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Biosynthesis of Oleanderaceae Represented by Vincristine and Clinical Applications

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Abstract: Vincristine, as a monoterpene indole alkaloid, has been widely used in various cancer treatments for its excellent antitumor efficacy.In this paper, we comprehensively review a comprehensive overview of the bisindole alkaloids from Oleaceae plants, exemplified by Vincristine, to explore their mechanisms of action, synthesis methods and clinical applications, as well as to look forward to their future development trends, aiming to provide references for Vincristine-related studies.

1. Introduction

The periwinkle of the Oleaceae family is rich in more than 130 alkaloids with medicinal value, among which indole terpenoid alkaloids (TIAs) are the most prominent. These alkaloids are structurally complex and are usually formed from two monoterpene indole alkaloid units by homo- or heterodimerization. Among them, Vincristine (Figure 1) is a typical representative of this type of alkaloids[1],Vincristine has been used in clinical use for more than 40 years and is widely used in the treatment of various cancers[2].

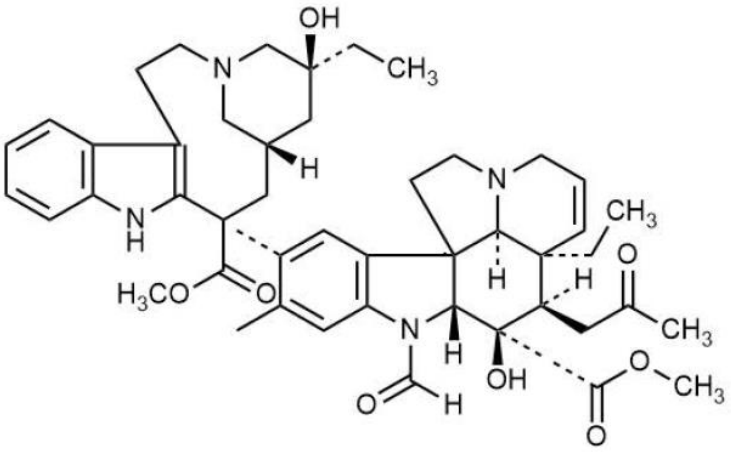

Figure 1 Vincristine

In addition, the extraction, isolation and purification, determination of the content, as well as biosynthesis and metabolism modulation of vinblastine have a significant impact on its efficacy. These findings provide an important theoretical basis for the large-scale production and effective utilization of vincristine[3].

1.1 The mechanism of action of vincristine

The mechanism of action of vincristine mainly stems from its high-affinity binding to microtubule proteins, which can effectively inhibit the polymerization process of microtubules and thus prevent the normal formation of spindle microtubules. As spindle microtubules play a key role in cell mitosis, their formation is blocked, leading to mid-mitotic arrest4 and ultimately preventing further cell proliferation[4].The inhibition of vincristine results in the inability of spindle formation, which leads to cell cycle arrest in the G2/M phase, potentially triggering programmed cell death (apoptosis), and thus suppressing the proliferation of tumor cells. In addition, vincristine inhibits the expression of the immunosuppressive molecule PD-L1 by tumor cells, reduces its binding to PD-1 on the surface of T cells, and enhances the body's anti-tumor immune response[5].It also inhibits cell proliferation by affecting the expression of cell cycle-related proteins and activates signaling pathways such as p53 to promote apoptosis[6].

Figure 2 Periwinkle

1.2 History of development of vincristine: from discovery to application to artificial synthesis

The discovery of periwinkle alkaloids originated from the systematic study of periwinkle. Native to Africa, periwinkle (Figure 2) extracts have been widely used in traditional medicine, but their anticancer activity was not recognized by the scientific community until the mid-20th century. 1958, Gordon Svoboda and Irving Johnson, while screening plant extracts for anticancer activity, found that crude extracts of periwinkle significantly prolonged the survival of mice implanted with the P1534 leukemia cell line. The survival of mice implanted with the P1534 leukemia cell line. Currently, the methods for extracting vincristine (Table 1) mainly include lipophilic organic solvent extraction method, supercritical fluid extraction method and biotechnology extraction method.

