Formation of 7-Hydroxymethotrexate Polyglutamyl Derivatives and Their Cytotoxicity in Human Chronic Myelogenous Leukemia Cells, in Vitro

Gerard Fabre and I. David Goldman

Departments of Medicine and Pharmacology, Medical College of Virginia, Richmond, Virginia 23298

ABSTRACT

The rapid synthesis of poly-γ-glutamyl derivatives of 7-hydroxymethotrexate (7-OH-MTX) and their selective intracellular retention are reported in human chronic myelogenous leukemia cells, K-562. After a 30-min exposure to 5 μM [3H]7-OH-MTX, three different polyglutamyl derivatives were detected by high-performance liquid chromatography. When extracellular 7-OH-MTX was removed, the 7-OH-MTX diglutamate level declined slowly in comparison to the monoglutamate, but the higher polyglutamyl derivative levels increased. Within 10 min after exposure of cells to 7-OH-MTX, the level of these polyglutamyl derivatives far exceeds the dihydrofolate reductase binding capacity. Gel filtration or charcoal binding analysis followed by high-performance liquid chromatography analysis of the bound component showed intracellular binding of virtually all 7-OH-MTX tetraglutamate at a level 4-fold higher than that of the dihydrofolate reductase binding capacity. No bound 7-OH-MTX diglutamate or triglutamate could be detected. Treatment of the 7-OH-MTX tetraglutamate:protein complex with 100 μM unlabeled methotrexate (MTX) for 15 min resulted in only a partial dissociation of this complex to an extent compatible with the dihydrofolate reductase level. The residual 7-OH-MTX tetraglutamate remained bound to a site with a molecular weight of approximately 25,000 to 35,000 as assessed by Bio-Gel P-60 analysis and could not be displaced by folic acid, 5-formyltetrahydrofolate, 7-OH-MTX tetraglutamate, or the tetraglutamate of 7-hydroxymethotrexate (7-OH-MTX) and MTX in cells growing in agar were 10⁻⁵ and 10⁻⁶ M, respectively. A 10-fold difference in cytotoxicity was also observed in cell-free systems (33, 34). Further, we demonstrated recently that the tetraglutamate of 7-OH-MTX, unlike the di- and triglutamates, binds to DHFR and is a better inhibitor of this enzyme than 7-OH-MTX. This raised the possibility that the 7-OH-MTX polyglutamates may play a role in drug action. This paper analyzes the formation, retention, and selective binding to DHFR of 7-OH-MTX polyglutamates and correlates these parameters with 7-OH-MTX cytotoxicity in a human chronic myelogenous leukemia cell line (K-562).

INTRODUCTION

The rapid synthesis of poly-γ-glutamyl derivatives of 7-hydroxymethotrexate (7-OH-MTX) and their selective intracellular retention are reported in human chronic myelogenous leukemia cells, K-562. After a 30-min exposure to 5 μM [3H]7-OH-MTX, three different polyglutamyl derivatives were detected by high-performance liquid chromatography. When extracellular 7-OH-MTX was removed, the 7-OH-MTX diglutamate level declined slowly in comparison to the monoglutamate, but the higher polyglutamyl derivative levels increased. Within 10 min after exposure of cells to 7-OH-MTX, the level of these polyglutamyl derivatives far exceeds the dihydrofolate reductase binding capacity. Gel filtration or charcoal binding analysis followed by high-performance liquid chromatography analysis of the bound component showed intracellular binding of virtually all 7-OH-MTX tetraglutamate at a level 4-fold higher than that of the dihydrofolate reductase binding capacity. No bound 7-OH-MTX diglutamate or triglutamate could be detected. Treatment of the 7-OH-MTX tetraglutamate:protein complex with 100 μM unlabeled methotrexate (MTX) for 15 min resulted in only a partial dissociation of this complex to an extent compatible with the dihydrofolate reductase level. The residual 7-OH-MTX tetraglutamate remained bound to a site with a molecular weight of approximately 25,000 to 35,000 as assessed by Bio-Gel P-60 analysis and could not be displaced by folic acid, 5-formyltetrahydrofolate, 7-OH-MTX tetraglutamate, or the tetraglutamate of 7-hydroxymethotrexate (7-OH-MTX) and MTX in cells growing in agar were 10⁻⁵ and 10⁻⁶ M, respectively. A 10-fold difference in cytotoxicity was also observed in cell-free systems (33, 34). Further, we demonstrated recently that the tetraglutamate of 7-OH-MTX, unlike the di- and triglutamates, binds to DHFR and is a better inhibitor of this enzyme than 7-OH-MTX. This raised the possibility that the 7-OH-MTX polyglutamates may play a role in drug action. This paper analyzes the formation, retention, and selective binding to DHFR of 7-OH-MTX polyglutamates and correlates these parameters with 7-OH-MTX cytotoxicity in a human chronic myelogenous leukemia cell line (K-562).

