Novel Actions of the Antitumor Drugs Vinflunine and Vinorelbine on Microtubules

Vivian K. Ngan, Krista Bellman, Dulal Panda, Bridget T. Hill, Mary Ann Jordan, and Leslie Wilson

Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, California 93106 [V. K. N., K. B., D. P., M. A. J., L. W.], and Division de Cancérologie Experimentale, Centre de Recherche Pierre Fabre, 81106 Castres Cedex, France [B. T. H.]

ABSTRACT

Vinflunine is a novel Vinca alkaloid presently in Phase I clinical trials. In preclinical studies, it exhibited superior antitumor activity to that of other Vinca alkaloids, including vinorelbine from which it was synthetically derived. Vinca alkaloids appear to inhibit cell proliferation by affecting the dynamics of spindle microtubules. Here we have analyzed the effects of vinflunine and vinorelbine on microtubule dynamic instability and treadmilling and found that these newer drugs exert effects on microtubule dynamics that differ significantly from those of the classic Vinca alkaloid, vinblastine. The major effects of vinflunine and vinorelbine on dynamic instability were a slowing of the microtubule growth rate, an increase in growth duration, and a reduction in shortening duration. In marked contrast to the action of vinblastine, they neither reduced the rate of shortening nor increased the percentage of time the microtubules spent in an attenuated state, neither growing nor shortening detectably. In addition, vinflunine and vinorelbine suppressed treadmilling, but less strongly than vinblastine. The diverse actions of these drugs on microtubules are likely to produce different effects on mitotic spindle function, leading to different effects on cell cycle progression and cell killing. Nontumor cells with normal checkpoint proteins may tolerate the relatively less powerful inhibitory effects of vinflunine and vinorelbine on microtubule dynamics better than the more powerful effects of vinblastine. Thus the unique constellation of effects of vinflunine and vinorelbine on dynamic instability and treadmilling may contribute to their superior antitumor efficacies.

INTRODUCTION

Vinca alkaloids, including the natural products vincristine and vinblastine and the semisynthetic derivatives vindesine and vinorelbine, are antimitotic drugs that are widely and successfully used in the treatment of cancer (1). Vinorelbine, the latest of the clinically approved Vinca alkaloids, has shown improved efficacy and reduced toxicity. It is effective in the treatment of non-small cell lung cancer, metastatic breast cancer, and ovarian cancer, and it shows promise in the management of lymphomas, esophageal cancer, and prostatic carcinomas (2–4). A fifth new Vinca alkaloid, vinflunine, is a semisynthetic bifluorinated compound, which is now in Phase I clinical trials in Europe. Vinflunine has demonstrated superior activity over vinorelbine, vinblastine, and vincristine in a range of murine tumors and human tumor xenografts (5–7). For example, vinflunine showed high activity against RXF944LX kidney cell and NCI-H69 small cell lung xenografts and moderate activity against PAXF546 pancreatic cell, PC-3 prostate cell, and TC37 colon cell human tumor xenografts, achieving an overall response of 64% (i.e., activity against 7 of 11 xenografts tested). In contrast, only a 27% (3 of 11) response was observed for vinorelbine tested concurrently with only moderate activity against RXF944LX kidney cell and TC37 colon cell xenografts (5).

Structurally, vinflunine and vinorelbine differ from vinblastine in the velbanamine moiety (“top” portion of the molecule (Fig. 1). Both drugs were synthesized by a novel method for coupling the precursor alkaloids catharanthine and vindoline, which resulted in the formation of an eight-membered rather than a nine-membered ring within the velbanamine portion of the molecule (8, 9). Vinflunine was derived by further modification of vinorelbine, using superacidic chemistry, which specifically introduced two fluorine atoms in the velbanamine moiety (10).