Table 1. Physical and chemical properties of vincristine

Molecular formula	C46H56N4O10
Molecular weight	824.9577
Appearance characteristics	White crystals, needle-like crystals when recrystallized in methanol
Melting point	211-216℃
Optical rotation	+42 ℃(Chloroform)
Density	1.4g/cm ³
Solubility	It has good solubility in organic solvents such as methanol, ethanol, DMSO, etc., and is slightly soluble in water
Stability	Stable, but may be heat sensitive. Incompatible with strong oxidants

Advances in synthetic biology have made it possible to synthesize periwinkle alkaloids from scratch using microbial cell factories. In 2018, O'Connor's group[7]used transcriptome analysis to identify two missing oxidoreductases in the periwinkle alkaloid biosynthetic pathway: precondylocarpine acetate synthase (PAS) anddihydroprecondylocarpine acetate synthase (DPAS). 2022, Keasling's group[8]successfully reconstituted 30 enzymatic steps in the periwinkle alkaloid biosynthesis pathway in highly engineered yeast cells, realizing the synthesis of the precursors of periwinkle alkaloid, vindoline and periwinkle plastocyanin, from scratch.In 2023, Lian Jia Chang's subject[9]group constructed a cell factory using Pichia pastoris, a yeast, to achieve the de novo synthesis of periwinkle alkaloids using a simple carbon source.This study demonstrated the feasibility of yeast cell factories for the production of high value-added periwinkle alkaloids and their derivatives.

2. Biosynthesis pathways of vincristine

2.1 Secondary metabolic synthesis pathways in plants

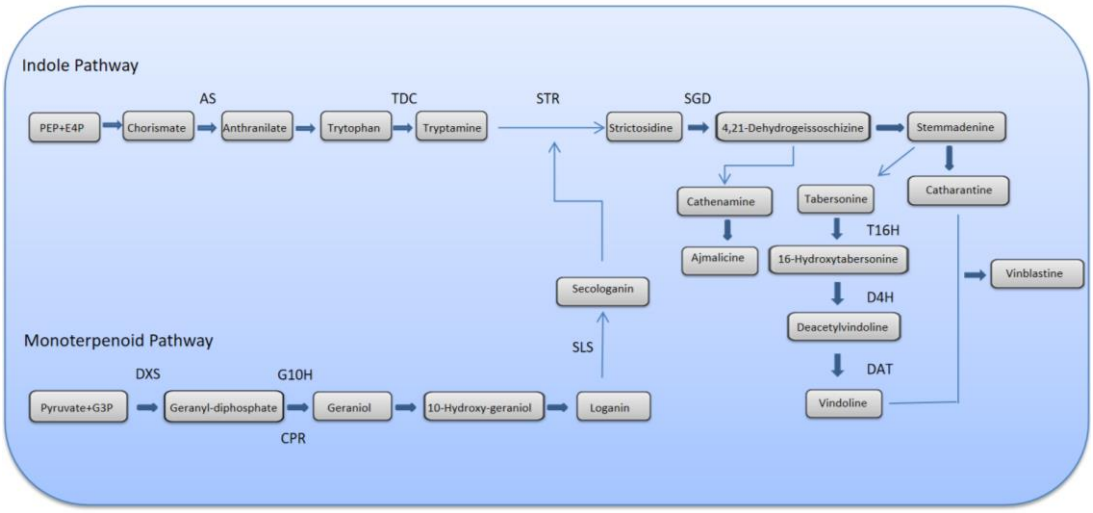

Figure 3. Biosynthesis pathways of vinca terpene indole alkaloids (TIAs)

The biosynthesis process of vinca terpene indole alkaloids (TIAs) (Figure 3) involves multiple stages, mainly including an upstream stage[10]and a downstream stage[11].

In the upstream stage, the synthesis of strictosidine, a common precursor substance of TIAs, is the central task.This process uses tryptamine and secologanin as the key precursor substances, which are generated by a reaction catalyzed by the enzyme strictosidine synthase (STR)[12]. In the downstream stage, strictosidine, as the common precursor, is progressively converted in a multistep enzymatic

reaction into vindoline and vincristine.vindoline and catharanthine[13]. In the final stage of this synthetic pathway, vindoline and catharanthine are coupled by type III peroxidase (CrPrx1) to produce catharanthine, which has important medicinal value[14-16].The reaction of vindoline and catharanthine in the final stage of the synthetic pathway is catalyzed by CrPrx1.

