Pharmacological Effects and Mechanism of Esculentoside A

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Abstract Extensive studies have found that Esculentoside a (EsA) has a variety of pharmacological effects, such as anti-inflammatory, antibacterial, anti-tumor and the treatment of arthritis. In the present paper, the pharmacological effects and related mechanisms of EsA in recent years were reviewed, in order to provide a theoretical reference for further research and development of EsA.

Key words Esculentoside A (EsA), Anti-inflammation, Anti-bacterium, Anti-tumor, Arthritis

1 Introduction

Phytolaccae Radix is the dry root of *Phytolacca acinosa* Roxb. or *Phytolacca americana* L. It is a perennial herb native to North America, mainly distributed in Jiangxi, Hunan and Hubei provinces of China[1]. Phytolaccae Radix has anti-inflammatory, antibacterial, anti-viral, anti-tumor and immunomodulatory effects[2]. Esculentoside A (EsA) is a triterpenoid saponin extracted from Phytolaccae Radix, with a molecular formula of $C_{42}H_{66}O_{16}$ and it is a solid powder at room temperature, soluble in water, ethanol and n-butanol, and insoluble in organic solvents such as acetone and ether. Studies have found that EsA has many effects such as anti-inflammatory, anti-cancer and arthritis treatment[3]. In this paper, we reviewed the research progress of pharmacological effects and mechanisms of EsA in recent years, in order to provide a theoretical reference for further research and development of EsA.

2 Pharmacological effects and mechanism of EsA

2.1 Anti-inflammatory effects Inflammation is a non-specific and complex physiological reaction of organisms caused by external injury, infection or stimulation. Inflammation usually involves vasodilation, increased vascular permeability, migration and activation of leukocytes, and edema of local tissues. The basic pathological changes in inflammation include degeneration, exudation and hyperplasia of local tissues. Its clinical manifestations are fever, tissue deterioration, exudation and tissue cell proliferation. Studies have shown that EsA has a good anti-inflammatory effect.

2.1.1 Anti-nephritis effect and its molecular mechanism. Nephritis is a disease caused by a variety of factors, including immune-mediated and inflammatory mediators, and its clinical manifestations are edema, hematuria and proteinuria. Glomerular mesangial cell (GMC) is an intrinsic cell in the glomerulus. Excessive proliferation of GMCs and the increased secretion of extracellular matrix (ECM) are the main causes of various proliferative glomerulonephritis. Zhang Xianggui *et al.*[3-4] found that EsA (2.5 - 5.0 mg/L) could significantly inhibit the proliferation of rGMC cells without obvious toxic and side effects through MTT assay, and they found that EsA could down-regulate the expression level of CDK2 protein and up-regulate the expression level of cell cycle protein p27, thus inhibiting the proliferation of rGMC cells. Through MTT test, Tang Yinjie *et al.*[5-6] found that the drug-containing serum of mice obtained after EsA treatment could significantly inhibit the proliferation of rGMC cells, and the inhibitory effect was the most significant when the concentration was 5.0 - 10 mg/kg, and they further found that EsA could down-regulate the expression of phosphorylated mitogen-activated protein kinase (p-ERK1/2) and transcription factor activated protein-1 (AP-1) by Western blot. These results indicate that EsA can inhibit the proliferation of rGMC through the ERK1/2-AP-1 signaling pathway. Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysfunction of the immune system. *Lupus nephritis* (LN) is an important clinical component of SLE, which is a common but difficult to treat nephritis. Through mouse experiments, Weng Xiaoxue[7] found that the urine protein/creatinine and serum creatinine values of LN mice treated with EsA were significantly reduced. The pathological changes of the kidney were observed under the microscope by HE and Masson staining. It was found that there were different degrees of glomerular mesangial cell proliferation, mesangial matrix proliferation and capillary endothelial cell proliferation in the kidney of MRL/Lpr lupus model mice. The renal pathological changes in the EsA-treated group were significantly improved compared with the model group. Further western blotting showed that EsA down-regulated the expression levels of pro-inflammatory factors such as TNF-α, IFN-γ and IL-17, and up-regulated the expression level of anti-inflammatory factor IL-2. Flow cytometry was used to detect the proportions of MAIT cells (mucosa-associated antibody T cells) in kidney tissues, and the proportions of MAIT cells in the normal group, model group, and drug group were 0.25% ±0.05%, 0.67% ±0.05%, 0.40% ±0.02%, respectively, indicating that EsA could significantly reduce the proportion of MAIT cells in LN mice. These results suggest that EsA can alleviate LN nephritis by down-regulating the expression levels of TNF-α, IL-6, Bcl-2, TNF-α, IFN-γ

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and IL-17, and up-regulating the expression levels of IL-2, Fas and FasL in lupus model mice.

