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Role of the Host in the Variable Chemotherapeutic Response of Advanced Ridgway Osteogenic Sarcoma

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Summary. *It is axiomatic that a given dose of an antitumor agent will not produce the same effect in 100% of the treated subjects. Numerous explanations regarding the sources of this heterogeneous response to drugs have been offered; however, there is a scarcity of experimental data allowing critical evaluation of the sources of variance. It is possible to study heterogeneous antitumor drug response in experimental, inbred animals. One animal model system, the advanced Ridgway osteogenic sarcoma, exhibits marked variation in its response to maximally tolerated doses of a number of clinically active antitumor agents. To evaluate the role of the host in the variable drug response, the tumor was bilaterally implanted into the flank regions of recipient AKR male mice. Treatment of the advanced tumor (200 mg–1,500 mg) with maximally tolerated doses of vincristine or* L*-phenylalanine mustard produced marked, but variable antitumor responses. Evaluation of a number of quantal and graded parameters of the chemotherapeutic response suggested that host heterogeneity contributes to variability. The host contribution was more apparent in this experimental model when the agent was noncurative. The underlying biological basis for the host heterogeneity is not known; however, it appears likely that pharmacological, immunological or other differences between the inbred animals account for the heterogeneity. Identification of these factors may be experimentally feasible in this animal model and help in the design of future studies in humans.*

Introduction

The literature is replete with suggestions and theories regarding the roles of host and tumor factors in the response to antitumor agents. However there is a scarcity of *experimental data* to establish the relative importance of such factors. It would be helpful to identify the major determinants of antitumor drug response so that appropriate means can be taken to identify patients who will or will not respond to a treatment with toxic drugs. For example, if host factors such as differences in rates of drug metabolism, immunogenic competence, etc., are major determinants, then more attention should be directed toward studies of the different hosts. On the other hand, if tumor cell heterogeneity, such as natural drug resistance [1, 2, 10, 11], is the major source of variance, then experiments should be designed to identify those tumor populations that will or will not respond to drug therapy.

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In an extensive series of experiments, Schabel et al. have demonstrated marked variability in the response of advanced Ridgway osteogenic sarcoma (ROS) to a large number of clinically active antitumor agents [20, 22]. Although it is clear that tumor cell heterogeneity may account for the variation in response observed between animals bearing this tumor, the possible role of the host has been largely ignored [20].

The purpose of this study was to establish the relative importance of host factors in the response of advanced ROS to anticancer agents. The experimental approach has been to bilaterally implant the tumor from a single donor into recipient mice. The purpose of bilateral implants is to test the importance of host factors. That is, if the host plays a major role in determining therapeutic response, there should be less variance between tumors within an animal than there is between tumors in different animals. The results obtained with vincristine and phenylalanine mustard (L-PAM) suggest that this tumor model system can be used to objectively demonstrate the role of host factors in the therapeutic response. A preliminary report of this work has appeared [15].

Material and Methods

Materials. L-PAM (Alkeran, brand of melphalan, Burroughs Wellcome Co., Research Triangle Park, NC, USA) and vincristine sulfate (Oncovin, Eli Lilly and Co., Indianapolis, In, USA) were obtained from the M.D. Anderson Hospital Pharmacy. The manufacturers' instructions for human parenteral use were followed in preparing the drug solutions.

Methods. ROS (kindly supplied by Mr Isodore Wodinsky, Arthur D. Little, Inc., Cambridge, MA, USA) was maintained by subcutaneous trocar implantation of approximately 40-mg fragments of tumors removed from mice implanted with the tumor 3–4 weeks earlier. Male AKR mice from the Jackson Laboratories, Bar Harbor, ME were used for all experiments. In chemotherapy studies, tumor fragments were bilaterally implanted into shaved flank regions of 2- to 3-month-old mice. Animal weight and tumor mass were monitored at least three times weekly, beginning approximately 15 days after tumor implantation. Caliper measurements of the subcutaneous tumors were made in two dimensions, and estimates of tumor mass were calculated using a formula that approximates the volume of a prolate spheroid [8]. Drug treatments were begun 20–23 days after tumor implantation, when the tumor size was between 200 and 1,500 mg. Data obtained when vincristine 1.0 mg/kg was given on

