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ABSTRACT

The purpose of this investigation was to determine whether antitumor selectivity of the third generation thymidylate synthase inhibitor 1843U89 could be enhanced by a combination of the drug with folic acid. The effects of folic acid on toxicity of 1843U89 to the dog and mouse and on antitumor efficacy of 1843U89 in the mouse were studied. These data were compared to the effect of folic acid on the *in vitro* cell culture antitumor activity of 1843U89. The sensitivity of eight cancer cell lines (three ovarian, one colon, one ileocecal, one epidermoid, one osteosarcoma, and one breast line) to 1843U89 was tested *in vitro* in the presence and absence of folic acid. Folic acid concentrations greater than 100 μM were required to decrease 1843U89 activity in seven of the cell lines. Only the activity in HCT-8, the ileocecal line, was reversed at folic acid concentrations below 100 μM. Oral folic acid given 30 min prior to an i.v. dose of 1843U89 increased the maximally tolerated dose and the lethal dose of 1843U89, both in dogs and in thymidine-depleted mice. In mice, oral folic acid produced little or no effect upon the antitumor efficacy of 1843U89 in two of three tumor cell lines *in vivo*. HCT-8, the line that was sensitive to folate reversal *in vitro*, was also sensitive *in vivo*. The results show that an oral dose of folic acid 30 min prior to i.v. 1843U89 can block mouse and dog intestinal toxicity without decreasing efficacy of 1843U89 in two of three human tumor lines in the nude mouse. Thus, the data reported here indicate that the antitumor selectivity of 1843U89 may be enhanced through a combination of 1843U89 with oral folic acid.

INTRODUCTION

1843U89 is a third generation TS[3] inhibitor under clinical development as an anticancer agent. The compound is cytocidal against a variety of cancer cell types in culture (1–4) and is active in several antitumor models in mice (3, 5–7). 1843U89 displays several attractive features compared to other antifolates. In human cells, the major metabolite of 1843U89 is the diglutamate (8). Both 1843U89 itself and the diglutamate metabolite bind to TS with noncompetitive kinetics (1). The affinities of 1843U89 and the diglutamate for TS are virtually identical (1). In comparison, the major cellular metabolites for a variety of other antifolates, including MTX, DDATHF, 5-deazacyclotetrahydrofolate, CB3717, and Tomudex, are the tri- to hexaglutamates (9–15). Furthermore, these polyglutamates are competitive or mixed inhibitors (with strong competitive components) of their respective enzymes (1, 9, 15–18). Lastly, the affinities of these other antifolate polyglutamates are greater for their respective enzymes than are those of the parent compounds (1, 9, 12, 15–18).

These properties of 1843U89 described above participate to produce another unusual feature of 1843U89. Compared to other antifolates (9, 12, 19, 20), 1843U89 is resistant to leucovorin reversal (3). Leucovorin can influence antifolate activity by direct competition for transport or by elevation of intracellular reduced folate levels (3, 21, 22). In turn, elevated intracellular folates compete directly with competitive and mixed inhibitors for binding to their respective target enzymes, which decreases inhibition of the target enzyme (1, 9, 15–18). The cellular folates also compete with the antifolates for polyglutamation, which decreases the extent of antifolate polyglutamation and cellular retention (9, 15, 23). Since the monoglutamates of most antifolates are poorer inhibitors than the polyglutamates, decreased polyglutamation directly decreases target enzyme inhibition (1, 9, 12, 14, 15, 17–20). The effects all decrease the efficacy of the antifolates in the presence of leucovorin. Since the mono- and diglutamate forms of 1843U89 are equipotent noncompetitive inhibitors of TS, the reduced folates do not compete with 1843U89 for binding to TS, and the decreased polyglutamation of 1843U89 has less influence. The main influence of leucovorin on 1843U89 efficacy then is through competition for transport on the reduced folate transporter (3).

