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Antitumor Activity of Macromomycin B (NSC 170105) against Murine Leukemias, Melanoma, and Lung Carcinoma[1]

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SUMMARY

Mice bearing either of the two rapidly growing mouse leukemias, L1210 or P388, or the slow-growing B16 melanoma responded to i.p. injections of Macromomycin B (NSC 170105) with significant increases in life-span. The maximal increases in life-span obtained in these experiments were 37% for L1210, 68% for P388, and 120% for B16. In addition, there were 7 of 30 cures for varying doses of Macromomycin in the B16 melanoma. Activity of over 50% increase in life-span in B16 was obtained with a daily i.p. injection on Days 1 to 9 of 16 to 40 mg/kg. Animals that had received s.c. implanted Lewis lung tumors responded to either single or repeated injections (8 to 16 mg/kg) given at the site of tumor implant by a marked reduction in growth of the primary tumor, increased life-span, and some cures. The same doses were without effect when administered i.p. The reported activity of Macromomycin against L1210, P388 leukemias, B16 melanoma, and Lewis lung carcinoma make it a good candidate for development for clinical trial against human solid tumors.

A new method of evaluating activity against solid tumors, "responder analysis," is also presented.

INTRODUCTION

Antitumor activity of MCR,[2] the polypeptide antibiotic isolated from *Streptomyces macromyceticus*, was first reported by Chimura *et al.* (2). They found a 50% increase in life-span in mice bearing L1210 tumors when daily i.p. injections of a highly purified preparation at 40 μg/mouse/day were given. They also noted activity against Sarcoma 180.

Kunimoto *et al.* (5, 6) demonstrated that the cytocidal action of this polypeptide on cell culture was a function of its adsorption to the cell surface. Their studies were carried out on HeLa and Yoshida sarcoma cells. The surface adsorption of MCR was confirmed in culture on L1210 and TA3 cells and by transplantation studies by Lippman *et al.* (7, 9). Among the 50 or more drugs presently used in the clinic, none is known to exert its action by adsorption to the cell surface. Since the mode of action appears to be unique, it seemed appropriate to test MCR against those experimental tumor systems that have demonstrated high predictability for good clinical activity. Appropriate levels of activity against L1210 leukemia for synthetic drugs and P388 for natural products have had demonstrably good records as predictive systems for human tumors (4, 10), such as leukemias. While not yet firmly established as predictive tools, it appears that the slower growing solid mouse tumors, the B16 melanoma, and the Lewis lung carcinoma also appear to be useful animal models for activity against human solid tumors. The Lewis lung carcinoma is extremely refractory to most chemotherapeutic agents. Substantial activity against it has been demonstrated so far by agents that have been among the most active against solid tumors in the clinic. Analysis and correlative studies for these tumor systems and clinical activity have been made by Venditti[3] and also by Carter (1).

The data presented in this report demonstrate the antitumor activity of MCR against these model experimental systems and provide substantial experimental basis for development of MCR itself for clinical trial and for further investigation of antitumor action of other surface-adsorbing polypeptides as well.

MATERIALS AND METHODS

Tumors

L1210 and P388 Tumors. These are in the ascites form and are transferred to C57BL × DBA/2 F_1 (hereafter called $BD2F_1$) or BALB/c × DBA/2 F_1 (hereafter called $CD2F_1$) mice by i.p. injection of 0.1 ml containing 10^5 (L1210) or 10^6 (P388) cells taken from animals bearing 6- to 7-day-old tumors.

B16 Melanotic Melanoma. This is implanted i.p. into $BD2F_1$ hosts in the form of a tumor brei prepared from a

[1] Studies on B16 melanoma were carried out under contracts to Southern Research Institute (1-CN-12098), Hazelton (1-CN-23704), and Arthur D. Little (1-CN-33727) from Division of Cancer Treatment, National Cancer Institute, NIH, Department of Health, Education and Welfare, under the expert supervision of W. R. Laster, L. Dudeck, and I. Wodinsky, respectively.

[2] The abbreviation used is: MCR, Macromomycin.

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[3] J. M. Venditti. Relevance of Transplantable Animal-Tumor System to the Selection of New Agents for Clinical Trial. *In:* R. W. Cumley (ed.), Pharmacological Bases of Cancer Chemotherapy. University of Texas, M. D. Anderson Hospital, Houston, submitted for publication.

