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Research paper

The potent microtubule-stabilizing agent (+)-discodermolide induces apoptosis in human breast carcinoma cells—preliminary comparisons to paclitaxel

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(+)-Discodermolide, a sponge-derived natural product, stabilizes microtubules more potently than paclitaxel despite the lack of any obvious structural similarities between the drugs. It competitively inhibits the binding of paclitaxel to tubulin polymers, hypernucleates microtubule assembly more potently than paclitaxel, and inhibits the growth of paclitaxel-resistant ovarian and colon carcinoma cells. Because paclitaxel shows clinical promise for breast cancer treatment, its effects in a series of human breast cancer cells were compared to those of (+)-discodermolide. Growth inhibition, cell and nuclear morphological, and electrophoretic and flow cytometric analyses were performed on (+)-discodermolide-treated MCF-7 and MDA-MB231 cells. (+)-Discodermolide potently inhibited the growth of both cell types ($IC_{50} < 2.5$ nM) at concentrations similar to those observed with paclitaxel. Complete inhibition of growth occurred with 10 nM or greater of each drug and was not reversed by removal. (+)-Discodermolide-treated cells exhibited condensed and highly fragmented nuclei. Flow cytometric comparison of cells treated with either drug at 10 nM, a concentration well below that achieved clinically with paclitaxel, showed both caused cell cycle perturbation and induction of a hypodiploid cell population. (+)-Discodermolide caused these effects more extensively and at earlier time points. The timing and type of high molecular weight DNA fragmentation induced by the two agents was consistent with induction of apoptosis. The results suggest that (+)-discodermolide has promise as a new chemotherapeutic agent against breast and other cancers. [© **1998 Rapid Science Ltd.**]

Key words: Apoptosis, discodermolide, flow cytometry, Hoechst 33342, paclitaxel, pulsed field gel electrophoresis.

Introduction

The antimitotic taxoids, paclitaxel (Taxol®) and docetaxel (Taxotere®), show great chemotherapeutic potential, and are currently in clinical use for the treatment of breast, ovarian and other cancers.[1,2] In contrast to most antimitotic agents, which inhibit the assembly of tubulin to microtubules, taxoids both enhance the stability of assembled tubulin and hypernucleate microtubule assembly, often under unfavorable conditions. We have recently found that the polyhydroxylated, polymethylated, C24:4 fatty acid lactone carbamate (+)-discodermolide, derived from the Caribbean sponge *Discodermia dissoluta*,[3] inhibits mitosis, induces formation of spectacular microtubule bundles in breast carcinoma cells, causes microtubule assembly under conditions in which paclitaxel is inactive and is the most potent known *in vitro* microtubule stabilizing agent discovered to date.[4] Further studies have shown that (+)-discodermolide competitively inhibits the binding of paclitaxel to tubulin polymers, enhances tubulin nucleation reactions more potently than paclitaxel, and inhibits the growth of paclitaxel-resistant (through altered β-tubulin expression) and multidrug-resistant P-

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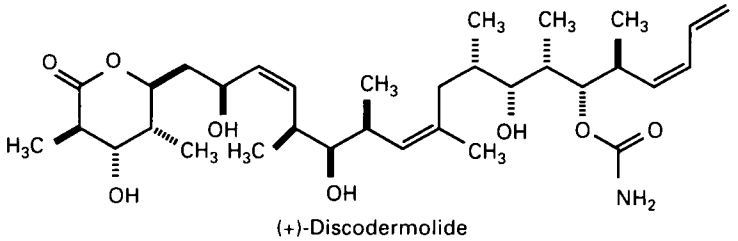
glycoprotein overexpressing ovarian and colon carcinoma cells.[5]

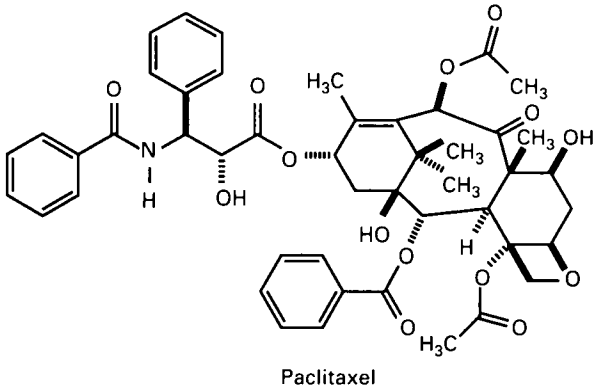
The microtubule stabilizing activity of (+)-discodermolide was discovered as a direct result of our computational studies on tubulin polymerization perturbing agents.[6,7] In an on-going effort to develop quantitative structure-activity relationships (QSARs) for such compounds and to find new potent antimitotic agents useful in the treatment of breast and other cancers, large, unrelated structural data bases were surveyed and the QSAR equations were used to predict antitubulin activity in previously described compounds not known to have this property. (+)-Discodermolide was predicted to have such antitubulin activity, and the responsible substructural features found in it were the same as those that led to accurate computational predictions of potent activity for taxoids and the only other known class of microtubule stabilizing agents, the epothilones.[7,8]

