Suppression of Centromere Dynamics by Taxol® in Living Osteosarcoma Cells

Jonathan Kelling, Kevin Sullivan, Leslie Wilson, and Mary Ann Jordan

INTRODUCTION
Taxol® is an important cancer chemotherapeutic agent that is effective for the treatment of many types of cancer (1). At high concentrations, Taxol® enhances microtubule polymerization and stabilizes microtubules against depolymerization (2–4), whereas at low concentrations, Taxol® strongly suppresses microtubule dynamic instability preferentially at microtubule plus ends along with only a modest increase in microtubule polymer mass (5–7). In many cells, Taxol® blocks mitosis at the transition from metaphase to anaphase (5, 8), leading ultimately to apoptosis (9, 10). The effects of Taxol® on mitosis appear due to an action on the spindle microtubules; however, the precise mechanism of action of Taxol® is not understood.

Microtubules are intrinsically dynamic polymers that undergo two kinds of dynamic behavior, “dynamic instability” and “treadmilling.” Dynamic instability is the stochastic switching of microtubule ends between episodes of prolonged growing and rapid shortening (11). Treadmilling consists of net growing at microtubule plus ends and net shortening at minus ends (12, 13). Both extensive dynamic instability and treadmilling (or flux) occur in mitotic spindles, and the rapid dynamics of spindle microtubules play a critical role in the intricate movements of the chromosomes (8, 14–16). Low concentrations of Taxol® potently suppress microtubule growth and shortening in human cells during interphase at concentrations that inhibit proliferation of the cells and block mitosis (17, 18). This observation has led us to hypothesize that suppression of spindle microtubule dynamics by Taxol® is responsible for its potent ability to inhibit mitotic progression and cell proliferation. However, it has not been possible to visualize the dynamics of individual microtubules attached to chromosomes (the “kinetochore microtubules”) in the central spindle of living cells because the microtubule density is too great. Thus we sought a novel method to analyze the effects of Taxol® on kinetochore microtubule dynamics and directly test the hypothesis that suppression of spindle microtubule dynamics is responsible for mitotic block.

During metaphase of mitosis, the duplicated chromosomes with their kinetochore-attached microtubules are aligned at the metaphase plate and oscillate toward and away from the spindle poles (19). The centromeres of sister chromatids also repeatedly separate from each other (they stretch apart) and then return to a relaxed position (referred to here as centromere dynamics (20)). Elastic heterochromatin lies between the sister centromeres; it is rich in α-satellite DNA and contains proteins that are involved in maintaining sister chromatid cohesion. The kinetochores containing the plus ends of microtubules embedded in them lie adjacent to the centromeres, and it is the dynamic kinetochore microtubules that appear responsible for centromere dynamics.

In the present study, we used GFP-labeled CENP-B (GFP-CENP-B), a centromere-binding protein, to examine the effects of Taxol® on the centromere dynamics in human osteosarcoma (U2OS) cells during metaphase of mitosis. We found that Taxol® (50–100 nM) suppressed the rates of stretching and relaxing of sister centromere pairs and significantly decreased the separation distance between the centromeres. The same concentrations of Taxol® that suppressed centromere dynamics also blocked mitosis, preventing progression from metaphase to anaphase. Together, these observations strongly support the hypothesis that the mechanism by which Taxol® inhibits mitosis and cell cycle progression into anaphase in U2OS cells is suppression of spindle microtubule dynamics.

MATERIALS AND METHODS

Cell Culture. U2OS human osteosarcoma cells (American Type Culture Collection HTB96) were maintained in DMEM with 1% penicillin-streptomycin (Sigma, St. Louis, MO) and 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA) at 37°C in a 5% CO2 atmosphere; doubling time was 28 h. Cells were transfected with a CENP-B-GFP plasmid (20). Expression of GFP-CENP-B was stable for 2–6 weeks; cells were periodically resected with 1.2 μg/ml G418 to maintain maximal GFP signal.

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The abbreviations used are: GFP, green fluorescent protein; CENP-B, centromere-binding protein B.

