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Cytokinetic differences in the action of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide as compared with that of amsacrine and doxorubicin

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Abstract. *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide dihydrochloride (DACA) is a topoisomerase II-directed DNA intercalator with high experimental solid-tumour activity. The effect of DACA on the cytokinetics of cultured Lewis lung adenocarcinoma cells was compared with those of two clinical drugs of this class, doxorubicin and amsacrine. Cells were exposed to drugs for a 1-h period at concentrations that reduced viability by approximately 99% as measured by clonogenic assays. Subsequent progress through the cell cycle was monitored by propidium staining of fixed cells and flow cytometry. DACA, amsacrine and doxorubicin did not inhibit the G_1- to S-phase transition but did delay progression through the S-phase. The effect was maximal in the late S-phase and, because of the differential rates of progress of cells in various cycle positions, led to the development of a synchronous S-phase peak. This peak moved to the G_2/M-phase position at 11 h after the removal of DACA or at 14 h after the removal of amsacrine or doxorubicin. The effects of the drugs on cells initially in the G_2-phase was measured by scoring mitotic cells in the presence and absence of colchicine. DACA had an immediate inhibitory effect on the progression of cells from the G_2-phase to mitosis. This effect was much greater for DACA than for the other two drugs, consistent with the greater effect of DACA on the G_2/M-phase to G_1-phase transition. The results suggest that DACA causes cell-cycle changes expected for a DNA-damaging drug but differs from doxorubicin and amsacrine mainly by its effect on the transition of G_2-phase cells to mitosis and the G_1-phase.

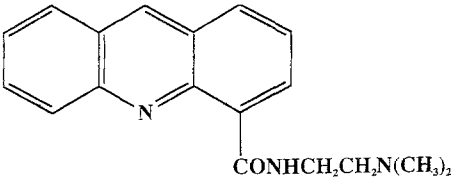

Fig. 1. Chemical structure of DACA as the free base

Introduction

DACA (acridine carboxamide, NSC 601316; see structure in Fig. 1) was synthesised in this laboratory as part of a programme to develop new acridine derivatives with increased activity against solid tumours [1]. DACA binds to double-stranded DNA by intercalation [21] and is an efficient inducer of protein-DNA cross-links in cultured L1210 cells [16], consistent with its action as a poison of the enzyme topoisomerase II (topo II). In contrast to available clinical topo II poisons, DACA is curative against the advanced Lewis lung carcinoma in mice [1, 5] and is also more active than these agents against advanced subcutaneous colon 38 tumours in mice (unpublished data). However, it displays only moderate activity against experimental leukaemia [1]. DACA is scheduled for clinical trial under the auspices of the Cancer Research Campaign, United Kingdom.

DACA has interesting in vitro properties that distinguish it from other topo II poisons such as amsacrine and doxorubicin. One is that the molecule is not easily oxidised or reduced in tumour cells and is stable in culture. Amsacrine has an electron-rich anilino side chain that is capable of forming electron-transfer complexes [2], while doxorubicin has the propensity to be reduced intracellularly to free-radical species that contribute to its cytotoxicity [17]. Secondly, DACA inhibits its own cytotoxicity at high drug concentrations and over short exposure periods [5, 10]. Thirdly, DACA is active against a series of Jurkat human leukaemia lines that display "atypical" (topo II-mediated) multidrug resistance [9].

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In this communication we compare the effects of DACA with those of amsacrine and doxorubicin on the progress of cultured Lewis lung adenocarcinoma cells through the cell-division cycle. For most experiments we used a DACA concentration of 2 μ*M*, which provides an approximately 99% loss of the viability of these tumour cells in clonogenic assays [5], and used equitoxic concentrations of the other two drugs based on clonogenic assay results [8]. We selected a 1-h exposure of cultured Lewis lung carcinoma cells to drug, in recognition of the observation that the plasma concentration of DACA in mice decreases to less than 2 μ*M* by 1 h after drug administration [14]. On the basis of the average results of a large number of assays for cell-cycle distribution as well as of detection of the incidence of mitoses, we could estimate the transition rates from one cell-cycle phase to another at various times after drug exposure.

Materials and methods

Materials and cells. DACA dihydrochloride and amsacrine isethionate were synthesised in the Auckland Cancer Research Laboratory. Other agents were obtained from the following sources: doxorubicin, Farmitalia Carlo Erba (Milan, Italy); penicillin, streptomycin, propidium iodide and pancreatic ribonuclease, Sigma Chemical Co. (St. Louis, USA); trypsin, Difco Laboratories (Detroit, USA).

LLTC, a tissue-culture-adapted line originally obtained from Parke-Davis Pharmaceutical Research (Ann Arbor, USA), was derived from the Lewis lung carcinoma [22]. Cells were cultured in α-modified minimal essential medium supplemented with 10% fetal bovine serum (FBS); Gibco BRL-Life Technologies Ltd., New Zealand), penicillin (100 U/ml) and streptomycin (100 μg/ml) as previously described [10].

