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CLINICOPHARMACOLOGIC STUDY AND THERAPEUTIC
EVALUATION OF ORAL METHOTREXATE GIVEN 24 HOURS
BEFORE 5-FLUOROURACIL IN CANCER PATIENTS

FOR USE IN PATIENTS WITH ADVANCED BREAST CANCER

ELLEN TATTELMAN

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Clinicopharmacologic Study and Therapeutic Evaluation
of Oral Methotrexate Given 24 Hours Before
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A Thesis Submitted to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
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Acknowledgements

ABSTRACT

Previous studies in breast, colon, and head and
neck cancer have indicated a benefit to one hour se-
quencing of methotrexate (MTX) and 5-fluorouracil (FUra).
However, in vitro studies in human breast cancer cell lines
suggest optimal tumor toxicity is achieved when $\geq$ 0.1μM MTX
precedes FUra by 18 to 24 hours. A clinical toxicity
study was carried out to assess whether 24 hour sequenced
MTX-FUra with leucovorin (LV) rescue could be tolerated
and to determine if oral MTX could sustain serum concentra-
tions of $\geq$ 0.1μM over 24 hours. Seven patients with ad-
vanced malignancy were treated with 21 courses of treatment;
MTX 50 mg/m^2 p.o. every six hours for five doses; FUra 600
mg/m^2 i.v. at one hour after the fifth does of MTX; LV
10 mg/m^2 p.o. every six hours for six doses begun six hours
after the fifth MTX dose. All patients had received prior
MTX and six out of seven had been previously treated with
one hour sequenced MTX-FUra plus LV. Performance status
in six out of seven patients was $\geq$ 2 (ECOG). No patient
was entered with leukopenia or creatinine clearance < 65ml/min.
Serum MTX levels were measured during all 21 courses of
therapy. Mean concentrations one hour prior to the fifth
dose of MTX were 1.07 $\pm$.74μM and were 2.10 $\pm$.92μM one hour
after the fifth MTX dose. Four of seven patients received
more than two courses at an average interval of 23 days be-

tween courses. No toxicity occurred in 38% of treatment courses; mild to moderate leukopenia and mucositis occurred in 29% and 38% of courses, respectively. Toxicity was related to re-treatment interval and not cumulative drug dose or elevated serum MTX levels. Based on these clinical results, the treatment protocol appears to be well tolerated and a larger trial evaluating 24 hour sequenced MTX-FUra in advanced breast cancer patients seems warranted.

INTRODUCTION

In 1981, cancer of the breast was the third leading
cause of cancer death. Occurring almost exclusively in
women, (99% of cases), it is both the leading cancer type
and the major cause of cancer death among women (1).
Breast cancer kills more women ages 40-44 than any other
disease, and one out of 11 women can expect to develop
breast cancer in her lifetime (2).

Over the past 50 years, there has been no substantial
change in survival rates (2). In an effort to elucidate
breast cancer risk factors and to evaluate the various
treatment modalities, there has been a great amount of
epidemiological and experimental data collected. As a
result, new concepts of the disease are being developed
with new therapeutic approaches following. The most im-
portant new concept to emerge is the fact that breast cancer
is actually a heterogeneous group of diseases with vary-
ing natural histories and time courses (3).

The cause of the disease is unknown, but it most
likely depends on the relationships between a number of
factors including age, genetic and familial variables, diet,
geographic area of residence, reproductive history, virus
exposure, radiation, and endocrinological environment. There
is a steady rise in breast cancer risk during premenopausal
years beginning in the third decade of life, a temporary
dip at menopause, and a continued rise at a slower rate

after menopause (4). Women with a previous personal history or a familial history of breast cancer have an increased risk of developing the disease (5,6). Also associated with an increased risk are early menarche and late natural menopause, nulliparity or a delay of first full term pregnancy until after age 30, excessive irradiation to the whole body or breast, fibrocystic disease of the breast, and large body size especially with increased animal fat intake (7). Bilateral oophor-ectomy before age 35 decreases the incidence of breast cancer in parous and nulliparous women (8), whereas estrogen replacement therapy and oral contraceptive use increases the risk, although this is still being debated (9,10).

As stated previously, despite extensive research and new developments in technology and pharmacology there has been little change in breast cancer mortality rates over the past several decades. Five-year survival rates for all stages of breast cancer in the 1950's were 60% while they were 65% in the 1970's. Between the 1940's and the 1970's, five-year survival rates for breast cancer diagnosed in the localized stage have increased from 78% to 85%. Cancer that has spread to the axillary lymph nodes now has a five-year survival rate of 56% up from 50% in 1950, while breast cancer with distant metastases has a five-year survival rate of 10% as opposed to 21% in the early

1950's. Ten-year survival rates parallel these trends (2).

Prognostic variables that influence these survival rates include tumor size, histologic findings and the presence or absence of metastases in axillary lymph nodes. For patients treated with radical surgery, the most important single prognostic factor is the absolute number of axillary lymph nodes involved with tumor as shown in Table 1 (3). The probability of node involvement increases with increasing tumor size, but tumor size affects survival rates independently as well. In patients with one to three positive nodes, the five-year recurrence rate in those with lesions one to two cm in diameter on physical examination is 44% as opposed to 63% in patients whose lesions measure six cm in diameter (11). However, there is a group of patients with very large lesions who delayed seeking treatment more than one year who show no evidence of nodal metastases and have a comparatively low recurrence rate. Although it seems reasonable to expect that the duration of symptoms would correlate with progression, this does not seem to be the case. This suggests a varied population of patients with breast cancer--those with tumors that grow large within the breast but do not metastasize and thus remain curable even with delayed consultation, and those with tumors that metastasize very quickly before there is much growth in the breast and therefore become incurable even with early consultation (3).

