# Long-Duration Intracavitary Infusion of Methotrexate with Systemic Leucovorin Protection in Patients with Malignant Effusions

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ABSTRACT 18 patients with malignant effusions were treated with continuous intraperitoneal, intrapleural, or intrapericardial infusion of methotrexate (MTX) 30 mg/m² per d combined with simultaneous intravenous administration of leucovorin at a dose rate calculated to yield an equimolar concentration in the serum. In the serum the geometric mean steady-state MTX concentration was 0.95 μM, whereas it was 24 μM in the peritoneal, 213 μM in the pleural, and 434 μM in the pericardial cavities. Mean clearance was 6.6 ml/min from the peritoneal cavity, 2.6 ml/min from the pleural cavity, and 0.14 ml/min from the pericardial cavity. Leucovorin provided sufficient protection to allow the duration of infusion to be escalated from 24 to 120 h before myelosuppression was encountered. Marrow thymidylate synthetase activity was inhibited by an average of 46% compared to 86% inhibition in malignant cells in the effusions. Flow cytometric analysis showed no perturbation of the cell cycle phase distribution of marrow cells. All eight of the evaluable patients have responded: three received no other form of therapy, five also received systemic hormonal or chemotherapy. This study demonstrated that tumors confined to third space body fluids can be given very high concentration × time exposures to MTX with minimal systemic toxicity.

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## INTRODUCTION

Methotrexate (MTX)[1] is a cell cycle phase-specific agent that only causes cytotoxic damage to cells when they are in S phase (1). The number of cells killed and toxicity are exponential functions of both MTX concentration and duration of exposure (2–5). This suggests that the optimal dose schedule for cell cycle phase-specific agents such as MTX would involve high concentration exposures of sufficient duration to allow a significant proportion of tumor cells to enter S phase and be damaged (6). This principle is firmly established for cytosine arabinoside, whose therapeutic ratio against acute leukemia increases with duration of infusion (7). The general application of this principle is limited by the fact that most human tumors proliferate much more slowly than normal marrow and gut cells (8–10), and in the case of MTX, bone marrow exposure must be limited to 36–40 h to avoid serious myelosuppression (11, 12).

Under certain circumstances differential exposure of normal and malignant tissues can be achieved when the malignant cells are confined to an extravascular fluid or body cavity. The most common example of this is the successful treatment of meningeal leukemia with intrathecal MTX (13–15). Pharmacologic modelling (16) suggested that the clearance of MTX from the peritoneal cavity would also be sufficiently less than total body clearance to allow significant differential

[1] *Abbreviations used in this paper*: [³H]dUrd, tritiated deoxyuridine; 5fTHF, 5-formyltetrahydrofolate; 5mTHF, 5-methyltetrahydrofolate; MTX, methotrexate; WBC, leukocyte count.

exposure for tumor cells in the peritoneal cavity, and recent studies of MTX administered via peritoneal dialysis confirmed that at steady state the peritoneal concentration of MTX was 10- to 30-fold higher than in plasma (17). However, at peritoneal MTX concentrations high enough to guarantee good drug penetration, enough MTX leaked into the systemic circulation to be toxic to marrow, and the duration of peritoneal dialysis had to be limited to 48 h.

MTX and reduced folate compete with each other for transport across cell membranes to gain access to both normal and malignant cells (18, 19), and the ability of reduced folate to protect or rescue cells from MTX is dependent on the concentration ratio of the two compounds (20–22). In the presence of high concentrations of MTX, reduced folate is unable to block the cytotoxic effect of MTX, and this has been documented in both murine tumor systems (22–24) and in man (25). We have exploited this competitive relationship between MTX and reduced folate, and the ability of intracavitary infusion to maintain high concentrations of MTX in third spaces, to expose malignant cells confined to third spaces to a MTX/reduced folate ratio high enough to permit cytotoxicity while the normal tissues of the body were exposed to a ratio low enough to permit the reduced folate to block MTX toxicity. By simultaneously infusing MTX directly into the third space and leucovorin (5-formyltetrahydrofolate, 5fTHF) intravenously we were able to achieve tumor exposures of up to 120 h without significant bone marrow or gastrointestinal toxicity.

