Cancer Chemother and Pharmacol (1995) 36: 244–248

ORIGINAL ARTICLE

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Experimental solid tumour activity of *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide

Received: 24 September 1994/Accepted: 22 December 1994

Abstract *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA), a DNA intercalator that exerts its antitumour action through the enzyme topoisomerase II, has previously been shown to be curative against the transplantable Lewis lung adenocarcinoma growing as lung tumour nodules in mice. On the basis of this finding as well as its high in vitro activity against multidrug-resistant cell lines, DACA has been chosen for clinical trial under the auspices of the Cancer Research Campaign, United Kingdom. In the present study the activity of DACA was assessed against advanced (5-mm diameter) s.c. colon 38 adenocarcinomas in BDF_1 mice using tumour-growth delay as an end point. Its activity was found to be related positively to the total dose given and negatively to the total duration of the dose schedule. Adoption of a split-dose i.p. administration schedule or slow i.v. infusion allowed the administration of large doses without toxicity. The activity of DACA was comparable with that of 5-fluorouracil and superior to that of doxorubicin, cyclophosphamide and the experimental amsacrine analogue CI-921. Mitoxantrone, amsacrine, etoposide, teniposide and daunorubicin showed minimal activity. DACA also demonstrated significant activity against the NZM3 melanoma human cell line growing as a xenograft in athymic mice.

Key words Acridine · Antitumour activity · Intercalation · Colon 38 adenocarcinoma · Lewis lung adenocarcinoma

Introduction

Topoisomerases are the targets of a number of clinically important anticancer drugs, including doxorubicin and etoposide, as well as for a large number of experimental agents [6, 13]. *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA, see Fig. 1 for structure) is undergoing clinical trial under the auspices of the Cancer Research Campaign, United Kingdom. A DNA intercalator and topoisomerase II poison [18], DACA has curative activity against the transplantable Lewis lung adenocarcinoma grown as lung tumour nodules in mice and is much more active than doxorubicin and etoposide [1]. The maximum tolerated dose of DACA, particularly following i.v. administration, is limited by central nervous system effects [10, 17].

The Lewis lung tumour model, with drug treatment being initiated 5 days after i.v. tumour inoculation, differs from clinical tumours in that insufficient time is allowed for the tumour to establish its blood supply [19]. Such a tumour blood supply is disorganised and may provide an important barrier to drug diffusion. In the present study we used the advanced s.c. murine colon 38 tumour as a model for human tumours. We carried out a schedule dependence study of DACA and compared its activity with that of a number of antitumour agents. We also examined the activity of DACA against a human melanoma xenograft.

Materials and methods

Chemicals

DACA dihydrochloride was synthesised in the Auckland Cancer Research Laboratory [1] and was dissolved in either water or 30% (v/v) aqueous ethanol for i.p. injection and in water for i.v. injection or oral administration. Amsacrine isethionate and CI-921 isethionate (the 4-methyl-5-[*N*-methyl]-carboxamide derivative of amsacrine) [4] were provided by the Parke-Davis Division of Warner-Lambert Ltd. and were dissolved in 30% (v/v) aqueous ethanol for injection. Doxorubicin and daunorubicin (Farmitalia Carlo Erba), mitoxantrone (Lederle) and 5-fluorouracil (Roche) were dissolved in water and cyclophosphamide was dissolved in normal saline. Etoposide and teniposide (Bristol-Myers Squibb) were obtained as clinical formulations in solution and were used directly.

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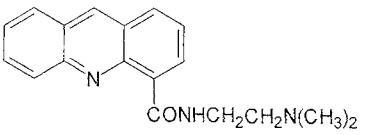

Fig. 1 Chemical structure of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) as the free base

Mice and tumours

C_3H/HeN and $(C_{57}Bl/6 \times DBA/2)F_1$ (BDF_1) mice were bred in the laboratory facility [5], whereas athymic C57Bl/6J-nu mice were supplied by the Animal Laboratories, University of Auckland School of Medicine. Breeding stocks were obtained from Jackson Laboratories (Bar Harbor, Me. USA). Colon 38 tumour stocks were obtained in 1981 from Mason Research Institute (Worcester, USA). NZM3 melanoma cells were grown from a clinical sample as described previously [14, 15].

