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EVALUATION OF CHEMICAL AGENTS AGAINST THE PLASMA CELL TUMOR LPC-1 IN MICE

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Abstract—Utilizing the advanced stage of an ascitic plasma cell tumor LPC-1 in mice, thirty-one drugs, plus cyclophosphamide employed as a positive standard, were tested for their ability to prolong the survival time of the animals. A determination was also made of the effect of the drugs on the biochemical parameter, abnormal protein production. Four drugs had an effect on both of these indices, i.e. tryptophan mustard (NSC-62403), aniline mustard (NSC-18429), cyclophosphamide (NSC-26271) and 5-fluorouracil (NSC-19893). Two of these, tryptophan mustard and cyclophosphamide, have been shown to be active clinically against plasma cell tumors. Nine additional compounds caused a significant increase in survival time but did not cause the abnormal protein to disappear.

The current study indicates that the LPC-1 tumor offers a suitable test system for screening compounds for clinical trial against myeloma. A comparison is also made of the relative activities of the compounds in the L1210 leukemia, Walker 256 carcinosarcoma (IM) and LPC-1 systems in relation to clinical experience.

TRANSPLANTABLE animal tumors have been proposed as models for detecting antitumor compounds,[1] for studying structure activity relationships,[2] and for evaluating the effects of drugs on biochemical parameters.[3] To date there has been only minimal evidence that animal tumors may select antitumor drugs active against their specific histologic counterparts in man. However, compounds with a high order of activity in L1210, an acute lymphoblastic leukemia of mice, have been in general active against patients with acute leukemia.[4] With the recognition of plasma cell tumors in mice and accompanying abnormalities in serum and urine proteins[5, 6] and kidney[7] and bone lesions,[8] several authors have suggested that these tumors, employed in chemotherapy screening trials, may select drugs which will be active against human plasma cell malignancies.[9, 10] The reported studies have utilized changes in tumor weight or volume and sometimes protein production as indices of drug response against a subcutaneously implanted tumor. In the present study, using an advanced ascites form of a Balb/c plasma cell neoplasm LPC-1,[11] survival time and protein production were evaluated after treatment with a variety of antitumor agents over a wide dose range.

METHODS

The experiments were conducted in 18–30 g Balb/c or CAF-1 male mice. The mice were inoculated intraperitoneally with 0·2 ml of LPC-1 ascites plasma tumor

cells that were propagated in Balb/c mice. The concentration of the tumor inoculum in the various experiments ranged from 150,000 to 580,000 cells. In addition to treated and control mice that received the experimental inoculum, each experiment included groups of untreated mice that received serial dilutions of the experimental inoculum. The groups of mice inoculated with $\frac{1}{10}$ of the experimental inoculum generally succumbed with tumors.

By day 14 the animals have an established ascitic tumor and the abnormal protein can be detected in the serum. In the current experiments treatment was begun on the 14th to 19th day after tumor implantation and was continued daily for 28 days. All compounds were injected subcutaneously in the constant volume of 0·01 ml/g body weight over a wide range of logarithmically spaced dosage levels. The day of death of each animal was recorded and the median survival time (MST) determined. The MST for those groups of animals which included mice sacrificed for bioassay studies is based on the assumption that the sacrificed animals would have succumbed in the same pattern as the animals which died after the day of sacrifice. Thus, the death was distributed proportionately among the remaining mice. In each experiment, cyclophosphamide (Cytoxan, Endoxana) was employed as a positive standard. The compounds are rated relative to (a) median survival time (MST) of untreated controls, and (b) cyclophosphamide efficacy in increasing the survival time of the mice.

