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Effect of AT1727 on Growth and Metastasis of Murine Tumours

XUE-TANG LI,* GILLIAN E. HUTCHINSON,† BARBARA A. PYM,† MAVIS FINCH† and KURT HELLMANN†‡

Department of Pharmacology, Cancer Institute (Hospital), Chinese Academy of Medical Sciences, Peking, China and †Cancer Chemotherapy Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Abstract—*AT1727 was tested on 5 different murine tumour systems. Compared with its analogue, razoxane, AT1727 was less effective at multiple low doses against sarcoma 180 and L1210 leukaemia. A single high-dose treatment with AT1727 was, however, more active than razoxane. AT1727 inhibited the growth of the Lewis lung primary tumour and significantly reduced the number of pulmonary metastases. Although AT1727 showed a slight inhibitory effect on the growth of the B16 primary, it had no effect on the metastases.*

INTRODUCTION

AT1727, bis(*N*-morpholinomethyl-piperazinedine 1,2-bis(3,5-dioxopiperazinyl)-ethane), is a new derivative of ICRF 154 and was developed in China in 1980 [1]. It had been assumed that very little alteration of the basic structure of ICRF 154 could be made without loss of antimitotic activity and that the intact dioxopiperazine ring was essential and could not be modified [2]. However, when the hydrogen in the imino groups of the dioxopiperazine ring of ICRF154 is substituted by morpholinomethyl, the active compound AT1727 (Fig. 1) results. This compound not only maintains antitumour activity, but also gives a higher chemotherapeutic index than ICRF154 against the sarcoma S37 [3]. Some ICRF154 analogues are potent antimetastatic compounds, but AT1727 does not as yet appear to have been investigated for this effect.

In the present studies, AT1727 was compared with razoxane, an analogue of ICRF154, on five murine tumours. Four of the systems involve blood or lymphatic spread. Different treatment schedules were used since *in vitro* assays showed that there were some differences of the cytotoxic stability and blood clearance patterns between AT1727 and razoxane [4].

MATERIALS AND METHODS

Animals and tumours

Tumours used in these experiments are set out in Table 1. In the experiments with the L1210

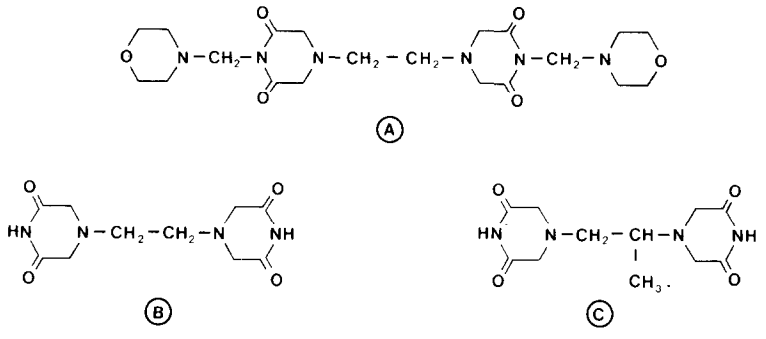

Fig. 1. Structures of AT1727 (A), ICRF154 (B) and razoxane (C).

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‡To whom reprint requests should be addressed.

Table 1. Animals and tumours

Tumour	Mouse strain	Sex
Sarcoma S180	Swiss Schneider	Male
Leukaemia L1210	BDF1	Male
Lymphoma TLC5	CBA	Female
Melanoma B16	C57/BL	Female
Lewis lung carcinoma 3LL	C57/BL	Female

leukaemia 5×10^4 to 5×10^5 cells were injected subcutaneously into the flanks of the recipient mice and for the TLC5 lymphoma 8×10^6 cells were similarly injected. The tumours for other systems were homogenised by forcing them through a syringe before subcutaneous inoculation into the flanks of the animals. No less than 8 mice were randomly allocated to each group, fed with standard laboratory diet and allowed to drink tap water *ad libitum*.

Drugs

AT1727 was kindly donated by Professor Y. F. Ren, Institute of Materia Medica, Shanghai, China, and razoxane was obtained from Imperial Chemical Industries Ltd., Macclesfield, U.K. Both drugs were made up as suspensions in 0.5% carboxymethylcellulose in isotonic saline and were usually injected intraperitoneally unless otherwise stated.

Evaluation of effects

The effects of AT1727 and razoxane on L1210 and TLC5 were evaluated by comparing mean survival times (MST) and were expressed as MST of treated mice/MST of control mice × 100 (T/C). A T/C of less than 125 is considered inactive.

