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Schedule Dependence of Activity of the Amsacrine Analogue CI-921 towards P388 Leukaemia and Lewis Lung Carcinoma*

BRUCE C. BAGULEY,† CHERRY D. GRIMWADE and ANTOINETTE R. KERNOHAN
Cancer Research Laboratory, University of Auckland School of Medicine, Auckland, New Zealand

Abstract—*The 4-methyl-5-(N-methyl)carboxamide derivative (CI-921; NSC 343499) of the clinical antileukaemia agent amsacrine is highly active towards P388 leukaemia and Lewis lung carcinoma in mice. When administered intraperitoneally at the optimal schedule and dose, CI-921 provided 5/6 50-day survivors in leukaemic mice and 10/11 60-day survivors in mice previously inoculated intravenously with Lewis lung cells. An intermittent (every 4 days × 3) schedule was superior to single dose, daily × 5 or daily × 9 schedules. Although intraperitoneal dosage was superior to intravenous or oral dosage for the treatment of intraperitoneally inoculated P388 leukaemia, all three routes of administration provided similar results with intravenously inoculated Lewis lung or subcutaneously implanted P388 cells. Daily intraperitoneal dosage schedules provided sharper dose-response relationships than intermittent schedules, and with daily schedules 1.5-fold rather than 2-fold dose increments were necessary for reliable detection of activity against Lewis lung carcinoma.*

INTRODUCTION

AMSACRINE is a derivative of 9-aminoacridine which was first synthesized and found to have experimental antileukaemic activity by Cain and Atwell [1]. The drug was developed for clinical trial by the National Cancer Institute (NCI), U.S.A., and was found to have clinical activity in early trials [2]. Subsequent phase II trials have indicated that although activity is restricted to acute leukaemias and possibly lymphomas, amsacrine, particularly in combination with other drugs, is a useful clinical agent [3]. Over the past few years, our laboratory has been concerned with the development of analogues of amsacrine with a wider spectrum of clinical antitumor activity. The Lewis lung carcinoma, an experimental tumour which is relatively resistant to many clinical and experimental drugs including amsacrine, has been used to seek such agents [4]. The most active analogue identified so far, the 4-methyl-5-(*N*-methyl)-carboxamide derivative of amsacrine (CI-921; NSC 343499; structure in Fig. 1) has now shown high activity in a number of antitumour systems [5, 6] as well as providing good activity towards human carcinoma cell lines [5]. Like amsacrine, CI-921 binds to DNA by intercalation, and arrests cycling cells in the G_2 phase of the cell cycle [5]. CI-921 is now a candidate drug for clinical trial.

In the course of early testing of CI-921, a discrepancy was found between the Lewis lung carcinoma testing results of the NCI and our own laboratory. Since a number of differences existed in protocol, we have conducted a schedule- and route-dependence study on the activity of this drug.

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†To whom requests for reprints should be addressed at: University of Auckland Medical School, Private Bag, Auckland, New Zealand.

MATERIALS AND METHODS

Materials

The amsacrine analogue CI-921 was either synthesized as described previously [5] or obtained from the Warner-Lambert Company as the isethionate salt. It was injected intraperitoneally

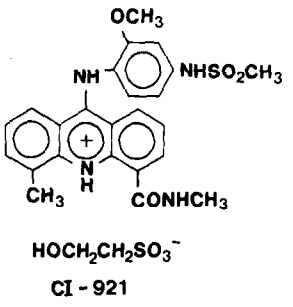

Fig. 1. Structure of CI-921.

(i.p.) or intravenously (i.v.) as a solution (0.1 ml) in 5% dextrose-water, and was administered orally (p.o.) by stomach tube (without prior fasting) as a solution (0.1 ml) in water.