The abnormal protein in the blood of mice inoculated with LPC-1 tumor cells was determined by agar electrophoresis prior to the initiation of therapy and 24 hr after the last injection of the compound. The procedure employed for the electrophoresis of the mouse sera was that of Potter and Kuff.[12] Briefly, glass slides were coated with approximately 15 ml of Ionagar (1%) in a barbital buffer at pH 8·6. Aliquots of the test samples (0·03 ml) were placed in the small wells on the slides. Two of these glass slides were then placed side by side in a Buchler electrophoresis tray and connected to the barbital buffer in the tray by gauze wicks. A p.d. (35–40 V) was applied across the ends of the plates and the electrophoresis was allowed to proceed for 90 min. The protein on the plates was then precipitated with 2% acetic acid, washed in distilled water, and dried overnight at 37°. The plates were then stained with Amido-Schwartz (0·1%) dye, rinsed, and either photographed, or stored for future evaluation. With this technique, the LPC-1 abnormal protein migrates distinctly away from the normal mouse γ-globulin (Fig. 1). The visible absence of abnormal protein is classified as a zero result. The average range of concentration of of abnormal protein at the onset of treatment was between 0·5 and 1·5 g%. The minimal visually detectable quantity was determined to be 0·05 g%.

The effect of food restriction on the survival time of mice inoculated with LPC-1 tumor was determined. Ten Balb/c mice were used for each level of food offered. All mice were caged individually following intraperitoneal injection of the tumor. The animals were offered an *ad libitum* diet of 6·0 g per day of pulverized Purina mouse chow during the first 14 days following intraperitoneal inoculation of the tumor. Commencing with day 15, the mice were offered either 6·0, 4·0, 3·0, 2·5, 2·0, 1·5, 1·0 or 0·50 g of food per day. The food was contained in small glass powder jars with an opening in the metal top of sufficient diameter so that the food was readily accessible to the mice. Wire screens were used on the floors of all cages to keep the mice from eating spilled feed and fecal material. The animals were observed daily, and the

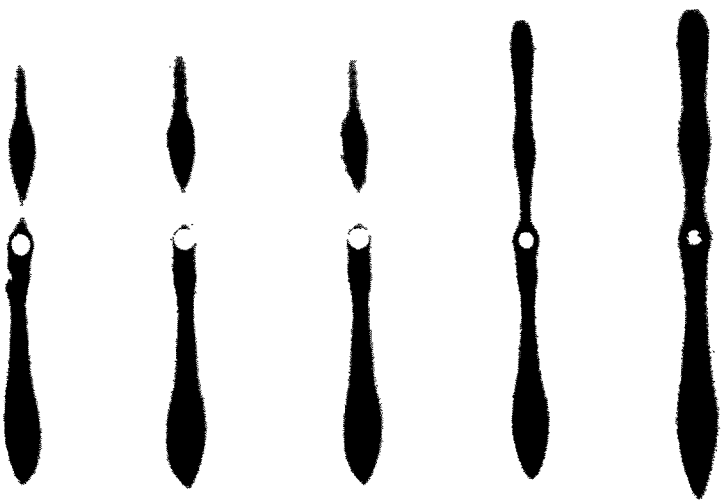

FIG. 1. Agar gel electrophoresis of sera from a Balb/c mouse. Anode at the bottom. Samples from left to right: day 6, 9, 12, 15, 18 and purified LPC-1 abnormal protein.

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day of death of the individual mice was recorded. The animals were weighed daily, and the amount of food consumed daily by each mouse was recorded.

RESULTS

Table 1 summarizes data from eleven experiments on the effectiveness of cyclophosphamide against the advanced ascites form of the plasma cell neoplasm LPC-1 in mice. In these experiments where the untreated control animals succumbed with a MST of 19–29 days, treatment with cyclophosphamide increased the median lifespan of the animals to 38–65 days.

Table 2 summarizes the effectiveness of thirty-one compounds. In each of the eleven experiments, cytoxan was employed as a standard for therapeutic effectiveness. The test compounds are related relative to: (a) MST of the untreated controls and (b) to the cyclophosphamide standard. Table 2 shows, for each compound tested in each experiment, the range of daily doses, the optimal daily dose level, the individual days of death and the MST observed at the optimal dose level. This table also shows the effect of treatment on the presence of abnormal protein in the sera of the treated mice 24 hr after the last injection.