Flow cytometric analysis. Cultures of LLTC cells were established at 10^5 cells/ml (5 ml/25-cm^2 tissue-culture flask) in growth medium and incubated at 37° C overnight to allow attachment. After 17–18 h the supernatant was removed and replaced with new medium plus cytotoxic agents (DACA, amsacrine or doxorubicin) and the cells were incubated for 1 h at 37° C. Concentrations of DACA (2 μ*M*), amsacrine (5 μ*M*) or doxorubicin (1 μ*M*) were selected to give 90%–99% killing over a 1-h incubation period. Drug-containing suspensions were aspirated, the cells were washed once with warm medium and incubation was continued such that the total time of incubation from the initial addition of cytotoxic agents ranged from 2 to 24 h. At the end of incubation the cells were harvested by removing the medium (which was retained to ensure that any floating cells were included), adding 0.07% trypsin in citrate-saline, then neutralising the trypsin with cold medium. The trypsinised cells and retained supernatant for each sample were combined, centrifuged and washed once. The cells were resuspended in 100 μl ice-cold phosphate-buffered saline (PBS), fixed in 1 ml 100% ethanol and stored at –20° C until analysis.

Analysis methods were similar to those used previously [12]. Cells were washed in PBS supplemented with 2% FBS and resuspended at 5×10^5/ml. Samples (4 ml) were centrifuged and resuspended in 2% FBS/PBS (200 μl) plus RNAase (50 μl; 1.0%, w/v) and propidium iodide (20 μl, 1 mg/ml), incubated at 37° C for 5 min and analysed on an Epics Profile (Coulter Electronics, Florida, USA) flow cytometer. Data were analysed using MCYCLE software (Phoenix Flow Systems, San Diego, USA) into G_1-, S- and G_2/M-phase fractions by a second-order S-phase fitting procedure. Data from a number of experiments were averaged to provide the percentages of each phase. Cycle phase-transition times were calculated using the principles discussed by Steel [19] for exponentially growing populations, assuming the frequency of cells that have just divided is twice that of those which ar just about to divide.

Measurement of mitotic indices. LLTC cells were plated at 10^5 cells/ml in 100-mm dishes containing α-modified minimal essential medium supplemented as described above. After 18 h, cells were detached from the plates by treatment with 0.07% trypsin, collected by centrifugation and resuspended to 10^5 cells/ml in growth medium in plastic tubes (5 ml/tube). Colchicine (0.4 μ*M*) was added to the appropriate tubes before the addition of either DACA, amsacrine or doxorubicin and the samples were incubated for 1 h. Cytotoxic agents were added over a range of concentrations selected to provide at least 90%–99% killing. At the end of the incubation period the samples were centrifuged (5 min, 1000 rpm), washed in PBS (5 ml), resuspended in pre-warmed KCl (5 ml, 75 m*M*) and incubated at 37° C for 9 min. The samples were cooled on ice, Carnoy's fixative (methanol and acetic acid at 3 : 1, v/v; 5 ml) was added dropwise, and the samples were centrifuged (5 min, 1000 rpm). After aspiration, more fixative was added (5 ml) and the samples were then kept on ice for at least 30 min. After further centrifugation, cells were suspended in 1 ml fixative and the cells were dropped onto alcohol-cleaned slides from a height of 20 cm. The slides were allowed to dry overnight and were stained with Giemsa stain (3.3 m*M*, dissolved in 3.3 m*M* Na_2HPO_4/3.3 m*M* K_2HPO_4, pH 6.8).

Results

Effects of DACA, amsacrine and doxorubicin on G_1-phase and G_2/M-phase content

Asynchronous cultures of LLTC cells were exposed for 1 h to DACA, amsacrine or doxorubicin at the respective concentration sufficient to cause an approximately 99% loss of viability. This was determined by clonogenic assay (data not shown), yielding results similar to those previously published [5, 8]. After drug exposure, cells were washed twice to remove the drug and then grown for various periods before the analysis of DNA content by flow cytometry. Results of multiple profiles were used to calculate the proportions of cells in each phase of the cell cycle at various times after drug removal. Control profiles (no drug) were analysed for each time point and showed no evidence for synchronisation of untreated cells over this time course.

Typical profiles for DACA, amsacrine and doxorubicin are shown in Figs. 2, 3, and 4, respectively. No significant increase in the content of cells with lower than G_1-phase DNA content was noted in the course of these experiments for any of the three drugs. Furthermore, within the errors of the experiment, no loss of cells occurred from the cultures with increasing time after drug exposure, indicating that essentially all of the cells in the cultures could be accounted for in the profiles.

The proportion of cells in the G_1-phase decreased from 31% to <5% over 3 h (DACA) or 5 h (amsacrine and doxorubicin; Fig. 5), indicating a block of the entry of cells into the G_1-phase that was more rapid for DACA than for the other two drugs. In contrast to the G_1-phase results, the proportion of cells in the G_2/M-phase increased only slightly over a 5-h period for all three drugs (Fig. 6), indicating that entry into as well as exit from the G_2/M-phase was inhibited. Profiles were also analysed at either 5 or 11 h following a 1-h exposure to each of the drugs at lower concentrations. The results obtained at 11 h are shown in Fig. 7 and indicate that at these lower concentrations, amsacrine and doxorubicin were more effective than DACA in arresting cells in the G_2/M-phase.