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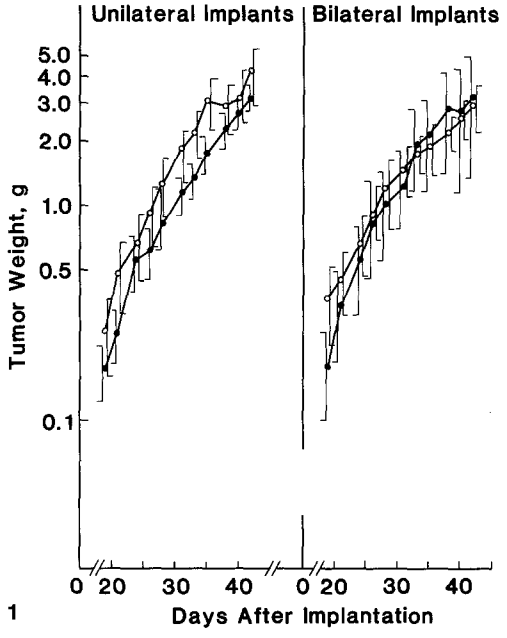
days 20, 24, and 28 after tumor implantation were combined from two experiments using 16 animals each. Two animals were excluded from the analyses of variance due to drug-related deaths (day 4 after first dose), and two animals were excluded because one tumor in each was less than 200 mg at the start of therapy. Similarly, data from three separate experiments (7, 8, or 10 animals) were combined for L-PAM treatments. One of the 25 animals died 76 days after tumor implantation without detectable tumors and he was considered among the group of 'bilateral cures'. The other two animals with bilateral cures were sacrificed 160 days after tumor implantation. There were no apparent drug-related deaths in the animals given L-PAM 10 mg/kg. With both drugs, the variability in chemotherapeutic response was similar in each separate experiment, and the qualitative interpretation of the results is not changed by combining the data. Following drug treatment, tumor growth was monitored until the bilateral tumors regrew to at least twice their original mass. Although AKR mice develop spontaneous and lethal leukemia-lymphoma between the 6th and 18th months of life [19], the animals used in this study were younger and no deaths were attributed to this spontaneous tumor.

Evaluation of Drug Response. The following characteristics of the possible tumor and host responses to drug treatment were evaluated: (1) animal weight; (2) tumor mass; (3) partial regression, i.e., reduction of tumor mass to $\leq 50\%$ of original mass; (4) complete regression, i.e., reduction of tumor mass to ≤ 20 mg (similar results were obtained whether 0 mg or 32 mg was used as endpoint); (5) time to reach partial remission, i.e., days to reach 50% of original tumor mass; (6) duration of remission; (7) days for the tumor to regrow to a particular size; and (8) cures, i.e., failure of the tumor to regrow to a detectable mass during the observation period (at least 70 days after tumor implantation). Graded responses were evaluated by the Kruskal-Wallis nonparametric analysis of variance. Quantal responses were evaluated using the binomial distribution to calculate probabilities.

Results

In tumors ranging from 100 mg to approximately 3 g, the presence of a contralateral tumor in mice did not markedly impede the growth of ROS (Fig. 1). The growth of this tumor was very predictable in AKR mice, and the data given in Fig. 1 are representative of several similar control experiments. The same tumor source was used for all the animals whose results are given in Fig. 1. Since ROS is nonmetastatic, evaluation of chemotherapy was limited to tumor mass measurements in these studies, i.e., for humane reasons animals were not always followed until death. There were no spontaneous regressions noted in these and other control animals, and the tumor 'take' rate was virtually 100%. Histological examination of the tumor 20 days after implantation revealed an undifferentiated sarcoma with numerous mitotic figures, many of which were atypical. Nuclei were highly pleomorphic, and either contained coarsely clumped chromatin or were vesiculated with large, prominent nucleoli. A more extensive discussion of the history and properties of this tumor has been presented [17, 18].

To evaluate the bilateral, advanced ROS as a model to study the role of host factors in variable antitumor drug response, the experiment illustrated in Fig. 2 was performed.



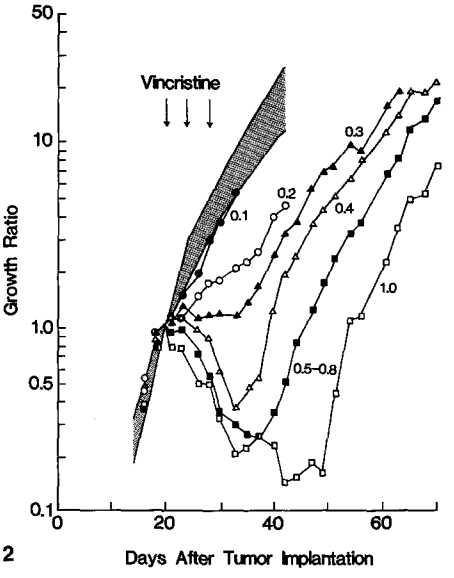


Fig. 1. Growth of bilateral and unilateral implants of ROS in AKR mice. Tumor fragments (approximately 40 mg) were implanted from a single donor on day 0 as described in *Methods*. Measurements of the subcutaneous tumors were made on the days indicated. The results shown are mean values ± SE for five animals per group (○–○) right side; (●–●) left side

Fig. 2. Dose-related inhibition of the growth of ROS. Mice bearing bilateral implants of ROS were treated IP with vincristine on days 20, 24, and 28 after tumor implantation. The *shaded curve* represents the growth of control, unilaterally implanted tumors in two separate experiments performed 1 year apart (99% confidence interval is given by the *shaded area*, $n = 37$). Growth of ROS in drug-treated animals represents the average values for at least four tumors (i.e., two mice at least for each dose level except for the 0.1 mg/kg dose, which represents the average value for two tumors in a mouse). Results are expressed as a 'growth ratio', i.e., relative to tumor mass measured on day 20 after implantation. *Numbers* associated with the *curves* indicate the daily dose of vincristine (mg/kg)

