Biochemical and Pharmacokinetic Effects of Leucovorin after High-Dose Methotrexate in a Murine Leukemia Model

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SUMMARY

The administration of calcium leucovorin, either concurrently or after high dosages of methotrexate in L1210 leukemic mice, has both pharmacokinetic and biochemical effects in tumor cells and drug-limiting proliferative normal tissue in small intestine. A reduction in the maximal level of accumulation and retention of exchangeable drug (unbound to dihydrofolate reductase) in tissue could be demonstrated when calcium leucovorin was given simultaneously with methotrexate at equal or greater dosages than the latter. The dose dependence for calcium leucovorin-induced drug loss is similar in both tissues and showed the expected variation when the time interval between methotrexate and calcium leucovorin doses was increased. With 400 mg methotrexate per kg, >96 mg calcium leucovorin per kg were required maximally to affect overall drug retention in tissue 2 hr after drug, whereas only 24 mg calcium leucovorin per kg were required 16 hr after drug.

Calcium leucovorin, given after methotrexate, induced synchronous recovery of DNA synthesis (measured by labeled deoxyuridine incorporation) in both small intestine and L1210 cells. An initial cycle of synthesis was induced in the presence of exchangeable levels of drug. Two hr after methotrexate, 12 mg/kg, calcium leucovorin induced an immediate but only partial (20 to 25% of control rate) recovery of synthesis with dose dependence from 3 to 12 mg calcium leucovorin per kg. Less synthesis was induced after 96 mg/kg and almost none after methotrexate, 400 mg/kg. With calcium leucovorin, 24 mg/kg, given 2 hr after methotrexate, 12 or 96 mg/kg, a major cycle of synthesis occurred when total drug levels approached the equivalence of the dihydrofolate reductase content. The magnitude of this cycle of synthesis in both L1210 cells and small intestine was the same as that seen in animals recovering from methotrexate alone. However, this is based on the assumption that an approximately equivalent relationship between DNA synthesis and labeled deoxyuridine incorporation in each tissue during the period of maximal incorporation within the cycle. The major effect of calcium leucovorin, then, was to induce an earlier resumption of DNA synthesis as a consequence of the pharmacokinetic effect in each tissue. With calcium leucovorin, 24 or 400 mg/kg, given 16 hr after methotrexate, an identical effect on drug retention was observed in both L1210 cells and small intestine. Although there was a difference in the time course for recovery in small intestine at each dosage of calcium leucovorin, the recovery of DNA synthesis as drug levels approached the dihydrofolate reductase content was similar in magnitude. In L1210 cells, however, substantial recovery of synthesis to a comparable level and with a similar time-course occurred only after leucovorin, 400 mg/kg. Little or no recovery of DNA synthesis was observed after calcium leucovorin, 24 mg/kg, during the same time period. This dosage schedule (methotrexate, 400 mg/kg, s.c. followed 16 hr later by calcium leucovorin, 24 mg/kg s.c.) administered once gave a 2-log leukemia cell kill and prevented lethal toxicity in most of the animals.

INTRODUCTION

Continuing reports of progress in the treatment of various forms of human cancer using high-dose methotrexate with calcium leucovorin (5, 6, 18, 20, 27, 28) have prompted further interest in animals (3, 4, 21, 22, 24, 25, 30–33, 41) and man (7, 15, 26, 27, 37–39) in the pharmacological and biochemical aspects of therapy with this agent [many earlier studies are cited in the companion report (35)]. Furthermore, the basis of the selective "rescue" effect observed with calcium leucovorin, either in laboratory models (8–11, 16) or clinically (5, 6, 18, 20, 27, 28), is not understood.

In approaching this question, we recognize the relevance of numerous in vitro studies that have elucidated various aspects of the molecular pharmacology of this agent, including work on membrane transport of naturally occurring folicates and inhibitory analogs as well as biochemical studies related to folate metabolism in general. (See Refs. 1, 2, 13, 14, 19, and 23 for a review of recent progress in these areas.) However, an understanding of leucovorin rescue in animal models, which can be usefully extrapolated to the clinical level, requires an examination of both pharmacokinetic and biochemical effects in target tumor and drug-limiting tissues within the pharmacological context of the in vivo situation. Only then can such aspects as the absolute dosage and temporal relationships between methotrexate and leucovorin administration providing maximum therapeutic benefits be meaningfully analyzed.