2.2.1 Vincristine semi-synthesis

The semi-synthesis of periwinkle alkaloids relies heavily on precursors obtained from periwinkle flowers, such as vindoline with periwinkle plastocyanin. These precursors undergo a chemical coupling reaction to form periwinkle bases, which are then converted to periwinkle alkaloids by a reduction reaction. Here is how the process works: The first step is to extract and purify vindoline and periwinkle alkaloids, which is complex and costly. However, with the help of optimized extraction and purification techniques, such as the use of supercritical fluid extraction (SFE) and high performance liquid chromatography (HPLC), the extraction efficiency and purity can be greatly improved. Next, the purified vindoline and periwinkle alkaloids are mixed in a suitable solvent, and a catalyst is added to carry out a chemical coupling reaction to produce periwinkle alkaloids. By screening different catalysts and optimizing the reaction conditions, the yield and purity of vincristine could be significantly improved. Finally, the generated vincristine was reduced in a suitable solvent, usually using a chemical reducing agent to reduce it to vincristine, and the reaction time and solvent system were adjusted to optimize the reduction reaction conditions.

2.2.2 Artificial synthesis of vincristine

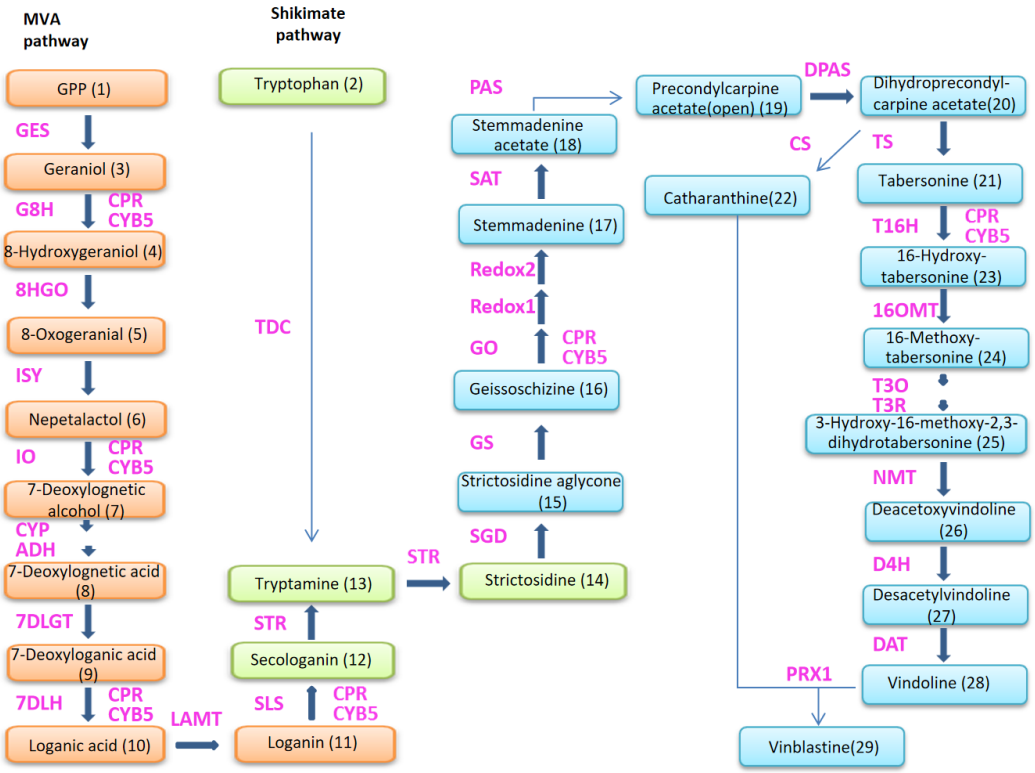

Figure 4 Complete biosynthesis pathway for vinblastine production in yeast

The artificial synthesis of vincristine (Figure 4) is mainly based on microbial cell factories, among which Saccharomyces cerevisiae and Pichia cerevisiae are commonly used host strains. This synthetic pathway involves 30 enzymatic steps from tryptophan and geranyl pyrophosphate (GPP) to vincristine and vindoline, which are distributed in multiple organelles including the cytoplasm, plastids, endoplasmic reticulum, nucleus, and vesicles. To reconstruct this complex biosynthetic pathway in microorganisms, the researchers divided it into three main modules: the strict carvacrol module, the vincristine/ventolin module, and the vincristine module. To enhance the yield of vincristine and vindoline, the researchers optimized the fermentation conditions, covering measures such as the continuous addition of glucose and galactose. Under the optimized conditions, the yield of vincristine reached 91.4 μg/L and the yield of vindoline reached 13.2 μg/L. Vinblastine was then successfully synthesized by chemically coupling vindoline with vincristine and purified by thin-layer chromatography and high-performance liquid chromatography (HPLC), and 23.9 μg/L vinblastine was detected[17].

3. Clinical application of vincristine

3.1 Pharmacokinetics of vincristine

Vinpocetine has complex pharmacokinetic properties, mainly administered by intravenous injection, rapidly enters the blood circulation and is widely distributed in the body, especially in the higher concentration in the nerve cells, but the blood-brain barrier permeability is low, and the binding ability with tumor tissues is relatively weak. Its protein binding rate is about 75%, mainly metabolized in the liver and excreted through bile, the fecal excretion rate is about 70%, and the urinary excretion rate is 5% to 16%.The half-life of vincristine is multiphasic, with an α-phase half-life of less than 5 minutes, a β-phase half-life of 50 to 155 minutes, and a γ-phase terminal elimination half-life of up to 85 hours in adults.

