REVIEW ARTICLE

THE MOST IMPORTANT MICROTUBULE NATURAL INHIBITORS

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Summary: Natural microtubule inhibitors represent chemically very variegated family of structures with strong effect on cytoskeletal functions and the use of them is one of the most frequent therapeutic strategies for carcinoma treatment. The survey of the most important natural microtubule inhibitors is summarized in this paper.

Key words: *Cytoskeleton; Microtubule; Inhibitor; Carcinoma; Minireview*

Introduction

The cytoskeleton of eukaryotic cells is a filamentous network formed by microtubules, microfilaments and intermediate filaments. The cytoskeletal network is responsible for the mechanical properties of the cell that modulate functions such as cell shape, locomotion, cytokinesis, and translocation of organelles. Experimental evidence suggests that there are many important functions of dynamic cytoskeletal network besides the regulation of cellular mechanics. The cytoskeleton also provides connections between cellular structures and presents a large surface area for interactions of various proteins and signaling molecules. Modulation of cytoskeletal network may influence cell signaling, ion channels and intracellular calcium levels. The reorganization or degradation of all cytoskeletal filaments is associated with apoptosis. Cytoskeleton is thus essential for regulation of cellular functions, cell integrity, and viability. The relationships between direct mechanical effects of modulations of cytoskeletal structures and cellular functions remains to be elucidated.

The aim of this minireview is to.characterize the most important compounds of natural origin which interact with microtubules. Microtubules are tubulin polymers involved in many cellular functions (10), one of which being the formation of the mitotic spindle required for chromosome moving to the poles of the new forming cells during cell division (2). The importance of microtubules to cellular functions makes them a sensitive target for biological microtubule poisons. All compounds which interact with microtubules in the sense of their stabilization or disorganization are called microtubule inhibitors. They have cytotoxic effect and may kill the cell. Since microtubules are required to carry out mitosis in cell proliferation, microtubule inhibitors would primarily attack cancer cell which divides more frequently than healthy cell. Therefore many of them are very important anti-cancer compounds. The use of this poisons is one of the most frequent therapeutic strategies for carcinoma treatment. In addition to well known microtubule poisons such as vinblastin, colchicin, and taxol, already now many new natural toxic compounds are used as outstanding scientific tools in biological experiments and serve the purpose of model structures for synthesis new compounds with expected effect.

Microtubule system

Tubulin is a protein whose quaternary structure is composed of two polypeptide subunits, α- and β-tubulin. Several isotypes have been described for each subunit in higher eucaryots. Microtubule functions are based on their capacity to polymerize and to depolymerize. This process is a very dynamic and is attend with rapid shortening or elongation of this cell structures. Tubulin is a GTP-binding protein and the binding of this nucleotide to the protein is required for microtubule polymerization, whereas the hydrolysis of the GTP bound to polymerized tubulin is required for microtubule depolymerization. Microtubule stability in healthy cell is regulated by the presence of some proteins called microtubule-associated proteins (MAP) which facilitate microtubule stabilization. The cellular mechanisms regulating microtubule assembly is highly sensitive to the concentration of Ca^{2+}. The low cytosolic Ca^{2+} level characteristic of the resting state of most eucaryotic cells promotes microtubule assembly, while the localized increase in Ca^{2+} cause microtubule disassembly (13). Microtubules forms through polymerization of protein dimers, consisting of one molecule each of α- and β-tubulin. Dimer and polymer are in a state of dynamic equilibrium, so that the network can respond flexibly and quickly to functional requirements. The polymer forms a fine, unbranched cylinder, usually with internal and external diameters of 14 and 28 nm, respective-

ly, the so called microtubule (Fig. 1) (22). Assembly is initiated by the binding together of α, β-dimers to form short protofilaments, 13 of which subsequently arrange themselves side by side to form the microtubule. Subsequent growth of the microtubule is polar, occurring mainly at the so-called plus end of the protofilaments through the addition of further dimers. Addition involves GTP, which is bound to the dimer, being cleaved to GDP, which remains attached to the tubulin. The binding site for GTP is on the β-subunit. When the cell becomes enriched with GTP-tubulin dimers, hydrolysis to GDP-tubulin falls behind the rate of assembly and an α, β-tubulin-GTP cap forms at the plus end of the protofilaments blocking further growth of the microtubule.

