Mechanisms of multidrug resistance: the potential role of microtubule-stabilizing agents

T. Fojo1* & M. Menefee2
1National Cancer Institute, Bethesda, MD; 2Division of Medical Oncology, Mayo Clinic, Rochester, MN, USA

Antimitotic agents that target the dynamic equilibrium between the microtubule polymer and tubulin heterodimers are key components of chemotherapeutic regimens for various solid tumors. These agents can be divided into two major classes based on their effect on microtubule polymerization and the mass of microtubule polymers: those that inhibit polymerization, such as the vinca alkaloids and those that stabilize microtubules, such as the taxanes and epothilones. The taxanes paclitaxel (Taxol) and docetaxel (Taxotere) were the first antimicrotubule agents approved for use in solid tumors, but their usefulness is often limited by development of drug resistance. The epothilones are distinguished from the taxanes structurally and functionally and have been shown in vitro and in preclinical models to have superior potency to the taxanes. The epothilones are not susceptible to P-glycoprotein-mediated efflux and have shown activity against taxane-resistant tumors. Other natural-product microtubule-stabilizing agents also have promising pharmacologic profiles. This article discusses mechanisms of drug resistance and summarizes scientific and clinical data supporting the potential of novel microtubule-stabilizing agents for achieving broad antitumor efficacy without the emergence of drug resistance. The ability to reduce the development of resistance with the epothilones and other microtubule-stabilizing agents may provide additional treatment options at the time of presentation and in the setting of taxane resistance.

Key words: epothilones, microtubulin, paclitaxel, P-glycoprotein, taxanes, tubulin

introduction

The effectiveness of many chemotherapeutic agents used in cancer therapy is limited by drug resistance, which may be intrinsic or acquired during the course of treatment. In the case of acquired drug resistance, tumors may become resistant to drugs other than those initiating the resistance, despite the fact that these drugs may have different mechanisms of action. Drug resistance plays a role in the initial treatment and in the adjuvant setting and has been estimated to cause treatment failure in >90% of patients with metastatic disease [1]. The development of drugs that are less sensitive to known resistance mechanisms may result in broader antitumor activity than that of the molecules from which they were derived, and this, in turn, could result in improved rates of survival.

Since the original description of paclitaxel (Taxol, Bristol-Myers Squibb, New York) as an antimicrotubule agent in the 1970s, structurally complex alkaloids and synthetic compounds that disrupt microtubules, and hence mitosis, have been the subject of intense investigation; these compounds have had a significant impact on the treatment of human malignancies [2, 3]. In particular, the prototypical taxane paclitaxel, the first natural product shown to promote tubulin assembly, as well as the semisynthetic derivative docetaxel (Taxotere, Sanofi-Aventis, Bridgewater, NJ), stabilize microtubules and have demonstrated activity against a broad spectrum of malignancies, including those of the ovary, breast, lung, and head and neck, as well as Kaposi’s sarcoma [4]. However, the enormous clinical success of taxanes has been compromised by the emergence of drug resistance, derived from several mechanisms [5]. Other potential shortcomings of the taxanes include numerous side-effects, including neutropenia and neurotoxicity, as well as the presence in the formulation of the nonionic surfactant Cremophor® EL (BASF, Ludwigshafen, Germany), a potential trigger for hypersensitivity reactions [6].

The impressive activity that the taxanes exhibit in a variety of clinical settings has led to a search for new antimicrotubule agents with superior therapeutic indices and broader antitumor activity. In this review, we summarize our current state of knowledge of the pharmacologic and chemical properties of the class of targeted antimitotic agents referred to as microtubule-stabilizing agents, which constitute a diverse group of compounds that promote the assembly of mammalian tubulin and stabilize the microtubule polymer. We also review molecular mechanisms of drug resistance operative in cancer cells that help to reduce their drug sensitivity.

microtubules: a target for anticancer therapy

Microtubules are intracellular tubular structures found in all eukaryotic cells. Microtubules have various functions including...
organization of intracellular structure, cell division, and intracellular transport. Tubulin heterodimers consist of two closely related globular proteins (molecular mass, ~50 000 kDa; 450 amino acids), the α- and β-tubulins, which are encoded by separate genes and represent the basic structural components of microtubules. The three-dimensional structure of the α,β-tubulin heterodimer has been determined by electron crystallography [7].