The tissue pharmacokinetics for exchangeable drug, the effects on DNA synthesis in drug-limiting host tissue and tumor, and the net effect of both factors on tumor cell "kill" following extremely high doses of methotrexate alone in tumor-bearing mice have been examined in the companion report (35). This work is an extension of earlier studies from...
this laboratory (25, 30, 32) and elsewhere (22). We now consider here the effects in the same model of administering calcium leucovorin simultaneously or after methotrexate. Our results reveal both pharmacokinetic and biochemical effects of calcium leucovorin in drug-limiting host (epithelium of small intestine) and tumor tissue. Moreover, differences in the biochemical effects (resumption of DNA synthesis) achieved in each tissue may account for the selectivity of rescue, at least in this model.

**MATERIALS AND METHODS**

The experimental methodology has been described in detail in the companion report (35). The transplantation procedure for the L1210 murine leukemia in C57BL × DBA/2 F1 (hereafter called BD2F1) female mice was given earlier (17). Beginning 2 to 4 days after 10⁶ cells were given i.p., tumor cells were harvested from the peritoneal cavity as suspensions in cold (0°) 0.14 M NaCl-0.01 M potassium phosphate (pH 7.4), and drug was extracted by heating (29, 36) after the cells were washed. Surgically removed small intestine was sectioned, washed, and homogenized in cold (0°) 0.14 M NaCl-0.01 M potassium phosphate (pH 7.4) and drug was also extracted by heating (25). The estimation and the removal of extracellular drug in tissue were considered in the companion report (25). Drug content in tissue extracts was determined by a routine enzyme assay (29), and the dihydrofolate reductase-binding content of tissue was determined by titration with methotrexate (40).

A determination of the number of tumor cells in ascites fluid was obtained microscopically (35) and expressed as total number of cells per animal. Measurements of DNA synthesis were estimated from determinations of [³H]UdR³ incorporation into DNA (25). Radioactivity was extracted (25) from acid-insoluble fractions and measured by scintillation spectrophotometry. Methods of data analysis were described in Ref. 35. Also, the extent of [³H]UdR incorporation may not always be a precise measure of DNA synthesis, since some differences in the size of the dUMP pool may occur following drug treatment of animals [see further discussion in the companion report (35)]. Methotrexate and calcium leucovorin were provided by Dr. Harry B. Wood, Jr., Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Dosages indicated for each agent represent the total amount administered.

**RESULTS**

Data on the effects of calcium leucovorin administered simultaneously with methotrexate on the initial rate of accumulation of drug in tumor cells (both L1210 and Sarcoma 180) and in drug-limiting tissue in small intestine have been presented (35). The reduction in the rate of drug accumulation observed in each tissue is in general agreement with notions of carrier-mediated transport established in in vitro systems (12, 13, 34) and the competition between methotrexate and leucovorin for the carrier component. Calcium leucovorin given with methotrexate at a suitable dosage ratio would be expected to have detectable effects on maximal accumulation and retention of drug in these tissues and, in fact, this has been demonstrated during this study. The data in Chart 1 show the effect of calcium leucovorin on drug retention in L1210 cells and small intestine when administered s.c. with methotrexate, 400 mg/kg. As already discussed in the companion report (35) and earlier (30, 32), the fraction of total drug accumulating above the dihydrofolate reductase content is fully exchangeable. At calcium leucovorin dosages of 24 and 96 mg/kg, there is no detectable effect on the time course for accumulation (not shown) or persistence of drug in either L1210 cells or small intestine. At 400 mg/kg, maximal accumulation of calcium leucovorin in both tissues was diminished, and exchangeable levels of drug persisted for shorter periods of time. A greater effect of calcium leucovorin at 400 mg/kg was seen in L1210 cells than in small intestine. Similar results were obtained when lower (12 and 96 mg/kg s.c.) equivalent dosages of calcium leucovorin and methotrexate were given.

Prior studies in mice (8-11, 16) indicated that high methotrexate dosage regimens incorporating delayed administration of leucovorin (12 to 24 hr following methotrexate) prevented most toxicity and were more therapeutically effective than regimens incorporating simultaneous calcium leucovorin administration. In this study, therefore, experiments were initiated in which the effects of calcium leucovorin administration were compared at 2 and 16 hr after methotrexate.

