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Variability of tumor response to chemotherapy* I. Contribution of host heterogeneity

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Summary. Host factors that might be associated with the variable response of tumors to effective chemotherapy were studied in B6C3F1 mice bearing transplanted mammary adenocarcinoma 16/C tumors and treated with melphalan. Tumor response ranged from regression to an unpalpable size to growth under treatment. That biochemical resistance of the cell population was not primarily responsible for the variability was demonstrated by passage of responsive and nonresponsive tumors into new hosts followed by treatment with melphalan. When the implanted subcutaneous tumor weighed 1.0 g or less (usually 12 to 13 days postimplant), both the plasma levels of melphalan and the variability in plasma levels were similar to those observed in tumor-free mice. With tumor progression beyond 1.0 g, an increase in mean plasma levels and in variability, but not in plasma half-life, was observed. A correlation between the dose of melphalan administered, the schedule, and the percentage of tumor responses was found. There was no correlation between the plasma levels in individual mice following a given dose of melphalan and subsequent tumor response. Also, there was no correlation between the plasma levels of melphalan in individual mice following the second, third or fourth treatment in a multiple-dose treatment schedule and the response of the tumor in that mouse to previous treatments. Prior therapy (1, 2 or 3 doses administered 4 days apart) either prevented the increase in plasma levels that occurred in mice bearing untreated advanced tumors or reduced the plasma level (and the variability) to approximately that found in tumor-free mice. Whether this was a direct result of the effects of melphalan on the host or an indirect result of tumor inhibition is not known. A similar study in tumor-free mice indicated that prior treatment had only minimal effects on subsequent plasma levels. These studies indicate that heterogeneity of the host was not a major factor in variable tumor response if therapy was initiated when the tumors weighed 1.0 g or less.

Introduction

Variable response to therapy by tumors of similar size and histology is a major problem in treatment of human malignancies. Frequently, the variability was assumed to be characteristic of cancer in man but not characteristic of transplantable tumors in animals. However, variable responses to chemotherapy [39] and to radiation [37] have been reported in staged, transplantable murine tumors derived from a single tumor and transplanted into inbred mice. The variability of response in experimental systems has been attributed to heterogeneity of the hosts [30, 39] or of the tumor cell populations [18, 26, 44]. Any factors in the host or tumor that produce variations in the concentration of drug, in the duration of exposure, in the fraction of the stem cells exposed to cytotoxic concentrations of drugs, or in the sensitivity of tumor cell populations to drugs may contribute to heterogeneity of tumor response. Dose escalation to increase the concentration of drug that reaches tumor cells is generally not feasible due to the life-threatening toxicity produced by most effective antitumor agents.

These studies were undertaken to explore the host factors that may be responsible for variable tumor response to a standardized chemotherapeutic regimen in individual mice. These mice, of similar genetic background, had tumors from the same tumor source and implanted in the same anatomic site. Further, the tumors were studied at selected sizes by the same techniques. Preliminary data were reported by Noker et al. [32].

Materials and methods

A. Biological systems. The transplantable mammary adenocarcinoma 16/C (mam ad 16/C) was derived from a spontaneous mammary tumor that arose in a C3H mouse in 1974. During early transplant generations, the tumor was propagated by s. c. passage of lung metastases into the host of origin [12]. The tumor line is now routinely maintained by s. c. implantation of tumor fragments into C3H female mice.

Fragments from at least three donor tumors from a single passage group of C3H mice were pooled for single or bilateral implantation for each experiment except when a single, previously treated donor tumor was used. The hybrid B6C3F1 mouse was selected because of the extensive drug response data available in that system [12, 39] and because the hybrid mouse was better able to withstand the experimental procedures, including surgery, that were required for these studies.

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Tumor growth was followed by caliper measurements of two perpendicular diameters of the tumor. The weight in mg was estimated from the formula for a prolate ellipsoid, i. e., ½ (length × width^2), assuming the specific density to be 1. Studies were initiated either on a designated day following tumor implantation, or on a day when there were sufficient tumors within a given range of calculated weights to conduct the study. Mice were randomized to control and treated groups either immediately after tumor implantation or after selection of mice bearing tumors of appropriate size.

