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Antitumor Drug Toxicity in Tumor-free and Tumor-bearing Mice

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Summary. *This report summarizes studies of the toxicology of two antitumor drugs, L-phenylalanine mustard (L-PAM) and 5-fluorouracil (5-FU), administered singly and in combination to tumor-free and tumor-bearing mice. The purpose was to obtain data that might reveal the effect of disease on standard endpoints of drug toxicity. Young adult female B6C3F1 mice free of tumor or bearing murine mammary adenocarcinoma 16/C were treated with various dosages or combinations of L-PAM and 5-FU. All experiments included diluent control groups, and treatments were all administered IP daily for 5 days. Tumor size, body weight, hematology, clinical chemistry, and histopathology data were obtained on days 19, 21, 24, 28, 32, and 39 (day of tumor implantation = day 1; treatment on days 14–18), or on the corresponding post-treatment days in tumor-free mice. Tumor-bearing mice exhibited notable abnormalities in peripheral hematologic values and in concentrations of plasma urea nitrogen. If no drug treatment was administered, persistent reticulocytosis, granulocytosis, and uremia occurred in these mice. These abnormalities seemed to be related to tumor growth; drug treatment that produced partial regressions temporarily moderated cell counts and urea nitrogen concentrations. In general, these effects were observed in tumor-bearing mice receiving the highest doses in each experiment, suggesting that tumor-bearing mice are less sensitive than tumor-free mice to the effects of drugs on vital normal cells. Our data suggest that in the case of the particular tumor, host strain, and drugs studied here, distinct qualitative and quantitative differences in toxicity are obserbed when responses of tumor-free and tumor-bearing mice are compared.*

Introduction

Continued evaluation of the reliability of preclinical studies in mice for predicting clinical toxicity of antitumor drugs has resulted in encouragement of greater dependence on toxicity studies in mice [6, 15]. Advantages commonly ascribed to the mouse as a model are ease and economy of studying large numbers of individuals, minimization of drug requirements, and the possibility of comparing toxicity in healthy and tumor-bearing animals. Studies in our laboratories have explored the usefulness of the mouse for toxicologic predictions beyond the determination of lethal dosages [4, 8–10]. These have included the principal hematology, histopathology, and clinical chemistry evaluations traditionally obtained only in larger animal species [17]. In the present study we have used the mouse model to determine how the presence of a tumor might affect host physiology and sensitivity to drug treatment.

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Materials and Methods

L-PAM and 5-FU were obtained from the Developmental Therapeutics Program, DCT, NCI (Bethesda, MD). Each drug was dissolved in aqueous sodium chloride (0.9 g NaCl/100 ml) and diluted so that 0.1 ml/10 g body weight provided the desired dosage. All drug treatments were IP.

Young, adult female C57B1/6 × C3H(B6C3F1) mice about 7 weeks old and weighing 22–24 g were obtained from Simonsen Laboratories, Gilroy, CA and Frederick Cancer Research Center, Frederick, MD. The mice were housed in individual stainless steel cages with hardwood bedding (Betta-Chip™, Northeastern Products Corp., Warrensburg, NY) and received Wayne Lab-Blox® F6 (Allied Mills, Inc., Chicago, IL) and tap water ad libitum. All the mice were weighed individually prior to each treatment.

The isolation, biology, and propagation of mammary adenocarcinoma 16/C have been described by Corbett et al. [3]. Tumor samples were minced to provide fragments weighing approximately 25 mg each. Fragments were implanted by trocar SC in 460 mice, and the tumors were allowed to grow for 14 days (day of implantation = day 1). On or before the first day of treatment (day 14 postimplant), tumors were measured with a caliper, and 350 mice with the narrowest tumor size distribution were selected and randomized for treatment. The calculated tumor mass [3] ranged from 500 to 3000 mg at the start of treatment.