## METHODS

*Subjects and treatment.* 18 patients with a histopathologically proven diagnoses of cancer and free flowing malignant effusions (12 pleural, 2 peritoneal, 2 pericardial) gave their written informed consent to enter this phase 1 trial. The tumor types included six bronchogenic lung, five breast, two ovarian, two melanomas, one pancreas, and one carcinoma of unknown primary. Entrance criteria included a creatinine clearance of >60 ml/min, leukocyte count (WBC) > 3,000/mm$^3$, platelet count > 100,000/mm$^3$, and Karnofsky status > 40%. All effusions were demonstrated to be free flowing by X-ray or ultrasound examination before treatment. Patient ages ranged from 28–71 with a median of 61 yr, and the median Karnofsky performance status was 50%.

Treatment was initiated by placing two plastic catheters through the body wall into the effusion at different points (one catheter was used on the two patients with pericardial effusions). MTX 30 mg/m$^2$ per d was administered as a constant infusion at 10 ml/h through one catheter, samples were obtained for determination of MTX concentration through the other. In the case of the two pericardial effusions, the MTX infusion was periodically interrupted for sampling. Concurrent with the start of MTX infusion, leucovorin 15 mg/m$^2$ was injected as an intravenous bolus, and leucovorin was continued at a dose rate of 10 mg/m$^2$ i.v. every 4 h for the duration of the MTX infusion, and then decreased to 10 mg/m$^2$ i.v. every 6 h until the serum MTX concentration was <50 nM. No attempt was made to quantitate the volume of the effusions because of the difficulty of obtaining accurate results and because knowledge of the volume was not essential to determination of clearance. Fluids were administered to maintain urine output at > 2 liters/d, but urinary alkalinization was not used. Each patient received only one course of treatment, and infusion duration was escalated from 6 to 24 h, and then in 24 h increments to 120 h in subsequent patients. At the end of the MTX infusion, the effusion was drained as completely as possible and the catheters were removed. All patients who received any MTX were considered evaluable for toxicity, but only patients who survived for more than 1 mo after treatment were considered evaluable for response. Response was defined as disappearance of the effusion, and the characteristics of each response are outlined in Table IV.

*Drugs and drug assays.* MTX and leucovorin were obtained from the Division of Cancer Treatment, National Cancer Institute. MTX concentrations were determined by radioimmunoassay using a kit generously provided by Diagnostic Biochemistry, Inc. (San Diego, Calif.). Serum reduced folate was measured with a microbiologic assay as described elsewhere (26). Drug concentrations were averaged using the geometric mean rather than the arithmetic mean, both to reduce the variance of the data, and because biologic responses to MTX and reduced folates are log normally rather and normally distributed.

*Cytokinetic measurements.* Bone marrow samples were obtained from the posterior iliac crest before treatment and again at the end of the MTX infusion. Patients receiving 120-h infusions also had samples taken at 72 h. All samples were anticoagulated with EDTA. The activity of thymidylate synthetase was determined by measuring the rate of incorporation of tritiated deoxyuridine ([$^3$H]dUrd) into acid insoluble material in one part of the undiluted sample as described elsewhere (25). Another part of each sample was subjected to Ficoll-Hypaque separation, and the recovered mononuclear cells were fixed, stained, and analyzed on a Phywe ICP 22 flow cytometer (Ortho Instruments, Westwood, Mass.) as described (27). Chicken cells were added as an internal standard (28, 29), and the proportion of cells in each phase of the cell cycle was determined by using the approximation method of Holley and Kiernan (30). The rate of [$^3$H]dUrd incorporation into cells in the malignant effusions was measured by the same technique (25) in selected patients whose effusions contained sufficient malignant cells.

## RESULTS

*Dose rate considerations.* The major circulating form of reduced folate in man is 5-methyltetrahydrofolate (5mTHF) (26). When injected intravenously, leucovorin (5fTHF) is converted to 5mTHF with a half-life of 4.5 h (31), and the two forms of reduced folate are equipotent with respect to protecting against MTX. Fig. 1 shows the ability of various concentrations of 5fTHF to protect freshly aspirated human marrow cells against inhibition of thymidylate synthetase activity by two concentrations of MTX, 1 and 10 $\mu$M. Thymidylate synthetase activity was measured by the rate of incorporation of [$^3$H]dUrd into acid-insoluable material after 1 h of MTX exposure. 1 $\mu$M 5fTHF afforded a small degree of protection against 1 $\mu$M MTX, but no protection against 10 $\mu$M MTX. Since only a small proportion of normal marrow thymidylate synthetase activity may be required for marrow proliferation in vivo, it was

