Antivascular Actions of Microtubule-Binding Drugs
Edward L. Schwartz

Abstract
Microtubule-binding drugs (MBD) are widely used in cancer chemotherapy and also have clinically relevant antiangiogenic and vascular-disrupting properties. These antivascular actions are due in part to direct effects on endothelial cells, and all MBDs (both microtubule-stabilizing and microtubule-destabilizing) inhibit endothelial cell proliferation, migration, and tube formation in vitro, actions that are thought to correspond to therapeutic antiangiogenic actions. In addition, the microtubule-destabilizing agents cause prominent changes in endothelial cell morphology, an action associated with rapid vascular collapse in vivo. The effects on endothelial cells occur in vitro at low drug concentrations, which do not affect microtubule gross morphology, do not cause microtubule bundling or microtubule loss and do not induce cell cycle arrest, apoptosis, or cell death. Rather, it has been hypothesized that, at low concentrations, MBDs produce more subtle effects on microtubule dynamics, block critical cell signaling pathways, and prevent the microtubules from properly interacting with transient subcellular assemblies (focal adhesions and adherens junctions) whose subsequent stabilization and/or maturation are required for cell motility and cell-cell interactions. This review will focus on recent studies to define the molecular mechanisms for the antivascular actions of the MBDs, information that could be useful in the identification or design of agents whose actions more selectively target the tumor vasculature.

Microtubule-Binding Drugs Have Antitumor and Antivascular Actions
Microtubule-binding drugs (MBD) have documented antitumor activity and are widely used in curative and palliative cancer chemotherapy regimens. Some MBDs are well established clinically and are active against solid tumors, including breast, lung, prostate, and ovarian cancers (Taxol and Taxotere) and leukemias and lymphomas (vincristine and vinblastine; ref. 1). Newer MBDs are in phase II and III clinical trials, where they have shown activity against refractory or advanced carcinomas of the breast, lung, prostate, ovary, and thyroid (epothilones and combretastatins; refs. 1, 2). The large number of ongoing trials that include a MBD make it likely that their clinical spectrum of activity will continue to expand.

Microtubules are key components of the cytoskeleton and are composed of heterodimers of α- and β-tubulin (proteins of ~50 kDa molecular weight), which assemble into linear, hollow, cytoplasmic filaments. MBDs can be broadly classified by their effect on microtubule polymer stability (they can promote polymerization or depolymerization) or based on their binding site on microtubules (to the Vinca, colchicine, or taxane binding domains). All classes of MBDs, those that stabilize microtubules and stimulate their polymerization (e.g., taxanes and epothilones) and those that destabilize microtubules and cause their depolymerization (e.g., colchicine, Vinca alkaloids, and combretastatins), can interfere with mitotic spindle formation in tumor cells, block proliferation by cell cycle arrest, and cause cell death via the induction of apoptosis, actions that undoubtedly contribute to their clinical activity (1).

More recent studies have shown that most MBDs also have antiangiogenic or vascular-disrupting activities or both; in this review, these will be referred to collectively as antivascular effects (Table 1). Targeting of the tumor vasculature as a therapeutic approach has a compelling theoretical rationale, is strongly supported by preclinical studies, and has been validated by clinical trials (34). There are now data to suggest that MBDs would be a particularly useful class of drugs for this purpose, most notably, evidence that MBDs have multiple direct actions on endothelial cells (discussed in detail below) and that they can produce a much greater reduction in blood flow in tumors than in normal tissues (35). A critical unanswered question is whether the antivascular actions of the MBDs contribute to their clinical efficacy, relative to their direct cytotoxic actions against tumor cells. In this regard, there are data from mouse models to suggest that the antivascular actions of the MBDs are therapeutically important (36). In this study, Taxotere was found to retain much of its antitumor activity against an ovarian cancer xenograft tumor that had been established by inoculating mice with Taxotere-resistant ovarian cancer cells (36). Because these cells were partially or completely resistant to the cytotoxic actions of Taxotere, the antitumor effect was attributed to the antiangiogenic actions of Taxotere that were observed in these tumors (36). This conclusion is in agreement with similar observations made in experiments done by treating mice with cyclophosphamide-resistant lung cancers with cyclophosphamide, using different