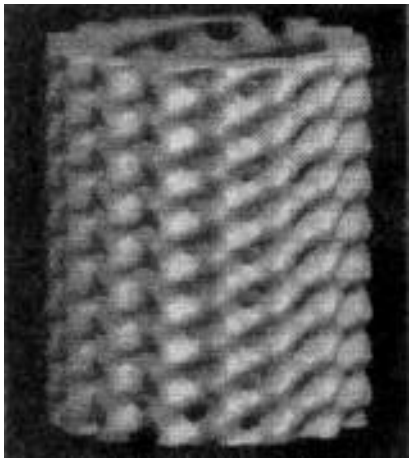


Fig. 1: The structure of microtubule polymer cylinder, usually with internal and external diameters of 14 and 28 nm, respectively.

The classification of microtubule natural inhibitors

Microtubule inhibitors represents chemically very variegated group of compounds from different biological sources with strong effect on cytoskeletal functions and strong toxicity. Microtubule functions in cell depend on the capacity of tubulin to polymerize or the capacity of microtubules to depolymerize.

Compounds which are able to influent these processes, i.e. microtubule inhibitors (also anti-tubulin agents, antimitotic agents, etc.), can be divided into four group according to their mechanism of action. 1. Compounds which bind to GTP site, 2. compounds which bind to colchicine site, 3. compounds which influence as microtubule-stabilizing agents, and 4. compounds which do microtubule network disorganization.

1. Compounds bind to GTP site

Typical representatives of this group of microtubule poisons are vinca alkaloids, compounds derived from *Catharanthus roseus*, a plant from warm climate. The most important compounds of this group are **vinblastine** and **vincristine**, compounds composed from a tetracyclic structure of catharantine and a pentacyclic structure of vindoline (41). Both structures appear to be important for both **vinblastine** and **vincristine** activity. The analysis of the localization of vinblastine-binding site on tubulin has indicated that it occurs at the central region of the beta-tubulin subunit (37). In this region is also GTP binding site and it has been shown that vinca alkaloids and other related molecules can prevent the binding of GTP to tubulin. **Vinblastine** is mainly useful for treating lymphocytic and histiocytic lymphoma, Hodkin's disease, Kaposi's sarcoma, and advanced breast or testicular cancer. **Vincristine** is used mainly to treat acute leukemia, neuroblastoma, rhabdomyosarcoma, Hodkin's disease and other lymphomas. Semisynthetic derivatives of vinca-alkaloids with lower toxicity are now at different phases of clinical trial, for example **vindesine** or **vinorelbin**, which are tested in breast cancer. Other microtubule inhibitors are **dolastin** isolated from the sea hare (*Dolabella auricularia*), compound with both pyrrolidine and thiazoline moiety in the molecule, **griseofulvin**, an antibiotic produced by *Penicillium griseofulvum*, **maytansine**, a macrolide compound from rainforest plant *Maytenus serrata* and others family Celastraceae (34), famous ethnomedicine known in western Amazonia as chuchuhuasi.

Halichondrin B is the most potent compound of a class of polyether macrolides isolated in low yield from four different sponge genera - *Axinella, Halichondra, Lissodendoryx, and Phakellia* (35). **Halichondrin B** acts on tubulin by similar mechanism as **vinblastine** (4,11). From the fungus *Rhizopus chinensis* was isolated other cytotoxic macrolide compound, **rhizoxin**, with significant antineoplastic activity in several murine and human tumor models (6). **Cryptophycin A** is a new antimicrotubule agent, active against some drug-resistant cells (44) and with potent antiproliferative effect and with excellent antitumor activity against mammary, colon, and pancreatic adenocarcinomas (33). A highly cytotoxic macrocyclic lactone polyether has been isolated from a Spongia species and named **spongistatin**. This compound inhibited the glutamate-induced polymerization of tubulin and it is a potent inhibitor of the binding of vinblastin and GTP to tubulin (3).

2. Compounds bind to colchicine site

Colchicine is alkaloid found in the autumn crocus (*Colchicum autumnale*) and also in other plants. Autumn crocus was used in the antiquity for the treatment of gout, but the main interest for the study of colchicine came when it was observed that this drug could stop cell proliferation in mitosis, by preventing the formation of the mitotic spindle. Structurally, colchicine is a tropolone derivative with three rings A, B and C. Ring A is a six-carbon ring with three metoxy groups, whereas B and C are seven-carbon rings (19). Several unfavorable characteristics have been observed for

the binding of colchicine to tubulin. The reaction is very slow, temperature-dependent and essentially irreversible. The binding of colchicine to tubulin becomes faster and reversible when a methyl group replaces the acetyl group present on the amine of the B ring, yielding the compound known as colcemide.