These striking differences of 1843U89 from a variety of other antifolates led us to seek a means to use the differences to increase antitumor selectivity. In the present studies, we have investigated the potential of combination chemotherapy with 1843U89 plus folic acid to provide the additional selectivity. We report here the use of oral folic acid to block the dose-limiting gut toxicity of 1843U89 but not its antitumor effects. A similar strategy was reported previously for the competitive inhibitor DDATHF (24, 25). Differences between the two strategies are discussed. The combination of folic acid with 1843U89 may provide a mechanism for enhanced clinical antitumor selectivity.

MATERIALS AND METHODS

Chemicals. 1843U89 was synthesized at the Wellcome Research Laboratories as described (2). It was solubilized with approximately 2 molar equivalents of NaOH in endotoxin-tested Dulbecco's PBS without calcium or magnesium (Sigma Chemical Co., St. Louis, MO), adjusted to pH 6.8–7.2, and was sterile filtered prior to use. Folic acid (Sigma Chemical Co.) for oral administration was dissolved in water by adjusting the pH to 7.2 to 7.4 with NaOH.

Cell Culture. Human tumor cell lines were obtained from the following sources: breast adenocarcinoma MCF7, ileocecal adenocarcinoma HCT-8, ovarian carcinomas A2780, OVCAR-3 and SK-OV-3 and osteosarcoma 143 B TK$^-$ (American Type Culture Collection, Rockville, MD); colon carcinoma GC3TK$^-$ (J. Houghton, St. Jude Children's Research Hospital, Memphis, TN); ileocecal adenocarcinoma HCT-8/TK$^-$ (Y. Rustum, Roswell Park Cancer Institute, Buffalo, NY); and human epidermoid carcinoma KB3–1 (Dr. Michael Gottesman, National Cancer Institute, Bethesda, MD). Cells were propagated as monolayer cultures as described (3). The basal medium for all cell lines was folate-free RPMI 1640 (GIBCO-BRL) supplemented with 10 nM calcium leucovorin and 10% dialyzed FCS [except A2780 and OVCAR-3, which contained 10% heat-inactivated (30 min at 56°C) FCS that had been depleted of thymidine by an incubation for 15 min at 37°C with 1 unit/ml *Escherichia coli* thymidine phosphorylase]. MCF7, A2780, and OVCAR-3 media contained 10 μg/ml insulin (Sigma I-1882); the plates for these three cell lines were coated with 10 μg/ml PepTite-2000 (Telios Pharmaceutical Research Products) for 1 h to aid cell attachment.

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[3] The abbreviations used are: TS, thymidylate synthase; MTX, methotrexate; DDATHF, 5,10-dideazatetrahydrofolate, lomotrexol; diglutamate of 1843U89, 1843U89 with one additional glutamate; PEG-TPase, polyethylene glycol-thymidine phosphorylase; RTCB, residual tumor cell burden; MTD, maximum tolerated dose.

***In Vitro* Protection Studies.** Cell growth inhibition and reversal experiments were done as described (3). The ability of folic acid or leucovorin to block the cytotoxicity of 1843U89 in several human tumor cell lines was also assessed by a clonogenic assay. Log phase cells were allowed to attach overnight to 60-mm plates before a 24-h exposure to 1843U89 ± folic acid or leucovorin (0.01–100 μM). After the 24-h drug exposure, drug was removed, the plates were washed with 3 ml of sterile Dulbecco's PBS without calcium or magnesium, and 3 ml of fresh media containing the appropriate levels of folic acid or leucovorin (but not 1843U89) were added. The plates were incubated 10–14 days at 37°C in 95% air/5% CO_2, the media was removed, and the plates were stained with a solution of 10 mg/ml crystal violet in 10% formaldehyde, 5% acetic acid, and 60% methanol (5-min stain, water rinse, and air dry). Colonies of >0.1 mm were counted on an image analyzer (Artek Systems Corp.; Model 982B). All determinations were run in triplicate. The inoculum density was adjusted to obtain 200–300 colonies on the control plates, and the 1843U89 concentration was chosen from a previous titration to yield ~80% inhibition of colony formation.