1:10 weight/volume homogenate of the tumor in balanced salt solution. The range of control survival times varies from narrow to very broad, depending upon the number of donor tumors pooled in preparing the brei. For this reason a true picture of the data on B16 melanoma is best seen by showing individual days of death rather than in the tabular form used for L1210 and P388 tumors. Percentage of increase in survival time and/or cures were the parameters used to measure the effectiveness of the agent. The details of the protocols used have been reported for all 3 systems used (3).

Lewis Lung Carcinoma. This is recovered from C57BL/6 hosts bearing 13- to 15-day-old tumors, cut into 35- to 50-mg fragments and inserted s.c. into the axillary region of $BD2F_1$ mice. Several individual host tumors are required for transplant to control and test animals for a single experiment. In order to minimize the time between excision of the donor tumor and its subsequent transplant in new hosts, the tumors of several donor animals are not pooled prior to transplant; rather, the animals are randomized following transplant of the tumors. In some cases wide ranges of survival times and bimodal distributions of survival times are observed that reflect differences in individual donor tumors used within an experiment. For this reason, in the case of the Lewis lung tumor the effect of the drug on survival time, as in the case with the B16 tumor, is also more adequately shown by indicating individual death days rather than by simple summary data of median survival time, range, and percentage of increase in life-span.

Two parameters are measured for each experiment, tumor size and survival time. Tumor size is determined by making a 2-dimensional measurement of the palpable tumor every 3rd day and converting these measurements to a mass equivalent based on calculations described elsewhere (3). Survival times are recorded and compared for individual animals on dead-day charts. A new method of analysis of this type of data is discussed in the text.

Test Materials

The MCR was a partially purified preparation (not greater than 30% purity) made available to the National Cancer Institute by Bristol Laboratories and designated Macromomycin B. It was prepared in aqueous solution immediately prior to use. Injections were either i.p. or local s.c. at the tumor site. Day 1 is the day following implantation of the tumor.

RESULTS

L1210 Tumor. Table 1 shows 3 treatment schedules for the L1210 tumor. The median survival times were increased by 2 to 3 days with a maximal increase in survival time of 37%. These findings mirror unpublished results obtained at Bristol Laboratories in which maximal percentage of increase in life-span was + 36% for 9 daily injections of 8 mg/kg. Since MCR proved to be a very potent cytotoxic agent against L1210 cells established in cell culture, this modest level of activity was unexpected. In order to determine whether the limited *in vivo* responses were due to failure of the MCR to reach the cells, an experiment was carried out in which L1210 cells were incubated from 2 to 180 min at varying concentrations of MCR prior to their transplant into $BD2F_1$ hosts. These data show that incubation up to 3 hr did not significantly influence the survival time (Table 2). Concentrations of 1, 2, or 4 μg/ml gave progressively increasing survival times. The average increase in survival time was still, however, only 37% for 4 μg/ml, a concentration that is lethal to L1210 cells in culture.

P388 Tumor. A series of treatment schedules similar to those used against L1210 were used for the P388 tumors (Table 1). Multiple injections, whether daily or every 4th day, gave a comparable increase in survival time with a maximum of 65% increase in life-span. At the highest dose on these schedules animals began dying 1 or more days beyond the range of the controls.

B16 Tumor. While a single i.p. injection of MCR produced a relatively small increase in median life-span (+ 22%), repeated injections were highly effective.

A series of 3 experiments were carried out (in 3 different laboratories) at varying dose levels in which animals were treated on a daily 1- to 9-day schedule. In all 3 experiments daily doses of MCR at levels at 16 to 40 mg/kg produced an increase in life-span greater than 50%, demonstrating unquestionably the significant antitumor activity of MCR against the B16 melanoma.

