Chapter 25

Anti-Microtubule Drugs

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Abstract

Small molecule drugs that target microtubules (MTs), many of them natural products, have long been important tools in the MT field. Indeed, tubulin (Tb) was discovered, in part, as the protein binding partner of colchicine. Several anti-MT drug classes also have important medical uses, notably colchicine, which is used to treat gout, familial Mediterranean fever (FMF), and pericarditis, and the vinca alkaloids and taxanes, which are used to treat cancer. Anti-MT drugs have in common that they bind specifically to Tb in the dimer, MT or some other form. However, their effects on polymerization dynamics and on the human body differ markedly. Here we briefly review the most-studied molecules, and comment on their uses in basic research and medicine. Our focus is on practical applications of different anti-MT drugs in the laboratory, and key points that users should be aware of when designing experiments. We also touch on interesting unsolved problems, particularly in the area of medical applications. In our opinion, the mechanism by which any MT drug cures or treats any disease is still unsolved, despite decades of research. Solving this problem for particular drug–disease combinations might open new uses for old drugs, or provide insights into novel routes for treatment.

Key words Microtubules, Vinblastine, Vincristine, Nocodazole, Paclitaxel, Colchicine, Microtubule drugs, Combretastatin A4

1 Introduction

Natural substances that bind to Tb or MTs have been isolated as extracts from many plants. They have been used as drugs for millennia and in the last five decades, they have been of utmost importance both as cancer therapeutics and as experimental tools to understand microtubule biology. Today, despite the advent of targeted therapies, their importance in cancer treatment remains undiminished. Drugs like paclitaxel, vinblastine, and vincristine are a fundamental pillar of chemotherapy protocols for most solid and hematopoietic neoplasms. Moreover, new, clinically effective, MT binding compounds are continuously discovered and new therapeutic indications are approved. The drugs we discuss in this article are listed in Table 1. They were chosen for their importance in basic research and/or medicine (Table 2). They represent most of

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Table 1
Summary of drugs discussed in this chapter

Drug[a]	Binding site[a]	In vitro IC_{50}[b]	In vivo C_{max}[c]
Colchicine	Colchicine	250 nM (to inhibit migration) [1] 13–250 nM (cell proliferation) [2]	3–10 nM [3]
Colcemid	Colchicine	~100 nM (cell proliferation) [4]	Currently not used in man, despite early successes in cancer and low toxicity
Combretastatin A4	Colchicine	7 nM [5]	5 μM [6]
Nocodazole	Colchicine (?) [7]	50 nM	Not used in man
Benomyl		5 μM [8]	Not used in man
T138067	Binds covalently to Cys-239 of β-tubulin	11–165 nM [9]	Not used in man
Vinblastine	Vinca	0.45 nM [10]	100–500 nM [3, 11]
Vincristine	Vinca	1–5 nM [12]	100–500 nM [3, 11]
Paclitaxel	Taxane	2.5–7.5 nM [13]	3–5 μM [3]
Docetaxel	Taxane	5–43 nM [14]	~2 μM [3]
Eribulin	Vinca	0.09–9.5 nM [15]	300 nM [16]

[a]sensitive Tb binding sites were traditionally defined based on competition studies, and, because Tb is notoriously difficult to crystallize, few structural studies exist [17–20]. Thus, the binding sites for some compounds are rather poorly defined and this classification can only serve as a rough approximation. For Benomyl the binding site to human tubulin has not been studied and T138067 does not fit into the traditional binding site classification.
[b]Concentration causing 50% of cells to die in a few days, or for causing mitotic arrest in cell culture. These are approximate data for "typical" human cell cultures, and are provided as an approximate starting point for experimental design. Note the reported values may differ widely, and particular cell lines may be much less, or more, drug sensitive.
[c]Maximum concentration in human plasma following a typical therapeutic dose.

the mechanisms of action that have been shown for small molecules binding to Tb, and are commercially available in most cases. For longer reviews that address biochemical and medical mechanisms see [3, 25, 28–30]. A full list of anti-MT drugs would be very long. Here, we focus only on those commonly used for lab research and representatives of classes used clinically (Table 1).

