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Combination Chemotherapy with AMSA on L1210 Leukaemia and B16 Melanoma*

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Abstract—*Clinical interest in AMSA, [4'-9(acridinylamino)methanesulfon-m-anisidide] has grown recently because it has shown remarkable activity in the treatment of adult myelogenous acute leukaemia.*

In the present investigation on two experimental murine tumours, namely L1210 leukaemia and B16 melanoma, m-AMSA was administered in association with BCNU, melphalan (MLP) and DTIC. The therapeutic activity of the AMSA–MLP–BCNU combination in murine L1210 leukaemia was compared to that of the MLP–BCNU–DTIC combination. These two associations proved to be very active (30% cures were recorded on day 60). The dose levels of the drugs used in the combination represent only 30–50% of the optimal dose of the same drugs given separately.

When DTIC was added to the AMSA–MLP–BCNU combination, considerable lengthening of survival time as well as up to 7 cures out of 10 mice on day 60 were recorded. However, none of the combination chemotherapy was able to induce 10 cures (out of 10 mice) like treatment with BCNU, at optimal dose.

INTRODUCTION

In 1974, *m*-AMSA (NSC 249992) or methanesulfon-*m*-anisidide [4'-9(acridinyl amino)] was shown by Cain and Atwell [1] to have an antitumour activity against murine L1210 leukaemia, B16 melanoma and C3H/HeJ spontaneous mammary tumours. Other authors subsequently demonstrated that the drug might be useful against the colon adenocarcinoma [2] and human colon cancer xenografts [3]. *m*-AMSA was found to react with DNA by intercalation between AT and GC base pairs [4].

On the basis of these good results obtained with experimental models, *m*-AMSA was selected for further studies on human tumours. In 1977 it entered phase I clinical trials. Therapeutic activity was observed in some patients with acute leukaemia, lymphoma or adenocarcinomas [5]. Because of its minimal myelosuppressive toxicity and good general tolerance, it went into phase II trials [6] in which antileukaemic activity was estimated. According to a review of AMS activity [7], the best results were obtained with AMSA in combination with other cytotoxic agents.

The present study reports on the results obtained on murine L1210 leukaemia and B16 melanoma with combinations of *m*-AMSA with known cytotoxic agents, melphalan, BCNU and DTIC.

MATERIALS AND METHODS

(A) *Experimental tumours and animals*

L1210 leukaemia is maintained in our laboratory by weekly transfers in DBA/2 mice (20–22 g). Ascitic fluid is removed from the peritoneal cavity of the donor, leukaemic cells are counted and then suspended in 0.85% saline solution. Male CDF_1 (22–24 g) mice were inoculated i.p. with 10^5 L1210 leukaemic cells on day 0.

Melanoma B16 is transferred from C57B1/6 mice in $B_6C_3F_1$ mice (20–22 g) as a cell suspension obtained by homogenizing 1 g of tumour in 10 ml of saline (0.85%).

Treatment started 24 hr after tumour implantation. Animals were weighed on days 1 and 5 after tumour implantation.

Animals were supplied by Charles River Breeding Labs, Wilmington, Mass. and tumour lines by Dr. A. Bogden, Mason Research Institute, Worcester, Mass.

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(B) *Drugs*

All drugs were dissolved in saline except AMSA that was dissolved in distilled water + Tween 80. They were administered i.p. following different schedules: BCNU was given only once on day 1, MLP and DTIC were given once a day on days 1, 5 and 9 and AMSA was given daily during 9 days.

All materials were provided by the National Cancer Institute, Bethesda, Maryland.

(C) *Evaluation of activity*

Synergism of action is said to occur when the total effect of two or more drugs administered together is higher than or equal to the sum of the effects of each drug given alone. If the total effect is just equal to the sum of individual effects, the effects are additive; if the total effect is higher, then there is potentiation. To select the doses in our study we followed the methodology described by A. Goldin [8] avoiding the use of the minimal active dose of each drug. For this reason, the theoretical global toxicity of the combination is usually higher than the toxicity of each individual drug used at optimal schedule. The experiment was continued for 60 days and survivors at that point were recorded as cures.

The parameter commonly used to evaluate activity of an anticancer agent is the median survival time expressed in number of days after transplant. With combination chemotherapy, it is generally considered that a more convenient and realistic parameter is the median survival time of dead animals only, excluding cures from calculation. Therefore, the latter parameter was used and cures were recorded on day 60.