Closer inspection of the data presented in these 3 separate experiments reveals the inadequacy of using percentage of increase in survival time as the sole criterion for comparison of activity with this tumor system. Since the tumor is transplanted as a brei with aggregates of cells rather than single cells, accurate counting of the number of viable tumor cells injected is difficult and will vary from experiment to experiment. Furthermore, it can be plainly seen in Charts 1, 2, and 3 that the variability in range of survival time of control groups in the different laboratories is considerable. While the range of survival was 10 days in 1 experiment and as much as 35 days in another, the median varied by only 8 days. Neither the median nor range, alone or together, gives a clear picture of the survival time pattern of treated individuals compared to their respective control groups. Another way of considering the results is on the basis of "responder analysis." In this method "unequivocal responders," defined here as those individuals surviving beyond 95% of their own control group, are scored. For example, in Chart 1 there is a clear-cut shift in survival at all dose levels, and at all levels 80 to 90% of the treated groups were unequivocal responders. In Chart 2 the control range is somewhat broader, and only 40% of the total treated population, at the 3 highest doses, survived beyond 95% of the controls. In Chart 3 there were 10 of 30 unequivocal responders that died by Day 85 and there were another 7 cures, giving 56% unequivocal responders. In the case of Chart 1 the 80 to 90% unequivocal responders show the

dramatic nature of the treated population shift much more than a 58% increase in life-span. In Chart 3 the 56% unequivocal responders modulates the inordinately high 120% increase in life-span, which does not express the large number of individuals who did not respond at all.

A further refinement of the responder analysis would be to group the unequivocal responders with regard to how long they lived beyond the 95% cut-off control point. This should also be expressed relative to the control range. The control range is divided in half to determine the duration of each subgroup. In the case of Chart 3 the control range is from Day 16 to Day 43, or 27 days; the halfway mark would be 14 days. Therefore, Group 1 consisted of animals that died within 14 days of the 95%-control cutoff day, Group 2 animals died within 28 days, Group 3 animals died within 42 days, Group 4 animals died beyond 42 days, and Group 5 consisted of cures.

The unequivocal responders and cures for each dose range of the 3 separate B16 experiments are shown in Table

Table 1

Effect of NSC-170105 on survival time of mice bearing L1210 or P388 ascites tumors

Experiment	Schedule (Day)	Dose (mg/kg)	Median survival time	Range (days)	% increase in life-span
1-L1210	1, 5	48	11.0	(9-12)	+37
		24	11.0	(11-11)	+37
		12	11.0	(7-11)	+37
		6	9.0	(9-11)	+12
		0	8.0	(7-9)	
2-L1210	1, 5, 9	8	12.0	(11-13)	+33
		4	11.0	(10-12)	+22
		2	9.5	(9-11)	+5
		0	9.0	(9-12)	
3-L1210	1-9	8	11.0	(11-13)	+22
		4	11.0	(8-12)	+22
		2	11.5	(6-12)	+22
		1	11.0	(10-12)	+22
		0	9.0	(8-12)	
1-P388	1	16	16.5	(14-20)	+37
		8	15.0	(13-18)	+25
		4	15.0	(14-18)	+25
		0	12.0	(7-21)	
2-P388	1, 5, 9	8	16.0	(13-18)	+68
		4	15.0	(15-16)	+57
		2	15.0	(13-18)	+57
		0	9.5	(9-12)	
3-P388	1-9	8	16.0	(14-17)	+68
		4	15.0	(12-16)	+63
		2	15.0	(15-16)	+57
		1	15.0	(12-16)	+57
		0	9.5	(9-12)	

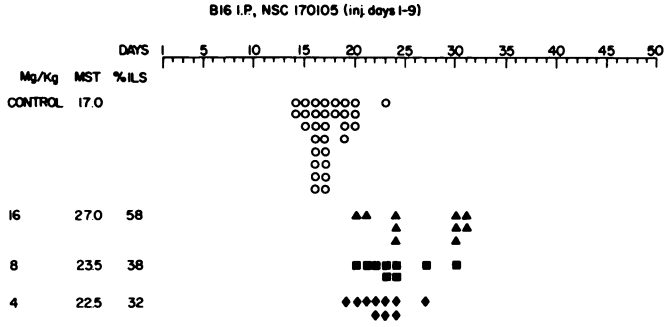

Chart 1. Survival time of animals bearing i.p. B16 tumors following daily i.p. injections (Days 1 to 9) of MCR at dose levels as indicated. *inj.*, injection; *ILS*, increase in life-span; *MST*, median survival time.