On the same site as colchicin bind also **podophyllotoxin**, plant compounds obtained from *Podophyllum peltatum.* **Podophyllotoxin** is a tetracyclic compound with four rings A, B, C, and D, linked to an aromatic ring with three methoxy groups. This alkaloid is, like **colchicine**, a drug that prevents microtubule polymerization. It has been used for topic treatment of some benign skin tumors. Some synthetic derivatives of podophyllotoxin apear to be more active than podophyllotoxin alone in the treatment of leukemias and solid tumors.

3. Microtubule-stabilizing compounds

Among these compounds, the best known one is **taxol** (paclitaxel), tetracyclic compound obtained from the bark of the Pacific yew (*Taxus brevifolia*). In the structure of taxol there are two aromatic rings and a tetracyclic-structure containing an oxetane ring which is required for the activity of the drug (18). The primary action of this compound is to stabilize microtubules, preventing their depolymerization. In this way taxol should block proliferating cells between G_2 and mitosis, during the cell cycle. The binding of **taxol** appears to occur at different localizations at the amino terminal of β-tubulin, but binding to the middle region of a alpha-tubulin has also been reported (28). **Taxol** has been used mainly for the treatment of breast and ovarian cancer but also it has been tested for other types of tumors such as lung cancer, head and neck cancer and melanoma. Several disadvantages have been indicated for application of this very actual anti-cancer compound. One of them, the relative low amount that can be obtained from the bark of the Pacific yew and its relatively rare incidence restrict to the forests of the Pacific Northwest of the USA and Canada. This problem has been partially solved by chemical synthesis of this compound (32). Another disadvantage is the low solubility of **taxol** in water, thus, this drug must be delivered dissolved in oil and this solvent could effect to cardiad functions or promote allergic reactions. Also, this problem has been partially solved by synthesizing some **taxol** analogs with a higher solubility in water (32). Interesting semisynthetic analogue of **taxol** with clinical use is **docetaxel** (Taxotere), compound which contains a taxane ring linked to an oxetan ring at positions C-4 and C-5 and to an ester side chain at C-13.

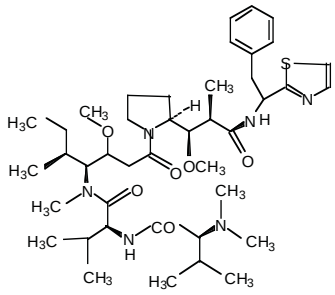
A new class of microtubule-stabilizing compounds have been isolated from the bacterium *Sorangium cellulosum*. These macrolide compounds were caled **epothilones**, because their typical structural units are epoxide, thiazole, and ketone (27,42). **Epothilone** occurs in two structural variations, **epothilone A** and **epothilone B**, the latter containing an additional methyl group (23) . **Epothilone A** is the main product of bacteria metabolism, the yield of **epothilone B** amounting to 20-30 per cent of the yield of epothilone A. Despite the small different in chemical structure, in most test systems **epothilone B** has been approximately ten-time more effective. These compounds show a striking effect on stabilizing polymerization of microtubules and they are easily obtained on large scale by a fermentation process (14). Both **epothilones** show a very narrow spectrum of activity (19) and halts cells, as does **taxol**, in the G_2-M phase (23). The Total synthesis of **epothilones** was reported in many laboratories (5,43,45,48).

Of recent interest is the discovery of the marine-derived compound, **discodermolide**, whose anti-mitotioc mechanism of action includes the polymerization and stabilization of microtubules in a method analogous to that observed with the structurally unrelated compound **taxol** (29,30).

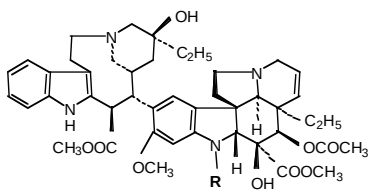
4. Compounds with disorganization effect on microtubule network

Some natural marine compounds with anti-tumoral activity were found to disorganise the microtubule network (12). There are **ecteinascidin 743**, tetrahydroisoquinoline alkaloid isolated from the marine ascidian, *Ecteinascidia turbinate,* (15,24), several members of the family of **lamellarins,** for example **lamellarin Q**, polyaromatic alkaloids isolated form marine tunicates belonging to the genus *Didemnum* (36), as well as cyclic depsipeptides of the family **didemnins**; the most known compound of this family is **didemnin B** (31). **Didemnins** were isolated from the marine tunicates *Tridemnum solidum* and *Aplidium albicans* (47) and many very biologically active compounds of this family were prepared also synthetically or semisynthetically (40).