Author’s Affiliation: Department of Oncology, Albert Einstein College of Medicine, Bronx, New York
Received 10/17/08; revised 12/29/08; accepted 1/6/09; published OnlineFirst 4/7/08
Grant support: National Cancer Institute, NIH grants RO1 CA 89456 and RO1 CA 89352.
Requests for reprints: Edward L. Schwartz, Department of Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY 10467. Phone: 718-920-4015; Fax: 718-882-4464; E-mail: eschwart@aecom.yu.edu.
© 2009 American Association for Cancer Research. doi:10.1158/1078-0432.CCR-08-2710

www.aacrjournals.org Clin Cancer Res 2009;15(8) April 15, 2009 2594
The neovascularization process and the maintenance of actions on endothelial cells that are more specifically related to the MBDs, recent reports indicate that these drugs have other apoptosis undoubtedly contribute to the antivascular effects of 40). Although the inhibition of proliferation and induction of more than five times higher than other cell types (28, 39, cells to accumulate certain MBDs intracellularly at levels observation that maybe due in part to the ability of endothelial endothelial cells are more sensitive to growth inhibition by are cytotoxic toward endothelial cells and can inhibit their proliferation (3, 4, 9, 18, 19, 28). In fact, in some instances, endothelial cells are more sensitive to growth inhibition by MBDs than are cancer cell lines or other primary cells, an observation that may be due in part to the ability of endothelial cells to accumulate certain MBDs intracellularly at levels more than five times higher than other cell types (28, 39, 40). Although the inhibition of proliferation and induction of apoptosis undoubtedly contribute to the antivascular effects of the MBDs, recent reports indicate that these drugs have other actions on endothelial cells that are more specifically related to the neovascularization process and the maintenance of the microvasculature. Several in vitro antivascular actions occur at MBD concentrations that are substantially lower than those required to block mitosis, produce cell cycle arrest, or induce apoptosis (9, 18, 28, 41). Similar observations have been made in vivo: a hallmark of vascular-disrupting MBDs such as the combretastatins is the induction of a rapid collapse in tumor blood flow, often first detected within 5 min of drug treatment in animal models and leading to complete vascular shutdown by 20 min (42). Tumor imaging studies have identified similar effects in phase I clinical trials of these agents (Table 2). Drug-induced effects on endothelial cell proliferation and/or endothelial cell apoptosis occur too slowly to account for these antivascular actions in vivo; rather, morphologic and functional changes in the endothelial cells are more likely to cause the tumour vasculature collapse (35, 40).

MBDs Inhibit Multiple Functions of Endothelial Cells

Tumor neovascularization occurs primarily (but not exclusively) by the sprouting of established capillaries. This process involves several steps, beginning with the cell-mediated proteolytic degradation of the surrounding basement membrane, the migration of endothelial cells out of an existing vessel into the surrounding extracellular matrix, the proliferation of the endothelial cells, and the organization and morphogenesis of the cells into tube-like structures (47). Aspects of these processes can be studied in tissue culture; thus, when plated on extracellular matrix, endothelial cells form structures that morphologically resemble capillary-like vessels, with lumens and an anastomosing and branching tubular network (48, 49). MBDs potently inhibit vessel formation in vitro, and this is likely due to their ability to disrupt one or more of the individual components of the process, including endothelial cell adhesion, migration, and cell-cell interactions (3, 5, 6, 9–11, 14, 15, 21, 25, 28, 30, 41). MBDs have antivascular effects in vivo as well, blocking angiogenesis in assays using subcutaneous Matrigel plugs, chick embryo chorioallantoic membranes, and corneal micropockets; they also have antivascular effects in tumor xenografts (9, 10, 19, 21, 26, 29, 41, 50). In vivo they can produce morphologic