Alkylating agents

Eight alkylating agents out of 12 (DL-tryptophan mustard, TEM, thio-TEPA uracil mustard, merphalan, aniline mustard, melphalan, and TEPA) were at least 59 per cent as effective as cyclophosphamide in increasing the survival time of the tumor bearing mice. DL-tryptophan mustard, the most active of the alkylating agents tested, produced two survivors and a 134 per cent increase in median survival time relative to the cyclophosphamide standard (Table 2). Chlorambucil, nitrogen mustard, and epoxypropidine gave moderate increases, being 28, 20, and 19 per cent as active as cyclophosphamide, respectively. Myleran (busulphan) was ineffective in this test system.

Nitrosourea derivatives

Of the two derivatives tested, BCNU, which has both nitrosourea and alkylating agent moieties, elicited the greater effect, being 77 per cent as active as cyclophosphamide. Methyl nitrosourea was 24 per cent as effective as cyclophosphamide.

Pyrimidine derivatives

5-FU was markedly active in increasing the lifespan of the LPC-1 tumor bearing mice. The activity observed was 152 per cent relative to the cyclophosphamide standard. This represented the greatest increase in survival time for the thirty-one compounds studied. With this compound, there were four mice that survived more than seventy-six days from the time of tumor inoculation. Moderate activity (65 per cent of that found with cyclophosphamide) was observed with 5-fluorotic acid, while 5-FUdR was only slightly active (29 per cent relative to cyclophosphamide).

Antibiotics

Streptonigrin was relatively inactive and the methyl ester derivatives of streptonigrin produced no effect with the LPC-1 tumor.

Table 1. Effect of cyclophosphamide survival of mice with advanced LPC-1. Summary of eleven experiments

	Cyclophosphamide					Untreated controls	
Expt. No.	Daily dose range (mg/kg)	Optimal daily dose (mg/kg)	Individual survival time (days)	MST	% Maxim. Increase in MST over controls	Individual survival time (days)	MST
PC-1	64–2·0	32·0	21, 23(2)*, 44, 47†(2), 53, 55, 62, 72, >76(2)	58	100	19†(2), 24, 26(6), 27(2), 28, 29(7), 30, 32(2), 33(4)	29
PC-4	64–8·0	32·0	18, 34, 41, 42†(2), 47, 49, 52, 56, 57, 58(2)	52	112	18, 20, 22, 23(4), 24(5), 25(3), 26, 27, 30, 31(2), 32, 39(2), 42†	24·5
PC-5	65–8·4	39·0	17, 19, 20, 22, 31, 39(2), 42, 44(2)	38	85	17, 18(4), 19, 20(3), 21(5), 23(2), 24, 31	20·5
PC-6	108–14	14·0	48†(2), 52, 53, 54, 60(2), >135	56	107	24, 25(2), 27(5), 28(4), 30, 36(2), 48†	27·5
PC-7‡	108–14	23·0	18, 47†(2), 47, 49, 63, 68, 81	56	154	16(2), 20, 21(4), 22(6), 23(2), 25	22
PC-12	128–8·0	16·0	38, 42†(2), 46, 47, 48, 50(2), 52, 74	50	112	20(2), 22(3), 23(5), 24(2), 25(3), 26, 27, 29, 30, 43†	23·5
PC-13	108–14	23·0	19, 42†(2), 48, 51, 54†(2), 64, 66, 69	65	210	19(2), 20(6), 21(3), 22(4), 23(2), 24(2), 26	21
PC-15	108–14	23·0	22, 42, 48, 49, 50(3), 54, 56, 60	50	127	20(3), 21(5), 22(4), 23(3), 24(4), 25	22
PC-16	108–14	14·0	20, 28, 38, 41, 42†(2), 42, 45, 49, 50	47	123	18(2), 19, 20(4), 21(5), 22(3), 23(2), 24(2), 26	21
PC-21	39–14	14·0	21, 30, 42, 43(2), 46, 47, 49, 52, >53	44·5	102	17(2), 19(2), 20, 22(7), 23(2), 24(3), 25, 28, 34	22
PC-23	65–8·4	23·0	18, 19, 26, 37, 39, 40, 41(2), 42†(2), 42, 45	40·5	113	16(2), 17(2), 18(5), 19(10), 20, 21(2), 22, 23	19

* ( ) No. of mice.
† Animals sacrificed.
‡ Treated for 32 days.