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Six experiments were carried out with 350 mice each. Each group of 350 mice was divided into five treatment groups of 70 mice each. One treatment group in each experiment received drug diluent IP. In the three experiments with tumor-bearing mice, these groups served as a control for the growth of untreated tumors. Drug dosages used in tumor-free mice were: L-PAM, 2.7, 4.1, 5.4, or 9.5 mg/kg/day; 5-FU, 12, 19, 26, or 39 mg/kg/day; L-PAM (1.7 mg/kg/day) plus 5-FU, 19, 26, 39, or 50 mg/kg/day. Drug dosages used in tumor-bearing mice were: L-PAM, 1.7, 3.4, 8.0, or 9.5 mg/kg/day; 5-FU, 19, 26, 39, or 50 mg/kg/day; 3.4 mg L-PAM/kg/day plus 19, 26, or 39 mg 5-FU/kg/day, or 1.7 mg L-PAM/kg/day plus 50 mg 5-FU/kg/day. All treatments were given daily for 5 days. The two drugs in combination were administered separately within 10 min.

Tumor size (in three experiments) and body weight were recorded twice each week after treatment began. Ten mice from each treatment group were killed on posttreatment days 1, 3, 6, 10, 14, and 21. Counting the day of tumor implantation as day 1, these study days were days 19, 21, 24, 28, 32, and 39. Ten mice from each treatment group were allocated as lethality controls. These mice were observed for 30–45 days posttreatment, and signs of morbidity and incidents of mortality were recorded daily.

Individual blood samples for hematologic evaluation and tissue samples for histopathologic evaluation were collected from five mice of each group killed. The remaining five provided individual blood samples for clinical chemistry analyses. Our methods of sample collection and analysis have been described elsewhere [8].

Samples of liver, kidney, spleen, lung, stomach, duodenum, jejunum, ileum, colon, sternum, and femur were collected and preserved in buffered formalin (10 g formaldehyde/100 ml phosphate buffer, pH 7). Tissue samples were embedded in Paraplast®, sectioned at 4–6 μm, and stained with Harris' hematoxylin and eosin [12].

Results

Dosages of L-PAM and 5-FU chosen for these studies were equivalent fractions of the historical LD10 values of each drug (unpublished data recorded at Southern Research Institute). L-PAM dosages were in the range of clinical usefulness [5]. Because mice are more sensitive than humans to the effects of 5-FU [9, 11], 5-FU dosages were necessarily lower than those used clinically. From each individual mouse we obtained either nine hematologic values and ten tissues for histopathologic evaluation, or 17 clinical chemistry values. These data revealed a number of differences between tumor-free and tumor-bearing mice. In general, lethality data were not useful for comparative purposes, because all tumor-bearing mice ultimately died of their disease regardless of drug treatment. Interpretation of comparative body weight changes was complicated by tumor growth. Noteworthy differences between tumor-free and tumor-bearing mice and the influence of these differences on measurement of drug effects are reflected in Tables 1 and 2 and in Fig. 1.

Changes in reticulocyte counts were similar in tumor-free mice regardless of the treatment (Table 1). Dose-dependent reticulocytopenia developed 24 h posttreatment and persisted 2–9 days before reticulocyte counts returned to normal. In contrast, tumor-bearing mice ex-

Table 1. Comparison of drug effect on reticulocyte counts of tumor-free and tumor-bearing mice
Reticulocyte counts at nadir[a]