Various doses of vincristine (0.1–1.0 mg/kg given on days 20, 24 and 28) were administered to a group of 15 tumor-bearing mice. The different doses serve as an *experimental method* to increase the variance between animals. The dose range used encompassed the complete dose-response relationship for this drug on this schedule and for the tumor stage treated (Fig. 2). Evaluation of two graded responses (time for tumor growth to twice original mass and percentage of original tumor weight at the nadir) indicated that the variance of responses within animals was significantly less than that between animals (Table 1). Similarly, evaluation of the quantal response (partial regressions) indicated that response and failure to respond occur more frequently within animals than is expected were the response to occur randomly (P value = 0.05). Thus, this bilateral tumor model appears capable of detecting a host contribution toward the variable drug response, at least when the between-animal variance is large as was artificially produced in this test of the model.

At the maximally tolerated dose in male AKR mice (1.0 mg/kg), vincristine produced a marked antitumor effect in most mice bearing advanced ROS (Table 1). That is, there were 56 of 56 partial and 37 of 56 complete regressions of the tumors in the 28 animals treated with the LD_{10} dose of vincristine. Thus, if complete regressions were to occur randomly, one would anticipate that bilateral responses would occur in 12 animals and bilateral failures in three animals (Table 1). The observed numbers of responses and failures within the animals were greater than these predicted values (P = 0.09 and P = 0.05). Although most of the tumors were highly responsive, considerable variability was observed with this drug treatment (Fig. 3). For purposes of illustrating the *range* of responses seen in the 28 mice, the *least* and *most* responsive tumors were identified without regard to whether they were on the left or right side of the animals. The growth patterns of these selected tumors and that of the contralateral tumors in the same animals were plotted. Each number associated with the curves in Fig. 3 refers to a given mouse. From inspection of Fig. 3, it appears that marked response or failure to respond tends to occur in the same animal. Statistically, this apparent host contribution to the variance is tested by a Kruskal-Wallis analysis of the variance. This type of analysis does not assume that the measured response is normally distributed and it is less biased than a simple one-way analysis of the variance (a one-way analysis of variance always gave lower P values). The within-animal variance was significantly less than that observed between animals when several objectively measurable responses were evaluated ($P < 0.02$). Perhaps the most pharmacologically important index of the therapeutic effect is the easily measured regrowth of the tumors to a given size, such as the time required for the

Table 1. Analysis of the variance in chemotherapeutic response of bilateral ROS to vincristine

Dose of vincristine, mg/kg on days 20, 24, and 28	Graded responses				Parameter evaluated	Quantal responses			
	Days to grow to 2× original mass	Days to regress to $^1/_2$ of original mass	Duration of regression	% of original weight at nadir		Response within animal		Failure within animal	
						Expected	Observed	Expected	Observed
	Value of P[a]								
Various, Fig. 2	0.014	–	–	0.058	PR	5	8*	2	5*
1.0, Fig. 3	0.020	0.016	0.018	–	CR	12	15**	3	6*

Male AKR mice bearing bilateral tumors were treated IP on days 20, 24, and 28 after tumor implantation. Tumor size was monitored at least 3× weekly and until the tumor regrew to twice its original mass. The range of responses observed is illustrated in Figs. 2 and 3

[a] Kruskal-Wallis nonparametic anaysis of the variance within animals versus animals

*P = 0.05, binomial distribution

**P = 0.09, binomial distribution

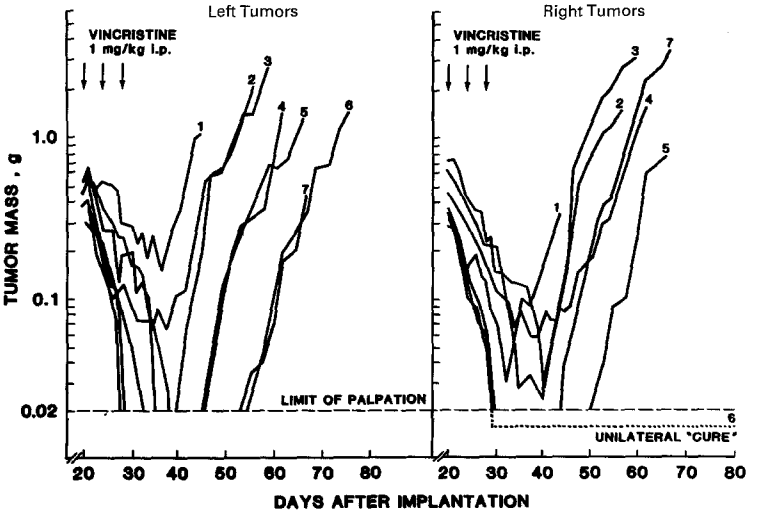


Fig. 3. Variation of the response of ROS to vincristine: Antitumor response between animals. Mice bearing bilateral tumor implants were treated with vincristine (1 mg/kg) on days 20, 24, and 28 and tumor mass was estimated by caliper measurements on the days shown. The least and most responsive (left or right tumors) were selected from 28 treated mice to illustrate the range and variation of response to this maximally tolerated dose of vincristine