Nevertheless, generally speaking, the size of the tu-

mor significantly affects prognosis. Eighty percent of
patients who have tumors less than 1 cm in diameter, as
compared to 45% of patients with 5-7.5 cm tumors, are
alive ten years after diagnosis (3). Early detection
while the tumor is small and before distant metastases occur
is at present the most important hope for breast cancer
control.

The percentage of breast cancers diagnosed in a
localized stage has increased from 38% to 47% between
the 1940's and 1970's. Breast self-examination and mam-
mography have contributed to this earlier detection.
Both the Health Insurance Plan of New York study and the
National Cancer Institute/American Cancer Society Breast
Cancer Detection Demonstration Projects showed the importance
of the combination of physical examination and mammography
in breast cancer screening. In the Breast Cancer Detection
Demonstration Project the number of breast cancers diag-
nosed in the localized stage reached greater than 70% (2).
Mammographic equipment and techniques have developed to
require lower radiation dose exposure, but there are still
concerns over the possible danger of radiation-induced
breast cancer. At present, indications for mammographic
screening include evaluation of high risk patients and pa-
tients with prior breast cancer (12).

Breast self-examination at monthly intervals is
crucial to cancer detection. Over 90% of breast cancers
are discovered by women themselves (2). Since 50% of masses
0.6-1.0 cm are palpable (13) they are often discovered

while still small. It is through breast self-examination
that the rapidly growing cancers that develop between annual
mammograms and are most likely to metastasize will be de-
tected (14,p.209). The screening of asymptomatic women
and early breast cancer detection has shown preliminary im-
provement in survival rates, but it is as yet unclear
whether this represents the result of lead time bias or in
fact will lead to a decrease in the number of deaths from
breast cancer(3).

A variety of treatment options are available to women
with breast cancer. Local therapy is the oldest form of
treatment with radical resection of the breast, pectoralis
muscles, and regional lymph nodes being the standard and
traditional treatment since Halsted's and Meyer's radical
mastectomy results in 1894 (14,p.257). This approach as-
sumed an orderly spread from the tumor to lymph nodes and
then to distant sites of the body. However, a biological
conception of cancer spread has superseded this anatomical
approach with the realization that tumor cells can dissemi-
nate through the blood and lymph systems in a variety of
less orderly ways. It is also true that some cancers may be
diagnosed in a very early phase, before the invasiveness
that makes radical surgery appropriate.

These new concepts of growth and spread of cancer
coupled with the fact that mortality rates have not decreased
despite radical surgery have sharpened the focus on thera-

peutic alternatives. These alternatives have been statistically evaluated in terms of morbidity of treatment method, the rate of local recurrence, the duration of symptom-free survival, and the total duration of survival. It should be noted that extreme variation in the natural history of breast cancer in terms of growth rate and length of patient survival makes evaluating new therapies very difficult.

Alternative approaches to breast cancer treatment include less radical surgery--the modified radical mastectomy, simple mastectomy and lumpectomy--all with or without radiation therapy. When analyzed in a series of prospective trials, no single approach or combination of surgery and radiation therapy proved more valuable in terms of survival results than any of the others (3,15,14,p.283). The obvious conclusion is that the more radical and deforming surgery no longer needs to be accepted as the standard treatment. Radiation therapy results show a decrease in local recurrence but do not show a survival advantage. However, surgical resection of lymph nodes and radiation of nodal metastases seem to be therapeutic equivalents (16).

Failure of therapy seems to correlate with the stage of disease rather than with a particular form of treatment. Thus the extent of the disease must be considered a major determinant of prognosis. It is not possible, however, to determine which patients have systemic disease at the

time of diagnosis since micrometastases are as yet unde-tectable. One third of patients without palpable nodes will have histologic evidence of tumor invasion in lymph nodes and one third of patients with palpable nodes will have no evidence of tumor there (3). Even patients with histo-logically negative axillary lymph nodes cannot be assumed to have local disease since in these cases there is at least a 24% treatment failure rate (17). Therefore, prophylactic treatments beyond local therapy have been developed to destroy the presumed occult metastases.

Adjuvant endocrine therapy, including ovarian radia-tion and oophorectomy, has been used for a long time pri-marily in premenopausal women. It has generally shown marginal value in premenopausal patients in lengthening the time to first recurrence and, in some studies, in prolongation of survival. The addition of prednisone seems to enhance the value of ovarian ablation (18). These studies were done before estrogen receptors were taken into account. Con-trolled trials correlated with estrogen receptor status are now underway to evaluate adjuvant endocrine therapy including ovarian ablation, estrogens, and anti-estrogen drugs in pre- and postmenopausal women (19).

Adjuvant chemotherapy has generally been used in pa-tients with operable disease and histologically positive axillary lymph node involvement. Regimens administered one to two years post mastectomy have shown impressive re-

sults in the past five years. The most dramatic benefits are seen in premenopausal women given a combination of cyclophosphamide (CTX), methotrexate (MTX), and 5-fluoro-uracil (FUra), monthly for one year. After four years these patients had a statistically significant advantage in disease-free and absolute survival. Nevertheless, almost 50% of the treated premenopausal patients with four or more positive nodes have relapsed within four years (20,21). It may be that adjuvant chemotherapy is merely delaying recurrence rather than increasing cure rate. The combination of CTX, MTX, FUra with vincristine and predni-sone (CMF-VP) has benefited both pre- and postmenopausal patients with four or more positive axillary nodes (22). There is at this point no established role of early sys-temic cytotoxic chemotherapy in the treatment of patients with negative nodes. The potential late complications of adjuvant chemotherapy are as yet unknown.

Other adjuvant therapies such as immunotherapy or the combination of endocrine-, immuno-, and chemo-therapies have yet to be studied conclusively. The combination of endocrine- and chemo-therapy seems promising; cytotoxic chemotherapy could be active against both those hormone-sensitive cells stimulated into a cycling state by endocrine therapy and the overgrowth of hormone-insensitive cells that result from the endocrine therapy (18). Adjuvant therapy is important as a therapeutic back-up to experi-

mentation with less extensive surgery.

