

CHEMOTHERAPEUTIC AGENTS: TAXOL AND VINCRISTINE ISOLATED FROM ENDOPHYTIC FUNGI

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ABSTRACTS

Endophytic fungi are abundant and have been found asymptotically in most plants species. Endophytic fungi are considered as hidden members of the microbial world and represent an under-utilized resource of natural bioactive compounds for agricultural, industrial and medicinal uses. As reported previously, several valuable natural products with anticancer, antimicrobial, and insecticidal properties have been successfully extracted from the endophytic fungi. Chemotherapy yields the first promise of destroying cancer cells that has distributed into the body. This is due to the fact that chemotherapeutic agents overlap with the process of dividing cancer cells, which in turn leads to establishing new cells. Vincristine (vinca alkaloids) and podophyllotoxin prohibit the microtubules polymerization at high drug concentrations whereas Taxol™ and Taxotere stimulate the microtubules polymerization and stabilize the microtubules as well at high concentrations. In This review, we critically attempt to summarize the mechanisms of the cancer chemotherapeutic drugs, namely, Taxol and Vincristine (vinca alkaloids) on the need for more the research in the field of cancer chemotherapy.

KEY WORDS: Cancer, Chemotherapy, Endophytes, Paclitaxel, Vincristine.

INTRODUCTION

Microorganisms are rich sources for natural products with large possibility for discovering of new molecules towards using them as anticancer drugs^{1,4}. The popularity of complementary and alternative medicine have increased in recent decades⁵. A significant role of drugs, derived from natural sources, has been detected for prevention and treatment of diseases. More than 70% of the antimicrobial and 60% of the anticancer drugs currently in clinical use are natural products or have been developed from natural products⁶. In the recent years, researchers are directed towards investigation the different novel natural bioactive products from plants⁷. According to Lam⁸, there was declining in the discovery and development of novel chemical entities. Therefore, the recent trends are to maximize the revelation of new bioactive metabolites⁹.

During the last 20 years, the majority of novel biochemistry and secondary metabolites were obtained from microorganisms which reside in the plant tissues^{1,3}. Endophyte term was used by mycologist for fungi that live in the intercellular spaces

of healthy host tissues of plants without causing over symptoms on the plants ^{10,12}. No doubt, exploiting a variety of new natural products from endophytic fungi has become a hot spot of new drug research. Endophytic fungi have potential applications value in various fields such as medicine, food industry and agriculture ^{13,14}. After discovering paclitaxel in 1993 ¹⁵, there have been a growing interest in the studying of endophytic fungi by scientists as potential producers of various types of biologically active compounds. Many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic, and anticancer activities have been successfully discovered from endophytic fungi. These bioactive compounds are categorized as lactones, alkaloids, terpenoids, quinones, steroids, phenols and lignans ^{16,17}. Taxol, Camptothecin, Podophyllotoxin, Torreyanic acid, Galioccladicillins A and B and Vincristine (vinca alkaloids) isolated from *Taxus brevifolia*, *Camptotheca acuminota*, *Trametes hirsute*, *Pestalotiopsis microspore*, *Alternaria alternata* and *Fusarium oxysporum* accordingly have been used as anticancer agents for treating various kinds of cancer.

Cancer is a class of disease by which abnormal growth of cells are able to divide uncontrollably and invade other tissues. Cancer was reported to cause about 13% of all deaths worldwide in 2007, making it a leading cause of death worldwide. Liver, Lung, colon, stomach and breast cancer are considered as the main kinds of cancer that cause to the total death worldwide. Described as one of the principle methods of treatment for cancer patients, Chemotherapy is the process of using anti-cancer agents to block cells from multiplying and dividing ¹⁸. Chemotherapeutic agents are defined as the drugs that block the growth and spread of cancer cells by interfering with specific molecules involved in tumour growth and progression. The primary goal of targeted therapy is to fight cancer cells with more precision and potentially fewer side effects ¹⁹. The use of chemotherapy to treat cancer began at the start of the 20th century with attempts to narrow the universe of chemicals that might affect the disease by developing methods to screen chemicals using transplantable tumors in rodents. Recently, chemotherapy has changed as important molecular abnormalities are being used to screen for potential new drugs as well as for targeted treatments ²⁰. Chemotherapeutic drugs are classified by their pharmacologic action and their interference with cellular reproduction. They are divided into cell cycle phase-specific and cell cycle phase-nonspecific. In this review, the mechanism of action of Taxol and Vincristine (vinca alkaloids), chemotherapeutic agents, are discussed.