tumors to regrow to twice their original mass. Most investigators would accept the premise that such regrowth is, among other factors, a reflection of the residual viable tumor mass following the chemotherapy (the 'clonogenic' population). Importantly, there was *no significant correlation* (correlation coefficients giving P values much greater than 0.10) between the tumor mass at the start of chemotherapy and the time required for the tumor to regrow to twice its original mass in these experiments. Thus, as Schabel et al. have reported [20, 22], the chemotherapeutic response is relatively independent of the original mass if the tumors are advanced and *similarly staged*, as they were in these experiments. Since the antitumor response was dramatic in most of the animals treated with a maximally tolerated dose of vincristine, comparison of the variance in measurements of tumor mass at the nadir is not given in Table 1. Similarly, since tumors in a number of the animals treated with *various doses* of vincristine failed to achieve partial regression, the analysis of variance regarding these graded responses is also not given in Table 1.

ROS, even in the advanced stage, is extremely sensitive to the alkylating agent L-PAM. Partial regressions were observed in all 25 of the animals with bilateral tumors; consequently, complete regressions and cures were the appropriate quantal responses to use to evaluate the possible host involvement in response to this drug. The numbers of the within-animal quantal responses or failures to respond that occurred did not exceed the expected frequencies, assuming that the tumors responded or failed to respond in a random manner (Table 2, $P > 0.10$, binomial distribution). Evaluation of the graded responses (days required for tumor regrowth and duration of remission) in animals that were not cured, either unilaterally or bilaterally, indicated that there was significantly less variance within animals than there was between animals. The variance in the rates of regression of all tumors was not significantly less for tumors within animals than it was for tumors in different animals ($P = 0.283$, Table 2).

To illustrate the *range* of antitumor effect observed in animals treated with L-PAM, the tumor mass measurements for the *most* and *least* responsive tumors were plotted (Fig. 4). Among the most and least responsive tumors in animals that experienced bilateral failures, there was an apparent host-related contribution; the response on one side tended to parallel

Table 2. Analysis of the variance in chemotherapeutic response of bilateral ROS to a maximally tolerated dose of L-PAM (10 mg/kg IP)

Graded responses			Parameter evaluated	Quantal responses			
				Response within animal		Failure within animal	
Days to grow to 2× original mass[b]	Days to regress to $^1/_2$ of original mass	Duration of regression[b]		Expected	Observed	Expected	Observed
Value of P[a]							
0.050	0.283	0.069	CR	14	15*	2	2*
			Cures	2	3*	13	14*

Male AKR mice bearing the advanced tumors were treated with L-PAM 23 days after tumor implantation. Tumor size was monitored at least 3× weekly until all tumors regrew to twice their original size. The range of response to this treatment is illustrated in Figs. 4 and 5

[a] Kruskal-Wallis nonparametric analysis of variance

[b] Excludes 'cures', unilateral or bilateral

* $P > 0.10$, binomial distribution

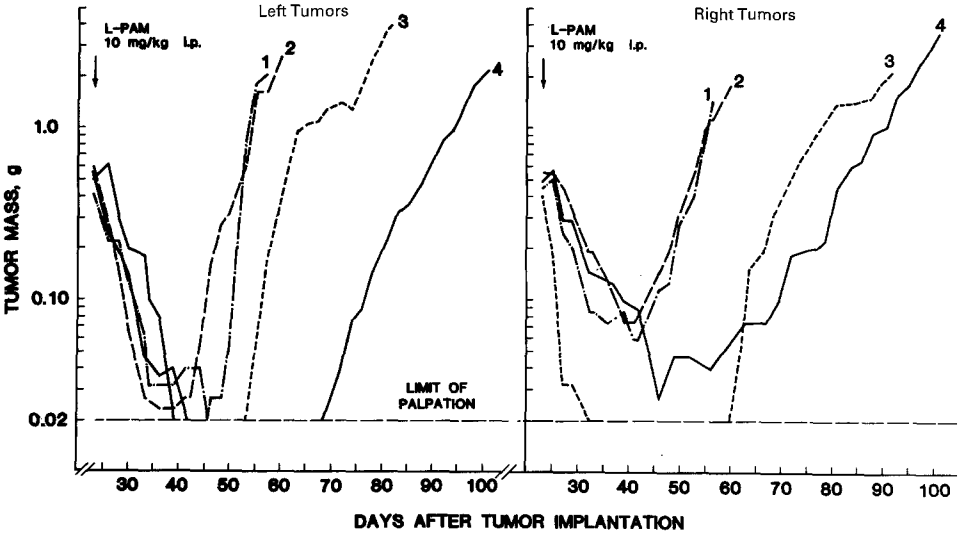


Fig. 4. Variation in the response of ROS to L-PAM: Antitumor response between animals. Mice bearing bilateral tumor implants were treated with L-PAM (10 mg/kg) on day 23 after tumor implantation. The least and most responsive (left or right tumors) were selected from 13 animals who were 'bilateral failures' (tumor regrowth occurred bilaterally) to illustrate the range and variation of response to this maximally tolerated dose of L-PAM