**Pharmacokinetic and Biochemical Effects of Calcium Leucovorin Given 2 hr after Methotrexate.** The effect of calcium leucovorin given 2 hr after the administration of methotrexate is shown in Charts 2 and 3. The initial accumulation of drug in these experiments is not shown, since this occurred in a manner already shown in the companion paper (35). Following 400 mg methotrexate per kg s.c., no effect of calcium leucovorin on the egress of drug was observed in either L1210 cells or small intestine at s.c. doses of 48 mg/kg or less (Chart 2). The period during which exchangeable drug persisted was unaltered in L1210 cells (>70 hr) and small intestine (>50 hr). At calcium leucovorin dosages of 96 mg/kg and above, the rate of drug loss was more rapid, and exchangeable drug levels persisted for much shorter periods of time in both tissues.

In a similar experiment (Chart 3), calcium leucovorin, 24 mg/kg s.c., was given 2 hr after varying doses of methotrexate (12, 96, and 400 mg/kg s.c.). Again, no effect was detectable on the rate of exchangeable drug loss after 400 mg/kg. However, some effect of calcium leucovorin at this dosage was observed in both tissues after methotrexate, 96 mg/kg, and a greater relative effect was seen after methotrexate, 12 mg/kg, particularly in L1210 cells. In this same experiment, measurements of DNA synthesis as determined by the incorporation of [³H]UdR were also made.

At the dosages of methotrexate used, maximal inhibition of incorporation of precursor in L1210 cells and small intestine occurred within 2 hr after drug administration and recovery occurred in a synchronous fashion (35). Since these data have already been presented (35), only the initial
High-Dose Methotrexate with Leucovorin

Chart 1. The retention of methotrexate (mtx) in L1210 cells and mouse small intestine after s.c. administration alone and simultaneously with leucovorin (CF). Values shown are averages of 3 separate experiments (2 to 4 individual determinations for every time point in each experiment). Standard deviation did not exceed ±20% of each mean value. FAH₂, dihydrofolate.

Chart 2. The effect of varying dosages of leucovorin (CF) given 2 hr after methotrexate (mtx) on the retention of drug in L1210 cells and mouse small intestine. Leucovorin and methotrexate were given s.c. Values shown are averages of 3 separate experiments (2 to 4 individual determinations for every time point in each experiment). Standard deviation did not exceed ±20% of each mean value. FAH₂, dihydrofolate.

Chart 3. The effect of leucovorin (CF) given 2 hr after varying dosages of methotrexate (mtx) on the retention of drug in L1210 cells and mouse small intestine. Leucovorin and methotrexate were given s.c. Values shown are averages of 3 separate experiments (2 to 4 individual determinations for every time point in each experiment). Standard deviation did not exceed ±20% of each mean value. FAH₂, dihydrofolate.

Chart 4. The effect of leucovorin (CF) given 2 hr after varying dosages of methotrexate (mtx) on the inhibition of DNA synthesis in L1210 cells and mouse small intestine. Leucovorin and methotrexate were given s.c. Values shown are averages of the relative rate of [³H]UdR incorporation into DNA of drug-treated versus control animals obtained in 3 separate experiments (2 to 4 individual determinations for every time point in each experiment). The standard deviation did not exceed ±20% of each mean value for absolute rate of incorporation.

time course for recovery of DNA synthesis after each dosage of methotrexate is shown in Chart 4. The effect of calcium leucovorin, 24 mg/kg s.c., when given 2 hr after methotrexate on the inhibition and recovery of [³H]UdR incorporation is also shown in Chart 4. Following calcium leucovorin, an earlier recovery, which was also synchronous, was seen in L1210 cells and small intestine. A cycle of incorporation was initiated within 30 min after administration. This was barely detectable (L1210 cells) or not detectable (small intestine) after methotrexate, 400 mg/kg, but occurred to a greater extent after 96 mg/kg and to an even greater extent after 12 mg/kg. Maximum levels of incorporation within this cycle of synthesis occurred at 4 to 6 hr after calcium leucovorin and never exceeded 25% (only after methotrexate, 12 mg/kg) of control levels of incorporation in each tissue. By 9 hr (small intestine) or 10 hr (L1210 cells) after calcium leucovorin, incorporation of label returned to an undetectable level (<0.5%) of control values. In animals that received methotrexate, 12 mg/kg, another cycle of synthesis reaching 150% of control level began 15 hr after calcium leucovorin.
in both L1210 cells and small intestine. In animals that received methotrexate, 96 mg/kg, a 2nd cycle of synthesis of approximately the same magnitude as the 1st cycle was also observed 15 hr after calcium leucovorin in L1210 cells. In this same group of animals, a greater cycle of synthesis reaching 150 to 200% of control levels of incorporation began at about 35 hr after calcium leucovorin in both L1210 cells and small intestine. No incorporation of label other than that occurring during the initial cycle of synthesis in L1210 cells was observed for up to 100 hr in both tissues in animals receiving 400 mg/kg.