### Table 1. MBDs with antivascular actions

<table>
<thead>
<tr>
<th>Agents that stimulate microtubule polymerization and increase their stability</th>
<th>Clinical status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel (Taxol)</td>
<td>Approved</td>
<td>(3–8)</td>
</tr>
<tr>
<td>Docetaxel (Taxotere)</td>
<td>Approved</td>
<td>(5, 9, 9–11)</td>
</tr>
<tr>
<td>Epothilones (Ixabepilone, Patupilone, KOS862)</td>
<td>Phase III</td>
<td>(6, 12, 13)</td>
</tr>
<tr>
<td>Laulimalide</td>
<td>Preclinical</td>
<td>(14)</td>
</tr>
<tr>
<td>IDN 5390</td>
<td>Preclinical</td>
<td>(15)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Agents that destabilize microtubules and cause their depolymerization</th>
<th>Clinical status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>Nonneoplastic use</td>
<td>(6, 16–18)</td>
</tr>
<tr>
<td>Combretastatins (Zybrestat, AVE8062, OXI4503)</td>
<td>Phase II</td>
<td>(19–22)</td>
</tr>
<tr>
<td>2-Methoxyestradiol (Panzem)</td>
<td>Phase II</td>
<td>(6, 18, 23)</td>
</tr>
<tr>
<td>JG-03-14</td>
<td>Preclinical</td>
<td>(24)</td>
</tr>
<tr>
<td>N-acetylcysteimol (ZD6126)</td>
<td>Preclinical</td>
<td>(25–27)</td>
</tr>
<tr>
<td>Vinblastine (Velban)</td>
<td>Approved</td>
<td>(6, 17, 28, 29)</td>
</tr>
<tr>
<td>Vincristine (Oncovin)</td>
<td>Approved</td>
<td>(29)</td>
</tr>
<tr>
<td>Vinflunine</td>
<td>Phase III</td>
<td>(30, 31)</td>
</tr>
<tr>
<td>Tubulysin A</td>
<td>Preclinical</td>
<td>(32)</td>
</tr>
<tr>
<td>XRP44X</td>
<td>Preclinical</td>
<td>(33)</td>
</tr>
</tbody>
</table>
alterations of the neovasculature, increase tumor vascular permeability, and can cause near complete vascular shutdown (19–27). As noted above, many of these inhibitory effects are observed at MBD concentrations that may be 100-fold lower than those required to produce cell toxicity (3–5, 8, 9, 15, 18, 25, 28). For example, docetaxel inhibits endothelial cell proliferation in vitro, with reported IC50 values ranging from 5 to 21 nmol/L, but inhibits endothelial cell tube formation and cell migration at much lower IC50 values of 0.3 to 0.8 nmol/L and 0.01 nmol/L, respectively (6, 10, 14). Similar results, although less pronounced, were observed with combretastatin, where the inhibition of endothelial cell migration and tube formation occurred at concentrations that were 8- to 16-fold lower than those that inhibited endothelial cell proliferation (41).

What actions of the MBDs might be responsible for these effects on endothelial cells? It has been known for some time that MBDs such as colchicine, nocodazole, vinblastine, and paclitaxel can interfere with the migration of fibroblasts, monocytes, and carcinoma cells (50–55). These early reports, in which the MBDs caused near complete microtubule breakdown, concluded that the actions of drugs were due to the loss of the ability of cells to polarize their actin activity, thereby preventing cell motion (54, 56). As microtubules play a critical role in regulating the organization of actin into stress fibers, agents that interfere with microtubule function can also cause the loss of cell polarity, interfere with the formation of cell protrusions such as lamellipodium, and interfere with cell contractility (54). However, the high concentrations of MBDs used in these early studies make it unclear whether these mechanisms are relevant to the actions of the drugs at concentrations that occur clinically. More recent studies have identified novel additional actions of the MBDs that occur at lower concentrations and therefore could contribute to any clinical antiangiogenic actions of the MBDs. These include the blockade of critical cell signaling pathways [including from the vascular endothelial growth factor (VEGF) receptor] and the interference with the proper functioning of transient subcellular assemblies (focal adhesions and adherens junctions) whose subsequent stabilization and/or maturation are required for cell adhesion, cell motility, and cell-cell interactions (11, 14, 20, 21).