Regardless of the initial treatment for women with breast cancer, the majority will eventually have disseminated disease (14,p.361). Once distant metastases are present, cure is rare with the present treatments available. Survival can be prolonged, however, and some of the symptoms of advanced cancer can be relieved with hormonal therapy, chemotherapy, or a combination of these (3).

Hormonal manipulation has been used for advanced breast cancer since 1895 when oophorectomy was found to produce tumor regression (14,p.367). The recent addition of antiestrogens and aminoglutethimide plus glucocorticoid, with their limited toxicity, may render the more traditional forms of endocrine ablation, (oophorectomy, adrenalectomy, and hypophysectomy), unnecessary. Premenopausal women benefit most from ablative therapy followed by antiestrogens or androgens. Postmenopausal women show the best response to antiestrogen or estrogen therapy (14,p.396,23,24).

The ability to assay for estrogen receptors has made the response to hormonal therapy more predictable. While the response rate to endocrine therapy for the general patient prior to selection by estrogen receptor status was approximately one third, the reponse rate in estrogen receptor positive women is now 50% to 60% and is less than 10% in estrogen receptor negative women (25). The women who would be expected to respond to endocrine therapy

by their clinical characteristics are also those who are more frequently estrogen receptor positive and who have higher receptor levels. This includes postmenopausal more than premenopausal women, older women in both the pre- and postmenopausal group, those women with long disease-free intervals (slower growing disease) , and those women with metastatic disease in bone or soft tissue as opposed to the viscera (3).

Cytotoxic chemotherapy is effective against advanced breast cancer and should be considered in women who are estrogen receptor negative, in women who fail to respond to endocrine therapy, and in women whose disease is rapidly progressing and life-threatening. Cytotoxic drugs are active against rapidly dividing cells which include the tumor as well as host tissues such as bone marrow, gastrointestinal tract lining, skin, and hair. The acute toxicities of bone marrow suppression, nausea, vomiting, diarrhea, stomatitis, alopecia, and increased fatiguability are the chief limitations to the use of chemotherapy. Short-term side effects are generally related to dose; however, dose reduction is often limited because of the narrow therapeutic index. Long-term side effects such as chromosomal breakage, fetal abnormalities, and increased incidence of second tumors, as well as the unknown effects of prolonged immunosuppression are of concern and are under investigation (14, p.408-409).

Although one of the experimental conditions for chemotherapeutic effectiveness is a rapid growth rate, pooled data show that cytotoxic drugs benefit women with slowly or rapidly progressive breast cancer. Patients respond to chemotherapy regardless of estrogen receptor status or previous response to endocrine therapy. Premenopausal women and women with a prior response to hormonal therapy more often respond to chemotherapy (3) as do women with soft tissue metastases (14,p.420).

Breast cancer responds to all major classes of cytotoxic drugs and 15 single agents were found to be effective in more than 20% of patients (26). The most frequently used drugs whether singly or in combination are the cell-cycle-specific agents, MTX and FUra (antimetabolites) and vincristine (a mitosis inhibitor) and the cell-cycle-non-specific agents, CTX (an alkylating agent) and adriamycin (an antitumor antibiotic) (14,p.412-413). These drugs have independent mechanisms of action with non-additive toxicities so they lend themselves to combination chemotherapy.

The Cooper regimen of CMF-VP, reported in 1969, first demonstrated the increased effectiveness of combinations. Many variations of this regimen have been studied in an effort to decrease the doses and number of drugs and, therefore, decrease the toxicity. The results show that vincristine does not contribute to the response rate (27), whereas prednisone may give a slight advantage (28,29).

Changes in dosage and scheduling do not seem to affect the results (3,14,p.416). Adriamycin is one of the most active single agents in breast cancer,but in combination it does not seem to affect significantly the response rates (3,14,p.419). Further randomized trials are being undertaken.

The response rates for drug combinations were 40-70% for a duration of 7 to 11 months as compared to the 20-30% response rates for 3 to 8 months in the cases of single agents. Complete response rates, however, were still only approximately 15% (30). There has been no clear improvement in survival or palliation with combinations as opposed to single agents (31). These disappointing results with combinations of drugs have led to laboratory studies attempting to devise synergistic combination schedules.

The use of MTX and FUra in combination with leucovorin (LV) rescue for the treatment of breast cancer is the basis for the present study. In vitro studies with murine leukemic cells, L1210, human colon adenocarcinoma cells, HCT-8, and hormone dependent human breast cancer cells, 47-DN, and in vivo studies in rodents have shown a greater than additive cytotoxic effect when FUra follows MTX pretreatment (32-36).

This synergism is in contrast to the theoretical antagonism between MTX and FUra on their abilities to inhibit

deoxythymidylate (dTMP) synthesis. The presumed major cyto-
toxic effect of FUra is DNA synthesis inhibition as a result
of inactivation of thymidylate synthetase by 5-fluoro-2'-
deoxyuridylate (FdUMP) when it forms a ternary complex with
$N^{5,10}$ methylenetetrahydrofolate($N^{5,10}$CHTHF) and thymidylate
synthetase. MTX, on the other hand, inhibits dihydrofolate
reductase and, therefore, prevents the regeneration of
$N^{5,10}$CHTHF needed for FUra effect. This is the proposed
evidence against the use of MTX preceding FUra (34).

However, experimental studies, as indicated above, have
demonstrated that when FUra follows MTX there is an enhance-
ment of intracelluar accumulation of FUra and its nucleo-
tide derivatives associated with an increase in 5-phospho-
ribosyl-l-pyrophosphate (PRPP) levels and synergistic antitumor
effect. This does not occur when MTX and FUra are given simul-
taneously or when FUra precedes MTX. In the human breast can-
cer cell line, 47-DN, accumulation and phosphorylation of FUra
was increased up to four fold following MTX pretreatment
resulting in synergistic cell kill (35).