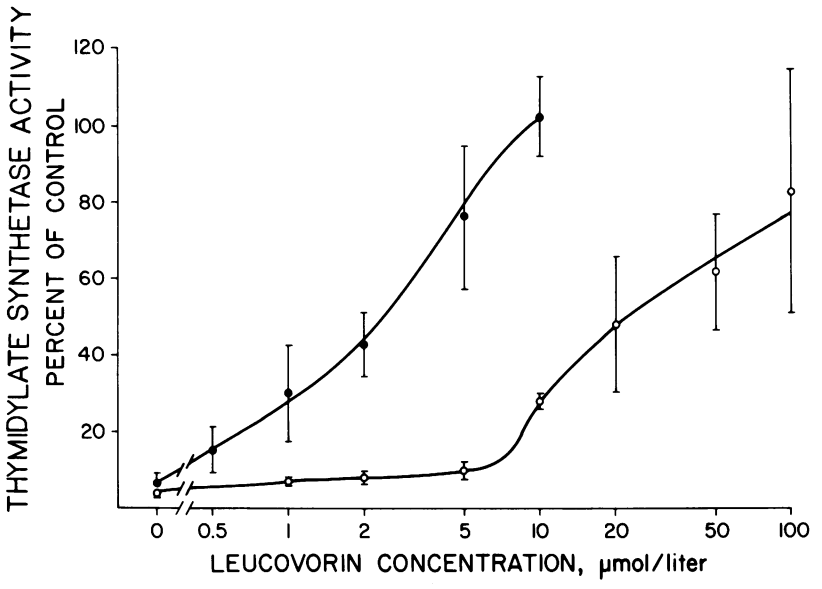


FIGURE 1 Protection by 5fTHF of human bone marrow mononuclear cells against MTX-induced inhibition of [$^3$H]dUrd incorporation. Cells were incubated with either 1 $\mu$M (●) or 10 $\mu$M (○) MTX for 1 h, and then pulsed with [$^3$H]dUrd for another hour. Mean±SD.

reasoned from the data in Fig. 1 that 1 $\mu$M reduced folate would be just sufficient to protect against the cytotoxicity of 1 $\mu$M MTX in vivo, wherease this concentration of reduced folate would be unable to protect against the toxicity of a 10-fold higher concentration of MTX (MTX/reduced folate ratio 10:1). A serum concentration of 1 $\mu$M MTX was selected for clinical trial, and it was estimated from Eq. 1 that this steady-state concentration ($C_{ss}$) would be achieved by infusion of 30 mg/m$^2$ per d assuming a renal clearance of 45 ml/m$^2$ per min.

$$C_{ss} = \frac{\text{infusion rate}}{\text{clearance}}. \quad (1)$$

The dose schedule of leucovorin (5fTHF), consisting of a loading dose of 15 mg/m$^2$ followed by 10 mg/m$^2$ i.v. every 4 h, was estimated to yield a mean concentration of 1 $\mu$M total reduced folate based on calculations using Eq. 2 with a dose interval (T) of 4 h and values for the coefficient of elimination ($\beta$) and the volume of distribution ($V_d$) for leucovorin reported by Mehta et al. (26), and assuming complete conversion of leucovorin to 5mTHF.

$$\bar{C}_{ss} = \frac{\text{dose}}{\beta \cdot V_d \cdot T}. \quad (2)$$

*Pharmacokinetics.* Table I shows the distribution of patients by type of effusion and duration of MTX administration. 14 of the 18 courses of therapy were evaluable for steady-state pharmacokinetic analysis: in two subjects steady state was never reached, and in two subjects a very rapid rise in effusion MTX concentration indicated that the drug was not being distributed throughout the cavity. Simultaneous measurements of effusion and serum MTX concentration in the peritoneal, pleural, and pericardial effusions are shown in Figs. 2, 3, and 4, respectively. Steady-state MTX levels were reached in the effusions within 3 h during pericardial infusion, 12 h during peritoneal infusion, and only after 24 h during pleural infusion. The explanation for the slow rise in MTX concentration in one of the two patients receiving a pericardial infusion after steady state was achieved is not known; this was not observed in any other patient. The geometric mean steady-state MTX concentration was 434 $\mu$M in the pericardial effusions, 213 $\mu$M in the pleural effusions, and 24.2 $\mu$M in the peritoneal effusions (Table II). The serum MTX concentrations were very close in value for all three types of infusions, and the geometric mean