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**Immunofluorescence Microscopy.** Localization of microtubules and chromosomes was performed on cells that were fixed in 10% formalin in PBS (20 min, 25°C) followed by 10 min in methanol (4°C), washed with PBS containing 1% BSA, and incubated with rabbit anti-α-tubulin (DM1a; Sigma) and mouse antihuman histone monoclonal antibody (or 4',6-diamidino-2-phenylindole) to stain nuclei and chromosomes followed by incubation with a goat antirabbit rhodamine-conjugated secondary antibody and CY5-conjugated immunofluorescence microscopy after fixation and staining of microtubules and chromatin. UOS cells were incubated with 1 μM vinblastine for 6 h, which completely depolymerized all of the microtubules (data not shown). The mean center-to-center separation between sister centromeres (seven pairs in three cells) in the absence of microtubules was 0.66 ± 0.03 μm, and the average rates of increasing and decreasing separation were 0.55 ± 0.03 and 0.58 ± 0.03 μm/min, respectively. We then determined the total distance that a centromere moved toward and away from its sister for each interval of 5 s, 10 s, 15 s, . . . , 300 s (the sum of all stretching and relaxing distances for each interval). These distances were averaged for all seven pairs of centromeres. The mean distance moved plotted against the length of interval was linear, with a slope of 0.42 μm/min (τ = 0.999). Thus, we concluded that 0.42 μm/min is the mean rate of movement in the absence of microtubules. Any movement less than this was considered to be background or diffusional movement and was classified as a “pause” or a movement that was so attenuated that it could not be reliably measured. We note that a slightly different value was used in (33) for determination of a “pause,” which resulted in different values for the control parameter.

**RESULTS**

**Effects of Taxol® on Proliferation and Mitosis in U2OS Cells.** The goal of these experiments was to analyze centromere dynamics in living U2OS tumor cells at the lowest Taxol® concentrations that significantly inhibited proliferation and slowed or blocked mitosis. Thus, we first needed to determine the effects of Taxol® on proliferation, mitotic progression, and spindle microtubule organization in the cells. Cells were incubated with Taxol® (5–300 nM), and the increase in the number of live cells after 28 h (one cell cycle) was compared with the increase in the absence of Taxol®. As shown in Fig. 1 (○), proliferation was inhibited by 50% at 9 nM Taxol® and by 100% at 50 nM Taxol®.

The effects of Taxol® on mitosis were measured by determining the mitotic index and the ratio of cells in anaphase to cells in metaphase. As shown in Fig. 1 (□), at Taxol® concentrations greater than 15 nM (60 frames). Image stacks were then imported into UTHSCSA Image Tool for Windows Version 2.00 (University of Texas, Austin, TX) for frame-by-frame analysis. The x-y position assigned to a centromere was determined by the brightest pixel at the center of the fluorescent signal and recorded on a spreadsheet (Microsoft Excel; Microsoft Corp., Redmond, WA). The distance between centromeres of a pair was calculated by triangulation. Three independent determinations were made of the position of each centromere in each frame of the movie and averaged. The series of separation distances was used to determine rates and durations of separation and coming together (relaxing) and the frequencies of transition between stretching and relaxing.

**Criteria for Selection of Centromere Pairs for Measurement of Dynamics.** Taxol® induced several spindle abnormalities, including lagging (uncongressed) chromosomes and multipolar spindles. For determination of centromere dynamics, only cells in which the majority of chromosomes had congressed to a well-formed and distinct metaphase plate were used, and only centromere pairs of congressed chromosomes were analyzed. Preliminary comparison of tripolar spindles and bipolar spindles indicated that the dynamics of centromeres in tripolar spindles were somewhat greater than in bipolar spindles. For this reason, we included only bipolar spindles for measurement at 10–50 nM Taxol®. However, bipolar spindles were relatively difficult to find at higher Taxol® concentrations; thus, at 100 nM Taxol®, all spindles were included in the reported measurements (7 pairs from 2 bipolar spindles and 13 pairs from 9 tripolar spindles). Suppression of centromere dynamics in all spindles was virtually complete at 100 nM Taxol®, and because the inclusion of tripolar spindles would only increase the measured dynamics, their inclusion did not affect the conclusions drawn.