MATERIALS AND METHODS

Materials and Chemicals. [3',5',7-³H]MTX (10 to 20 mCi/μmol) was obtained from Amersham (Arlington Heights, IL) and purified by HPLC (15). Unlabeled MTX was purified by DEAE-cellulose chromatography (18). [³H]7-OH-MTX was prepared enzymatically from [³H]MTX by incubation with a crude preparation of fresh mature rabbit liver and purified by HPLC. When 100,000 counts of [³H]7-OH-MTX were chromatographed, there were essentially no counts above background in the marker nonlabeled MTX peak, indicating essentially no contamination of 7-OH-MTX with MTX; the compound was at least 96% pure. Similar purity was established for the nonlabeled 7-OH-MTX. Authentic 7-OH-4-NH₂-10-CH₃-PteGlu₄ (18) was prepared by incubating radiolabeled 4-NH₂-10-CH₃-PteGlu₂ to -Glu₄ by incubating radiolabeled 4-NH₂-10-CH₃-PteGlu₂ to -Glu₄, respectively, with a crude preparation of fresh mature rabbit liver, and retention times were determined by HPLC as described in detail previously (9). Bio-Gel P-6 (200 to 400 mesh) was purchased from Bio-Rad Laboratories (Richmond, CA).

Cloning Assay. The K-562 human chronic myelogenous leukemia cell line (30) was maintained in continuous culture in Roswell Park Memorial Institute Medium (RPMI) 1640 (Grand Island Biological Co., Grand Island, NY) supplemented with 10% heat-inactivated undialyzed fetal calf serum and 2 mM L-glutamine. For cloning assay, cells were harvested during exponential growth (10⁶ cells/ml), washed twice in ice-cold RPMI, and exposed to the drug in this medium at 37°C in the presence of 5 mM L-glutamine. At the end of the incubation interval, cells
were washed twice with the same medium and resuspended in RPMI supplemented with 2 mM l-glutamine, 20% heat-inactivated dialyzed fetal calf serum, 15 mM N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid, and 0.35% Bacto agar (Difco Laboratories). Two ml of this medium were added to 35-mm 8-well plates and incubated at 37° in a 5% CO₂ humidified atmosphere. Colonies were scored after 10 to 14 days using a Bélico projection screen (Vineland, NJ) at ×17.5. Aggregates of 50 or more cells were considered colonies. Viability was defined as follows:

\[ \text{Viability} = \frac{\text{no. of colonies in drug-treated cultures}}{\text{no. of colonies in control cultures}} \times 100 \]

Each experimental point was performed in quadruplicate and repeated at least 2 times.

Preparation of Cells for Analysis of Intracellular Radiolabeled Constituents. Freshly harvested cells in exponential growth were washed twice with ice-cold 0.85% NaCl solution and then resuspended into RPMI medium supplemented with 5 mM l-glutamine and 2 mM glucose, and following this, the antifoam was added. The pH was maintained at 7.4 by passing warm and humidified 95% O₂,5% CO₂ over the cell suspension. The suspension was stirred with a Teflon paddle in flasks immersed in a 37°C water bath. Transport fluxes were stopped by the injection of the cell suspension into 10 volumes of 0°C 0.85% NaCl solution, following which the cell fraction was separated by centrifugation and washed an additional 2 times with the same solution. The washed pellet was then aspirated into the tip of a Pasteur pipet, extruded onto a polyethylene tere, and dried overnight at 70°C. The dry pellets were weighed on a Cahn Model 4700 electrobalance (Cahn Instruments, Irvine, CA), and radioactivity was determined in a Beckman LS-230 liquid scintillation spectrometer. Counting efficiencies were established with [³H]toluene internal standards.