By a comparison of these data to those shown in Chart 3, it can be seen that the initial cycle of synthesis with calcium leucovorin after methotrexate, 12 and 96 mg/kg, in both L1210 cells and small intestine occurred during a time when tissue levels of drug were well above the dihydrofolate reductase content. It can also be seen that the major cycle of synthesis observed in animals receiving these dosages occurred about the time drug levels approached the dihydrofolate reductase content in each tissue. These results permit the same correlation between recovery of DNA synthesis and the persistence of exchangeable drug obtained in experiments (Charts 3 and 4; Ref. 35), in which animals were given drug alone.

Also, simultaneous administration of calcium leucovorin, 24 mg/kg s.c., with methotrexate, 12 mg/kg s.c., induced a time course for recovery of [3H]UdR incorporation in both L1210 cells and small intestine, which was similar (data not shown) to that obtained when a 2-hr interval was used (Chart 3). Although the rate of incorporation within the 1st 2 hr was reduced to only 1 to 5% of the control level, the rate in both tissues increased to 20 to 25% in an initial cycle of synthesis 4 to 6 hr after administration. A 2nd and larger cycle of synthesis had an onset coincident with a return of drug levels to the dihydrofolate reductase equivalence in both tissues which reached a magnitude of 150 to 200% of control incorporation rates.

In another experiment shown in Charts 5 and 6, we evaluated the calcium leucovorin dose dependence for inducing recovery of DNA synthesis in the face of exchangeable tissue levels of methotrexate. Dosages of calcium leucovorin of 3 to 24 mg/kg were given s.c. to animals 2 hr after methotrexate, 12 mg/kg s.c. This dosage of methotrexate was used because not only did it allow the highest initial rate of recovery of DNA synthesis by 24 mg/kg (Chart 4), but also recovery occurred during a period in which drug levels in tissue were still above the dihydrofolate reductase content (Chart 3). In both L1210 cells and small intestine, the [3H]UdR incorporation rates during the initial cycle of recovery of DNA synthesis exhibited dose dependence to leucovorin, 12 mg/kg (Charts 5 and 6). As seen in the previous experiment, peak levels of incorporation at each dosage were observed somewhat earlier in small intestine. In both tissues, the rate and maximal level of incorporation increased to a dosage of 12 mg/kg, but at calcium leucovorin, 12 and 24 mg/kg, the effect on incorporation was essentially the same. Peak levels of incorporation never exceeded 20 to 25% of control values. In both L1210 cells and small intestine, a 2nd cycle of synthesis was observed (Chart 5) after calcium leucovorin, 12 and 24 mg/kg. These were
similar in magnitude (peak levels of incorporation were 100 to 140% of control values) and showed an onset (14 to 16 hr in L1210 cells and 10 to 12 hr in small intestine) that was approximately coincident with the return of exchangeable drug levels to the dihydrofolate reductase equivalence in mice receiving calcium leucovorin, 24 mg/kg, 2 hr after methotrexate, 12 mg/kg (Chart 3). At calcium leucovorin levels of 3 and 6 mg/kg, recovery in small intestine was indistinguishable from that seen with methotrexate, 12 mg/kg alone, and a barely detectable cycle of synthesis was observed in L1210 cells. These latter observations could be explained by the assumption, based on an expected continuum of effects between 3 and 24 mg/kg, that leucovorin doses of both 3 and 6 mg/kg had a minimal or no effect on the egress of exchangeable drug. Other results (not shown) related to data given in Chart 3 have, in fact, been obtained which validates this assumption for the lower doses (3 to 12 mg/kg).

Pharmacokinetic and Biochemical Effects of Calcium Leucovorin Given 16 Hr after Methotrexate. The effect of calcium leucovorin on tissue levels of drug when given 16 hr after methotrexate, 400 mg/kg s.c., is shown in Chart 7. The administration of calcium leucovorin, 400 mg/kg s.c., resulted in a rapid loss of exchangeable drug in L1210 cells and small intestine. In both tissues, drug levels approached the dihydrofolate reductase content by 30 hr. The same effect of calcium leucovorin was obtained in both tissues at dosages as low as 24 mg/kg s.c. (only data for leucovorin, 24 and 400 mg/kg s.c., are shown in Chart 7). At calcium leucovorin levels of 12 mg/kg s.c., drug loss was not as rapid in L1210 cells or small intestine and drug levels did not approach enzyme content until 50 hr.