Natural Products in Cancer Chemotherapy

There are many ways to treat cancer medicinally. Surgery, radiotherapy, chemotherapy and immunotherapy are the most common treatments to choose. Among these cancer treatment methods, chemotherapy is a relatively new one that can destroy cancer cells by stopping them from growing or multiplying ²¹. Healthy cells can also be affected by chemotherapeutic agents, especially the rapidly dividing cells. However, these cells has own repair mechanisms and self-editing. Occasionally, combination chemotherapy of drugs has been used at the same time due to the strong efficiency against many types of cancer. Chemotherapy agents, classified into several broad categories according to chief mechanism of action, could be used for patients either before “neoadjuvant” or after surgery “adjuvant” ²¹.

Identified as DNA interactive agents, Alkylating agents are medicines that directly damage the DNA inside cancer cells. These agents act against non-Hodgkin’s lymphoma, leukemia, multiple myeloma, Hodgkin's disease and specific cancer types such as breast, lung and ovary in all phases of the cell cycle. Cisplatin, busulfan, cyclophosphamide, mechlorethamine, carboplatin, ifosfamide, chlorambucil, melphalan and dacarbazine (DTIC) have been denominated as examples of alkylating agents ²².

Antimetabolites are able to disrupt nuclei acid synthesis either by falsely substituting for biosynthetic precursors of DNA/RNA or by inhibition of normal precursor biosynthesis. These agents could be applied during the S phase for many kinds of cancers such as leukemia, tumors of the breast, ovary and the gastrointestinal tract. Examples of antimetabolites include methotrexate, 5-fluorouracil, gemcitabine, capecitabine, fludarabine and cytarabine ²³.

Antitumor antibiotics interfere with DNA by stopping enzymes needed for cell division or by altering the membranes that surround cells. These drugs can further work in all phases of the cell cycle. Thus, they are excessively used for diversity types of cancers. Daunorubicin, doxorubicin, dactinomycin, mitoxantrone and idarubicin have been determined as antitumor antibiotics agents ²⁴.

Hormonal agents, which are often natural or synthetic hormonal substances such as steroids, steroid analogs or hormone-like compounds, interact with hormone receptors to reduce tumors whose growth are sensitive to hormonal controls. Tamoxifen is one of the hormone receptor targeting drugs that is applied in the treatment of both early and advanced ER+ (estrogen receptor positive) breast cancer ^{25,26}. Food and Drug Administration (FDA) has been approved to use Tamoxifen for the prevention of breast cancer in women at high risk of developing the disease. This agent works in G0 and G1 phases.

Antimitotic agents are essentially mitotic inhibitors and act by interfering with the cellular mechanism of mitosis. These drugs work during the M phase of the cell cycle. Paclitaxel, docetaxel, etoposide, vinblastine, vincristine, and vinorelbine have been characterized as Antimitotic agents ²⁷. This class will be discussed in more details in the following text.

Endophytic fungi could be alternative approaches for discovery of novel drugs ²⁸. Chemotherapeutic agents obtained from endophytic fungi have been examined and they showed acute efficiency against diverse kinds of cancer. Following, the mechanisms of the two cancer chemotherapeutic agents isolated from endophytic fungi.

Paclitaxel and Vincristine Mechanisms of Action

Due to its unique mode of action compared to other anticancer drugs, the diterpenoid taxol, known as paclitaxel, isolated from endophytic fungus *Taxus brevifolia*, have attracted more attention than any other new drug since its discovery ^{29,31}. Paclitaxel (Taxol) is the world's first billion dollar anticancer drug and is used to treat a number of human tissue proliferative diseases ³². Paclitaxel product has been approved by the Food and Drug Administration (FDA) for use in the treatment of breast, lung and resistant ovarian cancer ³³. Several researches regarding anticancer properties of paclitaxel were reported since its discovery as well as the production of paclitaxel has been investigated in the last decade [34-36]. Paclitaxel (Fig 1) is a complex diterpene having a taxane ring with a 4-membered oxetane ring and an ester side chain at position C-13 ³⁷.

Described as major fundamental components in cells, microtubules are among the most successful targets for anticancer therapy agents ³⁸. Microtubules are formed through the lateral association of between 12 and 17 tubulin protofilaments, which arrange to form a very stiff and hollow filament structure. Microtubules are also highly dynamic, undergoing rapid cycles of polymerization and depolymerization ³⁹

Microtubules are not static but they are highly dynamic polymers and exhibit two kinds of dynamic behaviors: dynamic "instability" and "treadmilling"(Fig 2 A, B) ⁴⁰. These dynamic actions are essential to mitosis phase by which new cells are formed from chromosomal division. Microtubule dynamics are highly regulated during the cell cycle by endogenous cellular regulators ⁴¹.

