Pharmacological Action and Molecular Mechanism of Lithospermoside

Anqi WANG, Jiazhu LI, Jingjing WEN, Changtao CAI, Jundong GUAN, Chenghao JIN*

College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing 163319, China

Abstract Lithospermoside is a kind of alkaloid compound existing in *Semiaquilegia adoxoides*, with anticancer, anti-inflammatory, antibacterial, hypoglycemic, antioxidant and other pharmacological effects. This paper reviews the pharmacological action and molecular mechanism of lithospermoside, in order to provide a theoretical basis for clinical application of lithospermoside.

Key words Lithospermoside; Anticancer; Anti-inflammatory; Antibacterial; Hypoglycemic; Antioxidant

1 Introduction

Semiaquilegia adoxoides has been used medically in China for a long time, and it is mainly produced in Sichuan, Guizhou, Hubei and Hunan. *S. adoxoides* is a perennial herbaceous plant, with strong effects of clearing heat and detoxifying, reducing swelling and dissolving knot, and relieving stranguria by diuresis. Lithospermoside ($C_{14}H_{19}NO_8$) is a kind of white flocculent crystal compound existing in the Chinese herbal medicine *S. adoxoides*, soluble in water, methanol and other organic solvents. Lithospermoside has a variety of pharmacological activities, among which anticancer, anti-inflammatory, antibacterial and other pharmacological activities have been applied in clinical treatment, and have a wide range of medical prospects[1]. This paper reviews the pharmacological action and molecular mechanism of lithospermoside, in order to provide a theoretical basis for the research and development of antitumor drugs.

2 Pharmacological action and molecular mechanism of lithospermoside

2.1 Anticancer effect Cancer is a malignant cycle of cloning transformation of normal cells in the human body that causes a large number of gene mutations. When these mutated genes constantly multiply and replicate, abnormal proliferation of local normal cells in the body is transformed into cancer cells, thus forming tumors. At present, the conventional chemotherapy drugs used to treat cancer have many disadvantages, such as unobvious efficacy, strong adverse reactions and high price, so it has become an urgent problem to develop anticancer drugs with obvious efficacy, low side effects and low price. Studies have found that compared with conventional chemotherapy drugs, lithospermoside has the advantages of high efficiency, safety and low cost, and can effectively inhibit the proliferation of various cancer cells and the migration and invasion of cancer cells[2].

2.1.1 Inhibiting the proliferation of cancer cells. Cancer cells grow rapidly, and their proliferation and spread greatly increase the mortality rate of patients. It has become the primary task of anticancer therapy by effectively inhibiting the proliferation and spread of cancer cells. Studies have proved that lithospermoside can inhibit the proliferation of a variety of cancer cells, including esophageal cancer, liver cancer and breast cancer.

Niu Feng[3] tested the anti-proliferation effect of lithospermoside on human hepatocellular carcinoma cell line Bel7402, human breast cancer cell lines Hela and MCF7 by MTT assay, and the results suggested that lithospermoside could inhibit the growth of Bel7402, Hela and MCF7 tumor cell lines in a concentration-dependent way. With the extension of treatment time and the increase of drug concentration, its ability to inhibit the proliferation of tumor cell lines was enhanced until the clone cell line disappeared completely. Niu Feng[3] also tested the anti-proliferation effect of lithospermoside on mouse hepatocellular carcinoma H22 ascites type tumor strain by intradermal injection, and concluded that with the increasing injection dosage of lithospermoside (0, 5 and 10 mg/kg) and the prolongation of administration time, the inhibitory ability of lithospermoside on the proliferation of mouse hepatocellular carcinoma H22 ascites type tumor strain was continuously enhanced. The above results manifest that lithospermoside has a good inhibitory effect on the growth of H22 ascites type tumor strains in mice.

Meng Zhaolian *et al.*[4] detected the effect of lithospermoside on the cell survival rate and clonal formation number of human glioma U251 by CCK-8 assay. With the increase of treatment concentration of lithospermoside (0, 2 and 4 μmol/L) and the prolongation of treatment time, the number of human glioma U251 cells constantly decreased, and the cell survival rate gradually decreased. In addition, colony formation assay demonstrated that lithospermoside inhibited the growth of human glioma U251 cells in a concentration-dependent manner. The above results suggest that lithospermoside can effectively inhibit the proliferation of cancer cells.