Mice were housed five to a cage, earmarked and followed individually for the course of the study. Animal rooms were maintained at 71 ± 2° F under slight negative pressure with 12-h alternating light and dark cycles. When surgical excision was indicated, the primary implanted tumors were removed by blunt dissection from mice anesthetized with Nembutal. To reduce shock, mice were kept at 82° F during the postoperative period.

B. Drug treatment. Melphalan (L-PAM, NSC 8806) was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, NIH or from Sigma Chemical Co., St. Louis, Mo. The drug was administered in H_2O on a body weight basis at the doses and on the schedule indicated for individual studies. Treatment solutions were prepared immediately prior to use and were administered i. p.

For the measurement of plasma levels of L-PAM, mice were first anesthetized with diethyl ether at selected times after dosing. A single, terminal blood sample was collected from the axillary region into a tube containing heparin. Plasma was obtained after centrifugation of cooled samples and immediately extracted with 4 volumes of methanol. Portions of each extract were assayed as described below.

Where replicate samples from individual mice during multidose treatment studies were desired, blood (approx. 50 µl) was taken from a tail vein [7] into a heparin-coated capillary tube and subsequently extracted as described for plasma.

C. Drug toxicity. Toxicity of L-PAM was determined in tumor-free B6C3F1 mice. The agent was administered every 4 days for four treatments. The doses ranged from 6.7 mg/kg (0 deaths) to 25 mg/kg (100% deaths). Mice were held for 30 days after the last dose of drug. From a probit plot the LD_{10} was estimated to be approximately 12 mg/kg.

D. HPLC analyses. HPLC analyses were accomplished with a Waters Associates (Milford, Mass) high-pressure liquid chromatograph equipped with a Model 6000 A high-pressure delivery pump, a Model 441 UV absorbance detector, a Model 710 automatic sample injector (WISP) and a Model 730 data module. The conditions for the analysis of L-PAM were as described by Chang et al. [11], except that the solvent was delivered at a flow rate of 1 ml/min and contained 10 m*M* acetic acid. For solutions of L-PAM in methanol, the intra- and interassay coefficients of variation were 1.4% (N = 7) and 3.6% (N = 5), respectively. Under our conditions of sample preparation, the lower limit of detection of L-PAM was 0.1 µg per ml plasma. Recoveries of L-PAM from plasma and whole blood were 90% and 95%, respectively. Standard deviations of the means are presented when appropriate.

E. Pharmacokinetics of L-PAM. Pharmacokinetic half-lifes ($t_{½}$) of L-PAM in plasma were estimated from HPLC data with a modified form of NONLIN [29] and CSTRIP [40]. The data were fitted to one-, two- and three-compartment open models. A model was accepted as best-fit if an additional term, or compartment, failed to reduce significantly ($P < 0.05$) the weighted sums of squared errors as estimated by the F test with appropriate degrees of freedom. Statistical weights were determined from the measured concentrations and were the same for each model.

Results

A. Variable tumor responses to L-PAM

Preliminary studies of the response of mam ad 16/C tumors in B6C3F1 mice to treatment with L-PAM at doses ranging from 8 to 16 mg/kg every 4 or 7 days indicated that 12 mg/kg given every 4 days for three treatments produced the highest percentage of responses. Improvement in response to treatment with increasing number of doses or amount of drug was limited by toxicity. The LD_{10} was reached when either the number of doses of 12 mg/kg was increased to four or the dose level was increased to 14 mg/kg for three doses. Tumor responses ranging from growth under treatment, partial regression (greater than 50% reduction in mass) to complete regression were observed when L-PAM was given at 10 mg/kg every 4 days for three doses, and this dose and schedule were used to study the host factors that might be related to the variable response.

Growth curves for individual control and treated tumors from one experiment are shown in Fig. 1 A, B. In this study, the pretreatment calculated tumor weights on day 14 after implant ranged from 0.5 g to slightly over 1.0 g. The responses to treatment with L-PAM at 10 mg/kg ranged from growth under treatment to regression below palpable size.