2 Colchicine and Colcemid

2.1 History and Medical Uses of Colchicine

Colchicine (Fig. 1) has been known far longer than any other anti-MT drug, indeed longer than most medicines, and it played a central role in the early development of the MT and molecular

Table 2
Summary of clinical aspects of therapeutically relevant drugs

	Textbook mechanism of action[a]	Clinical use, main indications	Most important side effects
Antiquity			
Colchicine	Inhibits neutrophil migration	Gout, rheumatoid arthritis, familial Mediterranean fever	Diarrhea
1960s—Vinca alkaloids			
Vinblastine	Antimitotic [21]	Germ cell malignancies and some types of advanced lymphomas	Neutropenia
Vincristine	Antimitotic [21–23]	Pediatric malignancies, ALL, blast crisis in CML, lymphomas (Hodgkin and Non-Hodgkin)	Neurotoxicity
1980s/1990s—Taxanes			
Paclitaxel	Antimitotic [24]	ovarian, breast, non-small-cell lung cancer, Kaposi sarcoma,	Neurotoxicity, neutropenia
Docetaxel	Antimitotic	Breast, non-small-cell lung, prostatic (hormone-refractory), head and neck squamous cell and gastric carcinoma	
1995			
Combretastatin A	Antiangiogenic	Cancer—experimental	Pain, cardiopulmonary toxicity
2010			
Eribulin	Antimitotic	Advanced breast cancer	Neurotoxicity, neutropenia

ALL acute lymphoid leukemia, *CML* chronic myeloid leukemia
[a]The mechanism of action according to most textbooks is given. The references given for vinca alkaloids and paclitaxel point to additional suggested mechanisms of action. Information is gathered from Refs. [3, 25–27]. For further references, see main text

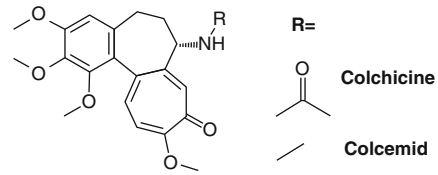

Fig. 1 Chemical structures of colchicine and colcemid

mitosis research fields. Its history and effects on cells were extensively researched by Pierre Dustin and his son Pierre Dustin Jr., and our comments are drawn in part from two seminal books written by Pierre Dustin Jr. [31, 32]. As a natural product extract of the autumn crocus (*Colchicum autumnale*), colchicine was mentioned in ancient Egyptian papyrus scrolls, and was well known to the ancient Greeks. It was the first drug known to arrest proliferating cells in mitosis [33], and this discovery started a whole new field of mitosis research in living organisms in the 1930s, when colchicine was used to estimate proliferation rates in many animals and plants. Tb was discovered, in part, as its binding partner [34] and codiscovered, and named, as a subunit of flagella [35]. Colchicine is still much used as a medicine, and there has been a recent resurgence in interest in its potential, following its recent approval for treatment of recurrent pericarditis [36]. Colchicine is effective treatment for gout, a common inflammatory disease caused by precipitation of uric acid crystals in joints. Taken daily, it also suppresses the symptoms of the relatively common inherited inflammatory disease, familial Mediterranean fever (FMF). Daily colchicine is effectively curative for most FMF patients, with few side effects and no disease progression, which represents a truly remarkable gene–drug interaction. FMF is caused by mutations in Pyrin, a protein that regulates inflammasome activation. It is currently unknown why colchicine is curative in FMF, but has no therapeutic action in apparently similar genetic diseases that cause inflammasome activation by other mechanisms [37]. How colchicine works as an anti-inflammatory drug is, in our view, an interesting unsolved problem. It is known to block only certain types of inflammation, specifically those caused by neutrophils and monocytes. It inhibits accumulation of these cells at sites of inflammation, and also inhibits release of pro-inflammatory cytokines such as IL1β [38]. These therapeutic actions are thought to result from inhibition of MT polymerization in neutrophils and monocytes, but it is unclear how this can occur without antimitotic side effects.

