Suppression of microtubule dynamics by discodermolide by a novel mechanism is associated with mitotic arrest and inhibition of tumor cell proliferation

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Abstract
Discodermolide is a new microtubule-targeted drug in Phase I clinical trials that inhibits tumor growth and induces G2-M cell cycle arrest. It is effective against paclitaxel-resistant cell lines and acts synergistically in combination with paclitaxel. Suppression of microtubule dynamics by microtubule-targeted drugs has been hypothesized to be responsible for their ability to inhibit mitotic progression and cell proliferation. To determine whether discodermolide blocks mitosis by an effect on microtubule dynamics, we analyzed the effects of discodermolide on microtubule dynamics in living A549 human lung cancer cells during interphase at concentrations that block mitosis and inhibit cell proliferation. We found that discodermolide (7–166 nM) significantly suppressed microtubule dynamic instability. At the IC50 for proliferation (7 nM discodermolide, 72 h), overall dynamicity was reduced by 23%. The principal parameters of dynamic instability suppressed by discodermolide were the microtubule shortening rate and length shortened. In addition, discodermolide markedly increased the frequency of rescued catastrophes. At the discodermolide concentration that resulted in 50% of maximal mitotic block (83 nM, 20 h), most microtubules were completely non-dynamic, no anaphases occurred, and all spindles were abnormal. The dynamicity of the remaining dynamic microtubules was reduced by 62%. The results indicate that a principal mechanism of inhibition of cell proliferation and mitotic block by discodermolide is suppression of microtubule dynamics. Importantly, the results indicate significant additional stabilizing effects of discodermolide on microtubule dynamics as compared with those of paclitaxel that may in turn reflect differences in their binding sites and their effects on tubulin conformation. (Mol Cancer Ther. 2003;2(12):1303–1311)

Introduction
Microtubule-targeted drugs, including the taxanes and Vinca alkaloids, are powerful chemotherapeutic agents used for treatment of many types of human tumors. However, the problem of the development of clinical resistance to taxanes and Vinca alkaloids, as well as the general clinical success of microtubules as a target for anticancer drugs, have highlighted the need for new microtubule-targeted chemotherapeutic drugs. Discodermolide (Fig. 1) is a new and structurally unique compound isolated from a marine sponge, Discodermia dissoluta. It was first described as an immunosuppressive agent (1) but more recently, it has been found to enhance the nucleation of microtubules and their stability to depolymerization by cold temperature or Ca2+ more potently than paclitaxel (2). The drug is particularly interesting because it is effective against paclitaxel-resistant cell lines and acts synergistically in combination with paclitaxel (2, 3). In the hollow fiber mouse model, discodermolide produced significant inhibition at tolerated doses against a panel of human tumor cells, including paclitaxel-resistant tumor cells. In human tumor xenograft models, it is efficacious at tolerated doses against non-small cell lung, breast, and colon tumor models, some of which are refractory to paclitaxel. Growth inhibitory activity is dose dependent and sustained for 5 weeks (or more) after administration of a single i.v. bolus dose of 15 mg/kg (4, 5). It is currently in Phase I clinical trials (6).

Discodermolide induces microtubule bundling at high concentrations, cell cycle arrest in the G2-M phase, aneuploidy, and apoptosis (7–9). It competitively and very strongly inhibits the binding of paclitaxel to microtubules, suggesting that the two drugs may have common or overlapping binding sites (2, 10), although it remains possible that discodermolide binds to more than one site on microtubules. The finding that discodermolide cannot substitute for paclitaxel in paclitaxel-requiring cells, where-as another microtubule-stabilizing drug, epothilone B, can substitute for paclitaxel (3), suggests that there are significant differences between the mechanisms of discodermolide, paclitaxel, and epothilones.

Inhibition of microtubule dynamics is a fundamental mechanism of action of several microtubule-targeted drugs (11, 12). One form of microtubule dynamics, called dynamic instability, is a behavior in which the ends of microtubules undergo frequent stochastic transitions between episodes of growing and shortening. Dynamic
instability appears to be essential for progression through mitosis. Suppression of microtubule dynamic instability by microtubule-targeted drugs is associated with aberrant organization of mitotic spindles and inhibition of progress into anaphase (13–17). We recently found that microtubule dynamics must be finely regulated for progress through mitosis, and that both excessively rapid dynamics as well as suppressed dynamics correlate with impaired mitotic spindle function and inhibition of cell proliferation (18). Information on the effects of discodermolide on microtubule dynamics in vitro or in cells is lacking. Whether, and if so, how, discodermolide blocks mitosis and inhibits cell proliferation by an effect on microtubule dynamics has not been determined.