### MBDs Target Focal Adhesions and Adherens Junctions in Endothelial Cells

MBDs have been reported to have effects on focal adhesions and adherens junctions in endothelial cells as well as on related pathways (Fig. 1). When stimulated to migrate, endothelial cells become elongated and polarized (they develop a front and back), with lamellipodium at their leading edge and a trailing cell body (57). The highly dynamic lamellipodium forms new contacts with the underlying extracellular matrix, a process mediated by focal adhesions and, more specifically, by the binding of cell surface integrins to the extracellular matrix (57). Focal adhesions are transient self-assembling protein complexes that are located at the cell surface and that serve as links to the intracellular cytoskeleton. The microtubule-stabilizing and microtubule-destabilizing MBDs caused decreased focal adhesion formation or defective focal adhesion assembly, respectively, in endothelial cells (11, 14, 20, 31). The microtubule-stabilizing MBDs blocked integrin activation and inhibited downstream signaling from the integrins along the FAK-paxillin-Akt pathway (11, 14). Inhibition of multiple heat shock protein 90-dependent signaling pathways, including reduced activation of endothelial nitric oxide synthase, was also observed. Interestingly, the microtubule-destabilizing MBDs appear not to affect FAK activation (24). They have been shown, however, to inhibit Ras-extracellular signal-regulated kinase-Net signaling and to increase myosin light chain phosphorylation and actinomysin contractility (20, 33).

### Table 2. Vascular response measurements from phase I pharmacodynamic clinical trials of combretastatin A4 phosphate

<table>
<thead>
<tr>
<th>Combretastatin A4 phosphate dose range (10 min intravenous infusion)</th>
<th>Vascular parameter</th>
<th>Measurement times</th>
<th>Effects observed (P)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-114 mg/m²</td>
<td>Blood flow</td>
<td>Pre, 0.5, and 24 h post</td>
<td>Mean changes: tumor: -49% (0.001), spleen: -35% (0.018), kidney: -6% (0.026)</td>
<td>(43)</td>
</tr>
<tr>
<td>5-114 mg/m²</td>
<td>Blood volume</td>
<td>Pre, 0.5, and 24 h post</td>
<td>Mean changes: tumor: -15% (0.007), spleen: -18% (0.022), kidney: -6% (0.020)</td>
<td>(43)</td>
</tr>
<tr>
<td>20-114 mg/m²</td>
<td>Perfusion</td>
<td>Pre, 0.5, and 24 h post</td>
<td>Mean changes: tumor: -37% (0.002), muscle: NS, kidney: -2% (NS)</td>
<td>(44)</td>
</tr>
<tr>
<td>52-75 mg/m²</td>
<td>Perfusion</td>
<td>Pre and 6-8 h post</td>
<td>Tumor: of 10 patients, 5 had modest and 3 had marked decreases</td>
<td>(45)</td>
</tr>
<tr>
<td>40-114 mg/m²</td>
<td>Perfusion</td>
<td>Pre and 4 h post</td>
<td>Tumor: of 21 patients, 12 had decreases ≥20%, of which 3 were significant</td>
<td>(46)</td>
</tr>
</tbody>
</table>

**NOTE:** NS, not significant.

*Relative to combretastatin A4 phosphate administration.

<table>
<thead>
<tr>
<th>Effect observed (P)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data for earliest post-combretastatin A4 phosphate measurement time. In all cases, the magnitude of change was reduced at later time points.</td>
<td>(43)</td>
</tr>
<tr>
<td>1mL blood/mL tissue/min. 15O-water positron emission tomography.</td>
<td>(45)</td>
</tr>
<tr>
<td>15O-carbon monoxide positron emission tomography.</td>
<td>(46)</td>
</tr>
<tr>
<td>Tumor perfusion is a measure of the tissue blood flow rate and the permeability of the vasculature wall. Measured by the transfer rate/min, Ktrans, using dynamic contrast-enhanced magnetic resonance imaging.</td>
<td>(46)</td>
</tr>
</tbody>
</table>

[Note: NS, not significant. ]

