Cancer Chemother Pharmacol (1994) 33: 399–409

Cancer Chemotherapy and Pharmacology

Accumulation of HL-60 leukemia cells in G2/M and inhibition of cytokinesis caused by two marine compounds, bistratene A and cycloxazoline

Diane J. Watters[1], Heather J. Beamish[1], Karen A. Marshall[1†], Robert A. Gardiner[2], Gregory J. Seymour[3], Martin F. Lavin[1]

[1] Queensland Cancer Fund Research Unit, Queensland Institute of Medical Research, The Bancroft Centre, 300 Herston Road, Herston, Brisbane, Australia 4029
[2] Department of Surgery, University of Queensland, Herston, Brisbane, Australia 4029
[3] Department of Dentistry, University of Queensland, St. Lucia, Brisbane, Australia 4072

Received: 14 June 1993/Accepted: 4 October 1993

Abstract. The effects on the cell cycle of two biologically active compounds, bistratene A and cycloxazoline, from the marine ascidian *Lissoclinum bistratum* were studied in HL-60 human leukemia cells using flow cytometry. Both compounds were shown to cause an apparent accumulation of cells in the G2/M phase. This effect was shown to be both time- and dose-dependent. At the longer time points (30 and 48 h after addition of the compounds) polyploidy was apparent. The fate of cells labeled in the S phase with 5-bromo-2′-deoxyuridine (BrdUrd) was analysed using a bivariate BrdUrd/PI (propidium iodide) technique. Bistratene A and cycloxazoline treatment prevented the majority of BrdUrd-labeled cells from progressing through to the G1 phase. Approximately 50% of the cells were delayed at G2/M, and a significant proportion of cells appeared to be polyploid. Light and electron microscopy revealed the presence of multinucleated cells accounting for the apparent polyploidy. The progression of cells out of the G1 phase was also examined by synchronising cells with mimosine and releasing them from mimosine block in the presence of bistratene A. There was no evidence of a block at the G1/S phase transition or through the S phase since DNA synthesis was not inhibited. The mechanism by which these compounds interfere with cytokinesis is presently unknown but, in the case of bistratene A, may be linked to altered phosphorylation of cellular proteins involved in cell-cycle control.

Introduction

Biologically active compounds from marine organisms are showing great promise as potential antineoplastic agents, for example the bryostatins from the bryozoan *Bugula neritina* [16] and didemnin B from the didemnid ascidian *Tridemnum solidum* [18]. Both of these compounds are in clinical trials for cancer chemotherapy [20]. Didemnin B is a potent inhibitor of DNA and RNA synthesis and inhibits the progression of B16 melanoma cells through the G1 and S phases of the cell cycle [3, 13]. An increasing number of cytotoxic compounds are being isolated from ascidians, particularly from species of the didemnid family, but little is known on their mode of action.

We have recently described a number of cytotoxic compounds from species of didemnid ascidians collected at Heron Island Reef, Great Barrier Reef, Australia, among them bistratene A and cycloxazoline [27]. Bistratene A (also known as bistramide A) is a polyether isolated from the marine colonial ascidian *Lissoclinum bistratum* [4, 8]. The structure has recently been revised [7]. It is toxic to normal and tumour cells in vitro with a 50% growth-inhibitory concentration (IC_{50} value) of 70 ng/ml (99 n*M*) for T24 transitional bladder carcinoma cells. In HL-60 cells bistratene A induces adherence to the culture dish, the appearance of a spindle-shaped morphology and the expression of monocyte-specific antigens, indicative of partial differentiation [26]. We have recently shown that this compound causes changes in protein phosphorylation patterns in HL-60 cells [28]. As little as 10 n*M* bistratene A causes the increased phosphorylation of a 20-kDa cytoplasmic protein, which reaches a maximum within 15 min of exposure. The phosphorylation pattern induced by the phorbol ester (TPA) in these cells is different to that induced by bistratene A, although there is some overlap.

Cycloxazoline is a symmetrical cyclic hexapeptide from the same organism and is composed of three residues each of L-threonine and L-valine, where the threonine hydroxyl group has condensed to form an oxazoline ring [9]. This compound has an IC_{50} value of 0.5 μg/ml (0.91 μ*M*) for T24 bladder carcinoma cells. An identical compound named westiellamide was isolated from a terrestrial blue green alga, *Westiellopsis prolifica*, and was reported to have an IC_{50} value of 2 μg/ml for KB cells [17].

This paper is dedicated to the memory of Karen Marshall, who died tragically in a car accident during the course of this study

