Characteristics of the Vincristine-induced Augmentation of Methotrexate Uptake in Ehrlich Ascites Tumor Cells*

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SUMMARY

Studies were undertaken to characterize the effect of vincristine on methotrexate uptake in Ehrlich ascites tumor cells and to define the mechanism of this interaction. Vincristine (10 μM) does not alter the unidirectional influx of methotrexate but slows the unidirectional efflux of methotrexate and leads to a large increase in the steady state level of exchangeable intracellular methotrexate. These effects of vincristine are rapid, but not completely, reversible. Vincristine stimulation of net methotrexate uptake occurs within 1 min of exposure of cells to this agent and increases over an extracellular vincristine concentration range of at least 5 to 50 μM. Vincristine does not alter the intracellular water content nor the chloride distribution ratio. The addition of glucose to the medium partially reverses the effect of vincristine and completely reverses the stimulatory effect of sodium azide on the net uptake of methotrexate. Vincristine (10 μM) increases net uptake of methotrexate in L1210 leukemia cells but does not have a significant effect on net methotrexate uptake in the L-cell mouse fibroblast over a 2½-hour interval of exposure even though replication of the L-cell is completely arrested.

The similarity between the effects of vincristine and metabolic poisons on methotrexate uptake and the partial reversal of the effect of vincristine by glucose suggest that this agent enhances methotrexate uptake by the inhibition of cellular energy metabolism with the consequent inhibition of an energy-dependent process which limits methotrexate accumulation within the cell. The data suggest further that this effect of vincristine represents an interaction with a cellular element(s) that is different from the interaction which results in the arrest of cell division in metaphase.

Studies from this laboratory have described a carrier-mediated transport process for the naturally occurring folates and their synthetic analogue, methotrexate,1 in several mammalian cells including the L1210 leukemia (1), Ehrlich ascites tumor (2, 3) rabbit reticulocyte (4), and the L-cell mouse fibroblast.2 Unique to this transport system inhibitors of anaerobic and aerobic metabolism enhance rather than inhibit the net uptake of methotrexate into these cells (5). In L1210 leukemia cells sodium azide produced a small increase in the unidirectional influx of methotrexate, a large fall in the unidirectional efflux of exchangeable intracellular methotrexate with a resultant increase in the apparent intracellular electrochemical potential for methotrexate to a level considerably greater than that of the extracellular compartment. The data suggested that metabolic poisons inhibit an energy-dependent process which drives methotrexate out of the cell (5) and a more extensive model was subsequently proposed to account for the over-all energetics of methotrexate transport (2). Recently, another laboratory reported that vincristine increases net uptake of methotrexate into the L1210 leukemia cell (6). The present study characterizes the mechanism by which vincristine augments methotrexate uptake in the Ehrlich ascites tumor cell and suggests that vincristine acts as a metabolic poison to inhibit an energy-dependent process which limits the intracellular accumulation of methotrexate. These findings relate to other studies from this laboratory which suggest that the augmented net uptake of methotrexate induced by vincristine is associated with an increase in the inhibition of DNA synthesis by methotrexate (7).

EXPERIMENTAL PROCEDURES

Cells and Media—Cells employed in these studies were: (a) the Ehrlich ascites tumor grown in CF1 mice; (b) Earles L-cell mouse fibroblasts maintained in cell culture in Y.L.E.2 medium supplemented with 5% calf serum; and (c) L1210 leukemia cells grown in R.P.M.I. 1630 medium (8) supplemented with 5% fetal calf serum (sera obtained from Grand Island Biologicals, Grand Island, N.Y.). Cells in culture were grown in spinner flasks and were washed twice with 0° buffer prior to experimentation.