2.1.2 Inhibiting the migration and invasion of cancer cells. The invasion and migration of tumor cells is the fundamental reason why it is so difficult to cure cancer. Once formed, tumor cells will

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* Corresponding author. E-mail: jinchenghao3727@qq.com

continuously invade and migrate from their primary site to other tissues and organs, and form tumors of the same type as the primary tumor, which greatly increases the difficulty of clinical treatment for cancer patients.

Epithelial-mesenchymal transformation (EMT) is associated with the migration and invasion of tumor cells. By real-time fluorescent quantitative PCR and western Blot assay, Meng Zhaolin *et al.* [4] detected the regulatory effect of lithospermoside on EMT. The results showed that the expression level of N-cadherin decreased after treatment of human glioma U251 cells with 0, 20, 40, 80 and 160 μmol/L lithospermoside for 24 h, while the expression level of E-cadherin was increased. In order to further detect the effects of lithospermoside on the migration and invasion of U251 cells, transwell migration/invasion test was conducted to detect the invasion and migration ability of lithospermoside on human glioma U251 cells. It was found that the number of human glioma U251 cells migration decreased significantly with the increasing concentration of lithospermoside (0, 1 and 2 μmol/L). These results indicate that lithospermoside can effectively inhibit the migration of human glioma U251 cells. Sun Jian *et al.* [5] tested the migration and invasion ability of lithospermoside on human breast cancer MDA-MB-231 cells by Western Blot assay and found that lithospermoside had a good inhibitory effect on the migration of human breast cancer MDA-MB-231 cells, eventually achieving the purpose of cancer treatment.

2.2 Antibacterial effect Bacteria are single-celled organisms. When bacteria overmultiply, get out of control, or harmful bacteria grow, most bacteria invade normal cells of the human body and cause great damage. The abuse of antibiotics has caused many microorganisms to develop resistance, so it has become a hot spot at home and abroad by looking for effective, safe and inexpensive drugs to fight fungal infections.

Liu Xueyong *et al.* [6] tested the antibacterial activity of lithospermoside against *Ralstonia solanacearum*, *Pseudomonas syringae*, *Erwinia carotorora* and *Pectobacterium aroidearum* by filter paper agar diffusion method, and figured out that lithospermoside had certain antibacterial effect when the drug loading capacity was 50 μg/tablet. Luo Kaimei *et al.* [7] tested the inhibitory activity of lithospermoside against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* by plate paper method, and found that lithospermoside had a good inhibitory effect on bacteria under specific pH and certain temperature conditions, especially on *S. aureus*. Yang Xiaoxia *et al.* [8] tested the inhibitory effect of lithospermoside on *E. coli*, *S. aureus*, *B. subtilis* and *Aspergillus niger* by double dilution method, and put foward that the activity of *S. aureus* was significantly decreased after treatment of lithospermoside at the lowest inhibitory concentration (0.25%). In order to further study the inhibitory effect of lithospermoside on *S. aureus*, the inhibitory effect on the activity of *S. aureus* was studied by filter paper diffusion method. It was found that under sterile conditions, the activity of *S. aureus* was significantly reduced after treated by 2 mL of lithospermoside extract solution. The above experimental results suggest that lithospermoside has a good inhibitory effect on some bacteria, providing a theoretical basis for the development of antibacterial drugs.

2.3 Anti-inflammatory effect Inflammation is the defensive response of the system to damaged tissue, consisting of infectious and non-infectious inflammation. Any living tissue injury can cause inflammation, which is a very complex and systematic pathological process. Inflammatory lesions mainly occur in local tissues, and the deterioration, exudation and hyperplasia of local tissues seriously threaten human health. However, local lesions and the whole affect each other, and there are often obvious systemic reactions when relatively severe inflammatory diseases occur in the human body.

