Different Effects of Vincristine on Methotrexate Uptake by L1210 Cells and Mouse Intestinal Epithelia in Vitro and in Vivo

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ABSTRACT

A comparative study of the effect of vincristine on the intracellular level of methotrexate in murine tumor (L1210 cells) and normal proliferative tissue (small intestine) in vitro and in vivo is presented. During in vitro studies, vincristine did not alter the initial rate of methotrexate uptake by L1210 cells, but did produce an increase in the steady-state level of exchangeable drug. The magnitude of the increase was dependent upon the concentration of Vinca alkaloid (0.1 to 10 μM). A maximum increase of 125% occurred with vincristine concentrations of 10 μM or higher. In parallel studies in vitro, vincristine concentrations up to 40 μM had no effect on methotrexate uptake by isolated intestinal epithelial cells.

For in vivo studies, mice were given injections of methotrexate, 25 mg/kg i.p., and the concentration of folate analog in L1210 ascites and small intestine was monitored for 24 hr. In both tumor cells and small intestine, peak levels of methotrexate were attained at 40 min to 1 hr after injection. Exchangeable methotrexate above that bound to dihydrofolate reductase persisted for >24 hr in tumor cells and <12 hr in small intestine. Vincristine, 0.5 to 5.0 mg/kg i.p., administered 2.5, 1.5, or 0.5 hr prior to methotrexate or 16, 24, or 32 hr after methotrexate had no effect on the pharmacokinetics for methotrexate in either tumor cells or normal tissue.

Treatment of mice bearing the L1210 leukemia with vincristine, 0.25 to 1.0 mg/kg i.p., prior to or together with methotrexate, 25 mg/kg i.p., produced no therapeutically gain other than that which could be explained from an additive effect of the two agents. However, delay of vincristine administration for several hr after methotrexate did produce therapeutic synergism. At a vincristine concentration of 0.5 mg/kg i.p., a maximum synergism (227% increased life span) was obtained when the interval between the two agents was 24 hr or longer. The synergistic effect was dose-dependent within the range of 0.25 to 1.0 mg/kg.

These data show that therapeutic synergism can result from the temporal use of vincristine and methotrexate. However, they raise the question of whether the mechanism for the synergism is related to the interaction between vincristine and methotrexate shown in vitro.

INTRODUCTION

In vitro studies with murine (8–10, 31) and human (1, 30) tumor cells showed that the addition of vincristine concomitant with methotrexate resulted in an increase in the intracellular steady-state level of methotrexate. Fyfe and Goldman (8) also showed that vincristine decreased the efflux of methotrexate from Ehrlich ascites carcinoma cells, suggesting that the basis of the effect on steady state was related to an effect on efflux. Bender et al. (1) showed a similar retardation of methotrexate efflux from acute myelocytic leukemia cells in vitro. These observations, together with data (31) showing that the administration of vincristine before methotrexate to BALB/c mice bearing the L1210 leukemia resulted in therapeutic synergism and some long-term survivors, prompted the inclusion of vincristine pretreatment in clinical protocols involving high-dose methotrexate therapy (13, 14). Despite the compelling nature of these observations, questions relating to the use of this regimen remained unanswered. First, there was no detailed evidence as to whether vincristine also increased the intracellular steady-state level for methotrexate in normal proliferative cells. Limited data obtained with cultured mouse fibroblasts (8) suggested a possible selective effect. Second, although a therapeutic synergism had been reported for the vincristine-methotrexate combination (31), there was little evidence that the mechanism for this synergism was actually related to an increase in methotrexate accumulation in tumor cells in vivo.

Answers to these questions were sought in an L1210 murine tumor model using Sprague-Dawley C57BL/6 × DBA/2 F1 (hereafter called B6D2F1) mice as the host. The possibility that vincristine selectively enhanced methotrexate accumulation in tumor cells was addressed through a parallel study of methotrexate transport by normal proliferative cells and tumor cells. Suspensions of epithelial cells isolated from the villi and the crypts of small intestine, the primary organ site of folate analog toxicity in mice (16, 18, 19), were used as representative normal cell populations containing drug-sensitive proliferative cells; L1210 ascites cells harvested from the peritoneal cavity served as the tumor cell population. The effect of vincristine in vivo was evaluated by measurements of methotrexate accumulation and retention in L1210 cells and small intestine in animals otherwise untreated or treated with the Vinca alkaloid. Finally, therapy trials were initiated with the same tumor model to provide additional documentation of the Vinca alkaloid-methotrexate synergism reported in mice by Zager et al. (31).

