Augmentation of the Therapeutic Activity of Lometrexol [(6-*R*)5,10-Dideazatetrahydrofolate] by Oral Folic Acid[1]

Teresa Alati, John F. Worzalla, Chuan Shih,[2] Jesse R. Bewley, Sidney Lewis, Richard G. Moran, and Gerald B. Grindey[3]

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285 [T. A., J. F. W., C. S., J. R. B., G. B. G.]; Department of Radiology, Wishard Memorial Hospital, Indianapolis, Indiana 46202 [S. L.]; and Department of Pharmacology and Toxicology and the Massey Cancer Center, Medical College of Virginia, Richmond, Virginia 23298 [R. G. M.]

ABSTRACT

Recent clinical trials with lometrexol [(6*R*)-5,10-dideazatetrahydrofolate] have revealed a level of toxicity in humans that was not predicted on the basis of previous *in vivo* preclinical studies. Because standard laboratory animal diets contain high levels of folic acid relative to human folate intake, the toxicity and therapeutic activity of lometrexol was studied in mice under conditions of restricted dietary folate intake. Remarkably, the lethality of this drug increased by three orders of magnitude in mildly folate-deficient mice, mimicking the unexpected toxicity seen in humans. Lometrexol had limited therapeutic activity in folate-deficient mice bearing the C3H mammary adenocarcinoma, compared with the substantial therapeutic index for treatment of this tumor in animals on standard diet. When folic acid was administered p.o. to mice that were mildly folate deficient, antitumor activity was again observed at nontoxic doses of lometrexol, and the range of lometrexol doses that allowed safe therapeutic use of this drug increased at higher dietary folate intake. At a fixed dose of lometrexol, the antitumor effects in animals were dependent on the level of dietary folate and went through a distinct optimum. Excessively high folate intake reversed the antitumor effects of lometrexol. Optimization of the folic acid content in the diet and of the lometrexol dosage are predicted to have substantial impact on the clinical activity of this class of drugs.

INTRODUCTION

Lometrexol[4] is a very potent folate antimetabolite that has no measurable activity against either of the two previously exploited targets for chemotherapy in the folate metabolic pathway, dihydrofolate reductase and thymidylate synthase (1, 2). Rather, it is active as an antiproliferative agent by virtue of inhibition of the first folate-dependent enzyme of *de novo* purine synthesis, *i.e.*, glycinamide ribonucleotide formyltransferase (3). Several lines of evidence indicate that metabolism of lometrexol to polyglutamate forms by FPGS plays a major role in the action of this compound. Enzyme kinetic studies have demonstrated that the long chain polyglutamates have much lower kinetically determined K_is (4). Cellular studies demonstrate that the cytotoxic events initiated by lometrexol exposure occur or continue after drug exposure, apparently as a result of the accumulation of polyglutamate metabolites (5). *In vitro* studies have shown that lometrexol is a very efficient substrate for human and mouse FPGS (3), and that polyglutamate metabolites accumulate rapidly and extensively in tumor cells exposed to drug (6). In addition, lometrexol binds very tightly to the folate receptor species studied thus far (7–9) and is an excellent substrate for the reduced folate transport system (9). Most of these characteristics distinguish lometrexol from the prototypical folate antimetabolite used in cancer chemotherapy, methotrexate.

Lometrexol was advanced to clinical trial as a result of its broad spectrum activity against a series of transplanted mouse tumors and of human tumor xenografts (10, 11), as well as a result of its novel mechanism of action and biochemical profile (1–9). The early Phase I clinical trials (12–15) revealed several unexplained patterns of behavior of this drug in humans. The most surprising was the extreme potency of the compound; toxicity was observed at doses that were a small fraction of those predicted from the previous extensive animal studies. Toxicity to platelets, upon onset, was prolonged, and there was a distinctly cumulative nature to the hematopoietic toxicity (12, 15). These toxicological characteristics have threatened the continued development of lometrexol; a schedule of lometrexol, used alone, suitable for a Phase II trial has not been found, despite evaluation of several schedules. Yet, in several of the reported Phase I trials of this drug, there have been anecdotal reports of substantial tumor responses (12–15).

Several years ago, we had noted that the administration of folic acid to animals protected against the toxicity of this compound without compromising its antitumor activity. The previous preliminary reports of this work (16, 17) have prompted second generation Phase I trials that now appear to have demonstrated the protective effects of folic acid against lometrexol toxicity in humans (18, 19)[5,6] and which are currently testing whether protocols involving the administration of folic acid will allow the toxicity of lometrexol to be clinically manageable. In this report, we described the preclinical evidence that initiated these trials and describe the rather startling relationship between the level of intake of folic acid and the toxicity of lometrexol.