Drug	Tumor-free mice				Tumor-bearing mice			
	Dosage mg/kg/day × 5	Mean ± SD (10^4/mm^3)	Nadir Day	Recovery Day	Dosage mg/kg/day × 5	Mean ± SD (10^4/mm^3)	Nadir Day	Recovery Day
L-PAM	0	8.2 ± 1.8	–	–	0	70.4 ± 34.5	–	–
	2.7	6.3 ± 2.7	1	6	1.7	28.1 ± 19.7	[b]	[b]
	4.1	3.2 ± 1.5	3	10	3.4	10.7 ± 3.2	[b]	[b]
	5.4	1.9 ± 1.1	1	10	8.0	0.5 ± 0.3	3	10[b]
	9.5	0.2 ± 0.4	1	10	9.5	0.8 ± 0.5	3	10[b]
5-FU	0	10.9 ± 2.6	–	–	0	46.9 ± 35.5	–	–
	12	5.8 ± 3.2	1	3	19	27.4 ± 15.4	[b]	[b]
	19	8.0 ± 4.2	1	3	26	11.1 ± 11.1	[b]	[b]
	26	4.2 ± 2.5	1	3	39	1.9 ± 2.9	1	3[b]
	39	0.3 ± 0.6	1	6	50	0.8 ± 0.8	1	[c]
L-PAM + 5-FU	0	12.8 ± 5.4	–	–	0	86.9 ± 45.9	–	–
	1.7 + 19	3.1 ± 2.0	1	10	3.4 + 19	5.9 ± 6.8	1	3[b]
	1.7 + 26	2.7 ± 1.0	1	10	3.4 + 26	5.6 ± 3.3	1	3[b]
	1.7 + 39	0.5 ± 0.7	1	10	3.4 + 39	0.7 ± 0.5	3	10[b]
	1.7 + 50	0.1 ± 0.3	1	[c]	1.7 + 50	0.3 ± 0.4	1	10[b]

[a] Reference range for reticulocytes = 1.9–38.3 (10^4/mm^3)
[b] Reticulocytosis
[c] Most of the mice were dead after Day 3 posttreatment

Table 2. Reticulocyte counts[a] of tumor-bearing mice treated with L-PAM, 5-FU, and L-PAM plus 5-FU[b]

Drug	Dosage mg/kg/day × 5	Days after tumor implantation					
		19	21	24	28	32	39
Diluent	–	86.9 ± 45.9	64.6 ± 28.7	41.8 ± 10.9	55.0 ± 39.1	52.3 ± 20.4	39.1 ± 36.3
L-PAM	8	1.0 ± 0.7	0.5 ± 0.3	18.8 ± 22.2	41.3 ± 27.7	93.4 ± 49.4	95.3 ± 24.7
5-FU	39	1.9 ± 2.9	43.6 ± 22.7	27.9 ± 25.4	30.3 ± 35.4	56.8 ± 46.1	23.7 ± 11.9
L-PAM + 5-FU	3.4 + 39	1.2 ± 1.0	0.7 ± 0.5	1.8 ± 2.0	72.7 ± 39.0	99.0 ± 54.7	58.5 ± 5.3

[a] Mean ± SD (10^4/mm^3); reference range = 1.9–38.3
[b] Data presented are for the optimal dosages determined from the tumor response data (Fig. 1)