The side-by-side assembly of tubulin heterodimers forms 13 protofilaments that surround a hollow core. In this parallel alignment of protofilaments, the β subunit of one dimer contacts the α-tubulin subunit of the next. An increasing body of evidence indicates that the function of microtubules can be regulated by differential expression of six α-tubulin and seven β-tubulin isotypes [8, 9]. In turn, this stability appears to be ‘tagged’ by posttranslational modification of the α-subunit, including acetylation of lysine 40, and a carboxypeptidase-catalyzed removal of the C-terminal tyrosine, two modifications that are considered markers of a more stable microtubule. Various microtubule-associated proteins (MAPs) may also regulate microtubule function [10–12].

Microtubules are dynamic polymers. A dynamic equilibrium exists between the intracellular pool of α,β-tubulin heterodimers and the microtubule polymer, and it is this equilibrium that is the target for microtubule-disrupting agents. Their dynamics consist mainly of stochastic phases of equilibrium that is the target for microtubule-disrupting agents. Their dynamics consist mainly of stochastic phases of growth and shrinkage due to polymerization and disassembly of tubulin dimers, a pattern of nonequilibrium behavior known as dynamic instability [13]. Dynamic instability can be considered to consist of phases describing growth, disassembly, the transition from growth to disassembly, and the transition from disassembly to growth [14, 15]. By changes in these dynamic instability parameters, cells rearrange the microtubule network and quickly respond to environmental and developmental stimuli [16].

mechanisms of multidrug resistance

Drug resistance can severely limit the effectiveness of cancer chemotherapy and affect patient survival. Drug resistance may occur at the level of the cell (e.g. evasion of apoptosis or changes in the target protein) or occur as a consequence of poor pharmacologic accessibility of the drug (e.g. changes in its rate of transport in and out of the cell or its metabolism, or at a more proximal level, reduced drug delivery secondary to poor perfusion). Acquired taxane resistance is considered to be mediated by multiple mechanisms including altered intracellular drug levels, variations in tubulin structure, altered signal transduction, and apoptotic pathways.

The overexpression of MDR-1 or P-glycoprotein (Pgp), a drug transporter belonging to the ATP-binding cassette (ABC) superfamily of proteins, is often the cause of resistance to antimitotic agents, including the taxanes, as demonstrated in vitro, in preclinical models and in patients. The ABC superfamily in humans includes genes whose products (such as Pgp) are membrane proteins involved in energy-dependent transport of a wide variety of substrates across the plasma membrane. In humans, several ABC proteins, including ABCB1 (Pgp/MDR-1), ABCC1 (MRP1), and ABCG2 (MXR/BCRP), participate in the blood–brain, the blood–testis, and the placental barriers. Currently, there are 48 known human ABC genes, which can be divided into seven distinct subfamilies, A through G, based on the organization of their transmembrane and ATP-binding domains and amino acid homology. The existence of ATP-binding domains composed of a signature sequence and two motifs, designated the Walker A and B motifs, are the basis for the classification of proteins as ABC transporters. The three ABC genes that are involved in the normal tissue barriers described above—ABCB1 (Pgp/MDR-1), ABCC1 (MRP1), and ABCG2 (MXR/BCRP)—are also thought to be primarily responsible for the drug-resistant phenotype in both human and rodent cells. These proteins actively extrude drugs out of cells, demonstrating a preference for many of the naturally occurring anticancer agents, including the taxanes, the vinca alkaloids, the anthracyclines, the epipodophyllotoxins, and other lipophilic natural products [17]. Each of these transporters has a unique drug resistance profile (Table 1). It is notable that none of the epothilones is transported by these or any other known drug transporter.

Pgp, a member of the ABCB subfamily, is arguably the most important of the ABC transporters since it confers the strongest resistance to the widest variety of compounds. Multidrug resistance is often associated with the overexpression of Pgp in leukemias and solid tumors, which frequently follows the administration of Pgp substrates [18, 19]. The prominent role of Pgp in multidrug resistance has led to a search for ABC transporter inhibitors to enhance intracellular drug accumulation. The use of both first- and second-generation Pgp inhibitors has led to poor clinical outcomes for various reasons, including unacceptable toxic effects, variable pharmacokinetic interactions that lead to greater toxicity for chemotherapeutic agents, and lack of specific molecular targeting [18, 19]. Superior potency, higher specificity, and reduced pharmacokinetic interactions exhibited by a third

| Table 1. Substrate specificity for the three principal ABC transporters involved in multidrug resistance in humans and rodents |
|---------------------------------|-----------------|-----------------|
| **Pgp/MDR-1** | **MRP1** | **ABCG2** |
| Vincristine | Vincristine | Mitoxantrone |
| Vinblastine | VP-16 | Topotecan |
| Vinorelbine | Irinotecan (SN-38) | Irinotecan (SN-38) |
| Taxol | Doxorubicin | SN-38-glucuronide |
| Docetaxel | MTX | Other camptothecins |
| VP-16 | MTX | MTX |
| Mitoxantrone | MTX polyglutamates | |
| Bisantrene | Flavopiridol | |
| Doxorubicin | PDT agents | |
| Daunorubicin | AZT | |
| Epirubicin | Geltitinib (Iressa) | |
| Rhodamine | STI571 (Gleevec) | |
| Topotecan | | |
| STI571 (Gleevec) | | |