2.2 Lab Uses of Colchicine

Colchicine remains a representative MT and antimitotic drug and can be used for those purposes in research. A major advantage is the huge wealth of published data on its actions at every level, from molecular effects on Tb to human and animal pharmacokinetic data. It is also relatively cheap, water soluble, and is thought to be highly specific, with no known targets other than Tb. Colchicine binds to β-Tb at a site near α-Tb in the heterodimer, and its binding forces the dimer into bent configuration that is not compatible with normal polymerization [39, 40]. The depolymerizing action of colchicine is sub-stoichiometric; only ~5 % of the Tb dimers in the cell need to be bound to colchicine to arrest cells in mitosis [41], and a similar fractional occupancy inhibits polymerization of pure Tb [42, 43]. A very important consideration when using colchicine as a tool

is its extremely slow association and dissociation rate constants for Tb binding. The association and dissociation rate constants for colchicine binding to Tb are ~100 M^{-1} s^{-1} and 10^{-5} s^{-1}, respectively [34, 44]. These values represent much slower binding and unbinding rates than those exhibited by most drugs, and they make colchicine binding slow, but effectively irreversible in most experimental contexts, including mitotic arrest of tissue culture cells [41]. If they are taken into account, they can provide experimental advantages. For example, Tb-colchicine complex can be prepared free of excess drug, and is relatively stable. However, mistakes can be made if the slow association rate constant is not taken into account. When adding colchicine to cells, it is important to keep track of the time of incubation as well as the concentration, since it is not safe to assume rapid equilibration of drug with target. The slow binding and unbinding of colchicine was the main reason most laboratories switched to other drugs, notably colcemid and nocodazole, for routine depolymerization of MTs in cells. In our opinion, combretastatin A4 may be the best drug for this purpose since it binds rapidly, and is more potent than nocodazole, and perhaps also more specific. Colchicine can be prepared as a stock solution in water or DMSO. It is stable at physiological pH, but is notably inactivated by long wavelength uv light (see below), so stock solutions should be protected from light.

2.3 Colcemid

Colcemid (Fig. 1), also called demecolcine, was isolated from the autumn crocus in 1950 [45] and commercialized by Ciba. Initially, it was explored as a cancer drug due to its low toxicity. Today, it is only used as a research tool mainly to overcome limitations of colchicine due its very slow association and dissociation rate constants. It binds to Tb at the same site as colchicine, but ~10-fold faster, and it also dissociates faster [46]. Its main use has been to arrest cells in mitosis for cytogenetic analysis, though to our knowledge it offers no special advantages over other drugs in this application. It shares the photosensitivity of colchicine.

2.4 Photoinactivation of Colchicine and Colcemid

Colchicine and close derivatives are efficiently converted by long wavelength UV light to a series of highly rearranged molecules called lumicolchicines [47]. Lumicolchicines have no known biological activity, and are sometimes used as negative controls in colchicine studies. Local photoinactivation of colchicine and colcemid under a microscope has allowed some creative experimental applications, and should be considered for modern experiments. In one classic example, Hamaguchi and Hiramoto used local photoinactivation of colcemid in echinoderm eggs to control the geometry of the MT aster nucleated by sperm centrosomes during pronuclear migration [48]. This elegant experiment provided the first evidence that a motor protein—now known to be cytoplasmic dynein—pulls on MTs from bulk cytoplasm to center asters.

3 Combretastatin A4

3.1 History and Medical Uses

The combretastatins were discovered, by Pettit and coworkers, through fractionation of extracts of *Combretum cuffrum* [49]. We will discuss only Combretastatin A4 (Fig. 2), which was the most potent derivative, and is also easily synthesized. Combretastatin A4 structurally resembles colchicine. It binds to an overlapping site on Tb, albeit much faster than colchicines, and has similar depolymerizing effects [50]. Combretastatin A4 phosphate ester, a water soluble prodrug, has strong antitumor action in animals, and has shown promise in clinical trials, though its clinical use is limited by high toxicity to multiple organs. The antitumor action of combretastatin A4 is thought to derive mainly from antivascular, rather than direct cytotoxic or antimitotic actions on cancer cells [51]. An enduring mystery is why colchicine and combretastatin A4 have very different clinical effects, given that they bind to similar sites on Tb and have similar effects on polymerization. Part of the answer may lie in their very different association rate constants, and consequent effects of pharmacokinetics and tissue distribution.

3.2 Lab Uses of Combretastatin A4

Combretastatin A4 is a potent and fast-binding drug that cleanly depolymerizes MTs through the same well-characterized mechanism as colchicine. It is easy to synthesize, ~10-fold more potent than nocodazole, has a fast association rate constant and is both chemically and photochemically stable. Combretastatin A4 is soluble in DMSO, and DMSO stock solutions do not tend to precipitate as they do with nocodazole. These properties make it an excellent choice as a routine depolymerizing drug, but for lab studies it is important to purchase the parent drug, and not the phosphate ester prodrug, which is inactive until hydrolyzed.