Tumours

P388 leukaemia and Lewis lung carcinoma cells were provided as frozen stocks in 1977 by the Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. After passage in mice of the appropriate strain, they were stored in liquid nitrogen. The P388 leukaemia was reinitiated from frozen stocks every 12 months and the Lewis lung tumour every 4 months. P388 cells were passaged weekly (i.p.) in DBA/2J mice. Cells were removed from carriers by peritoneal washing with phosphate-buffered saline (NaCl 8 g/l, KCl 0.2 g/l, KH_2PO_4 0.2 g/l, Na_2HPO_4 1.15 g/l, $CaCl_2$ 0.1 g/l, $MgCl_2$ 0.1 g/l). Carrier mice were inoculated i.p. with 10^6 cells in the same buffer. Lewis lung cells were passaged every 2 weeks as subcutaneous tumours in C57BL/6J mice. Minced tumours from carrier mice were passed through a 100 μm nylon monofilament mesh to prepare a single cell suspension. Cells were passed through a 26G needle and large nucleated cells were counted in 1% acetic acid with a haemocytometer. Cells (10^5 or 10^6) were inoculated s.c. in carrier mice.

Life extension assays

F_1 hybrid mice (DBA/2J male × C57BL/6J female) were bred under conditions of constant temperature and humidity, with sterile bedding, water and food. Each experiment consisted of a control group of 20–30 mice and a series of experimental groups with six mice. Groups were of either sex. Drug doses were calculated on the basis of mouse weight at the time of tumour inoculation, and groups were matched for body weight at intervals of 1 g body wt. Dose levels were set at 1.5-fold intervals. Deaths were recorded daily and experiments were evaluated on day 50 (P388) or 60 (Lewis lung). Mice surviving at this time were excluded from calculation and recorded separately. The mean survival time was calculated for control and treated groups. The ratio of mean survival times of treated and control groups was calculated and the percentage increase in lifespan defined as 100 × (ratio - 1.0).

RESULTS

Schedule-dependence for P388 leukaemia

F_1 hybrid mice inoculated i.p. with 10^6 P388 cells on day 0 were treated i.p. with CI-921 at single dose (day 1), intermittent dose (days 1, 5, 9) or daily (days 1–5 or days 1–9) schedules. The range of mean survival times in control animals was 10.1–12.8 days, with a mean of 11.5 days and a mean coefficient of variation of 14%. All schedules provided increases in lifespan of treated animals at the optimal dose of at least 90% (Fig. 2). Higher doses than those shown in Fig. 2 were toxic. Significant extension of lifespan was found over an approximately 10-fold dose range. The intermittent schedule was the most effective and at the optimal dose (30 mg/kg) 5/6 mice survived 50 days. Long-term survivors were also observed at the optimal dose using single dose (2/6 survivors), days 1–5 (1/6) and days 1–9 (1/6) schedules. The single dose administration provided a rather flat response curve, with no significant difference in ILS values between 8.9 and 45 mg/kg. Higher doses were toxic. In separate experiments amsacrine on an intermittent schedule provided a mean increase in lifespan of 78% [4] and on a single dose schedule, 39%.

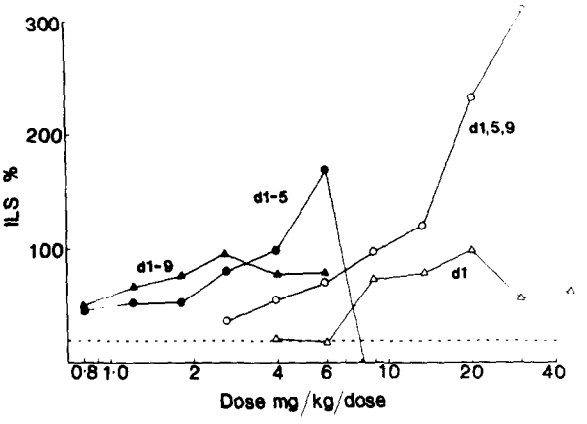

Fig. 2. Dependence of increase in lifespan (ILS) on drug administration schedule. Mice were inoculated i.p. with 10^6 P388 leukaemia cells on day 0. CI-921 was injected i.p. on day 1 (Δ), days 1, 5, 9 (○), days 1–5 (●) or days 1–9 (△).