S180 solid tumours were excised and weighed on the day following the last injection. The results were expressed as the ratio of the mean weight of treated tumours to the mean weight of control tumours (T/C). The 3LL mice were killed on day 21, the primary tumours were excised and weighed and the T/C calculated. The lungs of 3LL-bearing mice were filled with Indian ink and fixed in Fekete's solution. The total number of macroscopically visible colonies on the lung surface were counted and the T/C ratio calculated. In the case of B16 tumours, mice were treated with AT1727 before and/or after surgical removal of the primary on day 13.

The effect of AT1727 on B16 metastasis was evaluated by comparing the MST of the control and treated groups which had had their primary tumours excised. Each mouse that died was examined to ascertain whether the cause of death was due to the spread of the melanoma; if not it was excluded from the results.

RESULTS

Sarcoma 180

The S180 is sensitive to both AT1727 and razoxane (Table 2). When a dose of razoxane as low as 5–6 mg/kg (daily) was administered either parenterally or orally, a significant inhibition of tumour growth resulted (T/C = 0.47 i.p.; 0.35 p.o.), but the same dose of AT1727 seemed not to be as effective as razoxane (T/C = 0.64 i.p.; 0.64 p.o.) (Table 2).

Leukaemia L1210

The effects of AT1727 and razoxane against the L1210 were compared on different treatment schedules. These results are illustrated in Table 3. Both AT1727 and razoxane were effective in increasing the MST of L1210-bearing mice. At relatively low multiple doses (30 mg/kg × 4) razoxane appeared to be slightly more active than AT1727 in two experiments (T/C = 117–140 and 110–113 respectively). When the dose was increased to 60 mg/kg × 4, no difference between razoxane and AT1727 was found (T/C = 134 and 139 respectively). However, when treatment was by single i.p. injection of 100 mg/kg one day after tumour implantation, AT1727 yielded a greater increase in survival time than did razoxane (T/C = 131 and 121, respectively). This trend became more significant when the effects of AT1727 and razoxane were compared at a single dose of 150 mg/kg (T/C = 146 and 130 respectively, $P < 0.05$). This result was confirmed in a repeat experiment (T/C = 153 and 114 respectively, $P < 0.001$). In this experiment 150 mg/kg

Table 2. Effect of AT1727 and razoxane on the sarcoma S180

Drug	Dose (mg/kg/day)	No. of Doses	Route	Mean tumour weight (g)	T/C
AT1727	5	6	i.p.	0.90	0.64
Razoxane	5	6	i.p.	0.67	0.47
Control	—	—	—	1.42	—
AT1727	6	5	p.o.	0.64	0.64
Razoxane	6	5	p.o.	0.35	0.35
Control	—	—	—	1.00	—

Table 3. Effects of AT1727 and razoxane on the survival time of leukaemia L1210 and lymphoma TLC5 bearing mice

Tumour	Treatment schedule	T/C AT1727	T/C Razoxane
Leukaemia L1210	30 mg/kg i.p., days 1-3,6	113	140
	30 mg/kg i.p., days 1-3,6	110	117
	60 mg/kg i.p., days 1-3,6	139	134
	100 mg/kg i.p., day 1	131	121
	150 mg/kg i.p., day 1	146	130
	150 mg/kg i.p., day 1	153	114
Lymphoma TLC5	100 mg/kg i.p., day 1	124	109
	150 mg/kg i.p., day 1	125	114

AT1727 was slightly toxic. Animals that died of toxicity were excluded from MST calculations.

Another treatment schedule was designed as shown in Table 4, i.e. a one- or two-dose treatment was given on different days after tumour cell inoculation. In all cases a single dose of razoxane was inactive. The active single dose for AT1727 was 100 mg/kg or above. When treatment was delayed until day 3 no activity could be obtained. When two doses were used, 50 mg/kg of AT1727 significantly increased the mean survival time of L1210-bearing mice, while razoxane was inactive (T/C = 128 and 114 respectively). When the dose was increased to 100 mg/kg and 150 mg/kg, AT1727 further increased the mean survival time (T/C = 143 and 166 respectively), but the effects of razoxane at the same schedules were much less than those of AT1727 (T/C = 125 and 134 respectively). With these treatment schedules the differences between the effects of the drugs were statistically significant ($P < 0.01$ and < 0.02 respectively).