Correspondence to: Diane J. Watters

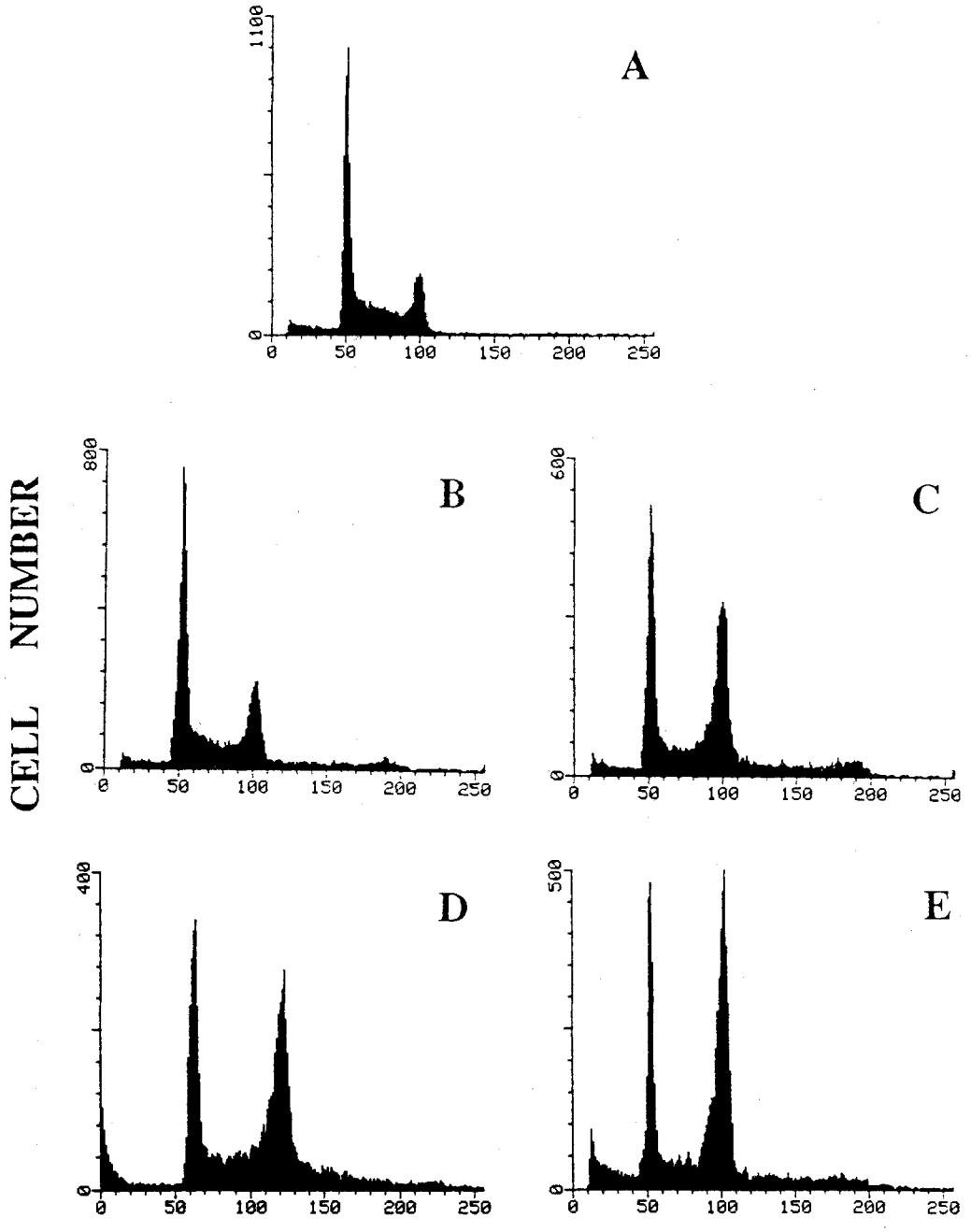

Fig. 1 A–E. Flow cytometric analysis of HL-60 cells treated with various concentrations of bistratene A for 18 h: **A** untreated, **B** 7.1 n*M*, **C** 14.2 n*M*, **D** 71 n*M*, **E** 142 n*M*

The control of cell proliferation and differentiation is linked to the cell cycle, and a study of the effects of biologically active drugs on the cell cycle often provides information on the possible mechanism of action. In this study we examined the effects of bistratene A and cycloxazoline on the cell cycle of human leukemia (HL-60) cells.

Materials and methods

Compounds. Bistratene A and cycloxazoline were isolated from the ascidian *Lissoclinum bistratum* as previously described [4, 9]. The purity of the compounds was assessed using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy. Mimosine was obtained from Aldrich Chemical Co. 5-Bromo-2′-deoxyuridine and fluorescein isothiocyanate (FITC)-labeled anti-BrdUrd (5-bromo-2′-deoxyuridine) antibody were obtained from Boehringer Mannheim. MTT (3,[4,5-dimethylthiazol-2-yl]-2,5-

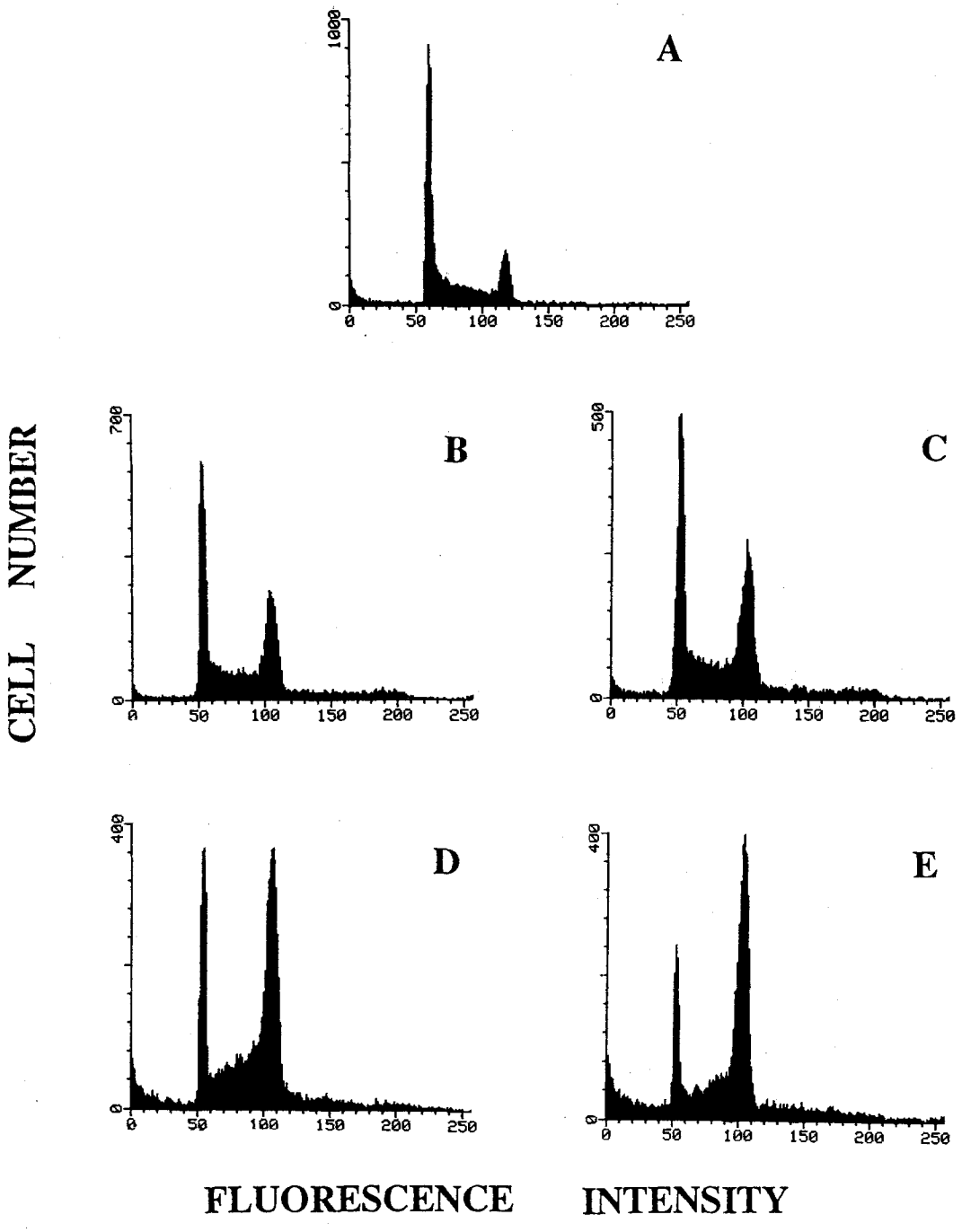

Fig. 2A–E. Flow histograms of HL-60 cells treated with various concentrations of cycloxazoline for 18 h: **A** untreated, **B** 0.91 μ*M*, **C** 1.83 μ*M*, **D** 9.1 μ*M*, **E** 18.3 μ*M*

diphenyltetrazolium bromide) was obtained from Sigma Chemical Co. (St. Louis, USA).

Cell culture. HL-60 human leukemia cells were obtained from the American Type Culture Collection and cultured in RPMI 1640 medium containing 10% foetal calf serum in an atmosphere of 5% carbon dioxide in air. The cell-doubling time was approximately 20 h. The cytotoxicity of the compounds was determined using the MTT colorimetric assay as previously described [26]. Briefly, cells (5000 per well of a 96-well plate) were cultured in the presence of varying concentrations of the compounds for 3 days, after which MTT was added for a further 4 h. The resulting formazan product was dissolved in buffered dimethylsulphoxide (DMSO) and quantitated by reading the absorbance at 540 nm.