**Determination of Background Movement.** To determine how much of the observed centromere movement was attributable to simple diffusion or to electronic noise rather than to microtubule dynamics and/or motor proteins, we measured centromere movements in the absence of microtubules. U2OS cells were incubated with 1 μM vinblastine for 6 h, which completely depolymerized all of the microtubules (data not shown). The mean center-to-center separation between sister centromeres (seven pairs in three cells) in the absence of microtubules was 0.66 ± 0.03 μm, and the average rates of increasing and decreasing separation were 0.55 ± 0.03 and 0.58 ± 0.03 μm/min, respectively. We then determined the total distance that a centromere moved toward and away from its sister for each interval of 5 s, 10 s, 15 s, . . . , 300 s (the sum of all stretching and relaxing distances for each interval). These distances were averaged for all seven pairs of centromeres. The mean distance moved plotted against the length of interval was linear, with a slope of 0.42 μm/min (τ = 0.999). Thus, we concluded that 0.42 μm/min is the mean rate of movement in the absence of microtubules. Any movement less than this was considered to be background or diffusional movement and was classified as a “pause” or a movement that was so attenuated that it could not be reliably measured. We note that a slightly different value was used in (33) for determination of a “pause,” which resulted in different values for the control parameter.

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The effects of Taxol® on mitosis were measured by determining the mitotic index and the ratio of cells in anaphase to cells in metaphase. As shown in Fig. 1 (□), at Taxol® concentrations greater
than 10 nM, cells accumulated in mitosis, reaching a maximum of 66.0 ± 3.2% at 300 nM Taxol® (28 h). To determine whether mitosis was only slowed or whether it was blocked at a particular stage of mitosis, we determined the ratio of cells in anaphase (with segregated chromosomes) to those in metaphase [with unsegregated chromosomes (Fig. 1, ◆)]. In control cells, the ratio was 0.31, and at 5 nM Taxol®, the ratio decreased to 0.25, indicating a slight inhibition of progress into anaphase. Transition into anaphase was inhibited by 50% at 12 nM Taxol® and by 90% at 100 nM Taxol®. These results indicate that the primary effects of Taxol® on mitosis in the U2OS cells occurred between 5 and 100 nM Taxol®, and thus we examined centromere dynamics in this Taxol® concentration range.

Spindle and Microtubule Organization and Localization of GFP-CENP-B. The localization of GFP-CENP-B, microtubules, and chromosomes in fixed and stained U2OS cells in the absence and presence of Taxol® (28-h incubation) is shown in Fig. 2. In control cells in prometaphase, chromosomes were dispersed throughout the forming bipolar spindle (Fig. 2A). GFP-CENP-B appeared as round, paired, fluorescent dots (arrows in Fig. 2, A and B) on the chromosomes, with varying separation between the members of a pair, and with varying degrees of alignment with respect to the axis of the forming spindle. At metaphase (Fig. 2B), all of the chromosomes were congressed at the metaphase plate, and the centromere pairs were oriented parallel to the spindle axis. The GFP-labeled centromeres appeared as either round or elongated spots (~400–650 nm diameter). During anaphase, sister chromatids and their associated centromeres became physically separated, and their centromeres appeared as single, spherical dots (Fig. 2C). After incubation for 28 h in 10 nM Taxol®, some spindles appeared normal and bipolar with all chromosomes congressed to the metaphase plate (Fig. 2D), whereas others had one or more chromosomes that remained uncongressed and were located at one or both spindle poles (Fig. 2E, arrow). Most of the spindles (70.3 ± 4.7%) were tripolar or multipolar (Fig. 2F; Table 1). GFP-centromere pairs were similar in appearance to those in control cells. At higher Taxol® concentrations (50 nM to 1 μM), all spindles were abnormally organized with many uncongressed chromosomes (Fig. 2G, arrows) and with tripolar spindles predominating (>90%). Interphase microtubules were generally single and well-dispersed at 10–50 nM (Fig. 2I), but distinct bundles of microtubules formed at 100 nM Taxol® (data not shown).