Animals. Male CD-1 and female BALB/c athymic mice mice were purchased from Charles River Laboratories (Wilmington, DE) and used for all studies. Mice were housed in polycarbonate, filter-capped micro-isolator cages and given sterilized food and water *ad libitum*. Female purebred beagles at least 10 months of age were purchased from Marshall Farms, USA, Inc. (North Rose, NY) and used for all studies. Beagles were housed individually in cages and given Prolab Canine 1600 Certified Diet (Agway, Inc.) and municipal tap water *ad libitum*. All animals were maintained in a temperature (22°C ± 1°C)-, humidity (50 ± 10%)-, and photoperiod (12-h light/12-h dark)-controlled room with 10 to 15 air changes/h.

Dog Protection Studies. 1843U89 was administered i.v. by slow bolus infusion via the cephalic vein once daily for five consecutive days. From a 20 mg/ml solution of 1843U89, 0.3, 0.6 and 0.9 ml/kg were dosed to obtain 6, 12, and 18 mg/kg, respectively. Oral folic acid was given 30 to 40 min prior to 1843U89. To facilitate rapid ingestion of the entire dose, folic acid was given by gavage by #12 gelatin capsules as a 50 mg/ml solution. Animals were monitored during the dosing period and for 30 days after the dose.

Mouse Protection Studies. The ability of folic acid to block weight loss and death was determined in mice given 1843U89. The high plasma thymidine levels in mice, which prevent the toxicity of thymidylate synthase inhibitors, was reduced by treating the mice with a conjugate of PEG-TPase. PEG-TPase (Wellcome Research Laboratories, Research Triangle Park, NC) was administered at 2500 units/kg, i.p., on days 1 and 4, 1.5 h prior to 1843U89, which was dosed at 200 and 400 mg/kg, i.p., bid × 7 days. Folic acid was dosed p.o. at 300 mg/kg 30 min prior to each 1843U89 dose. In all cases, five mice were used per group. The mice were maintained as described above and monitored for weight change and death.

Subrenal Capsule Tumor Growth Assay. Prior to their use *in vivo*, all cell lines were verified to be free of *Mycoplasma* bacteria and adventitial murine viral pathogens by mouse antibody production test (Charles River Biotechnical Laboratories). Cell preparation, implantation, and evaluation procedures of cell lines and tumors in *in vivo* studies have been described elsewhere (3, 26). Briefly, cells were implanted as fragments under the renal capsule of 18–22 g male CD-1 athymic mice by published procedures (26). Length and width of implanted fragments were measured at the time of surgery and again at autopsy. 1843U89 was administered to mice by the i.p. route in volumes of either 10 or 20 ml/kg. At each dose interval, mice were weighed, and doses were adjusted according to individual body weight to the nearest gram. In all cases, five mice were used per group. Antitumor activity was assessed in three ways. Differences between various treatments were determined by Kruskal-Wallis analysis (SAS, Cary, NC) of relative tumor volumes of individual mice by group; values of $P < 0.05$ were used to indicate statistical significance. The percentage of growth inhibition was calculated from group median doublings of treated and control tumors, treatment initiation to sacrifice. Histological evaluation of tumors in early studies indicated that 1843U89 might effect lysis of tumor cells with varying degrees of replacement with scar tissue. Accordingly, the RTCB of each lesion was also assessed and scored as follows by an individual unfamiliar with the particular study: histological score = 0, no effect of therapy evident from histological evaluation of lesion (RTCB > 60%); histological score = 1, equivocal to minimal effect (RTCB 40 to 60%); histological score = 2, moderate to strong effect (RTCB 5% to 40%); and histological score = 3, strong effect (RTCB < 5%).