In the 5 years between the first report of (+)-discodermolide and the discovery of its microtubule stabilizing effects, it was shown to have apparent immunosuppressive action,[9-11] with potent antiproliferative activity. Growth IC_{50} values in the low nanomolar range have been observed with the drug in various cell lines—murine lymphoid, fibroblast, bone marrow stromal and T hybridoma cells, and human lymphoid, foreskin fibroblasts and osteosarcoma cells—as has its ability to arrest the hybridoma and osteosarcoma cells in G_2/M.[3,9-12] Each of these results is consistent with its likely mechanism of action against tumor cells—kinetic stabilization of spindle microtubules—which is also the chemotherapeutic mechanism of paclitaxel.[13,14] More recently, the antitubulin findings have been confirmed and further elaborated to show that (+)-discodermolide competes for the same binding site on microtubules as paclitaxel, and has the higher affinity of the two compounds.[5,15] The G_2/M block induced by (+)-discodermolide in human osteosarcoma cells is accompanied by delayed degradation of cyclin B_1 resulting in formation of micronuclei,[15] an observation predicted by our computational studies.[6]

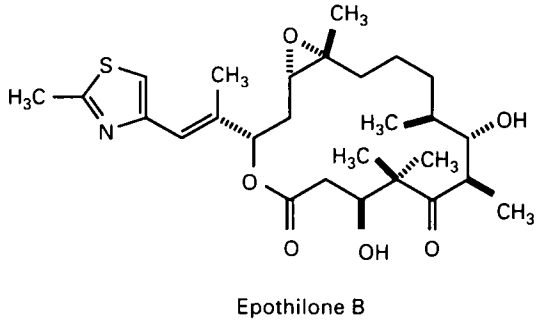
One important further difference noted between (+)-discodermolide and paclitaxel is that the former is at least 100-fold more water soluble.[4] Computational studies predict the aqueous solubility of (+)-discodermolide to be about 800 μM. The Pharmaceutical Resources Branch of the National Cancer Institute has determined the aqueous solubility of paclitaxel to be only 6-11 μM. Supplies of discodermolide thus far have been inadequate to perform exact measurements of its solubility, but as we observed during preparation of solutions used in these experiments, it is quite obviously considerably more water soluble than paclitaxel in our hands. Thus, a similar or like mechanism of action coupled with its greater water solubility in comparison to paclitaxel suggest that (+)-



(+)-Discodermolide



Paclitaxel



Epothilone B

discodermolide has potential clinical utility. Results from animal studies additionally show that it is not overtly toxic to mice.[10,11]

Herein we describe results of morphological, cell proliferation, electrophoretic and flow cytometric analyses that strongly indicate that (+)-discodermolide causes breast carcinoma cell death by the induction of apoptosis. (+)-Discodermolide caused nuclear condensation, DNA fragmentation and other hallmarks of apoptosis. Direct comparison of equimolar doses of paclitaxel and (+)-discodermolide was made. The results given here represent a survey performed within a confined scope of the actions of (+)-discodermolide. Since it is isolated from a Caribbean sponge, the supply of purified compound to date has been limited. A successful approach to its synthesis has been reported,[12,16,17] but synthetic material has yet to become available. Hence, this and our previous study of (+)-discodermolide's activity against breast cancer cells were necessarily restricted, as the experiments comprising them were performed with the 1 mg of compound available to us.

Materials and methods

Materials

(+)-Discodermolide was a generous gift from the Harbor Branch Oceanographic Institution. Paclitaxel was provided by the Drug Synthesis & Chemistry Branch of the National Cancer Institute. Both compounds were of 98% or greater purity as determined by C_{18} HPLC-diode array UV-electrospray mass spectrometric analyses. Drugs were dissolved in DMSO and control samples contained vehicle equivalent to that in drug-treated cultures. MCF-7 cells (28th passage) were a gift from Dr Marc Lippman (Vincent T Lombardi Cancer Center). MDA-MB231 cells were purchased from the American Type Culture Collection. IMEM culture medium was purchased from Biofluids. Fetal bovine serum (FBS) was from Hyclone. DMEM culture medium, Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS), glutamine, Trypan blue dye, the 123 bp DNA marker ladder standard and trypsin were obtained from Gibco/BRL Life Technologies. InCert and Pulsed Field Certified agarose were purchased from FMC and BioRad. Proteinase K was obtained from Boehringer Mannheim. *Saccharomyces cerevisiae* chromosomes and lambda phage ladders were from New England Biolabs. Cytological stains were from Fisher Scientific. All other chemicals and antibodies were purchased from Sigma.

Inhibition of cell growth

Cells were maintained in DMEM containing 5% FBS and 2 mM glutamine prior to experimentation. Growth curves were determined as described previously.[4,18] Cells were plated in triplicate in IMEM (without phenol red) with 2.5 - 5% FBS (no difference was noted). After a 24 h attachment period, cells were grown in the presence of test compounds or vehicle. At given time points, medium with non-adherent cells was removed and adherent cells were detached with trypsin-EDTA. The cells were combined, centrifuged, resuspended in HBSS and an aliquot was mixed with an equal volume of 0.08% Trypan blue in HBSS. After a 10 min period to allow for dye uptake in non-viable cells, cell counts were made on dye-excluding viable cells microscopically on a hemacytometer (in blinded triplicate from each sample; therefore $n = 9$).