Cell-cycle arrest. To arrest cells reversibly at the G1/S phase border the method of Hoffman et al. [10] and Watson et al. [25] was used, except that a lower concentration of mimosine was applied (80 μ*M* as compared with 300 μ*M*). Cells were seeded at 10^6/ml and 2 h later 80 μ*M* mimosine was added. The cells were incubated for 16 h, after which the mimosine was washed out by centrifugation in phosphate-buffered saline (PBS) followed by resuspension in complete medium. Bistratene A (71 n*M* or 0.50 μ*M*) was added immediately on release from mimosine, and the DNA content was analysed by flow cytometry at various times after release. The extent of apoptosis was determined by light microscopy of fixed cells stained with Hoechst 33258.

Flow cytometric analysis. Cells were harvested at the appropriate times by centrifugation and treated with 0.25 ml of propidium iodide (PI) at

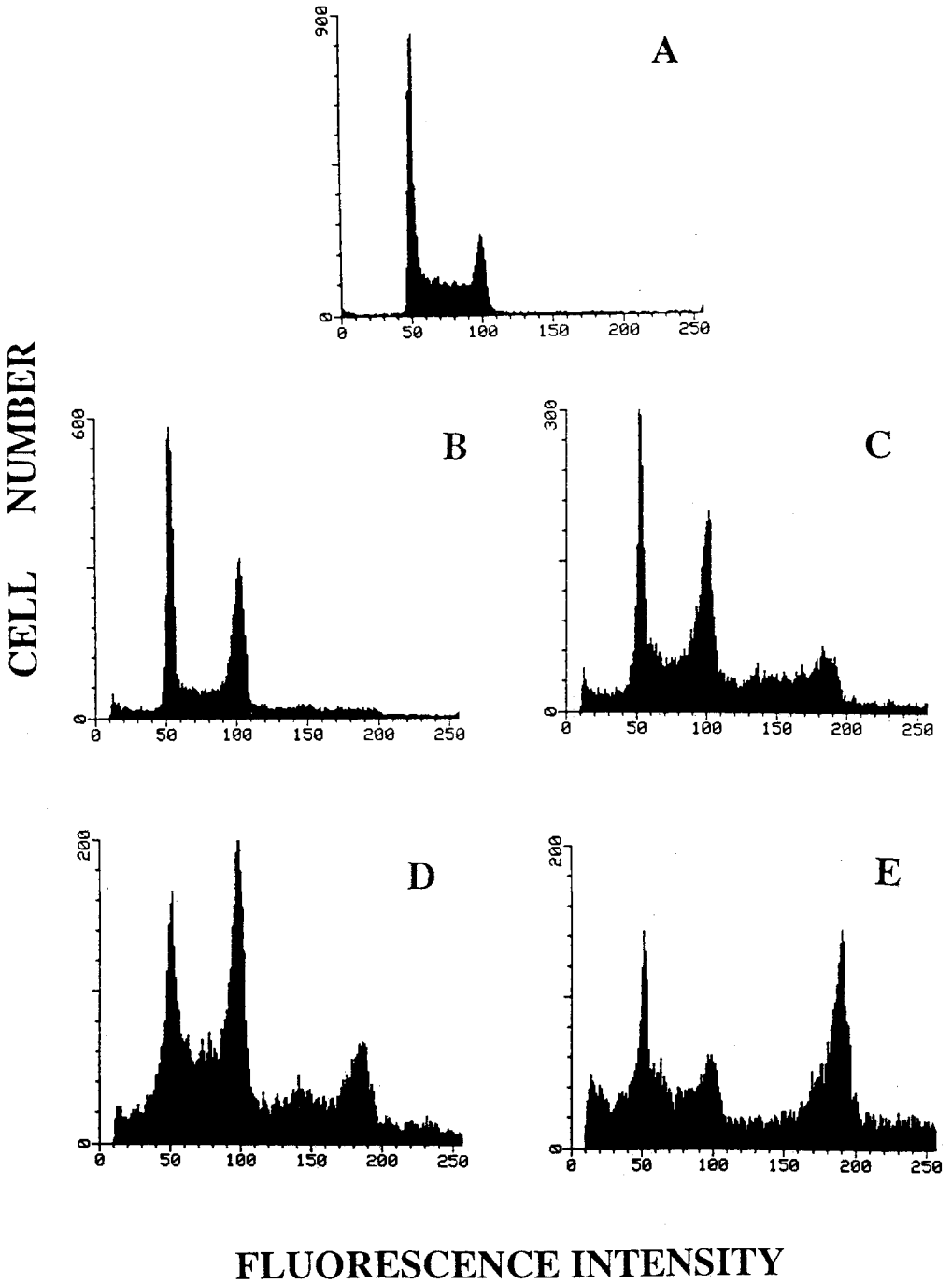

Fig. 3A–E. Flow histograms of HL-60 cells treated with 71 n*M* bistratene A for various times: **A** untreated, **B** 18 h, **C** 24 h, **D** 30 h, **E** 48 h

50 μg/ml containing 1-mg/ml RNase and 0.2% Triton X-100. After incubation on ice for 30 min, samples were analysed on a Becton Dickinson FACScan instrument [23]. The percentage of cells in each cell-cycle phase was estimated using Lysis II software (Becton Dickinson).

BrdUrd/PI analysis. To examine the fate of S-phase cells, a bivariate BrdUrd/PI flow cytometric technique was used [5]. HL-60 cells were plated at 5×10^5/ml and incubated for 24 h. They were treated for 30 min with 10 μ*M* BrdUrd at 37° C, washed twice with PBS and then incubated for either 24 or 48 h with bistratene A (71 n*M*) or cycloxazoline (9.1 μ*M*). After being washed with PBS, the cells were fixed in 3 ml of ice-cold 70% ethanol and left for at least 1 h (or overnight) at 4° C. The cells were then pelleted by centrifugation, washed with PBS, resuspended in 1 ml of 0.1-mg/ml pepsin in 0.1 *N* HCl. After a 5-min incubation at room temperature the cells were centrifuged and washed with 1-mg/ml bovine serum albumin (BSA) in PBS. The cell pellet was resuspended in 0.3 ml of 1 *M* HCl and incubated for 5 min at room temperature. The cells were centrifuged, washed with 2 ml of 0.1 *M* borax ($Na_2B_4O_7$) and resuspended in 50 μl of 0.5% Tween-20 in PBS. An equal volume of solution containing 0.1 μg of FITC-labeled anti-BrdUrd antibody and 0.5% BSA in PBS was added and the cells were incubated in a dark environment for 20–60 min. After centrifugation the supernatant was removed and the cells were treated with 0.4 ml of PI at 0.5 μg/ml and analysed on the FACScan as described above. The concentrations of pepsin and HCl used were much lower than those previously reported [5] due to the extreme sensitivity of HL-60 cells. This results in a smaller separation of the BrdUrd from PI-stained nuclei in the dot plots.

