Research Progress of Paclitaxel Drug Source Solution and Extraction and Separation Technology

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Abstract: Taxol, a natural secondary metabolite extracted from the bark of taxus chinensis, is one of the best natural anti-tumor drugs in the past decade and has great medicinal value. However, the content of paclitaxel in plant body is low, the extraction and separation are difficult, and the drug source is short. Based on the review of the literature on paclitaxel, this paper discusses and reviews the solutions of paclitaxel's drug sources in terms of its synthesis, plant cell culture, endophytic fungi fermentation and synthetic biology. The research progress of paclitaxel extraction and separation technology and the possibility of large-scale industrial production were considered and prospected, which provided a reference for the solution of the drug source problem of paclitaxel and further study.

1. Introduction

Paclitaxel (taxol) was first isolated from the bark of Pacific yew (Taxol brevifolia) [1]and was approved by the US FDA in late 1992. As a diterpenoid alkaloid compound with various unique anticancer activities [1], the sales volume of paclitaxel ranks first among anticancer drugs in the world. However, paclitaxel only contains 0.069% in the bark of Taxus brevifolia, the highest content at present, and it takes about 13.6kg of bark to extract 1g of paclitaxel. It takes 3-12 more than 100 years of paclitaxel to treat a patient with ovarian cancer [2]. So, extracting paclitaxel directly from yew can lead to significant environmental problems and is not efficient. Since the raw material of paclitaxel is in short supply for a long time, the development of extraction and separation technology with high utilization rate and high yield of paclitaxel and the method of discovering the solution of drug source can greatly increase the total output of paclitaxel raw material. Paclitaxel is one of the most widely used and effective antineoplastic drugs of natural origin. It has broad antitumor activity, particularly against ovarian, breast, non-small cell lung cancer, head and neck tumors, Kaposi's sarcoma, and urologic malignancies. It is a highly lipophilic compound with a log P value of 3.96 and very poor water solubility of less than 0.01 mg/mL. In addition, the compound lacks ionizable functional groups, which may cause its solubility to increase with changes in pH[17]. As a result, the delivery of paclitaxel presents significant challenges. Nanoparticles and liposomes are considered potentially better options for drug delivery, as they can minimize harmful toxicity while enhancing the therapeutic effects of paclitaxel[18].

The anti-cancer drug paclitaxel has a very unique mechanism of action by interfering with the breakdown of cell microtubules. Microtubules are proteins formed by cell mitosis. Paclitaxel drugs inhibit cell division, including tumor cells, by interfering with the normal circulation of microtubules. At present, paclitaxel drugs are known to have a good inhibitory effect on many tumor cells. Microtubules are a component of eukaryotic cells that are formed by microtubule dimers consisting of two similar polypeptide (a and p) subunits. Under normal conditions, there is a dynamic equilibrium between microtubules and tubulin dimers. Paclitaxel can lose this dynamic balance between the two, induce and promote tubulin polymerization, prevent depolymerization, and stabilize microtubules. These effects cause the cells to be unable to form spindles and spindle filaments during mitosis, inhibiting cell division and proliferation, thus exerting anti-tumor effects. Taxol has also been approved as one of the effective drugs for the combined treatment of lung cancer, esophageal cancer, pancreatic cancer, prostate cancer and Kaposi's sarcoma related to AIDS [3]. Paclitaxel, as well as other PTX-derived compounds, have been and continue to be very useful in fighting this disease. However, two major drawbacks of its use remain unaddressed: its production is expensive and unsustainable, and the mechanisms by which tumor cells develop resistance to it are not fully understood. The production of PTX by microbial fermentation is the most promising alternative to compete with chemical synthesis and plant extraction. However, there is still a need for a deeper understanding of microbial metabolism and the development of better genetic engineering techniques, to improve the efficacy of paclitaxel as well as other known anti-cancer drugs. For liquid chromatography, sample preparation, chromatographic separation, and analytical performance were compared. It can be used as a reference

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for the future development of liquid chromatography methods for the quantification of taxanes in various biological matrices to support preclinical and clinical research[19].(Figure 1)

In this paper, the ways to solve the drug source of paclitaxel in recent years, the extraction and separation techniques of paclitaxel are reviewed.