Cytological and nuclear morphological analyses

For cytological assays, treated and control (attached and floating) cells were separately fixed in 4% PBS-buffered formalin and stained with May-Grünwald for 10 min followed with Wright-Giemsa for 20 min.[19] One thousand cells were scored from each sample for nuclear condensation and DNA fragmentation. Cells were also stained with the fluorescent dye Hoechst 33342 for analysis of nuclear morphology and for identification of apoptotic bodies.[20] Floating and attached cells were separately fixed in 70% ethanol for 5 min, washed twice for 5 min with HBSS or 100 mM phosphate-buffered normal saline (PBS, prepared from Ca^{2+}- and Mg^{2+}-free water), then stained with 20 μg/ml Hoechst 33342 in PBS for 10 min to 1 h at room temperature. Cells were washed twice with PBS and analyzed by fluorescence microscopy.

Flow cytometry

Cellular DNA content was quantified by flow cytometric determination of propidium iodide intercalated into DNA of intact, fixed cells. Total cells (floating and attached) were collected, resuspended in HBSS, fixed in 70% ethanol (4°C for 30 min or longer, usually overnight), pelleted by centrifugation and the cell pellet was resuspended in 24:1 (v/v) 0.2 M Na_2HPO_4-0.1 M citric acid, pH 7.8, for 30 min at room temperature.[21] Cells were pelleted by centrifugation, treated with RNase A (100 μg/ml) and propidium iodide (100 μg/ml) for 30 min at 37°C, and stored at 4°C until

analyzed. DNA content was determined on a Becton-Dickinson FACScan flow cytometer equipped with a 488 nm argon laser by measuring forward and orthogonal light scatter, and peak and area red fluorescence. Cell cycle populations were quantified from a standard count of 10 000 cells using the Lysys II program.

Electrophoretic analyses of cellular DNA

Plugs for electrophoretic analyses were each made with 1×10^6 cells. Total cells were suspended in 10 mM Tris containing 20 mM NaCl and 50 mM EDTA, pH 7.2, equilibrated at 50 C. An equal volume of InCert agarose was added and plugs were formed in LKB Bromma molds. After solidification at 4 C, plugs were incubated overnight at 50 C in 4.8 mM sodium deoxycholate, 42 mM sodium dodecylsulfate, 10 mM EDTA, pH 8.0, containing 500 μg/ml Proteinase K, then washed four times with 50 ml of 20 mM Tris, 50 mM EDTA, pH 8.0. Static field gel electrophoretic analysis was performed on cell plugs loaded into slots of a 1.5% agarose gel and sealed in with molten agarose. Electrophoresis was performed in TBE buffer (89 mM Tris, 50 mM boric acid, 2 mM EDTA) at 75 V constant current for 6 h. A standard DNA marker ladder (123 bp multiples) was included in each gel. Gels were stained with ethidium bromide (1 μg/ml) and then visualized by UV transillumination. Pulsed field gel electrophoretic analysis of DNA from 10^6 cells per lane was performed on a LKB Bromma PulsaPhor Electrophoresis unit in 1.5% Pulsed Field Certified Agarose in TBE buffer at 10 C. Electrophoresis parameters optimized for 30–70 kb fragments were 60–100 s pulses for 40 h at 180 V (6 V/cm) from a LKB Bromma 2015 Pulsaphor Plus control unit. Marker lanes were loaded with *S. cerevisiae* chromosomes (225–1900 kb) and lambda phage ladders (48.5–1018 kb). After electrophoretic separation, gels were stained with ethidium bromide (1 μg/ml) for 1 h and then visualized with a UV transilluminator. Gel images were collected and digitized with a UVP Image-Store 5000 gel data documentation system. Band densities were estimated with the NIH Image (version 1.60) software.

Results

(+)-Discodermolide and paclitaxel have similar growth inhibitory effects on breast cancer cells

The growth inhibitory effects of (+)-discodermolide on human breast cancer cells were studied in comparison to those of paclitaxel. As we reported previously,[4] similar data were noted with the two drugs in estrogen receptor positive/estrogen responsive MCF-7 and estrogen receptor negative/estrogen non-responsive MDA-MB231 cells. The IC_{50} values obtained for (+)-discodermolide after 48 h of continuous exposure to the drug were 2.4 ± 0.3 nM for MCF-7 cells and 1.8 ± 0.5 nM for MD-MB231 cells, not significantly different from the 2.1 ± 0.2 and 2.0 ± 0.4 nM values, respectively, obtained for paclitaxel. Proliferation of the two cell lines was completely inhibited by either drug at 10 nM (i.e. growth curves with slopes of zero), apparently through pure cytostatic effects, as essentially no Trypan blue positive cells were observed at this or lower concentrations. Evidence of weak (10% or less) cell killing effects occurred at drug concentrations greater than 10 nM when exposure times were 48 h or longer.

The reversibility of the antiproliferative effects of (+)-discodermolide and paclitaxel was also studied. Cell numbers were determined after incubation with the drugs for 36 h at 1–100 nM, and at 12, 24 and 48 h after removal of the drugs by three washes with FBS-containing medium (floating and dislodged cells were returned to the cultures). The growth inhibitory effects of the drugs at 10 nM or higher were not reversed after the extensive washing of treated cells (data not shown). After exposure to 1 nM of either drug followed by washout, both cell lines slowly resumed growth, but never at the same rate as in control cultures.

