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The in vivo Cytotoxic Activity of Procarbazine and Procarbazine Metabolites Against L1210 Ascites Leukemia Cells in CDF_1 Mice and the Effects of Pretreatment with Procarbazine, Phenobarbital, Diphenylhydantoin, and Methylprednisolone upon in vivo Procarbazine Activity

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Summary. *An in vivo assay of the activity of procarbazine, N-isopropyl-∝-(2-methylhydrazino)-p-toluamide hydrochloride, and several metabolic intermediates against IP-implanted L1210 leukemia cells in CDF_1 male mice is described. Treatment of tumor-bearing mice with procarbazine at doses of 300–500 mg/kg IP increased the mean lifespan of treated mice by 29%–32% relative to that of untreated animals. Procarbazine treatment with doses of 200–400 mg/kg/day given IP for 3 consecutive days increased mean lifespan by 39%–46%. The major circulating metabolite, azoprocarbazine (N-isopropyl-∝-(2-methylazo)-p-toluamide), was as active as procarbazine when administered at equivalent doses for 3 consecutive days. A 2:1 mixture of azoxyprocarbazines (N-isopropyl-∝-(2-methyl-ONN-azoxy)-: and N-isopropyl-∝-(2-methyl-NNO-azoxy)-p-toluamide) was more active than procarbazine, increasing mean lifespan by 76% using the 3-consecutive-day dose schedule.*

The effects of pretreatment with procarbazine and drugs that are often co-administered with procarbazine, i.e., phenobarbital, diphenylhydantoin, and methylprednisolone, upon procarbazine anticancer activity against L1210 ascites leukemia cells was also determined. Pretreatment of CDF_1 male mice with phenobarbital and diphenylhydantoin for 7 days was found to increase the antineoplastic activity of procarbazine by 13%–24%. Pretreatment with methylprednisolone did not significantly alter procarbazine activity. The effects of pretreatment with procarbazine, which is often administered daily for a period of 2–4 weeks, on procarbazine antineoplastic activity were varied. The results of these preliminary pretreatment studies combined with the finding that procarbazine metabolites have antitumor activity that is equal to or greater than that of the parent drug suggest that current clinical protocols that use procarbazine along with agents capable of altering procarbazine metabolism may involve drug interactions that alter the efficacy of procarbazine as an anticancer agent.

Introduction

Procarbazine (*N*-isopropyl-∝-(2-methylhydrazino)-*p*-toluamide hydrochloride) is a clinically useful antineoplastic agent that was first reported in 1963 [17]. Procarbazine has been identified as a methylating agent [8] and methylation is thought to be a significant mode of cytotoxic action. The production of the methylating agent apparently results from the metabolic activation of procarbazine. Chemically, procarbazine oxidizes rapidly in aqueous solutions to azoprocarbazine [15, 17]. Recent studies in this laboratory have revealed that azoprocarbazine is selectively metabolized to a mixture of azoxy isomers, methylazoxyprocarbazine (*N*-isopropyl-∝-(2-methyl-*ONN*-azoxy)-*p*-toluamide), and benzylazoxyprocarbazine (*n*-isopropyl-∝-(2-methyl-*NNO*-azoxy)-*p*-toluamide). Both azoxy isomers are subject to further metabolism, although the isomers are metabolized at different rates. Azo- and azoxyprocarbazine were proposed as intermediates in the metabolic activation of procarbazine [15], by analogy with the metabolic activation sequence reported for another methylating agent, 1,2-dimethylhydrazine [5]. Prough and co-workers have found that the conversion of procarbazine to azoprocarbazine [3] and that of azoprocarbazine to azoxy isomers [16] are mediated by the cytochrome P-450 mixed function oxidase system. The rate of procarbazine metabolism and of subsequent metabolic conversions is increased by phenobarbital induction of hepatic microsomal enzymes [3, 15, 16]. Azoprocarbazine and both azoxy isomers are observed in the plasma of rats and man following administration of procarbazine [13].