TABLE I
*Distribution of Patients by Type of Effusion and Duration of MTX Infusion*

| | MTX infusion | | | | | |
|---|---|---|---|---|---|---|
| Effusion type | *h*....6 | 24 | 48 | 72 | 96 | 120 |
| Pleural | | 3 | 2 | 3 | 1 | 3 |
| Peritoneal | | 1 | | 1 | | 2 |
| Pericardial | 1 | | 1 | | | |

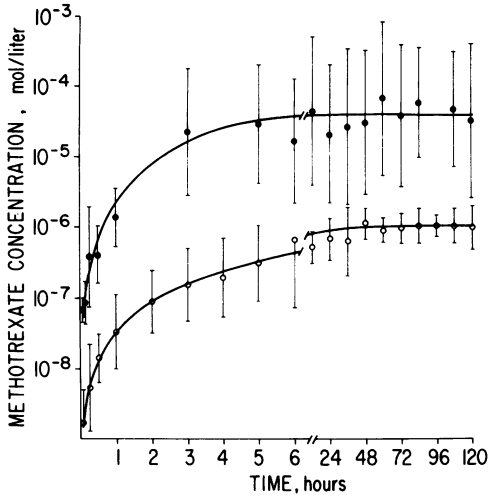


FIGURE 2 Geometric mean MTX concentrations in the serum (○) and effusions (●) of four patients with malignant ascites as a function of time after the start of intraperitoneal MTX infusion. Mean±SD.

MTX level for all courses of treatment was 0.95 μM (SD 0.38–2.3 μM). The effusion: serum MTX concentration ratios for the two pericardial infusions were 166 and 825 (Table II) The arithmetic mean effusion: serum MTX ratio for the eight evaluable pleural infusions was 157, and for the four peritoneal infusions was 92. The smallest steady-state ratio observed in any patient was 7.

Frequent measurements of 5fTHF and 5mTHF were

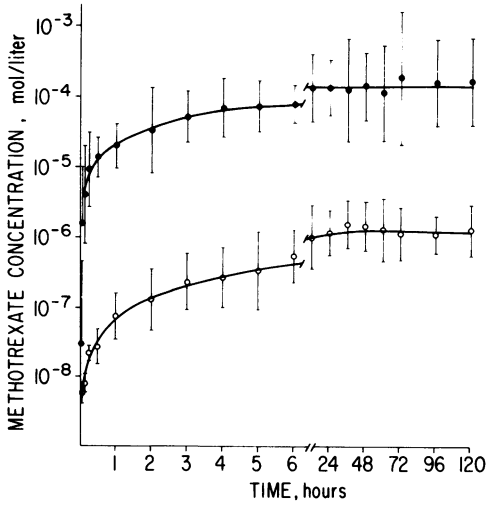


FIGURE 3 Geometric mean MTX concentrations in the serum (○) and pleural fluid (●) of eight patients with malignant pleural effusions as a function of time after the start of intrapleural MTX infusion. Mean±SD.

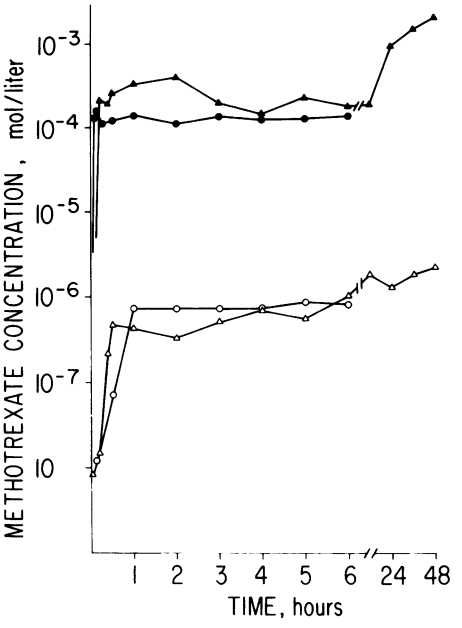


FIGURE 4 Geometric mean MTX concentrations in the serum (○) and pericardial fluid (●) of two patients with malignant pericardial effusions as a function of time after the start of intrapericardial MTX infusion. Mean±SD.

made at random times with respect to leucovorin doses during the steady-state portion of the infusion in the serum and effusion fluid of two patients receiving 120-h MTX infusions, and the data is presented in Table III. There were no significant differences between the steady-state serum and effusion fluid concentrations of 5fTHF or 5mTHF in either patient, indicating that both 5fTHF and 5mTHF equilibrated with the effusion fluid. In the serum the steady-state concentration ratio of MTX/reduced folate (5fTHF plus 5mTHF) was 2.1 and 2.5 in the two patients. However, in the effusion fluid the average concentration ratio was 57 and 211, and the minimum ratios that occurred in the two patients were 15 and 173. Thus, while the MTX/reduced folate ratio was low enough in the systemic circulation to permit some degree of protection by reduced folate, in the malignant effusion the ratio was high enough so that, despite equilibration of the reduced folate into the effusion, the reduced folate would not be anticipated to afford any protection.