For analysis of intracellular 7-OH-MTX and its polyglutamyl derivatives, portions of cells were injected into 10 volumes of ice-cold buffer and washed twice, and the cell pellet was extracted with 1 ml of 10% trichloroacetic acid. The trichloroacetic acid extract (0.7 ml) was then neutralized by adding 0.175 ml of 1 M KOH and 0.35 ml of 1 M K₂HPO₄ (pH 7.0) and chromatographed by HPLC as described previously (15). Intracellular 7-OH-MTX and 7-OH-MTX polyglutamyl derivatives were quantitated from the percentage of each derivative determined on the chromatographic analysis and the total ³H in each cell pellet as determined in units of nmol/g dry weight.

Analysis of Intracellular Binding of 7-OH-MTX and Its Polyglutamyl Derivatives. Portions of cells were injected into 10 volumes of 0°C NaCl solution and washed twice as described above. The cell pellet was suspended in 0°C buffer consisting of 0.05 mM citrate (pH 6.0), 0.15 M KCl, 0.05 M mercaptoethanol, 0.001 M EDTA, and 0.1 mM NADPH and disrupted with a model 300 probe sonicator (Artek, Farmingdale, NY) until over 95% of the cells were broken, as established by light microscopy. Following this, the sonicate was centrifuged at 40,000 × g for 30 min.

Two different methods were used to separate free from bound drug. (a) Samples subjected to sonic oscillation were injected on a Bio-Gel P-6 minicolumn which separates enzyme:ligand complex, which passes through the minicolumn in a small volume, from the free ligand which is retained completely within the column (14). (b) With the second method, sonicated samples were mixed with 5% activated charcoal for 30 sec and then centrifuged for 10 min at 14,000 × g. This procedure permits separation of the free drug in the charcoal pellet from the protein:ligand complex which remains in the supernatant fluid (28). Following both techniques, the enzyme:ligand complex was chromatographed on a column (1 × 50 cm) of Bio-Gel P-60 equilibrated with the above buffer and calibrated with appropriate molecular weight markers (31).

RESULTS

Formation and Retention of 7-OH-MTX Polyglutamyl Derivatives. After a 4-hr exposure of human chronic myelogenous leukemia cells to 5 μM [³H]7-OH-MTX in the presence of 5 mM l-glutamine and 2 mM glucose, 4 distinct radiolabeled compounds could be demonstrated by HPLC. One peak coeluted with 7-OH-MTX, and the 3 other peaks cochromatographed with 7-OH-4-NH₂-10-CH₃-PteGlu₂ to -PteGlu₄ and standards, respectively. Incubation with chicken pancreas conjugase resulted in the complete hydrolysis of all the peaks to 7-OH-MTX (9). The time course of accumulation of 7-OH-MTX and its polyglutamyl derivatives over a 4-hr exposure to 5 μM [³H]7-OH-MTX is illustrated in Chart 1. As observed previously in Ehrlich cells (9), as the total 7-OH-MTX polyglutamyl derivative level increased, there was an associated decline in the level of intracellular undervinylized 7-OH-MTX. Moreover, at each of the times examined beyond 10 min, the accumulation of 7-OH-MTX polyglutamyl derivatives far exceeded the DHFR binding capacity (1.32 ± 0.22 nmol/g dry weight (n = 5), as determined by gel filtration analysis (14, 15)).

The capacity of these cells to retain 7-OH-MTX and its polyglutamyl derivatives was also investigated. As depicted in the representative experiment of Chart 2, when cells exposed for 2 hr to 5 μM 7-OH-MTX were resuspended in drug-free medium, the major portion of intracellular 7-OH-MTX (Line 1) exited the cells within 15 min with a slower decline of the residual cell 7-OH-MTX by 90 min. Over the interval of observation, there was a substantial decline (approximately 6 nmol/g dry weight) in 7-OH-4-NH₂-10-CH₃-PteGlu₂ (Line 2); however, the slow concurrent rise in 7-OH-4-NH₂-10-CH₃-PteGlu₄ (Line 3) and -PteGlu₄ (Line 4) (approximately 2.5 nmol/g dry weight) resulted in a smaller net decline in the total level of cell 7-OH-MTX polyglutamyl derivatives.