A "caged" nitrobenzyl derivative of combretastatin A4 has been recently reported that may be useful for spatiotemporal control of MT polymerization, with the opposite properties of colcemid in that it is activated by UV light, rather than inactivated [52]. Photoactivation of caged combretastatin using a confocal

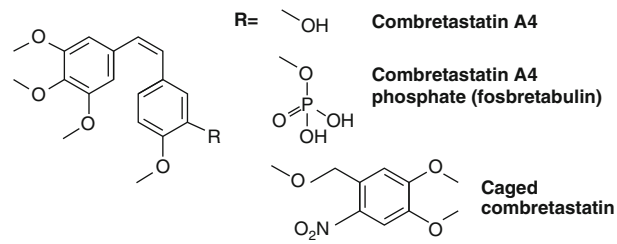

Fig. 2 Chemical structures of combretastatin A4, combretastatin A4 phosphate, and caged combretastatin

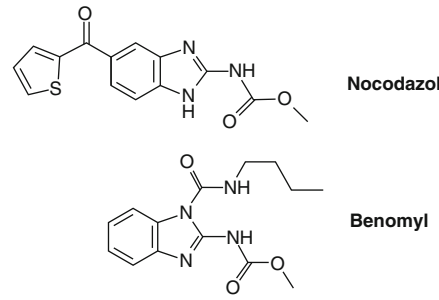

Fig. 3 Chemical structures of nocodazole and benomyl

microscope facilitated the local induction of MT depolymerization in zebrafish embryos, which revealed dynein-mediated pulling forces on centrosomes, in an update of Hiramoto's classic experiment using photo-inactivation of colcemid [48].

3.3 Nocodazole and Benomyl

Nocodazole (Fig. 3) was developed by DeBrabander and coworkers at Janssen in the 1970s [53], and is now perhaps the most used MT depolymerizing drug in basic cell biology research. It is not used clinically. Benomyl, a structurally related benzimidazole, was developed as a plant fungicide at DuPont in the 1960s, and is still widely used for that purpose. Benomyl is less active on mammalian Tb, and has been mostly used in budding yeast experiments. Nocodazole and benomyl bind to β-Tb and block polymerization, but their mechanism is less well understood than colchicine. Nocodazole came to favor in laboratory applications in part because its effects are more easily reversed in washout experiments than colchicine, which reflects faster drug binding and unbinding. Nocodazole addition is often used to arrest mammalian cells in mitosis, and washout to release them into cytokinesis and G1. Nocodazole at concentrations above ~500 nM arrests cells in mitosis with few or no MTs, which causes mitotic arrest, but also mis-regulation of mitotic kinases. For this reason, we prefer to arrest cells in mitosis with the Kinesin-5 inhibitor s-trityl-l-cysteine (STLC) [54] for biochemical analysis. STLC is cheaper than nocodazole, and arrested cells have normal, dynamic MTs. The presence of dynamic MTs significantly altered regulation of Plk1 in cells arrested in mitosis with STLC vs. nocodazole, with the STLC arrested state presumably more relevant to normal metaphase [55]. Nocodazole washout allows progression into cytokinesis and G1, but spindle reassembly tends to be slow and variable, leading to extensive loss of synchrony and high rates of chromosome mis-segregation. For biochemical analysis of cytokinesis, we prefer to override STLC mitotic arrest with purvalanol, a CDK1 inhibitor, which causes rapid and synchronous mitotic exit. The resulting cytokinesis is monopolar, but otherwise an excellent biochemical and morphological mimic of normal cytokinesis [56].

Nocodazole is relatively insoluble in water. It is prepared as a stock solution in DMSO (~10 mM) and stored frozen. Although nocodazole is chemically stable, DMSO stocks tend to precipitate when thawed, and care must be taken to make sure the correct amount of drug is added. Nocodazole precipitates in DMSO can be re-solubilized by warming to ~50 °C. Nocodazole added to tissue culture medium from a DMSO stock also precipitates, and it is important to mix well to ensure it redissolves. Nocodazole is less potent than many natural product anti-MT drugs. This, and its lack of water solubility, are reasons for considering an alternative drug such as combretastatin A4 or colcemid for depolymerizing MTs in tissue culture cells.