the response on the contralateral side. Another means by which the apparent host involvement in the antitumor response can be demonstrated is shown in Fig. 5. In the 13 animals (26 tumors) that experienced bilateral failures the antitumor effect, measured as a delay in the regrowth of the tumors, was less than that seen in the 'noncured' tumors in animals that were 'cured' on one side only (7 animals: 3 tumors on the right and 4 on the left side). The time required for tumor regrowth to twice its original mass was significantly less ($P < 0.05$) in bilateral failures than in the 'unilateral failures' (left side of Fig. 5). This result suggests that the noncured tumor in animals that were cured of the tumor on the opposite side achieved a greater chemotherapeutic response than did the tumors of those animals who experienced bilateral failures. The more dramatic antitumor effect in animals that experienced cures (unilateral or bilateral) was also reflected in a greater loss of body weight due to drug treatment (17.5% ± 2.6%, mean value ± SE, $n = 10$) compared with that seen in animals that experienced bilateral failures (14.0% ± 1.4%). Thus, the hosts also appeared to experience a greater effect of drug treatment when cures were observed. The rates of tumor regression in cured tumors were not markedly different from those in noncured tumors (Fig. 5). The initial weights of tumors that were cured did not differ significantly from those that were not cured (780 ± 149 and 609 ± 73 mg, respectively; mean values ± SE). Similarly, there was no significant correlation between initial tumor size and the time required for tumor regrowth to twice original size (correlation coefficient less than 0.16).

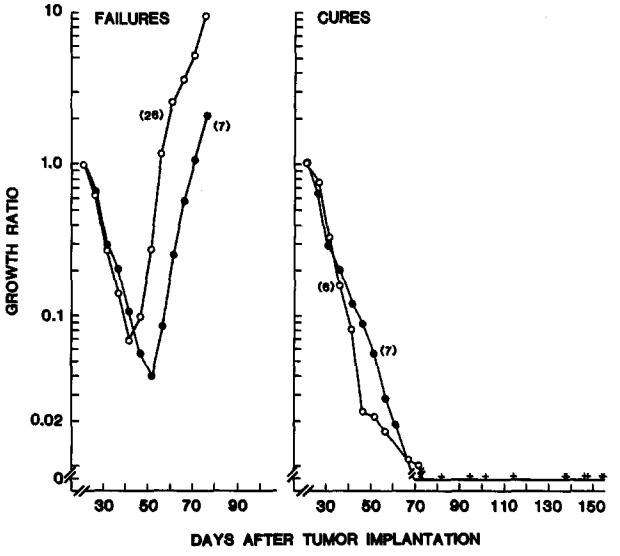


Fig. 5. Chemotherapeutic response of ROS to L-PAM. The mean responses of ROS to L-PAM 10 mg/kg administered on day 23 after tumor implantation are plotted. The figure to the *left* illustrates the response for tumors in 13 animals that were bilateral failures (tumor regrew during the time interval shown) or for seven animals that were unilateral failures (regrowth occurred on one side only). The figure to the *right* illustrates the response of cured tumors, either unilateral (7 tumors) or bilateral (6 tumors) (●——●) unilateral; (○——○) bilateral; (+ + +) day of death

Although much of the preceding data suggest a major contribution of the host toward the variability in antitumor drug response in this animal model system, marked variation in the response of tumors in the same animal was sometimes observed. Two examples of this within-animal variation are given in Fig. 6. The differential response of the two tumors in the animal treated with vincristine is *unique* in that this marked difference was only seen in one of 52 animals treated with vincristine at doses equal to or greater than 0.5 mg/kg on the schedule shown. The animal treated with L-PAM obviously experienced a very marked antitumor drug response in both tumors. As has been pointed out before [9], measurement of residual tumor mass at the nadir may not be indicative of the true viable tumor mass. The time required for tumor regrowth