---

**References**

(43) [www.aacrjournals.org](http://www.aacrjournals.org)
The homotypic interactions of endothelial cells are critical both for vessel formation in sprouting angiogenesis and for the maintenance of vasculature integrity in established capillaries (58). Endothelial cell engagement with other endothelial cells is mediated in part by adherens junctions, which, like the focal adhesions, are cell surface protein complexes. Adherens junctions are primarily formed by the cadherin adhesion proteins (predominantly VE-cadherin in endothelial cells) and other proteins...
that contribute to their function, including α- and β-catenins, Arp2/3, afadin, p120, plakoglobin, α-actinin, and vinculin (58). Recent reports suggest that the microtubule-destabilizing agents can disrupt VE-cadherin engagement and inhibit signaling along the VE-cadherin/β-catenin/Akt pathway (21, 24). Disruption of adherens junctions contributes to the rounding up of endothelial cells, which in vivo would lead to a direct increase in the geometric resistance to blood flow in capillaries and an increase in vasculature permeability. The latter would cause an acute leakage of plasma proteins from the vasculature, increasing the interstitial fluid pressure and reducing the oncotic pressure difference between the inside and the outside of the vessel, subsequently contributing to vascular shutdown (26, 59).

Is there a unifying mechanism for these cellular effects of the MBDs? As described above, the integrins and VE-cadherin play central roles in endothelial cell attachment, migration, and capillary tube formation and maintenance, and they share several similar properties: they provide a direct connection to the actin cytoskeleton, the driving force for cell motility and that also help to stabilize adherens junctions; they mediate signaling across the cell membrane and activate downstream signaling pathways; and they link these processes to the actions of cell surface receptors that regulate angiogenesis, notably those for VEGF and fibroblast growth factor (57, 58, 60). In this regard, it is noteworthy that both classes of MBDs are able to block signaling from the VEGF receptors, although, in the case of microtubule-stabilizing agents, this may be mainly directed toward VEGF-mediated effects on focal adhesions, whereas the adherens junctions may be the predominant target of the microtubule-destabilizing drugs. The actions of VEGF receptors are interconnected with those of the integrins and VE-cadherin, and the formation of complexes between VEGF receptor-2 and either an integrin or VE-cadherin appears to be important for the optimal outside-in and/or outside-out signaling of all three proteins. Thus, it is possible that MBDs have one or more cellular actions that ultimately prevent the endothelial cell from responding to and integrating multiple extracellular signals, whether they emanate from, or lead to the activation of, a VEGF receptor, an integrin, or VE-cadherin.

Can the Disruption of Microtubule Functions Account for the Noncytotoxic, Antivascular Effects of MBDs?

As discussed above, microtubules play a critical role in mitotic spindle function and mitosis, providing an attractive target for drug development for proliferating cells. These agents also affect the microtubule network in nonproliferating and interphase cells. The microtubules in endothelial cells in interphase are dynamic polarized structures, and during cell migration, the fast-growing plus ends of the microtubule polymer is targeted to and captured by the forming focal adhesions, whereas the stable minus ends are localized to the microtubule organizing center (MOC; also known as the centrosome), a structure typically found at a perinuclear position in cells (61). Although the role of microtubules in focal adhesion function is not fully understood, they have been shown to participate in the control of integrin clustering and avidity, actions associated with integrin activation and that allow their high-affinity binding to extracellular matrix ligands (62). Less is known about the role of the microtubules in adherens junctions, although it has been suggested that protein regulators of the microtubule cytoskeleton might localize at adherens junctions (63). In this regard, a recent study found that low noncytotoxic concentrations of vinflunine disrupted the localization of the protein EB1 at the microtubule plus ends (31). EB1 belongs to the family of microtubule plus end-tracking proteins, which mediate many microtubule-regulated actions, including cell migration, targeting and capture of microtubules at adhesion sites, and stabilization of microtubules at the cell cortex (64, 65). Further studies are needed to confirm this observation and to assess whether the function of other microtubule plus end-tracking regulatory proteins are altered by changes in microtubule dynamics due to MBDs.

An early event in directed cell migration that is dependent on the maintenance of microtubule plasticity is the reorientation of the MOC toward the side of the nucleus in the intended direction of movement (66). Extension of a new lamellipod in a migrating cell precedes MOC reorientation to the new leading edge of the cell. If MOC reorientation does not occur, the new lamellipod is retracted (66). Thus, although MOC reorientation does not direct cell motion, it has been postulated to be required for the maintenance of leading lamellipodia and for stabilizing a chosen direction of movement. Low concentrations of docetaxel have been reported to impair MOC reorientation in endothelial cells stimulated to undergo migration (9).