Benomyl (Fig. 3) has been much used to probe Tb and mitosis biology in budding yeast, and genetic tests proved it worked by inhibiting Tb polymerization [57]. It is also prepared as a DMSO stock and stored frozen [58].

4 T138067

The structurally simple sulfonamide T138067 (Fig. 4) is of interest because it reacts covalently with Cys-239 of β2- and β4-Tb, causing MT depolymerization and mitotic arrest in cancer cell lines [9]. This reaction was highly selective for Tb, as judged by analysis of whole cell lysate treated with a radioactive derivative. T138067 is useful for experiments where a completely irreversible inhibitor is needed. It was tested clinically, with the hope of being active on tumors that express drug efflux pumps. It exhibited the toxicities expected for an anti-microtubule drug (bone marrow, gut and neurotoxicity), but no useful clinical responses [59, 60]. The question why some anti-microtubule drugs are active in cancer, and others not, remains mysterious, as discussed below.

4.1 On the Mechanism of Action of MT Drugs in Cancer

The next three drug classes, vinca alkaloids, taxanes, and eribulin, are used to treat cancer (Table 2). The vincas and taxanes are widely used in combination chemotherapy, while eribulin has only been recently approved. They have different mechanisms of action on MTs, different pharmacodynamic properties, and different

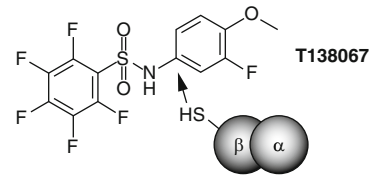

Fig. 4 Chemical structure of T138067

disease indications. In the last few years, we and others have become interested in the therapeutic mechanism of action of these drugs at the cellular and tissue level, problems that had long been considered solved. The basic concepts about how these drugs work against cancer and inflammation have been developed in the 1960s and 1970s and many of them are based on data obtained in highly artificial cell culture or mouse models with proliferation rates much higher than human cancers. Considering the physiological context in humans, these ideas contain many inconsistencies and contradictions [61–63]. Because mitotic arrest is such a prominent biomarker, especially in tissue culture of highly proliferating cells, mitotic effects of these drugs have been considered responsible for their antitumor efficacy and have been the main focus of interest in basic research [26]. A related concept is that these drugs do not kill cancers by mitotic arrest per se, but by promoting chromosome missegregation [64]. Recent extensive efforts to develop highly selective antimitotic drugs that do not interact with MT have been very disappointing as such drugs hardly show any effects in solid tumors [65]. Thus, it seems likely that interphase effects of MT drugs, which are numerous but have been mostly neglected as a therapeutic mechanism, are at least equally important for their antitumor effect. Interesting examples of such effects are:

- MT depolymerizers like colchicine have been shown to not only induce mitotic arrest, but also cause entry from G0 into the cell cycle, thus *increasing* rather than inhibiting proliferation both in embryos [66] and serum starved cultured cells [67 and references therein]. This effect is antagonized by stabilizing drugs like paclitaxel.
- MT drugs are known to affect a wide range of cellular processes like intracellular transport, signaling transmitted through the adhesion complex and cell migration [68–71] as a few examples. These effects have been regarded as mainly detrimental for therapeutical applications as they are responsible for side effects like neurotoxicity and increased susceptibility to infection, but it is likely that these effects are highly important for drug efficacy.
- MT drugs are known to activate signaling pathways that can cause cell death in particular contexts, such as the Jnk pathway [72, 73]

We believe that interphase effects of MT interacting drugs are therapeutically important and should receive more attention.

5 Vinca Alkaloids

5.1 History and Medical Uses

Vinca alkaloids (Fig. 5) were isolated in the 1950s by Beer, Cutts and Noble at the University of Western Ontario and were the first MT binding drugs to find their way into mainstream chemotherapy [74]. As with most cytotoxic chemotherapeutics, it was a serendipitous discovery. Tea made from the Madagascar periwinkle (formerly *Vinca rosea*, now *Catharanthus roseus*) was used as a diabetes remedy in Jamaica and Noble and Beer originally wanted to test its efficacy more systematically. Instead of finding effects on glucose levels in the blood, they noticed that rats injected with the plant extract suffered from severe depletion of white blood cells and became susceptible to lethal infections. A stepwise fractionation procedure, with depletion of white blood cells as a measure for activity, was used to isolate vinblastine, originally called vincaleukoblastine. Vincristine was also identified, but isolated in larger quantities only later at Eli Lilly. Systematic tests on solid and hematopoietic tumor models revealed that the vinca alkaloids are potent anticancer drugs, and they remain among the most used drugs in chemotherapy, even today. Interestingly, although vinblastine and vincristine differ only by substitution of a methyl group through a formyl group, they have very different clinical efficacy and side effect profiles. Vinblastine is considered effective in germ cell malignancies and some lymphomas in adults and its dominant side effect is white blood cell depletion, the hallmark effect which led to its discovery. Vincristine is mainly used in pediatric malignancies and some adult hematological cancers and its main side effect is peripheral neuropathy.