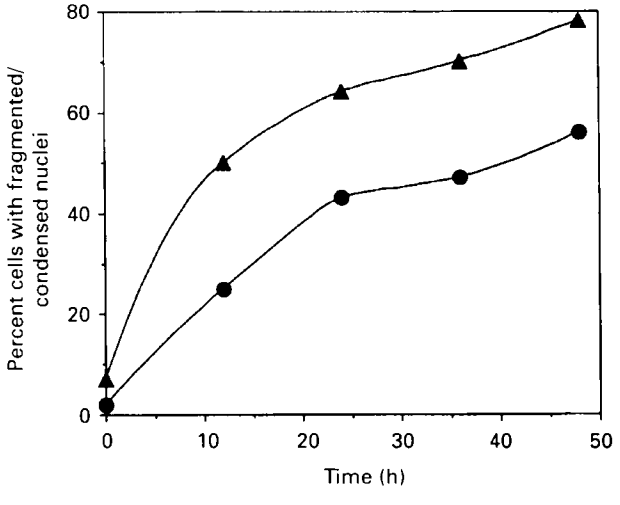


Figure 1. Percent of cells with fragmented or condensed nuclei after treatment with 100 nM (+)-discodermolide. Treated and control cells were fixed in buffered formalin, stained with May–Grunwald followed by Wright–Giemsa, and examined microscopically. Each point resulted from scoring 1000 cells: ●, MCF-7 cells; ▲; MDA–MB231 cells.

(+)-Discodermolide induces cytological and nuclear morphological changes indicative of apoptosis

Prior to our determination of the growth inhibitory concentrations of the drug, the morphological changes induced by continuous exposure to 100 nM (+)-discodermolide, a concentration near that achieved clinically for paclitaxel, were examined. Cells from both lines were grown in the presence of vehicle or 100 nM drug for 48 h, and examined microscopically after formalin fixing and staining with a May-