Tumour growth

Experiments were conducted according to institutional animal ethical guidelines. Colon 38 tumour fragments (1 mm^3) were implanted s.c. in one flank of anaesthetised mice (pentobarbitone, 90 mg/kg). NZM3 melanoma cell suspensions (5×10^6 cells/flank) were implanted bilaterally in each flank using anaesthesia. Drug treatment was started when tumour diameters were 5–6 mm (8 or 9 days following colon 38 tumour implantation and 3 weeks after NZM3 implantation). Groups of five to six mice were used for control and drug-treated groups. Drug solutions were either injected i.p. or infused i.v. with an infusion pump. Tumour diameters were measured with callipers three times weekly and volumes were calculated as $0.52 \times a^2 \times b$, where a and b were the minor and major tumour axes. Mean logarithmic tumour volumes were calculated as differences from the pretreatment volumes. The time required for tumours to reach a volume 4-fold their pretreatment volume was recorded, and tumour-growth delays were described as the difference in the corresponding times of treated and control mice. Statistics were calculated using SigmaStat (Jandel Scientific, USA)

Results

Schedule dependence of the activity of DACA against colon 38 tumours

Groups of mice with s.c. colon 38 tumours (5- to 6-mm diameter) were treated with DACA using an administration schedule (3 i.p. doses of 100 mg/kg given at 4-day intervals) similar to that used for P388 leukaemia and Lewis lung carcinoma [3]. Since only moderate activity was observed (5-day growth delay), a schedule dependence study was undertaken. When DACA was given as a single i.p. dose at the maximum tolerated dose (150 mg/kg), temporary sedation of the mice occurred and a tumour-growth delay of 4 days was found. Unexpectedly, when a dose of 200 mg/kg was given as two doses of 100 mg/kg separated by a time of 1 h, little sedation of mice was evident and a growth delay of 8.8 ± 1.1 days (SEM, $n = 6$ experiments) was obtained (Fig. 2). When a dose of 100 mg/kg × 2 was administered with an interval of 24 h, the growth delay was

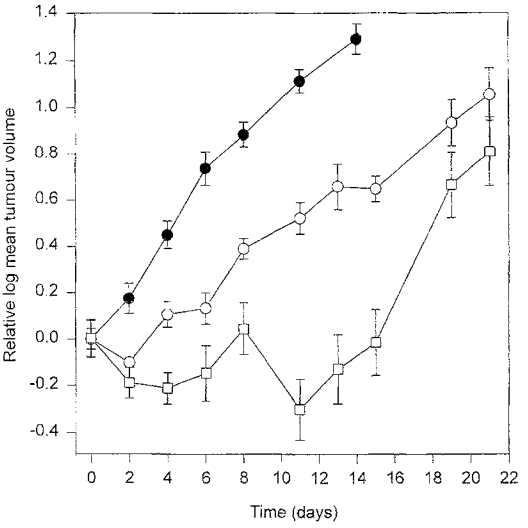

Fig. 2 Growth of colon 38 tumours either untreated (●) or treated with DACA in a split-dose administration schedule of 100 mg/kg i.p. × 2 with an interval of 1 h (○) or 100 mg/kg with the schedule repeated after 7 days (□). *Vertical bars* represent the standard errors of the logarithmic mean tumour volumes

5 ± 1 days. The administration of two courses of 2×100 mg/kg (each dose given 1 h apart) provided growth delays of 14 and 11 days in two experiments. One example is shown in Fig. 2. The addition of a third course of 2×100 mg/kg (12 h apart) produced a longer growth delay (19 days) but at a cost of 1/16 delayed toxic deaths. Similarly, a schedule of 65 mg/kg (loading dose) followed at 30-min intervals by maintenance doses (3×45 mg/kg) provided a 7-day growth delay, and when the same dose was given in three courses at 7-day intervals a growth delay of 22 days was found at a cost of 1/6 (delayed) toxic deaths.