MATERIALS AND METHODS

Lometrexol was synthesized and purified as described previously (1, 3). Folic acid was purchased from Sigma Chemical Co. (St. Louis, MO). C3H female mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA) and weighed 20–23 g when used in these experiments. Mice were housed in temperature- and humidity-controlled rooms and were fed either a standard laboratory rodent chow or a folic acid-deficient diet containing 1% succinylsulfathiazole; both diets were purchased from Ralston Purina Co. (St. Louis, MO). The average content of folates from natural sources in both diets was found to be 0.03 ppm, whereas the standard diet was analyzed to contain 7.3 ppm of added folic acid. It was estimated that mice on a standard diet ingested 1 to 2 mg/kg/day of folates, while mice on a low folate diet ingested 0.001 to 0.008 mg/kg/day. In some studies, folic acid was added to the drinking water and was solubilized with sodium bicarbonate; in other studies, solubilized folic acid was administered once a day by oral gavage. Food and water were provided *ad libitum*. Consumption of food (4 to 5 g/day per mouse) or water (4 to 5 ml/day per animal) was not appreciably different among groups of animals. The content of folates in serum and in washed RBC of mice fed each diet was determined by a competitive binding assay procedure (Quantaphase folate radioassay kit; Bio-Rad Laboratories, Hercules, CA) using folic acid as a standard.

Received 10/24/95; accepted 3/14/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by NIH Grant CA 27605 (to R. G. M.).

[2] To whom requests for reprints should be addressed, at Lilly Research Laboratories, Lilly Corporate Center, 307 East McCarty Street, Indianapolis, IN 46285-0540.

[3] Deceased November 16, 1993.

[4] The abbreviations used are: lometrexol, (6*R*)-5,10-dideaza-5,6,7,8-tetrahydrofolate; FPGS, folylpoly-γ-glutamate synthetase.

[5] A. Hilary Calvert, personal communication.

[6] J. Roberts, personal communication.

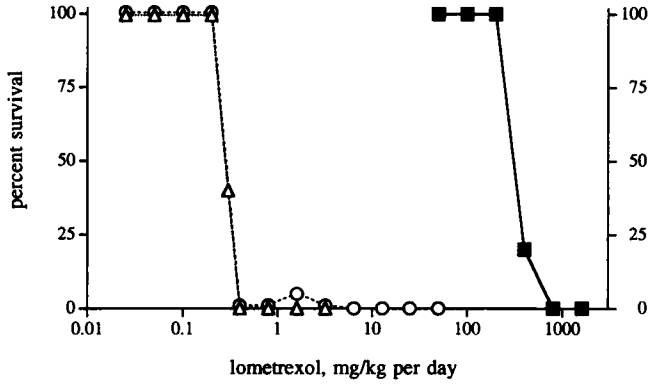

Fig. 1. The toxicity of lometrexol to C3H mice is increased by a folate-deficient diet. Mice were fed either a standard laboratory diet (■) or a folate-deficient diet for 2 weeks (△) or for 4 weeks (○) prior to the first dose of lometrexol and for the duration of the study. Groups of mice (10 animals/group) on each diet were given five daily doses of lometrexol i.p. at the indicated doses. The data present the percentage of animals alive 3 weeks after the last dose of lometrexol, and each point represents the cumulative results from two such experiments.

The C3H mammary adenocarcinoma was implanted s.c. in the lateral flank of each mouse 1 day prior to the first dose of drug treatment. Lometrexol was solubilized in either 2.5% GAF Emulphor EL620 (Warren-Graham, Cockeysville, MD) or PBS and was administered i.p. daily for 5 days, beginning 1 day after tumor implantation. Control mice received an equivalent volume of vehicle alone. In some experiments, mice were placed on a folate-deficient mouse diet for 2–4 weeks prior to tumor implantation. The growth of this tumor was not significantly different in animals fed the standard or folate-deficient diets. Tumor dimensions were estimated by external calipers interfaced with a computer, allowing tumor weights and inhibition of tumor growth to be calculated according to the formula (20): tumor weight (mg) = width^2 (mm) × length (mm) × 0.5, and stored electronically (21).

Tumor weights were measured 10 days after the first dose of lometrexol. In each experiment, there were at least 10 animals/treatment group, and each result was confirmed in at least two independent experiments performed several weeks apart. Animals were observed for at least 21 days after the last dose of lometrexol before scoring drug-induced lethality.