In the present work, we analyzed the effects of discodermolide on the dynamic instability of individual microtubules in living human A549 lung cancer cells. We found that discodermolide strongly suppresses microtubule dynamic instability in a concentration-dependent manner. At the concentration of discodermolide that inhibits cell proliferation by 50% at 72 h (7 nM), overall dynamicity was reduced by 23%. The principal parameters of dynamic instability that were altered include a decrease in the mean shortening rate and length together with an increase in the rescue frequency. When the concentration of discodermolide was increased to the concentration that further incubation with discodermolide for 4 h to allow incorporation of rhodamine-tubulin into microtubules and Injectman (Eppendorf AG, Hamburg, Germany). Injected cells were incubated for 2 h at 37°C before microinjecting into A549 cells using a Transjector (Rochester, NY), A549 cells were incubated with discodermolide at the indicated concentrations for 20 h. Immuno-fluorescence microscopy of microtubules and DNA staining were performed as previously described (20). Briefly, cells were fixed with 3.7% formaldehyde in PBS and stained with an anti-tubulin antibody, tetramethyl rhodamine isothiocyanate (TRITC)-conjugated secondary antibody and 4',6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO).

**Preparation and Microinjection of Rhodamine-Labeled Tubulin**

Rhodamine-labeled tubulin was prepared using carboxymethylrhodamine (Molecular Probes, Eugene, OR) labeling of microtubules assembled from phosphocellulose-purified bovine brain tubulin (18). Labeled tubulin was stored in 6-μl aliquots at −70°C until use. A549 cells were seeded 48 h before use at 0.5 × 10^6 cells/ml on glass coverslips coated with 10 μg/ml laminin and 20 μg/ml fibronectin (Life Technologies, Inc., Carlsbad, CA). Rhodamine-tubulin (5 mg/ml) was centrifuged in a Beckman (Palo Alto, CA) tabletop ultracentrifuge using a TLA 100.3 rotor at 38,000 × g for 14 min at 4°C to remove aggregates just before microinjecting into A549 cells using a Transjector 5246 and Injectman (Eppendorf AG, Hamburg, Germany). Injected cells were incubated for 2 h at 37°C to allow for incorporation of rhodamine-tubulin into microtubules and further incubated with discodermolide for 4 h to allow attainment of an equilibrium intracellular drug concentration.

**Time-Lapse Microscopy and Image Acquisition**

Microinjected cells were placed in RPMI culture medium lacking sodium bicarbonate and supplemented with 25 mM HEPES, 4.5 g/l glucose, and 30 μl Oxyarase/ml (Oxyrase Inc., Mansfield, OH) to reduce photodamage, in a double coverslip chamber, and observed using a fluorescence microscope (Nikon Eclipse E800) maintained at 37 ± 1°C, with a Nikon plan apochromat 1.4 N.A., 100× objective lens. Thirty to 41 images per cell were acquired at 3-s intervals using a Hamamatsu Orca II digital camera (Middlesex, NJ) driven by Metamorph software (Universal Imaging, Media, PA).

**Analysis of Microtubule Dynamics**

The positions of the plus ends of individual microtubules with time were recorded using Metamorph software, exported to Microsoft Excel, and analyzed by using Real Time Measurement software (21). The lengths of individual microtubules were graphed as a function of time (life histories). Changes in length ≥0.5 μm were considered growth or shortening events. Changes in length <0.5 μm were considered phases of attenuated dynamics or pause. The rates of growth and shortening events were determined by linear regression. Means and
SEs were calculated per event. The time-based catastrophe frequency was calculated by dividing the number of transitions from growth or pause to shortening by the total time growing and paused for each individual microtubule. The length-based catastrophe frequency was calculated by dividing the number of transitions from growth or pause to shortening by the total distance grown for each individual microtubule. The rescue frequencies based on distance or time were calculated similarly, dividing the total number of transitions from shortening to pause or growth by the length shortened or the time spent shortening, respectively, for each individual microtubule. Means and SEs of transition frequencies were calculated per microtubule (n = 30 for each experimental condition). Dynamicity is the total length grown and shortened divided by the life span of the population of microtubules (22).