An alternative mechanism has been proposed to explain the
interaction of MTX and Fura and is illustrated in Figure 1.
MTX inhibits dihydrofolate reductase needed for the regeneration
of tetrahydrofolate pools and thus inhibits de novo purine
synthesis. PRPP,that would have been used in purine synthesis,
accumulates and is now available for orotate phosphoribosyl
transferase to transfer a phosphoribosyl moiety to Fura to
form 5-fluorouridine monophosphate(FUMP) and other nucleotide

derivatives. The subsequent increased incorporation of FUra as 5-fluorouracil triphosphate (FUTP) into cellular RNA may explain the enhanced cell kill seen when cells are exposed to MTX before FUra (33-35).

The concentration of MTX and the time interval between drug administration were important factors in the degree of intracellular FUra accumulation and subsequent cytotoxicity. In the 47-DN cell line, concentrations of more than $0.1 \mu M$ MTX were effective in enhancing FUra accumulation and the effectiveness increased with greater MTX pretreatment concentration. In these breast cancer cells, with a doubling time of 30 hours, a 24 hour exposure to $10 \mu M$ MTX resulted in the greatest intracellular FUra accumulation and maximum cell kill. The 18 to 24 hour optimal MTX pretreatment interval in 47-DN cells is longer than that seen in HCT-8 (6-12 hours) and Ll210 (3 hours) cells which have proportionately shorter doubling times. The dependency of the MTX pretreatment interval on cellular growth rates may be explained by the fact that MTX inhibits purine synthesis only in those cells which are synthesizing DNA. Tumors with longer doubling times, like breast cancer whose time to double can vary from 3 to 745 days, may have fewer cells synthesizing DNA and, therefore, less opportunity for drug synergism during a treatment interval (14,p.36,35).

The timing of LV administration is also important since LV rapidly reverses the effects of MTX by decreasing

both intracellular PRPP and FUra accumulation. MTX-FUra synergism was only seen when LV followed FUra administration (35). In addition, hypoxanthine which utilizes PRPP was found to reverse totally the enhancement of FUra accumulation when it was added to cells pretreated with MTX (35).

Trials with sequential MTX and FUra have been undertaken in order to apply clinically this experimental data. Preliminary results of MTX followed one hour later by FUra and 24 hours later by LV in patients with advanced breast, head and neck, and colorectal cancers show favorable results suggestive of synergism withour major toxicity (37-40). However, the longer doubling time of breast cancer and the newer laboratory research with 47-DN cells suggest that an 18-24 hour MTX pretreatment interval is necessary to produce maximum MTX-FUra synergism. Clinical trials are needed to study the effect on cytotoxicity of prolonging MTX pretreatment intervals. Toxicity of the drugs in the new time sequence must first be assessed to see if patients can tolerate this drug schedule.

The present study of seven patients with advanced malignancy is a pilot project to evaluate the toxicity of the 24 hour MTX pretreatment interval followed by FUra and LV, and to determine if oral MTX can sustain effective serum levels of more than $0.1 \mu M$ over 24 hours. If this therapy can be tolerated, a further study comparing one hour and 24 hour sequenced MTX-FUra for use in advanced breast cancer can be undertaken.

METHODS AND MATERIALS

Patient Selection

Seven patients between the ages of 50 and 71 with advanced cancer were included in this study between July and November 1981. Four patients had disseminated breast carcinoma. One patient had locally advanced squamous cell carcinoma of the head and neck and two patients had mycosis fungoides, one of those with immunoblastic sarcoma of the T cell type. The patients all had measurable disease. Two had only locally advanced recurrence and five had dissemi-nated disease with at least two organ systems involved (Table 2). All patients had a creatinine clearance $\geq$65ml/min., a white blood cell (WBC) count $\geq$4500, platelets $\geq$130,000, and hemoglobin $\geq$9.5 gm%. Performance status was not used to select patients but six out of seven patients' status was two or greater on the Eastern Cooperative Oncology Group (ECOG) scale (41) (Table 2 and 3).

No patient was disqualified on the basis of prior therapy and six out of seven patients had responded to at least one form of previous treatment. Of the patients with breast cancer, three of the four patients were postmenopausal at diagnosis. These three patients had been treated with mastectomy and were all estrogen receptor positive. The fourth patient had not had surgery and estrogen receptor status was unknown. All four patients with breast cancer

had received prior anti-estrogen therapy. Six out of seven
of the patients had received prior radiation therapy and
all the patients had received chemotherapeutic trials with
multiple drugs. The amount of time since the last chemo-
therapy varied from 7 to 35 days with an average of 23 days.
Each of the patients had received MTX before this study
and only one of seven of the patients had not received FUra.
In fact, six out of seven of the patients had been pre-
viously treated with one hour sequenced MTX-FUra and LV
as part of an earlier study (37) with the number of courses
ranging from 1 to 40 (see Table 5). Four of the six patients
had received this one hour sequenced MTX-Fura as their last
chemotherapy before this study (Table 5).

Drug Regimen

The patients were treated with a 24 hour sequenced
schedule of MTX and FUra with LV rescue as follows: Oral
MTX 50 mg/m^2 every six hours for five doses, i.v. bolus
FUra 600mg/m^2 one hour after the fifth dose of MTX, and
oral LV 10mg/m^2 every six hours for six doses starting six
hours after the fifth dose of MTX. Three out of seven
patients received this exact drug regimen throughout their
entire treatment course. Two patients received FUra 1gm/m^2
and LV 25 mg/m^2 instead for their full course of therapy.
One patient started at a lower dose of MTX (33mg/m^2) but
the dose was increased to MTX 50 mg/m^2 for the second
course. Another patient required a dose reduction to

MTX 50 mg/m^2 and FUra 400 mg/m^2 for his third and final dose because of side effects (see Results).