Schedule-dependence with Lewis lung carcinoma

A similar experiment was carried out with the Lewis lung carcinoma, which had been inoculated i.v. (10^6 cells) on day 0. The range of mean survival times in control animals was 16.6–18.8 days, with a mean of 17.2 days and a mean coefficient of variation of 17%. All schedules provided

significant increases in lifespan at the optimal dose (Fig. 3), but there were clear differences in the efficacy of the different schedules. The intermittent schedule provided the highest increase in lifespan (228%), and at the optimal dose provided 10/11 survivors at day 60. Good activity was observed over three dose levels (13.3, 20 and 30 mg/kg). In contrast, the other schedules provided activity only at one dose level. Long-term (60 day) survivors were observed at the optimal dose with the single dose (1/6), the days 1-5 (3/11) and the days 1-9 (2/11) schedules. The single dose administration provided only borderline activity at the three doses tested.

In separate experiments amsacrine at the intermittent dosage schedule provided a mean increase in lifespan of 38% [4]. Single dose administration gave a value of 10%.

Route dependence of antitumour activity

In order to determine whether the difference in breadth of dose profile between P388 and Lewis lung tumours was a result of a proximity effect with P388 cells (i.e. i.p. injection of both drug and tumour cells), the activity of CI-921 was determined following i.v. or p.o. administration (Fig. 4). Activity against P388 leukaemia was

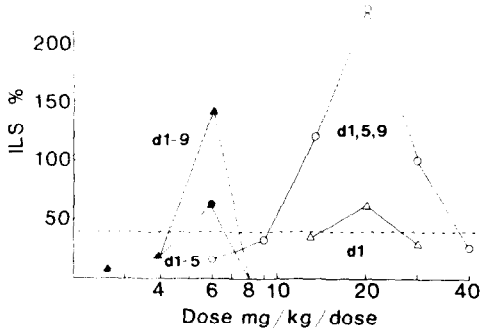

Fig. 3. Dependence on increase in lifespan on drug administration schedule. Mice were inoculated i.v. with 10^6 Lewis lung carcinoma cells on day 0. CI-921 was injected i.p. on day 1 (△), days 1, 5, 9 (○), days 1-5 (●) or days 1-9 (△).

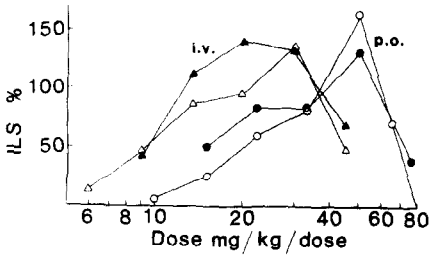

Fig. 4. Dependence of increase in lifespan on drug administration route. Mice were inoculated with 10^6 P388 cells (i.p., open symbols) or 10^6 Lewis lung cells (i.v., closed symbols) on day 0. CI-921 was injected i.v. (triangles) or orally (circles) on days 1, 5 and 9.

Table 1. Summary of results

Schedule	P388 Leukaemia R i.p./i.p.* TD† (mg/kg)	ILSm (%)	i.v./i.p. TD (mg/kg)	ILSm (%)	p.o./i.p. TD (mg/kg)	ILSm (%)	i.p./s.c. TD (mg/kg)	ILSm (%)	Lewis lung carcinoma i.p./i.v. TD (mg/kg)	ILSm (%)	i.v./i.v. TD (mg/kg)	ILSm (%)	p.o./i.v. OD (mg/kg)	ILSm (%)
Day 1	20	102					45	21	20	61(1/6)†				
Days 1-5	29.5	169(4/6)‡					29.5	29	29.5	68(1/12)				
Days 1-9	35	99(2/6)					53	36	66	142(3/12)				
Days 1, 5, 9	90	210(5/6)	90	136(1/6)	150	65	90	48	60	228(10/11)	60	134(9/12)	150	182

*Route of drug/route of tumour.
†TD: optimal total dose; ILSm: percentage increase in lifespan at optimal dose.
‡No. of long-term survivors/total in group are presented in parentheses.

considerably attenuated via either route (maximal life extension 136% with 1/6 survivors for i.v. dosage; 165% with no long-term survivors for p.o. administration) and the range of doses with significant activity was reduced. Although the dose potency of oral administration was lower, the therapeutic efficiency was very similar to that obtained with i.v. administration. In both cases the breadth of the dose profile was reduced in comparison to that obtained with i.p. dosage.