If the cell populations of these tumors that grew under treatment were biochemically resistant to L-PAM (thereby not responding to treatment) or if the cells that survived treatment were biochemically resistant, then the second-generation tumors should be less responsive than the parent line. To test this hypothesis, three tumors that either remained static or grew under treatment and one tumor that regressed below the palpable limit, but regrew after the cessation of therapy, were harvested on day 27 after implant and fragments of each tumor were implanted in 12–15 new mice. Growth data for two donor tumors and the second-generation implants are shown in Fig. 1 C–F. Beginning on day 11, all measurable tumors in each group of second-generation tumors were treated with L-PAM at 10 mg/kg, q 4 d × 3. Although the range of tumor weights at the time of initial treatment of the second-generation tumors was much broader than in the first group, the responses varied from growth under treatment to complete regression, whether the donor tumors had responded to treatment (Fig. 1 C) or not (Fig. 1 D). This study and replicate studies with similar results indicate that the variability of response of mam ad 16/C tumors to the initial treatments with L-PAM cannot be attributed to biochemical resistance of the tumor cell population. It should be noted that these studies do not rule out the possible emergence of L-PAM resistant cell populations during the course of extended therapy.

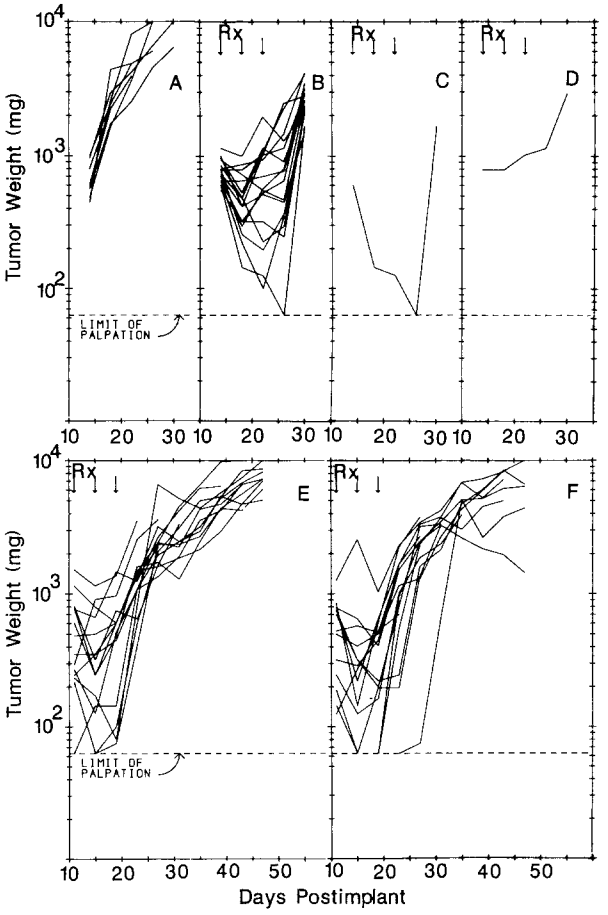

Fig. 1A–F. Growth curves for individual mam ad 16/C tumors in B6C3F1 mice. **A** Growth curves for untreated tumors; **B** growth curves for tumors treated with L-PAM at 10 mg/kg given at the times indicated; **C, D** individual tumor (donor) growth curves (from B) for two of the treated tumors that were used to implant the second generation; **E, F** response of two groups of the second-generation tumors (**C→E, D→F**) to treatment with L-PAM, 10 mg/kg, as shown

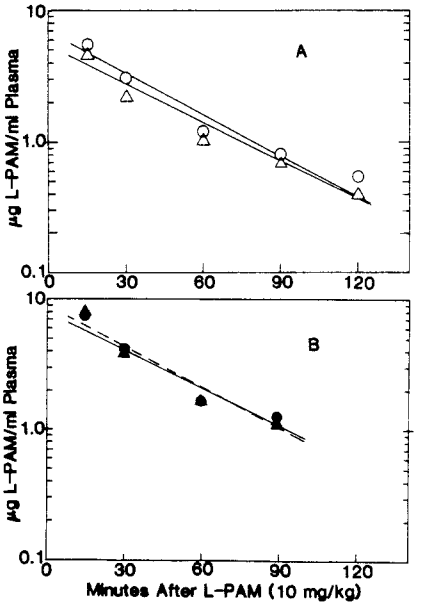

Fig. 2A, B. Elimination of L-PAM from plasma of mice bearing **A** > 2000 mg mam ad 16/C tumors (○) or tumor-free mice (△) and **B** mam ad 16/C tumors growing (●) or regressing (▲) during multiple-dose therapy with L-PAM. (Standard deviations did not exceed the size of the *symbols*)