A total of 21 courses of treatment were given with a mean of three courses per patient. The interval between doses varied according to individual patient tolerance but, in general, the re-treatment interval and not the drug dosage was altered.

Study Criteria

The patients were evaluated according to the ECOG toxicity criteria (41) in the categories of leukopenia, thrombocytopenia, anemia, nausea and vomiting, diarrhea, mucositis, renal function, infection, and fever as shown in Table 4. Patients were assigned a score of zero to five following each treatment. A toxicity grade of five indicates that the toxicity caused the death of the patient.

MTX serum concentrations were measured one hour before and one hour after the fifth dose of MTX by radioimmunoassay technique during all 21 courses of therapy.

Each patient was evaluated for response according to the following criteria. A complete response was defined as 100% disappearance of all metastatic disease at all sites. A partial response was regression of more than 50% but less than 100% of all measurable disease with no new lesions appearing. Advanced disease that showed neither signs of response nor progression was considered as stabilization.

The appearance of new lesions or 25% increase in size of existing lesions was considered as progression and treatment was then discontinued.

RESULTS

Serum MTX Concentration

Seven patients were treated with a total of 21 courses of therapy of 24 hour sequenced MTX-FUra with LV rescue. The serum MTX concentration one hour before the fifth dose of MTX varied from 0.21 M to 3.0 M with a mean of 1.07 M and a standard deviation of 0.74. The serum level of MTX one hour after the fifth dose varied from 0.52 M to 4.4 M with a mean of 2.10 M and a standard deviation of 0.92. These MTX concentrations were in the range that resulted in enhanced accumulation of FUra and synergistic cytotoxicity in laboratory studies. There was no clear relationship between the number of prior treatments with MTX or the cumulative amount of previous MTX and the present serum MTX levels.

Patient Response

Five of the seven patients in the study had dissemi-

nated disease, four with breast cancer and one with mycosis fungoides. One of these patients died as a result of disease progression. Four patients responded with disease stabilization. Two were removed from the study after one and two months, respectively, when their disease progressed. The other two patients continue to be treated with this drug regimen, each with three months of stabilization. Of the two patients with locally advanced disease (one mycosis fungoides, one head and neck cancer), one patient died secondary to disease progression. The other patient had a partial response lasting two and one half weeks but then progressed and was removed from the study.

The intervals between doses varied from 7 to 42 days; only one patient was initially re-treated after 7 days. Four of the seven patients received more than two courses (average of four) at a mean interval of 23 days. Of the patients who received two courses or less, two patients died and one progressed while on treatment. No patient died or was removed from the study because of toxicity.

Treatment Toxicity

The toxicity for the 21 courses of therapy is shown in Tables 5 and 6. Two of the 21 courses were associated with severe toxicity (ECOG grade 3-4) and in both patients this occurred after the second treatment course. Mild to moderate toxicity (ECOG grade 1-2) occurred in 11 courses,

usually after the first or second treatment, and no toxi-
city at all occurred in eight treatment courses.

No patient was leukopenic prior to treatment. Two
out of seven patients never developed any grade of leukopenia
and five of seven never developed a WBC count less than 2000.
One patient developed severe hematologic toxicity (WBC < 1000--
ECOG grade 4) on her second course of treatment, seven days
after the first. It was at this time that she developed
the one infection that occurred during the study. This
patient went on to receive four more courses of therapy
with the treatment interval increased from 7 to 14 days.
Her WBC count remained above 3700. One other patient's
WBC count fell significantly to 1500 but it rose quickly
and the patient suffered no consequences as a result. She
did not receive additional courses because of disease pro-
gression.

Three out of seven patients were anemic (ECOG grade 1)
at the start of treatment and anemia was therefore evaluated
separately as a toxicity. The anemia resulting from the
treatment was mild, both in patients who were and were not
anemic prior to treatment, with the exception of one patient
who developed severe anemia (Hb 8.8 gm%) and required a
transfusion. This was the same patient that developed grade 4
leukopenia and it occurred after the same course of treat-
ment. She had no further serious drop in RBC count once
the treatment interval was lengthened.

The nausea/vomiting and diarrhea side effects were infrequent and mild. Five of the seven patients experienced no nausea or vomiting following any of their treatments. Two patients vomited after their first treatment and one of these patients experienced nausea after the second. They had no difficulty with later treatments. Two of the seven patients had diarrhea after the chemotherapy but in each case it was mild and without gross bleeding.

Mucositis was a common side effect, experienced by four of the seven patients. Of the 21 courses of therapy, two courses resulted in mouth soreness and six caused ulcers. The ulcers were never so severe as to interfere with eating or the continuation of treatment.

One patient developed fevers ($\sim 101^{\circ}$ F) unrelated to infection on the second day of three out of six of her treatments. These fevers lasted one day and did not interfere with the patient's treatment.

One of the patients with mycosis fungoides experienced severe skin pain and erythema lasting three to four days after his second dose of therapy. This was relieved with a reduction to approximately two thirds of the original dose of MTX and FUra.

No patient developed thrombocytopenia or a deterioration in renal function during the course of treatment.

The more severe toxicity occurred after the first or second dose in each case. Later doses produced milder toxi-

city or none at all. In all but two cases no change in dose or interval between treatment was necessary to lessen toxicity. As previously noted, in the case of severe leukopenia the interval between treatments was lengthened and in the case of skin pain the dose was decreased.

The mean time since the patients were treated with chemotherapy was 23 days. The length of time since the last treatment was not related to the number of side effects experienced. Four out of seven patients had one hour sequenced MTX-FUra as their last chemotherapeutic regimen and these patients had less toxicity than the other patients. There was no clear relationship between the number of previous courses of one hour sequenced MTX-FUra and toxicity (see Table 5).