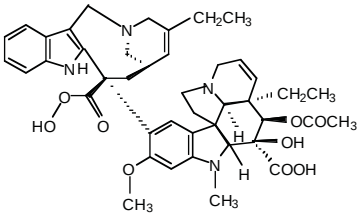
Microtubule-structures



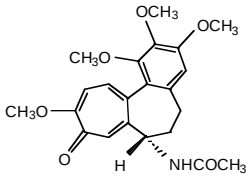
Dolastatin



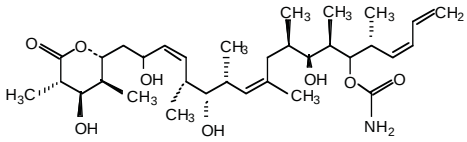
Vinblastine R = CH_3
Vincristine R = CHO



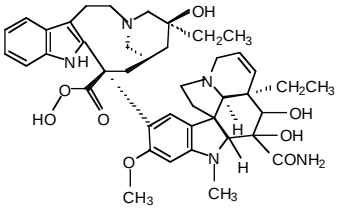
Vinorelbin



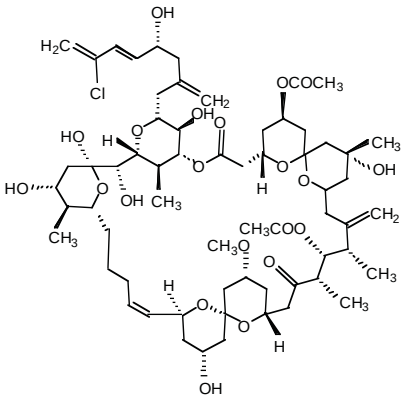
Colchicine



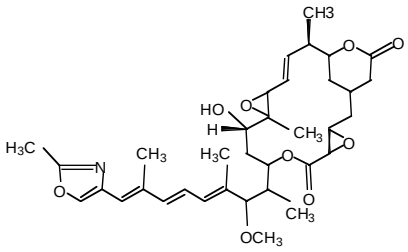
Discodermolide



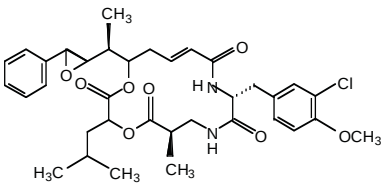
Vindesine



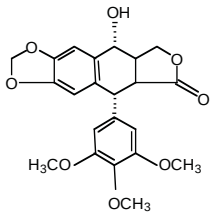
Spongistatin



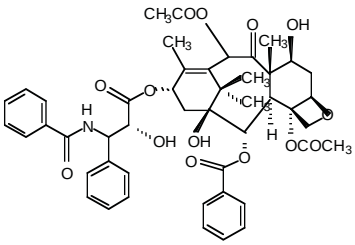
Rhizoxin



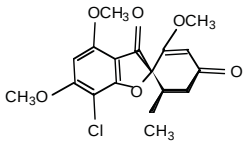
Cryptophycin A



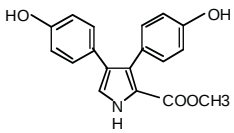
Podophyllotoxin



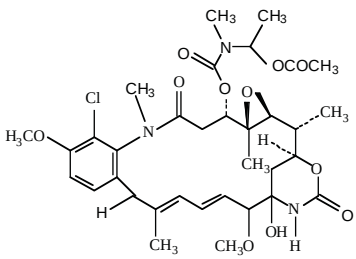
Taxol



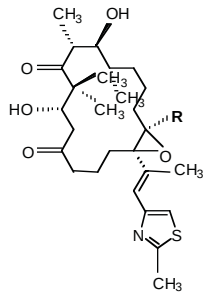
Griseofulvin



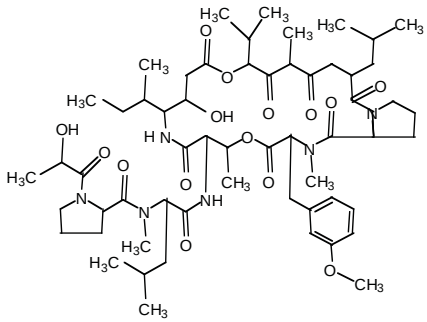
Lamellarin Q



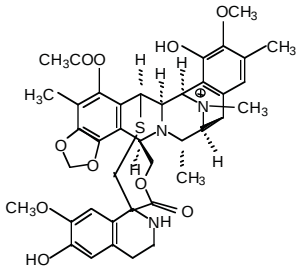
Maytansin



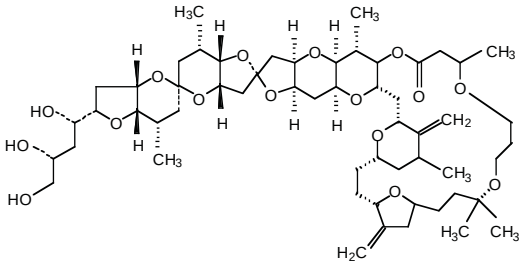
Epothilone A R = H
Epothilone B R = CH_3



Didemnin B



Ecteinascidin 741



Halichondrin B

Applications of microtubule inhibitors in future research

There are many demonstrations that mechanical forces mediated by cytoskeleton play a vital role in assembling cellular structures. Moreover, recent experimental evidence demonstrate the multiple interactions between cytoskeletal structures and ion channels, calcium fluxes, and events connected with signal transduction. The process of microtubule assembly proceeds in a cell-free system and the effects of various inhibitors can be thus studied even in the test tube. The use of various natural microtubule inhibitors provides the possibility to study the mechanisms of assembly and disassembly of cytoskeletal structures as well as the role of cytoskeleton in spatial and temporal integration of vital cell functions.

The regulation of microtubule assembly depends on the ability of tubulin heterodimers to bind GTP. The GTP bound to the β- polypeptide is hydrolyzed to GDP plus phosphate (26). The course of assembly and disassembly of microtubules is therefore affected by the action of GTPases. A number of heterotrimeric GTPases or small GTPases of the rho family move on the cytoskeleton after cell activation (9,46]. Moreover, a direct transfer of GTP from tubulin to the α subunit of the Gs and Gi protein has been reported (39). On the other hand it has been reported that microtubules can also assemble in the presence of nonhydrolyzable GTP analogues. These observations may demonstrate that such interactions do not entirely serve to microtubule reorganization, but may be also related with cell signaling pathways. The use of various microtubule inhibitors which bind to GTP site could contribute to the study of this suggested role of microtubules in eucaryotic cells.