RESULTS

***In vitro* Effects of Folic Acid or Leucovorin on 1843U89.** Based upon three-way mutual competition for transport among 1843U89, leucovorin, and MTX but not folic acid, we have reported that 1843U89 appears to enter MOLT-4 human T-cell leukemia on the reduced folate carrier (3). We have now also observed similar mutual transport competition among 1843U89, MTX, and leucovorin but not folic acid in MCF7 breast adenocarcinoma and SW480 and WiDr colon carcinoma cell lines, which suggests broader tumor use of this transporter for 1843U89 (data not shown). Since folic acid does not compete for this 1843U89 tumor cell transport and the products of cellular metabolism of folic acid, the reduced folates, do not compete for 1843U89 binding to TS (1), folic acid should not effectively reverse 1843U89 cytotoxicity in tumor cells. Table 1 shows the ability of folic acid and leucovorin to reverse the inhibition of clonogenic growth by 1843U89 in eight separate human tumor lines. HCT-8 was the most sensitive line to folic acid reversal, requiring 28 μM for 50% reversal of 1843U89 activity; all others required over 100 μM for 50% reversal. In contrast to folic acid, leucovorin, which does compete for 1843U89 transport in the μM range, reversed 1843U89 cytotoxicity more efficiently (Table 1). To further investigate this resistance of 1843U89 to folic acid or leucovorin reversal, reversal of 1843U89 and another TS inhibitor, Tomudex, by the two agents were compared in three cell lines (Table 2). Since Tomudex has been reported to be efficiently reversed by leucovorin (3), we expected Tomudex activity to be more sensitive to both folic acid and leucovorin. As can be seen in Table 2, both 100 and 10 μM leucovorin very efficiently reversed the activity of Tomudex but only weakly reversed the activity of

Table 1 *Reversal of 1843U89 cytotoxicity by folic acid or leucovorin in cells in culture*

		Concentration required for 50% reversal of 1843U89 cytotoxicity (μM)		% reversal of 1843U89 cytotoxicity with 1 or 10 μM reversal agent[a]			
				Folic acid		Leucovorin	
Cell line	Type	Folic acid	Leucovorin	1 μM	10 μM	1 μM	10 μM
SK-OV-3	Ovarian	>100	0.6	0	0	63	95
A2780	Ovarian	>100	5	0	13	31	63
OVCAR-3	Ovarian	>100	10	5	10	41	51
HCT-8	Ileocecal	28	0.07	25	44	94	98
KB3-1	Epidermoid	>100	3.7	0	0		
143B TK$^-$	Osteosarcoma	>100	5.0	0	4		
GC3/TK$^-$	Colon	>100	8	0	7	12	53
MCF7	Breast	>100	32	0	3	21	25

[a] Reversal of 80% inhibition by the drug; thus, 50% reversal leads to 40% inhibition remaining. Partial (80%) inhibition was chosen to be able to detect small reversal effects.

Table 2 *Effect of folic acid or leucovorin on cell growth inhibition by Tomudex and 1843U89*

			Reversal agent					
			100 μM leucovorin		10 μM leucovorin		25 μM folic acid	
	Drug	IC_{50} (nM)	IC_{50} (nM)	Fold increase	IC_{50} (nM)	Fold increase	IC_{50} (nM)	Fold increase
	Tomudex							
SW480		0.4	>55,000	>110,000[a]	10,000	25,000[a]	7	20
GC3TK$^-$		2.1	>1,000	>500	1,000	500	52.8	26
HCT-8		1.4					32	23
	1843U89							
SW480		1	30	30[a]	1	1[a]	1.2	1.2
GC3TK$^-$		0.34	11.6	34	3.4	10	1	3
HCT-8		0.82					2.5	3

[a] From Duch *et al.* (3).

1843U89 in either GC3TK$^-$ or SW480 cells. Furthermore, 25 μM folic acid increased the IC_{50} of Tomudex approximately 20-fold in all three of SW480, GC3TK$^-$, and HCT-8 cells, while these conditions only increased the 1843U89 IC_{50} 1- to 3-fold. Thus, the efficacy of 1843U89 in cell culture is less sensitive than Tomudex to reversal by either leucovorin or folic acid, and folic acid is the less effective reversing agent.