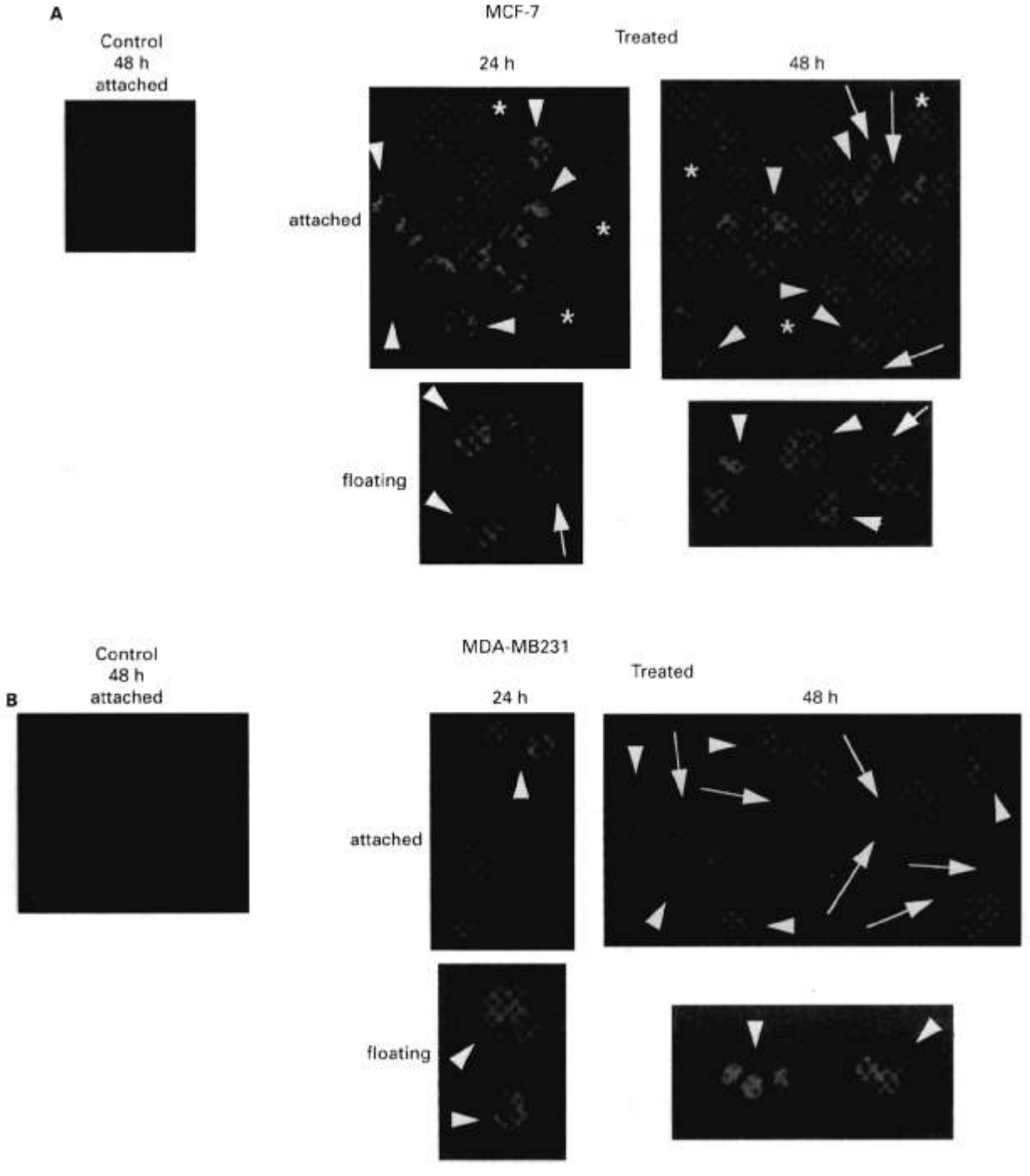


Figure 2. Perturbation of nuclear morphology and mitotic block induced by 10 nM (+)-discodermolide. Effects were visualized by fluorescence microscopic analysis of Hoechst 33342-stained MCF-7 (A) and MDA-MB231 (B) cells. Asterisks in treated cell panels denote normal appearing nuclei, arrowheads denote punctate nuclei and arrows point to apoptotic bodies.

Grünwald, Wright-Giemsa protocol.[19] Floating and attached cells were examined separately because drug concentrations above 10 nM caused a slight but significant increase of detached cells as compared to control cultures. It was apparent that the compound rapidly induced nuclear condensation and fragmentation, shrinkage of many cells, and micronucleus formation in some of the cells. One thousand cells were scored from each sample for these effects (Figure 1). The MDA-MB231 line was the more affected.

After determining that 10 nM of either drug caused full cytostasis, the remaining experiments were carried out at this concentration. Changes in nuclear morphology were examined next. Cells were treated with 10 nM (+)-discodermolide or an equivalent volume of vehicle, stained with the fluorescent DNA dye Hoechst 33342[20] and photographed. Examples of the perturbations of nuclear morphology and mitotic block induced by the drug are shown in Figure 2. Both the attached and the few detached cells were examined. Nuclei in control cells remained ovoid and intact throughout the experiment (nuclei with appearances equivalent to those seen in control cultures are denoted by asterisks in panels of drug-treated cells in the figure). Floating control cells were invariably mitotic (not shown). At both the time points examined, the vast majority of (+)-discodermolide-treated MCF-7 (Figure 2A) and MDA-MB231 (Figure 2B) cells, whether attached or detached, showed evidence of nuclear pyknosis (arrowheads) and fragmentation, all indications of apoptosis. Further, apoptotic bodies were evident in both lines at 48 h (arrows). Attached cells exhibited mitotic-like nuclei with points of dense staining at early (up to 24 h) time points suggestive of multiple microtubule organizing centers or spindle poles (Figure 2B). The nuclei in these cells progressed to a punctate but irregular staining pattern at 48 h, when apoptotic bodies also became readily apparent. Apoptotic bodies were most evident in attached cells.

(+)-Discodermolide more potently induces hypodiploidy than paclitaxel

The morphological changes effected by (+)-discodermolide suggested the agent was causing apoptosis in the breast cancer cells. Again, since based on the growth curves, 10 nM appeared to be sufficient for examination of the full cytostatic effects of the two compounds, this concentration was used in all following experiments. Cells were grown for 96 h in continuous presence of the compounds at this concentration and viable cell numbers were determined every 24 h. In both cell lines, the percentage of floating cells resulting from drug treatment was the same as in control cultures.

In addition to the microscopic techniques used above, there are several other methods that have been used to probe for the induction of apoptosis. Since part of the apoptotic process includes expression of one or more nucleases that cleave the DNA into regularly sized fragments that may or may not escape the cell before it loses membrane integrity, flow cytometric analysis of a population of cells treated with an apoptosis-inducing drug can sometimes reveal the appearance of a transient hypodiploid population during apoptosis. The breast cancer cell lines were examined for this effect after treatment with 10 nM (+)-discodermolide or 10 nM paclitaxel and simultaneously for accumulation into G_2/M as per previous reports on other cell lines tested with the two agents.[9-13]

Cells from complete cultures (attached cells combined with the few floating cells) were examined for DNA content by propidium iodide flow cytometric analysis at 24 h increments. Data was collected so that the *x*-axes (fluorescence/event) of the resulting DNA histograms could be displayed on a log scale. This was done to enhance detection and characterization of hypodiploid cell populations. Careful gating was employed to remove any trace of debris from the analyses. As can be seen in the example DNA histograms at 96 h of treatment shown in Figure 3(A), the cell cycle distribution of both cell lines was dramatically affected by the two drugs. The pattern of perturbation is easily recognizable as accumulation into G_2/M in response to both drugs in MCF-7 cells. The hypodiploid cell population in these cell lines appears as a shoulder on the G_1 peak. When there is a substantial population of hypodiploid cells, the G_1 peak is obscured. Careful quantitation, however, of the data from MDA-MB231 cells demonstrated that a significant G_2/M pileup occurred in this cell line in response to both drugs as well.