The characteristic block of cell proliferation during mitosis induced by all five of the Vinca alkaloids appears to be attributable to a specific action on mitotic spindle microtubules. The mechanism of action of the Vinca alkaloids was initially thought to involve the depolymerization of spindle microtubules and induction of paracrystalline tubulin-Vinca alkaloid arrays. At relatively high concentrations (μM levels), all five drugs inhibit microtubule polymerization in reconstituted microtubule systems and in cells (6, 11, 12). However, recent evidence indicates that at low concentrations vinblastine and vincristine exert a subtle but powerful action on microtubules; i.e., they inhibit their dynamic behaviors at concentrations below those required to significantly inhibit polymerization (13–15). For example, low vinblastine concentrations (nM levels) block mitosis in BSC-1 cells in association with suppression of microtubule dynamics but in the absence of appreciable changes in microtubule mass or spindle microtubule organization (13). The suppression of microtubule dynamics by vinblastine and other antimitotic drugs, including taxol, estramustine, and colchicine, appears to cause mitotic block by activating the metaphase-anaphase checkpoint (12, 16).

Microtubules display two types of unusual dynamic behavior, “dynamic instability” and “treadmilling,” which appear to be important for progression through mitosis and the cell cycle. Dynamic instability is a stochastic switching of microtubule ends between phases of relatively slow growth and rapid shortening (17). Treadmilling is a net addition of tubulin subunits at one end of a microtubule (the plus end) and the balanced net loss from the opposite (minus) end (18–20). The dynamics of microtubules are coordinated with the actions of an undefined number of molecular motors to bring about the equi-partitioning of chromosomes to the two daughter cells by the mitotic spindle. For example, microtubules emanating from each of the spindle poles at prometaphase make vast growing and shortening excursions, probing the cytoplasm until they “find” and attach to the kinetochores of the chromosomes. During metaphase, chromosomes aligned at the metaphase plate oscillate back and forth under considerable tension, most likely produced by a combination of motor molecules working in concert with the growing and shortening of kinetochore-attached microtubules. Superimposed on the chromosome oscillations is microtubule treadmilling or flux (21) where tubulin is continuously added to microtubule plus ends at the kinetochores and lost from the minus ends at the spindle poles in balanced fashion. The dynamics and the forces produced by them appear to be important in cell cycle signaling at the metaphase/anaphase checkpoint (22). Vinblastine has been shown to bind with high affinity to microtubule ends. This strongly suppresses both microtubule dynamic instability and treadmilling and appears to lead to a mitotic block at the metaphase/anaphase transition (12–15, 23, 24).

Vinflunine and vinorelbine share a number of properties with
ACTIONS OF VINFLUNINE AND VINORELBINE ON MICROTUBULES

Vinflunine

Vinorelbine

Vinblastine

Fig. 1. Chemical structures of vinflunine, vinorelbine, and vinblastine.

Vinblastine and other Vinca alkaloids. They inhibit polymerization of purified microtubule protein into microtubules (6). At high concentrations (≥50 μM), they inhibit mitosis in tumor cells in association with spindle microtubule depolymerization, and at very high concentrations (~50 μM), they induce tubulin paracrystal formation (6). Because of their superior antitumor activities as compared with the classical Vinca alkaloids, we wanted to determine the actions of these newer compounds on microtubule dynamic instability and treadmill- ing. Here we report that like vinblastine, both compounds suppress microtubule treadmillling and dynamic instability. However, we found that the actions of vinflunine and vinorelbine on microtubule dynamics are significantly different from those of vinblastine. The differences may contribute to the superior antitumor activities of these two semisynthetic drugs.

MATERIALS AND METHODS

Purification of Microtubule Protein and Tubulin. Microtubule protein preparations consisting of 70% tubulin and 30% MAPs were isolated from bovine brain by three cycles of polymerization and depolymerization. Tubulin was purified from the microtubule protein by phosphocellulose chromatography, drop-frozen in liquid nitrogen, and stored at ~70°C (20). The assembled polymer was then collected by centrifugation (150,000 × g; 1 h; 35°C). The supernatant was aspirated, the sedimented microtubules were depolymerized in an assembly buffer by incubation on ice (2 h), and the protein content was determined.