Miscellaneous compounds

Of the other twelve agents tested, urethane was the most effective, being 76 per cent as active as cyclophosphamide. MIH, stilbamidine, and Methotrexate produced moderate increases in survival time with effects relative to cyclophosphamide of 39, 28, and 23 per cent respectively. 6-MP, hydroxyurea, methyl-GAG, vincristine, prednisone, Cl-TIC, hadacidin and pyronine B showed little or no effect against the LPC-1 tumor.

Abnormal plasma protein

Cyclophosphamide, the standard employed in these studies, consistently increased the lifespan of the mice inoculated with the LPC-1 tumor (Tables 1 and 2). It should be noted that when these marked increases in survival time were observed with cyclophosphamide, abnormal protein was not detected in the sera of treated mice 24 hr after the last injection. Abnormal protein was not detected following treatment with the optimal dose of 5-FU, or tryptophan mustard, agents which were more effective than cyclophosphamide in increasing the lifespan of the mice. Of the ten agents which were between 50 and 100 per cent as effective as cyclophosphamide, only one, aniline mustard, caused the disappearance of the abnormal protein.

Caloric restriction

Caloric restriction, which produced animal weight loss, was not capable of increasing the survival time of the animals (Table 3).

DISCUSSION

The murine plasma cell tumors provide the unusual opportunity of evaluating the effect of chemotherapy not only on survival of the animal but also on a biochemical product of the malignant plasma cells, the abnormal serum globulin. Rosenoer and Whisson,[10] Whisson and Connors,[13] and Hayes and co-workers[9] have both utilized several of these tumors as laboratory models for human plasma cell tumors. In these studies the investigators evaluated primarily tumor weight or tumor volume utilizing solid tumor implants. In the present experiments, the ascitic form of the plasma cell tumor LPC-1 was employed. An attempt was made to mimic the clinical counterpart of advanced aspects of the disease in man by delaying treatment until the animals were all palpably positive for tumor. The ascitic form can be transplanted quantitatively and retains its ability to produce the abnormal serum protein. The advanced form of the disease does not appear to be sensitive to specific caloric restriction.

Of the compounds that produced marked increases in survival time of the mice only four were able to eliminate the abnormal protein. These were cyclophosphamide, DL-tryptophan mustard and 5-FU which produced the greatest increases in survival time; aniline mustard was also quite active. Nine other compounds caused an increase of >50 per cent in median survival time relative to cyclophosphamide but did not cause the abnormal protein to disappear.

What relationship does the activity of drugs against the LPC-1 bear to the human disease? Because several cooperative groups have been interested in treating human plasma cell disease, many compounds have received testing in the clinic. The clinically active compounds and their effects against the LPC-1 are shown in Table 4. Of the seven clinically active compounds listed, only prednisone and chlorambucil failed to

cause a >50 per cent increase in survival in the mice bearing LPC-1 tumor. While prednisone has antitumor activity against the human form of the disease, the effect is only partial and obviously of short duration.[14] Studies have indicated that while several clinical and laboratory parameters are improved, the lifespan of the patient is not increased. Chlorambucil has shown only limited activity in man.[15] The three most

TABLE 3. INFLUENCE OF FEED RESTRICTION ON THE SURVIVAL TIME OF MICE WITH ADVANCED PLASMA CELL TUMOR, LPC-1*

Daily amount of feed offered starting day 14 (g)	Average daily amount of food consumed† (g)	Average body wt. change‡ (g)	M.S.T. (days)	Individual survival times (days)
6·0	2·0	+3·6	19·5	16, 18(3), 19, 20, 23(2), 24, 28
(*ad libitum*)				
4·0	1·5	+4·2	19	17, 18(3), 19(2), 20(2), 21
3·0	1·8	+0·2	19·5	17, 18(3), 19, 20, 21, 24, 25, >31
2·5	1·6	−4·7	18·5	18(5), 19(2), 28, >31(2)
2·0	1·0§	−0·6¶	18	17(2), 10(5), 19, 21, 31
1·5	1·1§	−4·3§	18	16, 17(2), 18(4), 19, 20, 24
1·0	0·4¶	−0·4¶	17·5	16, 17(4), 18(4), 19
0·5	0·3‖	−2·6‖	16·5	16(5), 17(3), 18, 21
Non-tumored controls				
6·0	3·4**	−1·0**		