MATERIALS AND METHODS

Epithelial cells were isolated by hyaluronidase treatment of intestinal mucosa as described (5, 6). Small intestines were removed from male B6D2F1 mice and were everted on wooden sticks (Puritan applicators; Hardwood Products, Guilford, Maine) to increase the exposure of the immature proliferative epithelia in the crypts to the enzyme. The everted segments were incubated in a 37° salts medium (120 mm NaCl:20 mm Tris-HCl:3 mm K2HPO4:1 mm MgCl2:1 mm CaCl2, pH 6.9), 0.1% with respect to both bovine serum albumin and hyaluronidase (Sigma Chemical Co., St. Louis, Mo.; type 1, 460 N.F. units/
filtered cells were collected by centrifugation at 200 x g for 5 min, washed with medium minus hyaluronidase, and resuspended in a minimum volume of transport medium (107 mm NaCl:10 mm Tris-HCl:26.2 mm NaHCO3:5.3 mm KCl:1.9 mm CaCl2:1 mm MgCl2:10 mm glucose; pH 7.4). The final preparation consisted of 2 predominant cell types present in approximately equal amounts. The first had the distinct columnar appearance characteristic of mature adsorptive cells from the intestinal crypts. Each cell suspension also had a slight contamination (1 to 2%) of erythrocytes and lymphocytes. Since the ratio of crypts to columnar cells in the intact organ is approximately 1:5, the isolation procedure resulted in a significant purification of crypt cells.

L1210 tumor cells (line V) were maintained in female B6D2F1 mice as described (12). Four to 5 days after transplantation, cells were removed from the peritoneal cavity by flushing with transport medium (29) and were utilized in transport experiments.

The uptake of methotrexate (provided by Dr. Harry B. Wood, Jr., Drug Development Branch, Division of Cancer Treatment, National Cancer Institute) in the presence and absence of vincristine sulfate (Oncovin; Eli Lilly and Co., Indianapolis, Ind.) was measured by incubating suspensions (4 to 5 x 10⁷ cells) of tumor or normal intestinal cells with drug at 37° (5, 19, 21, 25). Uptake was terminated by placing cells on ice and rapidly diluting with cold (0-4°) 0.14 m NaCl:0.02 m potassium phosphate, pH 7.5. The diluted cells were centrifuged at 2000 x g for 5 min, washed twice with cold 0.4 m NaCl:0.02 m potassium phosphate, pH 7.5, and resuspended in a measured volume of the same NaCl solution. A aliquot was removed for determination of the cell number, and the remainder was subjected to heat treatment in order to extract and measure drug content (see below). The density of the cell suspension was determined from the absorbance at 600 μM. Using this, the cell number was calculated from a previously established correlation for each cell type (see Ref. 26) between absorbance and cell number as measured with a Neubauen improved counting chamber. (L1210 cells: A₆₀₀, 3.0 is equivalent to 2.4 x 10⁷ cells; intestinal epithelial cells: A₆₀₀, 3.0 is equivalent to 2.1 x 10⁷ cells). The wet and the dry weights of L1210 tumor cells and of intestinal epithelial cells were established from the weights of measured cell suspensions before and after drying in preweighed aluminum tares. Incubation at 0° followed by identical dilution and processing was used to estimate both a rapidly associating cell surface component of uptake (20) and passive diffusion. All in vitro uptake data were expressed as nmol of methotrexate taken up per min per g, dry weight, of cells.

Female B6D2F1 mice bearing the L1210 tumor were utilized for in vivo pharmacokinetic studies 3 and 4 days after transplantation of 10⁶ cells (12). Following i.p. injection of methotrexate, animals were sacrificed at the specified intervals, and both the tumor cell ascites and the small intestine were removed. The tumor cells were washed, and the cell number was determined as above; the small intestine was cleaned, weighed, and homogenized in cold 0.14 m NaCl:0.02 m potassium phosphate, pH 7.5, as described (16, 18, 21, 24, 27, 28). The folate analog was extracted from both the washed tumor cells and the homogenized tissue into 0.14 m NaCl:0.02 m potassium phosphate, pH 7.5, by heat treatment at 100° for 15 min. Cell debris was removed by centrifugation at 27,000 x g for 5 min, and the drug content of the supernatant was determined by titration of a Diplococcus pneumoniae dihydrofolate reductase (20). All data from the pharmacokinetic studies were expressed as ng of methotrexate per g wet weight of tumor cells or small intestine.

