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Potentiation by amphotericin B of the cytotoxicity of anticancer agents against MOPC-315 plasmacytoma and lewis lung carcinoma

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Summary. *The ability of amphotericin B (AmB) to potentiate the cytotoxicity of several different anticancer agents against two murine tumor models was examined. A spleen colony assay was used to quantitate the cytotoxicity of BCNU, CCNU, and* L-*PAM, either alone or in combination with AmB against the MOPC-315 plasmacytoma. A high level of potentiation of the effects of CCNU and* L-*PAM by AmB occurred, but AmB did not increase the cytotoxicity of BCNU. Tumor growth curves and calculation of cell survival demonstrated significant potentiation of the cytotoxicity of CCNU by AmB against SC Lewis lung carcinoma.*

Introduction

We have previously demonstrated that in vivo treatment of tumor-bearing mice with AmB can potentiate the cytotoxicity of a variety of anticancer agents [2, 3]. This potentiation of cytotoxicity occurred with both AKR and L1210 leukemia cells, but not with normal hematopoietic stem cells (unpublished data). This specificity of the action of AmB for tumor cells, as against normal host cells, has important implications for the clinical use of AmB in cancer protocols. We describe here our findings with two other tumor models: another hematological tumor, the MOPC-315 plasmacytoma, and a solid tumor, Lewis lung carcinoma. For both tumors, potentiation of cytotoxicity of CCNU and L-PAM by AmB was noted. The extent of potentiation was similar in magnitude, if not greater, than that noted previously for the AKR and L1210 leukemias (unpublished data).

Methods and materials

Drugs. Amphotericin B (amB), in the form of Fungizone, was purchased from E. R. Squibb and Sons, Inc., Princeton, NJ, and was dispersed in a 5% dextrose solution immediately before use. The dose of AmB refers to dry weight of the commercial preparation (AmB + deoxycholate + salts). 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-nitrosourea (CCNU), and phenylalanine mustard (L-PAM) were obtained from the Drug Synthesis and Chemistry Branch, NCI. BCNU was dissolved in ethanol. CCNU was dissolved in a 1 : 1 mixture of polyethoxylated oil (Protamine Chemical Co.) and absolute ethanol. L-PAM was dissolved in 1 ml acidified ethanol (5% conc. HCl in absolute ethanol). All agents were further diluted in 0.9% NaCl and administered IP in a volume of 0.5 ml. In all experiments, AmB was administered as four daily doses of 0.5 mg/mouse and the anticancer agent was administered within 5 min following the fourth dose of AmB. In all cases, the highest dose employed was below the maximum tolerated dose for the mouse.

Mice. Mice aged 7–9 weeks and weighing 20–24 g were used in these studies. The mice were bred in our animal facilities from stock supplied by the Jackson Laboratories, Bar Harbor, Me. Mice were allowed food and water freely.

MOPC-315 plasmacytoma. Balb/c mice received 10^6 MOPC-315 cells IV, and 11 days later received AmB IP for 4 consecutive days. On day 4 of therapy, which was 14 days after tumor inoculation, the mice then received different doses of designated anticancer agents IP. Twenty-four hours later their spleens were removed and assayed for plasmacytoma colony-forming units by a previously described assay [6]. Results were compared with those in an untreated control group.

Lewis lung carcinoma. The Lewis Lung cell line is maintained in our laboratory by SC passage of 10^6 viable tumor cells every 3 weeks in C57Bl/6 female mice. The experimental tumor-bearing mice used in this study were 7- to 9-week-old, female BDF_1 (C57Bl/6 ♀ × DBA/2 ♂) or C57Bl/6 mice, which had each received 10^6 viable Lewis lung cells SC. The diameter of the tumors was measured daily to the nearest 0.5 mm using a template. Three distinct size ranges of tumors were studied (4.5–7.5 mm; 8.5–12 mm and 13–16 mm). Treatment with AmB was initiated on the day the tumors reached these prescribed diameters. The mice received four daily doses of 0.5 mg AmB and on day 4 of therapy they also received the indicated dose of CCNU. Tumor size was then measured in the individual mice every 2 days thereafter in the treatment groups of 10 mice each. The mice were killed when the tumors reached 20 or 25 mm diameter. The measurements of the tumors were averaged and plotted; data points were fitted by eye.

