The Effects of Antibiotics and Cancer Chemotherapeutic Agents on the Cellular Transport and Antitumor Activity of Methotrexate in L1210 Murine Leukemia

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SUMMARY

The effect of the simultaneous addition of a number of clinically useful antibiotics and cancer chemotherapeutic agents on the transport of $2 \times 10^{-6}$ M methotrexate (4-amino-10-methylpteroyl-glutamic acid; MTX) was studied in L1210 murine leukemia cells in vitro. The results were correlated with the effects of these agents on the antitumor activity of MTX in BALB/cAnN x C57BL/KalWxF1 mice bearing L1210 leukemia cells using optimal dose levels and schedules.

Utilizing doses that are estimates of achievable peak blood levels in man after conventional single i.v. doses, corticosteroids, cephalothin, L-asparaginase, and the Vinca alkaloids showed the greatest effects on MTX uptake by L1210 leukemia cells, while hydroxyurea, bleomycin, penicillin, and kanamycin appeared to have lesser but significant activities. For example, hydrocortisone at $10^{-4}$ and $10^{-3}$ M inhibited MTX transport 20 and 65%, respectively. Competitive and dose-related inhibition was clearly evident for this agent. Vincristine and vinblastine at $10^{-5}$ M uniquely increased MTX transport by 66 and 49%, respectively, with the effect of vincristine still being significant at $10^{-9}$ M. Efflux of MTX-$^3$H from preloaded L1210 leukemia cells was not significantly altered suggesting little effect by any of these agents on intracellular binding of MTX to dihydrofolate reductase enzyme.

The transport inhibitors, hydrocortisone and L-asparaginase, antagonized the antitumor activity of MTX in L1210 leukemia-bearing mice. When hydrocortisone (175 mg/kg i.p. every 4 days, five times) preceded MTX (25 mg/kg i.p. every 4 days, five times) treatment by 180 min, the mean survival time was decreased by 7 days. Conversely, when treatment with the transport enhancer vincristine (1.5 mg/kg i.p. every 4 days, four times) preceded MTX (25 mg/kg i.p. every 4 days, four times) by 30 min, the mean survival time was increased from 23 to 43 days with 30% cures. The time course effects (interval between drug and MTX doses) appear to implicate other factors in addition to transport effects in the antagonism or enhancement of MTX antitumor activity in vivo. These studies suggest that the concurrent use of cancer chemotherapeutic agents and antibiotics may significantly alter cellular transport and ultimately antitumor activity of MTX.

INTRODUCTION

MTX$^2$ is widely used for the treatment of malignant tumors in man (1, 6, 10, 16, 17). However, with the exception of choriocarcinoma, relatively low response rates of most malignant neoplasms to MTX as a single agent in chemotherapy continues to be somewhat disappointing. Prompted by the successful results of combination chemotherapy in acute leukemia and the lymphomas (4, 9, 12), MTX is now being used in combination with many other cancer chemotherapeutic agents for the treatment of other human malignant neoplasms. Patients being treated with MTX often receive many other pharmacologically active compounds concomitantly. Little work has been done to determine what effect, if any, these other agents have on the transport of MTX into tumor cells and ultimately on its antitumor effect in vivo.

To examine this area, a series of experiments was performed to evaluate the effects of certain commonly administered "drugs" on MTX transport in the L1210 leukemia system. These studies have shown that certain chemotherapeutic agents and antibiotics can affect MTX transport and possibly MTX antitumor effect in this system.

MATERIALS AND METHODS

The methodology used in these experiments has been described in detail previously by Goldman et al. (8). It is reviewed only in outline form except where modifications have been made or where new methods have been used.

Cells, Media, and Incubation Techniques. L1210 leukemia cells were grown in spinner culture containing 10% fetal calf serum (RPMI 1630 media). For experiments, cells in log phase growth were prepared as previously described (8). Cells were suspended in a buffer solution (124 mM NaCl, 16 mM NaHCO$_3$, 4 mM KCl, 1.1 mM K$_2$HPO$_4$, 1.9 mM CaCl$_2$, and 1.0 mM MgCl$_2$) at pH 7.4 and placed in a glass incubation flask that was suspended in a water bath at 37$^\circ$ and continuously agitated by a mechanical stirrer. A stream of warmed humidified 95% O$_2$-5% CO$_2$ was passed over the incubation mixture.