Table 4. Effects of AT1727 and razoxane on the survival time of leukaemia L1210 bearing mice

Dose (mg/kg)	Injected on day:	T/C AT1727	T/C Razoxane
50	1	108	100
	2	114	101
	3	101	102
	1 and 2	128	114
100	1	130	115
	2	124	112
	3	114	108
	1 and 2	143	125
150	1	131	117
	2	132	119
	3	112	111
	1 and 2	166	134

Lymphoma TLC

The effects of AT1727 and razoxane on the TLC5 are shown in Table 3. When tumour-bearing mice were treated with a single dose of 100 mg/kg on day 1, AT1727 increased the MST (T/C = 124, $P < 0.01$), while razoxane was ineffective (T/C = 109). A dose of 150 mg/kg of AT1727 also appeared to be slightly more effective than the same dose of razoxane (T/C = 125, $P < 0.02$ and 114 respectively).

B16 melanoma

This melanoma was implanted subcutaneously on day 0 into 3 groups of 10 mice and on day 13 all primary tumours were surgically excised. One group was given AT1727 25 mg/kg/day from the 3rd day after implantation. Another group received AT1727 in the same dosage only after excision of the primaries on day 13, whilst yet another group received no drug treatment. AT1727 did not protect animals from death caused by metastases (Table 5). When treatment was given before excision, the growth of the primary B16 melanoma was inhibited by about 30% (T/C = 0.71). This inhibition was statistically significant ($P < 0.01$), but the T/C value is not sufficient to allow the drug to be classed as active against this tumour. When a dose of 20 mg/kg/day was used, razoxane showed no effect on the B16 primary tumour in two experiments. However, when animals were continuously treated with razoxane before and after excision of the primaries the mean survival time and the number of 60-day survivors were increased compared with controls (Tables 5 and 6).

Although the experiments with AT1727 and razoxane were done separately and are therefore not comparable, AT1727 had no effect on the MST, which was determined almost entirely by the growth of metastases, but had a slight inhibitory effect on the primary tumours, while razoxane gave the opposite result.

*Table 5. Effect of AT1727 and razoxane on the survival time of mice after removal of primary B16 melanoma**

Treatment†	T/C AT1727	T/C Razoxane	60-Day survival‡ AT1727	60-Day survival‡ Razoxane
Post-operative	98	113	3/10	1/7
Pre-operative	—	120	—	4/11
Pre- and post-operative	93	136	5/10	5/11

*Primary tumours were surgically removed on day 13.
†A daily i.p. dose of AT1727 and razoxane was 25 and 20 mg/kg respectively.
‡The control goup had 4 and 0 long-term survivors in the experiments with AT1727 and razoxane respectively.

Table 6. Effects of AT1727 and razoxane on the primary B16 melanoma

Drug	Dose (mg/kg/day)	No. of doses	Mean tumour weight (g)	T/C
AT1727	25	8	0.90	0.71
Control	—	—	1.27	
Razoxane	20	8	1.78	0.77
Control	—	—	2.31	
Razoxane	20	8	1.45	0.77
Control	—	—	1.89	

3LL carcinoma

Table 7 summarizes 3 experiments in which the effect of AT1727 on the 3LL primary tumour and pulmonary metastases was examined. The effect of AT1727 on both primaries and secondaries was dose-dependent. The response of the secondaries to the drug was greater than that of the primaries. After treatment with a low dose of 10 mg/kg (i.p. × 13), the mean weight of the primary tumour was only slightly reduced (T/C = 0.82), but the formation of lung metastases was significantly inhibited (T/C = 0.35).

A dose of 15–20 mg/kg (i.p. × 14) further decreased the number of lung metastases to a T/C ratio of 0.07–0.08, while the growth of the primary tumour was only mildly inhibited (T/C = 0.66–0.70). At a higher dose of 60 mg/kg (i.p. × 13) AT1727 greatly reduced the primary tumour weight of the 3LL (T/C = 0.34) and gave complete inhibition of lung metastases (T/C = 0). Although there was no overall change in weight of the groups of mice, the relative weight when allowing for the increase of the tumours and that of similar mice without tumours indicates that there was a weight reduction at this dose.