Figure 3(B) depicts the accumulation into hypodiploid populations caused by the drugs in the two cell lines over the course of the 96 h experiment. As stated above, the hypodiploid cell population in each line appeared immediately adjacent to the G_1 peak, averaging 20–29% (MDA-MB231) and 30–40% (MCF-7) of the DNA content of the G_1 population, with a broad distribution including cells with DNA content as small as 1% of G_1. Given this broad distribution, it is clear that the hypodiploid peak overlaps with the G_1 peak and therefore the quantities given in Figure 3(A) are an underestimation of the total hypodiploid population. This distribution is also consistent with apoptosis occurring in cells which cannot overcome the G_2/M block. The percentage of hypodiploid cells

in control cultures was similar in both cell lines and ranged from 1 to 5% during the 4-day measurement period, with no evidence of increase with time in culture. Hypodiploid cell populations appeared after 24 h in the MCF-7 and MDA-MB231 cells in response to both agents, and these populations grew over the course of the experiment. (+)-Discodermolide showed an advantage over paclitaxel in producing this effect.

Both (+)-discodermolide and paclitaxel cause high molecular weight DNA fragmentation at 10 nM

Since the flow cytometric studies indicated that both drugs caused loss of DNA from cells, static field agarose gel electrophoretic analyses were performed to determine if internucleosomal DNA fragments