Analysis of Microtubule Dynamics by Quantitative Video Microscopy. Purified tubulin (15 μM) was polymerized into microtubules as described above except that incubation was carried out at 37°C in 87 mM PIPES, 36 mM 2-(N-morpholino)ethane sulfonic acid, 1.4 mM MgCl₂, 1.0 mM EGTA, and 1.0 mM GTP (pH 6.8). Under the conditions used, the microtubules formed predominantly at the plus ends of the seeds, and little or no assembly occurred at the minus ends. The seed concentration was adjusted to yield three to eight seeds per microscope field. After 25 min, a 2–3-μl sample was placed between two coverslips and mounted on a prewarmed glass microscope slide (24). Microtubules were imaged by video-enhanced differential interference contrast microscopy using a Zeiss IM35 inverted microscope with a Zeiss Planapo n.a.1.4, ×63 oil immersion objective and a stage maintained at 35–37°C. Samples were imaged for a maximum of 35 min.

Microtubule images, from a minimum of 30 microtubules/condition, were captured in real time and recorded on video tape (24). Microtubule lengths were measured at 3–5-s intervals and analyzed using the Real Time Measurement (version 5.0) program (a kind gift from Neil Glikman and E. D. Salmon, University of North Carolina, Chapel Hill, NC). From these length measurements, “life history” plots of microtubule length versus time were generated, and the rates of microtubule growth and shortening were calculated by least squares regression. We considered a microtubule to be in a growth phase when its rate of growth was >0.15 μm/min and its length was changed by >0.2 μm. Similarly, a microtubule was considered to be in a shortening phase when its shortening rate was >0.30 μm/min and its length was changed by >0.2 μm.

Length changes of ≤0.2 μm were not detectable; thus a change of ≤0.2 μm over a duration of ≥30 s was considered to be a phase of attenuated dynamics, sometimes called a pause. A transition from a growth or attenuation phase to a shortening phase is termed a “catastrophe,” whereas a transition from shortening to a growth phase or to an attenuated state is called a “rescue.” The catastrophe frequency was calculated as the number of catastrophes divided by the sum of the total time spent in the growth and attenuation phases for all microtubules, whereas the rescue frequency was calculated as the number of rescues divided by the sum of the total time spent in the shortening phase for all microtubules. Dynamicity is the total rate of measurable tubulin exchange at microtubule ends (attributable to growth and shortening).

Determination of the Treadmilling Rate. Microtubule protein (2.5 mg/ml) was thawed and resuspended in PEM buffer [100 mM PIPES, 1 mM MgCl₂, 1 mM EGTA, 0.1 mM GTP (pH 6.8)] containing a GTP-regenerating system consisting of 10 mM acetyl phosphate and 1 IU/ml acetate kinase. The resuspended protein was polymerized into steady-state microtubules by incubation for 30 min at 30°C. Drug was added at the specified concentration, and incubation was continued for 30 min to allow re-establishment of steady state. [³H]GTP ([final specific activity, 167 mCi/mmol] was added, and 30 min later, duplicate samples of each reaction were removed and stabilized in microtubule stabilizing buffer (30% glycerol, 10% DMSO, 5.6 mM ATP in PEM buffer; 30°C). Stabilized microtubule samples were filtered through GFF glass fiber filters, which were then washed three times with an equal volume of stabilizing buffer to remove unincorporated [³H]GTP. The amount of [³H]GDP incorporated into microtubules, i.e., trapped on the glass fiber filters, was quantitated by scintillation counting in Beckman Ready Protein scintillation cocktail (26).

RESULTS

Reduction of Microtubule Polymer Mass by Vinflunine and Vinorelbine. We wanted to analyze the effects ofvinflunine and vinorelbine on the dynamic instability behavior of individual microtubules polymerized from purified tubulin onto axoneme seeds. Thus, we first determined the effects of vinflunine and vinorelbine on microtubule polymer mass in this system. Purified MAP-free tubulin (17 μM) was polymerized to steady state at the ends of axoneme seeds in the absence or presence of a range of vinflunine or vinorelbine concentrations (0–4 μM). The assembled polymer was then collected by centrifugation, and the protein content was determined (“Materials and Methods”). As shown in Fig. 2, vinflunine and vinorelbine re-
duced the microtubule polymer mass in a concentration-dependent manner. The concentration at which polymerization was inhibited by 50%, the IC$_{50}$, was 1.2 μM for vinflunine and 0.80 μM for vinorelbine. We previously reported an IC$_{50}$ of 0.54 μM for vinblastine under similar conditions (Ref. 15; data also shown in Fig. 2). Thus, the potencies of the three drugs were in the same concentration range, with a 2-fold difference between the strongest and the weakest. Hence, the relative potencies can be ranked as vinblastine > vinorelbine > vinflunine.