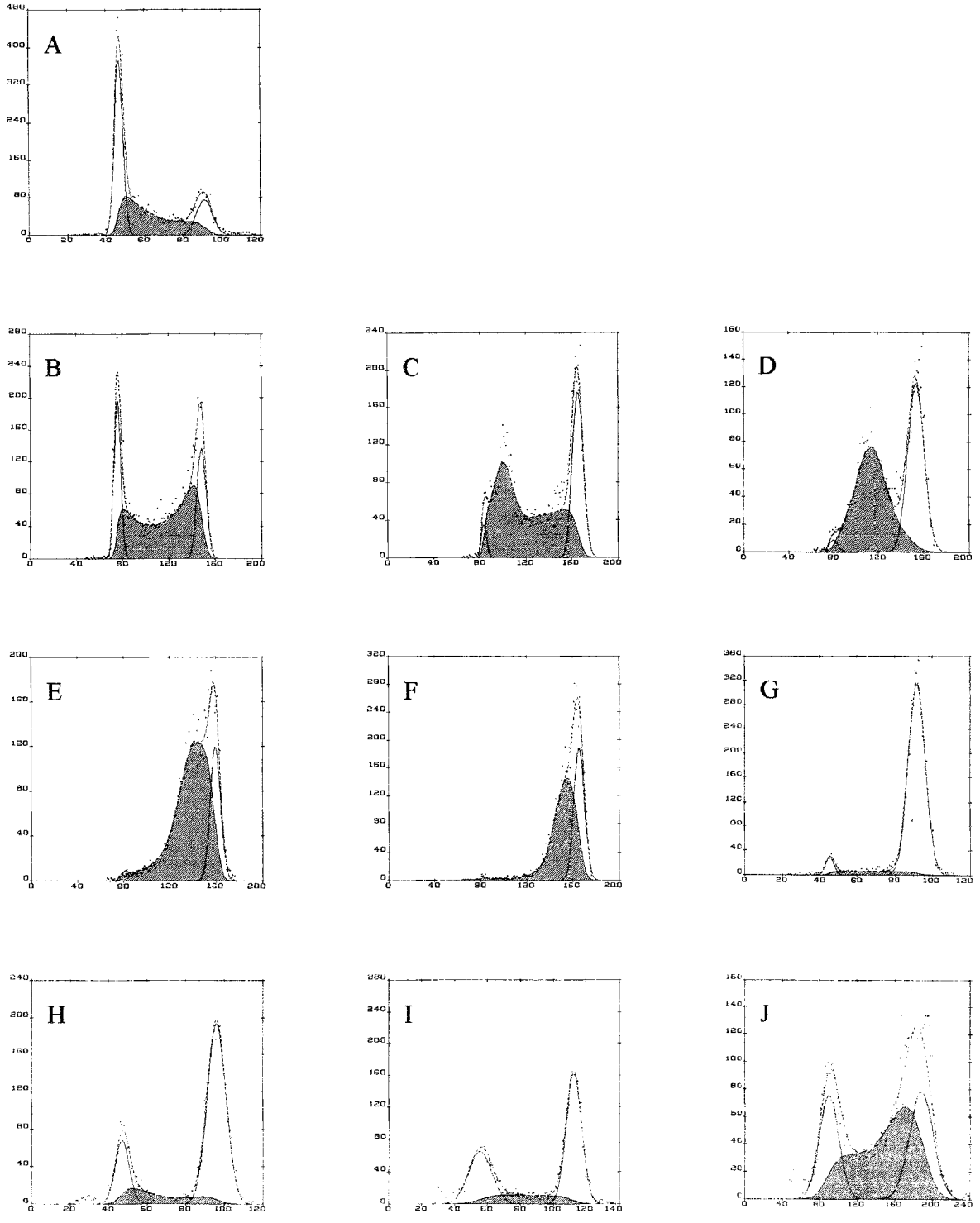

Fig. 2 A – J. Flow cytometric profiles (cell frequency versus DNA content) of propidium-stained LLTC cells either in the absence of DACA (**A**) or at 1 h (**B**), 3 h (**C**), 5 h (**D**), 7 h (**E**), 9 h (**F**), 11 h (**G**), 14 h (**H**), 17 h (**I**) or 23 h (**J**) after the removal of drug following a 1-h exposure to DACA (2 μM). Fitted G_1-, S- and G_2/M-phase components are shown for each profile, with the S-phase component being *shaded* for clarity

