenzyme, it might theoretically be superior to CF as a rescuing agent. Studies of MTHF, however, were hampered by its chemical instability. A new and stable preparation of MTHF has become available for clinical trials (Bio-Research, Milan, Italy). This preparation was used as a rescue agent in several clinical studies (1,2) in which intermediate (up to 1.5 g/m²) doses of MTX were given. These doses of MTX, however, were too low to establish the "rescue" efficacy of MTHF, because the 24-hour and 48-hour serum MTX levels in patients after such doses of MTX are usually < 10⁻⁶ M and 10⁻⁷ M, respectively. With such rapid clearance, the patients are at very low risk of sustaining MTX toxicity, even with minimal rescue (3) or no rescue at all. Thus, the lack of undue toxicity in these reports cannot be considered definitive evidence for the value of MTHF as rescue from MTX toxicity.

Our study was aimed at evaluating the rescue potential

of MTHF when otherwise lethal doses of MTX were used. The toxicity observed in this study is reported here.

PATIENTS AND METHODS

Fifteen patients were entered in the study. Criteria for entry included: wbc counts > 3000/mm³; platelet counts > 10⁵/mm³; serum bilirubin < 1.5 mg/dl; creatinine clearance > 40 ml/min; and a performance status (Eastern Cooperative Oncology Group) of 2 or better. Of the 15 patients, 12 were female and three were male. The mean age was 57 years (range, 35–67). Distribution of disease by category was as follows: breast cancer (six patients); soft tissue sarcomas (three); cancers of the colon, ovary, prostate, lung, and kidney (one each); and lymphoepithelioma (one). Ten of the patients had received chemotherapy prior to entering the study, five had had previous radiation therapy, and three had had both chemotherapy and radiation therapy. Informed consent was obtained from all patients.

MTX infusion.—Details of the MTX infusion procedure employed in this study were published elsewhere (4). The study was started with 1 or 3 g of MTX. The MTX dose was escalated, stepwise, to 6, 12, or 24 g, once the safety of MTHF rescue for a lower MTX dose was established. Serum MTX concentration was determined using the

¹Received Mar 20, 1984; revised May 13, 1985; accepted June 3, 1985.

²Supported in part by the Cancer Research Institute, Inc. and by the Barcelo and S. Buck Funds.

³Department of Research Oncology, Mercy Catholic Medical Center,

Philadelphia, PA.

*Reprint requests to: Avner Reggev, MD, New York Hospital, Westchester Division, 21 Bloomingdale Rd, White Plains, NY 10605.

EMIT (Syva, Palo Alto, CA) MTX assay system.

MTHF or CF rescue.—MTHF or CF rescue was administered by rapid iv infusion every 6 hours, at doses determined by the Equimolar technique (4).

RESULTS

The 15 patients entered in this study were given 18 separate infusions of HDMTX with MTHF rescue. These treatments included one infusion of 1 g of MTX, one of 3 g, six of 6 g, five of 12 g, and five of 24 g. Each infusion was evaluated independently.

MTX was cleared rapidly by all patients. Mean serum MTX level at 24 hours was 4.4×10^{-5} M (range, 0.52 – 19.5×10^{-5} M). At 48 hours, it was 1.24×10^{-6} M (range, 0.1 – 5.8×10^{-6} M). Average time for clearing MTX to a serum level of $\leq 10^{-7}$ M was 5.5 days (range, 3–12). The average length of MTHF rescue was 4.1 days (range, 3–12).

Table 1 outlines the frequency of side effects according to MTX dose administered. Once the MTX dose was > 3 g, there was no trend of increased toxicity with increased dosage. In fact, the subgroup receiving 6 g of MTX experienced a somewhat higher (statistically not significant) overall toxicity than the subgroups who received 12 and 24 g. For this reason, and because of the small number of patients in each subgroup, the toxicity observations were pooled and reported together. The most significant side effect was mild and brief bone marrow suppression. No infections or bleeding episodes were observed in any of the patients who had myelosuppression. The most severe bone marrow suppression was seen in a patient with metastatic breast cancer, ascites, and pleural effusion, who showed a marked delay in MTX clearance. This patient had a wbc count nadir of $900/\text{mm}^3$ and a platelet count nadir of $49,000/\text{mm}^3$, from which she recovered uneventfully.

The other side effects were mild and patients recovered from them, with regard to symptoms or laboratory value

abnormalities, within 2 weeks.

A comparison MTHF and CF rescue was possible in five of the patients in the study who received, at previous or subsequent occasions, infusions of HDMTX of identical dose, but with CF rescue instead of MTHF. Of these five patients, two received 12 g and three received 24 g of MTX, with MTHF rescue on one occasion and CF rescue on another. The toxicity was virtually identical with both folates.