RESULTS

Enhanced Lethality of Lometrexol to Mice with Dietary Restriction of Folic Acid. The toxicity of lometrexol seen in early clinical trials was observed at surprisingly low doses (6–15 mg/m^2; Refs. 12–15), considering the toxicity previously seen in mice (LD_{50} of greater than 175 mg/kg, when administered daily five times) and dogs (maximum tolerated dose of 5 mg/kg given once weekly for six weeks)[7]. Given that standard laboratory mouse diets contain high levels of folic acid, it was suspected that this unexpected poor prediction of the clinical toxicity pattern from previous animal studies was due to a difference in the folate status of laboratory chow-fed mice and that of patients with advanced cancer. Hence, the toxicity of lometrexol to C3H mice was studied after dietary deprivation of folic acid. In this study, mice fed a diet of laboratory chow *ad libitum* were found to have RBC folate levels of 836 ± 133 ng/ml and serum folate contents of 43.4 ± 14.1 ng/ml. After 2 weeks on a diet formulated without added folic acid, folate levels fell to 418 ± 61 ng/ml in RBC and to 10.2 ± 1.8 ng/ml in serum, levels comparable to those considered normal in humans (22, 23). Serum and RBC folate contents remained quite constant with longer periods on this folate-deficient diet up to at least 10 weeks; after 4 weeks, serum folates and RBC folates remained at levels (9.4 ± 1.4 and 396 ± 11 ng/ml, respectively) similar to those measured at 2 weeks.

[7] Unpublished toxicology studies on file (number D 00788; Eli Lilly and Company, Indianapolis, IN).

As in previous studies (10), lometrexol was toxic on a daily (five times) schedule only at rather high doses (LD_{50}, 300 mg/kg per day) for mice fed standard laboratory chow (Fig. 1). However, with frequent administration of lometrexol to animals that had been on a diet deficient of folic acid for 2 weeks, the toxicity of this drug was observed at 1000-fold lower doses (LD_{50}, 0.3 mg/kg per day; Fig. 1). Maintaining the mice on this folate-deficient diet for 2 additional weeks did not result in any further sensitization of the mice to lometrexol. This degree of increased toxicity is even more remarkable in view of the fact that 2 weeks of folate-deficient diet only decreased the content of folates in RBC, an indicator of tissue stores of folates, to about one-half of the control levels (see above).

The therapeutic activity of lometrexol against transplanted mouse tumors and human xenograft systems has been reported previously to be substantial in studies performed in animals fed standard mouse diets (10, 11). The therapeutic activity of this drug against the C3H mammary adenocarcinoma allowed complete suppression of the growth of tumor when measured 11 days after tumor inoculation (Fig. 2). The activity of lometrexol against the C3H tumor allowed complete suppression of the growth of this tumor at a range of doses that were without toxicity. However, when the antitumor activity of lometrexol was studied in mice with serum folate levels brought to the range of those in humans by 2 weeks of dietary restriction of folic acid intake, only limited therapeutic effects were observed, and the doses which partially suppressed tumor growth could not be increased without toxic effects (Fig. 2). In a series of experiments, even moderate inhibition of tumor growth was not observed in mice fed folate-deficient diets at any lometrexol dose without lethality to at least 2 of 10 animals (Fig. 2). The therapeutic index of lometrexol to animals fed a folate-deficient diet, measured by the shift between the dose-response curves for antitumor effects and toxicity, was less than a factor of two, whereas that for mice fed a diet with "standard," *i.e.*, high, levels of folic acid was more than a factor of 100.

Reinstatement of the Therapeutic Activity of Lometrexol with Orally-Administered Folic Acid. Increasing amounts of folic acid were administered to folate-deficient mice to reconstitute the therapeutic activity observed in standard laboratory animal diets and to study the relationship between intake of folic acid and the therapeutic ratio of lometrexol. In initial studies, folic acid was added to the drinking water of mice after they had been on a folate-deficient diet for 2–3 weeks. Mice bearing the C3H mammary tumor that were treated with folic acid in the drinking water at the lowest dose studied,

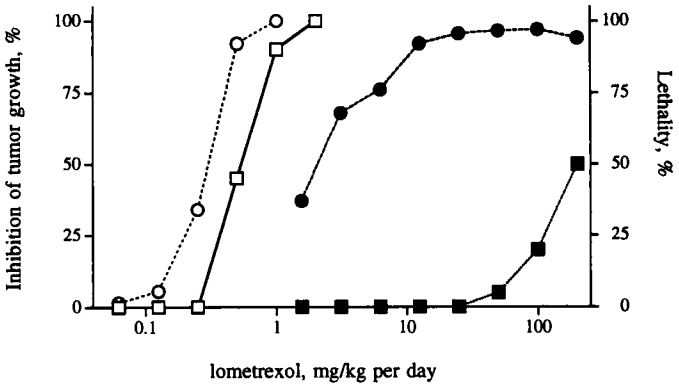

Fig. 2. Inhibition of the growth of the C3H mammary adenocarcinoma by lometrexol in mice fed a standard or a folate-deficient diet. Mice were fed either a standard diet (●, ■) or a folate-deficient diet (○, □) for 2 weeks prior to inoculation with tumor and for the duration of the study. Treatment with five daily doses of lometrexol was initiated on the day after tumor inoculation. Tumor dimensions were measured 10 days after the first dose of lometrexol. Each treatment group in an experiment contained 10 mice, and the data shown represents the sum of data from two such experiments. ○ and ●, tumor inhibition; □ and ■, lethality.