Results

Mitotic Arrest and Inhibition of Cell Proliferation and by Discodermolide

We determined the concentration dependence for the effects of discodermolide on the mitotic index and on cell proliferation in A549 cells (“Materials and Methods”). As determined by counting cells in mitosis and interphase after fixation and staining for microscopy, discodermolide induced the accumulation of cells in mitosis after one cell cycle (20 h) half-maximally at a concentration of 83 nM, and maximal mitotic accumulation (65%) occurred at concentrations of 166 nM and above (Fig. 2). Proliferation was determined by the sulforhodamine B assay after incubation with discodermolide for 72 h. Consistent with previous reports (3), discodermolide inhibited A549 cell proliferation at low nanomolar concentrations. The drug inhibited proliferation half-maximally at a concentration of 7 nM, whereas at concentrations ≥30 nM discodermolide, significant cell killing occurred (Fig. 2).

Suppression of Microtubule Dynamic Instability by Discodermolide in Living Cells

To determine the effects of discodermolide on the dynamic instability behavior of microtubules in living A549 cells, the cells were injected with rhodamine-tubulin, and the labeled tubulin was allowed to incorporate into microtubules for 2 h before adding the drug. Cells were observed by time-lapse fluorescence microscopy and images were collected (“Materials and Methods”). Fig. 3A shows a gallery of images of the lamellar region of a living, untreated A549 cell (control, top row) and a cell incubated with 83 nM discodermolide (4 h) (bottom row). In controls, the plus ends of microtubules (marked by arrowheads) alternated between prolonged phases of slow growing, rapid shortening, and a pause state (a state of attenuated dynamic instability). In contrast, 83 nM discodermolide markedly suppressed microtubule dynamic instability in cells as indicated by the unaltered positions of their plus ends during a 40-s time period (Fig. 3A, bottom panel). Fig. 3B shows several life history traces of the changes in length of individual microtubules in the presence or absence of 83 nM discodermolide. The life history traces of control microtubules in Fig. 3B (top panel) show extensive length changes in the microtubules, whereas the life history traces are nearly flat, indicating marked suppression of dynamics in the presence of 83 nM discodermolide (Fig. 3B, bottom row). The life history plots from 30 individual microtubules were used to determine the parameters of dynamic instability (Tables 1 and 2). In control cells, the plus ends of microtubules grew at a mean rate of 13.8 ± 1.4 μm/min, slower than their mean shortening rate of 24.0 ± 2.1 μm/min (P < 0.01). The mean lengths grown and shortened were 2.5 ± 0.2 and 5.0 ± 0.5 μm, respectively. Microtubules spent half of their time (52%) in a paused state, neither growing nor shortening to a detectable extent.

Discodermolide (7–166 nM, 4 h) suppressed microtubule dynamics in a concentration-dependent manner. The effects are shown at the IC50’s for cell proliferation (7 nM) and for mitotic block (83 nM) in Tables 1 and 2, and they are presented graphically for the entire concentration range in Fig. 4. At the relatively low concentration of 7 nM, several dynamic instability parameters were significantly altered. Notably, the shortening rate and length were decreased by 21% and 42%, respectively, and the rescue frequency calculated on the basis of length was increased by 93%. The dynamicity, a parameter reflecting the overall exchange of tubulin with the microtubule end, was reduced by 23%.

At the IC50 for mitotic accumulation (83 nM), significantly fewer microtubules were dynamic as compared with control cells and cells incubated with 7 nM discodermolide. Counts of stable and dynamic microtubules in three cells at each drug concentration during a

Figure 2. Effect of discodermolide on A549 cell proliferation at 72 h (●, left-hand axis) and induction of mitotic arrest at 20 h (○, right-hand axis). Cell number was determined at the time of discodermolide addition and 72 h later and mitotic arrest was determined after one cell cycle by counting 1000 cells after staining with 4,6-diamidino-2-phenylindole (“Materials and Methods”). Points, means of three independent experiments; bars, SD.
2-min recording period indicated that 31 ± 6% of the microtubules were stable in controls, 44 ± 7% were stable at 7 nM discodermolide, and 77 ± 5% were stable at 83 nM discodermolide. To determine the mechanistic effects of the drug on the individual parameters of dynamic instability, only dynamic microtubules were included in the measurements. All of the parameters affected by 7 nM discodermolide (shortening rates, lengths, and length-based rescue frequency, as well as dynamicity, discussed above) were more strongly altered by 83 nM discodermolide (−30%, −54%, +172%, and −62%, respectively). In addition, the growth rate and length were decreased by 30% and 34%, respectively; the time-based catastrophe frequency was decreased by 42%; and the time-based rescue frequency was increased by 88%. All of these changes indicate extreme stabilization of microtubule dynamics at the discodermolide concentration that resulted in half-maximal mitotic block at 20 h and also resulted in significant cell killing at 72 h (Fig. 2). As a result of the exclusion of completely stabilized microtubules from determination of the parameters, these values represent an underestimate of the suppressive effects of discodermolide.