Toxicities occurring in more than one system simultaneously were experienced by four patients and are shown in Table 7. The toxicities that most often occurred together were bone marrow suppression and mucositis. The simultaneous side effects first occurred after dose one (3 patients) or dose two (1 patient) yet the patients still tolerated an average of 3.5 courses at 20 day intervals. This compared well to the average of four courses at 23 day intervals for patients who received more than two courses of therapy. The mean serum MTX concentrations for the treatments that caused simultaneous toxicity were $0.89 \pm .6 \mu M$ before the fifth dose of MTX and $1.46 \pm .6 \mu M$ after the fifth dose, both less than the mean concentrations for the total 21 courses.

DISCUSSION

This small study of seven patients treated with a schedule of MTX, FUra, and LV was designed according to the sequencing schedule that produced optimal cell kill in vitro. The experimental data with 47-DN cells and the longer doubling times of breast tumors suggested that a 24 hour sequencing would be necessary for maximal synergism between MTX and FUra (35). A chemotherapeutic program was chosen that would give drug doses similar to that used in previous studies with one hour drug sequencing (37,38) but could be given out of the hospital. This MTX dose of 250 mg/m^2 was given orally in divided doses over 24 hours. The schedule maintained the MTX serum level above .1μM which is in the range that resulted in enhanced cytotoxicity in vitro.

The pharmacokinetic data of Henderson et al (42) for a single oral dose of MTX 50 mg/m^2 with decay can be extrapolated according to the dose schedule of MTX given in this study, 50 mg/m^2 p.o. every six hours for

five doses. Based on these data one would expect the serum MTX concentration to be 0.26 M at 23 hours (before the fifth dose) and 0.46 M at 25 hours (after the fifth dose) as shown in Figure 2. However, the actual mean concentrations in this study were well over two standard deviations above this model and were instead in the range of an i.v. bolus of MTX 250 mg/m^2 given 24 hours earlier. The fact that the serum concentrations were above that extrapolated from the Henderson curve for one dose of oral MTX 50 mg/m^2 suggests that repeated oral administration may alter MTX clearance. This oral dose schedule proved feasible for further use.

The present study also evaluated the effect on host toxicity of prolonging the MTX pretreatment interval according to in vitro studies. Prior studies with a lengthened pretreatment interval have shown increased toxicity that outweighed the therapeutic advantage of greater tumor cell kill.

Solan et al (43) reported on a small, uncontrolled trial in colorectal cancer patients using similar doses of intravenous MTX and FUra with four hour sequencing, given at weekly intervals. They found unacceptable myelosuppression and attributed it to the longer MTX pretreatment interval. Another study that compared MTX and FUra schedules in tumor-bearing mice found that although 24 hour sequencing resulted in maximal cell kill, it also increased early deaths from toxicity more than six times over the other two drug schedules (44).

In contrast, the present study shows that 24 hour sequenced MTX-Fura can be well tolerated with only mild and transient toxicity. No patient stopped therapy because of intolerable side effects and there were no drug related deaths. No toxicity occurred with 38% of the treatment courses. Bone marrow suppression and mucositis were the most common side effects with mild to moderate leukopenia and mucositis occurring in 27% and 38% of courses, respectively (Table 5). Those patients with more than two courses tolerated an average of four treatments with a mean treatment interval of 23 days. The patients with simultaneous toxicity developed this early on in treatment and were still able to tolerate an average of 3.5 courses at 20 day intervals. The occurrence of toxicity was not related to either cumulative drug dose or greater than average serum MTX concentration. Only one patient required a dose reduction and

one patient required a lengthening of the re-treatment interval from 7 to 14 days.

Why is there such a great difference between the toxicity results of the present study and that of the trial of Solan et al (43)? Unacceptable myelosuppression occurred in the study done by Solan et al when the interval between treatment courses was seven days. Only one patient in the present study experienced severe bone marrow suppression, and it was after the same seven day re-treatmennt interval schedule. This patient had no further problems once the interval was lengthened; no other patient had such a short interval between courses or had such severe side effects. Toxicity may be related to re-treatment interval rather than to the prolonged interval between MTX and FUra administration. Lengthening the interval between treatment courses to at least 14 days makes this chemotherapeutic regimen well tolerated.

Another possible explanation for the different results in the two studies is that Solan et al give no data on renal function; in this study no patient was accepted with a creatinine clearance less than 65. The patients in Solan's study may have had a decreased MTX clearance and thus a worsening of toxicity.

In addition, individual variations in circulating levels of thymidine and "salvage" purines may account for

differences in toxicities. Since MTX inhibits both thymi-
dylate synthesis and de novo purine synthesis, the addition
of thymidine or purines such as hypoxanthine can potential-
ly prevent the toxic effects of MTX. Howell et al (45)
found that the normal physiologic range of plasma hypoxan-
thine is within the concentration range of hypoxanthine
that reverses FUra accumulation in 47-DN MTX pretreated
cells (0.1-10μM) (35). They also found that the hypoxan-
thine concentrations in freshly aspirated bone marrow spec-
imens were so high that protection against MTX depended
only on thymidine availability. In culture, small changes
in thymidine concentrations resulted in large differences
in the degree of MTX toxicity. In two small uncontrolled
studies such as the present one and that by Solan et al,
differences in serum thymidine concentrations could account
for the variability in clinical toxicity. The patients in
this study may have thymidine and hypoxanthine concentra-
tions in the range to selectively protect bone marrow
precursors.