ABC, ATP-binding cassette; Pgp, P-glycoprotein; MRP, multidrug resistance protein; VP-16, etoposide; MTX, methotrexate; PDT, photodynamic therapy; AZT, zidovudine.
generation of ABC transporter inhibitors have provided the basis for the initiation of clinical trials examining their ability to modulate drug sensitivity [20]. The clinical effectiveness of these newer transport inhibitors is yet to be established.

Emphasis has been placed on the development of inhibitors to the ABC transporters, most notably Pgp, and this, in turn, has led to an emphasis on these transporters as putative mechanisms of resistance. However, abundant evidence indicates that other resistance mechanisms exist. In vitro, altered expression of MAPs may be involved in the development of resistance to cytotoxic drugs [10–12, 21]. Similarly, in vitro, several reports have described tubulin mutations as etiologic in drug resistance [22]. Some of these mutations have been shown to alter drug binding, while others have been observed to cause shifts in the equilibrium of the tubulin dimer and microtubule polymer. In the case of the latter, a shift in the equilibrium to more soluble tubulin dimer and less microtubule polymer has been shown to result in increased resistance to the taxanes; a shift to a more stable state with a greater fraction of tubulin in the polymerized form has resulted in resistance to the vinca alkaloids and other destabilizing agents [23]. Finally, altered expression of β-tubulin isotypes [24, 25] has been shown to confer altered sensitivity to microtubule-targeting agents, with both in vitro and clinical data implicating the microtubule composition as important in a cell’s drug sensitivity to microtubule-targeting agents. Thus, for example, expression of class III beta-tubulin in non-small-cell lung cancer is correlated with resistance to taxane chemotherapy.

**microtubule-stabilizing agents: overcoming drug resistance**

Antimicrotubule agents can be divided into two major classes based on their effect on microtubule polymerization and the mass of microtubule polymers at high drug concentrations. The first class is the microtubule-stabilizing agents, such as colchicine and the vinca alkaloids, which inhibit polymerization and, hence, decrease the mass of cellular microtubules. Through the 1980s, the vinca alkaloids represented the only class of antimicrotubule agents with significant antitumor activity. Research interest then focused on a second class of antimicrotubule agents, the microtubule-stabilizing agents, to which the taxanes, epothilones, and other compounds belong (Table 2). Compounds in this class stabilize microtubules, increase microtubule polymer mass, and induce the formation of microtubule bundles in cells. Many of these agents have been derived from natural marine and plant sources and appear to have self-protective mechanisms targeted to the microtubule.

Both classes of antimicrotubule agents function to dampen the dynamic equilibrium of the microtubules, which is the principal mechanism by which these antimitotic agents inhibit the proliferation of tumor cells. Consequently, the dynamics of the mitotic spindle are disrupted, resulting in an arrest of cells in mitosis through a block in the cell cycle at the metaphase–anaphase transition and leading to cellular apoptosis [26, 27]. It is noteworthy that the effects on mitotic spindle dynamics are achieved at drug concentrations lower than those actually required to inhibit the tubulin–microtubule polymer equilibrium. These agents may also target the interphase microtubule network, which plays an important role in cellular shape and intracellular transport.

**epothilones**

Other than the taxanes, the epothilones are the most advanced in development among the microtubule-stabilizing agents, with several being investigated in clinical trials. This class of cytotoxic macrolides includes various agents: epothilone A and epothilone B (patupilone; EPO906), which are the originally described naturally occurring epothilones; the synthetic ixabepilone (aza-epothilone B; BMS-247550); BMS-310705, a water-soluble epothilone derivative; KOS-862 (epothilone D; desoxyepothilone B); and ZK-EPO (ZK-219477). The epothilones were first identified as exhibiting antifungal activity in the fermentation broth of the myxobacterium Sorangium cellulosum and were later shown to have antimitotic and cytotoxic properties [3, 28]. The bacterial origin of the epothilones makes them relatively easy to manufacture, compared with naturally occurring compounds of plant or marine origin.