Fig. 1. Structure of paclitaxel (Taxol)

[42].

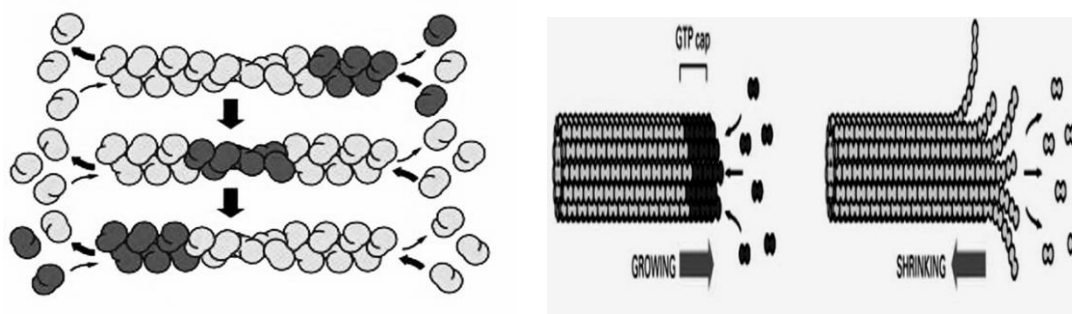
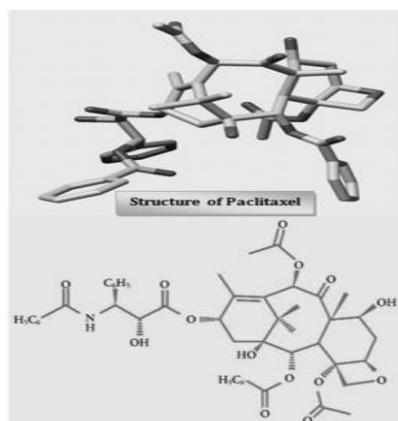


Fig. 2. (A) Dynamic Treadmilling. (B) Dynamic Instability

The action of mode for Paclitaxel (taxol™), taxotere™, laulimalide, sarcodictyins and eleutherobins have been recognized by stimulation microtubule polymerization and stabilization of microtubules at high concentrations (Fig 3), whereas the inhibition of tubulin-microtubule polymerization has been carried out by cryptophycins vincristine (vinca alkaloids), halichondrins, colchicine and estramustine ^{43,47}.

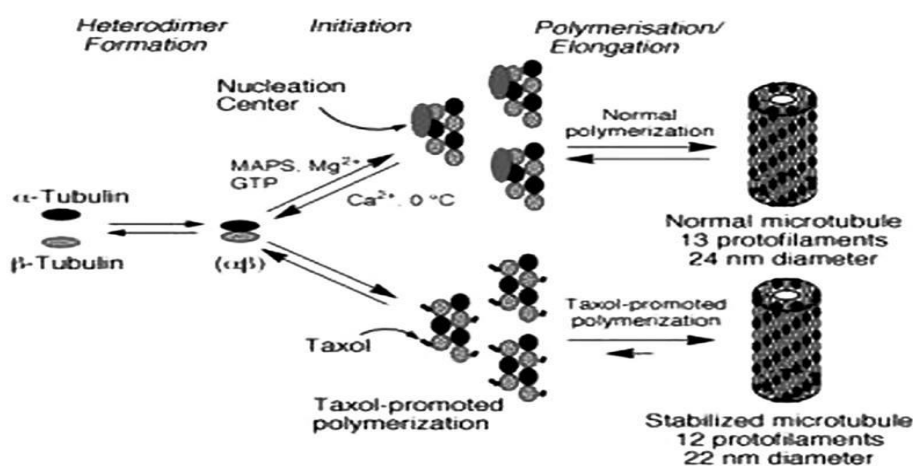


Fig. 3. Mechanism of action of paclitaxel

Regarding lower concentration, these agents have been characterized to possess a popular mechanism through suppression of the microtubules dynamics effectively without altering noticeably in the microtubules mass ⁴¹. Moreover, these drugs

bind to diverse sites on tubulin and at different positions within the microtubule, and they have diverse effects on microtubule dynamics⁴⁸. However, by their common action of suppression microtubule dynamics, these antitumor drugs can block mitosis at the metaphase or anaphase transition, and induce cell death⁴⁹.