Measurement of MTX Uptakes. For experiments involv-
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RESULTS

Effect of “Drugs” on MTX-3H Uptake by L1210 Leukemia Cells in Vitro. The “drugs” evaluated for their effect on MTX transport in L1210 leukemia cells are listed in Table 1. Drug concentrations are estimates of achievable plasma levels in man following conventional single i.v. doses. Corticosteroids, cephalothin, l-asparaginase, and the Vinca alkaloids showed the greatest effects, and they are discussed in more detail in subsequent sections. Other “drugs” that appeared to have a significant effect on MTX transport include hydroxyurea and bleomycin in the chemotherapeutic category and penicillin and kanamycin in the antibiotic group. The Vinca alkaloids were particularly interesting because of their unique ability to enhance MTX transport.

Inhibitors of MTX Transport in L1210 Leukemia Cells in Vitro. Cephalothin inhibited the transport of MTX into L1210 leukemia cells in vitro. Table 2 shows the dose-response of this effect. At 10^-3 M (418 µg/ml) inhibition was 46.5% of control uptake at 40 min of incubation. At 10^-4 M (4.18 µg/ml) little if any effect on MTX transport was seen.

Of the “drugs” evaluated, hydrocortisone was the most effective inhibitor of MTX transport in the L1210 system. At 10^-4 M (3.6 µg/ml) inhibition was 19.6% of control and at 10^-3 M (360 µg/ml) the inhibition reached 62.8%. The time course of this inhibition and its dose-response characteristics for hydrocortisone (sodium succinate) are shown in Chart 1. The same results were seen for the free base of hydrocortisone. As the concentration of hydrocortisone increased from 10^-4 to 10^-3 M progressively more inhibition of MTX transport was seen. Saturation of the intracellular DHFR binding sites with MTX (intracellular MTX level of approximately 5 to 6 nmoles/g dry cell weight) was achieved within 2 min in the control experiment (8). This time is gradually prolonged by the inhibitory action of hydrocortisone so that at a concentration of 10^-3 M saturation takes approximately 40 min. Experiments evaluating the effect of hydrocortisone on MTX transport during the period of unidirectional influx, before DHFR is saturated, clearly show that hydrocortisone can inhibit the unidirectional transport of MTX.

Methylprednisolone was also an inhibitor of MTX transport but it was less effective than hydrocortisone at equimolar concentrations. Dexamethasone was not an inhibitor in this system.

As shown in Table 2, l-asparaginase is a relatively weak inhibitor of MTX transport in L1210 leukemia cells. The L1210 leukemia used in these experiments showed no antitumor response to l-asparaginase therapy.

Enhancement of MTX Transport in L1210 Cells in Vitro. The effects of the Vinca alkaloids on MTX transport in L1210 cells were unique for the “drugs” examined in this study. VCR and VBL markedly increased the uptake of MTX in vitro to above control values. The dose-response of this effect can be seen in Table 2. At equimolar concentrations VCR is a more effective enhancer of MTX transport than is VBL. The VCR effect is still noted at 10^-3 M (0.00083 µg/ml). The enhancing activity of the Vinca alkaloids was observed at substantially lower concentrations than the previously discussed inhibitory effects of cephalothin, hydrocortisone, and l-asparaginase. However, this fact must be evaluated in light of the clinical fact that Vinca alkaloids are routinely administered in significantly smaller doses than are the above-mentioned inhibitors.