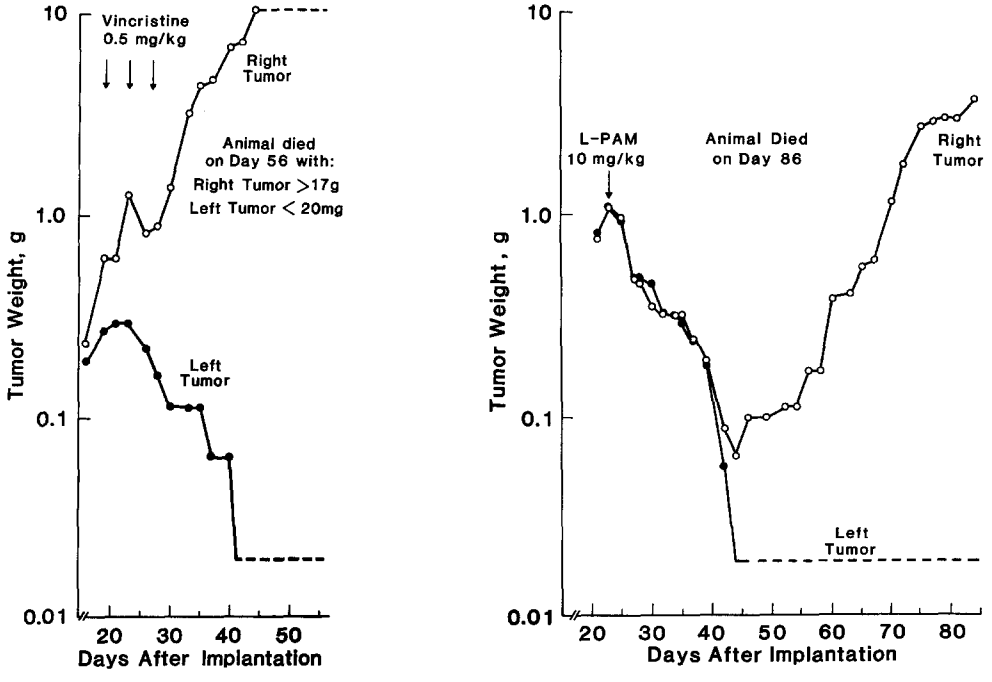


Fig. 6. Variation of the response of ROS to chemotherapeutic agents: Antitumor response within animals. Two animals, one treated with vincristine 0.5 mg/kg and the other treated with L-PAM 10 mg/kg as shown in Figs. 3 and 4, were selected to illustrate the large variation in drug response sometimes seen in tumors implanted in the same animal

Table 3. Chemotherapeutic response of bilateral ROS to maximally tolerated doses of vincristine or L-PAM

Parameter	Vincristine 1.0 mg/kg on days 20, 24, and 28		L-PAM 10 mg/kg on day 23	
	Left tumor	Right tumor	Left tumor	Right tumor
Initial weight of tumor (mg)	440 ± 35	411 ± 31	688 ± 89	626 ± 102
Days to regress to $^1/_2$ original mass	7.1 ± 0.6	7.4 ± 0.5	4.4 ± 0.3	5.7 ± 0.4
% of original weight at nadir[a]	4.5 ± 1.7	4.2 ± 1.3	2.4 ± 1.3	4.6 ± 1.3
Duration of regression, days[a]	23 ± 1	23 ± 1	29 ± 3	28 ± 2
Days to regrow to 2× original mass[a]	37 ± 1	36 ± 1	40 ± 3	43 ± 3

The values shown are the mean values ± SE for the experiments illustrated in Figs. 3 and 4. Time to regress to $^1/_2$ or regrow to 2× original mass refers to the days after the first dose of the drug treatment

[a] Cures, unilateral or bilateral, are not included in this calculation

on the right side of the animal treated with L-PAM suggests that this tumor underwent a 'near-cure'.

The results obtained in the measurement of chemotherapeutic response to the maximally tolerated doses of vincristine and L-PAM are summarized in Table 3, which reflects many of the observations discussed above. Also, the very similar antitumor response obtained in tumors implanted on the right and left sides is apparent.

Discussion

The potential factors which modify the effects of an anticancer agent (or any other drug for that matter) are numerous and have been the subject of many reviews. Identification of these factors is probably more important for cancer chemotherapeutic agents than for many other drugs because: (1) the slope of the dose–response relationship is steep [22] for the all-or-none effect, i.e., cell kill; and (2) the margin of safety or therapeutic index is generally small. It is the responsibility of the experimental chemotherapist to simplify the problem, that is, to identify those factors immediately relevant to the biological response. Our working hypothesis is: Some factors are host-related and others are tumor-related, and furthermore, for a given host/tumor/drug/schedule, some factors are more important than others in their effect on the eventual therapeutic outcome. The situation may be perceived as being analogous to a series of biochemical reactions that eventually lead to a product, the rate of formation being limited by only one of the reactions (the so-called Bottleneck step [12].

With the above thoughts in mind, we selected a simple animal model as an initial approach toward determining the relative importance of host factors toward chemotherapeutic response or failure. The model is the advanced ROS bilaterally implanted in AKR mice. Advantages of this tumor model for our purposes are as follows: (1) it is relatively nommetastatic [17, 18, 25], and therefore measurements of the subcutaneous mass are realistic estimates of the total tumor burden; (2) its growth is predictable (Figs. 1 and 2); (3) there are very few spontaneous regressions [17, 18]; and (4) the tumor is responsive to a large number of clinically active anticancer agents [17, 18, 20, 25].

The results obtained in the chemotherapy of advanced, bilateral ROS tend to support the working concept of many traditional chemotherapists. Specifically, the major factor for chemotherapeutic success or failure relates to the sensitivity of the tumor to the drug. For example, although there was an apparent role of the host in the quantal response of this tumor to a maximally tolerated dose of vincristine (Table 1), it must be appreciated that of 56 tumors in 28 animals, there was a single apparent cure. On the other hand, the within-animal quantal response to L-PAM did not suggest a strong host involvement; however, there were 13 cured tumors in 25 animals treated with an LD_{10} dose of the drug. Both agents are extremely effective against this tumor model; there were 37 of 56 complete regressions with vincristine and 31 of 50 complete regressions with L-PAM. It is obvious, therefore, that both agents produced significant, almost-curative therapy in animals treated with vincristine, and curative therapy in animals treated with L-PAM. Thus, although there are apparent host factors that reduce the variance of reponse between tumors within animals to therapy with vincristine, these factors do not improve the success of therapy in terms of tumor cure. In fact, it may be possible that the opposite occurs, that is, the host factors may play a role in promoting regrowth of the surviving fractions of tumor cells.