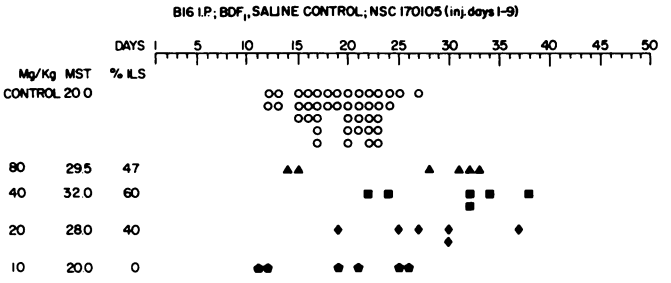

Chart 2. Survival time of animals bearing i.p. B16 tumors following daily i.p. injections (Days 1 to 9) of dose levels as indicated. *inj.*, injection; *ILS*, increase in life-span; *MST*, median survival time.

Table 2

Survival time (days) of BDF$_1$ mice following i.p. injection of L1210 cells preincubated for varying periods of time at different concentrations of MCR

Incubation time (min)	0 (control) survival time	Concentration of MCR (μg/10^5 cells) 1		2		4	
		Survival time	Significance[a]	Survival time	Significance	Survival time	Significance
2-5	9.50 ± 1.08[b]	10.60 ± 1.64	±	10.90 ± 0.99	++	12.88 ± 1.53	+++
30	12.77 ± 2.22	11.20 ± 1.39	–	15.70 ± 5.49	–	14.70 ± 1.94	+
60	9.30 ± 0.48	9.90 ± 0.87	–	11.40 ± 1.17	+++	15.30 ± 3.65	+++
180	9.00 ± 1.15	10.30 ± 1.05	–	11.80 ± 0.91	+++	12.90 ± 0.73	+++
Av. survival time	10.14	10.50 (+3)[c]		12.45 (+22)		13.94 (+37)	

[a] –, $p > 0.1$; ±, $0.5 < p < 0.1$; +, $0.2 < p < 0.05$; ++, $0.2 < p < 0.01$; +++, $p > 0.01$.
[b] Mean ± S.D.
[c] Numbers in parentheses, percentage of increase in life-span.

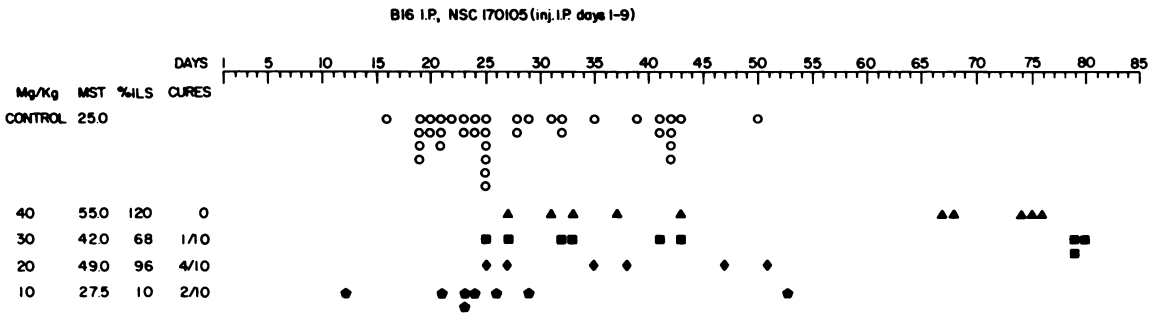

Chart 3. Survival time of animals bearing i.p. B16 tumors following daily i.p. injections (Days 1 to 9) of MCR at dose levels as indicated. *inj.*, injection; *ILS*, increase in life-span; *MST*, median survival time.

Table 3

Selection of optimal dose range based on data from 3 separate experiments, with combined total of at least 25 test individuals

Unequivocal responders are those surviving beyond the range of survival of 95% of the untreated individuals in their experimental group.

Dose range (mg/kg)	Unequivocal responders	Total	%	Cures
30-40	4/6, 5/10, 4/10	13/26	50	1
16-20	4/6, 6/10, 9/10	19/26	73	4
8-10	0/6, 3/10, 8/9	11/25	44	2

3. This analysis indicates that one can expect an average of 73% of treated individuals to be unequivocal responders at 16 to 20 mg/kg with some cures.