Can Cells Complete Anaphase with Impaired Dynamics?

To understand the effects of suppression of microtubule dynamics on mitosis, we examined the arrangements of microtubules and chromosomes in A549 cells incubated with the concentrations of discodermolide that suppressed microtubule dynamics (Figs. 5 and 6). In controls, the mitotic index was 5 ± 1.2%, and most spindles were normal bipolar spindles. For example, Fig. 5, A and B, shows a control metaphase spindle; it is bipolar and the chromosomes have all congressed to the metaphase plate. The ratio of cells in anaphase to cells in metaphase is a reflection of the times required to pass through metaphase and through anaphase; this ratio was 0.55 in control cells.

Table 1. Discodermolide suppresses microtubule dynamics at concentrations that inhibit A549 cell proliferation and induce mitotic arrest

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>7 nM</th>
<th>Change</th>
<th>83 nM</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean rates (μm/min ± SE)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Growth</td>
<td>13.8 ± 1.4</td>
<td>12.8 ± 1.0</td>
<td>−21%</td>
<td>9.6 ± 0.8**</td>
<td>−30%</td>
</tr>
<tr>
<td>Shortening</td>
<td>24.0 ± 2.1</td>
<td>19.0 ± 1.8*</td>
<td>−21%</td>
<td>16.98 ± 2.34**</td>
<td>−30%</td>
</tr>
<tr>
<td><strong>Mean duration (min ± SE)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>0.18 ± 0.02</td>
<td>0.19 ± 0.0</td>
<td>+1%</td>
<td>0.17 ± 0.01</td>
<td>−5%</td>
</tr>
<tr>
<td>Shortening</td>
<td>0.21 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>−26%</td>
<td>0.14 ± 0.02</td>
<td>−16%</td>
</tr>
<tr>
<td>Pause</td>
<td>0.34 ± 0.06</td>
<td>0.29 ± 0.03</td>
<td>−13%</td>
<td>0.40 ± 0.05</td>
<td>+23%</td>
</tr>
<tr>
<td><strong>Mean length (μm ± SE)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Growth</td>
<td>2.5 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>−42%</td>
<td>1.6 ± 0.1***</td>
<td>−34%</td>
</tr>
<tr>
<td>Shortening</td>
<td>5.0 ± 0.5</td>
<td>2.9 ± 0.2***</td>
<td>−42%</td>
<td>2.3 ± 0.2***</td>
<td>−54%</td>
</tr>
<tr>
<td>% Time spent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>20</td>
<td>26</td>
<td>34%</td>
<td>18</td>
<td>34%</td>
</tr>
<tr>
<td>Shortening</td>
<td>28</td>
<td>20</td>
<td>−23%</td>
<td>11</td>
<td>−23%</td>
</tr>
<tr>
<td>Pause</td>
<td>52</td>
<td>54</td>
<td>2%</td>
<td>71</td>
<td>3%</td>
</tr>
<tr>
<td><strong>Dynamicy (μm/min)</strong></td>
<td>9.45</td>
<td>7.23</td>
<td>−23%</td>
<td>3.57</td>
<td>−62%</td>
</tr>
</tbody>
</table>