5.2 Laboratory Uses

The interaction of vinca alkaloids with MTs is very complex. Tb crystals are notoriously hard to obtain and the binding mechanism of vinblastine has only been characterized at the structure level quite recently [19]. Like colchicine, it induces curved Tb assemblies but binds to a distinct binding site between heterodimers (at inter-dimer interfaces). This is an important difference from taxane and colchicine binding sites, which lie within a single heterodimer, on the interior of the MT and at the intra-dimer interface between monomers, respectively (ibid). In the 1960s and 1970s, colchicine

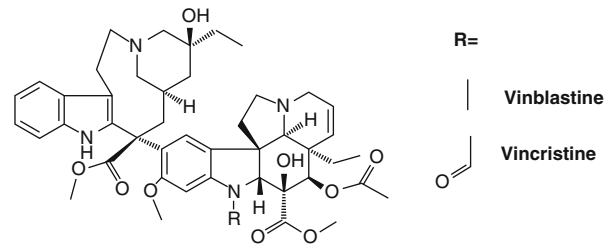

Fig. 5 Chemical structures of vinblastine and vincristine

and the vinca alkaloids were used to determine cell cycle dynamics in human and murine tissues in order to understand how antiproliferative cancer drugs work in vivo [75]. At the time, it was a relatively important field of research, now almost forgotten and superseded by the molecular revolution in cancer research. It seems that, among the classic vinca alkaloids and colchicine, vincristine is the best compound to estimate tissue proliferation by inducing mitotic arrest in vivo ("stathmokinetic agent"—σταθμός—station on a road), because it induces a relatively long lived mitotic arrest without affecting interphase cell cycle kinetics [76].

Three dose regimes have been described for treatment of tissue culture cells with vinblastine, and each has distinct uses. At the lowest active concentration (~2–20 nM) vinblastine causes mitotic arrest and suppresses MT dynamics without depolymerizing MTs [77]. This may be the best tool for blocking plus end polymerization dynamics without gross reorganization of MTs. Paclitaxel, in contrast, tends to promote reorganization, in part due its propensity to promote nucleation. Above ~10 μM vinblastine induces the formation of beautiful paracrystals [78]. In between, it mainly depolymerizes, though the nature of the soluble Tb species in cells in this regime is poorly characterized.

Vinblastine paracrystals have interesting potential as a research tool. They are formed from side-by-side aggregates of protofilaments [79], and thus are structurally related to the ends of growing and shrinking MTs. Comparison of binding affinity for MTs vs. vinblastine paracrystals might be useful to characterize proteins suspected to bind selectively to protofilaments. By forcing Tb dimer into paracrystals, high concentrations of vinblastine very effectively depolymerize MTs, but do not lead to buildup of soluble, drug-inhibited Tb dimers. In this respect, the cellular effects of vinblastine differ from the depolymerizers colchicine, combretastatin, and nocodazole, where soluble Tb bound to drug builds up, and also to the polymerizers paclitaxel and epothilone where most soluble Tb is forced into MTs. These differences between drug classes are useful for studies that probe possible regulatory roles of free Tb dimer, e.g., in experiments showing that translation of tubulin mRNA is regulated by levels of free tubulin dimer [80].