For therapy studies, female B6D2F1 mice were inoculated with 10⁶ L1210 cells (12) on Day 0 and randomly distributed among control and treated groups. All treatment was begun on Day 2, and all drugs were given by i.p. injection. Mice received methotrexate on Days 2, 6, 10, and 14; vincristine was given either prior to, together with, or after methotrexate. Mice were maintained on Purina laboratory chow and water ad libitum.

RESULTS

Although the effect of vincristine on L1210 cells in vitro had been reported by Zager et al. (31), experiments were necessary to demonstrate that vincristine could augment the intracellular level of methotrexate in the L1210 tumor cell line in this laboratory. The uptake of methotrexate (2.2 μM) at 37° in the presence and absence of vincristine (10 μM) is shown in Chart 1. All data of methotrexate uptake by L1210 cells at 37° are corrected for a rapidly associating cell surface component measured at 0° (20). Methotrexate uptake in the absence of vincristine was linear for 5 min, after which the rate slowed, and a steady state was attained by 20 min. Vincristine did not affect the initial rate of methotrexate uptake, but did increase the steady state level of intracellular drug. At 60 min, the total intracellular content of methotrexate in vincristine-treated cells was 70% greater than in nontreated cells (0.001 < p < 0.01, as analyzed by a paired t test). Vincristine had no effect on

![Chart 1](chart.png)

**Chart 1.** Time course of methotrexate (MTX) uptake by L1210 cells in vitro. L1210 cells were incubated at 37° with 2.2 μM methotrexate in the presence (○) and the absence (□) of 10 μM vincristine. Uptake was terminated at the indicated times, and the cells were analyzed for methotrexate content as described in Materials and Methods. All data are corrected for uptake at 0°. Each point, mean calculated from 3 separate determinations; bars, S.D.; FAH₂, dihydrofolate.
methotrexate uptake at 0° (data not shown). When these data were analyzed with respect to the concentration of free intracellular methotrexate, i.e., above that bound to dihydrofolate reductase [3.64 ± 0.39 (S.D.) nmol/g dry weight; see Ref. 26], the effect of vincristine was even more striking. At 60 min, there was a 125% increase in the level of free intracellular methotrexate in the presence of vincristine.

Because these data were obtained with 2.2 μM methotrexate, a concentration less than the Km (3 to 5 μM) for transport (6, 11, 20, 22, 26, 29), similar experiments were performed at a saturating concentration of 22 μM. In these experiments (data not shown), the presence of vincristine did not affect the initial rate of methotrexate uptake, but caused an approximate 30% increase in the total intracellular level of methotrexate at 60 min.

The relationship between the magnitude of the increase in the intracellular level of methotrexate and the vincristine concentration is shown in Table 1. At 60 min, a significant (p < 0.05) increase in total intracellular methotrexate was measurable after incubation with concentrations of vincristine of 0.1 μM or greater. The maximum increase in total intracellular methotrexate was obtained with a vincristine concentration of 10 μM.

The uptake of methotrexate by suspensions of isolated intestinal epithelial cells, as by L1210 cells (11, 20, 26, 29), is a saturable (Km = 87 μM), temperature-dependent process resembling carrier-mediated transport and exhibiting structural specificity (5, 6). To determine if the Vinca alkaloid would also alter the uptake of methotrexate by intestinal epithelial cells, suspensions were incubated with 22 μM methotrexate in the presence of 10 μM vincristine, as shown in Chart 2. Methotrexate uptake by cells not exposed to vincristine was linear for 15 min, after which the rate of uptake slowed and a transient steady state was attained. When the cells were exposed to vincristine, there was no significant difference in either the initial rate or the maximum level of methotrexate accumulation. No effect was seen even at a vincristine concentration of 40 μM (Table 1). This concentration was 200- to 400-fold higher than that necessary to produce an effect on methotrexate uptake by L1210 cells. When suspensions of intestinal epithelial cells were incubated with 2.2 μM (a level often used with L1210 cells) or 220 μM methotrexate (a level approaching saturation), vincristine (10 μM) had no effect on either the initial rate or the maximum level of accumulation.