Note: ***, P < 0.001; **, P < 0.01; *, P < 0.05, estimates of significance of difference from controls by Student’s t test.
At 7 nM discodermolide (a concentration that suppressed dynamics to a small but significant degree and also was the IC₅₀ for cell proliferation), there was a slight but significant increase in the mitotic index (to 5.29% from 1.12% in controls, \( P < 0.05 \)). The anaphase/metaphase ratio decreased from 0.55 to 0.09, indicating that the metaphase/anaphase transition was strongly slowed at 20 h of drug incubation. Thus, many cells appeared able to exit mitosis without proceeding through anaphase. At this concentration, significant numbers of the spindles were abnormal in appearance. We determined the frequencies of abnormal spindles using the classification previously developed by Jordan et al. (13, 14, 16). In controls, nearly all spindles were normal. With 7 nM discodermolide, 18% were normal metaphase spindles; 36% were abnormal spindle types I and II, with from a few to many un congressed chromosomes that remained at the spindle poles (Fig. 5, C, D and E, F, Fig. 6); 16% had many chromosomes that were arranged in a ball about the spindle (Type III) (Fig. 5, I and J, Fig. 6); and 20% were multipolar (Fig. 5, G and H, Fig. 6). Thus, although mitotic block was not prevalent, it appears that spindle morphology was distinctly abnormal which may have resulted in aberrant cytokinesis and either cell death or inability of cells to continue proliferating (8), thus leading to a decrease in cell number when examined at 72 h of drug incubation.

At 83 nM discodermolide, the IC₅₀ for mitotic accumulation (20 h), no cells progressed to anaphase and severely abnormal type III ball-shaped spindles and asymmetric bipolar spindles (37% and 43% of all spindles, respectively) predominated (Fig. 5, I and J, Fig. 6). The asymmetric bipolar spindles were similar to those observed in kangaroo rat (PtK2) cells treated with an extremely high concentration of discodermolide (10 µM) (2). One half-spindle was bigger than the other one, and chromosomes accumulated to the side of the spindle. In some cases, the spindle poles appeared to be unconnected and independent of each other.

Microtubules in control cells in interphase formed a fine meshwork emanating from the centrosome. At discodermolide concentrations ranging from 7 to 21 nM, there were no detectable changes in the microtubule network organization (Fig. 7, B and C). At 41.5 nM discodermolide, microtubule bundles occurred around the nucleus but were absent from the cell periphery (Fig. 7D). At high concentrations (i.e., 83–166 nM discodermolide) at which microtubule dynamics were nearly completely suppressed (Figs. 3 and 4; Tables 1 and 2), many small compact bundles of microtubules were formed throughout the cells and the cells were no longer flat, but were rounded (Fig. 7, E and F).

**Discussion**

We have found that discodermolide, a novel microtubule-targeted drug in Phase I clinical trials, acts to block mitosis and inhibit cell proliferation in concert with suppression of microtubule dynamics in living human tumor cells. At low concentrations (7 nM), discodermolide primarily decreased the rate and extent of shortening and

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**Table 2. Discodermolide increases the rescue frequency and suppresses the catastrophe frequency**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>7 nM</th>
<th>Change</th>
<th>83 nM</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catastrophe frequency (min⁻¹ ± SE)</td>
<td>1.68 ± 0.12</td>
<td>1.67 ± 0.09</td>
<td>0.97 ± 0.06***</td>
<td>−42%</td>
<td></td>
</tr>
<tr>
<td>Rescue frequency (min⁻¹ ± SE)</td>
<td>3.38 ± 0.30</td>
<td>4.62 ± 0.32</td>
<td>6.36 ± 0.30***</td>
<td>+88%</td>
<td></td>
</tr>
<tr>
<td>Catastrophe frequency (µm⁻¹ ± SE)</td>
<td>0.48 ± 0.03</td>
<td>0.44 ± 0.02</td>
<td>0.53 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rescue frequency (µm⁻¹ ± SE)</td>
<td>0.14 ± 0.01</td>
<td>0.27 ± 0.01**</td>
<td>+93%</td>
<td>0.38 ± 0.01***</td>
<td>+172%</td>
</tr>
</tbody>
</table>

Note: ***, \( P < 0.001 \); **, \( P < 0.01 \), estimates of difference from controls by Student’s \( t \) test.
increased the distance-based rescue frequency leading to 23% suppression of the overall microtubule dynamicity. These effects occurred in the presence of significantly aberrant mitoses, but in the absence of detectable microtubule bundling. At this concentration, discodermolide induced a 1.8-fold increase in the mitotic index and decreased the ratio of cells in anaphase to cells in metaphase by 82%, indicating that the transition from metaphase to anaphase was impeded. The slowed mitosis and the suppressed microtubule dynamics were associated with a 50% inhibition of cell proliferation after 72 h drug incubation.