3.2 Adverse reactions and preventive protection measures

In clinical practice, vincristine has encountered a number of difficulties. At high doses, the drug may trigger irreversible neurotoxicity, a side effect that greatly narrows its clinical utility. Although traditional injectable dosage forms are widely used in clinical practice, the lack of controlled release, non-specific biodistribution, and the resulting off-target side effects remain key issues that need to be addressed .In particular, at high doses, the neurotoxicity of vincristine may lead to peripheral neuropathy (e.g., numbness, abnormal sensation) and central neuropathy (e.g., encephalomyelopathy, seizures, etc.), which may be difficult to reverse once they occur, and in severe cases, may even be life-threatening[18].It is therefore important to precisely control the dose of vincristine in order to avoid the occurrence of these serious adverse effects. Therefore, it is important that the dose of vincristine be precisely controlled to avoid these serious adverse reactions.

3.3 Targeted medicine

In recent years, the application of nanotechnology drug delivery systems, including liposomes, nanoparticles, and microspheres, is being explored with the aim of enhancing their bioavailability, lowering the administered dose, and achieving highly effective, long-lasting therapeutic effects with fewer side effects[19].Taking liposomes as an example: liposomes[20](LP) are nanoscale drug carriers composed of phospholipids and cholesterol. Its structure is similar to the bilayer of a biological membrane. With their excellent biocompatibility, cellular affinity, targeting and slow release properties, liposomes can effectively prolong the retention time of drugs in vivo, promote the

distribution of drugs at the target site, and achieve sustained release of drugs[21].Researchers have used active drug-carrying techniques, such as pH-gradient or ammonium sulfate gradient, to prepare liposomes of pergolide, which have significantly increased the encapsulation rate (more than 80%), and were modified by PEG to enhance their stability and retention time in vivo. It can enhance its stability and in vivo retention time. Meanwhile, thermosensitive liposomes have become a research hotspot because of their non-toxicity, non-immunogenicity and temperature sensitivity to tumor foci, but their thermal sensitivity is easily affected by external factors, and it is difficult to accurately control the drug release and the local temperature in the process of thermotherapy.

4. Discussion and prospect

The research progress and future direction of vincristine involves a number of core areas, including the biological efficacy of alkaloids, metabolic pathways, synthetic biology and clinical applications. By optimizing the pharmacokinetic properties, metabolic process and drug delivery method, the clinical therapeutic effect of periwinkle alkaloids can be greatly improved and their side effects can be effectively reduced, so as to provide patients with safer and more efficient therapeutic solutions. Meanwhile, in-depth investigation of the biological functions of periwinkle alkaloids will help to elucidate the role of these alkaloids in the evolutionary history of plants and environmental adaptation mechanisms, and build a solid theoretical foundation for the development of new drugs.

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