The epothilones can be distinguished from the taxanes by several biologic and chemical criteria. The most important of these is that the epothilones are not susceptible to Pgp-mediated efflux; Pgp overexpression only minimally affects their in vitro cytotoxicity. Thus, epothilones represent a new strategy for overcoming Pgp-mediated resistance. In addition, the epothilones are functionally and structurally distinct from the taxanes and exhibit a greater potency than the taxanes. Examples of each of these points of differentiation are discussed briefly below.

**structure.** Members of the epothilone class are all very similar in structure, but differ markedly from the taxanes (Figure 1). For one thing, an unusual 16-member ring is characteristic of the epothilones but not the taxanes. In addition, the epothilone structure is flexible, as exemplified by the 180° rotation allowed around a number of bonds in the 16-member ring. The chemical structure of the epothilones lends itself to chemical modification whereby epothilone analogs can be produced that have enhanced therapeutic indices [29].

It is well established that, despite the dramatic structural differences between the epothilones and the taxanes, the
epothilones competitively inhibit the binding of paclitaxel to polymerized tubulin, indicating that the two compounds share a common binding site despite significant structural differences [30]. Studies of the structure of epothilone A bound to α,β-tubulin, determined by electron crystallography at 2.89-Å resolution, have now definitively shown that the epothilones bind at or near the paclitaxel binding site on the β-tubulin subunit in the interior of the microtubule [31]. However, these studies also concluded that docetaxel and epothilone A do not have a common pharmacophore, but rather that each ligand exploits the tubulin-binding pocket in a unique and independent fashion [31].

*function: overcoming Pgp resistance*. The pharmacologic profile of the epothilones confers significant functional advantage over that of the taxanes. In particular, cells that are resistant to taxanes are not cross-resistant to the epothilones, with the latter exhibiting cytotoxicity against taxane-resistant cells in both *in vitro* and *in vivo* preclinical models. The epothilones ixabepilone, patupilone, and KOS-862 are cytotoxic *in vitro* against Pgp-expressing cells in which the sensitivity to paclitaxel is significantly diminished, as exemplified by the increase in the median inhibitory concentration (IC50 values) for paclitaxel [32–34]. Similarly, the transfection of paclitaxel-sensitive lung cancer cells with a construct encoding the Pgp gene renders them resistant to paclitaxel, yet they remain sensitive to the apoptotic activity of the epothilones [35]. Although differences in response to Pgp overexpression exist among the various epothilones [32, 33], these differences are small compared with the differences in cytotoxicity between the epothilones and the taxanes against Pgp-overexpressing cells.

A number of epothilones have shown antitumor activity in animals when administered both parenterally and orally in various intermittent schedules. This activity has generally been superior to that exhibited by paclitaxel in both paclitaxel-resistant and paclitaxel-sensitive settings [32, 36, 37].

Paclitaxel is typically not active as an oral agent. Similarly, epothilones A and B have not demonstrated robust antitumor activities in human tumor xenograft models, which may have derived, at least in part, from metabolic instability in rodent circulation [32]. Ixabepilone (BMS-247550) is a semisynthetic lactam analog of epothilone B that is stable to enzymic degradation in rodent and human plasma. Parenterally administered ixabepilone has shown activity in human tumor xenografts against paclitaxel-sensitive and paclitaxel-refractory human and murine tumors, including breast, ovarian, pancreatic, and colon tumors [32]. Orally administered ixabepilone resulted in antitumor activity against both ovarian and colon cancer cells which was comparable to that produced following intravenous administration. Since the variable oral absorption of the taxanes is, at least in part, attributable to the overexpression of Pgp in the gastrointestinal tract [38], development of orally active microtubule-stabilizing agents that are poor substrates for Pgp is a desirable goal.

An interesting functional difference between the epothilones and the taxanes is their effect on tubulin derived from yeast (*Saccharomyces cerevisiae*). It has been demonstrated that epothilones A and B, but not paclitaxel, promote the formation of microtubules from purified yeast tubulin [39]. Studies using molecular models of paclitaxel and epothilone binding to mammalian microtubules identified three amino acid substitutions in the N-terminus and position 27 of β-tubulin that weaken the interaction between the 3′-benzamidophenyl group of paclitaxel and the tubulin protein [39].