Vinflunine and Vinorelbine Suppress the Dynamic Instability Behavior of Reconstituted Microtubules in Vitro. We chose to compare the effects of vinflunine, vinorelbine, and vinblastine on dynamic instability of individual microtubules polymerized from 15 μM tubulin at a concentration of 0.4 μM. This concentration was sufficiently high to reveal the effects of the drugs on the dynamic parameters by video microscopy, but not so high that it caused severe reduction of the microtubule polymer mass. An attempt was made to study the effects of the drugs at higher concentrations (0.6 μM), but for all three drugs, this concentration resulted in microtubules that were too few and too short for accurate analysis. As will be shown below, this concentration of all three drugs suppressed some parameters to the same extent, while affecting other parameters quite differently.

Life history traces showing the changes in length with time of representative microtubules at their plus ends in the absence (controls) and presence of the three drugs are shown in Fig. 3. The dynamic instability parameters determined from large numbers of such traces were quantitated and are shown in Tables 1 and 2. Consistent with previous data (15, 24), the plus ends of control microtubules grew slowly for long periods of time and occasionally transitioned to brief phases of rapid shortening (Fig. 3). Transitions to rapid shortening (catastrophes) occurred at a frequency of 0.18 ± 0.02 events/min, and episodes of shortening were “rescued” by the resumption of growth or pause at a frequency of 3.1 ± 0.4 events/min (Table 2). Sometimes the microtubules neither grew nor shortened detectably, but spent time in a phase of attenuated dynamics or “pause” (segments of traces between asterisks in Fig. 3). Overall, control microtubules spent 71% of the time growing, 5% shortening, and 24% in an attenuated (paused) state. Their dynamicity (the overall average rate of length gain and loss at the plus ends) was 1.3 μm/min (Table 1).

Vinflunine and vinorelbine (0.4 μM) clearly suppressed dynamic instability (Fig. 3; Table 1). The magnitude of their effects and the parameters affected were similar for the two drugs. Their major effects were a slowing of the microtubule growth rate, an increase in the mean duration of a growth event, and an increase in the percentage of time the microtubules spent growing. The rate of growth was slowed by 29–32% from 0.49 ± 0.04 μm/min in controls to 0.35 ± 0.01 μm/min and 0.34 ± 0.01 μm/min for vinflunine and vinorelbine, respectively. The growth duration was increased by 53–59% from 1.7 ± 0.2 min in controls to 2.7 ± 0.2 min and 2.6 ± 0.3 min for vinflunine and vinorelbine, respectively. The percentage of time the microtubules spent growing also increased by 16–20%, from 71% in controls to 85% and 82% with vinflunine and vinorelbine,
respectively. Interestingly, the drugs had little effect on the shortening rate, but greatly reduced the duration of shortening by 30–36%, from a mean of 0.27 ± 0.05 min in controls to a mean of 0.19 ± 0.03 min and 0.17 ± 0.02 min for vinflunine and vinorelbine, respectively.

### Vinflunine and Vinorelbine Affect Dynamic Instability Parameters Differently from Vinblastine

Interestingly, 0.4 μM vinflunine, 0.4 μM vinorelbine, and 0.4 μM vinblastine all reduced dynamicity to similar degrees; by 21%, 32%, and 32%, respectively. However, the three drugs affected several of the dynamic instability parameters differently. As reported previously (15, 24), vinblastine suppressed dynamics primarily by reducing the rate and extent of growth and shortening (Fig. 3 and Table 1). However, unlike vinblastine, vinflunine and vinorelbine did not reduce the shortening rate (Table 1). The effects of vinflunine and vinorelbine on the lengths grown and shortened differed significantly from those of vinblastine as shown in the histograms in Fig. 4. In the presence of vinflunine and vinorelbine, the percentage of microtubules that underwent long growing excursions (>2 μm; Fig. 4A) was greater than or equal to that of control microtubules, whereas with vinblastine, most of the growing changes were short (<1.2 μm). Similarly, vinblastine reduced the lengths of the shortening excursions much more strongly than vinorelbine or vinflunine (Fig. 4B). Furthermore, vinblastine increased the percentage of time microtubules spent in the attenuated state by 108%, whereas in contrast, vinflunine and vinorelbine reduced it by 52% and 40%, respectively.