Results

MOPC-315 plasmacytoma

In this series of experiments, mice received increasing doses of BCNU, CCNU, or L-PAM, either with or without AmB pretreatment, and the effects were measured by the spleen

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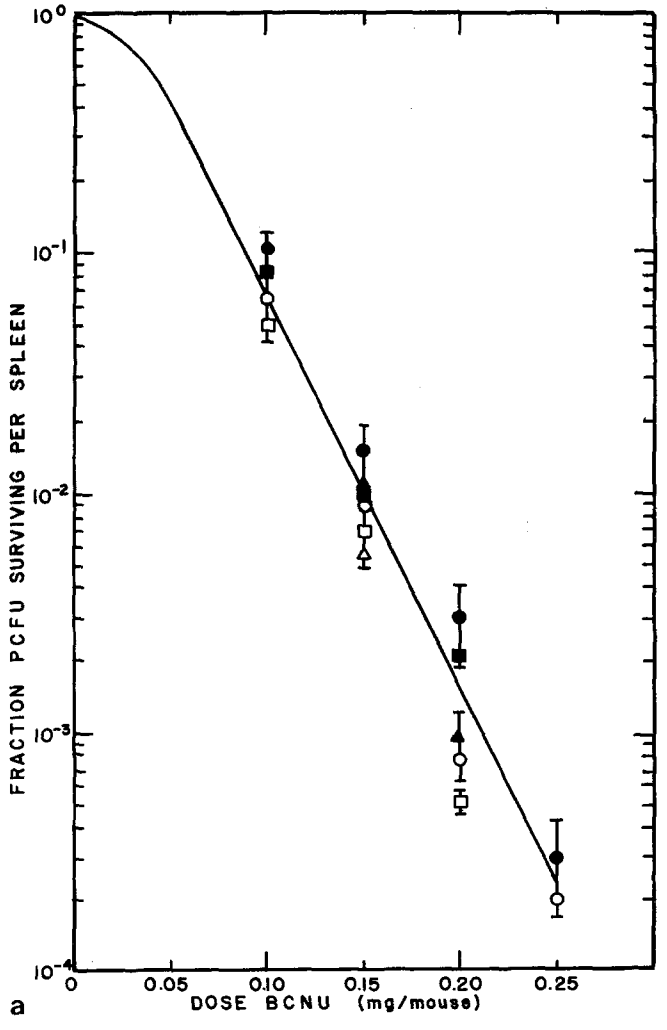
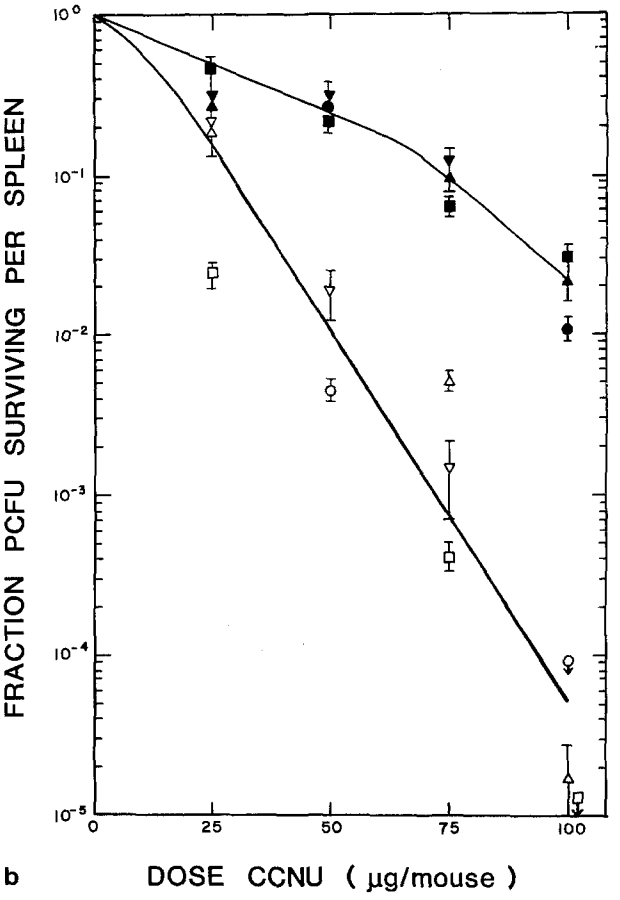
colony assay (Fig. 1). Figure 1a shows that AmB did not increase the cytotoxicity of BCNU. In contrast, there was a significant potentiation of the cytotoxicity of CCNU and L-PAM when the mice were pretreated with AmB (Fig. 1b, c). When the extent of potentiation is expressed in terms of the ratio of the slopes of the dose-survival curves, the potentiation index (PI) is obtained. For CCNU alone, the dose-survival curve was biphasic; thus, we calculated two PI values, 3.8 and 1.8, which represented the low- and high-dose segments, respectively. The results with L-PAM demonstrated a level of potentiation (PI = 2.4) that was intermediate between the two values calculated for CCNU.