6 Taxanes

6.1 History and Medical Uses

Paclitaxel (Fig. 6) was the first MT stabilizing drug to be discovered and is a hydrophobic compound isolated from the bark of the Pacific or Western Yew (*Taxus brevifolia*). Both clinically and for cell biology, it is a drug of eminent importance. Its unique structure and antitumor activity in multiple preclinical cancer models were discovered in the late 1960s by Monroe Wall and Mansukh Wani [81]. However, for at least a decade, it received relatively

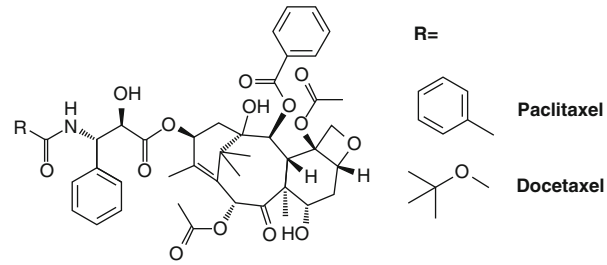

Fig. 6 Chemical structures of paclitaxel and docetaxel

little attention, in part because its complex structure was (correctly) considered an obstacle for production of large scale quantities for clinical use. Interest in paclitaxel as a drug rose again when Peter Schiff and Susan Horwitz published a seminal series of papers showing that paclitaxel was the first known compound to stabilize MTs, and that it allowed in vitro generation of polymerized MTs in absence of GTP or MT associated proteins [69, 82, 83]. The development of paclitaxel for clinical use was difficult. Due to its hydrophobicity, it was formulated with Cremophor EL, a substance known to cause hypersensitivity effects itself, and after one of the first patients treated with paclitaxel died from an anaphylactic reaction, clinical trials were put on hold for 5 years. The problem was alleviated by pretreating patients with anti-inflammatory drugs. A second problem was that a very large number of trees was needed to purify enough paclitaxel from the Pacific Yew and a complete synthesis proved to be difficult and highly inefficient. This problem was solved by semisynthesis approaches, utilizing 10-deacetylbaccatin III, a readily available precursor from the European yew, *Taxus baccata*. While developing this approach, Pierre Potier (who also developed the semisynthetic vinca alkaloid vinorelbine) and colleagues developed a semisynthetic taxane with higher potency and somehow better water solubility, docetaxel (formerly taxotere, Fig. 6) [84]. Paclitaxel and docetaxel seem to have similar antitumor activity characteristics. In the 1990s paclitaxel became one of the most successful cancer drugs to date, gaining initial FDA approval for breast, ovarian, and non-small-cell lung carcinoma. Taxanes have known activity against an impressive number of solid tumors, including sarcoma, melanoma, esophageal, gastric, endometrial, bladder, small-cell lung, hormone-refractory prostate, and germ-cell carcinoma [3]. Even today, in the era of targeted drugs, new clinical indications are approved.

6.2 Laboratory Uses

Taxanes bind to a unique binding site inside the MT lumen, and do not compete with exchangeable GTP, colchicine or vinca alkaloids [17, 85]. Other stabilizing drugs are thought to bind to similar sites [20]. Paclitaxel, which is historically called “Taxol” in the basic science literature (now the commercial name of this drug), is

very useful as a tool to polymerize MTs, and to keep them stable in the absence of Tb, GTP, or MT associated proteins. It has been instrumental in studying the function of motor proteins and other MT associated proteins in vitro. A lot of our knowledge about the ability of motor proteins to move along MTs, and to slide them against each other and generate forces in the mitotic spindle, was gained using assays based on paclitaxel-stabilized MTs [86].

Paclitaxel is soluble in DMSO, but only soluble in aqueous buffer below ~100 μM. The ester bond between the taxane ring system and the side chain is quite labile due to hydrolysis, which makes paclitaxel somewhat unstable storage, especially if the DMSO is impure. To obtain reproducible dose–response data is it important to dissolve paclitaxel in fresh, dry DMSO, to store stock aliquots frozen, and to minimize freeze–thaw cycles. Paclitaxel strongly promotes MT nucleation as well as stabilizing polymerized MTs. This property can make it difficult to assemble long, stable MTs from pure Tb, since addition of saturating drug to a concentrated solution of Tb tends to generate very short MTs and protofilament aggregates. To make long MTs we usually start by adding a low concentration of paclitaxel (~1 % compared to Tb) to a solution of Tb + GTP, incubate at 37 °C for ~20 min to induce polymerization, and only then add saturating paclitaxel (i.e., stoichiometric with the Tb) to fully stabilize the MTs. Alternatively, we induce polymerization by adding DMSO to ~5 %, and again add saturating paclitaxel only after the Tb is fully polymerized.