B. Pharmacokinetics of L-PAM

Variations in rate of uptake and elimination of L-PAM in individual mice appeared to be the host factors most probably associated with variable tumor response; that is, these rates would determine the concentration of drug and the time of drug availability to the tumor. The plasma half-life of L-PAM was determined in tumor-free mice, in mice bearing mam ad 16/C tumors at different stages of growth, and in mice bearing tumors that were growing or regressing under treatment (Fig. 2). The elimination half-life was 29.6 min in mice bearing 20-day old tumors about 2.0 g in weight, and 33.2 min in tumor-free controls. Studies in mice bearing mam ad 16/C tumors, growing or regressing under treatment with L-PAM, gave estimated elimination half-lives of 30.3 and 28.0 min, respectively. These results suggest that variation in the rate of L-PAM elimination is not a major factor in determining tumor response to treatment.

C. Variations in plasma L-PAM levels at different sampling times

The data presented in Fig. 2 illustrate that for plasma samples obtained from 15 to 120 min after administration of L-PAM to tumor-free mice, the interanimal coefficient of variation in L-PAM concentrations at any given time point was approximately 10%. Similar results were obtained in several additional studies that utilized different doses of L-PAM and other sampling intervals (data not shown). Thus the observed variability, or lack thereof, in plasma L-PAM levels was independent of the time of analysis over the linear portion of the decay curve.

D. Relationship between dose of L-PAM and plasma level

To determine whether the relationship between the dose of L-PAM administered and the plasma level achieved in individual animals was linear over the therapeutic range, plasma levels were measured 30 min after administration of the drug to tumor-free mice. The results of two experiments are shown in Fig. 3. A linear relationship between plasma level and dose administered was observed between 2 and 25 mg/kg (correlation coefficient 0.97), but the linear relationship no longer applied at a dose of 50 mg/kg. The variation in plasma levels among individual mice increased significantly at the highest dose. Data for mice bearing tumors of 1.0 g or less were similar and were omitted from the figure. Thus the plasma level of L-PAM in individual tumor-free mice or mice bearing tumors up to 1.0 g had a direct relationship to the dose administered over the range of therapeutic doses.

E. Plasma levels of L-PAM in individual tumor-free mice

Variation in plasma levels of L-PAM in individual mice, either as a result of host differences in disposition, dosing variation or previous therapy, would be expected to produce a spectrum of tumor responses, particularly in view of the steep dose response of L-PAM. To estimate the contribution of host heterogeneity, plasma levels in individual tumor-free mice from a single source and shipment were

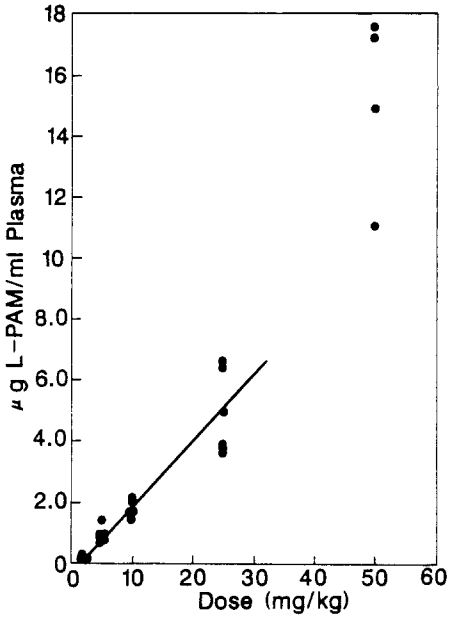

Fig. 3. Plasma levels in tumor-free, previously untreated B6C3F1 mice following administration of single doses of L-PAM varying from 2 mg/kg to 50 mg/kg. The line was fitted to the data points for doses 2 mg/kg through 25 mg/kg by linear regression analysis, and the correlation coefficient was 0.97