Niu Feng *et al.* [3] tested the effect of lithospermoside on ear swelling in normal rats by intradermal injection. With the increasing injection dosage of lithospermoside (0, 10 and 50 mg/kg) and the prolongation of administration time, the inhibition rate of ear swelling in normal rats gradually increased, and the inhibition rate of lithospermoside on ear swelling of normal rats was finally calculated to be 20%. The experimental results demonstrated that lithospermoside had a good inhibitory effect on ear swelling caused by intradermal injection. Wang Chuanlin *et al.* [9] tested the effect of lithospermoside on breast infection in patients with early non-suppurative mastitis through clinical oral single-taste therapy. When the patient took 6 g of lithospermoside orally, the pain was relieved and the redness and swelling were significantly relieved the next day. With the increasing dosage of lithospermoside, the anti-inflammatory effect became more obvious, and there was no case of toxic side effects, with a total cure rate of 100%. The experimental results indicate that lithospermoside has good anti-inflammatory effect.

Sun Jian[5] studied the effects of lithospermoside on inflammatory microenvironment where total alkaloids interfere with tumor *in vitro* by Western Blot assay, and suggested that the expression of pro-inflammatory factor TNF-α could be significantly reduced when the concentration of total alkaloids reached 60 μg/mL. The experimental results show that lithospermoside can interfere with the inflammatory microenvironment of tumor by regulating the expression level of inflammatory factors, and reduce the secretion of pro-inflammatory factors. Cheng Yaqun[10] studied breast infection in patients with surgical acute suppurative infection such as external blowing mammary abscess through nasal plugging therapy, and suggested that lithospermoside had a good therapeutic effect. Xu Yingna *et al.* [11] studied the cure of patients with mammary hyperplasia through external treatment, and found that the effective rate of applying ointment containing lithospermoside to the affected area was as high as 99.62%. The above results indicate that lithospermoside has a good inhibitory effect on inflammatory factors.

2.4 Hypoglycemic effect Diabetes mellitus is an endocrine disease caused by the lack of insulin secretion in the body, resulting in a series of metabolic disorders such as proteins, fats and electrolytes. The clinical treatment of diabetes mainly relies on in-

sulin or oral hypoglycemic drugs, and most patients need long-term or lifelong medication. Long-term medication will not only bring a heavy economic burden to patients, but also have large toxic side effects, which has a great impact on the body function of patients and brings great pain to patients with diabetes. The clinical manifestation of diabetes is "three more and one less" symptom, that is, polydipsia, polyuria, polyphagia and weight loss.

Wang Hongshan *et al.* [12] tested the hypoglycemic effect of lithospermoside on diabetic model rats by glucose oxidase method. The blood glucose level of rats in the model group increased significantly ($P<0.01$), and that in high dose group of lithospermoside decreased remarkably ($P<0.05$), indicating that high dose group of lithospermoside had certain hypoglycemic effect on diabetic model rats. With the increasing dosage of lithospermoside, the hypoglycemic effect became increasingly obvious. Matsui *et al.* [13] tested the hypoglycemic effect of lithospermoside on diabetic model rats by single oral administration method, and the results showed that after feeding lithospermoside to 8-week-old male rats for 30 min, the blood glucose concentration of rats decreased by 16.5% compared with that of the rats without lithospermoside. Hence, lithospermoside has a good hypoglycemic effect. Xie Hongdi *et al.* [14] tested the hypoglycemic effect of lithospermoside on human body by taking traditional Chinese medicine internally, and put forward that the total effective rate of reducing blood glucose in the body was as high as 88.89%. The result suggests that lithospermoside can effectively reduce blood glucose in the body. Zheng ZX *et al.* [15] tested the regulation effect of lithospermoside on blood glucose in rats by intravenous injection. Compared with the control group, lithospermoside had a short-term regulation effect on blood glucose no matter it was fasting blood glucose, random glucose, or glucose tolerance test.

2.5 Antioxidant effect As human body is constantly in contact with the outside world, free radicals are produced in the body. When the cells are exposed to external adverse environment, such as radiation, chemical stimulation, and external pollution, the content of reactive oxygen species will increase. The increase of these uncontrolled factors can lead to many chronic diseases in humans such as inflammation, viral infections, cancer, autoimmune, cardiovascular and digestive diseases. The antioxidant mechanism is to directly act on free radicals or indirectly consume substances that are prone to producing free radicals. The antioxidant system of the human body is a well-functioning and complex system, the stronger the antioxidant capacity of the human body, the healthier the human body, the longer the life span.