The response rate in this study is poor compared with
other reported drug regimens. Gerwitz and Cadman (37)
achieved a response rate of 53% (complete or partial
responses) with 1 hour sequenced MTX-FUra. In this study
only one patient (14%) achieved an objective response and
for only two and a half weeks. However,the patients in

this trial had far advanced malignancy and had received many prior chemotherapeutic trials; they were therefore less likely to respond to further treatment. The MTX-FUra synergism observed in tissue culture of human breast cancer was maximal with a 24 hour pretreatment interval, suggesting that this protocol may be very effective as an initial therapy in previously untreated women with advanced breast cancer. Nevertheless, the extreme variation in breast cancer growth rate may indicate that the optimal MTX pretreatment interval will vary from patient to patient.

Although this study is small, the clinical results show that the 24 hour sequenced schedule of MTX and FUra with LV rescue is well tolerated. This regimen can now be safely evaluated in a larger clinical trial and compared with one hour sequenced MTX and FUra. Advanced breast cancer is incurable; but the search for new and more effective chemotherapeutic programs is moving toward the goal of increasing the duration of symptom-free and absolute survival, while decreasing the morbidity of treatment. This study is a small step toward that goal.

Table 1   Survival of Patients with Breast Cancer Relative to Histologic Stage (3)

Histologic Staging	Crude Survival (%) 5 yr	Crude Survival (%) 10 yr	5-Year Disease-Free Survival (%)
All patients	63.5	45.9	60.3
Negative axillary lymph nodes	78.1	64.9	82.3
Positive axillary lymph nodes	46.5	24.9	34.9
1-3 positive axillary lymph nodes	62.2	37.5	50.0
>4 positive axillary lymph nodes	32.0	13.4	21.1

Table 2   Organs Involved with Metastatic Disease--Seven Patients

Primary Malignancy	Breast	Breast	Breast	Breast	Mycosis Fungoides	Mycosis Fungoides	Head and Neck
Performance Status	1	2	2	1	1	2	4
Bone	X	X	X	X			
Pleura		X		X			
Peritoneum				X			
Meninges			X				
Brain	X						
Liver		X					
Skin		X	X	X	X	X	X
Regional nodes		X	X				
Lymphoma					X		

Table 3   ECOG Performance Status Key (41)

Performance Status Scale	Number of Patients
0--Normal activity	0
1--Symptoms but ambulatory	3
2--In bed <50% of time	3
3--In bed >50% Of time	0
4--100% bedridden	1

From Yale Oncology
Clinic Form

ECOG TOXICITY CRITERIA (41)

TABLE 4

		0	1	2	3	4
Leuko-penia	WBC × 10³ Neut × 10³	≥4.5 ≥1.9	3.0−<4.5 1.5−<1.9	2.0−<3.0 1.0−<1.5	1.0−<2.0 0.5−<1.0	<1.0 <0.5
Thrombo-cytopenia	Plt × 10³	≥130	90−<130	50−<90	25−<50	<25
Anemia	Hgb gm% Hct % Clinical	≥11 ≥32	9.5−10.9 28−31.9	<9.5 <28 Sx of anemia	Req transfusions	
Infection		None	No active Rx	Requires active Rx	Debilitating	Life threatening
GU	BUN mg% Creatinine Proteinuria Hematuria	≤20 ≤1.2 Neg Neg	21−40 1.3−2.0 1+ Micro-Cult−positive	41−60 2.1−4.0 2+−3+ Gross-Cult−positive	>60 >4.0 4+ Gross+Clots	Symptomatic uremia c̄ obst uropathy
N & V		None	Nausea	N & V controllable	Vomiting intractable	
Diarrhea		None	No dehydration	Dehydration	Grossly bloody	
Skin & Mucosa	Stomatitis	None	Soreness	Ulcers—can eat	Ulcers—cannot eat	
Fever		≤37.5°C	≤38°C (≤100.4°F)	>38°C (>100.4°F)	Severe c̄ chills (>40°C)	Fever c̄ hypotension

Toxicity grade = 5 if that toxicity caused the death of the patient.

Table 5. Clinical Toxicity Study of 24 Hr Sequenced MTX and FUra with LV Rescue

Primary Malignancy	Patient Performance Status *	Number Previous Courses 1 Hour Sequenced MTX-FUra	Last Treated With 1 Hour Sequenced MTX-FUra	Patient Courses	*Toxicity Grade Per Course					
					leukopenia	nausea/vomiting	diarrhea	mucositis	fever	infection
breast	1	40	X	4	II,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0	0
breast	2	4		6	0,IV,I,0,0,I	II,I,0,0,0,0	0,I,0,I,0,0	0,II,I,II,0,0	0,0,II,II,II,0	0,II,0,0,0,0
breast	2	1	X	2	0,III	0,0	0,0	0,0	0	0
breast	1	1	X	3	0,0,0	0,0,0	1,0,0	II,I,0	0	0
mycosis fungoides	1	4		2	I,I	II,0	0,0	II,0	0	0
mycosis fungoides	2	0		3	0	0	0	0	0	0
head and neck	4	13	X	1	0	0	0	0	0	0

* ECOG criteria (41)

Table 6. Clinical Toxicity--21 Courses

	ECOG Grade				
Leukopenia	0	1	2	3	4
Nausea/ Vomiting	18	1	2	0	
Diarrhea	18	3	0	0	
Mucositis	13	2	6	0	
Fever	19	0	3	0	0
Infection	20	0	1	0	0
Anemia					
pts. not anemic at start	5	5	1	1	0
3 pts. anemic at start (grade 1)	4	4	1	1	0

Table 7.--Simultaneous Toxicity--Four Patients

Primary Malignancy	Breast	Breast	Mycosis Fungoides	Mycosis Fungoides
Occurred After Dose #	2. 3. 4.	1.	1.	1. 2.
Leukopenia/Anemia	X X		X	X X
Nausea/Vomiting	X		X	
Diarrhea	X X	X		
Mucositis	X X X	X	X	X X
Infection	X			

FIGURE 1

Proposed interaction between MTX and FUra metabolism
Adapted from Cadman, Heimer, and Benz (34)