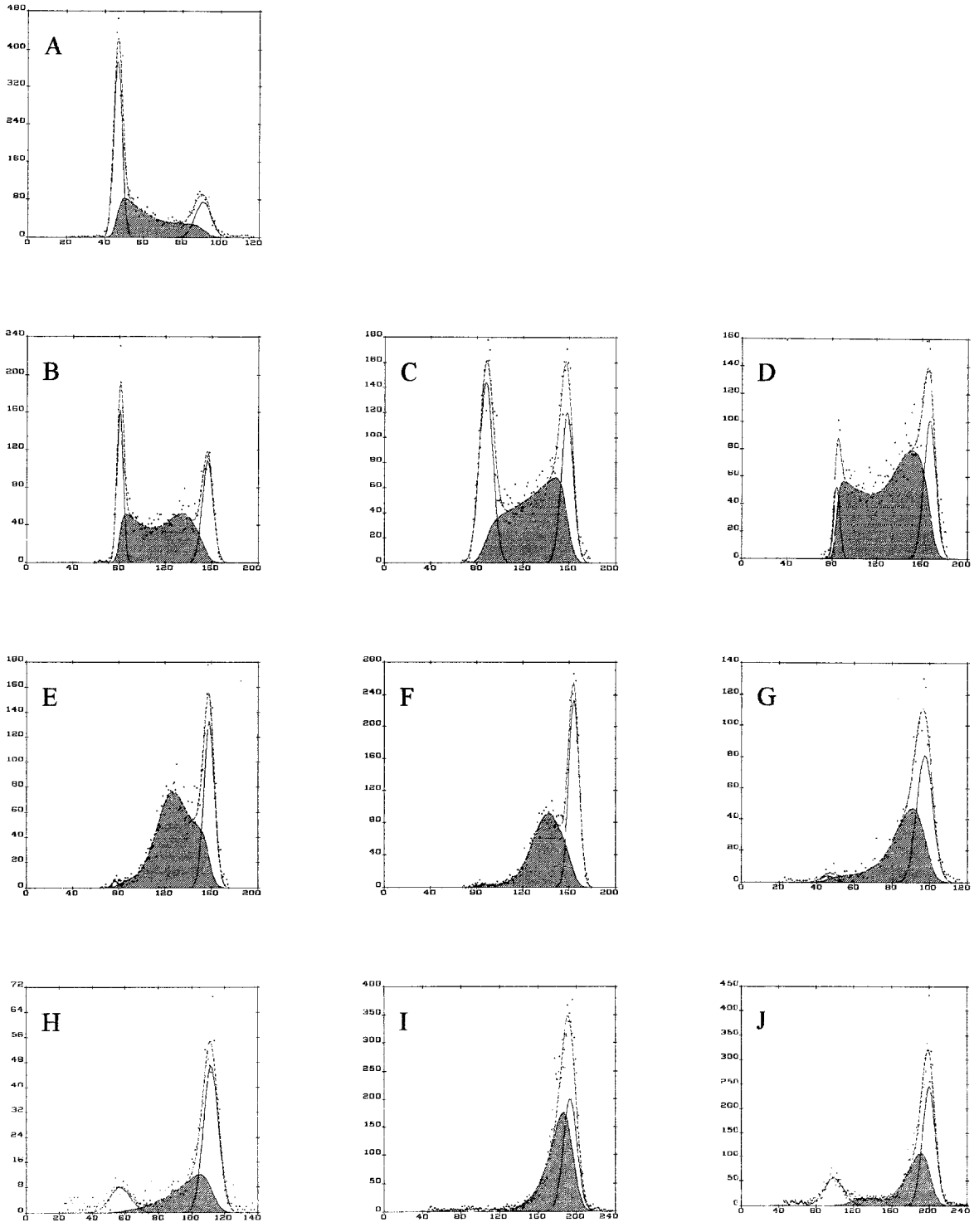

Fig. 3 A – J. Flow cytometric profiles (cell frequency versus DNA content) of propidium-stained LLTC cells either in the absence of amsacrine (**A**) or at 1 h (**B**), 3 h (**C**), 5 h (**D**), 7 h (**E**), 9 h (**F**), 11 h (**G**), 14 h (**H**), 17 h (**I**) or 23 h (**J**) after the removal of drug following a 1-h exposure to amsacrine (5 μ*M*). Fitted G_1-, S- and G_2/M-phase components are shown for each profile, with the S-phase component being *shaded* for clarity.