RESULTS

Combination chemotherapy on mice bearing L1210 leukaemia

A first experiment was performed with three drugs on male CDF_1 mice bearing L1210 leukaemia. Each drug was tested separately and only 30–50% of the respective optimal doses were used in three drug combination chemotherapy.

From results shown in Table 1, no combination of AMSA and other drugs was more effective than BCNU given alone. When comparing all combinations, it seems that AMSA–MLP–BCNU effectively enhanced lifespan and induced 30–40% of long-term survivors on day 60. The global toxicity of these combinations was very similar.

A four-drug association was performed, adding increasing doses of DTIC (60–100 mg/kg) to the above-mentioned combination. A considerable increase in the number of long-term survivors was recorded but this new combination did not significantly increase lifespan.

Combination chemotherapy on mice bearing B16 melanoma

We have repeated the same drug associations using $B_6C_3F_1$ mice bearing i.p. B16 melanoma. From results in Tables 3 and 4, both AMSA and MLP given separately considerably enhanced lifespan, but, however, did not induce long-term survivors. Three- and four-drug combinations have not prolonged median survival time as much as AMSA and MLP, although 10–55% of long-term survivors were recorded. AMSA–MLP–BCNU and AMSA–DTIC–MLP combinations seem to have been equally effective in terms of survival time; we did not observe any enhancement of survival by a four-drug association.

DISCUSSION

From results shown in Table 1, the combination of AMSA, DTIC and MLP did not show any potentiation under the conditions of the experiment and only showed an additive effect. The effect of this combination chemotherapy was similar to that of MLP given alone at a dose of 7.5 mg/kg/injection.

The therapeutic effect of the AMSA–DTIC–BCNU association was more than additive if we consider the increase in lifespan produced by this combination to that obtained with each drug given separately at similar doses. However, it never was so effective as the optimal dose of BCNU.

Of all these combinations, that of AMSA (2 mg/kg) plus MLP (2.5 mg/kg) and BCNU (10 mg/kg) was the most efficient against L1210 leukaemia. The percentage increase in survival time of animals treated by the latter combination was more than additive as compared to that of animals treated by separate administration of the compounds used in the combination (at same dose levels). Moreover, this combination induced 40% of long-term survivors while no cures were recorded on day 60 after separate administration of the drugs.

However, if we compare the activity of these combinations to that of BCNU administered alone at the optimal dose (32 mg/kg) where we recorded 100% cures, we can no longer speak of potentiation. Therefore, a question arises: why should we seek a therapeutic synergism

Table 1. Combination chemotherapy with AMSA, MLP, DTIC and BCNU on CDF_1 mice bearing L1210 leukaemia

Drugs	Dose mg/kg/inj.	B.W.C.* (g)	Med.S.T.† (days)	T.L.S (%)	Death pattern‡ (days on which animals died)
MLP	7.5	- 0.4	17.0	113	14,15,15,15,17,17,18,29,27 (1 long-term survivor)
	4.0	- 0.2	13.1	61	11,12,12,13,13,13,13,15,15,15
	2.5	+ 0.3	10.8	33	10,10,10,10,11,11,11,11,11,12
	2.0	+ 1.3	10.1	24	7,8,9,10,10,10,10,11,11,11
DTIC	180	+ 2.4	9.4	16	8,8,9,9,9,9,10,10,10,11
	80	+ 2.8	8.3	02	8,8,8,8,8,8,8,9,10,11
	60	+ 3.9	8.4	03	8,8,8,8,8,8,9,10,10,11
AMSA	4	- 1.4	13.0	60	10,11,12,13,13,13,13,13,14,14
	2	0	11.9	53	8,11,12,12,12,12,13,18,19,24
	1	+ 0.6	11.6	43	7,9,11,11,11,12,12,12,12,13
BCNU	32	- 3.0			10 long-term survivors
	15	- 0.5	14.1	74	12,13,13,14,14,14,14,15,15,16
	10	+ 0.1	11.9	46	9,11,11,11,12,12,12,12,13,13
	5	+ 1.3	9.2	13	9,9,9,9,9,9,9,9,10,10
AMSA 2 DTIC 80 MLP 2		- 0.2	14.4	77	8,9,14,14,14,14,15,16,16,16
AMSA 2 DTIC 60 MLP 2.5		- 0.7	14.0	72	8,12,13,13,14,14,14,15,16,18
AMSA 1 DTIC 60 MLP 4		- 0.1	16.7	106	14,14,14,16,16,17,17,17,18,19
AMSA 2 DTIC 80 BCNU 5		- 1.0	16.1	98	13,14,14,16,16,16,16,18,18,18
AMSA 2 DTIC 60 BCNU 10		- 0.5	18.0	122	14,15,15,17,18,19,30,32 (2 long-term survivors)
AMSA 1 DTIC 60 BCNU 15		- 0.6	19.0	134	12,17,17,18,19,22,23,28 (2 long-term survivors)
AMSA 2 MLP 2.5 BCNU 10		- 1.0	28.0	245	13,14,15,28,28,34 (4 long-term survivors)
AMSA 2 MLP 2 BCNU 15		- 1.7	19.3	138	14,16,19,19,20,21 (4 long-term survivors)
AMSA 1 MLP 4 BCNU 10		- 0.2	16.5	103	10,14,14,16,18,26,31 (3 long-term survivors)
Control	-	+ 3.5	8.1		7 (1 mouse), 8 (37 mice), 9 (4 mice), 10 (1 mouse)