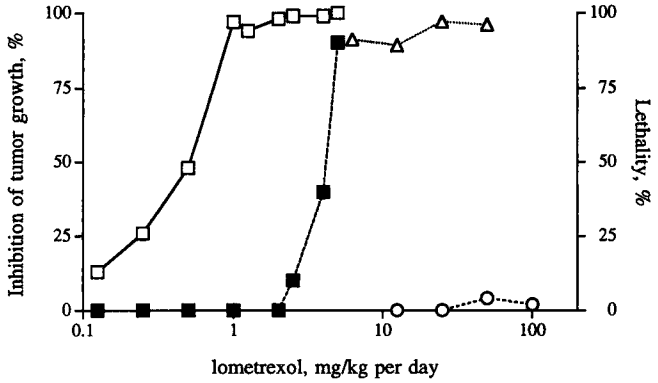

Fig. 3. Enhancement of the therapeutic effects of lometrexol against the C3H mammary adenocarcinoma in folate-deficient mice by oral folic acid. C3H female mice were fed a folate-deficient diet for 2 weeks prior to the inoculation of tumor and for the duration of the experiment. Folic acid was added to the drinking water 1 day prior to the first treatment with lometrexol and was continued for the duration of the experiment at 0.0003 (□, ■), 0.003 (△), or 1% (○) (w/v). Lometrexol was administered daily for 5 days i.p. at the indicated doses, and the size of each tumor was estimated 10 days after the first treatment with lometrexol. Each group consisted of 10 mice. ○, □, △: tumor inhibition; ■, lethality. All animals receiving 0.003% or 1% folic acid in drinking water tolerated lometrexol at any of the doses noted.

0.0003% (w/v), demonstrated higher tolerance of lometrexol given i.p. for 5 consecutive days (Fig. 3) compared to the toxicity of lometrexol in folate-deficient animals (Fig. 2). For instance, no lethality was observed at 1 or 2 mg/kg of lometrexol/day for animals with 0.0003% folic acid in the drinking water (Fig. 3), whereas doses as low as 0.4 mg/kg per day were lethal to some animals in the absence of folic acid in the drinking water (Fig. 2). This amount of folic acid intake, which was equivalent to a folic acid dose of 0.6 mg/kg/day, allowed complete suppression of tumor growth at lometrexol doses of 1 and 2 mg/kg/day, but higher doses (4 mg/kg/day) had substantial toxicity. At 0.003% folic acid in the drinking water, which resulted in an intake equivalent to 6 mg/kg of folic acid/day, a broad range of lometrexol doses allowed complete suppression of this tumor without toxicity (Fig. 3); this intake of folic acid in animals fed folate-deficient diets appeared to mimic the therapeutic response seen in animals on standard laboratory diets. However, at high levels of folic acid intake (1% in the drinking water, equivalent to 2000 mg/kg/day intake of folic acid), the antitumor activity of the drug was completely blocked at all doses of lometrexol studied (Fig. 3). The dependence of toxicity and antitumor activity on folic acid intake was studied more closely at a fixed dose of lometrexol (12.5 mg/kg/day daily five times). Again, this dose of lometrexol was uniformly lethal to animals fed a folate-deficient diet for 2 weeks, but 6 mg/kg of folic acid given daily by oral gavage completely protected the animals and allowed substantial antitumor activity (Fig. 4). Ten-fold increments in the dose of folic acid progressively reversed the antitumor activity of this fixed dose of lometrexol until, at very high doses of folic acid, the antitumor effects of this dose of lometrexol were negated. Hence, folic acid supplementation of animals allowed a substantial antitumor activity without toxicity, and the window of folic acid intake compatible with low toxicity and high antitumor activity appeared sufficiently wide to allow therapeutic use of the combination of lometrexol with oral folic acid.