Eq. 3 describes the mass balance for MTX in the effusion compartment under conditions where the volume of the effusion ($V_f$) does not change greatly and the efflux of MTX is by first order kinetics.

$$V_f \frac{dC_f}{dt} = I - (C_f - R \cdot C_s) \cdot PA. \quad (3)$$

TABLE II
*MTX Pharmacokinetics in Malignant Effusions*

| Type of effusion | Geometric mean steady-state MTX concentrations: Effusion mean (range) | Geometric mean steady-state MTX concentrations: Serum mean (range) | Arithmetic mean steady-state effusion/serum concentration ratio (range) | PA* mean (range) | PA/total body clearance mean (range) |
|---|---|---|---|---|---|
| | *μM* | | | *ml/min per m²* | % |
| Peritoneal | 24.2 (8–410) | 0.7 (0.2–1.4) | 92 (7–303) | 6.6 (0.2–11.4) | 5.5 (0.3–15.0) |
| Pleural | 213 (26–395) | 1.0 (0.1–3.8) | 157 (32–321) | 2.6 (1.4–4.4) | 3.9 (0.3–5.9) |
| Pericardial | 434 (120–1570) | 1.2 (0.7–1.9) | 470 (166–825) | 0.14 (0.06–0.2) | 0.6 (0.1–1.0) |

* PA = permeability × area, equivalent to effusion clearance, calculated for 50% binding of MTX.

This equation indicates that the rate of change of the MTX concentration in the effusion is a function of the effusion volume ($V_f$), the infusion rate (I), the concentration difference between the effusion and serum ($C_f - R \cdot C_s$), and the product of the permeability and the area of the limiting membrane (PA). PA is expressed in milliliters per minute and is equivalent to the clearance of MTX from the effusion (15). R represents the equilibrium distribution ratio of MTX between the effusion fluid and plasma, and can be calculated from the ratio of the free MTX fractions.

Once steady state has been achieved, the MTX concentration is no longer changing and Eq. 3 can be rearranged in the form of Eq. 4:

$$PA = \frac{I}{C_f - R \cdot C_s}, \quad (4)$$

from which PA can be readily calculated if R is known. The degree of MTX protein binding was determined on fluid obtained from three pleural and three peritoneal effusions. The mean±SD binding for the pleural effusions was 50±14% and for the peritoneal effusions 47±10%. There was no significant change in the fraction bound over the concentration range of 10 nM to 1 mM. These values are identical to those determined in this laboratory, and reported by other investigators, for the binding of MTX to plasma proteins using the same assay system (31–33). Thus R was very close to 1, and in the calculations reported here, R was assumed to be 1. Table II shows the clearances (PA) of MTX from the effusions calculated from Eq. 4 for the condition where MTX was 50% bound. The values of PA for each type of effusion were calculated using the actual MTX concentrations in each patient rather than the geometric mean values for the whole group. In keeping with their relative membrane surfaces, clearance was smallest for the pericardial effusions (0.14 ml/min per m²), intermediate for the pleural effusions (2.6 ml/min per m²), and largest for the peritoneal effusions (6.6 ml/min per

TABLE III
*5fTHF and 5mTHF Concentrations, and the Ratio of MTX to Reduced Folate, in the Effusion Fluid and Serum of Two Patients Receiving 120-h Intrapleural Infusions*

| | Steady-state 5mTHF concentration* | Steady-state 5fTHF concentration* | Steady-state ratio of MTX to reduced folate (5mTHF plus 5fTHF)‡ |
|---|---|---|---|
| | | *μM* | |
| Patient F.M. | | | |
| Pleural fluid | 0.58 (0.39–1.10) | 0.36 (0.29–0.56) | 57 (15–131) |
| Serum | 0.54 (0.16–1.15) | 0.21 (0.04–1.48) | 2.1 (0.2–4.5) |
| Patient D.O. | | | |
| Pleural fluid | 0.71 (0.55–0.90) | 0.40 (0.16–0.51) | 211 (173–250) |
| Serum | 0.55 (0.13–1.41) | 0.08 (0.03–0.21) | 2.5 (0.8–6.7) |

* Geometric mean of multiple measurements made between hours 24 and 120 after start of MTX infusion. Range in parentheses.
‡ Arithmetic mean of ratio derived from multiple simultaneous measurements made between hours 24 and 120. Range in parentheses.