Effect of Oral Folic Acid on Toxicity of 1843U89 in the Beagle Dog. As shown in Table 3, the MTD for i.v. 1843U89 on a daily 5-day schedule in the beagle dog was between 2 and 6 mg/kg/day; at 2 mg/kg, toxicity was mild, but 6 mg/kg was lethal. Gastrointestinal toxicity, including severe diarrhea and maturation arrest enteritis, was dose limiting. Hematological toxicity was observed but was consistently mild and never dose limiting (data not shown). Thus, a mechanism to decrease the gastrointestinal toxicity should result in an increased MTD for the drug.

Table 3 *Toxicity of five daily i.v. doses of 1843U89 in beagle dogs*[a]

	1843U89 dose (mg/kg/day)			
	2		6	
	M[b]	F	M	F
	0[c]	0[c]	2[c]	2[c]
Clinical signs: hypothermia, labored breathing, retching, righting reflex slow or absent, pale gingiva, prostration, salivation, and lacrimation	No	No	Yes	Yes
Diarrhea, emesis, and/or decreased activity, and/or dehydration	Yes	Yes	Yes	Yes
Body weight decrease	Yes	Yes	Yes	Yes
Food consumption decrease	Yes	Yes	Yes	Yes
Gross pathology: dark red discoloration of the lining and contents of intestinal tract, and/or severe, reddened mucosal lining or brown, watery contents in stomach; and/or longitudinal dark red streaking of the colon, and/or severe thymic hemorrhage; and/or minimal to severe injection site hemorrhage	No	No	Yes	Yes
Histopathology: maturation arrest enteritis of intestinal tract; severe thymus atrophy, congestion; and/or bile stasis in liver; minimal to severe involution of the white pulp of spleen; mild to moderate involution of cervical and mesenteric lymph nodes	No	No	Yes	Yes
Maturation arrest of myeloid elements in bone marrow	No	No	Yes	No

[a] Four dogs were treated at each dose level of 1843U89, two males and two females.
[b] M, male; F, female.
[c] Deaths.

The ability of folic acid to protect against the lethal toxicity of 1843U89 was tested in beagle dogs given a lethal dose of drug with or without prior treatment with oral folic acid. On a 5-day dosing schedule, 6 mg/kg/day 1843U89 i.v. was a 100% lethal dose in dogs (Table 3). By contrast, when 50 mg/kg oral folic acid was given 30 min prior to the i.v. doses of 1843U89 (Table 4), all of the animals survived both this dose and twice this dose (6 and 12 mg/kg/day 1843U89, respectively). However, even with the oral 50 mg/kg folic acid doses, five daily doses of 18 mg/kg/day 1843U89 i.v. were fatal to all dogs treated. Importantly, dose-limiting toxicity at 18 mg/kg 1843U89 with 50 mg/kg folic acid was intestinal and comparable to that seen with 6 mg/kg 1843U89 alone. Thus, oral administration of folic acid protects against the lethal toxicity of i.v. 1843U89, thereby raising the MTD for the drug more than 2-fold and potentially greater than 6-fold [MTD for 1843U89 alone is between 2 and 6 mg/kg (Table 3); MTD for 1843U89 plus folic acid is between 12 and 18 mg/kg].

In other experiments (data not shown), it was found that five daily doses of up to 100, 200, or 500 mg/kg/day of folic acid were well tolerated and effective in protection against a 10 mg/kg/day dose of 1843U89. The minimal dose of folic acid to provide protection to all dogs was between 10 and 50 mg/kg/day since a dose of 10 mg/kg/day of folic acid provided protection to three of four dogs receiving the 10 mg/kg/day 1843U89. However, the use of multiple daily doses of folic acid did permit the reliable use of lower individual doses; thus, 16.7 or 10 mg/kg folic acid three times daily (30 min before and 3 and 6 h after the 1843U89 dose) each protected four of four dogs receiving 10 mg/kg/day 1843U89.