DISCUSSION

We have demonstrated that oral folic acid dramatically decreases the toxicity of lometrexol to mice brought to serum and erythrocyte folate levels typical of the normal human population. Animals fed a low folate diet for a short period became more than 1000-fold more sensitive to the lethality of this drug than observed in animals fed standard laboratory diet, and the therapeutic index of lometrexol against mouse tumors was substantially reduced by such a dietary restriction. Clinical experience with lometrexol to date has indicated similar effects of administered folates on the toxicity of this drug as is documented herein in mice. Thus, it has been shown that patients who respond to lometrexol with progressive myelosuppression can be rescued from undue toxicity by the administration of the reduced folate, leucovorin (12, 15). In a clinical trial in which lometrexol was given without folic acid on a once-every-3-week schedule, cumulative hematopoietic toxicity was seen that would preclude repetitive dosing, even at the lowest dose studied, 15 mg/m^2 (12). However, an ongoing clinical trial[5] indicates that doses of at least 130 mg/m^2 can be repetitively administered every 3 weeks if patients are concurrently given oral folic acid at 5 mg daily for 7 days before and after each lometrexol dose. Previous clinical trials have found low tolerance of lometrexol on a once a week for a 3-week schedule, with even the dose found acceptable for three doses on this schedule (6 mg/m^2) poorly tolerated upon additional doses (15). A second ongoing trial[6] indicates that patients given oral folic acid daily at 5 mg/m^2 can tolerate eight weekly doses of lometrexol of at least 5 mg/m^2 with no obvious cumulative toxicity; this is the first time that frequent administration of lometrexol has been found possible in humans. Hence, repetitive administration of lometrexol in humans on either a weekly or on an every-3-week schedule appears to be feasible with (but not without) the coadministration of folic acid, and higher doses of lometrexol appear practical with folic acid administration. Although it remains to be determined whether the events responsible for these major shifts in the toxicity of lometrexol caused by folic acid are the

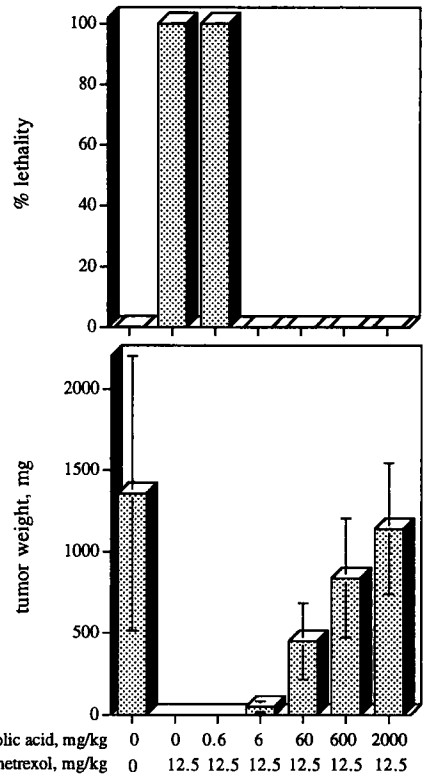

Fig. 4. Reversal of the lethality (*top panel*) and antitumor activity (*bottom panel*) of lometrexol by oral folic acid. Mice were maintained on a folate-deficient diet for 2 weeks prior to the inoculation of the C3H mammary adenocarcinoma and for the duration of the experiment. Treatment with lometrexol began 1 day later and continued for five daily i.p. injections at 12.5 mg/kg/day. Folic acid was administered by oral gavage beginning the day of tumor inoculation and continuing until tumor size was estimated, 6 days after the last dose of lometrexol.

Table 1 *Folate content of diet and maximum tolerated dose (MTD) of lometrexol*

Species	Diet	Daily folate ingestion (mg/m^2)	MTD lometrexol (mg/m^2)	Schedule	Ref.
Mouse	Standard mouse chow	3–6	600	Daily × 5	Fig. 1
			3000	Days 1, 4, 7, 10	Unpublished data
	Folate-deficient for 2 weeks	0.005–0.025	0.6	Daily × 5	Fig. 1
Dog	Standard lab chow	1–2	100	Once a week × 6	24
Man	Recommended daily allowance	0.25	?		
	Early Phase I trials	Normal diet	None found (<3)	Once a week × 3, followed by a 2-week rest, then redose	15
			None found (<15)	Every 3 weeks	12
	Ongoing trials	Normal diet + 5	>5	Weekly × 8	21
		Normal diet + 3–5	>130	Every 3 weeks	20

same in mice and humans, the folate-deficient mouse and the folate-repleted mouse are clearly important model systems for the study of this class of drugs and appear to predict clinical behavior of lometrexol.