Lewis Lung Tumor. A single i.p. injection of MCR at 4, 8, or 16 mg/kg was without effect on survival time, while a local s.c. injection increased the life-span of animals receiving the 2 higher doses by 41 and 58% (Chart 4). When a 16-mg/kg dose was given as local s.c., 90% of the animals were unequivocal responders. A local s.c. injection of 0.9% NaCl solution produced no increase in survival time. The tumor weight data (Chart 5) show that, while growth of the primary tumor was significantly retarded for 5 days following the single local s.c. injection, the subsequent rate of growth was normal for the 2 lower doses but, at the 16-mg/kg dose, remained lower than the control.

Comparison of the survival effects of 7 daily injections of MCR given i.p. *versus* local s.c. in doses of 5, 10, 20, and 30 mg/kg showed that significant increase in survival time was not obtained with i.p. injection, while median survival time was increased for all but the lowest dose given local s.c. Measurement of the growth of the primary tumor showed a clear dose-dependent response with the local s.c. injection, while no effect on growth was observed with i.p. injections.

Chart 6 shows an experiment where injections were given on Days 1 to 9. The local s.c. injections produced a marked increase in survival time (90% increase in life-span) with 3 of 10 cures for the 16 mg/kg dose. All 10 animals in this group lived beyond the 28th day and were unequivocal responders. At the 8 and 4 mg/kg doses the survival time was also considerably increased. The tumor weight data dramatically support these results (Chart 5). For all 3 doses the tumor size remains negligible for the 1st 5 days, but at the highest dose, 16 mg/kg, this effect was prolonged through at least Day 12, when tumors in some individuals did begin to show measurable growth. In contrast to local s.c. injection, i.p. injection was not effective. Although a 36% increase in median life-span was obtained with a 16-mg/kg dose i.p., no accompanying size reduction was noted for the primary tumor. It is possible that i.p. injection at this level was able to produce an effect on metastatic lesions in the lung without producing an effect on the primary tumor.

The effect of either route of administration of MCR starting after the tumor is well established (Day 7) was examined. An increase of 35% in survival time was observed but was not impressive in light of the distribution of deaths and the lack of any observable effect on the primary tumor.

DISCUSSION

In this study, activity of MCR against L1210 and P388 leukemias has been shown at doses well below toxic levels. On the basis of the retrospective analysis of Venditti, the

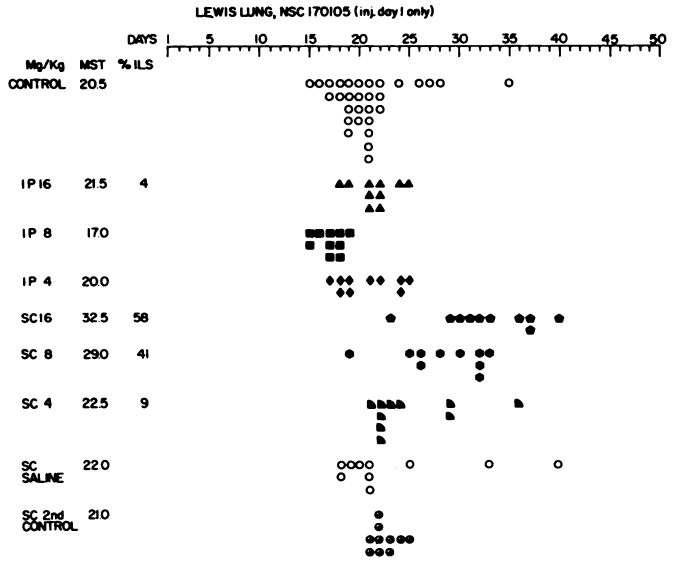

Chart 4. i.p. or local s.c. injection of MCR on Day 1 only into Lewis lung tumor. Controls at the top of the chart are uninjected. At the bottom of the chart a parallel set of controls injected locally with 0.9% NaCl solution (*SALINE*) and uninjected are shown. *inj.*, injection; *ILS*, increase in life-span; *MST*, median survival time.