† The trivial name used is: methotrexate, 4-amino-10-methylpteroylglutamic acid.
‡ I. D. Goldman, unpublished observations.
§ Yeast lactalbumin extract; yeast extract (Difco, Detroit, Mich.), 1 g per liter; lactalbumin hydrolysate (Nutritional Biochemical Corp., Cleveland, Ohio), 5 g per liter; NaCl, 0.8 g per liter; KCl, 0.4 g per liter; MgSO4, 0.2 g per liter; CaCl2, 2.0 g per liter; NaH2PO4, 1.5 g per liter; NaHCO3, 1.1 g per liter; glucose, 4.5 g per liter; neomycin sulfate, 5 mg per liter; phenol red, 10 mg per liter.
Ehrlich ascites tumor cells were passed by weekly intraperitoneal inoculation of 0.2 ml of undiluted ascitic fluid. Mice were killed 6 to 12 days after inoculation, and the ascitic fluid was suspended into 0° buffer. Contaminating erythrocytes were removed in the supernatant fluid after two to three washes with 0° buffer and separation of the tumor cell pellet was achieved by centrifugation for 1 min at 250 × g.

The buffer employed in these studies consisted of: 135 mM NaCl, 16 mM NaHCO3, 1 mM Na2HPO4, 4 mM KCl, 1.9 mM CaCl2, 1.0 mM MgCl2. The pH was maintained at 7.3 to 7.4 during incubations by passing warmed and humidified 95% O2-5% CO2 over the cell suspensions. Cytocrits were under 3% and the temperature was 37°.

Determination of Methotrexate Transport Kinetics and Intracellular Methotrexate Level—The unidirectional influx, efflux, and net uptake of [3H]methotrexate were measured as previously described (1, 5). Pertinent aspects of the experimental protocols are indicated in the legends. Flux reactions were halted by injection of the cell suspension into 10 volumes of 0° buffered 0.85% NaCl solution (pH 7.4) and the intracellular methotrexate level was determined as follows. The cell fraction was separated by centrifugation (2000 × g for 30 to 60 s) and washed twice with the 0° saline solution. The washed cell pellet was aspirated into the tip of a Pasteur pipette, extruded onto a polyethylene tare, and dried overnight at 60°. The dried cells were then peeled off the polyethylene tare. Pellets under 1 mg were weighed on the Cahn G-2 electrobalance (Cahn Instruments, Paramount, Calif.) and the other pellets which weighed in the range of 1 to 5 mg were measured on the Beckman LM 800 automatic microbalance (Beckman Instruments, Fullerton, Calif.). The dried pellets were placed in scintillation vials which were tilted at 45° in specially constructed racks so that the pellet would be totally encompassed by the subsequent addition of 0.1 ml of 1 n KOH. The cells were digested for 1 hour at 60° and cooled, 10 ml of scintillation mixture (1) was added, and radioactivity was determined with a Beckman LRI 800 automatic liquid scintillation spectrometer. All quench corrections were made employing [3H]- or [14C]toluene internal standards.

Determination of Intracellular Water and Chloride Distribution Ratio—Intracellular water was determined from the difference between the wet weight and dry weight of a cell pellet less the [14C]inulin space. This procedure as well as the determination of the chloride distribution ratio (the ratio of the concentration of chloride in the intracellular water to its concentration in the extracellular water) has been described in detail (1, 4). Chloride was measured on the Buchler-Cotlove chloridimeter (Buchler Instruments, Inc., Fort Lee, N.J.).

Purification of Methotrexate and Identification of Intracellular Radiolabel—[3H, 5'-3H]Methotrexate (Amersham-Searle, Arlington Heights, Ill.) and nonlabeled methotrexate (American Cyanimid Co., Lederle Laboratories Division, Pearl River, N.Y.) were purified by DEAE-cellulose-ion exchange column chromatography. The column was eluted with a linear gradient of 0.1 to 0.4 M ammonium bicarbonate at pH 8.3 with subsequent lyophilization of the methotrexate-containing fractions. For the identification of intracellular radiolabel, cells were incubated with radiolabeled methotrexate, then disrupted by sonic oscillation, and after a 9,000 × g centrifugation for 20 min at 4°, the supernatant was fractionated on a DEAE-cellulose column. These procedures have been published in detail (1).