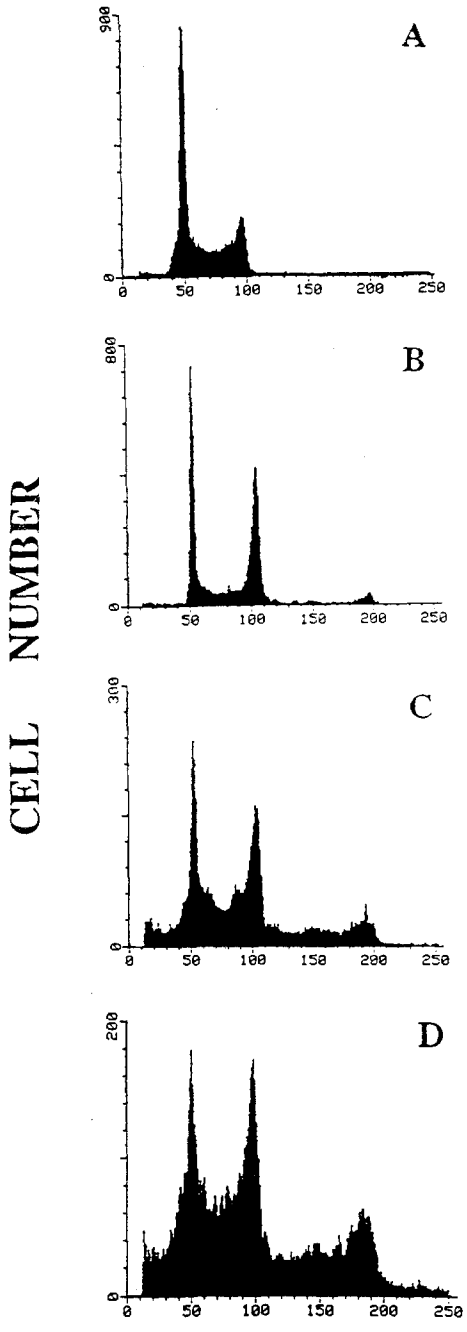

Fig. 4A–D. Flow histograms of HL-60 cells treated with 9.1 μ*M* cycloxazoline for various times: **A** untreated, **B** 18 h, **C** 24 h, **D** 30 h

Electron microscopy. Cells were incubated with either 71 n*M* bistratene A or 9.1 μ*M* cycloxazoline for 48 h. They were then centrifuged and washed once in PBS. The cells were resuspended in 3% glutaraldehyde in sodium cacodylate buffer (0.1 *M*, pH 7.2) and fixed overnight at room temperature. After centrifugation they were washed three times in sodium cacodylate buffer to remove glutaraldehyde, post-fixed in 1% osmium tetroxide in 0.1 *M* cacodylate buffer for 1 h, stained with 5% uranyl acetate for 30 min, dehydrated in graded ethanol and embedded in Epon-Araldite resin. Semi-thin sections were stained with toluidine blue. Ultra-thin sections were examined on a Jeol 1200 EX2 electron microscope for the presence of apoptotic and multinucleated cells.

Results

The IC_{50} values for bistratene A and cycloxazoline in HL-60 cells were 424 n*M* [26] and 5.5 μ*M*, respectively, as determined by the MTT assay. In asynchronous cultures of HL-60 cells, both bistratene A and cycloxazoline caused a marked block in the G2 phase of the cell cycle. Figure 1 shows a dose-dependent accumulation of cells in G2/M at 18 h after administration of bistratene A varying in concentration from 7.1 to 142 n*M* (5–100 ng/ml). There was also some evidence of polyploidy in treated cells. A similar dose-response pattern was observed for cycloxazoline at concentrations ranging from 0.91 to 18.3 μ*M* (0.5–10 μg/ml; Fig. 2). In this case also, cells were accumulating in G2/M. In Figs. 3 and 4 the time-dependent effects of both compounds can be seen. The percentage of cells in G2/M increased with time of exposure, and polyploidy was also apparent in both cases. Some variation occurred in the time of appearance of polyploidy. For cycloxazoline the polyploid peak was clearly present at 30 h after treatment.

The G2/M delay as measured by single-parameter DNA analysis does not provide information on the fate of cells in individual phases of the cell cycle. We therefore looked specifically at the progression of S-phase cells using a bivariate BrdUrd/PI flow cytometric technique that examines the fate of cells labeled in the S phase. At 24 h after pulsing with BrdUrd, untreated cells had progressed into the G1 phase and the next S phase (Fig. 5A). After treatment with bistratene, some of the S-phase cells had progressed to the G1 phase but the majority were blocked in G2/M (Fig. 5B). A similar pattern of delay of S-phase cells in G2/M was observed after treatment with cycloxazoline (Fig. 5C). Thus, the G2/M block is not absolute as some leakage into G1 does occur. Further incubation of S-phase-labeled cells led to an asynchronous pattern in untreated cells (Fig. 6A). As observed previously with bistratene, polyploidy was evident (Fig. 6B). At the concentration of cycloxazoline used, most of the cells were dead by 48 h.

Since it had previously been reported that high concentrations (0.35–1.42 μ*M*) of bistramide A (bistratene A) caused a block at the G1/S phase transition [19], we also investigated whether the concentrations used in this study caused a block in the G1 phase of the cell cycle. To achieve this, cells were synchronised at the G1 transition point with mimosine and subsequently released from mimosine in the presence of bistratene A. Progression of cells into the S phase was evident in both treated and untreated cells by 3 h post-release. At 71 n*M*, a concentration that caused significant blockage in G2/M, bistratene A had no effect on the passage of cells out of the G1 and into the S phase. After their passage through the S phase it was again evident that these cells accumulated in G2/M (Fig. 7), and there was evidence of polyploidy.

Polyploid cells were increasingly apparent at the longer time points after incubation with both compounds, being first evident at 24 h and very marked by 48 h. The polyploid peak was also apparent in BrdUrd-stained cells, indicating that cells that were in the S phase at the time of labeling progressed through the G2 phase and entered a second round of DNA replication without undergoing mitosis. It seems likely that polyploidy can be explained by