$m^2$). Not unexpectedly this group of patients with large third space fluid collections had relatively low total body clearances of free MTX, averaging 165 ml/min per $m^2$ for the patients with ascites, 78 ml/min per $m^2$ for the patients with pleural effusions, and 35 ml/min per $m^2$ for the patients with pericardial effusions. The ratio of PA to total body clearance ranged from 5.5% in the patients with peritoneal effusions to 0.6% in the patients with pericardial effusions (Table II).

*Cytokinetics.* To monitor patients for potential toxicity, frequent marrow aspirates were obtained and subjected to cytokinetic analysis. Fig. 5 shows the effect of infusions of varying duration on the distribution of marrow mononuclear cells in the $G_1$, S, and $G_2$/M phases of the cell cycle. During the first 48 h there was a gradual increase in the fraction of cells in S phase, and a proportional decrease in cells in $G_1$ and $G_2$/M. However, with longer infusions the pattern returned toward normal and none of the changes reached the level of significance. Inhibition of marrow thymidylate synthetase, as reflected by decrease in the incorporation of [$^3$H]dUrd into marrow mononuclear cells, averaged 46±20% (±SD) at the time of the first marrow aspirate at 24 h, and the degree of inhibition did not change significantly during the remainder of the infusion up to 120 h. In contrast, the incorporation of [$^3$H]dUrd into malignant cells obtained from the effusions of 10 patients was inhibited by mean±SD of 86±5%, reflecting a significantly greater MTX effect. As with marrow cells, the degree of inhibition in the malignant cells did not change with duration of MTX exposure beyond 24 h.

*Toxicity.* The only major toxicity associated with this study was thrombocytopenia. 16 of the 18 patients survived >2 wk after therapy, and were evaluable for the occurrence of myelosuppression (WBC < 2,000/$mm^3$ and/or platelet count > 100,000/$mm^3$). No suppression of peripheral blood WBC or platelet count was observed with infusion durations ≤ 96 h. However myelosuppression, consisting of thrombocytopenia alone, occurred on two of the five courses of 120-h infusion, with platelet nadirs of 31,000 and 87,000/$mm^3$ occurring on days 12 and 14, respectively. After the 120-h infusions the mean±SD change in the WBC was +10±67% and the mean±SD change in the platelet count was −32±45%. 2 of the 12 patients receiving intrapleural infusions had mild pleuritic pain for 2 d after the infusion that was easily controlled with pain medication, and 1 patient had 2 d of cough. One of the four patients receiving intraperitoneal infusion complained of mild peritoneal tenderness during the infusion, and another patient had 2 d of mild diarrhea after the infusion. However, all patients receiving the 120-h infusions had laboratory evidence of a chemical serositis: the mean effusion WBC rose from 680 to 2,500/$mm^3$, and the mean percentage of polymorphonuclear cells rose from 6 to 48%. Two patients had fluid leakage from the cavity at the site of catheter placement after the end of the infusion, and in one patient this resulted in the development of a metastatic deposit at that site.

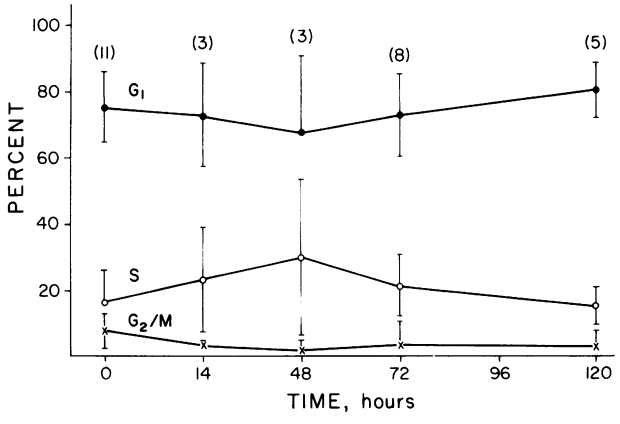


FIGURE 5 The fraction of marrow mononuclear cells in $G_1$, S, and $G_2$/M phases of the cell cycle, measured by flow cytometry, as a function of the duration of intracavitary MTX infusion. The numbers in parentheses indicate the number of marrow aspirates available for analysis at each time point. Mean±SD.