A number of single- and repeated-dose-schedule experiments over periods ranging from 2 to 96 h were carried out. The data for 28 growth delay experiments could be fitted by a regression equation expressing tumour-growth delay (in days) with a positive term for the total dose (in milligrams per kilogram) and a negative term for the total duration of treatment (in days):

$$(\text{growth delay}) = (0.065 \pm 0.007) \times (\text{total dose}) - (1.03 \pm 0.19) \times (\text{dose duration}) - 5.5.$$

The correlation coefficient was 0.92, with both of the variables entering at a significance level of $P < 0.0001$, and the standard error of the estimate was ± 1.9. The equation is depicted as a surface in Fig. 3.

DACA also showed antitumour activity following i.v. administration. The maximum tolerated i.v. dose was 30 mg/kg when infused over 30 s and 100 mg/kg when infused over 2 min. When given over 8 min, an i.v. infusion of 200 mg/kg was tolerated and the growth delay obtained was 3 days, whereas a 2-h infusion provided a growth delay of 6 days (Fig. 4). Within experimental error, the measured growth delays fitted

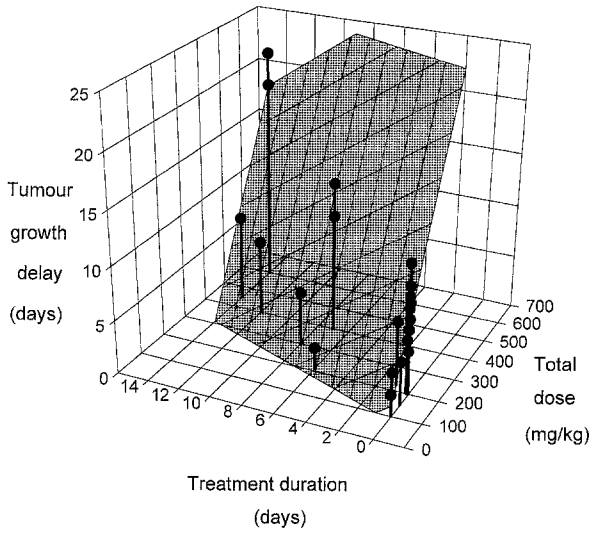

Fig. 3 Three-dimensional representation of the regression equation of a series of individual growth delay experiments (each represented by *one symbol*, projected to the X-Y plane by a *vertical bar*) incorporating single- and multiple-dose schedules and relating tumour-growth delay to a combination of the total dose delivered and the total duration of the administration schedule. The *shaded plane* represents the least-squares fit to the data ($r = 0.92$)

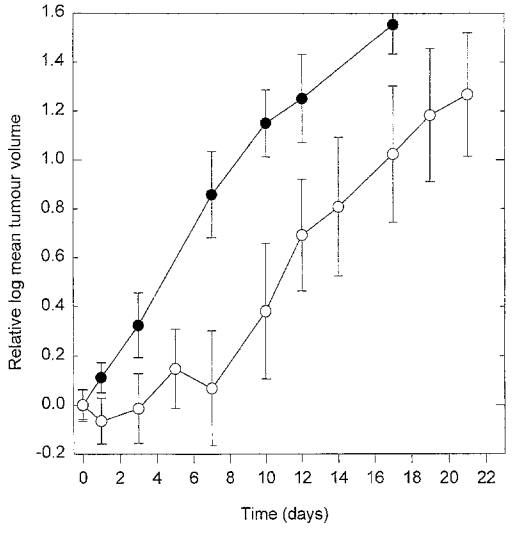

Fig. 4 Growth of colon 38 tumours either untreated (●) or treated with DACA by i.v. infusion (200 mg/kg) over 2 h (○). *Vertical bars* represent the standard errors of the logarithmic mean tumour volumes

well with the regression equation derived for i.p. administration. No activity was found following oral administration.