Vincristine (Oncovin®) (Fig 4) was extracted from *Fusarium oxysporum*. Described as antimetabolic agent that is widely used in cancer treatment, Vincristine belongs to the general group of chemotherapy drugs^{50, 51}. Food and Drug Administration (FDA) has approved to use vincristine as anticancer agent in 1963 and is still one of the most chemotherapeutic agents administered in the clinic⁵².

Vincristine possesses anti-tumor activity against a wide range of neoplasms including the Hodgkin's and non-Hodgkin's lymphomas, acute lymphoblastic leukemia, rhabdomyosarcoma, embryonal, neuroblastoma, breast carcinoma, and Wilm's tumor^{53, 54}.

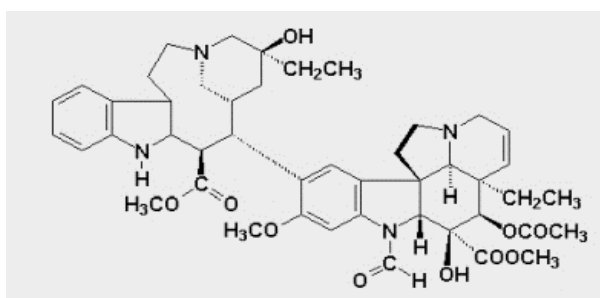


Fig.4. Structure of Vincristine (Vinca alkaloids) [55].

The vincristine (vinca alkaloids) binds to tubulin dimers at a specific recognition site on the protein. The formation of paracrystalline, which is realized by tubulin- drug complex, has a critical role in the reducing concentration of the dimers and pushes the equilibrium between growth and shrinking of the microtubules in favor of shrinking (Fig 5). Cells which are subjected to vincristine lose the ability to drive growth correctly in mitosis phase because of poorly formed mitotic spindles. The damaged cells then go through apoptosis stage⁵⁶.

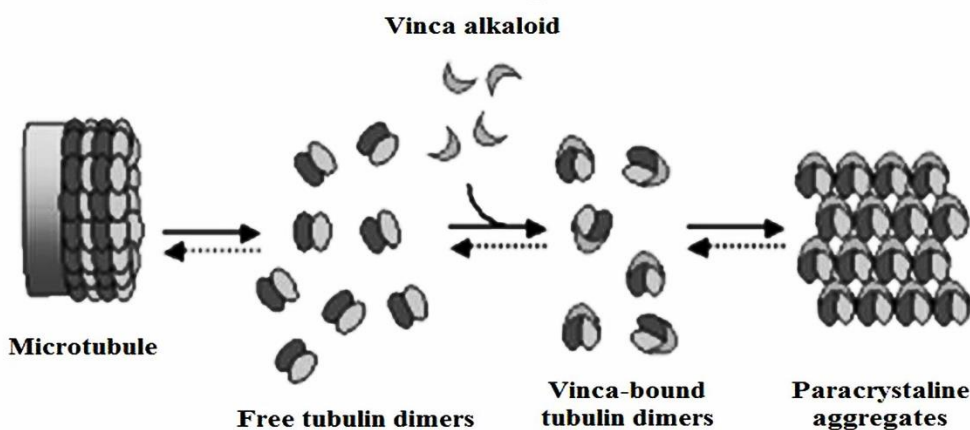


Fig.5. Mechanism of action of vincristine

CONCLUSION

Endophytic fungi produce natural products with a large diversity of chemical structures that would be useful for specific medicinal and biotechnological applications. Most of these secondary metabolites exhibit biological activities in

pharmaceutically relevant bioassay systems and represent potential lead structures which could be optimized to yield effective therapeutic and bioactive agents. Cancer chemotherapy is the use of drugs to kill cancer cells. Conventional chemotherapy targets have been the cell cycle, microtubules and DNA. Microtubules and tubulin have been classified as the single best cancer target for numerous of anticancer drugs. The mechanism of action of paclitaxel (taxol) has been recognized by stimulation microtubule polymerization and stabilization of microtubules whereas vincristine (vinca alkaloids) binds to tubulin dimers at a specific recognition site causing inhibition of the microtubules polymerization. Significant evidence indicates that the chemotherapeutic drugs have a common model of action; they suppress the microtubule dynamics without appreciably changing in the mass of microtubules resulting to block the cell cycle at the metaphase or anaphase transition and persuading apoptosis.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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