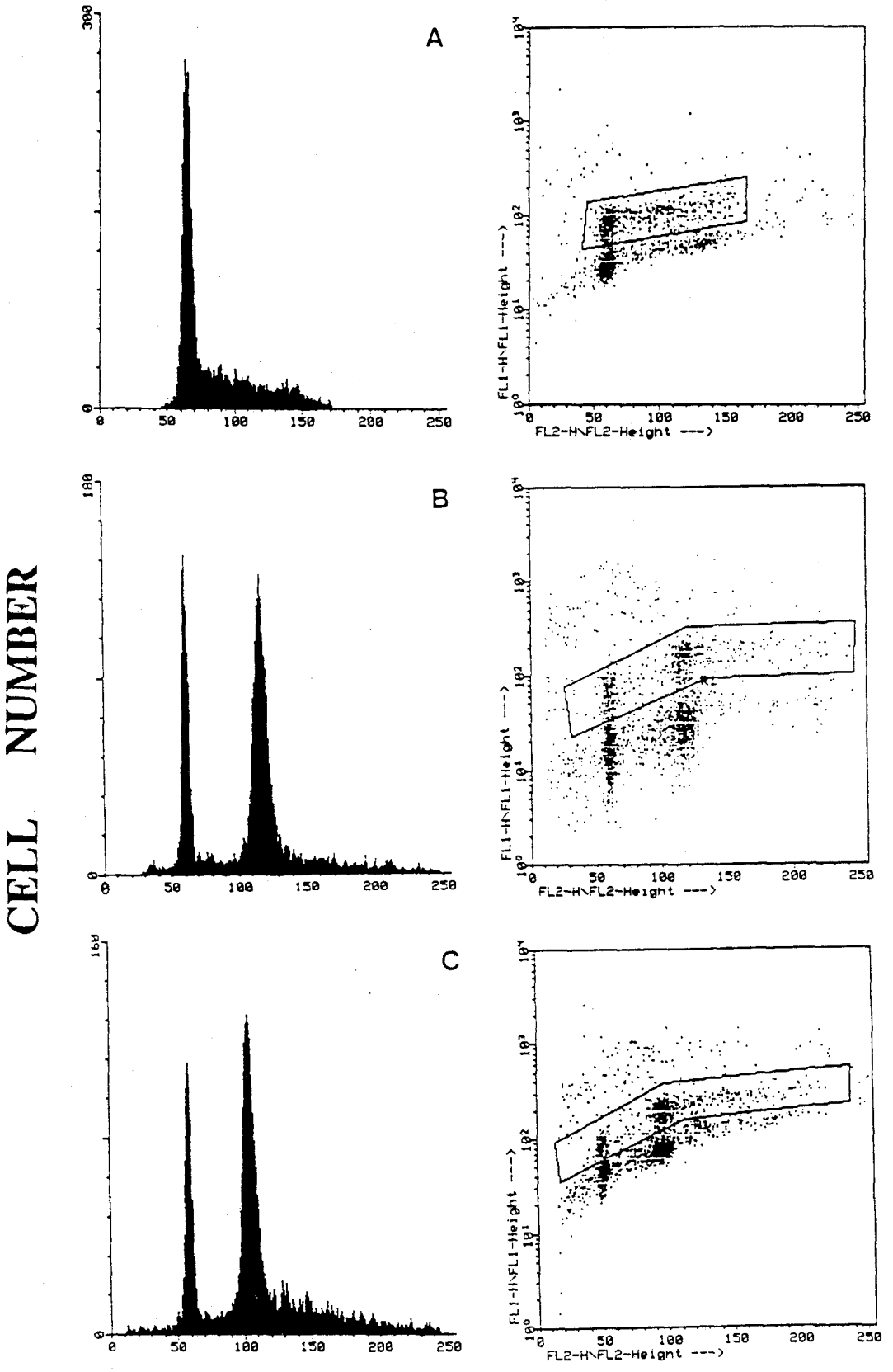

Fig. 5A–C. Bivariate BrdUrd/PI analysis of HL-60 cells. The histograms correspond to the regions outlined on the respective dot plots. The x-axis shows the relative DNA content based on linear propidium iodide fluorescence, and the y-axis shows relative BrdUrd staining based on logarithmic FITC fluorescence. *Cells in the box* were labled with BrdUrd, whereas those of lower fluorescence beneath were stained only with propidium iodide since they were not in the S phase at the time of pulsing with BrdUrd. **A** Untreated. Percentages of cells in the G1 and S phase were 61% and 32%, respectively. Since cells had not yet progressed through the cycle a defined G2 peak was not evident. **B** Treatment with bistratene A (71 n*M*) for 24 h. Percentages of cells in the G1, S and G2/M phases were 29%, 8% and 50%, respectively. **C** Treatment with cycloxazoline (9.1 μ*M*) for 24 h. Percentages of cells in the G1, S and G2/M phases were 23%, 7% and 55%, respectively.

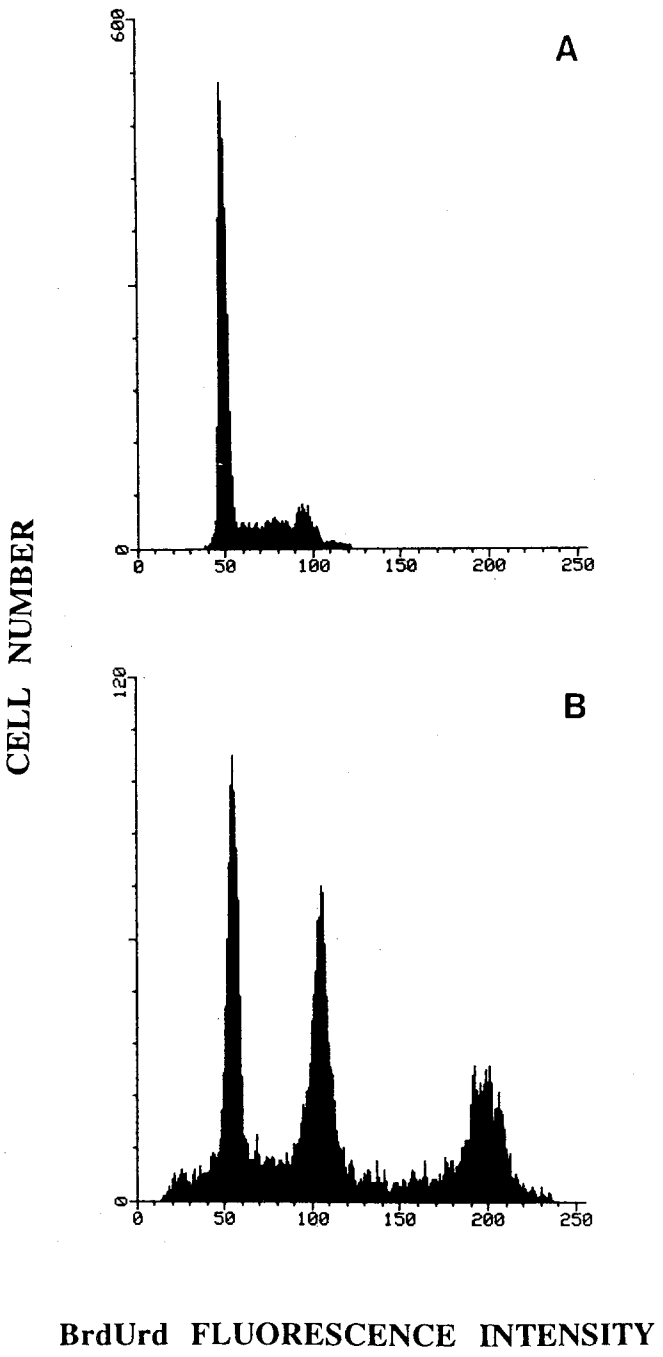

Fig. 6A–B. BrdUrd fluorescence histograms of HL-60 cells **A** untreated or **B** treated with bistratene A (71 n*M*) for 48 h. In **B** the percentages of cells in the G1, S, G2/M and polyploid peaks were 25%, 13%, 30% and 23%, respectively

nuclear division in the absence of cytokinesis since a significant number of multinucleated cells (15%–26%) were present in treated samples as determined by light and electron microscopy (Figs. 8, 9B, C). Cells contained either multiple nuclei or nuclei that had not completely separated. An increased level of apoptosis was also present after treatment with both agents (Fig. 9D). After 24 h of bistratene A treatment at 71 n*M*, 12%–25% of cells were apoptotic, whereas less than 4% of control cells were apoptotic. In all, 5%–11% of cycloxazoline-treated (0.91 μ*M*) cells were apoptotic at this time point. Bistratene A has recently been shown to induce apoptosis in a human Burkitt's lymphoma line, BM 13674 [22].

Discussion

Our results clearly show a pronounced block in G2/M for both of the compounds bistratene A and cycloxazoline. In addition, the presence of polyploidy and multinuclear cells as evidenced by electron microscopy point to an inability of the cells to undergo cytokinesis. There was no evidence of interference with cell progression from the G1 to the S phase or through the S phase for either compound. This is consistent with our earlier finding that bistratene A does not inhibit DNA synthesis [26].