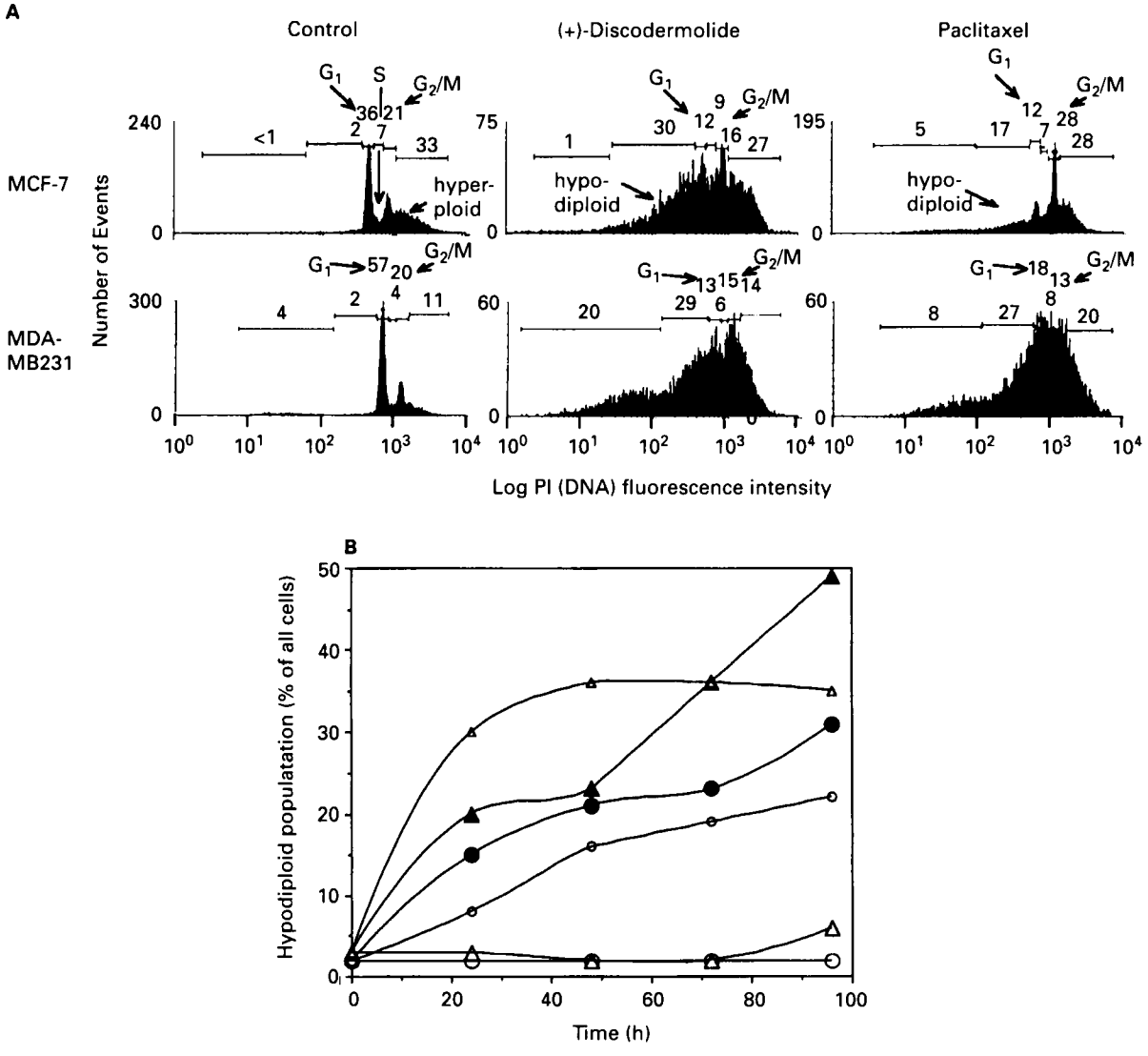


Figure 3. Flow cytometric analysis of MCF-7 and MDA-MB231 DNA content after treatment with vehicle, 10 nM (+)-discodermolide or 10 nM paclitaxel. (A) Example DNA histograms from the cell lines after 96 h of treatment. Ethanol-fixed cells were treated with RNase A and propidium iodide, and DNA content was determined with a 488 nm argon laser by measuring forward and orthogonal light scatter, and peak and area red fluorescence. Cell cycle populations were quantified from a standard count of 10 000 cells. Numbers above bars in the histograms give the percentage of cells in that region. Note that data were collected so that the histograms are presented with log scale *x*-axes (fluorescence/event). The hyperploid cell populations seen in both cell lines center on octaploid DNA content and are routinely observed in established tumor cell lines. (B) Accumulation of cells into hypodiploid populations during treatment for 96 h. Control cells (larger open symbols): MCF-7 (○) and MDA-MB231 (△). Paclitaxel-treated cells (smaller open symbols): MCF-7 (○) and MDA-MB231 (△). (+)-Discodermolide-treated cells (closed symbols): MCF-7 (●) and MDA-MB231 (▲).

(multiples of 100–200 bp), one of the earliest recognized hallmarks of apoptosis, were formed. Such 'ladders' were not detected during short (6 days) courses of low dose (10 nM) treatment with either drug.

The morphological and flow cytometric evidence

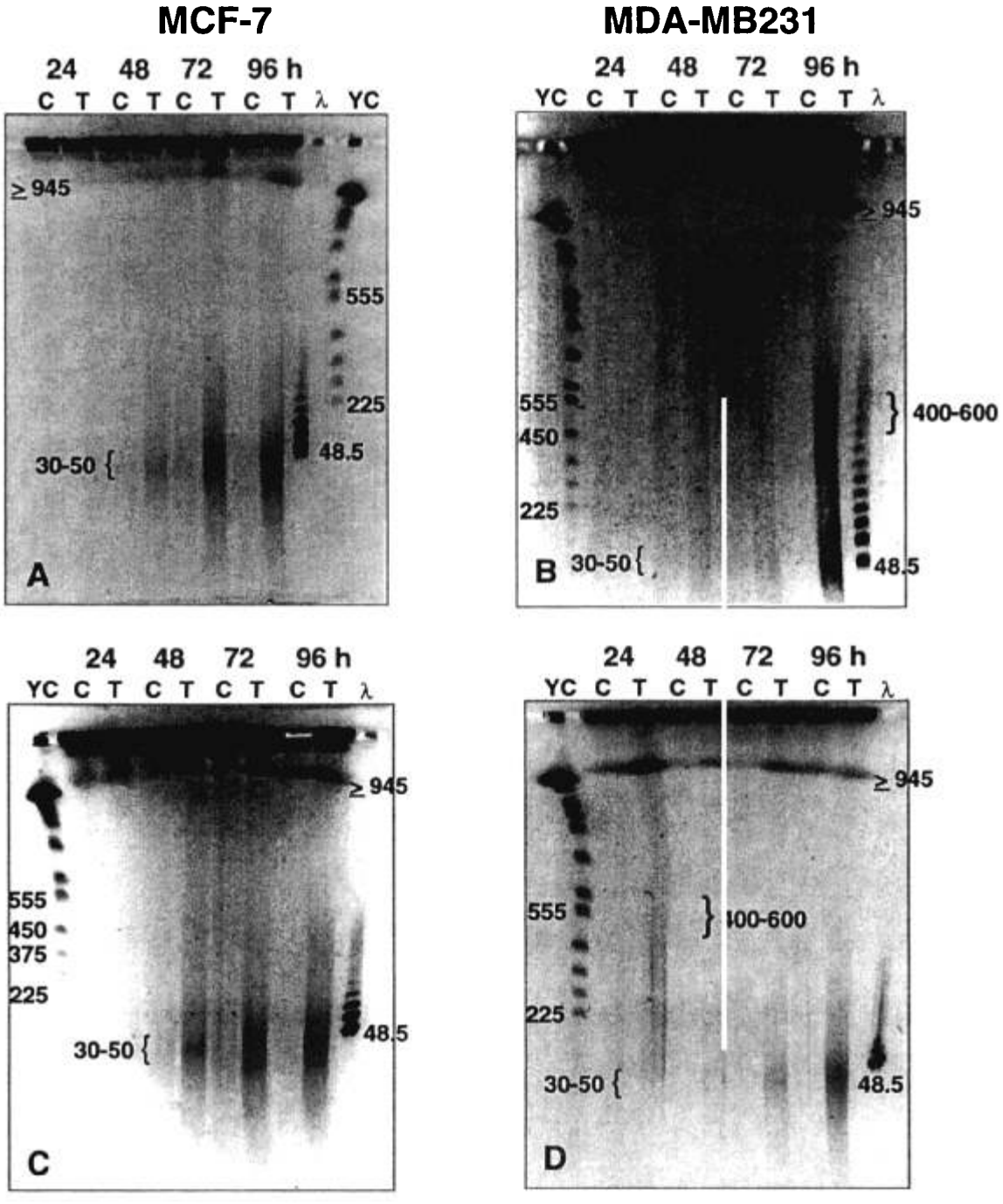


Figure 4. Timing and type of DNA fragmentation induced by 10 nM (+)-discodermolide (A and B) and 10 nM paclitaxel (C and D) in MCF-7 (A and C), MDA-MB231 (B and D) cells. High molecular weight fragments were detected by PFGE. Low molecular weight fragments were undetectable by normal, static field electrophoresis (not shown). All gels were 1.5% agarose. C, control cells; T, treated cells; λ, lambda phage markers; YC, *S. cerevisiae* chromosome markers. Gels are labeled at points to which the indicated markers migrated and at the regions where drug-induced fragments were noted. Equal numbers of cells were loaded in all of the lanes in the gels shown; thus, any apparent differences in densities between gels are an artefact of different photographic exposure periods.