Higher concentrations of discodermolide (83 nM) further suppressed dynamic instability parameters including significant reduction in the growing rates and lengths (by 30% and 34%, respectively), a significant reduction in the time-based catastrophe frequency (by 42%), and significant increases in the time-based and length-based rescue frequencies (by 88% and 172%, respectively). Microtubule dynamicity was decreased by 62%.

Discodermolide Suppresses Microtubule Dynamics Differently from Paclitaxel and Epothilone B

Many of the observed changes resemble the changes in microtubule dynamics induced by paclitaxel and epothilone B (13, 17, 23, 24). However, there is an interesting difference between the effects of discodermolide, paclitaxel, and epothilone B on microtubule dynamics. At the IC₅₀ for mitotic block (83 nM), discodermolide had no significant effect on the catastrophe frequency per length grown. In contrast, in the same cell line, paclitaxel at the IC₅₀ for mitotic block vastly increased the catastrophe frequency per length grown (+80%).¹ What this means is that discodermolide has an additional stabilizing effect on microtubule dynamics as compared with paclitaxel; microtubules in the presence of paclitaxel continued to undergo significant, even enhanced, catastrophes. In agreement with this distinction, in another human tumor cell line (MCF7), paclitaxel and epothilone B at the IC₅₀s for mitotic block also increased the length-based catastrophe frequency (by 38% at 7.5 nM paclitaxel and by 78% for 3.5 nM epothilone B) (24). Thus, there are significant differences between the mechanisms of discodermolide, on the one hand, and epothilone B and paclitaxel, on the other hand, which may relate to differences in their binding (10), in the number of binding sites on microtubules, and in their effects on the resulting conformation of tubulin in the microtubule. These

differences may be responsible for the inability of discodermolide to substitute for paclitaxel in paclitaxel-requiring cells, for its ability to act in synergy when combined with paclitaxel, and to its effectiveness in paclitaxel-resistant cell lines (2, 3). In addition, these results are consistent with the observation that discodermolide enhances nucleation of microtubules from purified brain tubulin more potently than paclitaxel (2). This phenomenon may reflect discodermolide’s ability to enhance the rescue frequency of shortening polymers, or, in other words, to inhibit the off-reaction of tubulin from small nucleating tubulin polymers.

Discodermolide also induced the formation of an incomplete or abnormal metaphase plate of chromosomes and an abnormal arrangement of spindle microtubules. At high concentrations (> 83 nM), the spindles became asymmetric. Interestingly, the asymmetric bipolar spindles were similar to those observed in kangaroo rat (PtK2) cells treated with an extremely high concentration of discodermolide (10 μM) (2). These asymmetric spindles have not been reported with other microtubule-stabilizing drugs, and may result from differences in the antimitotic mechanism of discodermolide as compared with paclitaxel or epothilones. The mitotic index was increased 6.6-fold (to 33.4% at 83 nM discodermolide) and no cells progressed to anaphase, suggesting a clear mitotic arrest at the metaphase/anaphase boundary. These effects were associated with complete inhibition of cell proliferation at 72 h.

To What Degree Is Suppression of Microtubule Dynamics by Discodermolide Responsible for Its Ability to Inhibit Cell Proliferation?

At the IC₅₀ for inhibition of cell proliferation (7 nM), we observed a 23% reduction in overall microtubule dynamics, a slight increase in the mitotic index (from 5% to 9%) and a significant decrease in the anaphase/metaphase ratio (from 0.55 in controls to 0.09 in 7 nM discodermolide). Fifty percent of all metaphase spindles were abnormal (Types I–III), indicating that chromosome congression was impaired. There are several mechanisms by which mitotic slowing could result from suppression of microtubule dynamics by discodermolide. During prometaphase, the plus ends of dynamic spindle microtubules appear to probe the cytoplasm until linkage with a chromosomal kinetochore is established. Reduced microtubule lengths shortened in cells treated with discodermolide may result in impaired probing of the intracellular space by microtubules and to the attachment of a less than normal number of microtubules to the kinetochores and/or a delayed attachment to the kinetochores, thus delaying chromosome congression to the metaphase plate. In addition, dynamic microtubules appear to be required to produce the tension required for the metaphase/anaphase

Figure 6. Frequency of spindle abnormalities induced in A549 cells by discodermolide at concentrations ranging from 7 to 166 nM (20 h). Metaphase, anaphase, and telophase consisted of normal bipolar spindles; types I and II were bipolar spindles with one or more uncongressed chromosomes; type III were monopolar spindles with chromosomes arranged in a ball around the spindle; multipolar spindles were spindles with more than two poles; asymmetric spindles were bipolar spindles, described in Fig. 5. Columns, means of three independent experiments and evaluation of at least 600 mitotic cells per condition; bars, SD.