Roussakis and co-workers [19] have previously described the effects of bistratene A (bistramide A) on a non-small-cell bronchial carcinoma line, NSCLCN6-L16. They used bistratene A concentrations ranging between 0.35 and 1.42 μ*M*, i.e. concentrations much higher than that used in most of our studies (71 n*M*). They concluded that there was a block in G1 based on a dose-dependent decrease in S-phase cells. At the lower concentration (0.35 μ*M*) they observed a partial blockade in the G1 phase and the appearance of polyploidy. At 1.42 μ*M*, polyploidy was not evident. In this study we found no evidence of a G1 block at 0.50 μ*M*. The apparent discrepancy between our results and theirs regarding the nature of the block can be explained to some extent by the use of very high concentrations by Roussakis et al. [19]. Although a G2 delay was also obvious in that study, it was considerably less marked than that observed by us at much lower concentrations of bistratene. The different cell types used in the two studies could also help to explain the apparent discrepancy.

The effects of bistratene A on the cell cycle in HL-60 cells are similar to those recently reported for the phorbol ester TPA in Daudi Burkitt's lymphoma cells by Menaya and Clemens [14]. In these cells, TPA (1–10 n*M*) causes an accumulation of cells in the G2 phase, and within this population a significant proportion of cells undergo nuclear division but fail to carry out cytokinesis, giving rise to multinuclear cells. DNA synthesis was also shown to continue in cells that had ceased to divide in the presence of TPA. Thus, the inhibition of proliferation was attributed mainly to an inhibition of cytokinesis rather than DNA replication. We have previously shown that bistratene A does not cause an inhibition of DNA synthesis [26] and, thus, the inhibition of cell proliferation at low concentrations is likely to be due to inhibition of cytokinesis as seen with TPA. Concomitantly, some differentiation of the cells occurs [26]. In CHO cell lines overexpressing the δ subspecies of protein kinase C, TPA also induces a block at G2/M due to inhibition of cytokinesis [24].

Multinucleation in response to *Clostridium difficile* toxin A has been observed in a human leukemic T-cell line, JURKAT [6]. This phenomenon was reported to be time- and dose-dependent, as was observed in our study with bistratene A. Continuing nuclear division in the absence of cytoplasmic division was also seen with the *Clostridium* toxin. The mechanism of action of *C. difficile* toxin A is not

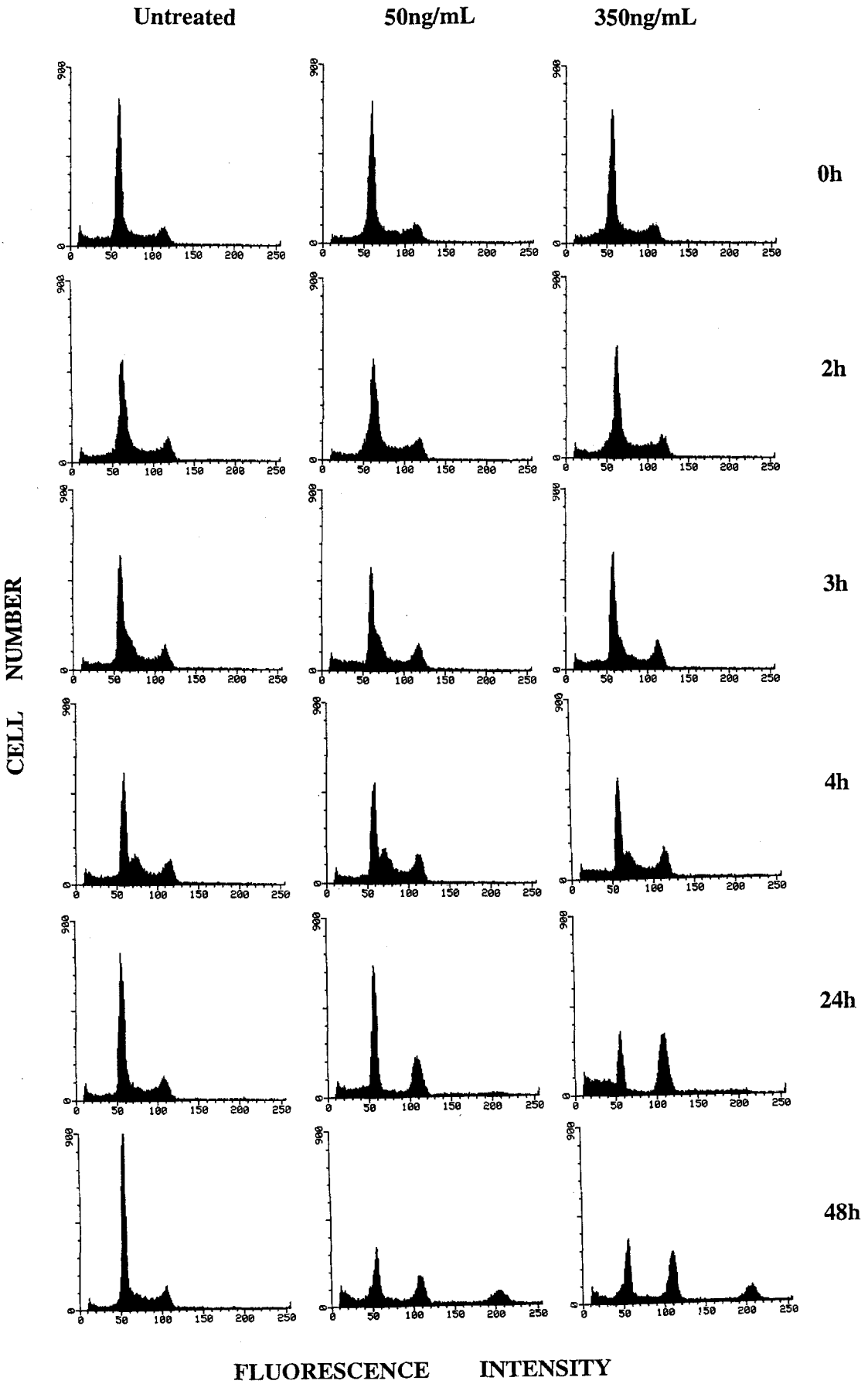

Fig. 7. Flow histograms of cells at various times after their release from mimosine block using high and low concentrations of bistratene A