Further experiments have revealed that preincubation of...
Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug concentration (µg/ml)</th>
<th>% Δ in uptake vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>36</td>
<td>-35 ± 0.4</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>250</td>
<td>-30 ± 1.4</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>75</td>
<td>-25 ± 0.5</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>200</td>
<td>-16 ± 1.1</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>50</td>
<td>-15 ± 1.7</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>37.5</td>
<td>-14 ± 1.1</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>2.5</td>
<td>-12 ± 1.9</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>35</td>
<td>-12 ± 1.5</td>
</tr>
<tr>
<td>5-Mercaptopurine</td>
<td>30</td>
<td>-8 ± 1.5</td>
</tr>
<tr>
<td>Neomycin</td>
<td>35</td>
<td>-7 ± 1.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>30</td>
<td>-4 ± 1.3</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>10</td>
<td>-3 ± 1.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>40</td>
<td>-3 ± 1.2</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>35</td>
<td>-3 ± 1.0</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>1.0</td>
<td>-3 ± 0.8</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>0.1</td>
<td>-1 ± 1.2</td>
</tr>
<tr>
<td>Cytoxan</td>
<td>150</td>
<td>-1 ± 1.2</td>
</tr>
<tr>
<td>Chloretocycline</td>
<td>2.5</td>
<td>+1 ± 0.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>15</td>
<td>+2 ± 1.5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>25</td>
<td>+2 ± 0.9</td>
</tr>
<tr>
<td>Cytosine arabinoside</td>
<td>23</td>
<td>+4 ± 1.6</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>20</td>
<td>+5 ± 0.8</td>
</tr>
<tr>
<td>VBL</td>
<td>9.12</td>
<td>+49 ± 0.9</td>
</tr>
<tr>
<td>VCR</td>
<td>8.25</td>
<td>+66 ± 0.9</td>
</tr>
</tbody>
</table>

“Drug concentrations are estimates of achievable plasma levels in man following conventional single i.v. doses.

*Negative values reflect inhibition of uptake. Positive values reflect enhancement of uptake. All values mean ± S.E. Values represent the mean of 3 to 4 separate experiments.

Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (moles/liter)</th>
<th>% inhibition of MTX-3H uptake</th>
<th>% enhancement of MTX-3H uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>1 x 10^-8</td>
<td>62.8 ± 1.6</td>
<td>93.7 ± 3.4</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>1 x 10^-3</td>
<td>46.5 ± 1.6</td>
<td>65.6 ± 1.7</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>1 x 10^-8</td>
<td>45.2 ± 1.6</td>
<td>65.6 ± 1.7</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>2000</td>
<td>28.0 ± 1.1</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

*All experiments performed with 2 x 10^-8 M MTX and for 40 min of incubation at 37°.

**Table 1**: Effect of chemotherapeutic agents and antibiotics on MTX-3H uptake in L1210 leukemia cells in vitro.

**Table 2**: Effect of "drugs" on MTX-3H uptake in L1210 leukemia cells in vitro.

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*Uptakes were done with MTX-3H at 2.0 µM with an incubation of 40 min at pH 7.4 and 37°.

Drug concentrations are estimates of achievable plasma levels in man following conventional single i.v. doses.

*Negative values reflect inhibition of uptake. Positive values reflect enhancement of uptake. All values mean ± S.E. Values represent the mean of 3 to 4 separate experiments.

Addition of "Drug" to Bath during Uptake in Vitro. Chart 3 demonstrates the effect of inhibitors and enhancers on the form of the control uptake curve when the "drugs" were added to the bath 20 min after initiation of the uptake process. It was seen that the inhibitors caused a fall in the intracellular MTX level in proportion to their previously discussed potency as inhibitors of MTX transport in vitro. For example, addition of hydrocortisone, the most potent inhibitor of MTX transport examined, resulted in the efflux of all the "free" MTX in the L1210 cell, leaving behind only that MTX that is presumably bound to DHFR. L-Asparagi...
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levels of this agent. Also, transport inhibitors can cause loss of cellular MTX from cells having intracellular levels greater than that which is presumably bound to DHFR.

Kinetics of Transport Inhibition. When the transport inhibitor hydrocortisone was present at constant external concentrations and MTX was added over a range of concentrations, the data can be plotted as the reciprocal of the initial uptake velocity as a function of the reciprocal of the concentration of MTX (see Chart 4). It was seen that the line for the inhibitor showed increased slope when compared to the control line and that the ordinate intercept was not significantly changed, indicating competitive inhibition. These experiments always measured unidirectional flux since the level of intracellular MTX never exceeded the level of DHFR in the cell.