Table 1 compares the intake of folates in the diet of mouse, dog, and humans and relates these levels to the maximal tolerated dose of lometrexol. The daily intake of laboratory mice and dogs is essentially the same when fed commercial laboratory chows, and the tolerance of both species for lometrexol appears more similar when expressed in a dosage per unit surface area than per kg of body weight. Nevertheless, the differences in schedules that have been studied in mice, dogs, and humans makes a direct comparison tenuous, given that the toxicity of lometrexol to mice is clearly schedule dependent, with the maximum tolerated dose in mice for an intermittent schedule five times higher than that for a daily schedule (Table 1). However, extrapolating between the dosage schedules used in dogs and mice, it appears that dogs are substantially more sensitive to lometrexol than mice, in spite of the similar daily intake of folate in the two species. The minimal daily intake recommended for humans is 400 μg which, for a 60-kg human, would amount to about 0.25 mg/m^2, substantially less than that ingested daily in mice or dogs fed standard laboratory diets. The fact that patients with active malignancies most often have plasma and RBC folate levels that are below normal and are often less than one-half the lower threshold of normal (24) suggests that folate intake in a population of patients on Phase 1 trials is likely to be less than that at recommended daily allowance, *i.e.*, less than 0.25 mg/m^2. The two ongoing trials are being performed at folate intake levels at least equivalent to those in experimental animals fed commercial laboratory diets (Table 1). However, it should be noted that all of the studies performed to date in mice and dogs have supplemented the diet with folic acid, similar to the conditions in the current clinical trials, but that folic acid is a "drug store artifact" not normally found in the human diet. The folate compounds ingested in normal human diets are typically fully reduced, methylated or formylated, polyglutamyl folates that are metabolized during adsorption and passage through the liver to, principally, 5-methyltetrahydrofolate (25). Folic acid follows a similar metabolic pathway during absorption and passage through the liver, but these processes are easily saturable, with free folic acid appearing in the circulation at higher oral intake (25, 26).

What events are responsible for the alteration in lometrexol toxicity induced by folic acid remains a critical and current question. Studies at Lilly Research Laboratories have shown previously that a gamma phase of elimination of lometrexol can be detected using a competitive particle concentration fluorescence immunoassay (27) in folate-deficient but not folate-replete mice. Using this assay, the levels detected for several days in mouse plasma following single bolus lometrexol were sufficient to inhibit the growth of most mammalian cells in culture. In addition, the accumulation of lometrexol in the livers of folate-deficient mice has been reported, and this accumulation was diminished by the administration of folic acid to these animals (28).[8] These observations lead to the hypothesis that the substantial and unexpected toxicity of lometrexol in humans not given concurrent folic acid and in folate-deficient mice is due to the sequestration of drug in hepatic tissue, with the subsequent slow release of drug to the circulation at toxicologically relevant concentrations. Thus, in animals not protected by folic acid preconditioning, a bolus administration of drug appears to result in a peak level of lometrexol in the blood, followed by the equivalent of a long-term infusion of parent drug.

The mechanism of this accumulation of lometrexol in liver involves metabolism to polyglutamate forms by the enzyme FPGS. Liver is rich in this critical enzyme (29), and hepatic lometrexol in folate-deficient mice has been found to be almost exclusively higher polyglutamates.[8] A recent report (30) has indicated that the metabolism of lometrexol to polyglutamates is controlled by the folate content of tissues, presumably by a direct feedback effect on FPGS. Hence, preadministration of folic acid to animals probably blocks the hepatic accumulation of lometrexol by causing an expansion of the folate content of hepatic tissue, an effect reported to occur at the dietary intake shown to block lometrexol toxicity (Fig. 4; Ref. 31). This competition between lometrexol and cellular folates for polyglutamation may be a general phenomenon; the accumulation of methotrexate as polyglutamates has been reported to result in lower folate pools in liver and erythrocytes of patients treated chronically with this drug (32, 33).

The administration of folic acid to folate-deficient animals clearly reverses both the lethal toxicity (Fig. 1) and the inhibitory effects of lometrexol on tumor (Figs. 3 and 4), but toxicity and therapeutics are affected at different doses of folic acid. There is a competitive relationship between the lethality of lometrexol and the dose of folic acid given, *i.e.*, the more folic acid given to mice, the higher the dose of lometrexol which is toxic (Fig. 3). Likewise, the more folic acid administered, the higher the dose of lometrexol required for therapeutic effects. Hence, it becomes of practical significance how to properly find the combination of drug and folic acid dosages for optimal therapeutic effect without toxic risk. It appears that the minimal folic acid conditioning needed to avert toxicity will allow the optimal therapeutic effects (Fig. 4).

ACKNOWLEDGMENTS

We thank Drs. A. Hilary Calvert, Sarah Freemantle, Franco Muggia, and John Roberts for their helpful critiques of this manuscript. We also thank Pamela Rutherford, Tracy Self, and Karla Theobald for their excellent technical assistance.