Figure 7. Microtubules of interphase A549 cells incubated with discodermolide (20 h). (A), control, irregular meshwork of microtubules. At 7 nM discodermolide (B) and 21 nM discodermolide (C), microtubule bundles occurred in the center of the cell around the nucleus and were absent from the periphery. At 83 nM discodermolide (E) and 166 nM discodermolide (F), compact bundles of microtubules formed throughout the cells and the cells were rounded. Scale bar, 10 μm.
transition (25–27), and for chromosome separation. Such slowing of mitotic progress may be in part responsible for the inhibition of cell proliferation at 72 h. We also observed that 20% of spindles were multipolar at 7 nm discodermolide, as compared with 2.5% in controls. It is conceivable that suppression of microtubule dynamics may be involved in the formation of multipolar spindles, but other mechanisms for their formation can also be envisaged. Interference with the function of centrosomes may contribute to aberrant mitosis and aneuploidy (8). Our results suggest that suppression of microtubule dynamics is one of the principal mechanisms for inhibition of cell proliferation by low concentrations of discodermolide while other mechanisms may coexist.

We have measured microtubule dynamics in cells in interphase at concentrations of discodermolide that block mitosis. The question arises as to whether these measurements accurately reflect the drug effects on microtubules in the mitotic spindle. While this question has not yet been directly addressed experimentally, studies of the effects of taxol and Vinca alkaloids on the dynamics of centromeres, kinetochores, and their attached microtubules in mitotic spindles of human osteosarcoma cells indicate that mitotic block is closely associated with suppression of centromere dynamics (26, 27). Likewise, examination of suppression of interphase microtubule dynamics in cell cultures containing high percentages of mitotically blocked cells indicates that for Vinca alkaloids, a halichondrin analog, taxol, and epothilone B, suppression of interphase microtubule dynamics occurs at the same drug concentrations that block mitosis (15, 17, 24).2 Thus, although dynamics of mitotic spindle microtubules are faster than those of interphase microtubules, the drugs appear to suppress microtubule dynamics relatively similarly, perhaps proportionally, regardless of the phase of the cell cycle.

Dynamics parameters and overall dynamics vary from cell type to cell type. Among the four human cancer cell types examined to date, measurements of overall dynamics in A498 kidney cells and CaOv-3 ovarian cells to a high of 10.4 ± 4.7 μm/min in MCF7 breast cells (17, 24). Values obtained for microtubule dynamics in non-human cell lines vary more widely, to both higher and lower values (28, 29). Microtubule polymerization and dynamics are regulated in cells by a number of factors including the tubulin isotype composition, tubulin posttranslational modifications, and a large number of microtubule regulatory proteins.3 Each cell type expresses a balance of these factors that appear to maintain its microtubule dynamics within a narrow range that supports mitosis, cell signaling, transport, and migration. Dynamics that are too fast or too slow appear to lead to mitotic block and ensuing cell death (18, 30). Regardless of the inherent cellular microtubule dynamics, the anti-tumor actions of the microtubule-targeted drugs appear to involve their suppression of microtubule dynamics to levels below those necessary to accurately form a mitotic spindle and to transit from metaphase to anaphase.

In summary, we have shown that discodermolide suppresses microtubule dynamics in living cells in a concentration-dependent manner. Slight to moderate suppression of microtubule dynamics is associated with mitotic slowing or block and inhibition of cell proliferation, and a greater suppression of microtubule dynamics is required to completely block mitosis. Our results confirm the importance of the regulation of microtubule dynamics for normal mitosis (18). Altogether, the results indicate that a major mechanism of mitotic block and inhibition of cell proliferation by discodermolide is the suppression of microtubule dynamics by mechanisms that differ from those of paclitaxel. Discodermolide has effects on some dynamic instability parameters that are different from those of other microtubule-stabilizing drugs. These effects may be involved in the synergism observed between discodermolide and paclitaxel (3), a hypothesis that is currently under investigation. In addition, these effects may be relevant to the clinical effectiveness of discodermolide, particularly in taxane-resistant cells.

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References

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Suppression of microtubule dynamics by discodermolide by a novel mechanism is associated with mitotic arrest and inhibition of tumor cell proliferation

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