Intracellular Binding of 7-OH-MTX and Its Polyglutamyl Derivatives. K-562 cells were exposed to 5 μM [³H]7-OH-MTX for 2 hr as described in Chart 1 and then washed twice, and cell pellets were isolated. A portion of the pellet was extracted with trichloroacetic acid, neutralized, and analyzed by HPLC, and the total cellular level of 7-OH-MTX and of each of its derivatives
CYTOTOXICITY OF 7-OH-MTX POLYGLUTAMATES

MINUTES

Chart 2. The intracellular retention of 7-OH-MTX and 7-OH-MTX polyglutamyl derivatives. Cells were exposed to 5 μM [3H]7-OH-MTX and 5 mm L-glutamine for 2 hr, separated by centrifugation, washed twice, and resuspended in drug-free medium at Time 0. Following this, portions of the cells were separated by centrifugation and analyzed as described under "Materials and Methods." Line 1, 7-OH-MTX; Unes 2 to 4, 7-OH-4-NH2-10-CH3-PteGlu2, -Glu3, and -Glu4, respectively.

Table 1
Analysis of intracellular radiolabel in K-562 cells

Cells exposed to 5 μM [3H]7-OH-MTX and 5 mm L-glutamine for 2 hr were washed twice, and total and bound intracellular radiolabel was analyzed as described in "Materials and Methods" and "Results."

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug accumulation (mmol/g dry wt)</th>
<th>Drug bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 1</td>
<td>8.00 ± 2.86^a</td>
<td>ND^b</td>
</tr>
<tr>
<td>n = 2</td>
<td>9.16 ± 2.23</td>
<td>ND</td>
</tr>
<tr>
<td>n = 3</td>
<td>2.39 ± 1.07</td>
<td>ND</td>
</tr>
<tr>
<td>n = 4</td>
<td>6.53 ± 1.10</td>
<td>5.35^c–6.13^d</td>
</tr>
</tbody>
</table>

^a Mean ± S.D.
^b ND, not detectable.
^c As assessed by charcoal assay.
^d As assessed by Bio-Gel P-6 analysis.

was determined. Another portion of the pellet was subjected to sonic oscillation and centrifuged, and the supernatant was either centrifuged through a Bio-Gel P-6 column or mixed with activated charcoal. As described in "Materials and Methods," both techniques permit the separation of free drug from the drug:protein complex. The drug:protein complex was then extracted by trichloroacetic acid, neutralized, and analyzed by HPLC. Table 1 indicates that, while appreciable amounts of 7-OH-MTX and its different polyglutamyl derivatives were present inside the cells under these conditions, only the tetraglutamyl derivative of 7-OH-MTX (7-OH-4-NH2-10-CH3-PteGlu4) was bound intracellularly. This is similar to what was observed in Ehrlich cells (9). Indeed, even over a 4-hr interval over which the tetraglutamate accumulates within cells, essentially all intracellular 7-OH-4-NH2-10-CH3-PteGlu4 is bound (data not shown). Table 2 focuses on the tetraglutamate derivative and illustrates that its total cell level (Line 1) was 4-fold greater than the DHFR binding capacity (Line 4) and that all intracellular tetraglutamate (Line 1) was bound as assessed by both assays (Line 2).

Treatment of the 7-OH-4-NH2-10-CH3-PteGlu4:protein complex with 100 μM unlabeled MTX for 15 min resulted in only a partial dissociation of this complex to an extent compatible with the intracellular DHFR level (Table 2, Line 3). The same extent of dissociation was observed after treatment of the 7-OH-4-NH2-10-CH3-PteGlu4:protein complex with 100 μM 4-NH2-10-CH3-PteGlu4 or 7-OH-MTX; however, neither folic acid nor 5-formyltetrahydrofolate at the same concentration had an effect on binding (data not shown).

The drug:protein complex was further analyzed by chromatography on a Bio-Gel P-60 column calibrated with appropriate molecular weight markers (Chart 3A). As illustrated in Chart 3B, 7-OH-4-NH2-10-CH3-PteGlu4 was bound to a protein(s) with a molecular weight of about 25,000 to 35,000, similar to the molecular weight of DHFR. No other peaks were observed. However, when the cell extract was treated for 15 min with 100 μM unlabeled MTX, there was partial displacement of bound 7-

Table 2
Analysis of intracellular 7-OH-4-NH2-10-CH3-PteGlu4 in K-562 cells

Cells exposed to 5 μM [3H]7-OH-MTX and 5 mm L-glutamine for 2 hr were washed twice, and total and bound intracellular 3H was analyzed. As indicated in the text and Table 1, only 7-OH-4-NH2-10-CH3-PteGlu4 was bound under these conditions.