Since procarbazine is apparently metabolized by the liver microsomal enzyme system to intermediates that may be responsible for cytotoxic activity, drugs that can either stimulate or depress these enzymes may influence procarbazine's duration of action, toxicity, and other therapeutic parameters. These interactions can become especially critical during clinical chemotherapy in man. Procarbazine is administered to brain tumor patients in multiple treatment protocols that continue for 14–28 consecutive days [10, 11]. Other drugs, including phenobarbital, diphenylhydantoin (Dilantin), methylprednisolone (Medrol), and dexamethasone, are commonly administered during the treatment of malignant brain tumors [11].

This paper reports the antineoplastic activity of procarbazine, azoprocarbazine, and azoxyprocarbazines. An in vivo assay of activity against intraperitoneal L1210 ascites leukemia cells in male CDF_1 mice was used. The high activity of these

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metabolic intermediates suggest that they may be precursors to the active species responsible for procarbazine activity. The effects of pretreatment with procarbazine, phenobarbital, diphenylhydantoin, and methylprednisolone upon in vivo procarbazine anticancer activity are also reported. Phenobarbital and diphenylhydantoin pretreatment increased procarbazine antineoplastic activity. Some procarbazine pretreatment protocols significantly increased procarbazine activity, while others did not. These changes in procarbazine anticancer activity in mice may have important implications in the clinical use of procarbazine in the treatment of malignant diseases in man.

Materials and Methods

The L1210 ascites leukemia tumor and male CDF_1 mice were obtained from the National Cancer Institute. This L1210 line was more sensitive to procarbazine than other L1210 cells. Procarbazine hydrochloride was obtained from Dr W. E. Scott, Hoffmann-LaRoche, Nutley, New Jersey and was found to be pure by mass-spectrometric, high-pressure liquid chromatographic (HPLC), and thin-layer chromatographic analyses. *N*-isopropyl-∝-(2-methylazol)-*p*-toluamide and a 2 : 1 mixture of *N*-isopropyl-∝-(2-methyl-*ONN*-azoxy)-*p*-toluamide and *N*-isopropyl-∝-(2-methyl-*NNO*-azoxy)-*p*-toluamide were synthesized, purified, and characterized as previously described [15].

Animal Tumor Implantation. The L1210 ascites leukemia tumor was carried in vivo by implantation of 1.5×10^6 cells in the peritoneal cavity of male CDF_1 mice. Cells were removed 4 days after implantation and diluted 1 : 6 by volume with Hank's balanced salt solution and 0.1 ml of the diluted cell suspension was injected into 20-g CDF_1 mice.

Drug Treatment and Survival Studies. L1210 ascites leukemia cells (1.5×10^6) in 0.1 ml Hank's balanced salt solution were placed in the peritoneal cavity of 20 g male CDF_1 mice 24 h before administration of the first drug dose. Drugs were administered by a 0.1-ml IP injection either as a single dose or as a course of single equivalent doses on 3 consecutive days. Procarbazine hydrochloride was administered as a solution in 0.9% NaCl (w/v). Azoprocarbazine and azoxyprocarbazine isomers were dissolved in Tween 80 with sonication. Procarbazine, azo, and azoxy isomeric mixtures were given as equimolar doses. An additional group of animals received the azoxy isomeric mixture at a 1.5 × molar amount. All drug colutions were prepared immediately before administration. Control animals received 0.1-ml IP injections of 0.9% NaCl or Tween 80. Groups of non-tumor-bearing mice received procarbazine, azoprocarbazine, and azoxyprocarbazine mixtures at the highest administered dose to determine the toxicity of the drugs alone. The animals were checked daily: the day of death counting from tumor implantation (lifespan) was noted, and the mean and median lifespans and % T/C (lifespan of treated animals divided by the lifespan of untreated tumor-bearing animals multiplied by 100) were calculated.