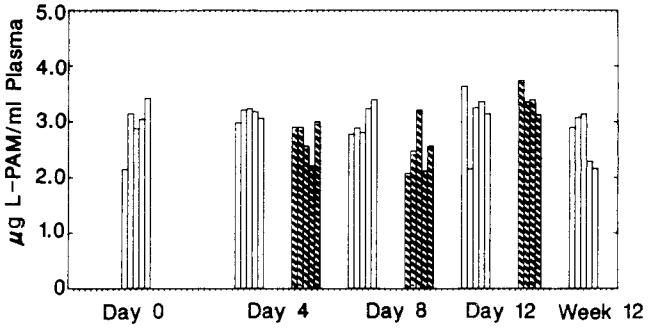

Fig. 4. Plasma levels of L-PAM measured in tumor-free mice from the same supplier and shipment. In control groups ▭, plasma samples were obtained 30 min after the initial dose of L-PAM at intervals up to 12 weeks. In the treated groups ▨, L-PAM, 10 mg/kg, was administered every 4 days and plasma levels were determined 30 min after the second dose (day 4), the third dose (day 8) and the fourth dose (day 12)

measured at intervals over a 3-month period. For previously untreated mice, the mean plasma level per group of 5 mice at 30 min after administration of L-PAM ranged from 2.7 ± 0.5 μg/ml to 3.1 ± 0.6 μg/ml (Fig. 4). The mean plasma level for all previously untreated mice over the 12-week period was 3.0 ± 0.4 μg/ml. The plasma levels in previously treated mice following the second, third or fourth treatment with L-PAM were slightly more variable, with a mean of 2.7 ± 0.7 μg/ml. This variation did not appear to be related to the number of previous doses of L-PAM.

That variation in the dose administered is not a major factor in tumor response is supported by these results and the results of an independent study which indicated a ±5% range in total radioactivity/carcass following administration of a radiolabeled drug (D. L. Hill, personal communication).

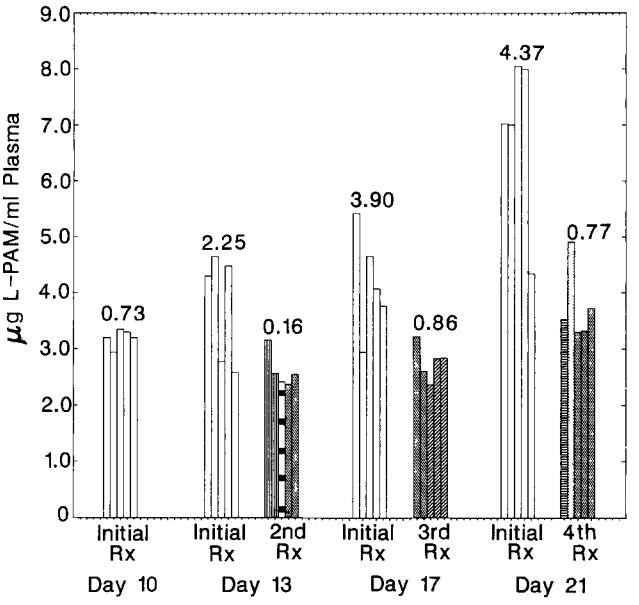

Fig. 5. Plasma levels of L-PAM in mice (bearing bilateral implants of mam ad 16/C tumor) 30 min after the initial treatment (▭) or at 30 min after second, third or fourth treatment. The tumor response to prior treatment was minimal regression (<50% reduction) of both tumors (▭▭); partial regression (>50% reduction but still palpable) of both tumors (▬); one partial and one complete regression (▬▭); one partial and one minimal regression (▭); mixed response, i.e., one tumor grew and the second tumor regressed (▬); or both tumors grew under treatment (▬). (Mean tumor weights in g for each group are indicated above the *bars)*

F. Plasma levels of L-PAM versus tumor age or tumor size

The effects of tumor age or tumor mass on plasma levels of L-PAM were investigated in mice bearing single or bilateral implants of mam ad 16/C tumors. The total weight of bilateral tumors was approximately equal to the weight of a single implant of equal age and the effects of single and bilateral implants on plasma levels of L-PAM were similar; thus only the data for bilateral implants are presented in Fig. 5.

These data indicate that the plasma levels of L-PAM (>2.0 to <4.0 μg/ml) following the initial treatment (control) in mice bearing tumors less than 1.0 g were similar to the plasma levels (3.0 ± 0.4 μg/ml) of tumor-free mice (Fig. 4). By day 13 after implantation of the tumors the plasma levels of L-PAM had become much more variable, with a trend toward higher values as tumors increased in size and age.

Also evident from the results shown in Fig. 5 is the fact on day 13 and later, plasma levels were lower in previously treated mice than in mice from the same tumor implant group that had not been previously treated. The measurable s. c. body burden of tumor was lower in the previously treated mice than the untreated mice but there was no direct relationship between plasma levels of L-PAM and tumor burden. Further, the plasma levels in 31/34 of the previously treated tumor-bearing mice studied before day 21 after implant (some data not shown) were lower than the mean plasma level in tumor-free mice (Fig. 4).