Leucovorin at 10 to 200 mg/kg/day could replace folic acid and protect all dogs receiving 10 mg/kg/day 1843U89. As will be shown below, leucovorin more effectively reversed 1843U89 antitumor efficacy and was, therefore, not pursued further.

Effect of Oral Folic Acid on Weight Loss and Death of Mice Administered 1843U89. In contrast to human cells, 1843U89 is not efficiently transported into mouse cells (3). In addition, high circulating thymidine levels in mice decrease the efficacy and toxicity of all TS inhibitors in mice (3, 4, 27, 28). As such, 1843U89 has very little toxicity in mice (data not shown; see also Refs. 3 and 4). Nonetheless, if mice are first depleted of thymidine by prior administration of a polyethylene glycol conjugate of thymidine phosphorylase (PEG-TPase), high doses of 1843U89 can produce weight loss and death (Fig. 1). This effect is presumably due to depletion, by PEG-TPase, of circulating thymidine available for salvage since circulating thymidine levels drop from 0.7–1.5 μM to <0.03 μM.[4] The toxicity produced by this regimen appears to be gut toxicity, as shown by bloody diarrhea and histopathology (data not shown). To test whether folic

[4] R. Ferone, unpublished data.

Table 4 *Effect of 50 mg/kg oral folic acid pretreatment on the toxicity of 5-day daily i.v. 1843U89 in beagle dogs*[a]

	1843U89 dose (mg/kg/day) Folic Acid dose (mg/kg/day)					
	6		12		18	
	50[b]	50[b]	50[b]	50[b]	50[b]	50[b]
	M[c]	F	M	F	M	F
	0[d]	0[d]	0[d]	0[d]	2[d]	2[d]
Severe clinical signs: altered stool with blood, frequent emesis, decreased activity, cool to the touch, blood and/or stains around anogenital area, salivation, labored breathing, body tremors, and nystagmus	No	No	No	No	Yes	Yes
Mild clinical signs: occasional altered stool without blood, occasional emesis, and salivation	Yes	Yes	Yes	Yes	No	No
Body weight decrease	Yes	Yes	Yes	Yes	Yes	Yes
Food consumption decrease	Yes	Yes	Yes	Yes	Yes	Yes
Histopathology: mild, minimal, or very minimal maturation arrest enteritis of the cecum, colon, and/or ileum	Yes	Yes	Yes	Yes	No	No

Gross pathological changes noted in the 6- and 12-mg/kg/day group: red streaking of the colon, approximately 5 mm raised reddened areas at the junction of the colon with the ileum.

Gross pathological changes noted in the 18-mg/kg/day group only: dark red discoloration and congestion of the mucosa of the duodenum, jejunum, ileum, cecum, and colon, minimal white streaking of the renal cortex.

Histopathological changes noted in the 18-mg/kg/day group only: moderate to severe maturation arrest enteritis of the duodenum, jejunum, ileum, cecum, and colon. Maturation arrest was noted in the bone marrow smears from two of the three dogs humanely sacrificed. Kidney congestion, minimal to mild dilated tubules, casts, very minimal focal tubular epithelial necrosis, and very minimal tubular epithelial regeneration.

Note: Effects in all parameters were reversed or reversing at the end of the postdose recovery period in all surviving animals.

[a] Four dogs were treated at each dose level of 1843U89, two males and two females.
[b] Folic acid dose (mg/kg/day).
[c] M, male; F, female.
[d] Deaths.

acid can protect in this model of 1843U89 toxicity, drug was administered to TPase-pretreated mice via i.p. injection twice daily for 7 days with or without oral 300 mg/kg folic acid 30 min prior to 1843U89. The data in Fig. 1 show that folic acid prevented the 20% weight loss caused by 200 mg/kg 1843U89. A higher dose of 1843U89, 400 mg/kg, was lethal to all mice by day 10 in the absence of folic acid, but in the presence of the protectant, 80% of the animals survived (Fig. 2). Thus, folic acid can protect against the lethal toxicity of 1843U89 in mice as well as dogs.