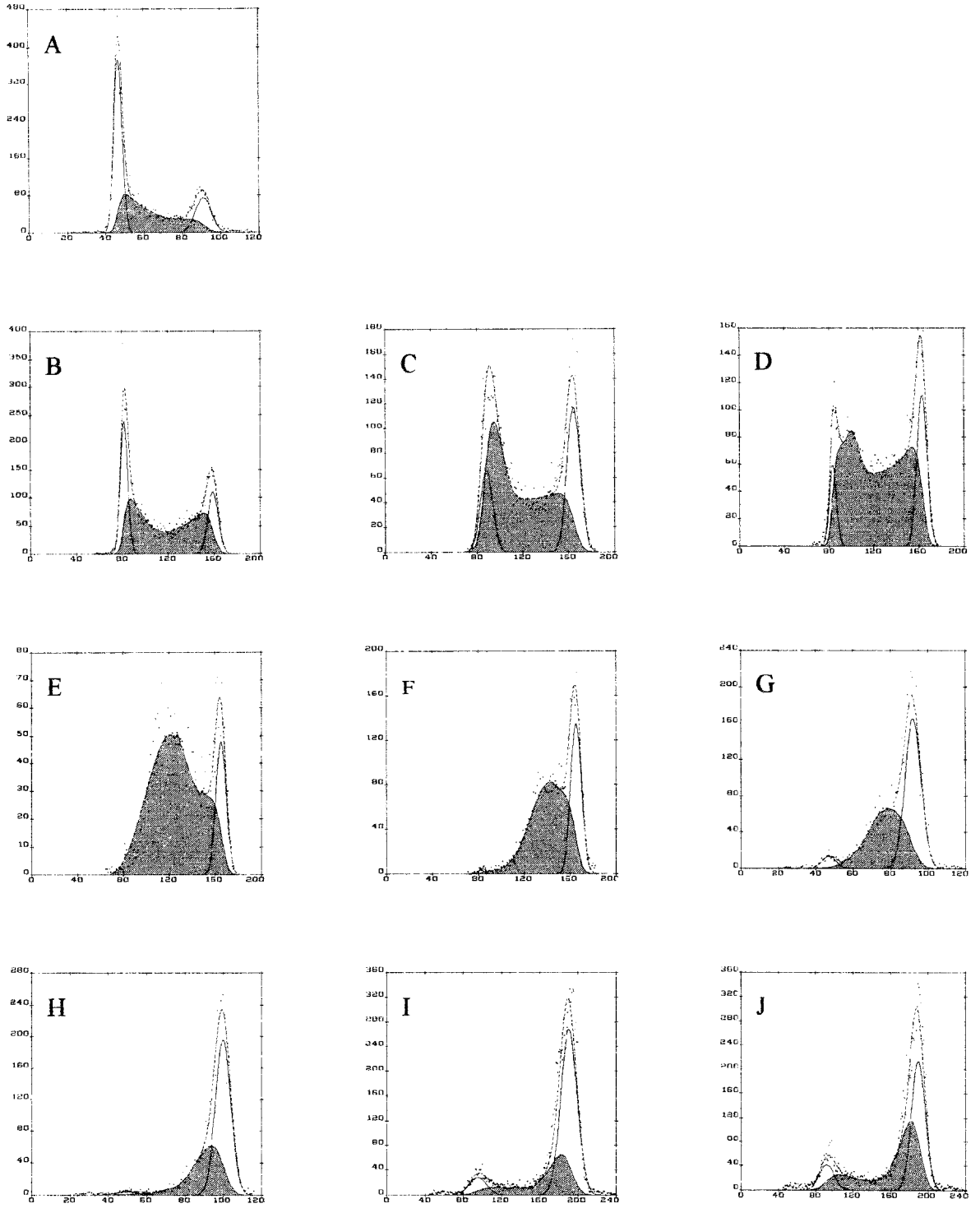

Fig. 4 A–J. Flow cytometric profiles (cell frequency versus DNA content) of propidium-stained LLTC cells either in the absence of doxorubicin (**A**) or at 1 h (**B**), 3 h (**C**), 5 h (**D**), 7 h (**E**), 9 h (**F**), 11 h (**G**), 14 h (**H**), 17 h (**I**) or 23 h (**J**) after the removal of drug following a 1-h exposure to doxorubicin (1 μ*M*). Fitted G_1-, S- and G_2/M-phase components are shown for each profile, with the S-phase component being *shaded* for clarity

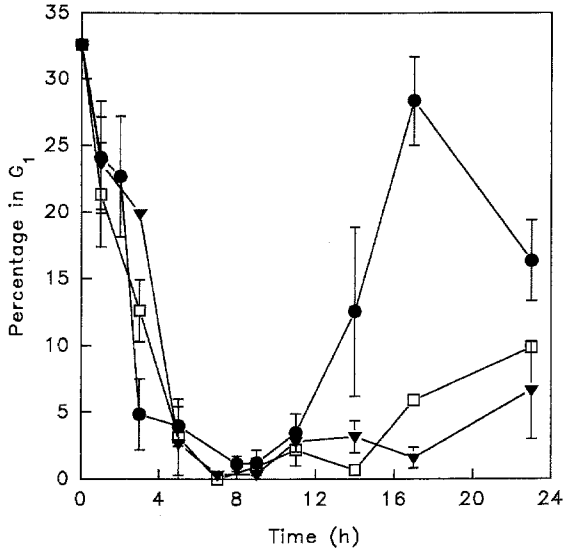

Fig. 5. Percentage of cells in the G_1-phase after the removal of drug following a 1-h exposure of LLTC cells to DACA (●), amsacrine (▼) or doxorubicin (□) at concentrations inducing approximately 99% cell death

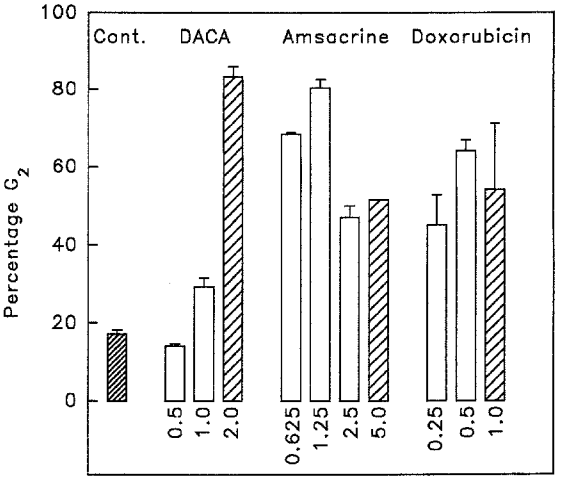

Fig. 7. Percentage of LLTC cells in the G_2/M-phase at 11 h after the removal of drug following a 1-h exposure to DACA, amsacrine or doxorubicin at the indicated concentrations (μ*M*). The bar with *fine hatching* represents cells incubated in the absence of drug. The bars with *coarse hatching* represent the drug concentration inducing approximately 99% cell death. *Vertical lines* represent the standard errors of the determinations, which were made from flow cytometric profiles