Since the antitumor response to L-PAM in these experiments was profound, it is not surprising that cures occurred as a seemingly random event (Table 2). This is because tumor cell kill is known to follow the law of mass action, and total cell kill may be required to achieve cure [13, 24]. On the other hand, evaluation of graded responses in animals that experienced bilateral failures indicated that there was also an apparent host contribution to the variability of response to L-PAM (Table 2). In other words, the possibility exists that there are pharmacological differences in the hosts that differ markedly in their response to L-PAM or vincristine (Fig. 3–5). In this regard, Struck et al. [26] reported that mice bearing ROS that differed markedly in their response to cyclophosphamide appeared to also exhibit measurable differences in their rates of metabolism of the drug.

Mendiondo et al. [14] recently demonstrated, in a similar animal model system, that host-associated factors are apparent in the response of an immunogenic tumor but not of a relatively nonimmunogenic tumor to radiotherapy. Of the two anticancer agents used in this study, vincristine is the less immunosuppressive. Thus, the possibility exists that the hosts' immunological defense mechanisms account for the greater-than-expected within-animal responses or failures when vincristine was used in our experiments (Table 1). Marked variation in immunological response between presumably identical, tumor-bearing animals has been clearly demonstrated by Suit et al. [27]. Assessment of immunological factors and pharmacological parameters, such as clearance or distribution of vincristine, in individual animals, and relation of these parameters to the therapeutic response are indicated experiments on the basis of our preliminary observations. To

date, antitumor drug monitoring has not been used to effectively predict clinical response. Such monitoring has been used to predict methotrexate toxicity in high-dose regimens and to establish appropriate schedules for arabinosyl cytosine therapy [4].

Tumor cell heterogeneity has resurfaced as a concept of importance in antitumor drug response. Clearly, within tumors there are subpopulations of cells with various degress of natural drug resistance, as has been shown by the work of Barranco [2], Heppner [11], Fidler [5], and others. In 1974, Hakansson et al. [10] performed experiments similar to those presented in this paper, using a mouse sarcoma transplanted serially or induced with a carcinogen. The sensitivities to antitumor drugs were assessed in fragments of the tumors in vitro. Marked differences in the abilities of drugs to inhibit DNA synthesis were observed in fragments taken from a given tumor nodule. This zonal behavior of tumors has been discussed by Fidler and Heart [6]. Fisher and Saffer [7] have recently reported results obtained with combined chemotherapy (cyclophosphamide) and immunotherapy (*C. parvum*) of bilaterally implanted C3H mammary carcinoma. These investigators interpreted their data to indicate that the tumors, rather than the hosts, were responsible for the observed variation in treatment effectiveness. In theory, use of human tumor stem cell assays in vitro to predict drug response or failure in vivo [16] would not be effective if the major determinants for success or failure were not tumor-associated. Evidence of tumor cell heterogeneity in the response of ROS to vincristine and L-PAM is presented in Fig. 6. The different response of the bilaterally implanted ROS to vincristine shown in Fig. 6 could very well be due to the right tumor being a predominantly vincristine-resistant line at the time that treatment was started. Spontaneous mutation to resistance among tumor cells is higher to vincristine than to any other current clinically useful anticancer agent [21]. In spite of this selected example of tumor heterogeneity, the striking observation reported in this paper is the objective demonstration, in the bilateral ROS model, of an apparent host contribution to the variable antitumor effect of these drugs. The underlying biological basis for this observation represents an area for future study, results of which may alter the manner in which we currently use these and other antitumor agents [3, 23].