Chemicals—Vincristine and vinblastine sulfate were supplied through the generosity of Dr. Robert Hosley of the Eli Lilly Research Laboratories, Indianapolis, Ind. Sodium azide was obtained from Fisher Chemicals. [COOH-14C]Inulin was obtained from Amersham-Searle.

RESULTS

Effect of Vincristine on Unidirectional Influx and Time Course of Methotrexate Uptake—Exposure of Ehrlich ascites tumor cells to 10 μM vincristine a few seconds prior to the addition of methotrexate results in a large increase in the steady state intracellular methotrexate level but the unidirectional influx of methotrexate is unchanged (Fig. 1). To assess the duration of the interval between addition of vincristine and the onset of stimulation of net methotrexate uptake, cells were first incubated with methotrexate until a steady state was achieved following which a portion of the cell suspension was exposed to vincristine (Fig. 2). Net methotrexate uptake ensued within 4 min after addition of this agent. Since the stimulatory effect of vincristine becomes manifest only after a 4-min delay, evaluation of a possible effect of this agent on the unidirectional influx of methotrexate required

![Fig. 1](http://www.jbc.org/)

**Fig. 1.** The effect of vincristine (VCR) on the time course of methotrexate (MTX) uptake. Cells were exposed to 10 μM vincristine a few seconds before addition of methotrexate. Extracellular methotrexate was 0.65 μM.

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** The effect of vincristine (VCR) on net uptake of methotrexate (MTX). Cells were brought to the steady state with 0.6 μM methotrexate. At the arrow, a portion of the cell suspension was transferred to another flask containing vincristine to achieve a concentration of 10 μM.
Fig. 3. The effect of vincristine (VCR) or azide on the unidirectional influx of methotrexate (MTX). Cells were incubated with 30 μM vincristine or 10 mM azide, or under control conditions for 8 min. Following this, each of the three suspensions was divided into five equal portions so that subsequent flux determinations could be done in quintuplicate. The cell fractions were isolated by centrifugation and the supernatant fluid was thoroughly aspirated. The tubes were gassed with 95% O₂-5% CO₂, capped, and the tips of the tubes with immersed in ice. For flux determination, the tubes were warmed in a 37°C bath for 20 s following which the cell pellets were dispersed in vincristine, azide or control buffer (respectively) but now containing 1 μM methotrexate and incubation was continued for 200 s. During this interval, methotrexate taken up by the cell did not exceed the intracellular binding capacity and influx of methotrexate was unidirectional (see Refs. 1 and 5). The bars reflect the mean ± S.E. of the average values from five separate experiments done on different days.

monitoring the 200-s influx of methotrexate following a >4-min preincubation with vincristine. In five such experiments (Fig. 3), 30 μM vincristine did not alter the influx of methotrexate while 10 mM sodium azide produced a 23% increase in methotrexate influx under the same conditions.

Effect of Vincristine on Unidirectional Efflux of Methotrexate—Cells were loaded with methotrexate to the steady state in the presence and absence of vincristine, then separated by centrifugation and resuspended into methotrexate-free buffer with or without vincristine, respectively, and the unidirectional efflux of methotrexate was monitored (Fig. 4). Intracellular methotrexate in the control cells consists of two components. There is an intracellular fraction (referred to as "exchangeable") that rapidly leaves the cell and another larger fraction that is bound within the cell. Vincristine may produce a small increase in a bound intracellular methotrexate component, as suggested from additional experiments in which the unidirectional efflux of methotrexate was monitored over longer intervals. The major effect of vincristine is a marked fall in the rate of exit of the exchangeable methotrexate fraction with a 2.8-fold increase in the efflux half-time.