The rescue and catastrophe frequencies and the lengths microtubules grow and shorten are important in the formation of a bipolar mitotic spindle. The two newer alkaloids affected the frequency of rescue quite differently from vinblastine at the conditions used. Vinflunine and vinorelbine increased the rescue frequency by 43% and 60% respectively, as compared with controls, whereas vinblastine had either no effect or only a minimal one (Table 2). The catastrophe frequency was virtually unchanged by any of the three drugs. In summary, although all three Vinca alkaloids suppress dynamic instability, the actions of vinflunine and vinorelbine differ from that of vinblastine, indicating that the mechanisms of action of the two newer drugs are different from that of vinblastine.

### Vinflunine and Vinorelbine Suppressed Microtubule Treadmilling in Vitro

We examined the effects of vinflunine and vinorelbine on the treadmilling rate in vitro by following [3H]GTP incorporation into MAP-rich microtubules (26). The MAPs in this system suppress dynamic instability so that treadmilling is the predominant dynamic behavior (20, 27). As shown in Fig. 5, vinflunine and vinorelbine, like vinblastine, suppressed the treadmilling rate in a concentration-dependent manner. The concentrations of each drug that inhibited the treadmilling rate by 50% differed greatly (over a 6-fold concentration range) with IC50 values of 0.42 μM, 0.10 μM, and 0.066 μM for vinflunine, vinorelbine, and vinblastine, respectively. Hence, the relative potency of the three drugs is vinblastine > vinorelbine > vinflunine.

### DISCUSSION

We have found that vinflunine, vinorelbine, and vinblastine all inhibited microtubule polymerization and, at relatively low concentrations, suppressed treadmilling, with vinblastine being the most potent and vinflunine the least. Additionally, dynamicity as well as growth rates were suppressed by the three drugs at 0.4 μM to similar extents (Table 1). However, vinorelbine and vinflunine affected other dynamic instability parameters differently than vinblastine, suggesting that the mechanisms of action of vinflunine and vinorelbine at microtubule ends differ from that of vinblastine. These differences may contribute to the superior antitumor activity of the two newer drugs as compared with that of vinblastine (5, 7).

The most prominent effects of vinflunine and vinorelbine on dynamic instability were a slowing of the microtubule growth rate, an increase in growth duration, and an increase in the percentage of time the microtubules spent growing. To illustrate these effects in comparison with those of vinblastine, the average growth and shortening events in the absence (control) and presence of 0.4 μM drug (from Table 1) are drawn in hypothetical life history plots in Fig. 6. Here the microtubule length during a growth or shortening event, relative to its length at the start of the event, is plotted against time such that the mean rate of microtubule length change is represented by the slope of each line. The X (horizontal) component of each line segment represents the duration of the average growth event (Fig. 6A) or shortening event (Fig. 6B), and the Y (vertical) component represents the length change in μm. For example, Fig. 6A shows that during an average growth event, a control microtubule grew 0.83 μm in 1.7 min. A comparison of growth events (Fig. 6A) reveals that although all three