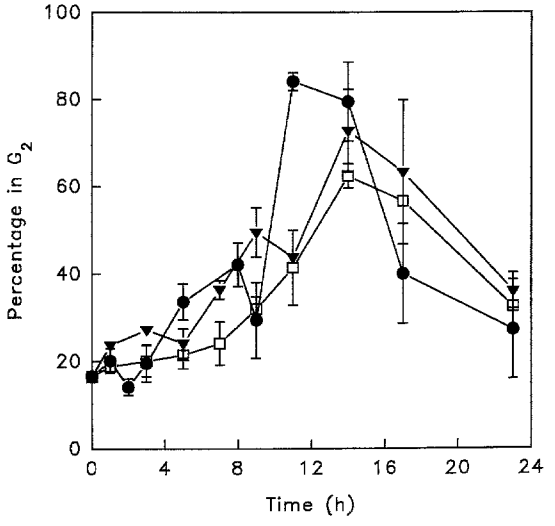

Fig. 6. Percentage of cells in the G_2/M-phase after the removal of drug following a 1-h exposure of LLTC cells to DACA (●), amsacrine (▼) or doxorubicin (□) at concentrations inducing approximately 99% cell death

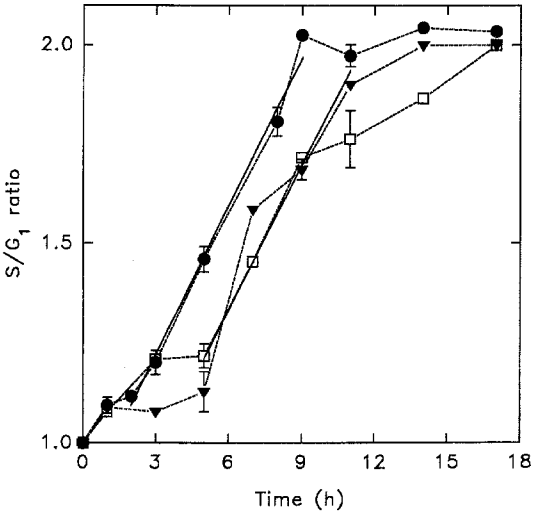

Fig. 8. DNA content of the mode of the synchronous S-phase peak at different times after a 1-h exposure of LLTC cells to concentrations of DACA (●), amsacrine (▼) or doxorubicin (□) inducing approximately 99% cell death

Formation of a synchronous S-phase peak

After exposure to DACA, G_1-phase cells had virtually disappeared by 3 h after drug removal, resulting in the appearance of a synchronous peak in the early S-phase (Fig. 2). In contrast, after exposure to amsacrine and doxorubicin, G_1-phase cells required 5 h from drug removal to decrease below 5% and form a synchronous S-phase peak. For all three drugs, the DNA content of the synchronous S-phase peak then increased with time. The modal position of the synchronous peak was identified and is plotted versus time after drug exposure in Fig. 8. A homogeneous G_2/M-phase peak was evident at 11 h after the removal of DACA and at 14 h after the removal of amsacrine and doxorubicin (Fig. 8).

Effects of DACA, amsacrine and doxorubicin on the G_2-phase to metaphase transition

The results shown in Fig. 5 suggested that DACA differed from the other two drugs in arresting the entry of mitotic or G_2-phase cells into the G_1-phase. This difference was also noted in the formation and progress of the synchronous S-phase peak (Fig. 8). For a comparison of the effects of the drugs on the entry of cells into mitosis, cultures of

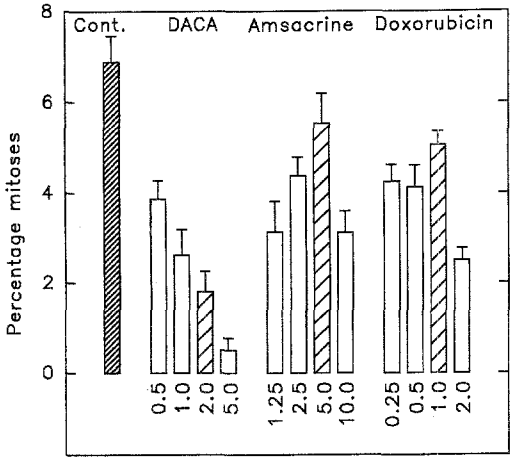

Fig. 9. Percentage of mitoses in cultured LLTC cells following a 1-h exposure to DACA, amsacrine or doxorubicin at the indicated concentrations (μ*M*) in the presence of colchicine (0.4 μ*M*). The bar with *fine hatching* represents cells incubated with colchicine alone. The bars with *coarse hatching* represent the drug concentration inducing approximately 99% cell death. *Vertical lines* represent the standard errors of the determinations

LLTC cells were exposed for 1 h to each of the three drugs at a concentration sufficient to cause an approximately 99% loss of reproductive viability. The cells were then fixed and metaphase spreads were analysed, providing the following mitotic indices: control cells, 4.5% ± 0.3%; DACA, 1.8% ± 0.3%; amsacrine, 4.1% ± 0.4%; and doxorubicin, 4.3% ± 0.7%. Thus, while no drug arrested cells in mitosis, DACA prevented the progress of cells from the G_2-phase to mitosis. Cells were also exposed to a range of drug concentrations in the presence of colchicine (0.4 μ*M*) to prevent mitotic cells from progressing into the G_1-phase. DACA was found to have a much greater inhibitory effect on progress into mitosis than had the other two drugs (Fig. 9).