* Feed restriction initiated 14 days after i.p. tumor inoculation (ascites), in BALB/c male mice, 20–28 g.
† Days 14 through 19.
‡ Average difference in body weight in animals between days 14 and 19.
§ Data for days 14 to 18.
¶ Data for days 14 to 17.
‖ Data for days 14 to 16.
** Data for days 14 to 19.
( ) No. of mice.

active compounds in man, L-phenylalanine mustard (melphalan),[16] DL-phenylalanine mustard (Merphalan)[17] and cyclophosphamide[18] were effective in increasing survival time in mice with the LPC-1 tumor, but only cyclophosphamide was capable of eliminating the abnormal protein. Although tryptophan mustard has significant activity both clinically and in the mouse tumor,[19] comparative studies with the other alkylating agents have not been done. Urethane was capable of prolonging survival in mice but was not effective in eliminating abnormal protein. Conflicting reports of clinical activity have been reported in man.[20, 21] Thus, only two compounds having significant antitumor response clinically, prednisone and chlorambucil, would be classed as inactive against the murine plasma cell tumor. Also listed in Table 4 are the increases in survival time over controls for Leukemia L1210 and the per cent inhibition of tumor weight relative to controls for Walker Carcinosarcoma 256 (IM).[22] Employing the criteria of 30 per cent or greater increase in survival time for L1210 bearing mice and 75 per cent or greater inhibition of tumor growth for Walker 256, the L1210–Walker combination would have selected all of the compounds but urethane as

TABLE 4. COMPARATIVE ACTIVITY OF DRUGS AGAINST LPC-1, L1210 AND WALKER 256: DRUGS CLINICALLY ACTIVE AGAINST PLASMA CELL TUMORS

NSC No.	Drugs	LPC-1*	Protein†	L1210‡¶	Walker 256§¶
26,271	Cyclophosphamide	100	0	80	90
8,806	Melphalan	60	+	75	95
14,210	Merphalan	71	+	60	87
62,403	Tryptophan mustard	134	0	100	90
3,088	Chlorambucil	28	+	31	84
10,023	Prednisone	0	+	0	80
746	Urethane	76	+	20	36

* Per cent increase in MST relative to cyclophosphide.
† (0) means abnormal protein eliminated by treatment.
(+) means abnormal protein not affected by treatment.
‡ Per cent increase in MST.
§ T/C per cent tumor weight inhibition.
¶ Ref. (22).

active. The battery of three tumors i.e. LPC-1, L1210 plus Walker 256 would have identified all seven compounds as active.

Of the eleven compounds listed as clinically ineffective against plasmacytic myeloma in man (Table 5), three compounds TEM,[23] 5-fluorouracil,[24] and thio-TEPA[25] were

TABLE 5. COMPARATIVE ACTIVITY OF DRUGS AGAINST LPC-1, AND WALKER 256: DRUGS CLINICALLY INACTIVE AGAINST PLASMA CELL TUMORS

NSC No.	Drugs	LPC-1*	Protein†	L1210‡	Walker 256§¶
35,605	Stilbamide	32	+	4	NT‖
740	Methotrexate	36	+	100	95
9,706	TEM	97	+	65	90
19,893	5-FU	152	0	60	72
762	Nitrogen mustard	20	+	55	62
755	6-Mercaptopurine	14	+	50	86
6,396	Thio-TEPA	74	+	45	96
32,065	Hydroxyurea	11	+	45	47
67,574	Vincristine	0	+	39	80
750	Busulphan	0	+	4	80
56,308	Epoxypropidine	19	+	NT	NT

* Per cent increase in survival relative to cyclophosphamide.
† (0) means abnormal protein eliminated by treatment.
‡ (+) means abnormal protein not affected by treatment.
‡ Per cent increase in MST over controls.
§ T/C per cent tumor weight decrease.
¶ Ref. (22).
‖ Not tested.

active in the LPC-1 system and could therefore be considered as false positives. However, none of these compounds has received extensive enough clinical trials to rule out definitely potential clinical usefulness.