pointed to induction of apoptosis even in the absence of internucleosomal fragmentation, however, so the DNA from cells treated with the drugs or vehicle was instead analyzed for the appearance of high molecular weight fragments, another indicator of apoptosis,[22,23] by pulsed field gel electrophoresis (PFGE) in order to account for the hypodiploid cells noted. DNA from 10^6 cells was examined in each lane (Figure 4). The timing and type of high molecular weight DNA fragmentation induced by the two agents was nearly identical in the MCF-7 line (Figure 4A and C), where larger than 1 Mb and 30–50 kb fragments formed at 2 days, and in the MDA-MB231 line (Figure 4B and D), where larger than 1 Mb fragments formed after 1 day, and 400–600 and 30–50 kb fragments were clearly evident after 4 days of continuous exposure to either agent. Densitometric estimation, based on the ratio of intensities of fragment band(s) to the sum of intensities of a DNA in a given lane, showed (+)-discodermolide to be approximately 2-fold more potent than paclitaxel.

Discussion

Both the flow cytometric and electrophoretic analyses indicate treatment of human breast carcinoma cells with 10 nM (+)-discodermolide or 10 nM paclitaxel induces nuclear fragmentation and specific chromatin digestion, suggesting an enzyme-catalyzed response to the drugs that results in hypodiploid cells, i.e. apoptosis. McCloskey *et al.* recently reported observation of high molecular weight DNA fragments by field inversion gel electrophoresis in MCF-7 and MDA-MB231 cells treated with 100 nM paclitaxel.[24] In this study, we observed that each drug at 10 nM induced similar types of DNA fragmentation in the cells, but with some differences. (+)-Discodermolide caused more large (larger than 1 Mb) DNA fragments to form in MDA-MB231 cells, but at a later time point than paclitaxel did. Additionally, (+)-discodermolide caused 400–600 kb fragment formation in both MDA-MB231 cells at day 4 of treatment, whereas paclitaxel apparently did so only weakly. In the two breast cancer cell lines studied, (+)-discodermolide appeared to be 2-fold more potent at causing large molecular weight DNA fragmentation than an equimolar dose of paclitaxel. Although the effects we saw with paclitaxel were quite reproducible, the data on (+)-discodermolide reported in this communication come from single experiments. Thus, accurate determinations of differences in potencies between the two drugs must await a more abundant supply of (+)-discodermolide.

We did not detect internucleosomal ladder formation in the breast cancer cells during 6 days of continuous exposure to 10 nM (+)-discodermolide or 10 nM paclitaxel. Others have shown some evidence of internucleosomal DNA fragment formation, likely resulting from a Ca^{2+}/Mg^{2+}-dependent endonuclease, in MCF-7 or MDA-MB231 cells in response to drugs, but only after prolonged treatment with DNA-damaging agents such as etoposide (8 days),[25] treatment with high doses of tamoxifen[26] or treatment with agents exerting direct physical effects on DNA like polyamines.[27] Published data[22,24–28] and our unpublished results indicate that internucleosomal DNA fragment formation in these two and other breast carcinoma cell lines is a late, difficult-to-observe event. It is also associated with treatments that cause a large percentage of detached cells, an event not observed after treatment with 10 nM (+)-discodermolide or paclitaxel.

In summary, (+)-discodermolide is highly cytostatic to breast cancer cells at low nanomolar concentrations and causes apoptosis in these cells. As gauged by flow cytometric analysis for hypodiploid cells and by PFGE analysis for high molecular weight DNA fragments, it is a more potent apoptosis-inducing agent on a molar basis than paclitaxel. Analysis of effects on tubulin assembly *in vitro*, on cellular microtubule arrays, on the growth of cells with altered expression of β-tubulin isoforms and the multidrug resistance phenotype,[4,5] as well as the high molecular weight DNA fragmentation kinetics presented here, show that the two compounds likely differ in their potencies and effects. Because of its mechanism of action, (+)-discodermolide holds promise as a new therapeutic agent for diseases such as breast cancer and other neoplasms, especially considering it has considerably greater water solubility than paclitaxel and cross-resistance has yet to be observed. Based on its unique structure and interesting physical and biological properties, more detailed studies of the actions of (+)-discodermolide appear warranted. As the compound becomes more available, determination of additional differences between its actions and those of the taxoids may be found. Furthermore, (+)-discodermolide is a structurally unique template for synthetic elaboration that could lead to improved compounds with microtubule-stabilizing properties.

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