Discussion

DACA, like other topo II poisons [3, 13, 23], induces G_2-phase arrest following drug exposure as demonstrated by a peak in G2/M on flow cytometry and an absence of M-phase cells. The present results provide a detailed sequence of the events leading up to cell-cycle arrest and delineate the subtle differences between the actions of DACA, amsacrine and doxorubicin. Because the LLTC cell line does not appear to respond to drug exposure by apoptosis, analysis can be conducted under the assumption that all cells are accounted for. Although a 1-h exposure of LLTC cells to DACA, amsacrine and doxorubicin results in eventual G_2-phase arrest, the initial effect of amsacrine and doxorubicin is exerted on the S-phase, whereas that of DACA is exerted on the S-phase plus the G_2-phase.

LLTC cells have a cell-doubling time of approximately 12 h [6]. The G_1-, S-, G_2- and M-phase transit times can be estimated from the observed proportions of cells in exponentially growing populations [19], assuming a cell-cycle time of 11.5 h [7], to be approximately 3, 6, 2 and 0.5 h, respectively. Following exposure to DACA, depletion of the G_1-phase compartment is virtually complete at 3 h after the removal of drug (Fig. 5), consistent with there being little inhibition of the G_1- to S-phase transition and complete inhibition of the G_2- to M-phase transition. However, if DACA acted purely by arresting cells in the G_2-phase, one would expect a monotonic increase in G_2-phase content with time. This is not the case, since it is evident from the kinetics of accumulation of G_2-phase cells that the initial accumulation of cells in the G_2-phase occurs at a rate of only about 1%/h (Fig. 6). It can be inferred from an analysis of the flow cytometric profiles that the cell-cycle delays that occur are longest for the G_2- and late S-phase, shorter for the early S-phase and even shorter for the G_1-phase. This has the effect of allowing G_1-phase cells to "catch up" with early S-phase cells to produce a synchronous S-phase peak (Fig. 8). This peak moves through the S-phase during a time that is only slightly slower (8 h) than the transit time for an unperturbed population (6 h). It is likely that the cycle delays are the direct or indirect results of drug-induced DNA damage. Since the content and activity of topo II increases during the S-phase and G_2-phase [11, 15], an attractive explanation for the results is that DACA causes DNA damage and a resulting cycle delay that is roughly proportional to the topo II activity.

The most dramatic difference between the action of DACA and those of amsacrine and doxorubicin is in its inhibition of the progress of G_2-phase cells into mitosis (Fig. 9). While DACA almost completely inhibits the entry of cells into mitosis, amsacrine and doxorubicin at equitoxic concentrations inhibit it only slightly. This effect is observed in either the presence or the absence of the mitotic inhibitor colchicine, but the addition of colchicine provides more mitotic events and, thus, greater statistical significance. In the case of amsacrine and doxorubicin, depletion of the G_1-phase population takes approximately 5 h from drug removal, consistent with a "run-through" of G_2- and M-phase cells into the G_1-phase but with little or no inhibition of the G_1. to S-phase transition. Thereafter, a similar course of events ensues, with a synchronous S-phase peak being formed that moves through the S-phase over the next 8 h. In cells exposed to doxorubicin there may also be slowing at later times (Fig. 8). Maximal G_2-phase arrest occurs at 11 h after treatment with DACA and at 14 h after treatment with amsacrine (Fig. 3) and doxorubicin (Fig. 4). At later times these populations divide to provide a new G_1-phase component (Figs. 2–4). It should be noted that approximately 99% of this population is non-viable.

In conclusion, it is clear that while all three topo II poisons induce S-phase delay and eventual G_2-phase arrest, DACA has a significantly greater effect on G_2-phase cells. The reason for this selective action of DACA may reflect its greater ability to induce topo II-mediated DNA damage in late S-phase and G_2-phase cells. It is known that the activity of topo II is increased by phosphorylation during the late S-phase and the G_2-phase [15] and that phosphorylation of topo II decreases its sensitivity to amsacrine and etoposide [4]. Therefore, a possible explanation for the results is that phosphorylated topo II remains

sensitive to DACA but resistant to amsacrine and doxorubicin. Such an explanation might also explain why DACA overcomes resistance to amsacrine and doxorubicin in multidrug-resistant Jurkat cell sublines [9], which is mediated by changes in topo II rather than in drug transport [18]. A common cause of such multidrug resistance involves increased overall phosphorylation of topo II [20]. If further studies do confirm that DACA is differentially active against phosphorylated topo II, DACA may become a useful tool for studying resistance mechanisms.