The corresponding results with the Lewis lung tumour were remarkably similar to those with P388 leukaemia (Fig. 4). Drug was administered on days 5, 9 and 13, and provided good activity following i.v. administration (maximal life extension 134%; 9/12 60-day survivors) and p.o. administration (maximal life extension 132%; no survivors). These results are similar to those previously published [5] for i.p. administration (maximal life extension 167%; 9/21 60-day survivors).

The effects of CI-921 on subcutaneously inoculated P388

As a further method for separating the sites of tumour and drug administration, P388 cells were inoculated s.c. and drug administered i.p. by various schedules. Control mice with advanced subcutaneous tumours died after 15.8 days (average coefficient of variation over five experiments 9.8%). CI-921, when administered i.p., was only moderately active, but the intermittent schedule (days 1, 5, 9) was clearly superior to either the single dose or daily schedules (Fig. 5). Administration of drug i.v. was also employed with the intermittent schedule and gave similar results (Fig. 5). There were no long-term survivors. Although four dose levels were found to be active using the intermittent schedules, only one or two dose levels showed significant activity on the other schedules.

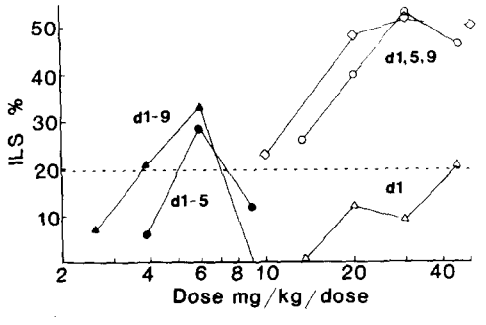

Fig. 5. Effects of CI-921 on s.c. inoculated P388 leukaemia. 10^6 cells were inoculated on day 0. CI-921 was administered i.p. on day 1 (Δ), days 1, 5, 9 (○), days 1-5 (●) or days 1-9 (▲). It was also administered i.v. on days 1, 5 and 9 (◇).

DISCUSSION

These results demonstrate the exceptionally high activity of the amsacrine analogue CI-921 towards both P388 leukaemia and Lewis lung carcinoma. Activity using dextrose-water as the drug solvent is similar to that using aqueous ethanol [5]. Both tumours used in these experiments are from the same source as those used in published results of the NCI [7]. Our studies indicate that the Lewis lung tumour is highly responsive to cyclophosphamide and CI-921, moderately responsive to 5-fluorouracil and teniposide, marginally responsive to amsacrine, methotrexate and etoposide, and unresponsive to daunorubicin, adriamycin and mitoxantrone ([4] and unpublished results).

For intermittent administration schedules, pronounced route-dependence of activity was observed for i.p. inoculated P388 cells but not for i.v. injected Lewis lung cells. The superiority of i.p. drug administration over i.v. or p.o. administration against i.p. inoculated P388 cells is almost certainly due to the high local drug concentrations produced when both tumour and drug have the same inoculation site.

When P388 cells are injected s.c. (Fig. 4) the difference between the efficacy of i.p. and i.v. drug administration (intermittent schedule) is negligible. However, the antitumour effect of i.v. administered CI-921 on s.c. tumour is diminished when compared to its effect on i.p. tumour. Since s.c. inoculated P388 cells take longer to kill control mice (15.8 days) than i.p. inoculated cells (11.3 days) it may be better to compare log cell kill, which is based on growth delay (and assumes that the doubling time of early tumour is the same at both sites). The maximal log cell kill for s.c. inoculated tumour is 5.1, considerably lower than that (9.7) for i.p. inoculated tumour. This result is consistent with the hypothesis that drug distribution to the s.c. inoculation site is inferior to that to the peritoneum.