The effect of calcium leucovorin in the same experiment on the inhibition of DNA synthesis by methotrexate is seen in Chart 8. During the period of the experiment (approximately 100 hr), the incorporation of labeled UdR remained undetectable beginning after the 1st 90 min following treat-

![Chart 7. The effect of varying doses of leucovorin (CF) given 16 hr after methotrexate (mtx) on the retention of drug in L1210 cells and mouse small intestine. Leucovorin and methotrexate were given s.c. Values shown are averages for 5 separate experiments (2 to 4 individual determinations for every time point in each experiment). Standard deviation did not exceed ±25% of mean values.](chart7)

![Chart 8. The effect of varying dosages of leucovorin (CF) given 16 hr after methotrexate (mtx) on the inhibition of DNA synthesis in L1210 cells and mouse small intestine. Leucovorin and methotrexate were given s.c. Values shown are averages for the relative rate of [3H]UdR incorporation in drug-treated versus control animals in 4 separate experiments (2 to 4 individual determinations for every time point in each experiment). Standard deviation did not exceed ±20% of each mean value for absolute rate of incorporation.](chart8)
dosage of calcium leucovorin also reached enzyme level at the same time.

The difference in the extent of recovery of DNA synthesis in L1210 cells following leucovorin, 24 and 400 mg/kg, given 16 hr after methotrexate is reflected in the data on tumor cell loss given in Chart 9. With drug alone at 400 mg/kg s.c., at least a 3-log decrease in cell number in the peritoneal cavity can be demonstrated during a period of 100 hr. Following calcium leucovorin, 400 mg/kg s.c., 16 hr after drug, the total cell loss did not reach 1 log. However, after treatment with leucovorin, 24 mg/kg s.c., total cell loss was almost 2 logs in magnitude.

We have also noted (Chart 10) that this regimen of therapy (methotrexate, 400 mg/kg s.c., followed 16 hr later by calcium leucovorin, 24 mg/kg s.c.) is appreciably effective in avoiding toxicity to methotrexate. Approximately 15 to 20% of the animals treated die of toxicity. This compares to 100% toxic deaths in animals receiving methotrexate, 400 mg/kg s.c., alone.

DISCUSSION

Both pharmacokinetic and biochemical effects have been associated with the administration of calcium leucovorin, either concurrently or after methotrexate, during this study (see also Ref. 35). The exact temporal relationship between methotrexate and calcium leucovorin administration influences the magnitude and dose dependence of each effect in the tissues studied. The pharmacokinetic effects of calcium leucovorin in L1210 cells and small intestine can be attributed to the same countertransport phenomenon described (12, 13) during transport experiments in vitro with tumor cell suspensions. As plasma levels of methotrexate continue to fall after administration, the net rate of exchangeable drug loss from tissue will reflect the magnitude of drug influx versus efflux. Following the administration of leucovorin [a naturally occurring folate which competes with methotrexate for the same membrane transport carrier (12, 13, 34)], influx is curtailed and the net rate of drug loss from tissue is greater. Differences in the leucovorin dose dependence for this pharmacokinetic effect at 2 hr (Charts 2 and 3) and 16 hr (Chart 7) after methotrexate are not unexpected in view of the drastic difference (3, 32, 35, 41) in plasma levels of methotrexate at these times.