Effect of Vincristine on Intracellular Water, Chloride Distribution Ratio, and Estimated Electrochemical Potential for Intracellular Methotrexate—While 10 μM vincristine has a profound effect on methotrexate uptake, it has little effect on other physical properties of the cells under these experimental conditions (Table I). Vincristine does not alter the ratio of the intracellular water to dry weight or the ratio of the extracellular water to wet weight. The dry weight to wet weight ratio of a cell pellet is unchanged. Vincristine does not produce significant change in the chloride distribution ratio, suggesting that this agent does not alter the membrane potential under these experimental conditions. Table II represents an analysis of the data of Fig. 4 to estimate the effect of vincristine on the steady state electrochemical potential for intracellular methotrexate. Assuming that the total intracellular methotrexate level less the bound fraction represents osmotically active intracellular methotrexate, it may be seen that this component of intracellular methotrexate is doubled by vincristine. Further, while the distribution ratio for methotrexate in the absence of vincristine was only 29% more than that ex-
Reversibility of Vincristine Effect—The reversibility of the effect of vincristine on methotrexate uptake was evaluated in terms of the alterations produced by this agent on the net uptake and the unidirectional efflux process. In the experiment of Fig. 6, cells were exposed to vincristine for 8 min and then were divided into two portions. The cell fractions were separated by centrifugation, one portion was suspended into vincristine-free buffer while the other was resuspended into buffer containing this agent. The time course of methotrexate uptake was then monitored in these cells as well as control cells which had not been exposed to vincristine. Stimulation of net methotrexate uptake by vincristine was markedly diminished after the agent was removed from the buffer, although a small increase in the net uptake of methotrexate did persist under these conditions.

Fig. 7 illustrates the reversibility of the vincristine effect on the unidirectional efflux of methotrexate. Cells were incubated with methotrexate in the presence and absence of 10 μM vincristine for 20 min. The vincristine-exposed cells were divided into two portions and the cell fractions were separated by centrifugation and then resuspended in the presence or absence of 10 μM vincristine. Both, as well as control cells, were resuspended into methotrexate-free buffer. The unidirectional efflux of exchangeable methotrexate was increased in the cells resuspended into vincristine-free buffer in comparison to the cells exposed to vincristine in the resuspension buffer but the efflux half-time remained higher, at least initially, than that of the control cells. The tightly bound intracellular methotrexate level reverted to that of the control upon exposure to the vincristine-free buffer.

Effect of Glucose on Stimulation of Methotrexate Uptake by Vincristine or Azide—Glucose alone produces a small, but significant, (p > 0.05 from five experiments) depression of net methotrexate uptake (Fig. 8). Sodium azide (10 mM) markedly enhances the net uptake of methotrexate but this effect is completely reversed by glucose. Unlike azide, the stimulatory effect of vincristine on net methotrexate uptake is only partially reversed by glucose; however, it is clear that the inhibitory effect of glucose on net methotrexate uptake is not reversed by vincristine.

### Table II

|                          | Control | Vincristine |
|--------------------------|---------|------------|
| Total cell MTX (nmoles/g dry wt) | 4.33    | 6.65       |
| Bound cell MTX (nmoles/g dry wt) | 2.78    | 3.33       |
| Exchangeable cell MTX (nmoles/g dry wt) | 1.55    | 3.32       |
| [MTX]i/[MTX]e (measured)     | 0.398   | 0.869      |
| [MTX]i/[MTX]e (expected)  | 0.558   | 1.17       |
| [MTX]i/[MTX]e (measured/expected) | 0.417  | 0.417      |

*a This analysis is derived from the data of Fig. 4. Methotrexate is abbreviated as MTX.
*b Determined by dividing [H2O]/DW, from Table I into “exchangeable cell MTX.”
*c The “expected” distribution ratio for MTX is derived from the Nernst equation (9) and refers to the distribution ratio for MTX (a bivalent anion) that would be expected if the transport system were passive (nonenergy-dependent). This calculation is based upon the reported membrane potential of 11 mv for Ehrlich ascites tumor cells (10). If the membrane potential were greater, as suggested in a recent study (11), the expected distribution ratio for MTX would be even lower and the electrochemical potential for methotrexate would therefore be even higher. [MTX]i/[MTX]e is the distribution ratio for MTX and refers to the ratio of the concentration of exchangeable MTX in the intracellular to extracellular water, respectively.