<table>
<thead>
<tr>
<th>Behavioral parameter</th>
<th>Control</th>
<th>Vinflunine</th>
<th>% change</th>
<th>Vinorelbine</th>
<th>% change</th>
<th>Vinblastine</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>0.49 ± 0.04</td>
<td>0.35 ± 0.01</td>
<td>−29</td>
<td>0.34 ± 0.01</td>
<td>−32</td>
<td>0.34 ± 0.04</td>
<td>−26</td>
</tr>
<tr>
<td>Shortening</td>
<td>20 ± 0.2</td>
<td>21 ± 0.02</td>
<td>+6</td>
<td>18 ± 0.2</td>
<td>−6</td>
<td>11 ± 1</td>
<td>−44</td>
</tr>
<tr>
<td>Mean duration (min)</td>
<td>1.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>+59</td>
<td>2.6 ± 0.3</td>
<td>+53</td>
<td>1.8 ± 0.2</td>
<td>+6</td>
</tr>
<tr>
<td>Attenuation</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>−20</td>
<td>1.5 ± 0.2</td>
<td>−1</td>
<td>2.4 ± 0.2</td>
<td>+60</td>
</tr>
<tr>
<td>% time spent</td>
<td>71</td>
<td>85</td>
<td>+20</td>
<td>82</td>
<td>+16</td>
<td>43</td>
<td>−39</td>
</tr>
<tr>
<td>Growing</td>
<td>5.0</td>
<td>3.6</td>
<td>−28</td>
<td>3.4</td>
<td>−32</td>
<td>6.8</td>
<td>+37</td>
</tr>
<tr>
<td>Shortening</td>
<td>24</td>
<td>11</td>
<td>−52</td>
<td>14</td>
<td>−40</td>
<td>50</td>
<td>+108</td>
</tr>
<tr>
<td>Attenuated</td>
<td>1.3</td>
<td>1</td>
<td>−21</td>
<td>0.9</td>
<td>0.03</td>
<td>0.9</td>
<td>−32</td>
</tr>
</tbody>
</table>

* Values that are statistically significant, at the ≥95% level, to the corresponding control value.

**Table 2 Effects of 0.4 μM vinflunine, vinorelbine, and vinblastine on steady-state microtubule transition frequencies**

<table>
<thead>
<tr>
<th>Transition frequency (events/min)</th>
<th>Control</th>
<th>Vinflunine</th>
<th>% change</th>
<th>Vinorelbine</th>
<th>% change</th>
<th>Vinblastine</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catastrophe</td>
<td>0.18 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>+2</td>
<td>0.19 ± 0.03</td>
<td>+3</td>
<td>0.20 ± 0.03</td>
<td>+13</td>
</tr>
<tr>
<td>Rescue</td>
<td>3.1 ± 0.40</td>
<td>4.4 ± 0.67</td>
<td>+43</td>
<td>4.9 ± 0.69</td>
<td>+60</td>
<td>2.6 ± 0.32</td>
<td>−17</td>
</tr>
</tbody>
</table>

* Estimates of variation and error are shown and were calculated as transition frequency divided by the square root of the sample size (41).
drugs slowed the growth rate to a similar extent, vinflunine and vinorelbine did not significantly reduce the length grown in a growth event, whereas vinblastine did. With regard to the average shortening event (Fig. 6B), vinflunine and vinorelbine, unlike vinblastine, had no effect on the shortening rate, but all three drugs reduced the length shortened to ~3–4 μm compared with 5.4 μm observed for controls.

**Possible Mechanisms for Suppression of Dynamic Instability by Vinflunine and Vinorelbine.** Free vinblastine appears to bind with high affinity to a maximum of 16–17 sites at microtubule ends without becoming incorporated into the core of the microtubules (28). Furthermore, the binding of one or two vinblastine molecules per microtubule is sufficient to inhibit the treadmilling addition of tubulin subunits by 50% (29). The binding of vinblastine to tubulin at the end of a microtubule may stabilize the end by inducing a conformational change that increases the affinity of the tubulin for its neighboring tubulin molecules (15, 24, 28–30). Differences in the effects of vinflunine, vinorelbine, and vinblastine on microtubule dynamic instability could be attributable to differences in the binding of the three drugs to the microtubule ends or in the extent to which the drugs induce stabilizing effects. The binding of vinflunine and vinorelbine to microtubule ends has not yet been determined. However, it is reasonable to assume that these two drugs, like vinblastine, also bind to the ends. If so, the block of growth could be a result of drug-induced conformational changes in tubulin at the microtubule end, or by simple steric hindrance. As suggested above, vinflunine and vinorelbine may be less able than vinblastine to increase the affinity of tubulin for its neighbors at the microtubule ends. This idea is supported by the observation that vinblastine binds more strongly to tubulin polymers than vinflunine or vinorelbine (31–33), and it would explain how growth, and not shortening, is inhibited by vinflunine and vinorelbine when the drug is bound to the microtubule end.