Table 1. Plasma levels of L-PAM in relation to body burden of tumor

Part A Tumors selected for size				Part B Surgical reduction of tumor burden			
Status	Days After implant	Tumor weight (g)	Plasma L-PAM (μg/ml)	Status	Days After implant	Tumor weight (g)	Plasma L-PAM (μg/ml)
Untreated	18	0.35	1.6	Surgery Day 10	19	0	2.2
		0.30	1.6			0.2	2.4
			1.6±0			0.7	2.0
							2.2±0.2
	18	1.9	1.7		19	3.8	4.0
		2.0	1.6			2.6	3.3
		2.0	1.4			2.6	4.1
		1.9	1.4				3.8±0.4
			1.5±0.2				
	18	6.5	2.6				
		5.0	1.9				
		4.5	2.3	No surgery	19	5.1	6.2
		3.9	2.4			5.1	8.4
		3.4	3.4			3.5	3.3
			2.5±0.5				6.0±2.6

The effects of tumor mass on plasma levels of L-PAM were investigated in mice bearing single tumors <0.5 g, approx. 2.0 g and >3.4 g selected from a large group of mice at 18 days afterimplant. The results are shown in Table 1, part A. The plasma levels were higher in the group bearing large tumors (3.4–6.5 g), suggesting that the body burden of tumor (and possibly the length of time that a large tumor had been present) determined the physiological state of the host and, ultimately, the plasma level of L-PAM.

In a second study, the tumor burden was reduced by surgical excision of the implanted s.c. tumor on day 10 to allow comparison with untreated mice from the same implant group. On day 19, mice from the surgically treated group were selected for tumor recurrences of 0–1.0 g and 2.0–4.0 g to compare with mice bearing tumors >3.5 g (no surgery). The results are shown in part B of Table 1. These results are consistent with an association between large tumor burdens and elevated plasma levels.

G. Relationship between plasma levels of L-PAM and response of single or bilateral tumor implants

To determine whether blood levels following the initial dose of L-PAM could predict for tumor response or for blood levels following successive doses, samples were taken from the tail vein of individual tumor-bearing mice 30 min after each of two treatments with L-PAM. Tumor growth was monitored in the interval between treatments.

The results of two studies are shown in Fig. 6A, B. In study A, mice bearing single implants of mam ad 16/C were treated on days 12 and 19 afterimplant. Following administration of 12 mg/kg L-PAM, blood levels ranged from 2.0 to 3.6 μg/ml after the first treatment and from 2.4 to 4.4 μg/ml after the second treatment. The initial blood levels did not predict for the subsequent blood level or for tumor response. In part B, L-PAM was given at 15 mg/kg on days 9 and 13 to mice bearing single implants. The blood levels were higher, as would be expected with the higher dose, and minimal or partial regressions were observed in all tumors. In all animals that received doses of 15 mg/kg, excessive weight loss (>5.0 g/mouse) occurred and probably contributed to the tumor regressions [27]. There were no complete responses, and all tumors subse-