Pretreatment and Survival Studies. Male CDF_1 mice were pretreated with phenobarbital, diphenylhydantoin, or methylprednisolone PO or with procarbazine by IP injection. Animals not receiving pretreatment received the appropriate carrier. Procarbazine was administered daily for 7 or 14 consecutive days by 0.1-ml IP injections at a dose of 30 or 60 mg procarbazine dissolved in 0.9% NaCl/kg body weight/day. Diphenylhydantoin, phenobarbital, and methylprednisolone were given PO for 7 days at doses of 60, 48, and 7.5 mg/kg/day, respectively, based upon the assumption that each mouse drank 6 ml/day. These oral solutions were freshly prepared every 3 days. The oral pretreatment was gradually withdrawn over a 3-day period following tumor implantation by decreasing the dose by 50% on each day. The animals were returned to normal drinking water. Tumor was implanted IP as a 0.1-ml injection of Hank's balanced salt solution containing 1.5×10^6 L1210 leukemia cells. Diphenylhydantoin-, phenobarbital-, and methylprednisolone-pretreated animals received tumor implantation on day 7 of pretreatment. Procarbazine-pretreated animals received tumor 48 h after the final pretreatment dose, to ensure that no tumor cell was killed as a result of residual procarbazine metabolites. The animals in the experimental group received a 0.1-ml IP injection of either 100 or 200 mg procarbazine in 0.9% NaCl/kg body weight/day for 3 consecutive days beginning 24 h after tumor implantation. Control animals received 0.1 ml 0.9% NaCl.

Plasma samples were taken from several procarbazine-pretreated mice prior to tumor implantation and no residual known procarbazine metabolites were present as determined using a reverse-phase HPLC assay described previously [13]. Plasma samples were also taken from several of the phenobarbital- and diphenylhydantoin-pretreated animals prior to procarbazine treatment and analyzed for phenobarbital or diphenylhydantoin using a standard HPLC assay [7]. Fasted phenobarbital- and procarbazine-pretreated animals and untreated control animals were sacrificed 24 h after tumor implantation. The livers of these animals were removed, the 100,000 *g* microsomal fractions isolated, and the cytochrome P-450 and total microsomal protein concentrations determined. Microsomal fractions were isolated using the procedure of Fouts [6]. Cytochrome P-450 levels were determined by the method of Omura and sato [12] using an Aminco DW-2 dual-beam recording spectrophotometer. The difference spectra from a reference sample of 1 ml microsomal preparation and a sample containing microsomal preparation and 2 mg sodium dithionite, both gassed with carbon monoxide for 20 s, were measured from 400 to 500 nm. The total microsomal protein concentration was determined using the Bio-Rad Protein Assay.

Table 1. In vivo activity of a single dose and of a 3-day course of procarbazine treatment against IP implanted L1210 ascites leukemia cells in CDF_1 mice

Protocol	Mean lifespan	Median lifespan	% Mean T/C
L1210 ascites[a]	8.5	8.5	–
Single dose, 300 mg/kg[b]	11.0	11.0	129
Single dose, 400 mg/kg	11.2	11.0	132
Single dose, 500 mg/kg	11.2	11.0	132
Multiple dose, 200 mg/kg for 3 days	11.8	12.0	139
Multiple dose, 300 mg/kg for 3 days	12.0	12.0	141
Multiple dose, 400 mg/kg for 3 days	12.4	12.0	146

[a] 0.1 ml of 1 : 6 dilution of IP L1210 cells removed 4 days after implantation

[b] All drug treatments were given by IP injection

Table 2. In vivo activity of three consecutive daily doses of procarbazine, azoprocarbazine and a 2 : 1 mixture of methyl/benzylazoxyprocarbazine against IP-implanted L1210 ascites leukemia cells in CDF_1 mice