2.1.2 Anti-hepatitis effect and its molecular mechanism. Hepatitis is a general term for liver inflammation. It is mainly caused by viruses, bacteria, alcohol and parasites. Its clinical manifestations are fatigue, vomiting, abdominal distension and loss of appetite. Through CCK-8 experiment, Zhang *et al*.[8] found that the content of tumor necrosis factor (TNF-α) in normal liver cells (L-02) increased sharply after treatment with carbon tetrachloride (CCl_4), while EsA could significantly reduce the expression level of TNF-α in L-02 cells. Further, Western blotting and real-time fluorescence quantitative PCR experiments indicated that EsA up-regulated the expression of peroxisome proliferator-activated receptor γ (PPAR-γ) in L-02 cells after CCl_4 treatment. The liver injury model and histopathological experiments in mice showed that the liver cells in the CCl_4 model group (mice intravenously injected with CCl_4 and olive oil) were severely damaged, the cells were balloon-like, and the liver became white, while the liver cells in the Es-A-treated group were normal, the cytoplasm was well preserved and the nucleus was clear and plump. The results showed that the pathological injury symptoms of CCl_4 model group were significantly alleviated after EsA treatment. By measuring liver function enzymes in serum, it was found that serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly down-regulated by EsA. These results indicate that EsA can attenuate CCl_4-induced acute liver injury in mice by inhibiting inflammatory response and oxidative stress. Through MTT experiments, Wang *et al*.[9] found that EsA significantly inhibited hepatotoxicity induced by acetaminophen (APAP) or hydrogen peroxide (H_2O_2). Through the GSH peptide detection experiment, it was found that EsA significantly inhibited the GSH consumption caused by APAP. In addition, by measuring the contents of H_2O_2 and O_2 in the cells, it was found that EsA significantly reduced the production of H_2O_2 and O_2 in the cells induced by APAP, indicating that EsA had a good inhibitory effect on oxidative stress. It was found by flow cytometry that EsA significantly reduced H_2O_2-induced apoptosis in a dose-dependent manner. Western blot analysis showed that EsA up-regulated the expression of phosphorylated Akt (p-Akt), phosphorylated glycogen synthase kinase 3β (p-GSK3β) and phosphorylated AMP-activated protein kinase (p-AMPK). These results indicate that EsA can protect the liver by inhibiting oxidative stress.

2.1.3 Anti-arthritic effect and its molecular mechanism. Osteoarthritis (OA) is a common acute or chronic inflammation of connective tissue with migratory pain in joints and muscles. Rheumatoid arthritis (RA) is a chronic, systemic disease characterized by inflammatory synovitis, with clinical manifestations of joint pain, fever, and swelling. Through CCK-8 experiment, Shao *et al*.[10] found that EsA had no obvious toxic side effects on chondrosarcoma cells (SW1353). Through fluorescence real-time quantitative PCR and ELISA kit detection, it was found that EsA can significantly inhibit the protein expression of MMPs (MMP2, MMP3 and MMP13) and pro-inflammatory cytokines (IL-6, IL-8 and TNF-α) of SW1353 cells, suggesting that EsA can prevent OA by inhibiting inflammatory response and catabolism. Western blot analysis showed that EsA could down-regulate the expression of p-P65, p-ERK, p-JNK and p-P38 proteins. EsA can alleviate the cartilage degeneration and inhibit the activity of osteoclasts in OA model mice. The above results indicate that EsA inhibits cartilage inflammation, matrix catabolism and osteoclast degeneration by regulating NF-kB and MAPK signaling pathways. Through thymocyte proliferation method and bioassay, Zheng Qinyue *et al*.[11] found that EsA could significantly inhibit interleukin-1 (IL-1) and tumor necrosis factor (TNF) produced by rabbit synoviocytes induced by lipopolysaccharide (LPS), indicating that EsA may help alleviate the symptoms of rheumatoid arthritis.