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References

1. Abe I, Sato S, Watanabe M, Sato H (1978) Mechanism of natural resistance of rat ascites hepatomas to 1-ß-D-arabinofuranosylcytosine. Gan 69: 557–564
2. Barranco SC, Drewinko B, Ho D, Humphrey RM, Romsdahl M (1972) Differential sensitivities of human melanoma cells grown in vitro to arabinosylcytosine. Cancer Res 32: 2733–2736
3. Carpenter JT, Maddox WA, Laws HL, Wirtschafter DD, Soong SJ (1982) Favorable factors in the adjuvant therapy of breast cancer. Cancer 50: 18–23
4. Erlichman C, Donehower RC, Chabner BA (1980) The practical benefits of pharmacokinetics in the use of antitumor agents. Cancer Chemother Pharmacol 4: 139–145
5. Fidler IJ (1978) Tumor heterogeneity and the biology of cancer invasion and metastasis. Cancer Res 38: 2651–2660
6. Fidler IJ, Hart IR (1981) Biological and experimental consequences of the zonal composition of solid tumors. Cancer Res 41: 3266–3267
7. Fisher B, Saffer EA (1981) Heterogeneity of tumor growth during chemoimmunotherapy: Observations in a murine model. In: Fidler IJ, White RJ (eds) Design of models for testing cancer therapeutic agents. Van Nostrand Reinhold, New York, pp 114–135
8. Geran RI, Greenberg NH, MacDonald MM, Schuhmacher AM, Abbott BJ (1972) Protocols for screening chemical agents and natural products against animal tumors and other biological systems, 3rd edn. Cancer Chemother Rep [3] 3: 1–87
9. Griswold DP Jr, Schabel FM Jr, Wilcox WS, Simpson-Herren L, Skipper HE (1968) Success and failure in the treatment of solid tumors. I. Effect of cyclophosphamide on primary and metastatic plasmacytoma in the hamster. Cancer Chemother Rep 52: 345–387
10. Hakansson L, Trope C (1974) On the presence within tumors of clones that differ in sensitivity to cytostatic drugs. Acta Pathol Microbiol Scand [A] 82: 32–40
11. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi P (1978) Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res 38: 3758–3763
12. Kacser H, Burns JA (1973) In: Davies DD (ed) Rate control of biological processes, no 27. University Press, Cambridge, p 65
13. Mendiondo OA (1981) Multiple concurrent tumors in the same host: A model for chemotherapy. Cancer Immunol Immunother 10: 257–259
14. Mendiondo OA, Suit HD, Sedlacek RS (1980) Concurrent and subsequent tumors in the same host: A model to evaluate the host tumor interaction. Int J Radiat Oncol Biol Phys 6: 193–198
15. Nelson JA, Hokanson JA and Jenkins VJ (1982) Host factors in the variable chemotherapeutic response of advanced Ridgway osteogenic sarcoma. (Abstract) Proc Am Assoc Cancer Res 23: 223
16. Salmon SE (1980) Cloning of human tumor stem cells. Alan R. Liss, New York
17. Schabel FM Jr (1975a) Animal models as predictive systems. In: Cancer chemotherapy: Fundamental concepts and recent advances. Year Book Medical Publishers, Chicago, pp 323–355
18. Schabel FM Jr (1975) In: Descriptions of systems used in experimental screening of anti-cancer preparations in sixteen countries (CAN/75.6), World Health Organization, Geneva, pp 80–82
19. Schabel FM Jr, Skipper HE, Trader MW, Laster WR Jr, Cheeks JB (1974) Combination chemotherapy for spontaneous AKR lymphoma. Cancer Chemother Rep 4: 53–72
20. Schabel FM Jr, Griswold DP, Corbett TH, Laster WR Jr, Mayo JG, Lloyd HH (1979) Testing therapeutic hypotheses in mice and man: Observations on the therapeutic activity against advanced solid tumors of mice treated with anticancer drugs that have demonstrated or potential clinical utility for treatment of advanced solid tumors of man. In: Methods in Cancer Research Vol XVII Eds. H. Busch and V. Devita Jr. Academic Press Inc. NY pp. 3–50
21. Schabel FM Jr, Skipper HE, Trader MW, Laster WR Jr, Corbett TH, Griswold DP Jr (1980) Concepts for controlling drug-resistant tumor cells. In: Mouridsen HT, Palshof T (eds) Breast cancer. Experimental and clinical aspects. Pergamon Press, Oxford, pp 199–211
22. Schabel FM Jr, Griswold DP, Corbett TH, Laster WR Jr, Lloyd HH, Rose WC (1981a) Variable responses of advanced solid tumors of mice to treatment with anticancer drugs. In: Fidler IJ, While RJ (eds) Design of models for testing cancer therapeutic agents. Van Nostrand Reinhold, New York, pp 95–113
23. Schabel FM Jr, Griswold DP Jr, Corbett TH, Laster WR Jr (1981b) Increasing therapeutic response rates to anticancer drugs

by applying the basic principles of pharmacology. Presented at: Cancer 1981/Cancer 2001 – An International Colloquium, sponsored by The University of Texas System Cancer Center, MD Anderson Hospital and Tumor Institute, Houston, Texas, Nov 11–14, 1981
24. Skipper HE (1964) Perspectives in cancer chemotherapy: Therapeutic design. Cancer Res 24: 1295–1302
25. Skipper HE (1979) Ridgway osteogenic sarcoma: Response at different stages to surgery, single drugs, combinations of drugs and surgery chemotherapy. Universal-Microfilms International, Ann Arbor (Monograph publishing sponsor series)
26. Struck RF, Rose WC, Schabel FM Jr (1977) Attempts to develop a predicitive method for response of Ridgway osteogenic sarcoma to cyclophosphamide therapy. (Abstract) Proc Am Assoc Cancer Res 18: 45
27. Suit HD, Sedlacek R, Wagner M, Orsi L, Silobrcic V, Rothman KJ (1976) Effect of *Corynebacterium parvum* on the response to irradiation of a C_3H fibrosarcoma. Cancer Res 36: 1305–1314

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