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The Chemosensitivity of a New Experimental Model—the M5076 Reticulum Cell Sarcoma*

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Abstract—*The M5076 reticulum cell sarcoma is a murine tumour of potential value in experimental chemotherapy. Experiments were conducted to ascertain the growth characteristics and chemosensitivity of this neoplasm in the BDF_1 mouse. The intramuscular tumour proved to be responsive to the alkylating agents, nitrosoureas, procarbazine, DTIC and treosulphan, yet insensitive to the antimetabolites and only weakly responsive to adriamycin. Analogues of the antitumour agents hexamethylmelamine and N-methylformamide were tested against this neoplasm. The patterns of activity determined for these analogues against this tumour were identical to those previously reported against other model systems.*

INTRODUCTION

THE APPLICABILITY of rodent tumours as test systems in the search for new antitumour drugs has been questioned increasingly. There are many examples of chemicals such as the nitrosoureas [1, 2] and the aryldimethyltriazenes [1, 3] which exert astonishingly selective activity against fast-growing neoplasms, such as the L1210 leukaemia and the Lewis lung carcinoma (i.v. model), but which are at best only moderately active when tested in cancer patients. The realization that rodent tumours appear to be profoundly different from their human counterparts in certain important biological respects (e.g. loss of heterogeneity, rapid doubling times and high proliferative fractions) has led to efforts to find more relevant tumour systems which may detect new agents with antineoplastic properties in humans. So far these attempts have yielded little, and rodent tumours, despite their failings, continue to make a significant contribution to screening systems in experimental chemotherapy research.

One of the murine tumours recently included in the screening panel of the National Cancer Institute is the M5076 (M5, M5076/73A) reticulum cell sarcoma [4–7]. Originally classified as a carcinoma, histological and immunological studies now indicate the tumour to be macrophagic in origin [5]. Further studies have demonstrated that the spread of the tumour is highly unusual, with metastases appearing primarily in the visceral organs, lung lesions being rare [6, 7]. The M5076 is attracting interest as a model for the study of tumour spread and colonisation [6, 7].

We have been using the M5076 line for some years, both as a screening tool to investigate the antitumour properties of novel chemical structures and for the study of structure–activity relationships with analogues of known active agents. Only brief reference has previously been made to the chemosensitivity of this tumour [8, 9] and in the present paper we report on a study of the activity of a variety of clinically established and investigational agents against it.

MATERIALS AND METHODS

Mice

Female BDF_1 mice (hybrids of the C_{57}/BL and DBA/2 mice; 18–20 g) were obtained from Bantin and Kingman Ltd, Hull, U.K.

Tumour

The M5076 tumour was obtained through the courtesy of Dr C. J. Rutty of the Institute of Cancer Research, Sutton, U.K. The tumour arose spontaneously in a C_{57}/BL mouse in Dr W. F.

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Dunning's laboratory, Papanicalao Cancer Research Institute, Miami, FL. The tumour was maintained subcutaneously (s.c.) in the flanks of female BDF_1 mice and passaged every 2–3 weeks. Tumour lines were run for no more than 10 serial passages, after which they were destroyed, with new lines being obtained from a large store of the initial line (kept banked in liquid nitrogen).

Antitumour testing

Fragments of the tumour were obtained from donor mice. These were pooled, homogenised and diluted with saline to produce a suspension of 10^7 cells/ml. Cells (10^6 in 0.1 ml) were then injected i.m. into the left hind legs of female BDF_1 mice. Drugs were administered i.p. according to the appropriate schedules, commencing 24 hr after tumour implantation. Untreated tumours became palpable at about day 12 post-implant and tumour volumes were measured every 4 days from day 12 until day 24. Measurement was made by vernier calipers and the tumour volume calculated by the standard method [10]. The mean tumour volume in each group of treated mice was then compared with the mean volume of the untreated control mice. Groups of either 5 or 10 mice were used for each dose level and at least 20 control mice were used for each experiment.