To further substantiate that vincristine did not alter methotrexate transport by these normal murine cells, the following experiments were undertaken (data not shown). First, to allow for possible differences in vincristine uptake between tumor and normal cells, intestinal epithelial cells were preincubated with vincristine (10 μM) for 30 min before being exposed to methotrexate (22 μM). No effect on either the initial rate of methotrexate uptake or the steady state was observed. Second, to determine whether the hyaluronidase treatment altered the responsiveness of cells to vincristine, L1210 cells were incubated in a hyaluronidase preparation identical to that used to release epithelial cells from the underlying intestinal mucosa. After 20 min at 37°, the tumor cells were processed in a manner identical to that used for intestinal epithelia in order to remove the proteolytic enzyme. The effect of vincristine on the time course of methotrexate uptake by these hyaluronidase-treated cells was the same as that seen with non-hyaluronidase-treated cells.

Although these in vitro data suggested an ideal situation, i.e., an apparent selective effect of vincristine on the uptake of methotrexate by tumor but not by normal cell populations, there remained the question of whether the enhancement of uptake by vincristine, selective or not, could be demonstrated in vivo. For this reason, a series of pharmacokinetic experiments (6, 21, 24, 25, 27, 28) were initiated. The effect of vincristine on methotrexate accumulation and persistence in L1210 ascites cells and in small intestine in vivo is shown in Chart 3. For these experiments, animals were given injections of methotrexate, 25 mg/kg i.p., with and without vincristine, 0.5 to 5.0 mg/kg i.p. In both L1210 tumor cells and small intestines from animals not treated with vincristine, intracellular levels of methotrexate reached a peak by 40 min to 1 hr and then declined. By 8 to 12 hr, the intracellular level of methotrexate in small intestine had declined to that assumed to be bound to intracellular dihydrofolate reductase (see Refs. 21, 24, and 25), while in L1210 cells levels of methotrexate above that bound to dihydrofolate reductase (see Refs. 21, 24 and 25) persisted for

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**Table 1**

Effect of vincristine on methotrexate steady state in vitro

<table>
<thead>
<tr>
<th>Vincristine concentration (μM)</th>
<th>% change in methotrexate steady state</th>
<th>L1210 cells</th>
<th>Intestinal epithelial</th>
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<tbody>
<tr>
<td>0.1</td>
<td>102.4 ± 1.0*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.5</td>
<td>116.6 ± 4.8*</td>
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<td>ND</td>
</tr>
<tr>
<td>1.0</td>
<td>120.5 ± 3.1*</td>
<td>98.3 ± 16.6*</td>
<td>ND</td>
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<td>5.0</td>
<td>126.2 ± 10.7*</td>
<td>94.6 ± 9.6*</td>
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</tr>
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<td>10.0</td>
<td>163.6 ± 7.9*</td>
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<td>ND</td>
</tr>
<tr>
<td>20.0</td>
<td>159.3 ± 23.1*</td>
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<tr>
<td>40.0</td>
<td>163.3 ± 6.9*</td>
<td>104.2 ± 13.4*</td>
<td>ND</td>
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</table>

* Each value represents the mean ± S.D. calculated from 3 to 8 separate determinations. Uptake in the absence of vincristine was taken as 100%.

+ p < 0.05 (paired t test).

- ND, not determined.

+ p > 0.1 (paired t test).

* p < 0.001 (paired t test).
Treatment with vincristine, 0.25 to 1.0 mg/kg i.p., every 4 days, tumor cells on Day 0 survived an average of 6.7 ±0.3 days. Combination with methotrexate on the survival time of leukemic shown in Table 3, untreated mice inoculated with 10^6 Li210 or small intestine. The representative data presented in Table 2 show that there were no significant differences (p ≥ 0.5, as analyzed by a nonpaired t test) in the levels of intracellular methotrexate (expressed as ng/g wet weight) in either the L1210 tumor cells or the small intestines removed from mice which received or did not receive vincristine at 24 hr after the folate analog. Moreover, no significant differences were found when experiments were repeated with vincristine concentrations of 1.0 or 5.0 mg/kg.