*†‡see legend in Table 2.

between drugs like AMSA, MLP and BCNU when there is evidence that BCNU, at optimal dose, is more effective? Nevertheless, considering the well known side-effects provoked by BCNU, it would be preferable to reach a high therapeutic activity with less toxicity. In our experiment, indication of lower toxicity is given by the body weight change on day 5 after treatment: there is an average weight loss of 3 g per mouse when BCNU is used alone at optimal dose as compared to 1 g per mouse when the above-mentioned combination is used. Although these combinations were not potent enough to totally eradicate cancer cells, they were able to induce a high percentage of long-term survivors which makes it difficult to conclude whether our target/potentiation has been attained.

With the B16 melanoma, if we compare the I.L.S. values, none of the combinations (Table 3) prolonged survival more than AMSA or MLP administered separately. However, the combination of AMSA, DTIC and MLP and that of AMSA, MLP and BCNU (at 2, 2.5 and 15 mg/kg) produced an increase in survival similar to that obtained with MLP and AMSA given alone and could induce 45–55% of long-term survivors, which might be due to a potentiation.

It should be pointed out that with four-drug combinations the I.L.S. of mice used in the calculation was lower, which indicates that treatment was toxic to a number of mice. However, the combination of AMSA (2 mg/kg)–DTIC(80 mg/kg)–MLP(2.5 mg/kg)–BCNU (10 mg/kg) induced 70% of long-term survivors.

Table 2. Combination chemotherapy with three and four drugs on CDF_1 mice bearing L1210 leukaemia

Drugs	Dose mg/kg/inj.	B.W.C.* (g)	Med.S.T.† (days)	I.L.S. (%)	Death pattern‡ (days on which animals died)
AMSA	2				
MLP	2.5	- 1.9	18.5	120	14,16,17,18,19,25,48
BCNU	10				(3 long-term survivors)
MLP	2.5				
BCNU	10	- 2.0	22.5	167	18,18,20,22,25,33,34
DTIC	80				(3 long-term survivors)
AMSA	2				
MLP	2.5	- 3.9	12.5	48	11,12,12,14,23
BCNU	10				
DTIC	60				(5 long-term survivors)
AMSA	2				
MLP	2.5	- 4.2	12.0	42	8, 12,12 (7 long-term survivors)
BCNU	10				
DTIC	80				
AMSA	2				
MLP	2.5	- 3.0	23.5	179	12,23,25 (7 long-term survivors)
BCNU	10				
DTIC	100				
AMSA	2				
MLP	2.5	- 1.9	21.3	153	2,16,20,21,21,21,23,25,26
BCNU	5				
DTIC	60				(1 long-term survivor)
AMSA	2				
MLP	2.5	- 2.1	21.5	155	15,18,20,21,26,26,28
BCNU	5				
DTIC	80				(3 long-term survivors)
AMSA	2				
MLP	2.5	- 2.0	20.3	141	18,18,10,20,20,21,22,23
BCNU	5				
DTIC	100				(2 long-term survivors)
CONTROl	-	+ 1.8	8.4	-	7 (1), 8 (15), 9 (8), 10 (5), 11 (1)

CDF_1 mice (20–22 g) were inoculated i.p. with 10^5 L1210 cells on day 0. Treatment started on day 1: AMSA was given once a day during 9 consecutive days, MLP and DTIC were given once every fourth day and BCNU once on day 1 only.

*B.W.C., the body weight change is the difference in average weight change of animals between day 1 and day 5 after tumour transplantation.