Alternative mechanisms involving drug-tubulin oligomers also exist. It is known that the *Vinca* alkaloids bind to free tubulin and to tubulin spiral oligomers, as well as to microtubules. Vinflunine, vinorelbine, and vinblastine bind to GTP-tubulin subunits with similar affinities (31, 32). The drug-liganded tubulin subunits then undergo isodesmic self-association and form drug-tubulin spiral oligomers. The equilibrium constants for *Vinca* alkaloid-tubulin self-association are larger than the equilibrium constants for *Vinca* alkaloid binding to tubulin, and they differ significantly among the three drugs with vinblastine > vinorelbine > vinflunine (33). Also, the spiral oligomers formed with the three drugs are different (32) and the equilibrium constants for binding to polymers differ for the three drugs (31–33). Thus there will be different proportions of free drug and
drug-tubulin oligomers present in suspensions of microtubules treated with the three drugs. Although the affinity of Vinca alkaloid-tubulin oligomers for microtubule ends is unknown, differences in the effects of the three drugs on dynamic instability could be attributable to differing affinities for binding of drug-tubulin oligomers to microtubule ends or to the effects of the different drug-bound oligomers at the ends.

**Effects of Vinflunine and Vinorelbine on Treadmilling.** Treadmilling has been postulated to play a role in inducing tension on the kinetochores during mitosis (18), a condition that may be critical for signaling the passage from metaphase to anaphase (22). A large number of antimitic drugs that block mitosis at the metaphase/ anaphase transition inhibit treadmilling at concentrations that have little effect on the mass of microtubule polymer (14, 23, 34–36). In the present study, we found that vinflunine inhibited the rate of treadmilling 4-fold less strongly than vinorelbine and 7-fold less strongly than vinblastine (Fig. 5). These potency differences on treadmilling may be an important determinant of antitumor activity. We recently modeled how the treadmilling rate can be greatly modified by small changes in the dissociation rate constant at microtubule minus ends (20). Vinblastine has been shown to stabilize microtubule plus ends and to destabilize minus ends (15). The effects of vinflunine and vinorelbine on dynamics at minus ends have not been determined, but it seems possible that all three drugs might destabilize minus ends differently. Such differential action at minus ends, which would result in differences in the abilities of the three drugs to affect treadmilling, may also be important in determining antitumor activity.

**Possible Significance for the Antitumor Efficacy of Vinflunine and Vinorelbine.** Vinflunine and vinorelbine have shown reduced toxicity in animal studies, and vinorelbine has shown reduced toxicity in the clinic as compared with vinblastine (4–6). Although differences in pharmacokinetics may play an important role, there are several other possible explanations for the reduced toxicity. One possibility involves the roles of microtubule dynamics during mitosis and the differences in the effects of the three drugs on the dynamics. Dynamic instability is important for congression of the chromosomes to the equatorial metaphase plate during prometaphase (37). It also plays an important role in the tension-associated oscillations of the chromosomes at prometaphase and metaphase (38). Treadmilling or poleward tubulin flux (21) may serve an important function during metaphase by mediating transport of signaling molecules from the kinetochores to the spindle poles, and also by creating tension (18). Vinblastine suppresses treadmilling more powerfully than vinflunine or vinorelbine (Table 1; Fig. 6), and it strongly suppresses the rate of shortening, whereas vinflunine and vinorelbine do not. These diverse actions of the three drugs are likely to have different effects during mitosis, which may lead to different effects on cell cycle progression and cell killing. Nontumor cells with normal checkpoint proteins could conceivably tolerate the relatively less powerful inhibitory effects of vinflunine and vinorelbine on microtubule dynamics but not the relatively more powerful effects of vinblastine. Furthermore, checkpoint mechanisms in tumor cells are frequently faulty (39, 40). Thus, cancer cells with defective checkpoint proteins may be more susceptible than normal cells to vinflunine and vinorelbine, yielding an overall effectiveness that is superior to that of vinblastine.

**ACKNOWLEDGMENTS**

We thank Herb Miller for preparing the tubulin used in this work.
Novel Actions of the Antitumor Drugs Vinflunine and Vinorelbine on Microtubules


Cancer Res 2000;60:5045-5051.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/18/5045

Cited articles
This article cites 33 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/18/5045.full#ref-list-1

Citing articles
This article has been cited by 16 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/18/5045.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/60/18/5045.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.