For Lewis lung tumours, i.p. inoculated P388 cells and s.c. inoculated P388 cells an intermittent (i.p.) administration schedule was clearly superior to single dose or daily schedules. The most likely explanation for this behaviour is that the intermittent schedule allows a higher total dose to be administered. The total optimal doses administered (summarized in Table 1) are in general greatest for intermittent schedules. This implies that host toxicity is greater for daily schedules than for the intermittent schedule. Thus, for P388 leukaemia the optimal total dose for the days 1, 5, 9 schedule is 4-5 times that for a single dose, 3 times that for the days 1-5 schedule and 2.6 times that of the days 1-9 schedule. It is the

sensitivity of dose-limiting normal tissues rather than the tumour itself which would therefore appear to impose the pronounced schedule dependence of CI-921.

It is difficult to compare the results of CI-921 with previously published data for amsacrine, because of the lower activity of amsacrine towards P388 leukaemia [5] and Lewis lung carcinoma [4, 5, 8]. Cain and Atwell [1] have reported a schedule dependence study for amsacrine using L1210 leukaemia. Daily and intermittent schedules were of similar efficacy. Greco *et al.* [8] showed that fractionated doses of amsacrine (daily or intermittent schedules) had similar (minimal) antitumour effects but lower host toxicity when compared to single doses.

The results indicate a potential problem in the screening of new drugs using a single administration schedule. If CI-921 is tested using a daily schedule (Fig. 3), activity against the Lewis lung carcinoma is critically dependent on the drug dose over a narrow range. If drugs are administered at 2-fold dose intervals, the activity could be missed. Our results have been confirmed by the NCI, which has found negative results for the Lewis lung carcinoma in earlier daily schedules but has found results similar to our own using an intermittent schedule (J. Plowman, personal communication). Although the generality of this observation is not known, it is worth considering that DNA binding antitumour agents should be tested at some stage with an intermittent administration schedule.

Although it is difficult to extrapolate from mouse results to humans, these results should be kept in mind in designing schedules for clinical trials of CI-921. If, as in the case of amsacrine, bone marrow toxicity is dose limiting, optimal solid tumour activity may be provided by intensive treatment periods over 1–2 days alternating with periods which allow host tissue recovery.

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REFERENCES

1. Cain BF, Atwell GJ. The experimental antitumour properties of three congeners of the acridylmethanesulphonanilide (AMSA) series. *Eur J Cancer* 1974, **10**, 539–549.
2. Arlin ZA, Sklaroff RB, Gee TS *et al.* Phase I and II trial of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide in patients with acute leukemia. *Cancer Res* 1980, **40**, 3304–3306.
3. Baguley BC. Amsacrine: a new antileukaemic agent. *Drugs Today* 1984 **20**, 237–245.
4. Baguley BC, Kernohan AR, Wilson WR. Divergent activity of derivatives of amsacrine (*m*-AMSA) towards Lewis lung carcinoma and P388 leukaemia in mice. *Eur J Cancer Clin Oncol* 1983, **19**, 1607–1613.
5. Baguley BC, Denny WA, Atwell GJ *et al.* Synthesis, antitumour activity, and DNA binding properties of a new derivative of amsacrine, *N*-5-dimethyl-9[2-methoxy-4-methyl-sulfonylamino)phenylamino]-4-acridine carboxamide. *Cancer Res* 1984, **44**, 3245–3251.
6. Leopold WR, ShilliS JL, Corbett TH, Jackson RC. *Proc AACR* 1984, **25**, 294.
7. Goldin A, Venditti JM, MacDonald JS, Muggia FM, Henney JE, De Vita VT. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. *Eur J Cancer* 1981, **17**, 129–142.
8. Greco C, Zupi G, Badaracco G. Influence of scheduling on therapeutic and toxic effect of AMSA in Lewis lung carcinoma. *Cancer Chemother Pharmacol* 1983, **11**, 38–42.