Mechanisms of action of microtubule stabilizing agents

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Cellular tubulin and microtubules exist in a constant and changing state of equilibrium. The most dramatic changes occur when the cell transitions from interphase to mitosis, with the interphase microtubule network rapidly disassembling to reform as the mitotic spindle, and with reformation of the interphase network. With a significant number of compounds used as chemotherapeutic agents (the vinca alkaloids vincristine, vinblastine, and vinorelbine; the taxoid site agents paclitaxel, docetaxel, carbazitaxel, and ixabepilone; the halichondrin B analog eribulin; and drug-antibody conjugates containing the dolastatin 10 analog monomethyl auristatin E or the maytansinoid mertansine), there has been continuous interest in identifying new antimitotic agents that target tubulin. Despite the well-characterized effects of antitubulin agents in dividing cells, the most significant source of tubulin for biochemical and pharmacological studies has been mammalian brain tissue, since as much as 30% of the soluble protein in brain is composed of tubulin, as opposed to about 1-2% in other tissues and cultured cells. It must be emphasized that tubulin is a structural protein, so that micromolar concentrations are required for most studies. There are multiple isotypes of both α- and β-tubulin, and both subunits undergo a variety of post-translational modifications. These vary between tissues and cultured cells. Nevertheless, no significant differences in drug sensitivity have been described for tubulin from different sources, and brain tubulin appears to be a valid model for the identification and characterization of different classes of antitubulin agents. Compounds that cause mitotic arrest through an interaction with tubulin either inhibit or induce the assembly of purified tubulin. It is the latter class of compounds that are the subject of this seminar. The first agent in this class to be identified was paclitaxel in 1979, with the next chemotype, the epothilones, identified in 1995. Subsequently, a growing number of potent natural products binding at the same site as paclitaxel were identified. The primary binding site for these compounds is on β-tubulin, on the interior of the microtubule, with access probably occurring through pores in the microtubule wall. The most recently identified natural products, cyclostreptin, zampanolide and the taccalonolides, have, as part of their mechanism of action, alkylation of amino acid residues of β-tubulin in the taxoid site. Cyclostreptin, in addition, alkylates an amino acid residue of β-tubulin adjacent to a microtubule pore. Finally, in 2002, the previously identified laulimalide was shown to induce microtubule assembly by binding to a different site on tubulin than the taxoid site. A second natural product binding at the same site, called peloruside A, was soon identified. The laulimalide binding site appears to be on the exterior of the microtubule. Compounds binding at the laulimalide and paclitaxel binding sites, under select conditions, can act synergistically in inducing tubulin assembly, and a similar effect can be observed in tissue culture experiments.