In cells, similar to the vinca alkaloids, the effects of paclitaxel vary with concentration and depend on the binding stoichiometry to Tb. At low nanomolar extracellular concentrations, paclitaxel stabilizes MTs without increasing MT mass. At higher concentrations (1 μM and above) it dramatically increases polymer mass and causes the formation of characteristic MT bundles [69]. The mitotic effects of paclitaxel are heterogeneous and strongly cell type and concentration dependent. Below 10 nM, perturbation of MT dynamics is often too weak to arrest cells in mitosis, but induces chromosome segregation defects which have been implicated in its antitumor effects [87]. Above 10 nM, it induces nucleation of MTs from acentrosomal nucleation centers and leads to formation of multipolar spindles and activation of the spindle assembly checkpoint (SAC). Starting around 500 nM, however, stabilization of chromosome mis-attachments seems to lead to inappropriate fulfillment of the SAC and mitotic exit [88]. Although the binding of paclitaxel to Tb is reversible, it accumulates and is concentrated up to 1000× inside cells [87] and dissociation is very slow. This makes paclitaxel an unreliable drug to synchronize cells in mitosis and its main use in cell biology is to study the consequences of MT stabilization and excess nucleation, and to purify MT binding proteins. Addition of paclitaxel to a cell lysate, followed by centrifugation, is one of the most convenient

methods for isolation of microtubule binding proteins [89]. Some motor proteins also co-sediment with taxol-stabilized microtubules, but their binding can be enhanced by adding nucleotide analogs and/or depleting ATP [86].

Paclitaxel derivatives labeled with several fluorescent dyes are commercially available that allow labeling of MTs in living cells from Molecular Probes [90–92]. Since these drugs also affect MT dynamics, they are only useful for correlating drug effects with localization rather than as a tool to observe MTs in living cells. To achieve this, in cells, transiently or stably expressed GFP-Tb has been used, but the effects of overexpression of fluorescent Tb are difficult to determine [93]. Recently, docetaxel derivatives labeled with silicon-rhodamine derivatives have been used to address these problems and look very promising [94]. These far red emitting compounds show a more than tenfold increase in fluorescence intensity upon binding to MT and seem to affect MT dynamics much less than unlabeled taxanes. Thus, they seem to be suitable for long term imaging of MT in living cells and do not require washout of unbound compound.

7 Eribulin

This synthetic analog of the marine sponge natural product halichondrin B was introduced by Eisai as a treatment for metastatic breast cancer in patients who have become resistant to other drugs including taxanes [15]. It is remarkably potent, with a dose in humans ~100 fold lower than a taxane. Eribulin (Fig. 7) binds to Tb dimer and prevents polymerization [95, 96]. This action leads us to question the hypothesis that MT stabilization plays a central role in the actions of MT drugs against carcinomas [26]. Another interesting clinical aspect of Eribulin is its relative lack of neurotoxicity [97]. One notable aspect as a laboratory tool is the free amine, which can be modified with a BODIPY fluorophore with little decrease in potency. BODIPY-Eribulin is a substrate for the MDR/P-glycoprotein drug pump, and has been recently used to probe drug efflux in tumor cells by intravital imaging in living mice [98].

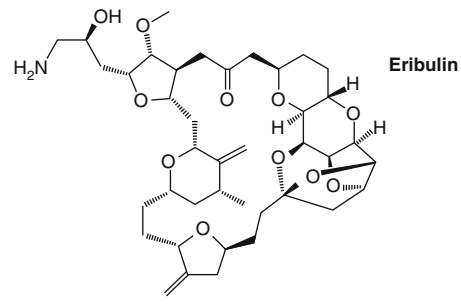

Fig. 7 Chemical structure of eribulin

Microtubule drugs have historically been central to advances both in medicine and basic science. Tubulin was discovered using these drugs, and we owe them a lot of our knowledge about microtubules and mitosis. Recent systematic attempts to develop antimitotic cancer drugs that do not bind to MT have failed rather dramatically [62, 63, 65], making the significant antitumor efficacy of MT drugs seem even more remarkable. It is likely that MT binding is essential for their antitumor effects and besides the induction of mitotic arrest in tumor cells, other, nonmitotic effects on tumor or immune cells are important. Understanding how these drugs kill tumor cells in patients is one of the great challenges ahead, and we expect that work in this direction will continue to result in new discoveries of great relevance both to biology and medicine.

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