[8] L. L. Habeck, S. H. Clay, R. C. Pohland, J. F. Worzalla, C. Shih, K. G. Bemis, and L. G. Mendelsohn. Whole body disposition and polyglutamate distribution of the GAR transformylase inhibitors LY30887 and lometrexol in mice: effect of low folate diet, submitted for publication.

REFERENCES

1. Taylor, E. C., Harrington, P. J., Fletcher, S. R., Beardsley, G. P., and Moran, R. G. Synthesis of the antileukemic agents 5,10-dideazaaminopterin and 5,10-dideaza-5,6,7,8-tetrahydroaminopterin. J. Med. Chem., *28:* 914–921, 1985.
2. Beardsley, G. P., Moroson, B. A., Taylor, E. C., and Moran, R. G. A new folate antimetabolite, 5,10-dideaza-5,6,7,8 tetrahydrofolate, is a potent inhibitor of *de novo* purine synthesis. J. Biol. Chem., *264:* 328–333, 1989.
3. Moran, R. G., Baldwin, S. W., Taylor, E. C., and Shih, C. The 6-*S* and 6-*R* diastereomers of 5,10-dideaza-5,6,7,8-tetrahydrofolate are equiactive inhibitors of *de novo* purine synthesis. J. Biol. Chem., *264:* 21047–21051, 1989.
4. Baldwin, S. W., Tse, A., Gossett, L. S., Taylor, E. C., Rosowsky, A., Shih, C., and Moran, R. G. Structural features of 5,10-dideaza-5,6,7,8-tetrahydrofolate that determine inhibition of mammalian glycinamide ribonucleotide formyltransferase. Biochemistry, *30:* 1997–2006, 1991.
5. Smith, S. G., Lehman, N. L., and Moran, R. G. Cytotoxicity of antifolate inhibitors of thymidylate and purine synthesis to WiDr colonic carcinoma cells. Cancer Res., *53:* 5697–5706, 1993.
6. Pizzorno, G., Sokoloski, J. A., Cashmore, A. R., Moroson, B. A., Cross, A. D., and Beardsley, G. P. Intracellular metabolism of 5,10-dideazatetrahydrofolic acid in human leukemia cell lines. Mol. Pharmacol., *39:* 85–89, 1991.
7. Brigle, K. E., Spinella, M. J., Westin, E. H., and Goldman, I. D. Increased expression and characterization of two distinct folate binding proteins in murine erythroleukemia cells. Biochem. Pharmacol., *47:* 337–345, 1994.
8. Jansen, G., Westerhof, G. R., Kathmann, I., Rijksen, G., and Schornagel, J. H. Growth-inhibitory effects of 5,10-dideazatetrahydrofolic acid on variant murine L1210 and human CCRF-CEM leukemia cells with different membrane-transport characteristics for antifolate compounds. Cancer Chemother. Pharmacol., *28:* 115–117, 1991.
9. Pizzorno, G., Cashmore, A. R., Moroson, B. A., Cross, A. D., Smith, A. K., Marling-Cason, M., Kamen, B., and Beardsley, G. P. 5,10-Dideazatetrahydrofolic acid (DDATHF) transport in CCRF-CEM and MA104 cell lines. J. Biol. Chem., *268:* 1017–1023, 1993.
10. Shih, C., Grindey, G. B., Houghton, P. J., and Houghton, J. A. *In vivo* antitumor activity of 5,10-dideazatetrahydrofolic acid (DDATHF) and its diastereomeric isomers. Proc. Am. Assoc. Cancer Res., *29:* 283, 1988.
11. Taylor, E. C. New pathways from pteridines. Design and synthesis of a new class of potent and selective antitumor agents. J. Heterocycl. Chem., *27:* 1–11, 1990.
12. Nelson, R., Butler, F., Dugan, W., Jr., Davis-Land, C., Stone, M., and Dyke, R. Phase I clinical trial of LY264618 (dideazatetrahydrofolic acid, DDATHF). Proc. Am. Soc. Clin. Oncol., *9:* 76, 1990.
13. Young, C., Currie, V., Baltzer, L., Trochanowski, B., Eton, O., Dyke, R., and Bowsher, R. Phase I and clinical pharmacologic study of LY264618, 5,10-dideaza-tetra-hydrofolate. Proc. Am. Assoc. Cancer Res., *31:* 117, 1990.
14. Sessa, C., Gumbell, L., Hatty, S., Kern, H., and Cavalli, F. Phase I study of 5,10-dideazatetrahydrofolic acid (LY264618, DDATHF) given daily for 3 consecutive days. Ann. Oncol., *1* (Suppl.): 38, 1990.
15. Ray, M. S., Muggia, F. M., Leichman, G. C., Grunberg, S. G., Nelson, R. L., Dyke, R. W., and Moran, R. G. Phase I study of (6R) 5,10-dideaza-5,6,7,8-tetrahydrofolate: a folate antimetabolite inhibitory to *de novo* purine synthesis. J. Natl. Cancer Inst. (Bethesda), *85:* 1154–1159, 1993.