References

1. Atwell GJ, Rewcastle GW, Baguley BC, Denny WA (1987) Potential antitumor agents. 50. In vivo solid tumor activity of derivatives of *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide. J Med Chem 30: 664–669
2. Baguley BC (1990) The possible role of electron transfer complexes in the action of amsacrine analogues. Biophys Chem 23: 937–943
3. Baguley BC, Finlay GJ, Wilson WR (1986) Cytokinetic resistance of Lewis lung carcinoma to cyclophosphamide and the amsacrine derivative CI-921. In: Hall TC (ed) Cancer drug resistance. Progress in clinical and biological research. vol 23. Allan R. Liss, New York, pp 47–61
4. Devore RF, Corbett AH, Osheroff N (1992) Phosphorylation of topoisomerase II by casein kinase II and protein kinase C – effects on enzyme-mediated DNA cleavage/religation and sensitivity to the antineoplastic drugs etoposide and 4′-(9-acridinylamino)methanesulfon-*m*-anisidide. Cancer Res 52: 2156–2161
5. Finlay GJ, Baguley BC (1989) Selectivity of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide towards Lewis lung carcinoma and human tumour cell lines in vitro. Eur J Cancer Clin Oncol 25: 271–277
6. Finlay GJ, Wilson WR, Baguley BC (1987) Cytokinetic factors in drug resistance of Lewis lung carcinoma: comparison of cells freshly isolated from tumours with cells from exponential and plateau-phase cultures. Br J Cancer 56: 755–762
7. Finlay GJ, Wilson WR, Baguley BC (1987) Response of cultured Lewis lung carcinoma cells to tiazofurin. J Natl Cancer Inst 79: 291–296
8. Finlay GJ, Wilson WR, Baguley BC (1989) Chemoprotection by 9-aminoacridine derivatives against the cytotoxicity of topoisomerase II-directed drugs. Eur J Cancer Clin Oncol 25: 1695–1701
9. Finlay GJ, Marshall ES, Matthews JHL, Paull KD, Baguley BC (1993) In vitro assessment of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA), a DNA-intercalating antitumor drug with reduced sensitivity to multidrug resistance. Cancer Chemother Pharmacol 31: 401–406
10. Haldane A, Finlay GJ, Gavin JB, Baguley BC (1992) Unusual dynamics of killing of cultured Lewis lung cells by the DNA-intercalating antitumour agent *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. Cancer Chemother Pharmacol 29: 475–479
11. Heck MMS, Hittelman WN, Earnshaw WC (1988) Differential expression of DNA topoisomerases I and II during the eukaryotic cell cycle. Proc Natl Acad Sci USA 85: 1086–1090
12. Holdaway KM, Finlay GJ, Baguley BC (1992) Relationship of cell cycle parameters to in vitro and in vivo chemosensitivity for a series of Lewis lung carcinoma lines. Eur J Cancer 28 A: 1427–1431
13. Minderman H, Brons PPT, Linssen PCM, Pennings AHM, Wessels JMC, Boezeman JBM, Haanen C (1991) Effect of doxorubicin exposure on cell-cycle kinetics of human leukemia cells studied by bivariate flow cytometric measurement of 5-iodo-2-deoxyuridine incorporation and DNA content. Exp Hematol 19: 1008–1012
14. Paxton JW, Young D, Evans SMH, Kestell P, Robertson IGC, Cornford EM (1992) Pharmacokinetics and toxicity of the antitumour agent *N*-[4-(dimethylamino)ethyl]-acridine-4-carboxamide after i. v. administration in the mouse. Cancer Chemother Pharmacol 29: 379–384
15. Saijo M, Ui M, Enomoto T (1992) Growth state and cell cycle dependent phosphorylation of DNA topoisomerase II in Swiss 3T3 cells. Biochemistry 31: 359–363
16. Schneider E, Darkin SJ, Lawson PA, Ching L-M, Ralph RK, Baguley BC (1988) Cell line selectivity and DNA breakage properties of the antitumour agent *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide: role of DNA topoisomerase II. Eur J Cancer Clin Oncol 24: 1783–1790
17. Sinha BK, Katki AG, Batist G, Cowan KH, Myers CE (1987) Differential formation of hydroxyl radicals by Adriamycin in sensitive and resistant MCF-7 human breast tumor cells: implications for the mechanism of action. Biochemistry 26: 3776–3781
18. Snow K, Judd W (1991) Characterisation of Adriamycin-resistant and amsacrine-resistant human leukaemic T-cell lines. Br J Cancer 63: 17–28
19. Steel GG (1977) Basic theory of growing cell populations. In: Steel GG (ed) Growth kinetics of tumours, 1st edn. Clarendon, Oxford, pp 56–85
20. Takano H, Kohno K, Ono M, Uchida Y, Kuwano M (1991) Increased phosphorylation of DNA topoisomerase II in etoposide-resistant mutants of human cancer KB cells. Cancer Res 51: 3951–3957
21. Wakelin LPG, Chetcuti P, Denny WA (1990) Kinetic and equilibrium binding studies of amsacrine-4-carboxamides – a class of asymmetrical DNA-intercalating agents which bind by threading through the DNA helix. J Med Chem 33: 2039–2044
22. Wilkoff LJ, Dulmadge E, Chopra DP (1980) Viability of cultured Lewis lung cell populations exposed to β-retinoic acid (40753). Proc Soc Exp Biol Med 163: 233–236
23. Zucker RM, Adams DJ, Bair KW, Elstein KH (1991) Polyploidy induction as a consequence of topoisomerase inhibition – a flow cytometric assessment. Biochem Pharmacol 42: 2199–2208