DISCUSSION

The data presented above demonstrate clearly that MTHF is an effective rescue for HDMTX. The patients in the study received doses of MTX which are very likely to produce substantial toxicity when used without adequate rescue. All patients showed MTX levels in excess of the previously established (5) safe concentrations. Therefore, they were susceptible to life-threatening toxicity. Instead, the toxicity observed was quite tolerable and similar to that expected following HDMTX with CF rescue. This could be attributed only to the efficacy of MTHF as a rescue agent. Since MTHF is the natural derivative of CF in the biochemical cascade by which reduced folate is made available as a coenzyme for thymidine synthetase, its effectiveness for HDMTX rescue is not surprising.

This study was limited in scope to the investigation of MTHF as an effective MTX rescue agent. No less intriguing, but not addressed by this study, is the question of whether MTHF, when compared to CF, can improve the therapeutic index of HDMTX with rescue. Could it, and under what conditions, affect higher rescue selectivity by protecting normal cells more effectively than malignant cells? Halpern et al (6) demonstrated in vitro such selectivity advantage for MTHF over CF. They attributed this advantage to the low levels of the enzyme 5-methyl-tetrahydrofolate homocysteine methyl-transferase (EC2.1.1.13) found in most malignant cells. Similarly, Smith (7) showed that MTX rescue with MTHF was supe-

prior to re
tion of st
treated w

The ab
could offi
cumstanc
vantage r
is rapidly
The relev
the theraj
tion.

REFERE

1. CORTELLI
folate res
terapia C

TABLE 1.—Toxicity by MTX dose*

Toxic effect	MTX dose					
	1 g (n = 1)	3 g (n = 1)	6 g (n = 6)	12 g (n = 5)	24 g (n = 5)	Combined (n = 18)
Leukopenia (cells/mm ³)						
< 3000	0	0	1	2	2	5 (28%)
< 2000	0	0	1	0	1	2 (11%)
< 1000	0	0	1	0	0	1 (6%)
Platelet count < $10^5/\text{mm}^3$	0	0	1	0	1	2 (11%)
Serum creatinine elevation	0	0	1	0	0	1 (6%)
Mucositis	0	0	2	0	0	2 (11%)
Diarrhea	0	0	1	0	1	2 (11%)
Nausea and vomiting	0	1	3	4	4	12 (67%)
Serum transaminase elevation	0	0	3	3	3	9 (50%)

* Values = No. of treatments.

rior to rescue with CF and yielded substantial prolongation of survival in L1210-injected BDF₁ mice who were treated with HDMTX and cyclophosphamide.

The above preclinical observations suggest that MTHF could offer, under proper and still-to-be determined circumstances, better rescue selectivity than CF. This advantage may not, however, be readily apparent, since CF is rapidly converted in vivo into MTHF and used as such. The relevance of these observations to improvement in the therapeutic index of HDMTX needs further investigation.

REFERENCES

1. CORTELLARO M. High-dose methotrexate with 5-methyl-tetrahydrofolate rescue in acute leukemia and non-Hodgkin's lymphoma. *Chemioterapia Oncologica*, Anno II, No. 2, Giugno, 1978. High-Dose Methotrexate International Workshop, June 13-14, 1978, pp 184-186.
2. NOVELLI A. Clinical data on rescue of high-dose methotrexate with N⁵-methyl-tetrahydrofolate in solid tumors. *Chemioterapia Oncologica*, Anno II, No. 2, Giugno, 1978. High-Dose Methotrexate International Workshop, June 13-14, 1978, p 183.
3. STOLLER RG, KAPLAN HG, CUMMINGS FJ, ET AL. A clinical and pharmacological study of high-dose methotrexate with minimal leucovorin rescue. *Cancer Res* 39:908-912, 1979.
4. DJERASSI I, KIM JS, and REGGEV A. Response of astrocytoma to high-dose methotrexate with citrovorum factor rescue. *Cancer* 55:2741-2747, 1985.
5. STOLLER RG, KENNETH R, HANDE M, ET AL. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. *New Engl J Med* 297:630-639, 1977.
6. HALPERN RM, HALPERN BC, CLARK BR, ET AL. New approach to antifolate treatment of certain cancers as demonstrated in tissue culture. *Proc Natl Acad Sci USA* 72(10):4018-4022, 1975.
7. SMITH RA. N⁵-formyl-tetrahydrofolate versus N⁵-methyl-tetrahydrofolate rescue in vivo and in vitro. *Chemioterapia Oncologica*, Anno II, No. 2, Giugno, 1978. High-Dose Methotrexate International Workshop, June 13-14, 1978, pp 136-140.