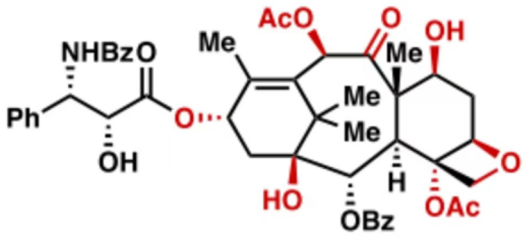

Figure 1. The structure of pacilitaxel

2. Pharmaceutical solutions of taxol

2.1. Chemical synthesis

In the early 1990s, researchers successfully synthesized paclitaxel, but the process is complex, the synthesis efficiency is low, the preparation cost is expensive, and it is not suitable for industrial production [4].

By the end of 2021, the research group of Professor Chuangchuang Li of the Department of Chemistry of Southern University of Science and Technology completed the asymmetric total synthesis of the famous anti-cancer natural drug paclitaxel through 21 steps efficiently and simply, which is the shortest total synthesis route of paclitaxel in the world so far. After eight years of clever substrate design, the team successfully closed the challenging eight-membered ring at the bottom of the molecule for the first time, using samarium diiodide-mediated pinacol coupling reaction as a key strategy [4].The total synthesis adopts a convergent diversity synthesis strategy, all the intermediates are new compounds, which provides a material basis for the development of better taxanes antitumor drugs.(Figure2)

Figure 2. Asymmetric total synthesis process

Table 1. Comparison between two groups

Total synthesis of paclitaxel	Baran team (JACS, 2020)	Li Chuangchuang team (JACS, 2021)
Total steps	24	21
Number of separated intermediates	22	19
Total recovery	0.001%	0.118%

However, paclitaxel drug itself also has defects, such as large toxic side effects, causing the emergence of drug-resistant cells, low bioavailability, difficult to dissolve in water, currently only through injection cannot be taken orally. The predecessors of paclitaxel have carried out many beneficial structural modifications, mainly to modify the functional group of its parent nucleus, but there are certain limitations. Therefore, new strategies for structural modification need to be developed in order to discover superior taxel drug moleculzes [4] .(Table 1)

2.2. Chemical semi-synthetic

In 1981, French chemists Pierre Potier isolated a substance called 10-DAB from the leaves of the British taxus [5], which is very similar to paclitaxel, and the content is high, and the leaves are more regenerative than the branches, and the damage to the taxus is also less. In the process of continuous research, scientists have developed a method of synthesizing paclitaxel from 10-DAB, which is no longer extracted by cutting.

Subsequently, through the structural study of paclitaxel, other similar chemical drugs such as docetaxel, albumin paclitaxel, etc. [6], have also been developed, bringing more therapeutic drugs for tumor patients. In addition, the chemical semi-synthesis of paclitaxel also avoids the complex master ring part of the synthesis of paclitaxel. Chemical semi-synthesis is cheaper than natural extraction and has become the main source of paclitaxel [7].(Figure 3)

CAS number	33069-62-4
Molecular mass	853.9
Molecular formula	C47H51NO14
Solubility information	DMSO, methanol, ethanol. Unstable in Methanol.
Consistency	White crystalline solid.
λmax	227, 273 nm
Melting point	(decomposition) 200-220 °C
Solubility	Clear colorless solution at 10 mg·cm-3 of methanol. Solubility in water is ~ 0.4 μg·cm-3.
Specific optical rotation	-49 ~ -55
Storage	-20 °C. Protect from light.
Warnings	Warning! May cause birth defects. Irritates eyes, skin, respiratory system
Classification	cyclodecane Antitumor agent

Figure 3. General characteristics of paclitaxel[20].

2.3. Plant cell culture

The main processes of Taxus cultured cells to produce paclitaxel include callus induction, screening of stable and high-yield paclitaxel cell lines, and using bioreactor for culture. To induce callus, it is necessary to consider the site of explants, the composition of the medium and the types of hormones. The results showed that although the stem, bud, arils, leaves, embryo and roots of Taxus taxus could be used to induce callus, the induction effect of different explants was different, and the cell line from the embryon-induced callus had higher yield of paclitaxel. Plant cell culture eliminates the ecological environment problems of natural extraction, facilitates the expansion of production, and is feasible. In the process of cultivating taxus

chinensis cells to produce paclitaxel, the yield can be increased from the following aspects.

2.3.1. Screening of high yield cell line

Relatively speaking, the content of paclitaxel is higher in the light-colored, blocky or granular cell masses [8]. Some high-yielding cell lines have taxol content of up to 0.1%, which is 10-100 times higher than taxol in natural bark [9].