As in studies (35) measuring onset of recovery of DNA synthesis following methotrexate alone, calcium leucovorin appears to effect recovery in a synchronous fashion. At 2 hr after drug (Charts 4 to 6), an initial cycle of synthesis occurred almost immediately after calcium leucovorin was given and before there was an obvious effect on tissue levels of drug. Since the magnitude of this cycle of synthesis was relatively small, it has doubtful pharmacological significance. This conclusion is tentative and relies on the assumption that, if there was an expansion in the dUMP pool in drug-treated animals, which would reduce the rate of [3H]UdR incorporation, it would not be large enough to affect the [3H]UdR incorporation rate for more than a brief portion of the cycle (see relevant discussion in Ref. 35). The extent to which this initial cycle of synthesis occurred depended upon the actual tissue level of drug at the time calcium leucovorin was given. The diminution of incorporation seen as methotrexate dosages were increased (Chart 4) could be related to the exclusion of calcium leucovorin from cells as plasma levels of drug are increased. At the lowest dosage of methotrexate used (12 mg/kg), a calcium leucovorin dosage of 12 or 24 mg/kg achieved the same maximal recovery of DNA synthesis. The onset of recovery apparently occurred prior to any appreciable pharmacokinetic effect, i.e., while tissue levels of drug are substantially above dihydrofolate reductase levels. Moreover, since the amount of calcium leucovorin entering cells should have been different at each dosage, calcium leucovorin transport could not
have been a limiting factor to the extent of recovery. A similar situation, with regard to the initial cycle of recovery of deoxyuridine incorporation, was observed during experiments in which calcium leucovorin was given 16 hr after methotrexate, 400 mg/kg. The pharmacokinetic (Chart 7) and biochemical (Chart 8) effects induced by calcium leucovorin, 24 or 400 mg/kg, were essentially identical. The transport of calcium leucovorin under these conditions, again, could not be limiting.

The reason for a relatively low maximum for incorporation in the cycle of synthesis occurring immediately after calcium leucovorin is given is not known. The fact that a pharmacokinetic effect is demonstrable (Chart 3) at most dosages would appear to suggest that substantial amounts of calcium leucovorin were transported into tissue and that the initial cycle of synthesis was mediated by enzymatic conversion of leucovorin in situ. The maximal level of incorporation observed, then, may reflect a limitation in the capacity for conversion of leucovorin to 5,10-methylene-tetrahydrofolate during a brief and transitory accumulation of intracellular leucovorin because of a rapid fall in plasma levels shortly after administration.

It is also possible that, shortly after each dosage of methotrexate used or for more prolonged periods of time after the higher dosages, intracellular levels of drugs are high enough to, at least, partially block the methylation of dUMP by a direct action on thymidylate synthetase. Estimated intracellular concentration of exchangeable drug 2 hr after the lowest dosage of methotrexate (12 mg/kg) is in the vicinity of 10⁻³ M in both small intestine and L1210 cells. Exchangeable intracellular drug 16 hr after 400 mg/kg is at a similar concentration in each tissue.

The major biochemical effect of calcium leucovorin administration appears to be a direct consequence of the pharmacokinetic effect achieved in tissue. When a more rapid rate of tissue loss occurred, an earlier onset of recovery of DNA synthesis at a substantial level was the result. In this respect, the effect of calcium leucovorin given at either 2 or 16 hr appears to be similar in both L1210 cells and small intestine with one important exception. At 16 hr after methotrexate, 400 mg/kg, the administration of leucovorin at 24 or 400 mg/kg appears to achieve an identical pharmacokinetic effect and a comparable biochemical effect in small intestine. Although the same dosages of calcium leucovorin achieve the same pharmacokinetic effect in L1210 cells, only with leucovorin, 400 mg/kg, does one see a major resumption in DNA synthesis. These results are in close agreement with data showing a far greater tumor cell kill (ca. 2 log) at the lower leucovorin dosage. Also, regimens with leucovorin doses of either 24 or 400 mg/kg s.c. 16 hr after methotrexate, 400 mg/kg, prevent lethal toxicity in approximately 80% of leukemic mice (Chart 10). In addition, in agreement with the data on tumor cell kill, the antileukemic effect of leucovorin levels of 24 mg/kg, demonstrable during therapy experiments (F. M. Sirotnak, unpublished results), is 2-fold greater than at 400 mg/kg leucovorin.

It would appear that some difference exists in L1210 cells versus small intestine, which determines the ability for resuming folate coenzyme synthesis after prolonged duration of inhibition. This determined the necessity 16 hr after methotrexate, 400 mg/kg, for high dosages of leucovorin for recovery in L1210 cells, even though leucovorin at 24 and 400 mg/kg mediates an identical rate of drug loss from tissue. These findings will probably require confirmation at a biochemical level before their exact significance can be determined in respect to the question of the basis of leucovorin rescue in general, particularly in the case of "normally" nonresponding tumors. However, the results would appear to provide some information as to the possible basis for rescue in this animal model. Additional data in other models of the type provided here and in the companion report (35), as well as therapeutic findings similar to that already reported (8-11, 16), should eventually provide a general pharmacological perspective of the high-dose methotrexate-leucovorin modality which can be extrapolated with some utility to the clinic.

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