Protocol	Number of mice	Mean lifespan	Median lifespan	% Mean T/C
L1210 ascites[a]	11[b]	7.6	8.0	–
Procarbazine-HCL				
50 mg/kg, 0.20 mmole/kg	7	8.7	8.0	114
100 mg/kg, 0.39 mmole/kg	7	9.9	10.0	130
200 mg/kg, 0.78 mmole/kg	7	10.3	10.0	136
Azoprocarbazine				
42.5 mg/kg, 0.20 mmole/kg	5[b]	9.2	9.0	121
85 mg/kg, 0.39 mmole/kg	6[b]	9.2	9.5	121
170 mg/kg, 0.78 mmole/kg	6[b]	9.8	10.0	129
Azoxyprocarbazine				
45.7 mg/kg, 0.20 mmole/kg	7	9.3	10.0	122
91.3 mg/kg, 0.39 mmole/kg	7	10.6	10.0	139
182.6 mg/kg, 0.78 mmole/kg	5[b]	13.4	13.0	176
273.9 mg/kg, 1.16 mmole/kg	7	5.1	5.0	67

[a] Mice received 1.5×10^6 cells by IP injection
[b] Mice dying within 5 days of tumor implant were omitted from experimental results. A single animal was omitted from the non-treated tumor-bearing group and each of the azoprocarbazine groups. The 0.78 mmole/kg azoxyprocarbazine group had two animals deleted

Table 3. Toxicity of three consecutive daily doses of procarbazine, azoprocarbazine, and a 2 : 1 mixture of methyl/benzylazoxyprocarbazine in CDF_1 mice

Drug	Dose[a]	15-day survivors[b]
Procarbazine HCl	200 mg/kg, 0.78 mmole/kg	6/6
Azoprocarbazine	170 mg/kg, 0.78 mmole/kg	4/6
Azoxyprocarbazine	182.6 mg/kg, 0.78 mmole/kg	3/5
	91.3 mg/kg, 0.39 mmole/kg	6/7

[a] All drug treatments were given by IP injection
[b] All animals sacrificed on day 15 after tumor implantation

Results

In vivo Activity of IP Administered Procarbazine Against L1210 Ascites Leukemia Cells in the Peritoneal Cavity of CDF_1 Mice

Daily administration of procarbazine beginning 24 h after tumor implantation and continuing for 3 consecutive days was more effective in prolonging the survival of L1210 ascites leukemia-bearing mice than administration of a single dose. The mean and median lifespans and % T/C for groups of animals receiving a single procarbazine dose of 200, 300, or 400 mg/kg, and for groups receiving only the carrier solution, 0.9% NaCl (w/v), are shown in Table 1. The median and mean lifespan of the untreated group was 8.5 days. Multiple-day procarbazine administration increased the mean lifespan by 39%–46%, while a single procarbazine dose increased mean lifespan by 29%–32%. The increase in mean lifespan by procarbazine was dose-dependent within the range of drug dose administered. There is a good correlation between T/C × 100 and total procarbazine dose for single administration or cumulative dose administered over 3 consecutive days.

In vivo Activity of Procarbazine, Azoprocarbazine, and Azoxyprocarbazine Against L1210 Ascites Leukemia Cells in the Peritoneal Cavity of CDF_1 Mice

Procarbazine, azoprocarbazine, and a 2 : 1 mixture of methyl : benzylazoxy-procarbazine were administered at equimolar doses of 0.2–0.78 mmoles drug/kg body weight of male CDF_1 mice. Intraperitoneal injections were begun 24 h after implantation of the L1210 ascites tumor cells and continued daily for 3 consecutive days. When administered according to this protocol, procarbazine, and azoprocarbazine increased the lifespan of the tumor-bearing mice to a similar extent. The azoxyprocarbazine mixture was significantly more active. The mean lifespan for untreated animals was 7.6 days, while treatment with either procarbazine or azoprocarbazine increased the mean lifespan by 29%–36% at a dose of 0.78 mmoles/day for 3 days. Azoxyprocarbazine administered at the same dose increased the mean lifespan by 76%. The median and mean lifespans and % T/C for L1210 leukemia-bearing CDF_1 mice are shown in Table 2. Toxicity data for these compounds are shown in Table 3.