To extend these investigations, the effect of vincristine in combination with methotrexate on the survival time of leukemic mice was also studied. All therapy was begun on Day 2. As shown in Table 3, untreated mice inoculated with 10^6 Li210 tumor cells on Day 0 survived an average of 6.7 ± 0.3 days. Treatment with vincristine, 0.25 to 1.0 mg/kg i.p., every 4 days until death produced a 30 to 46% increase in life span. Methotrexate, 25 mg/kg i.p., given every 4 days for a total of 4 doses, produced a 124% increase in life span. The therapeutic effect achieved when vincristine was given 30 min prior to methotrexate was no different from that achieved when the 2 agents were administered together. In neither case did the therapeutic gain exceed that which could be explained from an additive effect of the 2 agents. However, when vincristine was administered several hr after methotrexate, different results were obtained. At a vincristine concentration of 0.5 mg/kg, an 8-hr interval between the 2 drugs resulted in a slight synergism. This synergistic effect increased as the interval between the 2 drugs was expanded, with a maximum therapeutic gain being obtained with a delay of 24 hr or longer. That the vincristine effect was concentration dependent was shown by the lesser synergism (only with a minimum delay of 24 hr) obtained with 0.25 mg/kg i.p. and the greater degree of synergism obtained with 1.0 mg/kg i.p.

**DISCUSSION**

Our studies confirm earlier work by Zager et al. (31) that vincristine can augment the steady state for methotrexate in L1210 murine tumor cells in vitro. The availability of method-
ology for the isolation of suspensions of absorptive and proliferative intestinal epithelial cells also permitted an assessment of the effect of vincristine on a normal cell population. Vincristine concentrations as high as 40 μM had no effect on the time course of methotrexate uptake by suspensions of these cells. Levels 200- to 400-fold less were adequate to produce an effect in L1210 cells. Although the evidence is indirect, studies with L1210 cells preincubated with hyaluronidase indicated that the proteolytic enzyme used to release the epithelial cells from the intestinal mucosa did not alter the ability of vincristine to augment the steady state for methotrexate. These in vitro data indicate that there may be a greater specificity of the vincristine effect for neoplastic cells. Fyfe and Goldman (8) previously suggested a difference between tumor and normal cells when they reported that the threshold of the vincristine effect toward cultured mouse L-fibroblasts was 30 μM as compared to 5 μM (or lower) for Ehrlich ascites carcinoma cells. However, it must not be overlooked that these differences in both cases were observed under conditions in which exposure to different vincristine concentrations was sustained.

During the experiments which dealt with methotrexate pharmacokinetics in vivo, exchangeable methotrexate in excess of that bound to dihydrofolate reductase persisted for >24 hr and <12 hr in tumor cells and in small intestine, respectively. As complete inhibition of dihydrofolate reductase requires levels of exchangeable methotrexate (21, 23), this difference in persistence in tumor versus normal tissue appears to account for the selective toxicity and the therapeutic effect observed during therapy (6, 24–26). Administration of either of 3 concentrations of vincristine before methotrexate caused no significant alteration in the pharmacokinetic profile of methotrexate in either tumor cells or normal tissue. Attempts to augment the level of exchangeable methotrexate at times (6 to 32 hr) after methotrexate injection were also unsuccessful. These negative results in situ may be due to a combination of the rapid clearance of vincristine and the reversibility of the effect. The volume of ascitic fluid in the peritoneal cavity of B6D2F1 mice 3 to 4 days following inoculation of 10⁶ L1210 tumor cells is approximately 1.0 ml.² For a 20-g mouse, the i.p. injection of vincristine equivalent to 0.5, 1.0, or 5.0 mg/kg will result in estimated initial i.p. concentrations of 10, 20, and 100 μM, respectively. These estimates are slightly higher than the

<table>
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<th>MTX³ (mg/kg)</th>
<th>VCR (mg/ kg i.p.)</th>
<th>VCR administration</th>
<th>Total mice</th>
<th>MST (days)</th>
<th>ILS (%)</th>
<th>Final wt² (g)</th>
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<td>221</td>
<td>17.7 ± 2.8</td>
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</tbody>
</table>

¹ MTX, methotrexate; VCR, vincristine; MST, mean survival time; ILS, increase in life span.
² Administered on Days 2, 6, 10, and 14.
³ Weight at death (Initial weight, 20.0 ± 0.5 g).
⁴ Number of experiments times mice per experiment.
⁵ Mean ± S.D.
⁶ Administered on Day 2 and every 4 days thereafter until death.