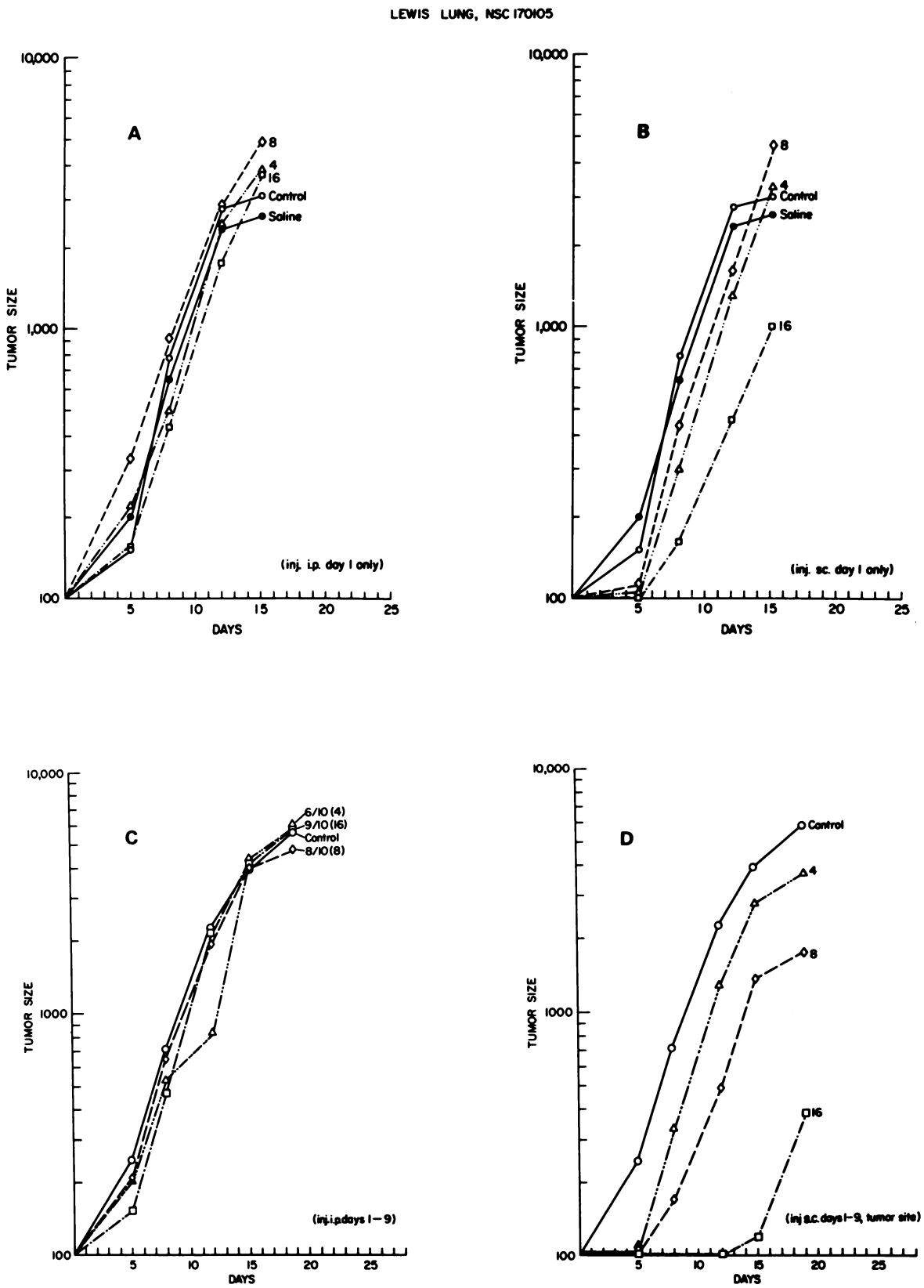

Chart 5. Lewis lung tumor size in mg. i.p. injection (*A*) *versus* local s.c. injection (*B*) of MCR, Day 1 only. i.p. injection (*C*) *versus* local s.c. injection (*D*) of MCR, Days 1 to 9.