Dynamic Behavior of Centromeres in Living Cells. Sister centromeres, as observed by confocal microscopy in living mitotic cells, alternated between phases of increasing and decreasing separation; they stretched apart and then relaxed back together (20). The change in separation distance of a typical pair of centromeres over a period of 90 s in a control cell is shown in Fig. 3. In the first panel, at time 0, the sister centromeres were separated by 0.9 μm, in the second panel (55 s), they were separated by 1.4 μm, and in the third panel (90 s), they relaxed back together and were only 0.7 μm apart.

Table 1 Spindle and nuclear abnormalities induced by Taxol® in U2OS cells

<table>
<thead>
<tr>
<th>Taxol® concentration (nM)</th>
<th>Multipolar spindles, 6-h incubation (%)</th>
<th>Multipolar centromere pairs, 6-h incubation (%)</th>
<th>Multipolar centromere pairs, 28-h incubation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>1.9 ± 1.98</td>
<td>0.8 ± 0.8</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>7.0 ± 4.9</td>
<td>3.6 ± 3.6</td>
<td>61.9 ± 12</td>
</tr>
<tr>
<td>50</td>
<td>9.1 ± 5.1</td>
<td>9.4 ± 5.1</td>
<td>94.1 ± 0.3</td>
</tr>
<tr>
<td>100</td>
<td>9.1 ± 1.3</td>
<td>7.7 ± 0.5</td>
<td>96.1</td>
</tr>
</tbody>
</table>

U2OS cells were incubated with Taxol® for 6 or 28 h. After fixation and staining for microtubules and chromatin (see “Materials and Methods”), the numbers of mitotic cells with multipolar, bipolar, or monopolar spindles as well as the number of interphase cells with multiple or single nuclei were counted by immunofluorescence microscopy. Values are the mean ± SE of two experiments and represent counts from 100 to >500 cells per concentration and time.
Fig. 3. Dynamic behavior of centromeres in living U2OS cells in the absence of drug. Images of GFP-centromeres were collected from a single plane by confocal microscopy at 5-s intervals. Arrowheads indicate the positions of sister centromeres at time 0 (0.9 μm separation), 55 s later (when they are maximally stretched apart to a distance of 1.4 μm), and at 90 s [when they have relaxed back together (0.7 μm separation)].

Fig. 4 shows a time course for stretching and relaxing of two pairs of sister centromeres as determined from images like those shown in Fig. 3. The top trace is the distance in micrometers between the two members of a centromere pair in a control cell, and the bottom trace is the distance between the two members of a pair in the presence of 100 nM Taxol®. Such graphs are similar to “life history” plots of microtubule dynamics (for example, see Ref. 17). The rate, duration, and extent of each stretching or relaxing episode were determined from such graphs (“Materials and Methods”). For the centromere pair shown in the top trace in Fig. 4 (in the absence of Taxol®), the pair was separated by 0.8 μm at time 0. At 40 s, the pair was separated by 1 μm (the maximum separation observed for this pair), and 10 s later, the separation was 0.7 μm. For some periods of time, ranging from 5 to 60 s, pairs often displayed little or no detectable change in separation distance, a phase that we called a pause (defined as movement that is not significantly different from background, (diffusional movement, which was determined to be 0.42 μm/min; see “Materials and Methods’)). We also determined the frequency of transitions between phases of stretching and relaxing. The members of the lower centromere pair (100 nM Taxol®) were closer together than they were in the absence of Taxol®, and both the mean frequency and the mean amplitude of their movements relative to each other were reduced as compared with those of control centromeres.