### Fig. 5
The steady state intracellular methotrexate (MTX) level as a function of the extracellular vincristine (VCR) concentration. Cells were incubated with 1.0 μM methotrexate for 20 min following which aliquots of the cell suspension were transferred to other flasks containing vincristine. Incubation was continued until the steady state for methotrexate was achieved following which four measurements of the intracellular methotrexate level were obtained. The bars indicate the mean ± S.E. of four replicate measurements from a representative experiment. The extracellular vincristine levels are indicated below the bars. The difference between control cells and cells exposed to 5 μM vincristine is significant at p < 0.02.

Expected for a passive (nonenergy-dependent) process, the distribution ratio for methotrexate in the presence of vincristine exceeds that predicted for a passive process by a factor of 2.8.

### Fig. 6
Reversibility of the effect of vincristine (VCR) on the net uptake of methotrexate (MTX). Cells were exposed to 10 μM vincristine for 8 min then divided into two portions. The cell fractions were separated by centrifugation, then resuspended into vincristine-containing buffer (•) or vincristine-free buffer (○) with 1 μM methotrexate. Control cells (□) were treated similarly except for the omission of vincristine during the incubation.

### Fig. 7
Illustrates the reversibility of the vincristine effect on the unidirectional efflux of methotrexate. Cells were incubated with methotrexate in the presence and absence of 10 μM vincristine for 20 min. The vincristine-exposed cells were divided into two portions and the cell fractions were separated by centrifugation and then resuspended in the presence or absence of 10 μM vincristine. Both, as well as control cells, were resuspended into methotrexate-free buffer. The unidirectional efflux of exchangeable methotrexate was increased in the cells resuspended into vincristine-free buffer in comparison to the cells exposed to vincristine in the resuspension buffer but the efflux half-time remained higher, at least initially, than that of the control cells. The tightly bound intracellular methotrexate level reverted to that of the control upon exposure to the vincristine-free buffer.
in L-Cell

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sions can be distinguished (p < 0.05 in five experiments). However,
over a 2.5-hour exposure to 10 μM vincristine, changes in net methotrexate uptake are negligible. Ten determinations of the cell methotrexate level were obtained after control and vincristine-exposed L-cells were at the steady state with methotrexate. The averages of these values from five such experiments, done on 5 different days were, in turn, averaged. Vincristine resulted in a 5% increase in the total cell methotrexate level, a value that showed poor statistical significance (p > 0.1) when the differences between the control and the vincristine-treated cells were analyzed by a two-tailed t-test. At higher vincristine levels (30 μM), a significant increase in the cell methotrexate level could be detected. Vincristine at 10 μM produced a prominent stimulation of net methotrexate uptake into the L1210 leukemia cell consistent with a report from another laboratory (6).

Recovery of Intracellular Radiolable in Presence of Vincristine—
To exclude the possibility that vincristine-induced alterations in net uptake of methotrexate are related to chemical modifications of the methotrexate molecule, DEAE-cellulose column chromatography was performed after sonic oscillation of cells exposed to methotrexate for 1 hour in the presence and absence of 10 μM vincristine. In both cases more than 98% of the radiolabel added to the column was recovered in the methotrexate peak.