Comparison of the activity of DACA with that of other agents

DACA was compared with a number of other agents in the advanced colon 38 assay and the results are shown

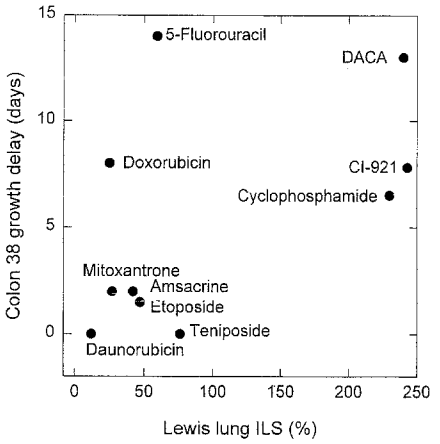

Fig. 5 Comparison of the antitumour activity of DACA and that of a number of other agents against advanced s.c. colon 38 tumours, as reported in this study, with published [1, 3, 4] and unpublished data for percentage of increase in life span (ILS) induced by the same agents against in inoculated. Lewis lung carcinoma. DACA, CI-921 and cyclophosphamide are curative in this tumour model; to facilitate comparison, life extensions have been calculated assuming that a cure corresponds to a 60-day survival

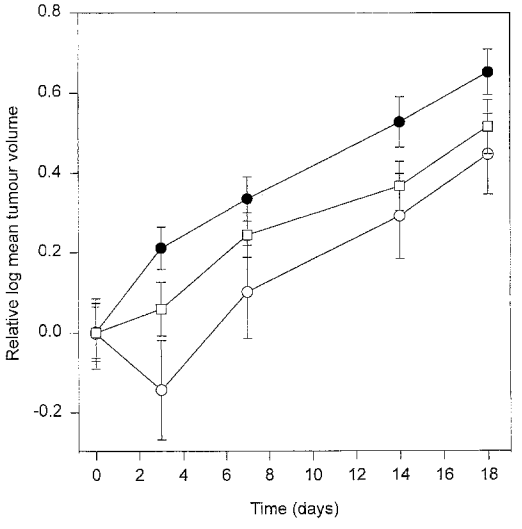

Fig. 6 Growth of NZM3 xenografts in athymic mice either untreated (●) or treated with DACA in a dose administration schedule of 100 mg/kg i.p. × 2 with an interval of 1 day (○) or in a schedule of 66 mg/kg × 3 at daily intervals (□). *Vertical bars* represent the standard errors of the logarithmic mean tumour volumes

in Fig. 5. 5-Fluorouracil (65 mg/kg given every 4 days × 3) had activity similar to that of DACA. The amsacrine analogue CI-921 (20 mg/kg given every 4 days × 3), doxorubicin (65 mg/kg given every 4 days × 3) and cyclophosphamide (220 mg/kg given as a single dose) appeared to be slightly less active than DACA. Amsacrine (13.3 mg/kg given every 4 days × 3), etoposide (45 mg/kg given every 4 days × 3), teniposide (13.3 mg/kg given every 4 days × 3), mitoxantrone (3.9 mg/kg given every 4 days × 3) and daunorubicin (3.9 mg/kg given every 4 days × 3) had little, if any, activity.

Activity of DACA against a melanoma xenograft

The NZM3 line developed in this laboratory [15] was grown as a xenograft and tested with three different administration schedules. Administration of DACA as two doses of 100 mg/kg given 1 h apart and repeated after 7 days (i.e. 400-mg/kg total dose) provided a growth delay of 14 days but produced several delayed deaths. Two doses of 100 mg/kg given 1 h apart provided a 5-day growth delay but also several delayed deaths. Two doses of 100 mg/kg given 24 h apart provided a growth delay of 6 days, whereas three doses of 67 mg/kg given at 24-h intervals provided a growth delay of 4 days (Fig. 6)

Discussion

The results show DACA to have high activity against advanced s.c. colon 38 tumours in mice when injected i.p. with an optimised dose schedule (Fig. 5). In this study we combined the results of a number of growth delay experiments into a single regression equation so as to identify administration schedules that provide high activity. The equation relates tumour-growth delay positively to the total dose given and negatively to the duration of the administration schedule (Fig. 3) and has a correlation coefficient of 0.92, indicating a good fit to the experimental data. The presence of a negative constant term (-5.5 days) indicates the presence of a "shoulder" (an inverted shoulder as depicted in the figure) on the dose-response curve, such that single doses of less than 65 mg/kg are ineffective. This shoulder increases (Fig. 3) as the duration over which the dose is delivered is extended (i.e. doses of 300 mg/kg given at intervals over 14 days would be ineffective), accounting for the negative term for duration of schedule in the regression equation.