Lewis lung carcinoma

Initially, we established that 1 mg CCNU/mouse was lethal in 100% of the treated mice. At 0.75 mg CCNU/mouse none of the animals died; however, 20% of the animals died when the AmB pretreatments were added to this dose of CCNU. We chose, therefore, to use dosages of 0.25, 0.50, and 0.75 mg CCNU in our studies. The effect of these doses of CCNU, either alone or in combination with AmB, was examined in the





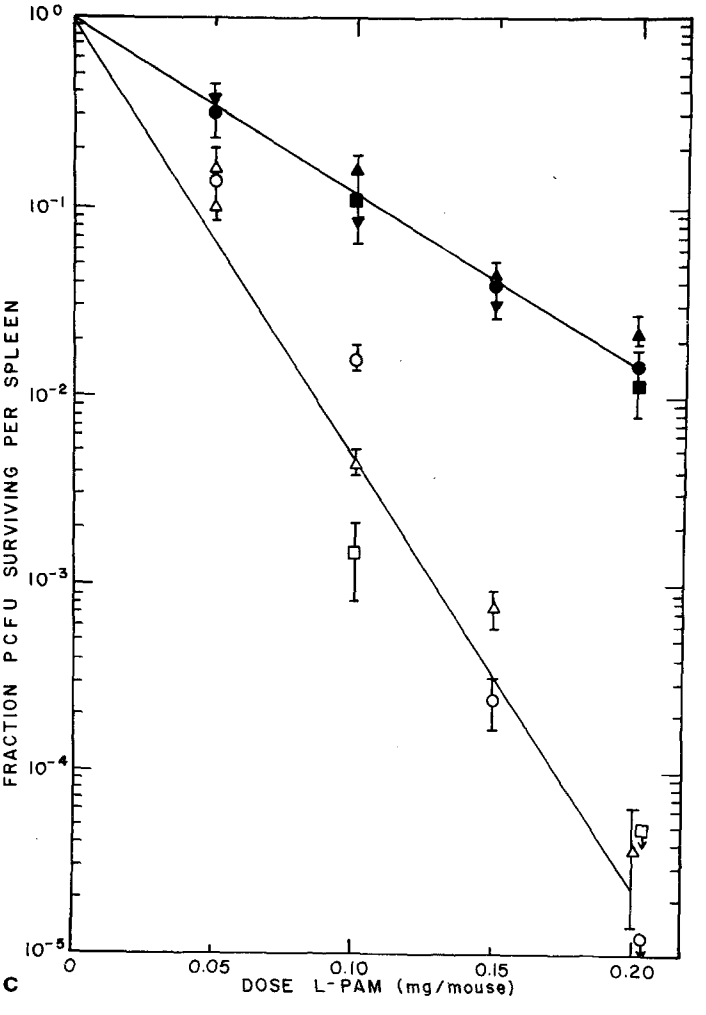


Fig. 1a–c. Survival of MOPC-315 plasmacytoma CFU following BCNU **(a)** CCNU **(b),** or L-PAM **(c)** alone *(closed symbols)* or in combination with AmB *(open symbols)*. Different symbols refer to different experiments. Errors are ± 1 SE

three different diameter groups of tumors. The averaged results of three separate experiments are presented in Fig. 2.

For the smallest tumors (4.5–7.5 mm), the average tumor size at the start of treatment with AmB (day 0) was 6.0 mm (Fig. 2a). The slope of the control growth curve yielded a doubling time of about 1.5 days during the time of treatment of the mice. After treatment with 0.25 mg CCNU per mouse there was no discernible effect on the growth rate of the tumor. Pretreatment of the mice with AmB, however, potentiated the cytotoxicity of CCNU sufficiently to yield a discernible effect on tumor growth. The extent of drug effect is quantitated in this study by defining a delay in tumor growth. A 'T-C' value for the difference between tumor (T) and control (C) growth curves was determined at a tumor size of 16 mm. This specific endpoint of tumor size was chosen because at this point the tumor in the control group was still in the exponential phase of growth and all treated groups had recovered from the therapy. For the AmB plus CCNU regimen, T-C equals 5.8 days; this and other T-C results are presented in Table 1. For the dose of 0.5 mg CCNU per mouse alone a definite effect on tumor growth (T-C = 5.0 days) was observed, while the combination of AmB plus CCNU had a very pronounced effect (T-C = 25 days). With 0.75 mg CCNU alone the T-C was 12.5 days; however, the majority of the animals receiving the combination died from toxicity and a T-C value could not be calculated. All but one of the mice died within 8 days following this combination.