As noted above, the plus ends of microtubules exhibit dynamic instability (random changes between periods of growth and shortening), and this dynamic instability is essential for migration of some cell types. It may also contribute to the interactions between the microtubule and actin microfilament systems that are critical for cell migration (1, 67). The reorganization of actin into stress fibers and many aspects of cell locomotion are regulated by members of the Rho family of small GTPases. Thus, Cdc42 and Rac1 induce formation of filopodia and lamellipodia, respectively. RhoA has been shown to regulate the formation of actin stress fibers and focal adhesions, and the reorientation of the MOC has been reported to depend on the activation of Cdc42 (68). Although the Rho proteins are regulated by several different upstream factors, direct evidence for microtubule-dependent regulation of Rho GTPases has come from biochemical studies that found that the depolymerization of microtubules resulted in an increase in the level of GTP-bound RhoA, whereas polymerization of microtubules resulted in activation of Rac1 (67, 68).

The linkage between microtubule dynamic instability and Rho GTPase regulation and actin reorganization is likely central to understanding how specific MBDs affect endothelial cell function. The predominant cellular actions of both classes (stabilizing and destabilizing) of MBDs can vary dramatically depending on the concentration at which they are studied. Although, at high micromolar concentrations, the microtubule-stabilizing and microtubule-destabilizing agents have quite different phenotypic effects on microtubules and on cells in vitro, at lower antiproliferative concentrations, both classes inhibit microtubule dynamics; this ultimately interferes with mitotic spindle function and leads to cell cycle arrest and/or apoptosis (1). Paradoxically, it has been reported that, at even lower concentrations, MBDs can actually increase microtubule dynamics (7, 30, 31). These intriguing studies had several
noteworthy aspects: increased microtubule dynamic instability was observed at drug concentrations that were 50- to 100-fold lower than those which inhibited endothelial cell proliferation, the effect was selective for endothelial cells and was not observed in tumor cells, and it was observed with both microtubule-stabilizing (Taxol) and microtubule-destabilizing (vinflunine) agents (7, 30). Consistent with these observations, both polymerizing and depolymerizing MBDs have been reported to cause altered Rho-GTPase signaling, including activation of RhoA and inhibition of Rac1 and Cdc42, and to produce an inhibition, stimulation, and/or misassembly of actin into stress fibers and focal adhesions (6, 20, 69). These observations raise the possibility that, at some level, the microtubule-stabilizing and microtubule-destabilizing classes of agents share similar or overlapping molecular mechanisms of action. It should be noted that different MBDs varied in the nature of their specific effects on the Rho signaling pathway. Interestingly, direct disruption of actin microfilaments in endothelial cells by cytocholasin B had a much weaker effect on microvessel structure than did the MBDs (70).

In addition to being required for cell signaling and migration, microtubules are also involved in the intracellular transport of proteins and vesicles, the maintenance of the composition of the plasma membrane, the regulation of cell shape, and the development of cell polarization (9, 20, 21, 25, 69, 71). The disruption of one or more of these by MBDs could interfere with the formation and maintenance of the tumor vasculature.

**Do Differences in the Location and Consequences of the Binding of a Specific Agent to Tubulin Influence Their Effect on Endothelial Cell Function?**

Despite the similarities in cellular actions noted above, studies to date have tended to differentiate the two classes of MBDs based on their predominant antivascular actions, with the microtubule-stabilizing agents having antiangiogenic actions that occur at concentrations well below their cytotoxic IC50 values versus the microtubule-destabilizing drugs that more prominently cause vascular collapse in vivo, generally at concentrations that are closer to those that at which they are cytotoxic. As noted above, however, the microtubule-destabilizing drugs are also potent inhibitors of endothelial cell migration and tube formation and thus are likely to also be antiangiogenic. Thus, although the classification of an agent as either antiangiogenic or vascular disrupting is somewhat arbitrary, these distinctions may be helpful in discerning either broad patterns of antivascular effects or as a way of categorizing therapeutically relevant drug actions on endothelial cells.