<table>
<thead>
<tr>
<th>Drug</th>
<th>nmol/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total cell 7-OH-4-NH2-10-CH3-PteGlu4</td>
<td>5.98 ± 1.77^a</td>
</tr>
<tr>
<td>2. Bound cell 7-OH-4-NH2-10-CH3-PteGlu4</td>
<td>6.13 ± 1.11</td>
</tr>
<tr>
<td>Bio-Gel P-6</td>
<td>5.35 ± 0.47</td>
</tr>
<tr>
<td>3. Bound level in the presence of 100 μM MTX</td>
<td>4.36 ± 0.73</td>
</tr>
<tr>
<td>4. DHFR binding capacity</td>
<td>1.32 ± 0.22</td>
</tr>
</tbody>
</table>

^a Mean ± S.D. of 3 experiments.
OH-4-NH$_2$-10-CH$_3$-PteGlu$_4$ (Table 2) and the appearance of a second radiolabeled peak which coeluted with free 7-OH-MTX and its polyglutamyl derivatives, which are not distinguished on Bio-Gel P-60 (Chart 3C).

**Effects of 7-OH-MTX on Cell Growth.** Cells in logarithmic growth were exposed to medium containing different 7-OH-MTX concentrations for 4 hr. The cells were then washed twice, resuspended in drug-free fresh medium at 37°C, and counted every 24 hr for 4 days. As illustrated in Chart 4, there is a concentration-dependent inhibition of cell growth. A 4-hr exposure to 50 µM 7-OH-MTX inhibited the growth of K-562 cells by 89 ± 1% (n = 3) after 3 days, comparable to the inhibition of 87 ± 1% (n = 3) observed 3 days after a 4-hr exposure to 5 µM MTX (not shown).

**Clonogenic Growth of Cells Exposed to 7-OH-MTX.** K-562 cells were exposed to increasing concentrations of MTX or 7-OH-MTX for 2 hr, and effects on colony formation were determined as described under “Materials and Methods.” MTX and 7-OH-MTX concentrations correspond to the spectrum of plasma levels for the drug and its catabolite observed after high-dose MTX infusions (2, 8, 29). K-562 cells showed a 10-fold-greater sensitivity to MTX than to 7-OH-MTX with ID$_{50}$s of approximately $10^{-6}$ and $10^{-5}$ M, respectively (Chart 5).

**Effects of GAT Protection on 7-OH-MTX Inhibition of Clonal Growth.** Continuous exposure to the end products of folate-dependent reactions, GAT, completely protects cells from a continuous exposure to 5 µM 7-OH-MTX, a level which produced complete inhibition of colony formation in the absence of GAT. The requirement for continuous exposure to GAT to prevent the expression of toxicity of even brief exposures to 7-OH-MTX is illustrated in Chart 5. In this experiment, cells were exposed to 5 µM 7-OH-MTX with or without GAT for 30 min to 5 hr, following which the cells were plated in 7-OH-MTX-free agar in the presence or absence of GAT. If GAT was present only during the exposure to 7-OH-MTX (△), or if cells were not exposed to GAT at all (□), comparable increasing cytotoxicity was expressed with increasing exposure to 7-OH-MTX. However, the presence of GAT during both the period of exposure to 7-OH-MTX and over the subsequent 10- to 14-day period of clonal growth completely preserved the viability of these cells (○).

**DISCUSSION**

Recently, 7-OH-MTX was shown to be metabolized to 7-OH-MTX polyglutamyl derivatives in human T-lymphocytes (11), Ehrlich cells (9), and cell-free systems (33, 34). Furthermore, 7-OH-4-NH$_2$-10-CH$_3$-PteGlu$_4$ was observed to bind to and to be a more potent inhibitor of Ehrlich ascites cell DHFR than the monoglutamate (9). This paper demonstrates that 7-OH-MTX also undergoes rapid polyglutamylation in chronic myelogenous leukemia cells, K-562. After a 1-hr exposure to 5 µM 7-OH-MTX, approximately 60% of the intracellular drug was metabolized to polyglutamyl derivatives; by 4 hr, only 20% of intracellular 7-OH-MTX remained unchanged. Within 30 min, 7-OH-MTX polyglutamyl derivatives far exceed the DHFR binding capacity.
Cytotoxicity of 7-OH-MTX Polyglutamates