Effect of Folic Acid on the Antitumor Efficacy of 1843U89. To determine the effect of folic acid or leucovorin on the *in vivo* antitumor efficacy of 1843U89, three human tumor lines were grown under the renal capsule of mice and treated with 1843U89 with or without prior oral doses of folic acid or leucovorin. These tumor lines (GC3TK$^-$ colon carcinoma, HCT-8/TK$^-$ ileocecal adenocarcinoma, and 143B TK$^-$ osteosarcoma) all lack thymidine kinase, preventing thymidine salvage.

GC3TK$^-$ is the most sensitive of the three cell lines to 1843U89. Table 5 shows that 3 to 10 mg/kg of 1843U89 twice daily for 5 days greatly inhibited tumor growth and led to cell kill and regression of GC3TK$^-$ (all measured on day 10, experiments 1 through 4). In this table, the term histological score (measured as described in "Materials and Methods") is introduced and used as a measure of the extent of cell kill caused by drug; values for histological score of 2 to 3 indicate extensive tumor cell kill. As can be seen in Table 5, most treatment schedules produced histological scores of 2 to 3, indicating a substantial antitumor effect. The effect of folic acid upon therapy of GC3TK$^-$ by 1843U89 is also shown in Table 5. Addition of 50 to 500 mg/kg folic acid p.o. 30 min prior to 1843U89 did not change either growth inhibition or tumor cell kill (Table 5, experiments 1 through 4). Since *in vitro* leucovorin reversed 1843U89 activity in GC3TK$^-$ only poorly, although more efficiently than folic acid, leucovorin was also

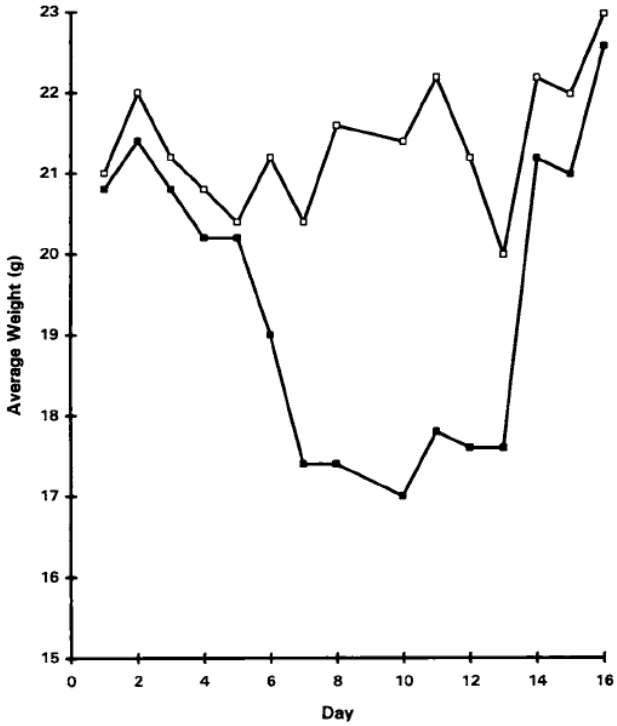

Fig. 1. Effect of oral folic acid on the weight loss of mice given 200 mg/kg 1843U89. BALB/C female mice were dosed, i.p., on days 1 and 4 with 2500 units of PEG-TPase/kg. On days 1 through 7, the mice were dosed, i.p., with 200 mg 1843U89/kg, twice daily, 5.5 h apart alone (■) or along with oral predoses of folic acid (□). For animals receiving the oral folic acid protection, 300 mg folic acid/kg were dosed 0.75 to 0.5 h prior to all 1843U89 doses.

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