No patient developed mucositis, skin rash, nephrotoxicity, vomiting, or changes in liver function tests. The only infection encountered was a *Clostridium perfringens* peritonitis which was later documented by culture to have been present at the time of initiation of the MTX infusion. The pericardial infusions were not associated with any rhythm disturbances attributable to the MTX. The serosal surfaces of two patients dying 3 and 21 d after their MTX infusions were examined in detail for MTX induced changes. Although some old adhesions were present in both, no evidence of sclerosis could be found.

*Responses.* 10 of the 18 patients were considered unevaluable because they died of causes unrelated to their MTX infusions in <1 mo. The characteristics of the remaining eight patients are presented in Table IV. Three of these patients received no other therapy after their MTX infusion and are thus fully evaluable: one effusion due to bronchogenic carcinoma resolved completely except for minimal blunting of the costophrenic angle, and did not recur before death at 13 mo; another due to bronchogenic carcinoma resolved completely and has not reaccumulated for 20+ mo; a peritoneal effusion due to ovarian carcinoma resolved to the point where it could not be detected by physical examination and remained controlled for 10 mo after which it reappeared. Five patients received

TABLE IV
*Characteristics and Responses of Patients Surviving >1 mo after Treatment*

| Patient | Tumor type | Effusion types | Subsequent therapy | Response | |
|---|---|---|---|---|---|
| | | | | Effusion | Other sites |
| F.A. | Undifferentiated lung | Pleural | None | Complete disappearance for 20+ mo | Progressive intra-abdominal disease after 18 mo |
| G.S. | Adenocarcinoma lung | Pleural | None | Residual blunting of costophrenic angle until death due to mediastinal disease at 13 mo | Progressive mediastinal disease |
| W.D. | Ovarian | Peritoneal | None | Complete disappearance, reaccumulation at 10 mo | Progressive pleural disease |
| J.S. | Breast | Pleural | Tamoxifen | Small residual effusion visible on X ray, stable for 16+ mo | Progressive pulmonary disease |
| G.H. | Breast | Pleural | Tamoxifen | Residual blunting of costophrenic angle, reaccumulated at 7 mo | Progressive boney and lymph node disease |
| P.W. | Ovarian | Pleural | 1-phenylalamine mustard methotrexate 5-fluorouracil | Small residual effusion, reaccumulated at 7 mo | Stable pelvic and mediastinal disease |
| H.B. | Breast | Pleural | Tamoxifen | Small residual effusion visible on X ray, stable for 11+ mo | Stable pulmonary disease |
| D.O. | Melanoma | Pleural | DTIC* | Small residual effusion, stable for 5+ mo | Progressive skin and lymph node disease |

* 5-(3,3-Dimethyl-1-triazeno)-imidazole-4-carboxamide.

systemic therapy after completion of their MTX infusion and are thus not evaluable for the effect of MTX alone. In two of these patients effusions reappeared after 7 mo, whereas the effusions have remained controlled in the other three patients for 5+, 11+, and 16+ mo.

## DISCUSSION

The rationale for intracavitary chemotherapy has been reviewed by Dedrick et al. (16). Pharmacologic modeling suggested that during intraperitoneal drug administration differences in peritoneal and total body clearance would result in large differences in concentration × time exposure to intraperitoneal tumor and normal tissues in contact with the systemic circulation (16). Studies of intraperitoneal MTX and doxorubicin (17, 35–37) and of intraperitoneal 5-fluorouracil (38), confirmed large differences in clearance, but the concentration × time exposure to the intracavitary tumor was still limited by leakage of active drug into the systemic circulation (17, 38). The phase one trial reported here extends observations on the clearance of MTX to two additional cavities in which malignant effusions frequently cause morbidity, and introduces the technique of concurrent intracavitary injection of MTX and intravenous administration of leucovorin to achieve very long durations of exposure.

The leucovorin dose rate, selected on the basis of pharmacokinetic parameters reported for leucovorin (26), clearly provided enough protection to allow an otherwise lethal concentration of MTX to be maintained in the systemic circulation for up to 120 h. The general lack of clinical toxicity was accurately reflected by marrow cytokinetic measurements. Whereas a 46% decrease in incorporation of [$^3$H]dUrd into marrow cells did occur, this degree of thymidylate synthetase inhibition was not sufficient to block cell cycle progression as evidenced by the absence of accumulation of cells at the $G_1$/S interface that is characteristic of the MTX effect on marrow in vivo (25).