*potency*. The taxanes and the epothilones share a common mechanism that results in a mitotic block which prevents dividing cells from progressing out of metaphase, thus leading to apoptosis. However, there are significant differences in potency between compounds in these two classes. In cell culture studies on malignant breast, lung, colon, prostate, and ovarian cell lines, IC50 values for the epothilones ixabepilone, patupilone, and KOS-862 were in the low nanomolar range, which is approximately one order of magnitude lower than the IC50 for paclitaxel [32, 34, 37]. Thus, the epothilones appear to have a substantial potency advantage over the taxanes. Patupilone was generally the most potent among the epothilones tested in these cell culture models.

The potency with which ixabepilone and paclitaxel polymerize tubulin isolated from calf brain has been evaluated [32]. In these studies, the epothilones are 2.5–3.3 times more potent than the taxanes in an *in vitro* polymerization assay with

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**Figure 1.** Chemical structures of the basic components of two distinct classes of structurally unrelated microtubule-stabilizing agents: taxanes and epothilones. Ph, phenyl; tBuO, tert-butoxy; Ac, acetyl.
purified tubulin. The potency of the epothilone B analog ixabepilone was similar to that of the two naturally occurring epothilones A and B, but it was ~2.5-fold more potent than paclitaxel in polymerizing tubulin.

other marine and plant microtubule-stabilizing agents

Many marine and plant species appear to have evolved a structurally diverse array of antimicrotubule agents as self-protective mechanisms. Among the more promising marine products is discodermolide, which was originally isolated from extracts of the sponge Discodermia dissoluta and can now be produced synthetically as both the parent molecule and analogs [40, 41]. Like the epothilones, discodermolide is active in Pgp-expressing malignant cells and has demonstrated more potent activity than paclitaxel [29, 42]. Discodermolide has also demonstrated cytotoxicity against cell lines, in which the resistance to paclitaxel is mediated by acquired mutations in β-tubulin [43, 44], and, in contrast to paclitaxel, can induce cell senescence [45]. Although discodermolide competes with paclitaxel for binding to β-tubulin, its effect on microtubule nucleation is more rapid, and it produces shorter microtubules than paclitaxel [43, 46]. In addition, paclitaxel can act synergistically with discodermolide to affect apoptosis, mitotic arrest, and the inhibition of microtubule dynamics, further indicating that their mode of binding to microtubules and their effects on microtubule function are different [44, 47]. In contrast, the amplification of the cytotoxicity of paclitaxel achieved with discodermolide was not observed with either the epothilones or eleutherobin [44]. Based on these promising preclinical data, a completely synthetic form of discodermolide (AAA296A) was entered into clinical trials, where it showed unexpected pulmonary toxicity [48].

Sarcodictyins A and B and eleutherobin are marine soft coral-derived natural products that have a similar mechanism of action to paclitaxel in stabilizing microtubules (Table 2) [49]. The marine sponge-derived microtubule-stabilizing agents laulimalide and isolaulimalide are poor substrates for Pgp and the ABC transporters and have shown activity against Pgp-expressing cell lines [50]. Taccalonolides A and E, steroids derived from the plant Tacca chantrieri, disrupt microtubules, cause G2/M arrest, are poorer substrates for Pgp transport than in paclitaxel, and have potent cytotoxic activity in vitro [51].

microtubule-destabilizing agents

The most prominent antimicrotubule agents that interact with tubulin in the vinca alkaloid binding domain are the dolastatins, a series of oligopeptides isolated from the sea hare Dolabella auricularia [2, 3, 52]. These potent cytotoxic agents inhibit tubulin polymerization and tubulin-dependent guanosine 5'-triphosphate hydrolysis. Dolastatin-10 and semisynthetic analogs have entered clinical development. Other compounds that competitively inhibit vinca alkaloid binding and are being evaluated in preclinical or early clinical studies include nocodazole, cryptocophycin, hemiasterlin, halichondrin B/homohalichondrin B, phomopsin, and spongistatin 1 [2, 3, 53]. In general, these compounds can disrupt mitotic spindle formation and induce mitotic arrest; hence, they exhibit potent growth inhibitory effects.

conclusions

The ongoing development of microtubule-stabilizing agents has opened up new opportunities to overcome the drug resistance associated with the taxanes. These advances will facilitate the rational design of chemotherapy regimens, in which newer, targeted antimitotic agents are used in combination with conventional cytotoxic drugs to enhance clinical activity and minimize the development of resistance. The ability to modulate the emergence of drug resistance with targeted antimicrotubule therapies will hopefully offer additional treatment options for cancer patients.

disclosures

TF has reported no financial relationships with companies whose products are mentioned in this supplement.

references

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