5-Fluorouracil, which was the most active compound against the plasma cell tumor for the daily schedule employed here received scattered and relatively inadequate clinical trials.[24] With respect to drugs clinically inactive against plasma cell

tumor, the LPC-1 would appear to have been somewhat more discriminating than the L1210 or Walker 256 systems (Table 5). In contrast to the three out of eleven actives (false positives) for LPC-1, the L1210 system indicated eight out of ten compounds as active and the Walker system indicated six out of nine compounds as active. Making the requirement for activity higher (i.e. increasing the survival time increase from 30 per cent to 50 per cent in L1210 mice and tumor weight inhibition from 75 to 90 per cent for Walker 256) reduces the number of false positives to five out of ten for L1210 and to three out of nine for Walker 256.

TABLE 6. COMPARATIVE ACTIVITY OF DRUGS AGAINST LPC-1, L1210, AND WALKER 256: DRUGS NOT TESTED CLINICALLY AGAINST PLASMA CELL TUMORS

NSC. No.	Drugs	LPC-1*	Protein†	L1210‡¶	Walker 256§¶
34,462	Uracil mustard	71	+	40	90
18,429	Aniline mustard	66	0	NT‖	NT
9,717	TEPA	59	+	40	90
409,962	BCNU	77	+	150	98
23,909	Methyl nitrosourea	24	+	87	NT
31,712	Fluororotic acid	65	+	46	40
27,640	5-FUdR	29	+	52	75
45,383	Streptonigrin	14	+	20	90
45,384	Streptonigrin, methylester	2	+	26	NT
77,213	MIH	39	+	45	75
32,946	Methyl-GAG	6	+	60	44
60,339	Chlor-Tic	0	+	95	58
72,962	Hadacidin	0	+	0	0
44,690	Pyronine B	0	+	8	0

* Per cent increase in survival relative to cyclophosphamide.
† (0) means abnormal protein eliminated by treatment.
(+) means abnormal protein not affected by treatment.
‡ Per cent increase in MST over controls.
§ T/C per cent tumor weight decrease.
¶ Ref. (22).
‖ Not tested.

For the compounds that were tested in the mice but not in man (Table 6), the LPC-1 system suggests that five compounds are likely candidates for clinical trial. Aniline mustard was the only one which both increased the survival time and eliminated the abnormal protein. This compound has been shown to cure mice bearing advanced plasma-cell tumor.[13] Uracil mustard, TEPA and BCNU were active against LPC-1, L1210 and Walker 256. Fluororotic acid was active against LPC-1 and L1210.

Thus, the LPC-1 tumor by virtue of biological and biochemical parameters of response appears to offer a suitable test system for screening compounds for clinical trial. There are, however, certain points that must be raised. First the clinical disease in man, like the disease in mice, consists of at least seven different protein producing types. Bergsagel[26] has suggested that patients producing type K light chain Bence–Jones protein respond to L-phenylalanine mustard better than type L Bence Jones protein producing patients. This has not been the experience of other investigators (P. P. Carbone, personal communication).[27] However, in the murine plasma cell tumors, the relationship of protein type produced to response to treatment is not

known. Toward this end a program is in progress to test several active and inactive compounds against various types of immunoglobulin producing tumors in mice.

In addition, only a daily treatment schedule was used in the experiments reported here. It has been clearly shown that drug schedules may influence the response to treatment for L1210.[28] Changes in schedule may also alter drug effectiveness against the LPC-1 tumor. Finally until more quantitative data can be obtained in the clinic for comparison with the results obtained in the animal tumor systems, empiric screening of compounds in man must continue.

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