The ID_{90} refers to that dose which produces 90% inhibition of the mean tumour volume of control mice on day 24. To determine lethal dose values, groups of tumour-bearing mice received dose levels of the drug ranging from non-lethal to 100% mortality. A graph of mortality vs log dose was then plotted and the LD_{10} and LD_{50} values estimated. The ID_{90}, LD_{10} and LD_{50} values refer to the schedules described.

The optimal dose is taken as that dose which is nearest to the LD_{10} value except where an agent exerts maximum activity at a dose lower than the LD_{10} value, when the optimal dose is taken instead to be that dose.

Drugs

All drugs were obtained from commercial sources with the exception of the following compounds: 1-11, 15-17 and 20 were synthesized by the authors and their colleagues R. J. Simmonds, E. N. Gate and M. D. Threadgill.

RESULTS

Growth characteristics of the M5076 sarcoma

The death days of 212 BDF_1 mice injected i.m. with 10^6 M5076 cells in the left leg are illustrated in Fig. 1. These represent the total of 7 experiments (Table 1), with the range of medians lying between days 31 and 39 post-implant. No animals survived until day 60.

The tumour growth curves of the M5076 sarcoma in BDF_1 mice after implantation of increasing numbers of cells (10^2–10^6) i.m. are illustrated in Fig. 2.

The mass doubling time of the M5076 sarcoma is compared in Table 2 with those of other widely used murine models.

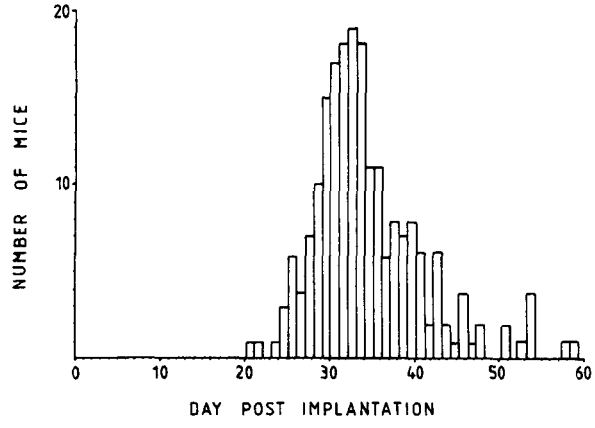


Fig. 1. The death days of BDF_1 mice injected i.m. with 10^6 M5076 cells in the leg.

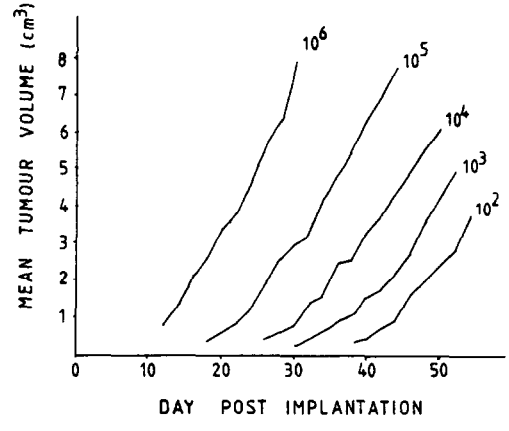


Fig. 2. The tumour growth curves of the M5076 sarcoma in BDF_1 mice after implantation of differing numbers of cells (10^2–10^6).

Table 1. Days of death of BDF_1 mice implanted with 10^6 M5076 sarcoma cells (i.m.)