Effect of "Drugs" on MTX Antitumor Activity in Vivo. The effects of previously discussed inhibitors and enhancers of MTX transport on the antitumor activity of MTX in the L1210 murine leukemia were evaluated. Table 3 shows the effect of pretreating the L1210 leukemia-bearing mice with hydrocortisone i.p. prior to each dose of MTX. Hydrocorti-

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nase, a relatively weak inhibitor of MTX transport, caused only a slight decrease in the level of intracellular MTX. VCR, an enhancer of MTX transport, caused a rapid rise in the intracellular MTX to a level greater than twice control values. From this experiment it was seen that VCR (and VBL) has the potential to increase the level of free intracellular MTX in cells that already have substantial

Chart 2. Effect of "drugs" on MTX efflux in L1210 leukemia cells in vitro. Variations of initial MTX level is noted because experiments were done on different days with variations in the level of nonexchangeable MTX. In all experiments, L1210 cells were preloaded for 20 min at 37° with an extracellular concentration of MTX of $2 \times 10^{-6}$ M. Each curve represents the average of 3 experiments.

Chart 3. Effect of "drugs" on MTX transport in L1210 leukemia cells in vitro. Drugs were added to bath 20 min after initiation of the MTX uptake process. Each curve represents the average of 3 experiments in which the extracellular MTX was $2 \times 10^{-6}$ M.

Chart 4. The effect of hydrocortisone on the unidirectional influx of MTX in L1210 leukemia cells. Each point represents 3 experiments. At each concentration of MTX only unidirectional flux was measured because the maximum MTX concentration did not exceed the DHFR binding capacity.
Table 3

Effect of hydrocortisone (sodium succinate) and VCR sulfate on the survival of L1210-bearing CDF+ mice treated with MTX

Mice were inoculated on Day 0 with 10^6 L1210 cells i.p. Hydrocortisone or VCR was always given prior to MTX by stated interval. The difference in mean survival between 60- and 180-min experiments (hydrocortisone) is significant (p < 0.01). Ten mice were used in each group.

<table>
<thead>
<tr>
<th>MTX treatment</th>
<th>“Drug” treatment</th>
<th>Interval between “drug” and MTX</th>
<th>Days of treatment</th>
<th>Mean survival time (days)</th>
<th>Cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>9.1 ± 0.1*</td>
<td>0</td>
</tr>
<tr>
<td>25 mg/kg i.p., 5 times</td>
<td>Hydrocortisone (175 mg/kg) i.p., 5x</td>
<td>60 min</td>
<td>2, 6, 10, 14, 18</td>
<td>25.2 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>25 mg/kg i.p., 5 times</td>
<td>Hydrocortisone (175 mg/kg) i.p., 5x</td>
<td>180 min</td>
<td>2, 6, 10, 14, 18</td>
<td>18.3 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>25 mg/kg i.p., 4 times</td>
<td>VCR (1.5 mg/kg) i.p., 4x</td>
<td>2</td>
<td>2, 6, 10, 14</td>
<td>12.0 ± 0.0</td>
<td>0</td>
</tr>
<tr>
<td>25 mg/kg i.p., 4 times</td>
<td>VCR (0.5 mg/kg) i.p., 4x</td>
<td>30 min</td>
<td>2, 6, 10, 14</td>
<td>43.3 ± 1.9</td>
<td>3/9</td>
</tr>
<tr>
<td>25 mg/kg i.p., 4 times</td>
<td>VCR (1.5 mg/kg) i.p., 4x</td>
<td>30 min</td>
<td>2, 6, 10, 14</td>
<td>24.0 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>25 mg/kg i.p., 4 times</td>
<td>VCR (1.5 mg/kg) i.p., 4x</td>
<td>24 hr</td>
<td>2, 6, 10, 14</td>
<td>33.7 ± 0.8</td>
<td>1/7</td>
</tr>
</tbody>
</table>

*Cures = Number of long-term survivors/Number inoculated. Cures are defined as animals surviving greater than 90 days.

*Mean ± S.E.