16. Alati, T., Shih, C., Pohland, R. C., Lantz, R. J., and Grindey, G. B. Evaluation of the mechanism of inhibition of the toxicity, but not the antitumor activity, of lometrexol (DDATHF) by folic acid. Proc. Am. Assoc. Cancer Res., *33:* 407, 1992.
17. Grindey, G. B., Alati, T., and Shih, C. Reversal of the toxicity but not the antitumor activity of lometrexol by folic acid. Proc. Am. Assoc. Cancer Res., *32:* 324, 1991.
18. Young, C. W., Currie, V. E., Muindi, J. F., Saltz, L. B., Pisters, K. M. W., Esposito, A. J., and Dyke, R. W. Phase I study of lometrexol (LY264618 or LTX)-improved clinical tolerance with folic acid. Proceedings of the 7th NCI-EORTC Symposium on New Drugs in Cancer Therapy, p. 136, 1992.
19. Wedge, S. R., Laohavinij, S., Taylor, G. A., Boddy, A., Calvert, A. H., and Newell, D. R. Clinical pharmacokinetics of the antipurine antifolate (6*R*)-5,10-dideazatetrahydrofolate (lometrexol) administered with an oral folic acid supplement. Clin. Cancer Res., *1:* 1479–1486, 1995.
20. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M., and Abbott, B. J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother. Rep. Part 3, *3:* 51, 1972.
21. Worzalla, J. F., Bewley, J. R., and Grindey, G. B. Automated measurement of transplantable solid tumors using digital electronic calipers interfaced to a microcomputer. Invest. New Drugs, *8:* 241–251, 1990.
22. Sauberlich, H. E. Detection of folic acid deficiency in populations. *In:* H. P. Broquist, C. E. Butterworth, Jr., and C. Wagner (eds.), Folic Acid: Biochemistry and Physiology in Relation to Human Nutritional Requirements, pp. 213–231. Washington DC: National Academy of Sciences, 1977.
23. Herbert, V. Making sense of laboratory tests of folate status: folate requirements to sustain normality. Am. J. Hematol., *26:* 199–207, 1987.
24. Hoogstraten, B., Baker, H., and Gilbert, H. S. Serum folate and serum vitamin B_{12} in patients with malignant hematologic diseases. Cancer Res., *25:* 1933–1938, 1965.
25. Halsted, C. H. Intestinal abruption and malabsorption of folates. Annu. Rev. Med., *31:* 79–87, 1980.
26. Olinger, E. J., Bertino, J. R., and Binder, H. J. Intestinal folate absorption. II. Conversion and retention of pteroylmonoglutamate by jejunum. J. Clin. Invest., *52:* 2138–2145, 1973.
27. Taber, L. D., O'Brien, P., Bowsher, R. R., and Sportsman, J. R. Competitive particle concentration fluorescence immunoassay for measuring 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) in serum. Clin. Chem., *37:* 254–260, 1991.
28. Pohland, R. C., Alati, T., Lantz, R. J., and Grindey, G. B. Whole-body autoradiographic disposition and plasma pharmacokinetics of 5,10-dideazatetrahydrofolic acid in mice fed folic acid-deficient or regular diets. J. Pharm. Sci., *83:* 1396–1399, 1994.
29. Moran, R. G., and Colman, P. D. Measurement of folyl polyglutamate synthetase in mammalian tissue. Anal. Biochem., *140:* 326–342, 1984.
30. Tse, A., and Moran, R. G. Control of the polyglutamation of 5,10-dideazatetrahydrofolate by intracellular folate pools: a novel mechanism of resistance to antifolates. Proc. Am. Assoc. Cancer Res., *35:* 304, 1994.
31. Schmitz, J. C., Grindey, G. B., Schultz, R. M., and Priest, D. G. Impact of dietary folic acid on reduced folates in mouse plasma and tissues. Relationship to dideazatetrahydrofolate sensitivity. Biochem. Pharmacol., *48:* 319–325, 1994.
32. Kamen, B. A., Nylen, P. A., Camitta, B. M., and Bertino, J. R. Methotrexate accumulation and folate depletion in cells as a possible mechanism of chronic toxicity to the drug. Br. J. Haematol., *49:* 355–360, 1981.
33. Kremer, J. M., Galivan, J., Streckfuss, A., and Kamen, B. Methotrexate metabolism analysis in blood and liver of rheumatoid arthritis patients. Arthritis Rheum., *29:* 832–835, 1986.