Polymerization of tubulin heterodimers is regulated by Ca^{2+} concentration. Under low Ca^{2+} concentration characteristic for the cytoplasm of most eucaryotic cells, much of the tubulin is assembled into microtubules. Localized increases in Ca^{2+} concentration cause microtubule disassembly (13). The alteration of microtubule structure by colchicin has been reported to enhance the activity of Ca^{2+} channels in Lymnaea neurons and mammalian hippocampal pyramidal neurons (25). An intact microtubule system is required for the IP_3 - dependent Ca^{2+} release from intracellular stores (17). The inhibitory effect of colchicin in saponin-permeabilized platelets has been reported (8).

Disruption of microtubules by colchicin has been reported to increase conductance of Cl^- channels in skeletal muscle (21), and decreased conductance of snake twich fibre end plates (20). The mechanism of this functional change and the role of cytoskeletal structures in the transmembrane ion transport is not known. On the other hand, taxol had no effect on the ion channels in hippocampal neurons (38).

Disruption of microtubular structures by taxol leads to increased phoshorylation and to the cell death (7,16). Degradation of tubulin can occur very early in the course of apoptosis. It has been reported in neuronal cells treated with glutamate (1). Although the relation between the microtubule system and the apoptotic program remains unclear, the disruption of microtubule turnover undoubtedly leads to cell death.

Conclusions

Microtubule inhibitors from different natural sources represents chemically very variegated group of compounds with strong effect on cytoskeletal functions and strong toxicity. The use of this poisons is one of the most frequent therapeutic strategies for carcinoma treatment. Drugs like **vinblastine** and **taxol**, have wide clinical use, although they have some drawbacks. The discovery of new compounds such **epothilones**, **halichondrins**, **didemnins**, etc., could overcome some of the problems found with the use of the earlier drugs. In addition, already now these natural toxic compounds are used as outstanding scientific tools in biological experiments and serve the purpose of model structures for synthesis new compounds with expected effect.

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