Hydrocortisone alone (175 mg/kg, 5 times) did not prolong survival beyond control mice with tumor. The control mean survival of mice receiving only MTX at 25 mg/kg every 4 days for 5 doses was 25.2 days. When hydrocortisone (175 mg/kg) preceded MTX, by 60 min, no change in survival was noted. However, when treatment with MTX and hydrocortisone was separated by 180 min, the mean survival time decreased by 7 days. The difference between mean survival for 180 min and the MTX control was statistically significant (p < 0.01).

The Vinca alkaloids are unique in their ability to enhance transport of MTX in the L1210 leukemia cell in vitro. In vivo experiments to evaluate the effect of VCR on MTX antitumor activity can also be seen in Table 3. Control survival of 9.1 days was prolonged only slightly by VCR at 1.5 mg/kg given i.p. on Day 1 or 0.5 mg/kg i.p. every 4 days, 4 times. MTX treatment with 25 mg/kg every 4 days, 4 times, increased mean survival to 23.5 days and resulted in no long-term survivors (defined as animals surviving greater than 90 days). VCR given at 0.5 mg/kg i.p. 30 min prior to MTX markedly increased mean survival to 43.3 days with 30% cures. Even giving the VCR as a single dose (1.5 mg/kg) 24 hr before the 1st dose of MTX increased survival to 33.7 days with approximately 15% cures. However, when a single dose of 1.5 mg of VCR per kg was given 30 min prior to MTX (25 mg/kg every 4 days, 4 times) on Day 2 only, no increase in survival was noted.

Additional in vivo experiments involved determining the number of viable ascites cells per mouse following i.p. L1210 tumor inoculation. The results of these quantitative ascites experiments can be seen in Chart 5 for L-asparaginase or VCR given prior to MTX. Tumor cells were given i.p. on Day 0 and MTX was given i.p. on Day 3.

L-Asparaginase (3500 units/kg) or VCR (1.5 mg/kg), when given alone to L1210-bearing mice, resulted in no change in the number of ascites cells per mouse when compared to control mice inoculated with tumor cells. When given 24 hr before MTX, VCR (1.5 mg/kg) caused a substantial curve shift to the right of the L1210 plus MTX curve illustrating its synergistic effect with MTX in the L1210 tumor system. Giving the VCR only 30 min before MTX resulted in a reduced synergistic effect.

Giving the L-asparaginase (3500 units/kg) i.p. 30 min before MTX resulted in slight antagonism of MTX antitumor activity. However, when the interval between MTX and L-asparaginase was extended to 3 hr (Chart 5) more marked antagonism was seen. Quantitative ascites experiments with 175 mg of hydrocortisone per kg given 30 min prior to 2 mg of MTX per kg resulted in slight antagonism of MTX antitumor activity (not shown in Chart 5).

The survival data presented and the killing effect on the number of ascites cells per mouse seem to support the conclusions that MTX transport inhibitors can antagonize the antitumor activity of MTX in the L1210 leukemia system and the Vinca alkaloids, transport enhancers, can act synergistically with MTX to improve its antitumor activity. However, the time course of effects in these experiments indicates that factors other than transport effects may be involved in producing changes in MTX antitumor activity.
Chart 5. Effect of L-asparaginase and VCR on MTX inhibition of L1210 murine leukemia cell proliferation in vivo. Each point represents the average of 4 mice. Doses: MTX, 15 mg/kg; VCR, 1.5 mg/kg, and L-asparaginase, 3500 units/kg.

DISCUSSION

MTX transport in the L1210 murine leukemia cell has been shown to be an active, carrier-mediated uphill process (8). Studies have shown that MTX tightly binds DHFR, with depletion of folate coenzymes (14, 15), cessation of thymidylate synthesis (2, 20), and ultimately disruption of DNA synthesis. Clearly, MTX must enter the cell to have an antitumor effect. In support of this fact, it has been shown that inability to transport MTX into a cell, in certain animal tumor systems, is associated with resistance to this agent (5, 18). This information has prompted our examination of the effects of other "drugs" on MTX transport.