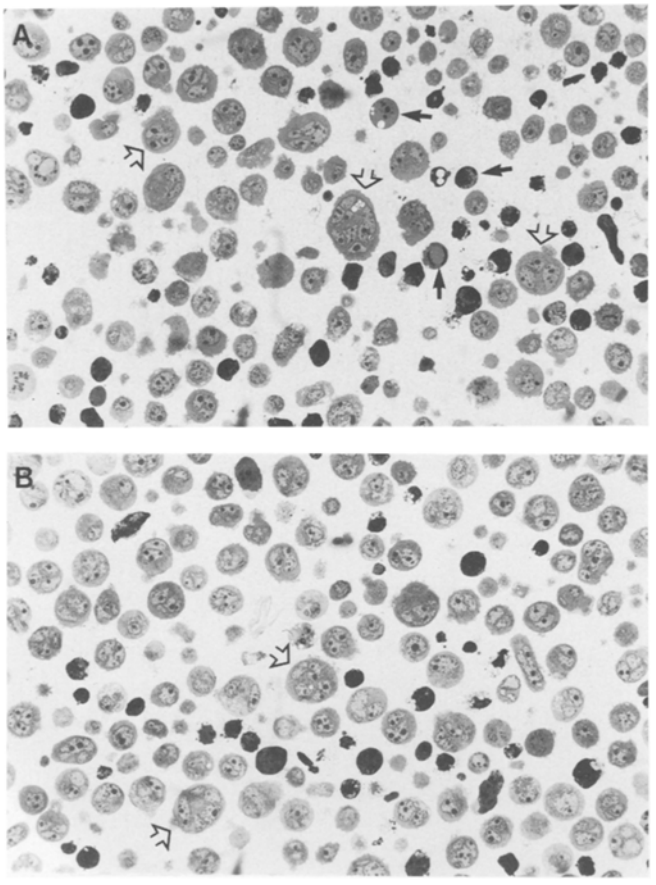

Fig. 8A–B. Toluidine blue-stained sections of cells treated with **A** bistratene A (71 n*M*) or **B** cycloxazoline (9.1 μ*M*). *Solid arrows*, cells undergoing apoptosis; *open arrows*, multinuclear cells.

known; however, it must be internalised for toxicity to occur, and one of the earliest events is disruption of the cytoskeleton, particularly the microfilament system. *C. difficile* toxin B has also been shown to induce multinucleation of transformed cells [21]. It disrupts the microfilament cytoskeleton and has been shown to affect the phosphorylation of cellular proteins [2]. Cytochalasin B, a fungal metabolite that disrupts the cytoskeleton, induces multinucleation through inhibition of contractile ring formation during mitosis [1]. Interferon treatment of Ehrlich ascites tumor cells has also been shown to result in a significant proportion of multinucleated cells through multiple mitoses and incomplete cytokinesis [15].

It is interesting to speculate on the mechanism for inhibition of cytokinesis. We have shown that bistratene A induces protein phosphorylation changes in HL-60 cells [28], and it is tempting to suggest that the compound alters the state of phosphorylation of proteins involved in cell-cycle control or cytokinesis. These proteins could be kinases, phosphatases or part of the cytoskeletal machinery. Altered phosphorylation of a microfilament component could conceivably lead to inability of the cell to divide properly. Bistratene A does have dramatic effects on the cytoskeleton (Watters et al., unpublished observations). In particular, actin microfilaments are disrupted within 15 min of exposure to nanomolar concentrations of the compound, most likely due to a phosphorylation event, although this remains to be proven. No evidence for an interaction of bistratene A with tubulin has been obtained. At present we have no data on protein phosphorylation changes or tubulin interaction in the presence of cycloxazoline, and further work is necessary before we can speculate on the possible mechanism of action for this compound.

Vinca alkaloids are widely used as anti-cancer drugs and bind tubulin directly, resulting in inhibition of polymerisation and block in the G2 phase of the cell cycle. These drugs have been shown to produce metaphase arrest and induce the formation of multinucleated interphase cells [11, 12]. Thus, there are at least two possible mechanisms by which multinucleated cells can occur: via a phosphorylation-type process as seen with phorbol ester or via direct binding to cytoskeletal components and interference with the dynamics of mitotic spindle and contractile ring formation. It is likely that the first mechanism applies to bistratene A in view of our previous data [28], and it remains to be determined which mechanism applies to cycloxazoline. Current work is aimed at investigating these possibilities.

Acknowledgements. The authors wish to thank Clay Winterford, Department of Pathology, University of Queensland, for the electron microscopy and Prof. Kay Ellem and Dr. Ian Taylor for helpful discussions. This work was supported by grants from the National Health Medical Research Council of Australia and the Queensland Cancer Fund.

References

1. Carter SB (1967) Effects of cytochalasins on mammalian cells. Nature 213: 261
2. Ciesielski-Treska J, Ulrich G, Baldacini O, Monteil H, Aunis D (1991) Phosphorylation of cellular proteins in response to treatment with *Clostridium difficule* toxin B and *Clostridium sordellii* toxin L. Eur J Cell Biol 56: 68
3. Crampton SL, Adams EG, Kuentzel SL, Li LH, Badiner G, Bhuyan BK (1984) Biochemical and cellular effects of didemnins A and B. Cancer Res 44: 1796
4. Degnan BM, Hawkins CJ, Lavin MF, McCaffrey EJ, Parry DM, Van den Brenk AL, Watters DJ (1989) Novel cytotoxic compounds from the ascidian *Lissoclinum bistratum*. J Med Chem 32: 1354
5. Dolbeare F, Gratzner H, Pallavicini MG, Gray JW (1983) Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. Proc Natl Acad Sci USA 80: 5573
6. Fiorentini C, Chow SC, Mastrantonio P, Jeddi-Tehrani M, Thelestam M (1992) *Clostridium difficile* toxin A induced multinucleation in the human leukemic T cell line JURKAT. Eur J Cell Biol 57: 292
7. Foster MP, Mayne CL, Dunkel R, Pugmire RJ, Grant DM, Kornprobst J-M, Verbist JF, Biard JF, Ireland CM (1992) Revised structure of bistramide A (bistratene A): application of a new program for the automated analysis of 2D INADEQUATE spectra. J Am Chem Soc 114: 1110
8. Gouiffes D, Moreau S, Helbecque N, Bernier JL, Henichart JP, Barbin Y, Laurent D, Verbist JF (1988) Proton nuclear magnetic study of bistramide A, a new cytotoxic drug isolated from *Lissoclinum bistratum* Sluiter. Tetrahedron 44: 451
9. Hambley TW, Hawkins CJ, Lavin MF, Van den Brenk A, Watters DJ (1992) Cycloxazoline: a cytotoxic cyclic hexapeptide from the ascidian *Lissoclinum bistratum*. Tetrehedron 48: 341
10. Hoffman BD, Hanauske-Abel HM, Flint A, Lalande M (1991) A new class of reversible cell cycle inhibitors. Cytometry 12: 26