Effects of Procarbazine, Phenobarbital, Diphenylhydantoin, and Methylprednisolone Pretreatment upon the in vivo Activity of Procarbazine Against L1210 Ascites Leukemia Cells

Procarbazine, phenobarbital, diphenylhydantoin, and methylprednisolone were administered at doses chosen to simulate those clinically given to man. Male CDF_1 mice received pretreatment with one of the above drugs for 7 or 14 days prior to IP implantation of L1210 ascites leukemia cells and treatment with 100 or 200 mg procarbazine/kg/day for 3 consecutive days. Phenobarbital and diphenylhydantoin pretreatment significantly increased procarbazine's in vivo anticancer activity at the doses studied, while methylprednisolone did not significantly alter procarbazine cytotoxicity towards L1210 ascites leukemia cells at either procarbazine dose.

The effect of procarbazine pretreatment on procarbazine activity varied from not significant to significant, depending upon the pretreatment and procarbazine treatment protocols used. The survival curves of pretreated versus non-pretreated animals were analyzed for significant differences using a computer program for Wilcoxan-Gehen matched-pair signed-rank statistical analysis test.

Phenobarbital pretreatment at a dose of 48 mg/kg/day resulted in a statistically significant 18% and 13% increase in antineoplastic activity of procarbazine at the 200 and 100 mg/kg doses, respectively (Table 4). The phenobarbital plasma level attained after 7 days of pretreatment was 6 μg/ml and cytochrome P-450 levels were increased by 52%. The 60 mg/kg/day diphenylhydantoin pretreatment schedule increased procarbazine anticancer activity by 24% (Table 4). Plasma concentration of diphenylhydantoin was 2 μg/ml after 7 days of pretreatment. The 7.5 mg/kg/day methylprednisolone pretreatment had no effect upon the activity of procarbazine in the L1210 ascites leukemia system (Table 4).

Procarbazine pretreatment with 30 or 60 mg/kg/day for 14 consecutive days significantly increased the antitumor activity of the 3-day 100 mg/kg/day procarbazine treatment. Increases of 18% and 14%, respectively, made the 100 mg/kg/day schedule as effective as the 200 mg/kg/day schedule for non-pretreated and pretreated animals. Seven-day pretreatment at 30 or 60 mg/kg/day was less effective in increasing procarbazine activity. Only marginal 10%–11% increases were observed following the higher pretreatment dose. Total cytochrome P-450 concentrations in liver microsomes decreased by 2% and 13% following the 7-day 30 and 60 mg/kg/day pretreatment schedules, respectively. Increases of 4% and 6% were observed following the corresponding 14-day pretreatment. No clear correlation was found between dose, protocol, total of the pretreatment and treatment doses or total cytochrome P-450 content and in vivo anticancer activity against L1210 ascites leukemia.

Discussion

The antineoplastic activity of procarbazine and major metabolites was determined against IP-implanted L1210 ascites leukemia cells in male CDF_1 mice. Procarbazine has been shown to be active against several transplanted animal tumors and through several routes of administration, although IP-implanted L1210 ascites leukemia cells have been reported to be non-responsive to IP administration of procarbazine [9]. In contrast, this report demonstrates that procarbazine administered IP is active against IP-implanted L1210 ascites leukemia cells using a single dose of 300–500 mg/kg or three consecutive doses of 200–400 mg/kg/day (Table 1). This discrepancy may be due to the fact that some L1210 strains have a greater sensitivity to procarbazine than others.

Procarbazine was found to increase the lifespan of IP L1210 ascites leukemia-bearing mice to a T/C × 100 of 136–139 at a dose of 200 mg/kg/day (0.78 mmole/kg/day) for 3 consecutive days (Tables 1 and 2). Azoprocarbazine produced essentially the same increase in lifespan as procarbazine at the three doses tested (Table 2). The mixture of azoxy isomers was significantly more active than its metabolic precursors, as a T/C × 100 of 176 was produced at a dose of 0.78 mmole/kg/day for 3 days (Table 2). Some acute toxicity was detected for both azoprocarbazine and azoxyprocarbazine at this dose. Bollag et al., in an early communication, reported activity of azoprocarbazine and an undefined azoxyprocarbazine isomeric mixture against Walker carcinosarcoma and Ehrlich carcinoma