2.3.2. Select the appropriate bioreactor

The mechanical properties and shear forces of different bioreactors are different. It was found that the airlift bioreactor had low shear force and good gas transfer [10], and it was more suitable for cultivating taxus cells than the stirred bioreactor.

2.3.3. To improve the dynamical process of suspension culture

By monitoring the cell growth process and yield changes in real time, optimizing the ventilation and nutrient content of the bioreactor can improve the yield of paclitaxel.

2.3.4. Adjust the metabolic processes

The yield of paclitaxel can be increased by adding intermediates and inducers of paclitaxel biosynthesis. For example, it has been found that adding glycine, serine and other intermediates of paclitaxel biosynthesis in bioreactors can improve the ability of cells to synthesize paclitaxel. For another example, inducers can regulate the synthesis of plant secondary metabolites. The commonly used bioinducers are endophytic fungi (Such as beautiful Fusarium), methyl jasmonate, coronin, cyclodextrin, etc. When growing taxus cells in stirred bioreactor, the secretion rate of paclitaxel can be increased by 6.8 times if Fusarium meliflorum is added[9].

2.4. Endophytic fungi fermentation

In theory, the natural product can be obtained more quickly and on a large scale by isolating the endophytic fungi, Yew Andrew, which can produce paclitaxel independently from Yew bark. The production of paclitaxel by endophytic fungi has many advantages such as short cycle, controllable conditions and low cost, and can obtain paclitaxel or key precursors stably and on a large scale [11], which opens up a new way for the synthesis of paclitaxel.

2.5. Extract paclitaxel from cotton

In 2022, it was reported that Li Fuguang, a scientist from Henan province and director of the Cotton Research Institute of the Chinese Academy of Agricultural Sciences, and his team found that the substrate of gosvenol and the substrate of the anti-cancer natural product paclitaxel are the same kind of substances, and cotton has raw materials for the synthesis of paclitaxel, but it lacks an anabolic pathway [12]. Li Fuguang told reporters that they are currently carrying out the use of synthetic biology to put the "raw material" into the "factory", so that cotton can also synthesize paclitaxel research. In the early stage, they used the similarity principle between cotton callus and animal tumor cells for rapid proliferation and lasting regeneration to find out the mechanism of the cotton-specific tumor suppressor G protein, which can target the proliferation of common human cancer cells such as ovarian cancer, esophageal squamous cell cancer, and gastric adenocarcinoma [12]. In addition to clothing and food, cotton is also expected to be used in the future to treat cancer and play a big role in the field of medicine and health.(Figure 4)

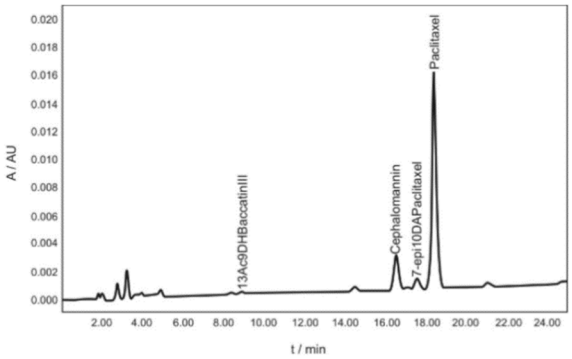

Figure 4. Chromatogram of crude plant extract

3. Drug taxol sample pretreatment techniques

In the process of paclitaxel production, each sample must be pre-treated, which mainly includes sample extraction and separation.

3.1. Sample extraction

The crude extraction of paclitaxel mainly used methane, ethanol, methanol and methylene chloride (1:1), but the impurity quality in the extractum was higher. It was found that in ethyl acetate, ethyl ether and other solvents, the mixture of ethyl acetate and acetone (1:1) was the best to extract paclitaxel, and the content of paclitaxel in the extract was up to 3 times that of paclitaxel [13]. Yu Guangbie et al. found that methanol had the best extraction effect among seven single solvents: methane, ethanol, acetone, dichloromethane, trichloromethane, ethyl acetate and ether.

The commonly used methods include cold immersion, percolation and the introduction of ultrasonic and microwave technology in the process of Soxhlet extraction, which greatly reduces the extraction time [14].