†Med. S.T., the median survival time expressed in days is evaluated from the dead animals; survivors are excluded from the calculation.

‡10 mice were used per test group; the figure between parentheses in the control group indicates the number of dead animals on each mentioned day.

Table 3. Activity of AMSA–BCNU–DTIC–MLP combination against i.p. melanoma B16

Drugs	Dose mg/kg/inj.	B.W.C. (g)	Med.S.T. (days)	I.L.S. (%)	Death pattern§ (days on which animals died)
AMSA	4	+ 1.2	39.0	122	26,31,33,35,39,45,46,50
	2	+ 1.6	25.3	44	19,21,25,25,25,35,36,41
DTIC	180	+ 0.9	21.0	20	15,16,19,20,21,24,31,32
	80	+ 0.8	18.0	02	6,16,17,18,19,20,21,26
	60	+ 1.1	21.0	20	15,16,16,17,21,26,31,40
MLP	7.5	+ 0.4	42.0	140	10,34,35,35,42,43,44,46
	4.0	+ 1.0	36.0	105	25,25,30,35,36,36,37,38
	2.5	+ 0.5	32.0	82	20,25,28,31,32,38,41,46
	2.0	+ 1.5	29.0	65	21,22,24,26,29,29,31,36
BCNU	32	- 0.4	27.0	54	25,25,26,26,27,29,43,51
	15	- 0.1	24.0	37	4,22,23,23,24,26,29,37
	10	+ 0.9	21.0	20	16,17,17,19,21,23,23,24
AMSA	2				
DTIC	80	+ 0.1	48.0	174	18,28,41,46,48,50,50,50
MLP	2				(1 long-term survivor)
AMSA	2				
DTIC	60	- 0.5	40.0	128	7,10,38,40,42,46
MLP	2.5				(3 long-term survivors)
AMSA	2				
DTIC	80	- 1.0	43.0	145	31,32,40,42,43,47,48,52,55
BCNU	10				
AMSA	2				
DTIC	60	- 1.6	30.5	74	6,10,30,38,53
BCNU	15				(4 long-term survivors)
AMSA	2				
MLP	2.5	+ 0.3	26.5	51	9,15,26,38,52
BCNU	10				(4 long-term survivors)
AMSA	2				
MLP	2.5	- 0.9	40.3	130	26,40,40,43
BCNU	15				(5 long-term survivors)
CONTROL	-	+ 1.5	17.5	-	15 (3), 16 (2), 17 (6), 18 (3) 20 (1), 21 (2), 23 (1), 26 (1) 29 (1)

Male $B_6C_3F_1$ mice (22–25 g) received i.p. 0.5 ml of a 1/10 B16 cell suspension on day 0. I.p. treatment started on day 1 and following schedules were used: AMSA day 1–9; MLP and DTIC days 1,5,9; BCNU on day 1 only.

§8 animals were used when drugs were administered alone and 9 in combination groups. The figure between parentheses in the control group indicates the number of dead mice on each specified day.

Table 4. Three- and four-drug combinations against i.p. melanoma B16

Drugs	Dose mg/kg/inj.	B.W.C. (g)	Med.S.T. (days)	I.L.S. (%)	Death pattern (days on which animals died)
MLP DTIC BCNU	2.5 80 15	+ 0.7	37.3	103	34,34,35,37,37,44,46,54 (2 long-term survivors)
MLP DTIC BCNU	2.5 80 10	+ 0.4	29.3	60	13,18,20,26,29,29,35,35,44 46
MLP DTIC BCNU AMSA	2.5 80 10 2	+ 0.4	46.0	151	10,36,38,43,46,48,49,52 (2 long-term survivors)
MLP DTIC BCNU AMSA	2.5 80 15 2	- 1.6	43.5	137	10,24,43,46,48 (4 long-term survivors)
Control	-	+ 2.1	18.3		15 (3), 16 (4), 17 (4), 18 (5) 19 (5), 20 (1), 21 (2), 25 (1) 27 (1), 28 (2), 29 (1)

With the B16 melanoma, no significant difference appeared between three- and four-drug combinations therapy. Our results emphasize the fact that we should be cautious in the choice of the model used in combination chemotherapy and have no clear-cut position since the same combination of AMSA, MLP and BCNU has shown a synergism of action against the B16 melanoma but not against the L1210 leukaemia. The fact that there were no signs of any antoganism between the drugs used in our combination chemotherapy makes the study of clinical interest.

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