A related question is whether differences in the nature of the binding of the MBDs can be exploited in future drug development to design agents that have the specific desired antivascular actions. As noted above, both classes of MBDs can cause an increase in microtubule dynamics; however, differences between them have been noted. Thus, although both the stabilizing agent Taxol and the destabilizing agent vinflunine were associated with increased microtubule growth, increased shortening rates, and decreased pause times, only Taxol also altered the time-based microtubule transition frequency (7, 30). Differences in the microtubule binding site can also influence selected molecular actions on endothelial cells. The phosphorylation of Net (a transcription factor involved in angiogenesis) was strongly inhibited in endothelial cells by combretastatin-A4 but only weakly blocked by vincristine and not at all by Taxotere (33). Laulimalide, which stabilizes microtubules in manner indistinguishable from docetaxel but binds to a site distinct from that of the taxanes, was much more potent in blocking the phosphorylation of paxillin than was Taxotere in endothelial cells (14). Whether these and other potential subtle differences affect the broader antivascular actions of these agents is not known.

**Future MBD Development**

If the salient mechanistic aspects of the antivascular actions of the MBDs could be determined with some degree of confidence, these could then be used in structure-activity analyses to search for links to the way in which different MBDs interact with microtubules in endothelial cells. A practical consideration in the identification of therapeutically useful compounds would be the selection of appropriate and progressively biologically complex drug screening approaches. Most of the studies discussed in this review have examined the effects of the MBDs on cultured endothelial cells, either primary human umbilical vein or microvascular endothelial cells or immortalized lines derived from them. There are little data to indicate which, if any, of these cell types would be the most appropriate for evaluating the actions of the MBDs on the tumor vasculature. Interestingly, endothelial precursor cells derived in vitro from human AC133+/CD34+ bone marrow progenitor cells have been found to express genes that were more similar to those expressed by endothelial cells isolated from fresh surgical specimens of human tumors when compared with human umbilical vein or microvascular endothelial cells, suggesting that these cells could be valuable models (72). Assessment of drug effects on the architecture of vessel formation and maintenance, using, for example, in vitro and in vivo Matrigel tubule assays, are likely to be particularly important for evaluating the effects of the MBDs. Nascent tumor vessels are often deficient in pericyte coverage, and these vessels have been found to be more sensitive to the disrupting effects of combretastatin than were more mature, pericyte-enwrapped vessels (pericytes themselves were found to be resistant to combretastatin relative to endothelial cells; ref. 21). This study suggests that MBDs could have some selectivity for tumor versus normal vasculature. Ultimately, this hypothesis will have to be tested using pharmacodynamic measurements of tumor vascular parameters in preclinical and clinical studies.

Hundreds of analogues of MBDs have been synthesized and studied in detail in cell-free and cellular assays, and as would be expected, varying chemical substitutions affect the nature of the interaction of the agents with tubulin and microtubules, leading to wide variations of potencies in tubulin polymerization and depolymerization assays (73–75). However, for both agents that stabilize and destabilize microtubules, attempts to correlate the effects on microtubule polymerization with inhibition of proliferation or cytotoxicity are often not successful (73, 74). One might anticipate that the potencies to produce cytotoxicity, which presumably incorporates any differences in the uptake, metabolism, nonspecific binding, etc., of the agents in intact cells, could be used as a surrogate for...
their antivascular effects. Limited data, however, would suggest that this is not the case. In a study of over fifty 2-aroylindole derivatives that bind to and destabilize microtubules, there seemed to be little relationship between the IC_{50} to inhibit the proliferation of multiple cancer cell lines and the antivascular activity in a chorioallantoic membrane assay (75). Thus, although we are far from understanding the relationship between specific effects on microtubules and antivascular activity, this study does support the premise that the antivascular effects of the MBDs can be separated from their cytotoxic actions. Identification of MBDs with greater therapeu- tic antivascular selectivity, relative to their cancer cell cytotoxicity, would be an important objective for the next generation of MBDs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

39. Kubota Y, Kleinman HK, Martin GR, Lawley TJ. Role