Table 7. Effect of AT1727 and razoxane on the Lewis lung primary tumour and pulmonary secondaries

Drug	Dose (mg/kg/day)	No. of doses	T/C 1°	T/C 2°
AT1727	10	13	0.82	0.35
	15	14*	0.66	0.08
	20	14*	0.70	0.07
	60	13	0.34	0
Razoxane	2.5	11	1.04	0.18
	5	10	0.90	n.d.
	5	11	0.72	0.07
	10	10	0.95	n.d.
	20	10	0.98	n.d.
	30	10	1.14	0
		10	0.91	0
	40	10	0.98	n.d.
	50	10	0.67	0
		10	0.36	0
	80†	10	0.90	n.d.

Daily treatment (i.p.) was started from day 1 or day 3(*) and evaluation of the drug activity was usually performed on day 21. † = toxic; n.d. = not done.

The results for razoxane listed in Table 7 were obtained from several separate experiments in which the number of doses was 10–11. The results therefore are not completely comparable with those of AT1727. However, it seems that razoxane preferentially inhibits the development of lung metastases rather than the growth of the 3LL primary tumour. Of these 11 experiments using a wide range of doses, only one with a dose of 50 mg/kg/day showed marked inhibition of the primary tumour, while a dose of razoxane as low as 2.5 mg/kg/day considerably reduced the number of secondaries to a T/C ratio of 0.18. At a dose of 30 mg/kg/day or above, secondaries were completely inhibited by the drug.

DISCUSSION

Of the antimitotic bisdioxopiperazines, razoxane has been most widely studied. Its effects against various animal and human tumours have been described [5, 6]. In attempts to find more soluble analogues with greater activity, a number of bisdioxopiperazine compounds have been synthesized and this has recently culminated in the successful synthesis of AT1727 in China. Confirmation of the antitumour effect of AT1727 has shown that the assumption that the dioxopiperazine ring cannot be modified without

loss of activity is invalid. This would also imply that this series of antitumour agents can be further developed.

The *in vitro* results showed that both AT1727 and razoxane had the same mode of action in the cell cycle [4], but in the present *in vivo* studies, two differences between the effects of AT1727 and razoxane were found: (a) at low multiple doses AT1727 was less effective than razoxane on the S180 and the L1210. However, when a single high dose was used, AT1727 was more active than razoxane. When all results with a single dose of 100 and 150 mg/kg on the L1210 and the TLC5 were added, AT1727 was active (T/C $\geqslant$ 125) in 7/11 experiments, while razoxane was active in only 1/11. This advantage of AT1727 might be partly due to its lower rate of degradation and metabolism [4]; (b) when the two drugs were compared on two blood-borne metastasizing tumour systems, the B16 and 3LL, razoxane seemed to be more active in preventing the formation of secondaries than against the primary tumour, while AT1727 showed more effect on the primary tumour. However, at a dose which did not influence the growth of the primary 3LL, AT1727 also considerably reduced the number of lung metastases. Whether AT1727 will also show the other biological effects that have been discovered for razoxane, including the angiometamorphic effect, is still unknown.

The effects of AT1727 and razoxane in these experiments were compared on a weight-for-weight basis. However, since AT1727 has a higher molecular weight (452) than razoxane (268), a more realistic comparison would be on an equimolar basis. When the effects are recalculated on this basis, AT1727 seems to be more active than razoxane in almost all experiments.

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REFERENCES

1. ZHANG T, CHEN Z, LIN C. Pharmacological studies on bimolane (AT-1727), a new antineoplastic agent. *Acta Pharm Sin* 1980, **15**, 577–583.
2. CREIGHTON AM, JEFFERY WA, LONG J. Bisdioxopiperazines. In: SIMKINS MA, ed. *Medical Chemistry VI: Proceedings of the 6th International Symposium on Medicinal Chemistry*. Cotswold Press, Warrandyte, 1979, 281–288.
3. ZHANG T, CHEN Z, LIN C. Kangai xinyao yishuangmalin (AT-1727) yu yiyaan, bingyaan de zuoyong bijiao. *Henan Yixueyuan Xuebao* 1980, **15**, 145–150.
4. LI X-T, HELLMANN K. Comparative studies on the cytotoxicity, stability and blood clearance of AT1727 and razoxane. In preparation.
5. BAKOWSKI MT. ICRF 159, (±),1,2-di(3,5-dioxopiperazin-1-yl)-propane, NSC-129,943; razoxane. *Cancer Treat Rev* 1976, **3**, 95–107.
6. BELLET RE, ROZENCWEIG M, VON HOFF DD, PENTA JS, WASSERMAN TH, MUGGIA FM. ICRF-159: current status and clinical prospects. *Eur J Cancer* 1977, **13**, 1293–1298.