Of particular interest was the observation that, while the di- and triglutamates are not bound within these cells, virtually all intracellular 7-OH-4-NH₂-10-CH₃-PteGlu₄ was bound, but only a small portion of bound drug was associated with DHFR. Hence, treatment of the 7-OH-4-NH₂-10-CH₃-PteGlu₄:protein complex with a high concentration of MTX for 15 min results in only a small displacement of bound drug to an extent comparable with the DHFR binding capacity. The remaining 7-OH-4-NH₂-10-CH₃-PteGlu₄ not associated to DHFR was bound to a protein(s) with a molecular weight of approximately 25,000 to 35,000, which is comparable to that of DHFR, but for which neither MTX, folic acid, 4-NH₂-10-CH₃-PteGlu₄, 7-OH-MTX, nor 5-formytyetahydrofolate competes. This does not appear to be a folate-binding protein of the kind that has been reported to be present in chronic granulocytic leukemia cells (38), because the 7-OH-4-NH₂-10-CH₃-PteGlu₄ complex is not disassociated by folic acid. This second site could correspond to an altered form of DHFR with the same molecular weight but a marked reduction in affinity for MTX, as observed in L5178Y cells resistant to MTX (6). The identity of this binding site(s) remains unclear and is presently under further investigation.

These studies indicate that 7-OH-MTX is cytotoxic. Clonogenic assay in soft agar indicates an ID₅₀ about one-tenth of that of MTX after a 2-hr exposure to the drug. A factor of one-tenth the activity of MTX is also observed in cell growth inhibition. After a 4-hr exposure, the same growth inhibition (ID₅₀) is achieved with 5 µM MTX as with 50 µM 7-OH-MTX. The data suggest further that the cytotoxicity of 7-OH-MTX is related to the formation of 7-OH-4-NH₂-10-CH₃-PteGlu₄ in these cells. First, as illustrated in Chart 7, there is a linear relationship (r = 0.999) between the cytotoxicity of 7-OH-MTX in agar and the level of 7-OH-4-NH₂-10-CH₃-PteGlu₄ that accumulates within these cells. Indeed, the intercept on the X-axis is equal to an intracellular 7-OH-4-NH₂-10-CH₃-PteGlu₄ level of 1.6 nmoi/g dry weight of cells, comparable to the DHFR binding capacity. Hence, the data suggest that to express cytotoxicity, the 7-OH-4-NH₂-10-CH₃-PteGlu₄ level must exceed the DHFR binding capacity. Since virtually all 7-OH-4-NH₂-10-CH₃-PteGlu₄ in excess of DHFR binding capacity in these cells is bound, the data suggest that this second bound component plays a role in cytotoxicity. Of course, the di- and triglutamyl derivatives of 7-OH-MTX also persist in these cells in a free form and could contribute to cytotoxicity. However, because these derivatives do not bind to DHFR, their effects, if present, would likely be related to an interaction with another site(s).

A role for 7-OH-MTX polyglutamyl derivatives in 7-OH-MTX cytotoxicity is further supported by the observation that continuous exposure to GAT is required to protect cells from even a short pulse of 7-OH-MTX. This is a phenomenon also observed after exposure of cells to MTX and is attributed to the build-up of polyglutamyl derivatives which are retained in cells after the monoglutamate has been removed, to produce a sustained pharmacological effect (1, 12, 13, 16, 17, 27, 36, 37).

This proposed increased binding of the tetraglutamyl derivative of 7-OH-MTX to DHFR, as compared to the monoglutamate in this and the previous study with Ehrlich cells (9), is consistent with the observation that polyglutamyl derivatives of a variety of natural folates and MTX (26) have increased affinity for folate-dependent enzymes (5, 32) but is unlike the observation that polyglutamyl derivatives of MTX have affinities for DHFR comparable to that of the monoglutamate (19).

Earlier studies suggested that 7-OH-MTX could have a role in modulating MTX action by its inhibitory effects on MTX transport and net cellular MTX accumulation, and on this basis, as well as possible direct competition at the level of the polyglutamate synthetase, could reduce the formation of active MTX polyglutamyl derivatives. These studies suggest, in addition, a possible direct cytotoxic role for 7-OH-MTX polyglutamyl derivatives that are formed within cells particularly after high-dose MTX protocols, when high levels of 7-OH-MTX are generated, and high plasma ratios of 7-OH-MTX to MTX are sustained for long intervals.

REFERENCES


Formation of 7-Hydroxymethotrexate Polyglutamyl Derivatives and Their Cytotoxicity in Human Chronic Myelogenous Leukemia Cells, in Vitro

Gerard Fabre and I. David Goldman


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/45/1/80

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.