A major concern with the use of simultaneous MTX and leucovorin is the possibility that, when concentrations of reduced folate just sufficient to protect normal tissues are present in the blood, enough reduced folate will enter the effusion to reduce the toxicity of MTX to the malignant cells. However, pharmacologic considerations argue strongly against this possibility. A concentration of leucovorin that afforded 50% protection of human marrow exposed to 1 $\mu$M MTX provided

essentially no protection against a 10-fold higher concentration of MTX. These studies, whose results are in agreement with those of Bertino (39), were done with relatively short MTX exposures and measured only inhibition of [$^3$H]dUrd incorporation. However, leucovorin/MTX ratios of 100:1 were required to protect other sensitive cells in assays that measure proliferative ability (20–22, 40). Thus, under conditions where the reduced folate concentration in serum is just sufficient to protect against the toxicity of 1 $\mu$M MTX for 120 h, even if the plasma reduced folate equilibrated completely with the effusion it would be unable to afford any protection for the malignant cells as long as the effusion/serum MTX ratio exceeded ~10. In both patients in whom measurements of reduced folate were made, the concentration of reduced folate in the effusion was less than one-tenth of the MTX concentration. The lowest mean effusion:serum MTX ratio actually observed for any of the three types of effusion was 92. This was the value for the peritoneal cavity; the mean ratios for the pleural and pericardial cavities were 157 and 434. Although the concentration of reduced folate undoubtedly fluctuated because of the intermittent dose schedule, we chose a leucovorin dosing interval that approximated the elimination half-life, so that fluctuation in the concentration should have been approximately less than twofold. The large MTX/reduced folate ratios in the effusions suggest that even much higher peak concentrations of reduced folate would not compromise the activity of the intracavitary MTX. Resistance of the malignant cells to MTX due to deficient transport would only tend to decrease the effectiveness of any reduced folate reaching the effusion (21) since the reduced folate relies upon the same deficient transport system for entry into the cells.

Intracavitary MTX infusions of even 120 h were generally well tolerated. Although the influx of polymorphonuclear WBC into the effusions indicated that MTX induced an acute inflammatory response, symptoms of serotitis were mild and transient. One possibility to be considered is whether MTX was acting simply as a sclerosing agent. This is unlikely in the case of the peritoneal effusions, and post-mortem examinations in one patient who received an intrapleural infusion and one who received an intraperitoneal infusion did not show sclerosis. It is noteworthy that under conditions of partial protection by either leucovorin or thymidine (41), megakaryocytes were the most sensitive to long exposures to MTX, and that in this study thrombocytopenia was the dose-limiting toxicity. There were no episodes of bacterial infection that could be attributed to the catheters. This may in part be due to the antibacterial action of MTX itself, but larger numbers of patients are needed before the risk of bacterial infection can be adequately evaluated.

The MTX clearance was greatest for the peritoneal cavity and average clearances from the pleural and pericardial cavities were only 39 and 2% of the peritoneal clearance, respectively. The average peritoneal clearance of 6.6 ml/min per m$^2$ is close to the 8 ml/min predicted by pharmacologic modelling (16). To our knowledge there are no previous reports of the clearance of MTX from the pleural or pericardial cavities.

Other investigators (17, 36, 38) have used large volume drug containing dialyses to avoid the possibility of limited diffusion of drug in the peritoneal cavity. Although with the constant infusion of MTX in small volumes, a concentration gradient between the catheter tip and the periphery of the peritoneal cavity is to be expected even at steady-state, the concentrations of MTX at the tip of the sampling catheter were well above the cytotoxic range, and the long duration of MTX infusion achieved in these patients should favor adequate although not uniform drug distribution. In the case of pleural and pericardial infusions, there is less concern about distribution of drug within the free-flowing fluid volume because the mechanical action of the lungs and the heart would be expected to result in rapid drug mixing.

Intracavitary drug delivery is likely to provide useful exposure only for small tumor masses and tumor actually on the serosal surfaces due to limited drug penetration. The tumor for which this kind of treatment program may have the greatest impact is ovarian carcinoma. Although MTX is not the most effective drug against ovarian carcinoma, the principle of concurrent intracavitary infusion of a cytotoxic drug and systemic administration of a protective agent can now be extended to 5-fluorouracil, for which allopurinol provides concentration-dependent protection (42–44), and *cis*-platinum for which sodium thiosulfate provides concentration-dependent neutralization (45).

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