It is noteworthy that the in vitro survival curves for DACA also have slight shoulders, and the size of the shoulders increase as drug exposure times are extended from several hours to 3 days [12]. Although the cause of the shoulders in the in vivo and in vitro dose-response curves is not known, they may be related to each other and could reflect the ability of cells to repair limited amounts of DACA-induced DNA damage. It is also possible that changes in cytokinetics occur in response to DACA and that these protect cells to some extent from subsequent cytotoxicity. The in vitro dose-response curve for CI-921 does not have a shoulder [7], and preliminary experiments indicate that an intermittent (every 4 days $\times$ 3) administration schedule is superior to a single (split)-dose schedule (results not shown).

The implication of the data shown in Fig. 3 is that a high in vivo dose rate is necessary to counteract the dose "shoulder" effect for DACA mentioned above. Working against this requirement is the acute toxicity of DACA. The i.p. dose of DACA in mice is limited to 150 mg/kg (730 μmol/kg) by sedation rather than by haematological toxicity [10, 11], and following i.v. administration, central nervous system toxicity limits the dose to 40 mg/kg, with clonic seizures occurring at higher doses [16]. A high dose rate of DACA, which is highly lipophilic [1], has been reported to cause a rapid increase in the concentration of DACA in the brain [9]. This is likely to be the cause of the acute toxicity. The present results show that when the dose rate of DACA is reduced by use of an i.p. split-dose schedule or by i.v. infusion over periods greater than 15 min, the maximum tolerated total dose increases to 200 mg/kg. Therefore, this split-dose schedule has the advantage of reducing host toxicity, presumably by reducing both the maximal concentrations and the dose rate of DACA in the brain, while delivering drug over an interval that is short enough to satisfy the requirements of the regression equation illustrated in Fig. 3.

Preliminary work using a human melanoma xenograft in athymic mice has indicated significant activity of DACA against advanced s.c. tumours (Fig. 6). DACA toxicity was evident in this model when a split dose of 200 mg/kg was given on day 1, but this probably reflects the greater sensitivity of athymic mice to chemotherapy. No toxicity was evident with the 2- or 3-day schedules tested, and the growth delays obtained (6 and 4 days, respectively) were close to those predicted by the regression equation (6.4 and 5.4 days, respectively). However, it cannot be assumed that the regression equation for colon 38 tumours (Fig. 3) will apply to human tumours, which generally have much slower growth rates (NZM3 has a tumour-doubling time of approximately 8 days). It will be interesting to determine whether the optimal administration schedule for DACA in xenografts is related to that found in clinical trials.

In conclusion, when given using an optimised dose schedule against advanced colon 38 carcinoma (Fig. 5), DACA is as active as 5-fluorouracil and superior to doxorubicin and cyclophosphamide. One possible reason for the high activity of DACA is its ability to be retained in solid tumours [17]. Etoposide, mitoxantrone and teniposide have low activity in this system, in agreement with the results of other studies, which show a lack of effect of etoposide and teniposide against early-stage colon 38 tumours [8]. Although it can be argued that DACA has the advantage of having been tested in a greater variety of dosage schedules than have been used for other compounds, its experimental antitumour activity is nevertheless exciting. Moreover, DACA is extremely active against the Lewis lung carcinoma (Fig. 5). One possible reason contributing to this is that Lewis lung carcinoma cells exhibit some degree of transport-mediated multidrug resistance [6] and that DACA, as well as the amsacrine analogue CI-921, is capable of overcoming this resistance [2]. When the

antitumour activity detected in the colon 38 and Lewis lung models is compared with that of other agents, the activity of DACA is evident (Fig. 5). It will be interesting to determine whether this experimental activity is reflected in clinical trials.

Acknowledgements This work was supported by the Cancer Society of New Zealand, by its Auckland Division, and by the Health Research Council of New Zealand.

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