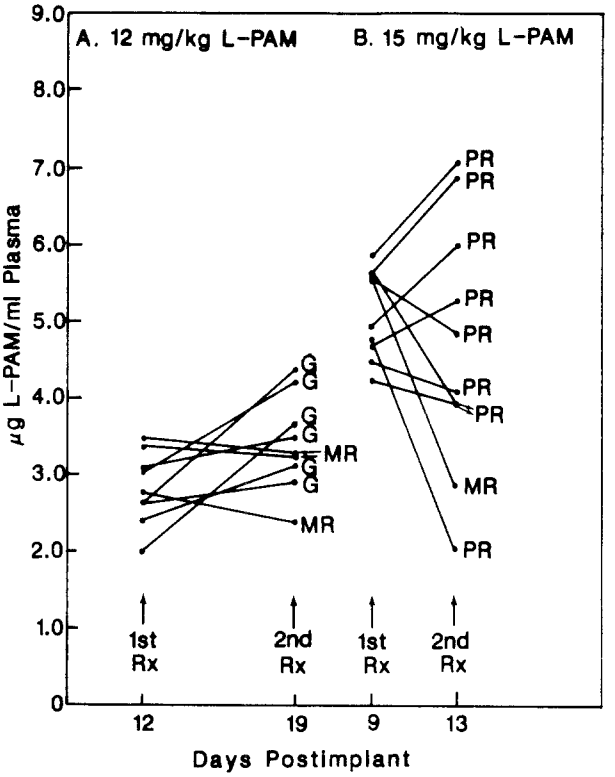

Fig. 6. Blood levels of L-PAM in individual mice (obtained from a tail vein) 30 min after the initial and second doses of L-PAM. The tumor response for each mouse is indicated by: MR, minimal response; PR, partial response; or G, growth (>25% increase in tumor mass). No complete regressions where observed

quently grew. Again, the initial blood level could not be used to predict the blood levels following subsequent treatments.

The mice bearing bilateral implants of mam ad 16/C were used for further investigation of the relationship between plasma level of L-PAM and tumor response. Multiple tumors in an individual host often exhibit different responses to treatment and, if the plasma level of drug is directly related to the tumor response, the highest plasma levels (following a given dose) should be associated with maximum response of bilateral tumors. Plasma levels were monitored at intervals between day 10 and day 21 in treated and previously untreated animals, and tumor growth or regression was followed during the study. The response of each tumor was determined in relation to its pretreatment size and the results indicated in Fig. 5 were determined on the day of sacrifice. There were no differences in the plasma levels of L-PAM in mice bearing tumors that both grew, that both regressed or that exhibited divergent responses during previous therapy. There was no correlation between the plasma level after the second, third or fourth dose and the response of the tumor to previous doses.

Discussion

These studies indicate that the heterogeneity of the host was not a major factor in the variable response of mam ad 16/C tumors to therapy with L-PAM if therapy was initiated when the implanted s. c. tumor weighed 1.0 g or less (usually 12–13 days afterimplant). Until that stage of tumor growth, both the plasma levels and the variability in levels were similar to those observed in tumor-free mice. With tumor progression beyond 1.0 g, increases in mean plasma levels and in variability, but not in plasma half-life, were observed.

A correlation between the dose of L-PAM administered, the schedule, and the percentage of tumor responses was found. There was no correlation between the plasma levels of individual mice following a given dose of L-PAM and subsequent tumor response. Also, there was no correlation between the plasma levels of L-PAM in individual mice following the second, third or fourth treatment in a multiple-dose treatment schedule and the response of the tumor in that mouse to previous treatments.

The presence of a large mam ad 16/C tumor (>1.0 g) in the B6C3F1 mouse introduced significant variation into the pharmacokinetics of L-PAM; the amount of variation was related to tumor age, tumor size and host health status. That the major factor influencing plasma level was tumor burden was suggested by the results of the study where the tumor was surgically excised and plasma levels were measured 9 days later. Both plasma levels of L-PAM and variation were lowest in the group with minimal recurrence at the site of excision. The tumor-associated host changes that occurred with increasing tumor burden and that were probably responsible for the variable pharmacokinetics of L-PAM are under study.

There are few reports in the literature on the relationship between pharmacokinetic parameters of anticancer drugs and therapeutic response [34]. In the absence of definitive data, the variable response of individual patients with solid tumors to chemotherapy is frequently attributed to host factors such as age, sex, genetic composition, and pathophysiology of the disease as well as drug absorption, distribution, metabolism, and elimination [8, 15, 41, 42] in addition to heterogeneity of the tumor cell population. Patient-to-patient differences are assumed to be responsible for the 2- to 3-fold variation in plasma levels of drug that result from the same dose of drug (on a body weight or area basis) [22].

That tumor response is directly related to pharmacokinetic parameters has not been well documented. Data on plasma pharmacokinetics have been useful in preventing toxicity of high-dose methotrexate and, to a lesser extent, other agents. Preisler et al. [36] reported that high plasma levels of adriamycin were associated with death during remission therapy and, for those patients who entered remission, with long remissions. In patients with adrenocortical carcinoma treated with 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethane, no therapeutic effect was observed if serum levels were <10 μg/ml; lasting remissions occurred in patients with serum levels >15 μg/ml [45]. Baguley and Falkenhaug [5] reported that plasma concentrations of cytosine arabinoside were significantly higher in patients with acute myeloblastic leukemia who responded to treatment than those who did not. However, Harris [24] found no such correlation. Garattini [22] reported a clear relationship between the peak concentration of doxorubicin in serum and tumor tissue and the calculated cell kill in mice bearing Lewis lung carcinoma tumors of unspecified size. Our data on the mam ad 16/C tumors treated with L-PAM indicate that there was a direct relationship between dose administered and the median response of a group of tumors, but there was not a direct relationship between plasma levels in an individual mouse and response of the tumor in that mouse.