Centromere Dynamics in Control U2OS Cells. The various centromere dynamics parameters we measured are shown in Tables 2 and 3 and Figs. 5 and 6. In the absence of Taxol®, centromere pairs in cells stretched and relaxed at similar rates and for similar durations of time. They stretched at a rate of 0.76 ± 0.04 μm/min for a duration of 13.8 ± 0.8 s and relaxed at a rate of 0.71 ± 0.03 μm/min for a duration of 16.6 ± 1.2 s (Table 2; Fig. 5). The mean separation distance was 0.73 ± 0.03 μm and ranged from a maximum of 0.89 ± 0.04 μm to a minimum of 0.59 ± 0.02 μm. Centromere pairs transitioned from a stretching phase to a pause or relaxing phase and from a relaxing phase to a pause or stretching phase at a frequency of 0.81 ± 0.14 transitions/min (in other words, once every 74 s). Centromere pairs in control cells spent 15.4 ± 2.3% of the time stretching, 19.3 ± 2.6% of the time relaxing, and 65.4 ± 3.8% of the time in a paused state. Here we use the term centromere “dynamicity” in a manner analogous to its use in describing microtubule dynamics (22), the sum of the all of the stretching and relaxing distances divided by the total time observed. The mean centromere dynamics in control cells was 0.25 μm/min.

Taxol® Significantly Suppressed Centromere Dynamics. We note that at the lowest Taxol® concentration examined (10 nM), the mean values of several centromere dynamics parameters increased slightly (Tables 2 and 3; Fig. 6), but none of the increases was significant at the 90% confidence level (Student’s t test). The most prominent changes in centromere dynamics at 50 and 100 nM Taxol® were reductions in the transition frequency, the dynamicity, and the separation distance between sister centromeres and an increase in the pause time. For example, 50 nM Taxol® reduced the transition frequency by 27% from 0.81 ± 0.14 to 0.59 ± 0.07 events/min. It also reduced the dynamicity by 24% and reduced the mean maximal and
minimal separation distances between centromeres (by 11% and 14%, respectively). Finally, it increased the percentage of time paused by 14%.

At 100 nM Taxol®, in addition to affecting the same parameters discussed above for 50 nM Taxol®, both the rates and durations of stretching and relaxation were also suppressed (Table 2; Fig. 5). For example, the rates of separation and relaxation were decreased by 15% and 18%, respectively. The durations of separation and relaxation also decreased, by 16% and 30%, respectively. There was also a large (56%) decrease in dynamicity at 100 nM Taxol® from 0.25 μm/min in controls to 0.11 μm/min in 100 nM Taxol® (Table 2; Fig. 6D). Thus, as shown pictorially in Fig. 5, centromeres did not stretch apart as fast or as far or as long or as frequently as they did in control cells. They also did not relax together as fast, as long, or as frequently as they did in control cells. Taken together, these results indicate that the movements of the centromeres were suppressed in a concentration-dependent manner by Taxol®. These results are consistent with a mechanism in which microtubule dynamics play a major role in centromere dynamics.

Abnormal Mitoses Resulted in Multinucleate Interphase Cells. Mitotic spindle abnormalities developed rapidly after addition of Taxol®, but abnormalities in interphase cells were detectable only after many hours of incubation with Taxol® and appeared to result from earlier mitotic block. After 6 h of incubation with Taxol®, most interphase cells appeared normal and had a single nucleus (>90%), similar to control cells (99.3% mononucleate; Table 1). However, after 28 h of incubation with 10 nM Taxol®, 61.9 ± 1.2% of interphase cells were multinucleate (compare control in Fig. 2E with Fig. 2F, 10 nM), and >94% were multinucleate at 50–100 nM Taxol® (Table 1). Thus, although many blocked cells eventually exited mitosis, cytokinesis was unsuccessful, multinucleate cells formed, and proliferation was inhibited (Fig. 1).

**DISCUSSION**

Using GFP-CENP-B as a marker for centromeres, kinetochores, and the plus ends of kinetochore microtubules, we have analyzed the effects of Taxol on centromere dynamics in living U2OS cells. We found that 50–100 nM Taxol® significantly suppressed the stretching and relaxing dynamics of centromeres on sister chromatids. The time that centromeres were in a paused state increased, and the separation distance between sister centromeres and the frequency of transitions between stretching and relaxing decreased. Taxol® (50 nM) reduced the centromere dynamicity by 24%, and 100 nM Taxol® reduced it by 55%. These effects on centromere/kinetochore dynamics were associated with mitotic accumulation (63%), a >90% block of the metaphase/anaphase transition, and complete inhibition of proliferation.