Zhang Wenzhan *et al.* [16] studied the scavenging ability of lithospermoside on DPPH free radicals by HPLC-Q-TOF-MS, DPPH and ABTS free radical scavenging methods. When vitamin C was used as a positive control, the DPPH scavenging ability of lithospermoside extract increased steadily. As the concentration of lithospermoside extract was 140 μg/mL, the clearance rate reached 90%. Thus, lithospermoside has a good antioxidant effect. Xu Shuli *et al.* [17] tested the inhibitory effect of lithospermoside on ABTS and DPPH free radicals by spectrophotometry, and found that with the increasing concentration of lithospermoside, the inhibitory effect on ABTS and DPPH free radicals became more obvious. Polyphenol compounds have good antioxidant effect and have certain material basis for the health promotion function of lithospermoside. Consuming large amounts of polyphenols can remove excess free radicals from the body, thereby preventing cell death and tissue damage. Chen Bo *et al.* [18] tested the scavenging ability of lithospermoside on DPPH free radicals through *in vitro* antioxidant test, and found that with the continuous increase of total phenol concentration, the absorbance of the reaction system at 700 nm gradually increased. With the increase of total phenol content, the scavenging ability of lithospermoside on DPPH free radical also increased. Ma Jingfan *et al.* [19] tested the scavenging ability of lithospermoside on DPPH free radicals through *in vitro* antioxidant test and found that the content of lithospermoside polyphenol increased from 14.73% to 45.21%. The scavenging ability against DPPH and ABTS free radicals increased with the increase of total phenol content. The above results provide data support for further development and utilization of antioxidant activity of lithospermoside.

3 Prospects

Lithospermoside is an alkaloid compound extracted from plant roots, widely used in basic research and clinical research in the fields of anticancer, anti-inflammatory, antibacterial, hypoglycemic and antioxidant, especially in anticancer. Compared with conventional chemotherapy drugs, it has the advantages of high efficiency, safety and low price, and has important application value. However, as a natural medicine, the specific molecular targets and signal transduction pathways of lithospermoside are still unclear, and there is a lack of detailed and accurate experimental data support. Therefore, more comprehensive and in-depth studies should be carried out at the molecular, cellular and animal levels in combination with modern biotechnology such as molecular biology, cell biology, immunology and experimental zoology, and its potential practical application value should be explored, in order to provide a solid theoretical basis for the development and application of lithospermoside.

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4.3.5 Applying ozonizer. The produced ozone purified water is sprayed on the surface of the tree, and the strong oxidation of ozone is used to kill pathogens, eggs and young nymphs, which is broad-spectrum and non-toxic. The concentration range of ozone is set by the R&D manufacturer. It will receive better application effect in closed facility fruit trees. However, it must be sprayed for many times, with an interval not more than 5 d, leading to high labor cost. Thus, it should be combined with pesticide application. It will receive little effect on open field fruit trees in an unenclosed environment. At present, ozonator is less applied in agriculture due to its high price.

4.3.6 Chemical control. Mineral-based, plant-derived and biological agents are preferred choice, while restrictive pesticides should be used as little as possible, and prohibited drugs are not allowed to be used. Before germination and after harvesting, 3 - 5° Br lime sulphur 200 times dilution is applied twice to clear the garden, kill bacteria and mites, and protect the branches. During the rainy season after bagging, Bordeaux mixture 200 times is applied once or twice to control fruit and leaf diseases. Before bagging, polyoxins combined with mancozeb (limited to 3 times) and thiophanate methyl (limited to 2 times) are administrated to control leaf and fruit diseases. Imidacloprid and acetamiprid are sprayed to control aphids, and avermectin and diflubenzuron are applied to control fruit moths and leaf folders. On the basis of applying lime sulphur twice before germination and after harvesting, spirodiclofen, azacyclotin or avermectin are used to control red spider before and after bagging. Matrine and petroleum emulsion are used to control woolly aphids and scale insects. Quicklime is broadcasted around root collar to prevent snails and slugs.

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