DISCUSSION

Previous studies from this laboratory demonstrated that the net uptake of methotrexate into many mammalian cells is op-

posed by energy-dependent processes and that, when these proc-

esses are blocked with metabolic poisons, net uptake of metho-

trexate is enhanced (2, 4, 5). This phenomenon has been related more specifically to the energetics of methotrexate transport with the proposal that metabolic poisons inhibit an energy-dependent process which drives methotrexate out of the cell (5). To ac-
count for continued uphill transport of methotrexate into the cell

even in the presence of metabolic poisons, a model was presented

which suggests that the asymmetrical distribution of organic

phosphates across the cell membrane results in a countertransport

in which the downhill flow of organic phosphates out of the cell via

the methotrexate carrier system drives methotrexate uphill into

the cell, a phenomenon which might be relatively unaffected by

the immediate consequences of metabolic poisons (2). These

studies were undertaken to clarify the mechanism by which the

vinca alkaloids enhance net uptake of methotrexate into tumor

cells (6) and to characterize the physical state of the intracellular

methotrexate accumulated in the presence of these agents.

Upon exposure to vincristine, a small tightly bound intracellular

methotrexate component may appear which is rapidly and com-

pletely reversed when vincristine is removed; this is under

further study. The major effect of vincristine is an increase in

the level of exchangeable intracellular methotrexate; this is to a

large extent, but not completely, reversed when vincristine is

removed. These changes occur without an alteration in the

intracellular water content. Since vincristine did not change

the chloride distribution ratio, the data suggest that this agent
did alter the membrane potential under these experimental con-
ditions. The chloride distribution ratio as measured in these

experiments may not reflect the absolute membrane potential

but is employed only to indicate relative changes in membrane

potential (11, 12). If the assumption is made that exchangeable

methotrexate is osmotically active within the intracellular water,

and not in part loosely bound (loose binding is, however, virtually

impossible to exclude), then the data suggest that vincristine

methotrexate uptake in the presence of vincristine is greater than
the inhibitory effect of glucose alone (p < 0.02 from five experimen-
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Effect of Vincristine on Methotrexate Uptake and Cell Replication
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L1210 Leukemia Cells—Following exposure of L-cells in their
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increased the intracellular electrochemical potential for methotrexate.

The effect of vincristine on the interaction between methotrexate and Ehrlich ascites tumor cells is comparable to that observed for azide, an inhibitor of aerobic metabolism, in these cells as well as in the L1210 leukemia cell (5). Both agents depress the unidirectional efflux of methotrexate, increase the exchangeable intracellular methotrexate fraction, and appear to increase uphill transport into the cell. However, while azide produces a small increase in the unidirectional influx of methotrexate, such a change could not be demonstrated for vincristine at levels up to 30 μM. Many structurally diverse compounds with different sites of action, iodoacetate, azide, antimycin A, dinitrophenol, inhibit energy metabolism and enhance net uptake of methotrexate (a similar effect was observed with hypotaurine) (5). The metabolic effects of agents which inhibit aerobic metabolism should be antagonized by glucose as a result of glycolytic production of ATP which compensates for impaired oxidative ATP formation. Accordingly, alterations in the sodium and potassium content of Ehrlich ascites tumor cells induced by antimycin A, and the reduction in the cellular ATP level induced by iodoacetate, azide, or dinitrophenol, are eliminated by glucose (13). Likewise, glucose completely reversed the effect of azide on methotrexate uptake in these studies. Hence, it is of particular interest that glucose partially reversed the vincristine-induced increase in methotrexate uptake. The similarity between the effects of azide and vincristine suggests that vincristine inhibits energy metabolism and that it is by this mechanism that this agent alters methotrexate uptake. Because glucose partially reverses the effect of vincristine, the data suggest that vincristine may inhibit aerobic energy metabolism; however, since glucose reversal is not complete, vincristine may also inhibit anaerobic energy metabolism. Additional evidence that vincristine may inhibit energy-dependent processes comes from the observation that this agent like other metabolic poisons reduces the uphill transport of the nonmetabolized amino acid analogue α-aminoisobutyric acid, into Ehrlich ascites tumor cells (14).