Pyrimidine Synthesis   Purine Synthesis

Glutamine + HCO_3^- + 2 ATP   PRPP pools   PRPP + Glutamine

PP_i

Orotate

FUra → FUMP → FUDP → FUTP → RNA $N^{5,10}CHTHF$ THF

OMP   THF Pools

FdUDP

FdUMP $N^{5,10}CH_2THF$ $N^{10}CHOTHF$

UMP

UDP → dUDP → dUMP   3 MTX

2   DHF   THF

dTMP

UTP   dTDP

dTTP

RNA

CTP - - → dCTP → DNA

FdUMP directly inhibits enzyme 2 in the
presence of THF. MTX inhibits enzyme 3
and dTMP synthesis when the THF pools are   IMP
depleted. The reduction of THF pools also
leads to an inhibition of purine synthesis   AMP   GMP
and then an accumulation of PRPP. PRPP is
used in the conversion of FUra to FUMP and   ADP → dADP → dATP DNA dGTP ← dGDP ← GDP
eventual incorporation into cellular RNA.
                                            ATP   RNA ←   GTP

Broken arrows represent multiple enzymatic steps
■ represents inhibition of step

Enzymatic Steps
    1. orotate phosphoribosyltransferase
    2. thymidylate synthetase
    3. dihydrofolate reductase
    4. glycinamide ribonucleotide transformylase
    5. aminoimidazole carboxamide ribonucleotide transformylase

DHF=dihydrofolate   THF=tetrahydrofolate

FIGURE 2   MTX Pharmacokinetic Data

The dotted lines represent the data of Henderson et al (42) for a single dose of MTX 50 mg/m^2 p.o. and 250 mg/m^2 i.v. with decay. The curve for the MTX dose schedule in this study, 50 mg/m^2 p.o. Q 6 hours x 5, has been extrapolated from the 50 mg/m^2 p.o. single dose curve. Serum MTX concentrations were measured one hour before and one hour after the fifth dose and mean values are shown here.
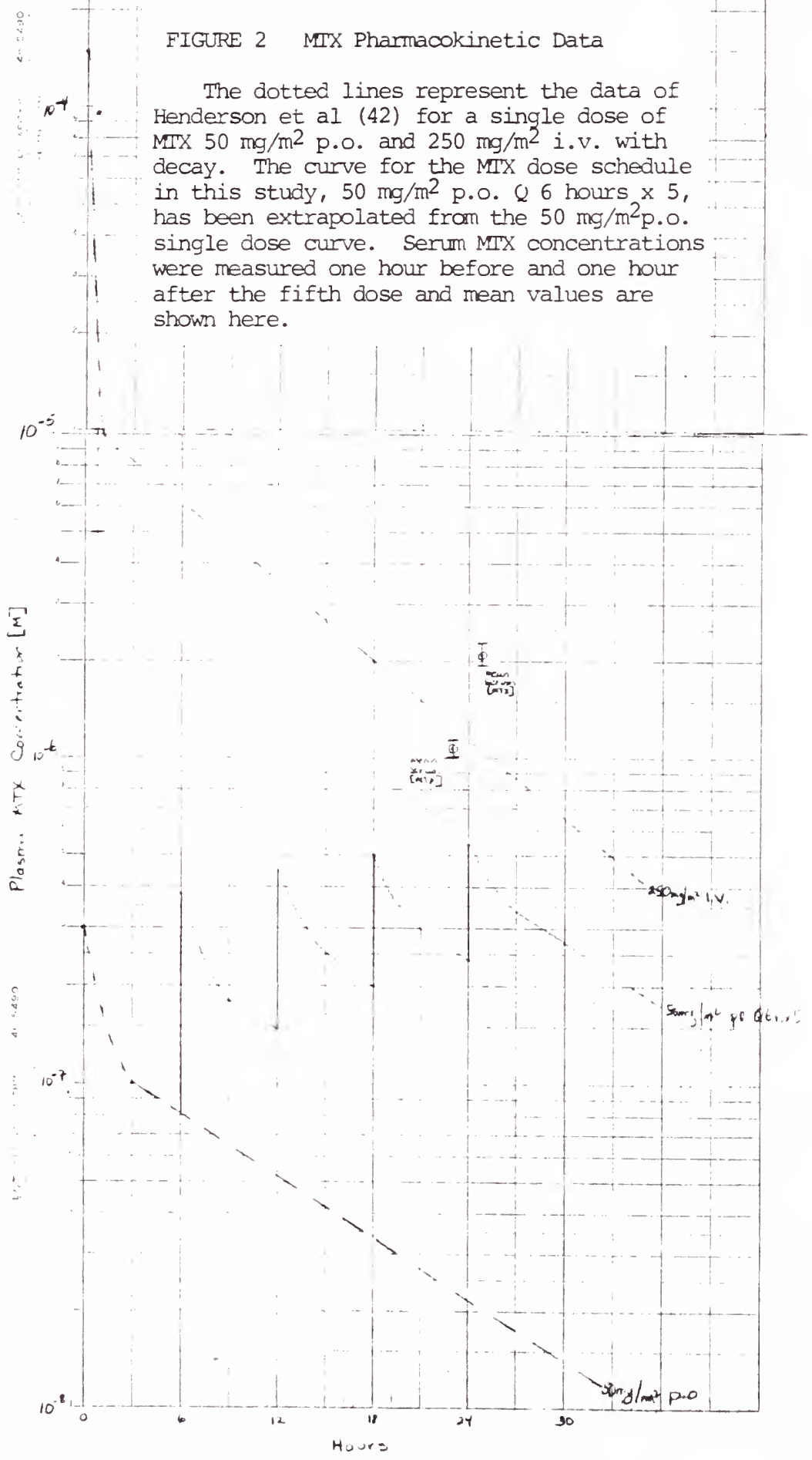
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