For the medium-sized tumors (8.5–12 mm), the average tumor size at the start of AmB treatment (day 0) was 10.3 mm (Fig. 2b). Response to therapy followed a pattern similar to that noted above for the smaller tumors, but in this case the

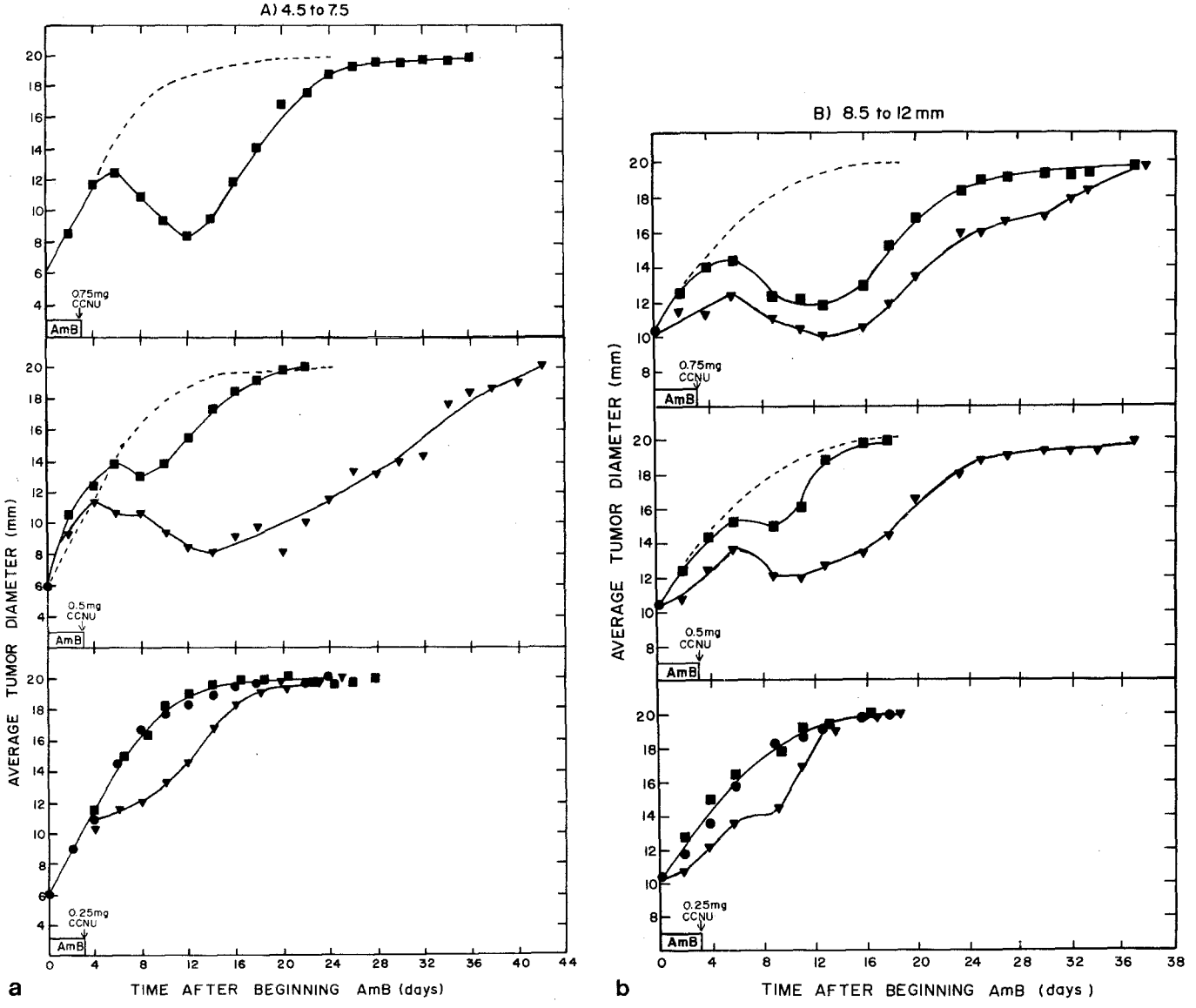


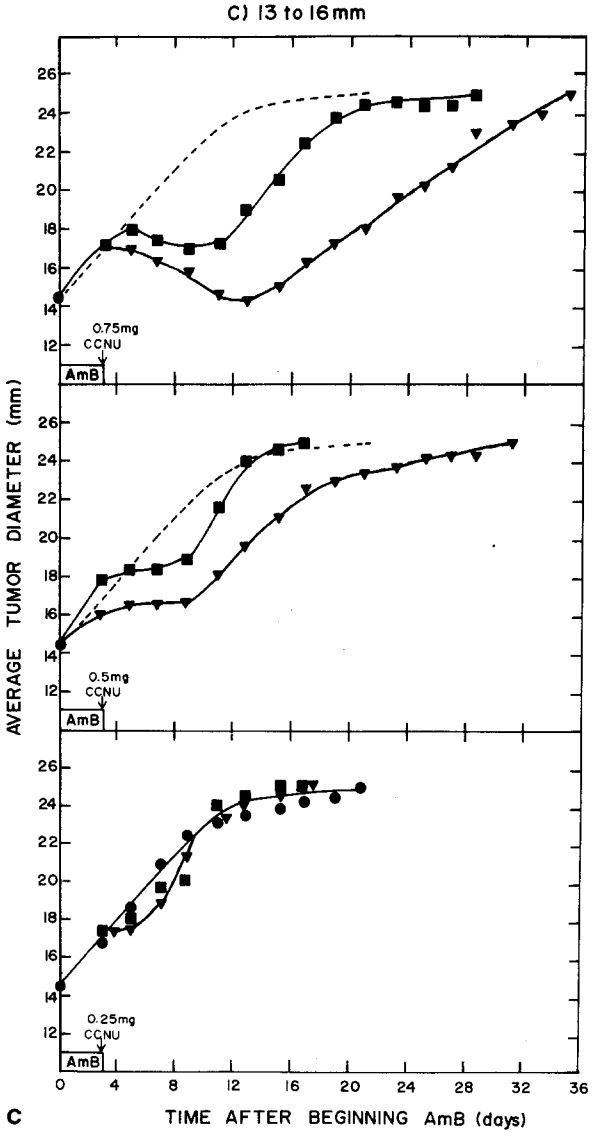
Fig. 2a–c. Response of small **(a)**, medium **(b)**, and large **(c)** SC implants of Lewis lung carcinoma to treatment with CCNU alone or AmB followed by CCNU. ●, . . ., controls; ■, CCNU alone; ▼, AmB plus CCNU. AmB was administered daily on days 0–3, with CCNU administered on day 3

combined effect of AmB and 0.75 mg CCNU was not toxic to the animals and potentiation was noted. Responses to all three of the regimens were less than when the smaller tumors were treated; this result was expected because of the larger tumor size at the time of initiation of therapy. T-C values were determined at a tumor size of 16 mm and the results are presented in Table 1.

The largest tumors (13–16 mm) averaged 14.5 mm at the start of AmB treatment (day 0) (Fig. 2c). The responses were similar to those observed above for the other tumor sizes; pretreatment with AmB potentiated the cytotoxicity of all three doses of CCNU. In this experiment, however, the T-C calculations were carried out when the tumors reached 20 mm; the results are presented in Table 1.

To relate the T-C data to cell survival, an estimate of the survival of tumor cells was made using the tumor doubling time of 1.5 days. These calculations are presented in Table 1. As expected, the tumor became less sensitive to both CCNU alone and AmB plus CCNU with increasing size. This has been demonstrated previously with this tumor using the nitrosourea BCNU alone [5]. For all tumor sizes and drug doses, however, significant potentiation was noted, although this was most significant for the small tumors.