Table 4. Effect of pretreatment with methylprednisolone, diphenylhydantoin, and phenobarbital upon procarbazine in vivo activity against IP-implanted L1210 ascites leukemia cells in CDF_1 mice

Protocol	Number of mice	Mean lifespan	Median lifespan	% Mean T/C	% change in % mean T/C due to pretreatment
L1210 ascites[a]	10	9.0	9.0	–	–
200 mg/kg procarbazine[b]	6	11.0	11.0	122	–
100 mg/kg procarbazine[b]	6	10.0	10.0	111	–
Methylprednisolone[c]					
7.5 mg/kg pretreatment	10	8.8	9.0	–	–
7.5 mg/kg pretreatment + 200 mg/kg procarbazine	9	11.1	11.0	126	+ 4
7.5 mg/kg pretreatment + 100 mg/kg procarbazine	9	10.2	10.0	116	+ 5
Diphenylhydantoin[c]					
60 mg/kg pretreatment	6	8.0	8.4	–	–
60 mg/kg pretreatment + 200 mg/kg procarbazine	6	11.7	12.3	146	+ 24[d]
Phenobarbital[c]					
48 mg/kg pretreatment	12	8.5	8.5	–	–
48 mg/kg pretreatment + 200 mg/kg procarbazine	10	11.9	12.0	140	+ 18[d]
48 mg/kg pretreatment + 100 mg/kg procarbazine	10	10.5	10.5	124	+ 13[d]

[a] Mice received 1.5×10^6 cells by IP injection

[b] Procarbazine was administered daily for 3 consecutive days, beginning 24 h after tumor implant by IP injection

[c] Methylprednisolone, diphenylhydantoin, and phenobarbital pretreatments were PO administered in the drinking water. The doses were determined assuming each animal drank an average of 6 ml/day for 7 days

[d] Increases in activity are significant at the 95% confidence limit

[1]. Procarbazine and azoprocarbazine inhibited the growth of subcutaneous Walker carcinosarcoma equally, while the unspecified azoxyprocarbazine mixture was 30%–50% less active at equivalent doses. In contrast, the 2 : 1 mixture of 2-methyl-*ONN*-azoxy-:2-methyl-*NNO*-azoxyprocarbazine used in the present study was 40% more active than equivalent doses of procarbazine and azoprocarbazine in prolonging the lifespan of leukemia L1210 ascites tumor-bearing mice. This apparent discrepancy in azoxyprocarbazine activity might result from differing compositions of the azoxyprocarbazine mixtures, assuming that both mixtures were chemically pure and that both azoxy isomers do not have equal therapeutic indices. The mixture used by Bollag melted at 128–132° C, while the 2 : 1 mixture melted at 95–103° C. Furthermore, Bollag's azoxyprocarbazine mixture was equitoxic with procarbazine (maximum tolerated doses were 75 mg/kg). In the present study, the azoxyprocarbazine was more toxic than procarbazine. Possible resolution of the differences in azoxyprocarbazine activity in these two studies must await the results of ongoing studies aimed at evaluating the antitumor activity of the separate azoxy isomers.

The L1210 ascites leukemia assay was used to assess the effects of several drugs that are commonly administered to cancer patients undergoing chemotherapy on the antitumor activity of procarbazine. Two of the agents studied were phenobarbital and diphenylhydantoin. Both of these agents are known to induce the synthesis of cytochrome P-450 enzymes in liver and other tissues and thereby affect the rate of metabolism of other drugs. More specifically, phenobarbital pretreatment has been shown to increase the rate of procarbazine and azoprocarbazine metabolism in vitro [3, 15, 16]. Phenobarbital pretreatment of rats has been reported to double the rate of procarbazine plasma clearance and to increase the rate of azoproxcarbazine metabolism [14]. Phenobarbital and, presumably, other drugs that induce cytochrome P-450 enzymes may affect procarbazine antitumor activity by increasing the rate of formation of active metabolites and metabolic intermediates. In this study, pretreatment with phenobarbital increased the procarbazine T/C × 100 from 122 to 140 (Table 4). Similarly, diphenylhydantoin pretreatment increased the T/C × 100–146. This drug interaction significantly increases the in vivo antitumor activity of procarbazine in rats, but it is not known whether a similar enhancement occurs in patients being treated with these drug combinations.