² F. M. Sirotnak, unpublished observations.
ascitic fluid concentrations of 1.4 and 16.5 μM measured (3) after the i.p. injection of 0.15 or 1.5 mg/kg into BALB/c × DBA/2 F1, (hereafter called CD2F1) mice, respectively. Although these concentrations approximate those used during folate analog transport experiments in vitro, clearance and metabolism of vincristine appears to prevent their maintenance. Bender et al. (3) found that the disappearance of vincristine from the ascitic fluid of CD2F1 mice was a rapid, monophasic function. The half-life of an i.p. injection of 0.15 or 1.5 mg/kg was 33.5 or 24.9 min, respectively. El Dareer et al. (7) showed that shortly after the i.p. injection of 3.0 mg/kg to B6DF1 mice, both vincristine and its metabolites were present in the serum. The serum level of vincristine was maximum (0.77 μg/ml) by 15 min and declined with an apparent half-life of 60 min. Vincristine metabolites increased to a maximum at 30 min and remained elevated for at least 3 hr. In other studies in which vincristine was given by i.v. injection into rats (4), dogs (4, 7), and monkeys (7), an initial and a secondary clearance phase for the disappearance of the drug from the blood were found. The half-life values for the 2 phases were 6 to 15 and 75 to 190 min, respectively. In conjunction with the rapid clearance and metabolism of vincristine in vivo, the in vitro transport experiments with tumor cells (1, 8) showed that the retardation of methotrexate efflux was reversed when the vinca alkaloid was removed. Finally, since vincristine had no significant effect on methotrexate transport by isolated intestinal epithelial cells in vitro, the lack of effect on methotrexate accumulation by small intestine was not unexpected.

In addition to the problems of rapid clearance and reversibility, the clinical use of vincristine to alter methotrexate pharmacokinetics may also be complicated by the maximum therapeutic level attained and the threshold concentration necessary to produce a response in tumor cells. In humans (2, 17), as in animals (4, 7), vincristine is rapidly cleared from the blood. Following the administration of 0.025 mg/kg i.v., Ow-ellen et al. (17) reported a biphasic clearance with half-life values for each phase of 3.4 and 155 min, respectively. In a parallel study using similar doses of 0.025 to 0.031 mg/kg i.v., Bender et al. (2) found a triphasic blood disappearance profile with half-life values for each phase of 0.85, 7.4, and 164 min, respectively. The maximum blood level observed was 0.36 μM. In our in vitro studies with L1210 murine tumor cells, we were able to produce a statistically significant 2.4% increase in the steady state for methotrexate with a vincristine concentration of 0.1 μM. However, the biological significance of such an increase is doubtful. Zager et al. (31) reported that vincristine concentrations from 0.01 to 1.0 μM produced a 30 to 40% increase in the steady state for methotrexate in their L1210 cells, while the L1210 cells used by Bender et al. (3) were less responsive. In both our study and that of Zager et al. (31), the maximum increase in steady state (60 to 70%) was obtained at a vincristine concentration of 10 μM. Although 0.1 μM vincristine produced a 33% increase in total intracellular methotrexate in acute myelocytic leukemia blast cells in vitro (1), this concentration of Vinca alkaloid did not enhance methotrexate uptake by 2 lines of cultured human lymphoblastoid cells (30). These in vitro data may reflect differences in the sensitivity of the various cell types to vincristine or differences in experimental conditions, such as the transport medium used. However, the suggestion that the threshold may vary between cell types, considered with the data on the maximum level of vincristine attained clinically and its rapid clearance, seriously challenges the feasibility of using vincristine to alter the steady state for methotrexate in tumor cells in situ.

In addition to the negative results during the pharmacokinetic studies, no synergism could be demonstrated during therapy trials when vincristine was administered prior to or together with methotrexate. Bender et al. (3) have reported similar findings. However, although no effect on the methotrexate pharmacokinetics in tumor cells or in normal tissue in situ could be shown when vincristine administration was delayed, therapeutic synergism was evident. The mechanism for the synergism may therefore be unrelated to an effect on transport, and the rapid clearance of vincristine may not be a compromising factor.

The clinical relevance of the therapeutic synergism obtained with i.p. injections of 0.5 to 1.0 mg/kg must be ascertained. However, since these concentrations of vincristine are not toxic to the experimental animals when given on this schedule and since the mechanism for the synergism may not be concentration dependent in the same way as the effect on methotrexate transport shown in vitro, it may eventually be possible to extrapolate these data to clinical regimens.

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Different Effects of Vincristine on Methotrexate Uptake by L1210 Cells and Mouse Intestinal Epithelia  *in Vitro* and *in Vivo*

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