Group	No. of mice	Median day of death	Range of days of death
A	30	39.0	25–58
B	27	32.4	26–59
C	30	32.7	21–46
D	32	32.9	24–54
E	28	33.3	26–46
F	28	35.5	25–51
G	37	31.3	26–48
Total	212	31.3–39.0	21–59

Chemosensitivity of the M5076 sarcoma

In Table 3 the response of the M5076 sarcoma to a range of clinically-established and experimental drugs is reported. Three types of treatment schedule were used: a single injection (day 1), an intermittent regime (days 1, 5, 9, 13 and 17) and a chronic daily treatment (days 1–17). The latter two schedules represent a treatment period corresponding to approximately half of the life span of control tumour-bearing animals. These were selected in an attempt to mimic the acute, intermittent and chronic schedules of day 1, days 1, 3 and 5 and days 1–5 respectively used against the murine L1210 leukaemia. Several of these agents (*cis*-platinum, treosulphan, hexamethylmelamine, pentamethylmelamine and *N*-methylformamide) were tested on all of these schedules against this tumour and the optimal schedule is given in Table 3. The schedules for the other agents were selected on the basis of data obtained against other tumours. Tumour volume inhibition was employed as the parameter of antitumour activity and day 24 was chosen as the day of evaluation. The parameter of therapeutic index (LD_{10}/ID_{90}) represents a function of both antitumour activity and toxicity. One of the major advantages of this coefficient is that it permits differentiation between an agent capable of eliciting complete inhibition at only one dose and another capable of eliciting the same response over a range of doses.

Chlorambucil and adriamycin elicited inhibitions (day 24) of only 41 and 31% respectively at their optimal dose levels (20 and 2.5 mg/kg respectively).

In Tables 4 and 5 the activity of analogues of hexamethylmelamine and *N*-methylformamide against the M5076 sarcoma is recorded.

DISCUSSION

When establishing a tumour model as a screening system, several parameters such as the host for the tumour and the endpoint of antitumour evaluation have to be determined initially. The M5076 sarcoma arose in a $C_{57}BL/6$ mouse and there have been several reports of this strain being employed for experiments with the tumour [5–7]. The BDF_1 mouse, a hybrid of the $C_{57}BL/6$ and DBA/2 mice, has also been used [14–17] and these were employed in this study. To confirm that this strain was an acceptable host for the neoplasm, the characteristics of cell growth in

Table 2. Mass doubling times of the experimental tumours

Tumour	Site	Tumour size	Doubling time (days)	Reference
L1210 leukaemia	i.p.	10^6 cells	0.5	[11–13]
B16 melanoma	s.c.	560 mg	1.9	[11–13]
Lewis lung carcinoma	s.c.	575 mg	2.9	[11–13]
M5076 sarcoma	i.m.	500 mg	4.0	*

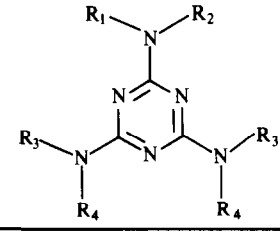
The doubling time value is for a tumour of the size shown.
*See Fig. 2.

Table 3. Chemosensitivity of the M5076 reticulum cell sarcoma

Compound	Schedule (days of injection)	LD_{10}*	LD_{50}*	ID_{90}*†	T.I.‡	Optimal dose*	%inhibition§
Cyclophosphamide	1	370	520	100	3.7	400	100
Chlorambucil	1	26	45	inactive	inactive	25	41
CCNU	1	43	57	17	2.5	20	100
cis-Platinum	1,5,9,13,17	4.8	9.6	1.8	2.7	4.0	100
Adriamycin	1,5,9,13,17	3.7	5.6	inactive	inactive	2.5	31
DTIC	1,5,9,13,17	185	260	32	5.8	50	100
Procarbazine	1,5,9,13,17	240	470	23	10.4	50	100
Methotrexate	1–17	2.6	3.8	inactive	inactive	2.0	0
5-Fluorouracil	1–17	10.7	14.2	inactive	inactive	10	2
Treosulphan	1–17	570	800	420	1.4	400	87
Hexamethylmelamine	1–17	99	110	69	1.4	100	100
Pentamethylmelamine	1–17	144	167	132	1.1	160	96
N-Methylformamide	1–17	220	300	100	2.2	200	100

*mg/kg/day.
†ID_{90} assessed on day 24.
‡Therapeutic Index = LD_{10}/ID_{90}.
§Assessed on day 24 for optimal dose.