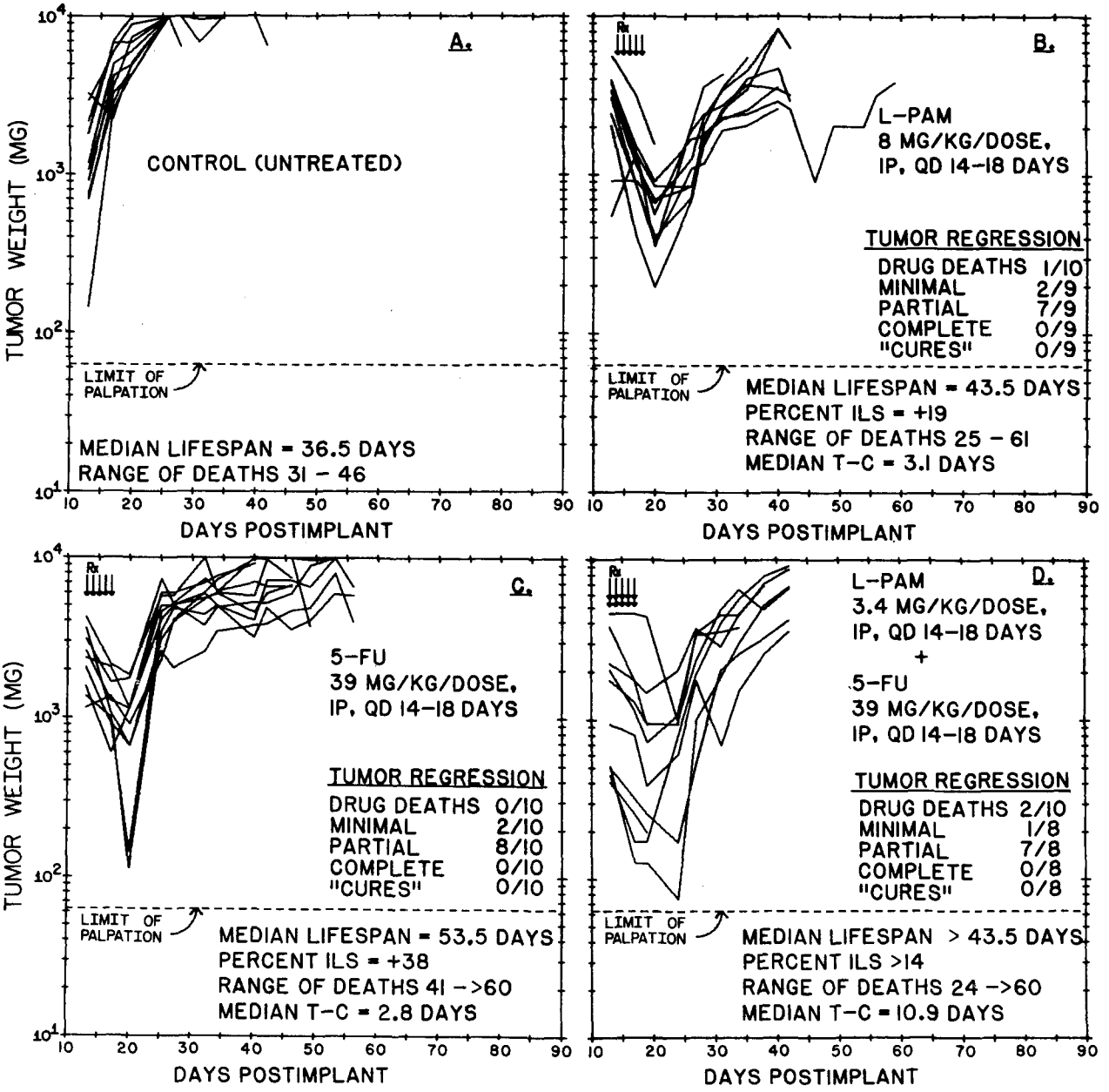

Fig. 1. Effect of treatment on tumor growth. These were the optimal dosages of L-PAM, 5-FU, and L-PAM plus 5-FU in the present studies. Each plot presents the changes in mass of ten individual tumors. Partial regression is regression to less than 50% of tumor mass at start of treatment.

Table 3. Comparison of drug effect on granulocyte counts of tumor-free and tumor-bearing mice
Granulocyte counts at nadir[a]