Chemical hydrolysis is a major determinant of L-PAM disposition [17] and half-life in vivo [2, 3]. The effects of the physiological changes in the host (associated with large tumor burdens) on this major route of elimination are unclear. Altered pharmacokinetics of drugs have been associated with metabolic changes [6, 28, 38], renal or hepatic dysfunction [4, 35] and altered hematocrit [24]. That altered metabolism is not a major factor in the elevated levels of L-PAM is suggested by the reports that L-PAM is not extensively metabolized in vivo [19, 21] or in vitro [16]. It is subject to both urinary and fecal elimination; the latter is probably a result of biliary excretion [17], and concentrations of L-PAM in the bile often exceed those in plasma. Byington et al. [10] reported a compensatory increase in biliary excretion under conditions of renal failure in rats. However, renal excretion does not appear to be a major route of elimination. Alberts et al. [2] reported that the mean 24-h urinary excretion of unchanged L-PAM was about 13% of the dose administered i.v. In children given high-dose L-PAM for advanced malignant disease, the contribution of renal clearance was low (5.8% over 12 h [31]. In the dog, $<2\%$ of the injected dose could be accounted for in the urine in 4 h; after 8 h, L-PAM was not detectable [20]. Alberts et al. [4] reported a 75% increase in terminal-phase half-life and renal clearance following surgically induced renal failure in dogs. Cornwell et al. [13] reported that patients with renal dysfunction experienced a higher incidence of leukopenia than patients with normal renal function, but the reason for the increased toxicity is not known. Adair et al. [1] reported a positive correlation between the elimination rate constant and renal function in patients with multiple myeloma treated with oral L-

PAM. In contrast, jaundice was associated with lower plasma level and shorter half-life of plasma level and shorter half-life of plasma radioactivity from L-PAM in one patient [43].

Broggini et al [9] reported that adriamycin concentrations in plasma of rats bearing Walker 256 tumors were inversely related to changes in the cellular fraction of the blood; i.e., more of the drug became available to the plasma. A similar observation was reported in cancer patients [33]. Preliminary data in the mam ad 16/C system suggest that the amount of L-PAM associated with the cellular fraction could not account for the increased plasma levels observed in mice bearing large tumors (Simpson-Herren, unpublished data).

Costa [14] suggested that abnormal expansion of one body compartment (most often water) at the expense of another frequently occurs in advanced malignant disease. Preliminary data from this laboratory suggest that alterations in the volume of distribution of L-PAM were not related to the change in plasma levels, but additional studies designed to further clarify the factors responsible are in progress.

Our observation that prior L-PAM therapy (1, 2 or 3 doses administered 4 days apart) prevented the increase in plasma levels that might be expected in mice bearing advanced mam ad 16/C tumors or reduced the plasma level (and the variability) to approximate that found in tumor-free mice may be associated with the report that patients undergoing cycles of treatment with adriamycin do not achieve as high plasma levels in later cycles as were observed in the first cycle [23]. We have not determined whether the observed lower plasma levels of L-PAM in previously treated tumor-bearing mice were a direct result of the effects of L-PAM on the host or an indirect result of tumor inhibition. A direct effect on the tumor or tumor-associated changes in the host was suggested by the study of tumor-free mice that showed minimal effects of prior treatment on subsequent plasma levels of L-PAM. Also, reticulocytosis and granulocytosis, as well as reduction in plasma urea, have been associated with L-PAM therapy of mice bearing large mam ad 16/C tumors [25].

Host-to-host heterogeneity was not a major factor in the variable response of mam ad 16/C tumors to L-PAM. Elevated plasma levels of the drug that were observed following the initial treatment to mice bearing very advanced tumors (> 1.0 g) were not present following successive treatments with the same drug. The relationship between the observed plasma levels of L-PAM and the levels of L-PAM that reach the tumor cell population is a function of the tumor-to-tumor heterogeneity. This aspect of variable tumor response will be reported separately.

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