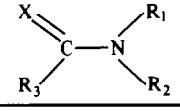
Table 4. Activity of analogues of hexamethylmelamine in the M5076 sarcoma model



Compound No.	R_1	R_2	R_3	R_4	Schedule (days)	LD_{10}*	LD_{50}*	Optimal* dose	% inhibition†
1	CH_3	CH_3	CH_3	CH_3	1-17	99	110	100	100
2	CH_3	H	CH_3	CH_3	1-17	144	167	100	81
3	H	H	CH_3	CH_3	1-17	154	180	160	92
4	CH_3	CH_2OH	CH_3	CH_3	1-17	86	114	80	75
5	CH_3	CHO	CH_3	CH_3	1-17	95	187	80	53
6	CH_3	OH	CH_3	CH_3	1-17	350	494	320	27
7	H	NH_2	CH_3	CH_3	1-17	148	208	160	22
8	N_2	-‡	CH_3	CH_3	1-17	45	72	40	0
9	CH_3	H	CH_3	H	1-17	170	225	160	20
10	H	H	H	H	1-17	960	1180	1000	24
11	C_2H_5	C_2H_5	C_2H_5	C_2H_5	1-17	820	-	750	39

*mg/kg/day.
†Assessed on day 24 for optimal dose.
‡2-Azido-4,6-bis(dimethylamino)-1,3,5-triazine.

Table 5. Activity of analogues of N-methylformamide in the M5076 sarcoma model



Compound No.	X	R_1	R_2	R_3	Schedule (days)	LD_{10}*	LD_{50}*	Optimal* dose	% inhibition†
12	O	CH_3	H	H	1-17	220	300	200	100
13	O	H	H	H	1-17	200	270	200	63
14	O	CH_3	CH_3	H	1-17	1130	1280	1000	60
15	O	C_2H_5	H	H	1-17	320	420	300	22
16	O	CH_2OH	H	H	1-17	1580	1930	1500	63
17	O	CH_2OH	CH_3	H	1-17	370	520	400	24
18	O	CH_3	H	CH_3	1-17	880	1240	800	0
19	O	CH_3	CH_3	CH_3	1-17	900	1420	800	22
20	O	CH_3	H	CF_3	1-17	800	-	800	5
21	O	CH_3	H	$NHCH_3$	1-17	1730	2260	1600	34
22	O	CH_3	CH_3	$N(CH_3)_2$	1-17	440	640	400	33
23	S	CH_3	CH_3	H	1-17	185	260	200	3
24	S	CH_3	H	$NHCH_3$	1-17	450	720	400	9
25	S	CH_3	CH_3	$N(CH_3)_2$	1-17	430	565	400	21
26	NH	CH_3	CH_3	$N(CH_3)_2$	1-17	107	142	100	0

*mg/kg/day.
†Assessed on day 24 for optimal dose.

the BDF_1 were examined and compared with reported data on growth in the $C_{57}BL/6$ mouse [7]. After implantation of cell numbers of between 10^2 and 10^6, take rates in the BDF_1 mouse were 100% and the growth curves of the solid tumour corresponded well with those reported for $C_{57}BL/6$ mice (Figs 1 and 2) [7].

One possible endpoint of antitumour activity that may easily be measured is the survival times of mice inoculated with the tumour. Alessandri *et al.* [7] have shown that the median survival time of tumour-bearing $C_{57}BL/6$ mice is proportional to the number of M5076 cells implanted. Our results with the BDF_1 mouse indicate a similar survival time after inoculation with 10^6 cells and that the range of death days is small enough for this parameter to be used as an endpoint (Table 1).