Drug	Tumor-free mice				Tumor-bearing mice			
	Dosage mg/kg/day × 5	Mean ± SD (10^3/mm^3)	Nadir Day	Recovery Day	Dosage mg/kg/day × 5	Mean ± SD (10^3/mm^3)	Nadir Day	Recovery Day
L-PAM	0	2.1 ± 0.9	–	–	0	7.1 ± 2.3	–	–
	2.7	1.2 ± 0.3	3	6	1.7	2.2 ± 0.5	3	7[b]
	4.1	1.2 ± 0.2	1	6	3.4	1.4 ± 1.0	3	7[b]
	5.4	0.8 ± 0.1	1	6	8.0	0.6 ± 0.5	3	7[b]
	9.5	0.7 ± 0.4	1	6	9.5	0.7 ± 0.5	1	7[b]
5-FU	0	1.4 ± 0.5	–	–	0	11.1 ± 4.0	–	–
	12	1.1 ± 0.6	1	3	19	1.5 ± 0.5	1	3[b]
	19	0.6 ± 0.4	1	3	26	0.9 ± 0.3	1	3[b]
	26	0.5 ± 0.2	1	6	39	0.6 ± 0.6	1	6
	39	0.1 ± 0.1	3	10	50	0.5 ± 0.3	1	[c]
L-PAM + 5-FU	0	1.3 ± 0.8	–	–	0	10.6 ± 4.4	–	–
	1.7 + 19	0.3 ± 0.1	3	6	3.4 + 19	0.04 ± 0.04	1	6
	1.7 + 26	0.1 ± 0.1	3	6	3.4 + 26	0.3 ± 0.2	1	3
	1.7 + 39	0.1 ± 0.1	3	10	3.4 + 39	0.06 ± 0.07	1	10[b]
	1.7 + 50	0.1 ± 0.1	3	[c]	1.7 + 50	1.0 ± 0.6	1	3[b]

[a] Reference range for granulocytes = 0.4–2.7 (10^3/mm^3)
[b] Granulocytosis
[c] Most of the mice were dead after Day 3 posttreatment

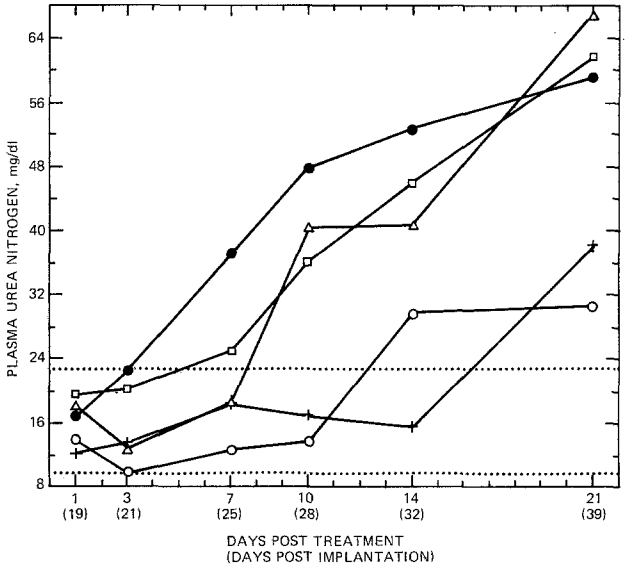

Fig. 2. Changes in plasma urea nitrogen concentrations of tumor-bearing mice treated with L-PAM on days 14–18 postimplant and studied at various times posttreatment. ●, control, diluent only; □, 1.7 mg/kg; △, 3.4 mg/kg; ○, 8.0 mg/kg; +, 9.5 mg/kg; ::::, reference range.

hibited reticulocytosis that was sustained throughout the 21-day observation period if no drug treatment was administered. Reticulocytopenia occurred only after the higher dosages of L-PAM, 5-FU, or the combination. The time required for onset of and recovery from reticulocytopenia was unaffected by the presence of tumor, but recovery was synonymous with reticulocytosis in tumor-bearing mice rather than with normal reticulocyte counts. Reduced reticulocyte counts in tumor-bearing mice (Table 2) coincided with regression of the tumors (Fig. 1).

In tumor-free mice, dose-dependent granulocytopenia occurred on days 1–3 posttreatment, and recovery was evident in most cases by day 6 (Table 3). Tumor-bearing mice that received no treatment exhibited granulocytosis that persisted throughout the observation period. Tumor-bearing mice were as sensitive as the tumor-free mice to drug-induced granulocytopenia, and the effect was dose-dependent (Table 3). Granulocyte nadirs occurred on days 1–3 posttreatment. Granulocytosis was reestablished by day 6.