3.2. Sample separation

The content of impurities in the crude extract of paclitaxel is generally large, which needs to be treated by separation technology. The general separation technology methods

include precipitation method, liquid-liquid extraction method (solvent extraction method), solid phase extraction method, supercritical fluid extraction, steam distillation method, dialysis method, chromatography and other methods. At present, liquid-liquid extraction and solid-phase extraction play a major role in sample separation. This paper mainly describes and compares the two methods, and summarizes and discusses the application of this technology in the production of paclitaxel.

3.2.1. Liquid-liquid extraction

Liquid-liquidextraction method (LLE) uses organic solution as extractant to extract certain components in the aqueous solution [15]]. Liquid-liquid extraction is a common separation technique used to separate the mixture from the organic solvent to obtain a higher purity product.

The basic principle of liquid-liquid extraction is to mix two incompatible solvents together, and use the difference in miscibility between the solvents to separate the mixture in one of the solvents [15]. When the two solutions are mixed, the interaction between them causes the organic and metal ions in the mixture to separate, forming two different solvent layers that separate the paclitaxel drug components needed for the study.

3.2.2. Solid phase extraction

Solid Phase Extraction (SPE) is a kind of sample pretreatment technology based on chromatographic separation, were divided into stationary Phase and mobile Phase. Mainly through the solid adsorbent to extract the selective desorption of taxanes, keep the active ingredient, after eliminating impurity in the sample, get the separation, purification and concentration of the sample. The process consists of four steps, namely stationary phase activation, sample loading, leaching, and sample elution [15].

The basic principle of solid phase extraction is that the liquid sample under the action of positive pressure, negative pressure or gravity, through the extraction device equipped with solid phase adsorbent, the taxane crude extract sample adsorbed on the fixed phase, and then the organic solvent such as n-hexane and dichloromethane to elute paclitaxel, in order to achieve the purpose of taxol separation.

3.2.3. Liquid Phase Microextraction

Liquid phase micro extraction (LPME) technology is since 1966, with the development of the environmental analysis technology and developed a kind of fast, accurate, high sensitivity, friendly environment sample preparation technology[16], which can be used to study trace substances as well as to analyze and test large molecule substances.

The basic principle of liquid phase microextraction is to use the solvent effect to separate the specified substance in the solvent. Specifically, the substance in the solvent is divided into two parts: one part enters the solvent, and the other part is isolated after a physical or chemical reaction.

3.2.4. Application of each method in paclitaxel separation

Compared with the traditional separation and enrichment methods such as liquid-liquid extraction, automatic solid phase extraction has the advantages of reducing the influence of impurities in organic solution on the pre-treatment technology and reducing environmental pollution. Therefore, in order to obtain more accurate paclitaxel content, after using liquid-liquid extraction technology, then using solid phase extraction technology can obtain more pure paclitaxel active ingredients [16]. Modern liquid phase microextraction technology can improve the solvent effect, and liquid phase microextraction technology has joined the ranks of drug pretreatment technology, which is an effective method to study and analyze organic and inorganic substances, and it is also used in the separation process of paclitaxel drug production.(Figure 5)

Oil	Solubility of paclitaxel (mg/g)
Tributyrin	9.62
Tricaproin	9.03
Tricaprylin	1.19
Corn oil	0.23
Soybean oil	0.18
Cotton seed oil	0.14
Mineral oil	Not detected

Figure 5 Solubility of paclitaxel in various oils

4. Summary and outlook

Paclitaxel is a targeted drug for the treatment of cancer patients, and is one of the most precious and rare therapeutic drugs. Biologists have made unremitting efforts and exploration in the study of paclitaxel drugs, and have studied the solution of the major problem of insufficient drug sources. Chemical semi-synthesis is the first development method with low cost and little damage to the environment. Cell culture technology solves the problem of ecological environment, so it is a good method. Paclitaxel has been found in cotton, opening up a new road for the development of paclitaxel. I believe that with the continuous progress of extraction technology, we can make it possible to use paclitaxel as a raw material for large-scale production of anti-cancer drugs, and the total output of paclitaxel original drugs will also make rapid progress. Liquid phase microextraction technology also stands out from many extraction technologies and becomes a new and effective synthesis method. In the future, the pre-treatment technology and biosynthesis of paclitaxel is bound to become the focus, on the road of research and development, ecological environment, sustainable development, supplemented by large-scale production, to develop more efficient and feasible production technology.

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