There are, however, disadvantages in using the survival time mode of evaluation in the case of the M5076 tumour. Firstly, mice die only when the

primary tumour is massive (*ca.* 7 g). At this point the tumour has often broken through the skin of the leg, leading to ulceration and infection, problems which might contribute to the death of the animal. These complications may also cause unnecessary suffering to the rodents. Secondly, survival time represents a composite function of both primary tumour growth and metastatic potential and thus is a very indirect measure of tumour cell kill. In our opinion these considerations militate against the use of this evaluation mode for establishing the endpoint of this antitumour test, if tumour volume or weight inhibition may be used instead.

In these experiments we have used tumour volume inhibition as a measure of tumour cell kill. This parameter also represents a complex interplay of variables. Oxygenated proliferating and non-proliferating cells and hypoxic cells are present, each with differing susceptibilities towards antitumour agents [18–21]. Further, dying and necrotic tissue awaiting removal contribute to the final volume of the tumour. The determination of tumour volume, although far from being a simple measure of viable tumour cell number, is, however, fast and simple and gives a reasonable measure of antitumour response.

In Table 2 the mass doubling times for several experimental tumours are shown. The doubling time for the M5076 sarcoma is seen to be longer than for the other experimental systems. Since long doubling times and low proliferative fractions are the characteristics of the most intractable human solid tumours, Venditti has suggested that these properties are desirable within a tumour model [11].

The tumour is clearly sensitive to the alkylating agents and the nitrosoureas (Table 3) but apparently unresponsive to the antimetabolites. Procarbazine and DTIC possess unusually marked activity against the tumour, considering their moderate clinical usefulness. Treosulphan and hexamethylmelamine (agents effective against human ovarian carcinoma [22–26]) demonstrate activity against the M5076 sarcoma, as do pentamethylmelamine and *N*-methylformamide. It is of interest that these compounds are amongst those active against this tumour since they are able to elicit, at best, only weak activity against most murine tumours. The brief reports of the chemosensitivity of the M5076 sarcoma that have appeared previously have been concerned with the possibility that this tumour might model for human ovarian carcinoma. These reports appeared before the histology of the M5076 sarcoma was fully understood. Our studies would indicate that the tumour successfully detects some of the lesser used agents (e.g. hexamethylmelamine and treosulphan) active towards the human disease. However, its failure to respond to methotrexate and 5-fluorouracil and its sensitivity to the nitrosoureas, procarbazine and DTIC would suggest that its predictive ability for what was once thought to be its clinical tumour counterpart is poor (Table 6).

The M5076 sarcoma has proved to be a convenient model for studying the structure–antitumor activities of analogues of hexamethylmelamine (1) and *N*-methylformamide (12). These results (Tables 4 and 5) complemented previous studies [44, 45] and allowed us to delineate the essential components required for

Table 6. Comparison of the chemosensitivity of the M5076 sarcoma model with human ovarian carcinoma

Compound	M5076* sarcoma (i.m.)	Human ovarian carcinoma†	Reference
Cyclophosphamide	++	++	[27, 28]
Chlorambucil	-	++	[29, 30]
cis-Platinum	++	++	[31, 32]
CCNU	++	+	[33, 34]
Methotrexate	-	+	[35–37]
5-Fluorouracil	-	++	[38, 39]
Adriamycin	-	++	[40, 41]
DTIC	++	-	[42]
Procarbazine	++	-	[43]
Treosulphan	+	++	[22]
Hexamethylmelamine	+	++	[23–26]
Pentamethylmelamine	+	?	-
N-Methylformamide	++	?	-

Criteria of activity: *M5076 sarcoma: ++ T.I. >2; + T.I. >1; - T.I. <1; †human ovarian carcinoma: ++ definite evidence of drug activity (overall response rate >25%); + evidence of drug activity but inadequate evaluation; ? evidence only of minimal activity after adequate evaluation (i.e. overall response rate <20%).

antitumour activity within these molecules. Thus the importance of both the formyl and the *N*-methyl moieties within the *N*-methylformamide molecule was recognised and the requirement of the *N*-methyl groups for activity within the analogues of hexamethylmelamine confirmed. However, whether this new tumour model is capable of indicating completely novel anti-neoplastic agents remains to be seen.

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