**Centromere Dynamics in the Absence of Taxol®.** The movements of sister centromeres in control cells were characterized by periodic transitions from stretching apart to relaxing back together. On average, a centromere pair transitioned 1.2 times/min from stretching to relaxing and vice versa. Each kinetochore and its adjacent centromere is the site of attachment of 20–30 individual microtubules (23, 24). When the centromeres are stretching, the attached microtubules on the sister kinetochores must be shortening. When the centromeres are relaxing, the attached microtubules must be growing. The transitions between centromere stretching and relaxation were abrupt and must represent simultaneous and tightly coordinated phases of growth and shortening of all of the microtubules attached to an individual kinetochore/centromere.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>10 nM Taxol®</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Time stretching (%)</td>
<td>15.4 ± 2.3</td>
<td>20.5 ± 2.6</td>
<td>+33%</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>Time relaxing (%)</td>
<td>19.3 ± 2.6</td>
<td>21.2 ± 2.8</td>
<td>+10%</td>
<td>14.0 ± 1.7</td>
</tr>
<tr>
<td>Time paused (%)</td>
<td>65.4 ± 3.8</td>
<td>58.4 ± 4.2</td>
<td>−11%</td>
<td>74.4 ± 2.5</td>
</tr>
<tr>
<td>Mean separation (μm)</td>
<td>0.73 ± 0.03</td>
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<td>0%</td>
<td>0.65 ± 0.01</td>
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<tr>
<td>Mean maximal separation (μm)</td>
<td>0.89 ± 0.04</td>
<td>0.93 ± 0.04</td>
<td>+5%</td>
<td>0.79 ± 0.01</td>
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<td>Mean minimal separation (μm)</td>
<td>0.59 ± 0.02</td>
<td>0.56 ± 0.02</td>
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* The mean for all tracings of the two maximal separations (peaks) or the two minimal separations (valleys) during each 5-min tracing for each condition.

b,c,d Values were significantly different from control values at the 90%, 95%, or 98% confidence level, respectively (Student’s t test).

between stretching and relaxing decreased. Taxol® (50 nM) reduced the centromere dynamicity by 24%, and 100 nM Taxol® reduced it by 55%. These effects on centromere/kinetochore dynamics were associated with mitotic accumulation (63%), a >90% block of the metaphase/anaphase transition, and complete inhibition of proliferation.

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separation distances are indicated. Chromatids in the presence of 100 nM Taxol right panels kinetochores in the absence of Taxol (20, 34). Vinblastine is a microtubule-binding drug that suppresses centromere dynamics (33) and reduce the intercentromere distance (35). Given the different binding sites for Taxol® and vinblastine indicates that during normal fluctuations of centromere stretching and relaxation, the individual centromere pairs transiently relax to a state in which no tension is apparent. However, the overall mean separation distance in the absence of drugs was 0.73 μm, indicating that, on average, centromeres attached to the spindle are under tension.

It is conceivable that when the centromeres are completely relaxed and there is no tension, this state may signal or facilitate the simultaneous transition of all of the attached microtubules to switch from a growing state to a shortening state, which then recreates increased tension. The gain and loss of a stabilizing GTP-tubulin or GDP-Pi-tubulin cap are thought to regulate the growing and shortening transitions associated with microtubule dynamic instability (27, 28).