The effect of pretreatment with methylprednisolone on procarbazine activity against IP L1210 ascites tumor was also investigated. Methylprednisolone is not known to induce cytochrome P-450 enzymes but is commonly administered to brain tumor patients along with procarbazine. Pretreatment with this drug did not produce a significant change in procarbazine activity.

Table 5. Effect of pretreatment with procarbazine upon procarbazine in vivo activity against IP-implanted L1210 ascites leukemia cells in CDF_1 mice

Protocol	Number of mice	Mean lifespan	Median lifespan	% Mean T/C	% change in % mean T/C due to pretreatment	Total pretreatment dose (mg)	Total Dose (mg)
L1210 ascites[a]	10	9.0	9.0	–	–	–	–
200 mg/kg procarbazine[b]	6	11.0	11.0	122	–	–	12.0
100 mg/kg procarbazine[b]	6	10.0	10.0	111	–	–	6.0
Procarbazine[c]							
30 mg/kg for 7 days	10	9.1	9.0	–	–	–	–
30 mg/kg for 7 days + 200 mg/kg procarbazine	10	11.6	11.5	127	+ 5	4.2	16.1
30 mg/kg for 7 days + 100 mg/kg procarbazine	10	10.1	10.0	111	0	4.2	10.2
60 mg/kg for 7 days	6	8.4	8.4	–	–	–	–
60 mg/kg for 7 days + 200 mg/kg procarbazine	6	11.2	11.2	133	+ 11	8.4	20.4
60 mg/kg for 7 days + 100 mg/kg procarbazine	6	10.2	10.2	121	+ 10	8.4	14.4
30 mg/kg for 14 days	5	8.8	8.4	–	–	–	–
30 mg/kg for 14 days + 200 mg/kg procarbazine	6	11.3	10.8	128	+ 6	8.4	20.4
30 mg/kg for 14 days + 100 mg/kg procarbazine	6	11.4	10.8	129	+ 18[d]	8.4	14.4
60 mg/kg for 14 days	6	8.8	8.4	–	–	–	–
60 mg/kg for 14 days + 200 mg/kg procarbazine	6	10.4	9.9	118	– 5	16.8	28.8
60 mg/kg for 14 days + 100 mg/kg procarbazine	6	11.0	10.5	125	+ 14[d]	16.9	22.8

[a] Mice received 1.5×10^6 cells by IP injection

[b] Procarbazine was administered daily for 3 consecutive days, beginning 24 h after tumor implant, by IP injection

[c] Procarbazine pretreatment was administered IP injection. Pretreatment was terminated 48 h prior to tumor implantation to ensure that no tumor-kill resulted from residual procarbazine metabolites

[d] The increase in activity is significant at the 95% confidence limit

Procarbazine itself affects the activity of hepatic cytochrome P-450 enzymes [2, 4]. Preliminary pharmacokinetic data shows that the rate of appearance and disappearance of the active metabolites, azo, and azoxyprocarbazines, in patient plasma is higher on day 14 of procarbazine therapy than on day 1. In this study a moderate increase in antitumor activity was found following 14 days of procarbazine pretreatment and procarbazine treatment at low doses (Table 5). Other schedules produced no significant change in antitumor activity. These experiments were designed to avoid any involvement of drug administered as pretreatment on implanted L1210 cell survival. As a consequence, 3 days elapsed between cessation of the pretreatment course and the first treatment dose. There may have been a significant diminution in the pretreatment effect during this interval. The enhancement of procarbazine activity by procarbazine pretreatment suggests that the increased antitumor effect observed following a 14- to 28-day course of therapy over single dose administration may be due, in part, to self-induced metabolic alteration.

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