Periwinkle alkaloids bind with high affinity (15) to protein subunits of microtubules to form protein-drug precipitates (16–18) which are thought to be the filamentous structures and crystals that are observed on electron microscopy (19–21). This interaction results in impaired formation of mitotic spindles (22–24) and in the arrest of cell division in metaphase (25, 26) which is considered to be the basis of the cytotoxicity of these agents. It is unlikely that impaired spindle formation can account for the effects of vincristine on methotrexate uptake. Hence, although 10 μM vincristine or vinblastine produce crystal formation on electron microscopy within 1/2 hour after exposure of L-cell mouse fibroblasts to these agents (19), over this interval there are no significant changes in methotrexate uptake. Further, although the L1210 leukemia and the Ehrlich ascites tumor cell lines are very sensitive to the stimulatory effect of vincristine on methotrexate uptake, vincristine negligibly affects survival of mice bearing these tumors (6, 27) and over an interval of exposure of L-cells to vincristine sufficient to arrest cell replication a significant change in methotrexate uptake is not demonstrable. Vincristine alkaloids interact with other microtubular elements have been noted beneath the plasma membrane of some cells and are eliminated by exposure to vincristine (28). Microfilamentous elements precipitable by vinblastine have been extracted from erythrocyte membranes and exposure of erythrocytes to vinblastine produced an increase in sodium flux and glycolysis (29, 30). These structures may be involved in the regulation of cellular energetics in general and/or the energetics of membrane transport processes in particular. However, if vincristine and vinblastine bind to these microtubular elements with the same affinity that they bind to other microtubular proteins, this would not account for the changes in methotrexate uptake. Hence, although vincristine or vinblastine bind rapidly to porcine brain microtubular protein, the rate of dissociation of the complex is slow (half-time, 5 hours) (15), while the effects of vincristine on methotrexate uptake are more rapidly reversible. Although periwinkle alkaloids interact with other cellular elements, this usually requires levels of these agents which are orders of magnitude higher than concentrations which alter methotrexate uptake (31).

Vincristine alkaloids produce other changes in membrane function. Vinblastine inhibits phagocytosis in rabbit polymorphonuclear leukocytes (32) and augments membrane internalization in erythrocytes (33). Although vincristine alkaloids may interact with cell membranes to alter membrane structure and function, an alteration in the passive permeability of the cell membrane to methotrexate is excluded as an important factor in these studies since a change in the passive diffusion of methotrexate should be accompanied by an alteration in bidirectional fluxes but the unidirectional influx of methotrexate is unchanged under conditions in which the unidirectional efflux process is markedly retarded. It is unlikely that the vincristine effect on methotrexate uptake is secondary to the metabolic consequences of the mitotic block (34–36), since the interval between exposure of cells to vincristine and stimulation of net methotrexate uptake is brief (4 min.).

The nature of the vincristine-induced alteration in the uptake of methotrexate may contribute to a further understanding of the mechanism(s) by which methotrexate inhibits DNA synthesis. Studies from this laboratory suggest that inhibition of deoxyuridine incorporation into DNA by methotrexate is influenced by the level of intracellular methotrexate in excess of that required for stoichiometric binding to dihydrofolate reductase. When methotrexate uptake is enhanced by vincristine in Ehrlich ascites tumor cells, inhibition of DNA synthesis is increased, while vincristine alone has no effect under similar conditions (7). This may account for the increased survival of mice bearing the L1210 ascites tumor when vincristine is administered with methotrexate (6) while vincristine alone has little effect (6, 27).

This interaction between methotrexate and the vincristine alkaloids should provide a rational basis for the design of synergistic chemotherapeutic regimens with these agents. Conversely, vincristine might antagonize the effectiveness of other cytotoxic agents for which the maintenance of a high intracellular drug level is dependent upon cellular energy metabolism. Further, the apparent difference between the effects of vincristine on net uptake of methotrexate in L-cells as compared to the tumor cells studied suggests specificity among different tissues and raises the possibility that vincristine may increase the cytotoxicity of methotrexate to the tumor without a comparable increase in toxicity to susceptible host tissues.

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