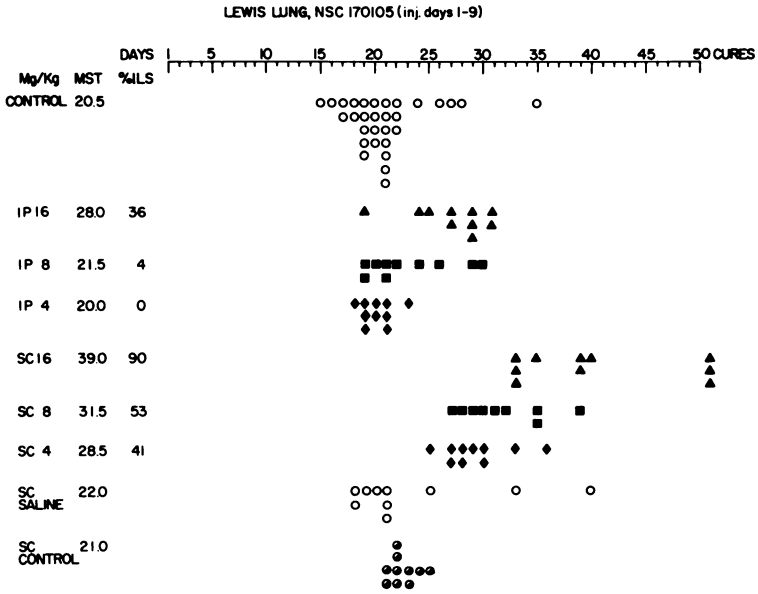

Chart 6. i.p. or local s.c. injection of MCR on Days 1 to 9 for Lewis lung tumor. Controls at the top of the chart are uninjected. At the bottom of the chart a parallel set of controls given local injections of 0.9% NaCl solution (*SALINE*) and uninjected are shown. *inj.*, injection; *ILS*, increase in life-span; *MST* median survival time.

level of L1210 and P388 activities obtained would predict about an 80% chance of showing good activity against human solid tumors. The B16 melanoma, a slower growing solid tumor representative of such tumors in humans, is also considered to be a useful predictive model system.

Carter (1) scored the activity of 54 known antitumor drugs against solid human tumors and rated 21 of them as highly active or active. The remaining 33 were marginally active, inactive, or inadequately tested. Venditti and Carter (1) showed that 15 of the 21 highly active and active drugs produced significant increase in survival time (over 30%) in the Lewis lung system; in contrast, none of the 33 drugs that were marginal or inactive against human solid tumors was effective against Lewis lung. Cyclophosphamide (NSC 26271), isophosphamide (NSC 109724), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037), 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962), 1-(2-chloroethyl-3-(4-methylcyclohexyl)-1-nitrosourea (NSC 95441), and tris(1-aziridinyl)phosphine sulfide (NSC 6396) were the only 6 agents of the 54 studied that had significant activity against all 4 tumor systems (L1210, P388, B16, and Lewis lung). All of these are highly active except isophosphamide, which was rated active. The inference is, therefore, that the anti-Lewis lung activity of MCR alone gives good indication of potential activity against human solid tumors. The graded activity against all 4 tumors according to the evaluation methods of both Venditti and Carter (1) places MCR in a category with such active drugs as vincristine, actinomycin D, and mitomycin C and in a higher category than methotrexate, 5-fluorouracil, or tris(1-aziridinyl)phosphine sulfide.

The results on the Lewis lung carcinoma show that activity against the primary tumor depends upon local injection of the MCR. Death from this tumor results from metastasis to the lungs that occurs by Day 7. The increase in survival time produced by local injection of MCR is probably associated not only with reduced size of the primary tumor but also with reduction in metastasis. This point will have to be established by further experiments. The need for local application of macromolecules is important as it is likely that activity of other high molecular weight compounds is obscured because of poor delivery.

The Lewis lung experiments involved single or daily injections. An apparent 5-day duration of reduced growth following a given injection suggests that an intermittent schedule might be as effective as daily injections.

Evidence presented elsewhere indicates that MCR arrests cells in the G_2 stage of the cell cycle (7, 8). Accordingly, combination of MCR with other drugs that act by other mechanisms and/or at other times in the cell cycle will be tested.

An analytical scheme for comparing drug effectiveness has been presented. This method focuses on the incidence of response to treatment rather than on the average response of the group. Further grading for level of activity within the responding group gives a detailed evaluation and presses much more information out of the same data than just median, range, and cures. In the light of any evidence to the contrary or until specific studies are available, it may be valuable to continue to assume that individual variation in response of experimental animals offers a suitable model for variable human response to drug therapy. Whether or not this assumption is valid is not known at this time; nevertheless, responder analysis is at least as informative and important a tool in evaluating drug performance as is increase in median survival time.

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