Drug-drug interactions have become increasingly important factors affecting the therapeutic response of a variety of diseases to drug therapy. Such examples as the antagonism between barbiturates and the anticoagulant effects of the warfarin compounds and the effect of allopurinol on 6-mercaptopurine metabolism are recognized in the daily use of these agents. For MTX, recent work has suggested that drug-drug interactions may affect its antitumor activity. Capizzi et al. (3) reported the antagonism of the antineoplastic effect of MTX by L-asparaginase in the L5178Y murine leukemia system both in vitro and in vivo. Most recently, working with the L5178Y murine leukemia system, Nahas and Capizzi (13) have shown a marked decrease in cellular uptake of MTX in vitro. This inhibition is dose related and competitive for hydrocortisone. In addition, for hydrocortisone and L-asparaginase, antagonism of MTX antitumor activity has been demonstrated in vivo.

The relationship between transport inhibition and antagonism of MTX antineoplastic activity by hydrocortisone in the L1210 system is yet to be fully explained. Since hydrocortisone does not prevent the eventual saturation of the DHFR binding sites in the L1210 cells, it would appear that its ability to antagonize the antineoplastic activity of MTX would be minimal. This conclusion is supported by the quantitative ascites data. However, in vivo survival experiments, using multiple doses of hydrocortisone and MTX, showed much greater antagonism. This suggests that, although transport inhibition may play a role in the antagonism of MTX antineoplastic activity, other factors are probably of greater importance.

The potency of L-asparaginase as a transport inhibitor does not reflect its antagonistic potential. Although it is a relatively weak transport inhibitor in the L1210 system, quantitative ascites experiments demonstrated substantial antagonism of MTX antitumor activity when the MTX was administered 3 hr after L-asparaginase. Lieberman et al. (11) has shown that inhibition of protein synthesis can protect against cell death induced by 1-β-D-arabinofuranosylcytosine, a cell-cycle-specific agent similar to MTX. Since L-asparaginase is a known inhibitor of protein synthesis and an interval of approximately 3 hr seems necessary to demonstrate maximum antagonism, protein synthesis inhibition may be the mechanism of antagonism of MTX antitumor activity. In the L1210 system, cephalothin, hydrocortisone, and L-asparaginase appear capable of interfering with MTX antitumor activity. The clinical significance of these observations for MTX chemotherapy in man is yet to be determined.

Vinca alkaloids uniquely enhance MTX transport in the L1210 leukemia system. L1210 cells preincubated with VCR or VBL showed enhanced MTX transport in vitro. This effect was seen with as brief as 1 min of preincubation and repeated washing after incubation does not prevent enhanced transport. This transport enhancement results in substantially higher levels of "free" MTX, not bound to DHFR, within the L1210 cell. The role of this fraction of the MTX in antitumor activity in the L1210 system has not been established. While VCR, at clinically achievable levels
in man, acts synergistically with MTX in L1210 murine leukemia in vivo, this phenomenon is schedule dependent and may involve interrelated factors other than drug transport such as cell-cycle kinetics (19) and the duration of effective drug levels. This was apparent when no increased survival was noted in L1210 leukemia-bearing mice given VCR (1.5 mg/kg) 30 min prior to MTX (25 mg/kg every 4 days, 4 times) on Day 2 only. This suggests that the enhancement of transport of MTX occurring on Day 2 only is not sufficient to give increased survival.

This study has described several commonly used "drugs" with an inhibitory effect on MTX transport in the L1210 leukemia system. We have also shown that they possess the ability to antagonize the antitumor activity of MTX in vivo. However, the relationship between these 2 phenomena is not established and further work, particularly in human malignant cell lines, is needed before the possible clinical significance of these interactions can be fully appreciated.

The enhancing effect of the Vinca alkaloids on MTX transport and antitumor activity in the L1210 system may be of clinical significance in the concomitant use of drugs in cancer chemotherapy. It is envisioned that enhanced therapeutic effect of MTX in certain tumor systems might be achieved through a drug administration schedule that takes advantage of the transport-enhancing effect. In particular, this technique might be useful in the therapy of malignant cell lines resistant by virtue of diminished MTX transport. Appropriate drug scheduling may take advantage of the transport-enhancing effect and the cell synchronization effect of the Vinca alkaloids to obtain maximum therapeutic effect from MTX.

REFERENCES


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