Microtubule Dynamics, Rather Than Microtubule Motors, May Be Primarily Responsible for Centromere Stretching and Relaxation. The rates of centromere separation and relaxation in control cells (~0.7 μm/min) are relatively slow as compared with the rates of growth and shortening of individual microtubules measured in living cells. For example, the rates of microtubule growth and shortening during interphase in A498 human kidney and CaOv-3 ovarian carcinoma cells are 10–17-fold faster (17) than the rates of centromere stretching and relaxation reported here. In addition, the dynamics of microtubules in mitotic asters is severalfold higher than that for interphase microtubules (31, 32). Centromere dynamics may be slowed to rates that are significantly slower than the inherent growth and shortening rates of individual microtubules by the arrangement of microtubules at the centromere/kinetochore. Bundles of microtubules from opposite spindle poles are attached to sister kinetochores. Individual microtubules in each bundle may not be perfectly synchronous in their transitions between growth and shortening and thus may work against each other. In addition, centromere dynamics are the result of competing growing and shortening of the opposing microtubule bundles, and thus the net rates may be suppressed by the competition between them. Although other possibilities exist, the slow rates of centromere stretching/relaxation movements indicate that centromere dynamics could be accounted for primarily by microtubule dynamics and may not require significant energetic contributions from microtubule motors. This suggestion is strengthened by the observation that two very different drugs, vinblastine and Taxol®, both suppress centromere dynamics (33) and reduce the intercentromere distance (20, 34). Vinblastine is a microtubule-binding drug that suppresses microtubule dynamics by binding with high affinity to the ends of microtubules rather than to their interior surfaces as Taxol® does (35–37). Given the different binding sites for Taxol® and vinblastine...
on microtubules, it is unlikely that both Taxol® and vinblastine suppress centromere dynamics by sterically blocking the interaction of motor proteins with microtubules. Rather, their suppression of centromere dynamics can be most readily attributed to their common suppression of microtubule dynamics. Thus, during metaphase, microtubule motor proteins may serve primarily to attach kinetochores to the dynamic microtubules or to directly modulate microtubule dynamics (38) rather than to act in a motoring capacity.

Low concentrations of Taxol® (10 nM) may induce slightly increased centromere dynamics in cells, whereas high Taxol® concentrations (50–100 nM) suppress dynamics. We found that 10 nM Taxol® slightly increased the rate and duration of stretching, the rate of relaxing, the transition frequency, the total time spent separating bipolarities, and complete inhibition of proliferation. Intercentromere/H11022 suppressed centromere dynamics and were associated with maximal ploidy, and cell death (39, 40).

*4 M. A. Jordan and L. Wilson, unpublished observations.

The Relationship between Mitotic Block and Inhibition of Proliferation by Taxol®. At low Taxol® concentrations, e.g., 5–10 nM, the mitotic index increased slightly (from 1.3% to 4.2%; Fig. 1), progress from metaphase to anaphase was slowed but not blocked, and proliferation was inhibited by 31% (Fig. 1). Thus, interestingly, at low Taxol® concentrations, mitosis was not blocked, even though cell proliferation was significantly inhibited. At low Taxol® concentrations, microtubules appear to be sufficiently dynamic that the mitotic checkpoint is eventually satisfied, and anaphase ensues. Thus, at low concentrations, Taxol® appears to inhibit cell proliferation not by a long-term block of mitosis but by another mechanism. This mechanism may involve the production of multipolar spindles (Table 1), which ultimately induces abnormal chromosome segregation and ablated cytokinesis. In some cells lines, anaphase in the presence of low concentrations of Taxol® (<10 nM) results in abnormal chromosome segregation, abnormal DNA content and cell size, aneuploidy, and cell death (39, 40).

**Summary.** High concentrations of Taxol® (100 nm) significantly suppressed centromere dynamics and were associated with maximal mitotic accumulation (63%), >90% block of the metaphase/anaphase transition, and complete inhibition of proliferation. Intercentromere distances were minimal; they were equivalent to the separations observed in the absence of attached microtubules. Thus, the tension on the centromeres in the presence of Taxol® was virtually nonexistent. These results are a direct demonstration that dynamic microtubules are necessary for the transition from metaphase to anaphase. The strict Taxol® concentration dependence between the degree of suppression of kinetochore microtubule dynamics and the degree of mitotic block strongly indicates that the primary mechanism of mitotic block by Taxol® is suppression of microtubule dynamics of kinetochore microtubules.

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**REFERENCES**


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