Clinical chemistry evaluations revealed progressive uremia in the tumor-bearing mice that received no treatment (Fig. 2). No histologic evidence of renal damage was noted in any of the mice, regardless of the treatment they received. The increase of plasma urea nitrogen concentrations in tumor-bearing mice paralleled the increase of tumor mass during the observation period (Fig. 1). A linear regression analysis of log BUN and log (tumor mass) was performed with data from the diluent control group of the L-PAM experiment (Fig. 2). This analysis yielded a correlation coefficient of 0.77.

The principal microscopic changes induced by L-PAM and 5-FU at sublethal dosages (≤ LD10) were in the gastrointestinal epithelium in tumor-free mice. These

changes consisted of mild to moderate epithelial hypertrophy, hyperplasia, and macronucleosis similar to that described previously [4, 9]. The presence of tumor had no apparent impact on the development and resolution of drug-induced histologic changes in gastrointestinal epithelia. Histologic examination of tissue samples revealed no evidence of infection that might have accounted for changes in the hematology of the tumor-bearing mice.

Discussion

We chose 5-FU and L-PAM for these studies because they are important chemotherapeutic drugs for surgical adjuvant treatment of premenopausal women who have mammary adenocarcinoma metastatic to the axillary lymph nodes at the time of mastectomy [1, 2, 5]. Use was made of the murine mammary adenocarcinoma 16/C to provide toxicology data on an animal model of this human disease. As might be expected, the presence of tumor can radically alter host physiology as reflected by a panel of clinical laboratory tests. However, evaluation of drug toxicity in a model of the disease for which the drug is intended may prove a useful extension of conventional toxicity studies.

Mice bearing mammary adenocarcinoma 16/C exhibited a marked hematologic imbalance. Reticulocytes and granulocytes responded to drug treatment in a way that seemed to parallel tumor cell destruction. These cell populations responded earlier to drug-induced cytotoxicity than did tumor mass, however. Posttreatment reticulocytosis and granulocytosis were not rebound effects in the usual sense, because cell counts were abnormally high before treatment began. Pazdernik and Uyeki [14] have reported that the effects of cyclophosphamide on murine myelopoiesis differ in the presence of L-1210 leukemia. The toxicologic consequences of tumor-induced changes in populations of peripheral blood cells will not become clear until other drugs and combinations representing the full spectrum of biochemical mechanisms of cytotoxicity have been studied with other tumors and other murine host strains. Extended studies of different antitumor drugs may reveal toxicologic differences attributable to altered drug metabolism in tumor-bearing mice [18].

In the present studies in tumor-bearing mice, elevated plasma urea nitrogen concentrations were not accounted for by renal lesions. Instead, the uremia correlated with tumor mass. The biochemical explanation for this observation is not clear. One possibility is that tumor growth is accompanied by increasing rates of protein synthesis [13, 16]. Increased protein catabolism might account for the plasma urea in tumor-bearing mice, but other nitrogenous metabolites associated with cell growth should accumulate as well. We observed no change, for example, in plasma uric acid concentrations. The implication that uremia was associated with tumor growth was strengthened, however, by the observation that temporarily effective chemotherapy reduced plasma urea concentrations while partial regressions of tumors were evident.

Of the subhuman species available for toxicology studies of anticancer drugs, only the mouse offers the advantage of a wide range of experimental tumors histologically similar to cancer in man. This is a unique addition to other advantages the mouse offers for preclinical toxicologic evaluations. Retrospective analyses of extensive lethality data have indicated that in the past, studies in mice have been characterized by considerable variability attributable to several sources, including experimental design [6, 15]. The presence of tumor would not be expected to lessen experimental variation. With careful control, however, consistent and reliable data on the quantitative and qualitative toxicity of antitumor drugs may be obtained with mice [7]. The qualitative and quantitative differences between tumor-free and tumor-bearing mice reported here strongly suggest that toxicity studies in tumor-bearing mice should be explored to determine whether these provide important information on the safety of anticancer drugs for use in humans. Additional work with other drugs, combinations, tumors, and mouse strains will be required to indicate how consistent these tumor-related toxicity differences are and to provide sufficient information for comparison with clinical data.

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