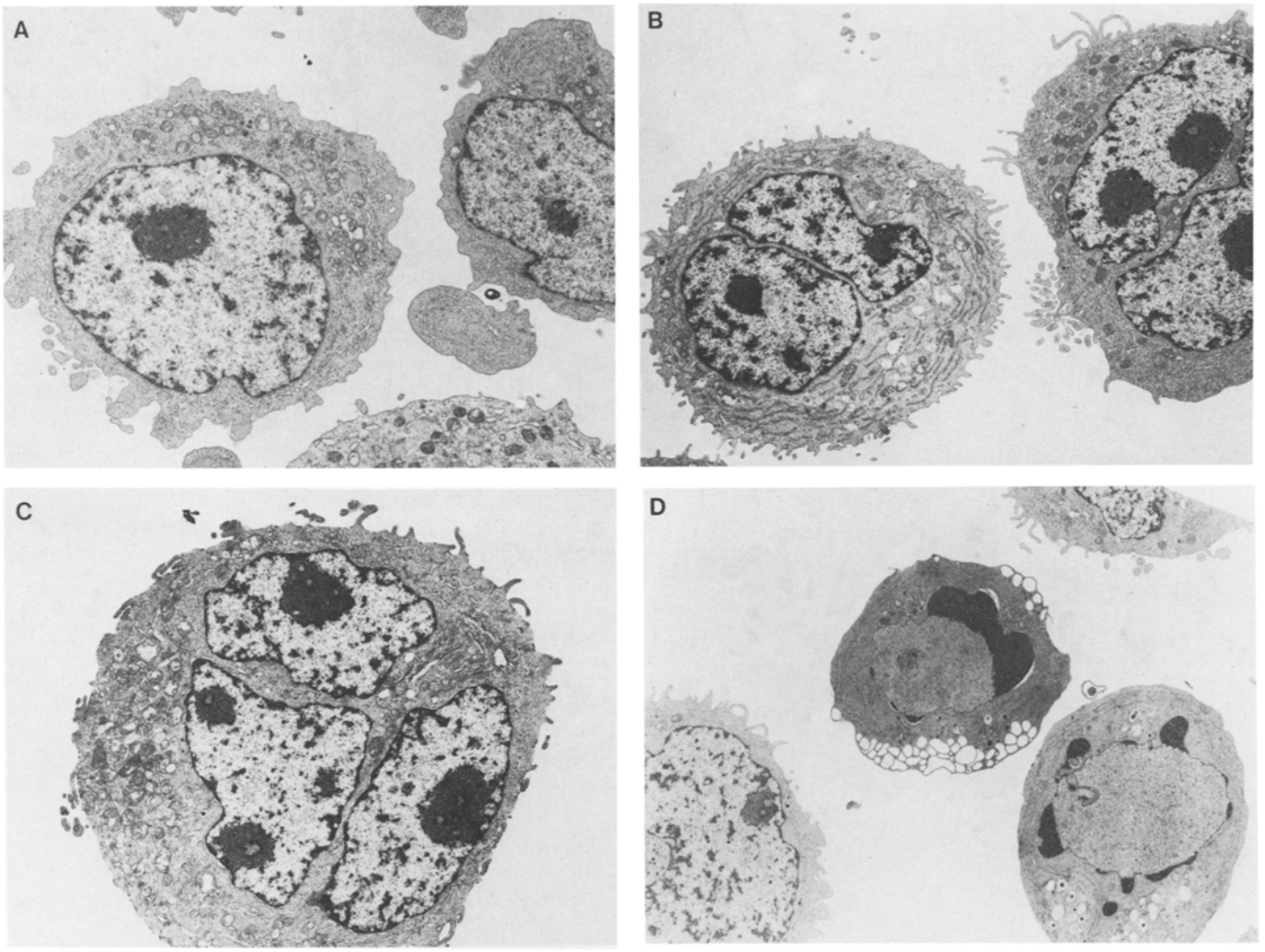

Fig. 9 A–D. Electron micrographs of HL-60 cells **A** untreated (×4000) or treated with **B** bistratene A (71 n*M*) for 48 h (×3000) or **C** cycloxazoline (9.1 μ*M*) for 30 h (×3000). **D** Apoptotic cells after cycloxazoline treatment (×2500)

11. Jordan MA, Thrower D, Wilson L (1991) Mechanism of inhibition of cell proliferation by *Vinca* alkaloids. Cancer Res 51: 2212
12. Kung AL, Zetterburg A, Sherwood SW, Schimke RT (1990) Cytotoxic effects of cell cycle phase specific agents: result of cell cycle perturbation. Cancer Res 50: 7307
13. Li LH, Timmins LG, Wallace TL, Krueger WC, Prairie MD, Im WB (1984) Mechanism of action of didemnin B, a depsipeptide from the sea. Cancer Lett 23: 279
14. Menaya J, Clemens MJ (1991) Phorbol-ester induced inhibition of proliferation of Daudi Burkitt's lymphoma cells by impairment of cytokinesis. Exp Cell Res 194: 260
15. Panniers LRV, Clemens MJ (1981) Inhibition of cell division by interferon: changes in cell cycle characteristics and in morphology of Ehrlich ascites tumour cells in culture. J Cell Sci 48: 259
16. Pettit GR (1991) The bryostatins. In: Herz W, Kirby GW, Steglich W, Tamm C (eds) Progress in the chemistry of organic natural products, vol 57. Springer, New York Berlin Heidelberg, p 153
17. Prinsep MR, Moore RE, Levine IA, Patterson GML (1992) Westiellamide, a bistratamide-related cyclic peptide from the blue-green alga *Westiellopsis prolifica*. J Nat Prod 55: 140
18. Rinehart KL, Gloer JB, Cook JC, Mizsak SA, Scahill TA (1981) Structures of the didemnins, antiviral and cytotoxic depsipeptides from a Caribbean tunicate. J Am Chem Soc 103: 1857
19. Roussakis C, Robillard N, Riow D, Biard JF, Pradal G, Piloquet P, Debitus C, Verbist JF (1991) Effects of bistramide A on a non-small-cell bronchial carcinoma cell line. Cancer Chemother Pharmacol 28: 283
20. Shin DM, Holoye PY, Murphy WK, Forman A, Papasozomenos SC, Hong WK, Raber M (1991) Phase I/II clinical trial of didemnin B in non-small-cell lung cancer: neuromuscular toxicity is dose-limiting. Cancer Chemother Pharmacol 29: 145
21. Shoshan MC, Åman P, Skog S, Florin I, Thelestam M (1990) Microfilament-disrupting *Clostridium difficile* toxin B causes multinucleation of transformed cells but does not block capping of membrane Ig. Eur J Cell Biol 53: 357
22. Song Q, Baxter GD, Kovacs EM, Findik D, Lavin MF (1992) Inhibition of apoptosis in human tumour cells by okadaic acid. J Cell Physiol 153: 550
23. Taylor IW (1980) A rapid single step staining technique for DNA analysis by flow microfluorimetry. J Histochem Cytochem 28: 1021
24. Watanabe T, Ono Y, Taniyama Y, Hazama K, Igarashi K, Ogita K, Kikkawa U, Nishizuka Y (1992) Cell division arrest induced by phorbol ester in CHO cells overexpressing protein kinase C-δ subspecies. Proc Natl Acad Sci USA 89: 10159

25. Watson PA, Hanauske-Abel HH, Flint A, Lalande M (1991) Mimosine reversibly arrests cell cycle progression at the G1-S phase border. Cytometry 12: 242
26. Watters DJ, Marshall K, Hamilton S, Michael J, McArthur M, Seymour G, Hawkins C, Gardiner R, Lavin MF (1990) The bistratenes: new cytotoxic marine macrolides which induce some properties indicative of differentiation in HL-60 cells. Biochem Pharmacol 39: 1609
27. Watters DJ, Hawkins CH, Lavin MF (1992a) Toxins from sea squirts. In: Watters D, Lavin M, Maguire D, Pearn J (eds) Toxins and targets. Harwood Academic Publishers, Chur, Switzerland, p 35
28. Watters DJ, Michael J, Hemphill JE, Hamilton SE, Lavin MF, Pettit GR (1992b) Bistratene A: a novel compound causing changes in protein phosphorylation patterns in human leukemia cells. J Cell Biochem 49: 417