Since the Lewis lung tumor arose in a C57Bl/6 mouse (the above experiments were carried out in a B602F_1 mouse), and AmB has been previously demonstrated in our laboratory to have a marked immune potentiating effect in an animal tumor system [4], the above results might reflect totally or in part an



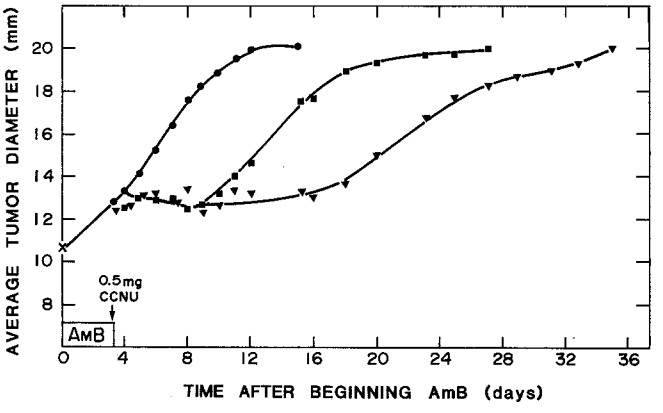


Fig. 3. Response of medium SC Lewis lung carcinoma grown in C57Bl/6 mice to CCNU alone or AmB followed by CCNU. ●, controls; ■, CCNU alone; ▼ AmB plus CCNU. AmB was administered daily on days 0–3, with CCNU administered on day 3

Table 1. Response parameters of Lewis lung carcinoma to CCNU and AmB plus CCNU

Treatment	Tumor size (mm)					
	4.5–7.5		8.5–12		13–16	
	T–C[a]	Survival[b]	T–C	Survival	T–C	Survival
0.25 mg CCNU	0	10^0	0	10^0	0	10^0
AmB + CCNU	6	6.3×10^{-2}	3	2.5×10^{-1}	1.5	5×10^{-1}
0.5 mg CCNU	5	1×10^{-1}	3	2.5×10^{-1}	3	2.5×10^{-1}
AmB + CCNU	25	9.5×10^{-6}	12.5	3×10^{-3}	7	3.9×10^{-2}
0.75 mg CCNU	12.5	3.1×10^{-3}	11.5	4.9×10^{-3}	8	2.5×10^{-2}
AmB + CCNU	–	–	16.5	4.9×10^{-4}	17.5	3.1×10^{-4}

[a] In days to the nearest ½ day

[b] Cell survival = $10^{-(\log \text{cell kill})} = 10 \exp\left[-\frac{\text{(T-C) days}}{3.32 \times 1.5 \text{ days}}\right]$

immunochemotherapeutic response. We examined one dose of CCNU (0.5 mg/mouse) and treated a medium-sized tumor in the syngeneic C57Bl/6 host. The data are presented in Fig. 3. The average tumor size at the start of therapy was 10.7 mm. T-C (determined at 16 mm) was 7.2 days for 0.5 mg CCNU alone and 15.2 days for the combined AmB-CCNU group. Thus, a pronounced potentiation of CCNU cytotoxicity by AmB was also found when the syngeneic C57Bl/6 mouse strain was used used for growth of the Lewis lung carcinoma.

Discussion

The data reported here extend our observations to two further murine models in which the potentiation by AmB of cytotoxicity of anticancer agents was noted. The MOPC-315 plasmacytoma is a transplantable myeloma model and its response is similar to those previously reported for the transplantable AKR and L1210 leukemias [7]. Table 2 summarizes the PI values calculated for these three hematologic tumors. Little if any potentiation of cytotoxicity was noted with BCNU, while with both CCNU and L-PAM a significant potentiation by AmB was demonstrated. The extent of potentiation of both of these anticancer agents by AmB was greatest for the MOPC-315 plasmacytoma and lowest against the L1210 leukemia.

The results of therapy against the Lewis lung carcinoma are interesting from a number of viewpoints. This is the first positive result of the AmB antitumor treatment against a solid tumor in vivo using a quantitative assay. This tumor is reasonably representative of most solid tumors in that it is insensitive to most anticancer agents but is responsive to CCNU [1]. The level of response of the Lewis lung carcinoma to CCNU which we achieved was similar to that reported by others [1]. In all our experiments, AmB plus CCNU caused more inhibition of cell growth and a greater level of cell killing than that found with single doses of CCNU alone. We presume that AmB increases the permeability of cell membranes, thereby enhancing the uptake of CCNU by the tumor cells.

Our results indicate that the use of AmB in combination with the appropriate chemotherapeutic agents may be effective against hematological malignancies and should be tested in clinical protocols. Moreover, the results with the Lewis lung carcinoma suggest that some solid tumors may also be responsive to combinations of certain anticancer agents with AmB.

Table 2. Summary of PI values for AKR and L 1210 leukemias and MOPC-315 plasmacytoma

Tumor type	Anticancer agent		
	BCNU	CCNU	L-PAM
AKR leukemia	1.2	1.8	2.3
L 1210 leukemia	–	1.6	1.8
MOPC-315 plasmacytoma	1.0	3.8	2.4
		1.8	

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