2.1.4 Anti-neuroinflammatory effect and its molecular mechanism. Alzheimer's disease (AD) is one of the most common diseases of senile dementia. It can affect the thinking, memory and independence of patients, and bring serious damage to the quality of life of patients. Through Morris water maze test and field test, He *et al*.[12] found that EsA could improve the cognitive deficit and anxiety of mice. By analyzing the changes in EsA levels in blood and brain of mice by liquid chromatography and tandem mass spectrometry, it was found that EsA could penetrate the brain-blood barrier, thus playing a therapeutic role in AD mice. Furthermore, immunofluorescence assay and Western blotting showed that EsA could up-regulate the expression of PPARγ, thereby reducing neuronal apoptosis. In cultures of primary neurons, the addition of the PPARγ inhibitor GW9662 reversed the therapeutic effect of EsA on AD pathology. These results show that EsA can penetrate the brain-blood barrier and play a neuroprotective role by regulating the expression level of PPARγ, thereby alleviating the cognitive deficit of AD mice. He *et al*.[13] found that EsA could alleviate the symptoms of memory deficit, recognition deficit and synaptic damage in AD mice through Y-maze test, novel object recognition test, Gallyas-Braak silver staining and transmission electron microscopy. Through quantitative proteomic analysis, it was found that EsA regulates the expression levels of brain-specific angiogenesis inhibitor 3, galectin-1, and Ras-related protein 24. It was further found by Western blotting that EsA could upregulate AKT/GSK3β expression levels, inhibit microtubule-associated protein (Tau) hyperphosphorylation, and promote autophagy to clear abnormally phosphorylated Tau. Inhibition of AMP-activated protein kinase (AMPK) activity by morphine abrogated the effects of EsA in hippocampus-derived primary neurons. These results indicate that EsA inhibits the hyperphosphorylation and autophagy clearance of targeted Tau through AMPK signaling pathway, attenuates the cognitive decline of mice, and then alleviates the cognitive deficits of AD mice.

2.2 Anticancer effects Cancer is a disease caused by uncontrolled growth, reproduction and division of cells under the influ-

ence of many factors. At present, chemical drugs commonly used in the treatment of cancer have many shortcomings, such as toxic side effects, strong drug resistance and high price. Therefore, looking for a natural anticancer drug with high efficacy, less side effects and low price is the focus of medical research. Studies have reported that EsA has a good anti-cancer effect.

2.2.1 Anti-colon cancer effect and its molecular mechanism. Colon cancer is a common malignant tumor and it mainly occurs in the mucosa and submucosa of the colon. Its clinical manifestations are dyspepsia, abdominal distension and mucopurulent bloody stool. Momenah *et al.* [14] used CCK-8 method to study and found that EsA had a good inhibitory effect on the proliferation of colon cancer cells HT-29, with an IC_{50} value of 16 μM. In addition, through cell colony formation assay experiments, it was found that EsA could significantly reduce the colony formation ability of HT-29 cells. Transwell assay showed that the migration rate and invasion rate of HT-29 cells treated with EsA decreased by 45% and 51%, respectively, when compared with the untreated group. The above results indicate that EsA has a good killing effect on colon cancer cells and effectively inhibits the migration and invasion of HT-29 cells.

2.2.2 Anti-breast cancer effect and its molecular mechanism. Breast cancer is a common female malignant tumor, which usually occurs in women's breast tissue, and is clinically manifested as a breast lump, and a few are accompanied by symptoms such as vague or stabbing pain in the breast. AO/EB staining experiments showed that the number of apoptosis of CSCs cells increased after EsA treatment. Furthermore, Annexin V-FITC/PI double staining method indicated that EsA induced apoptosis in mouse breast cancer cells, mouse breast CSC cells, human breast cancer cells and human breast CSC cells. Besides, Western blotting assays showed that EsA down-regulated the expression level of the anti-apoptotic protein B-cell lymphoma/leukemia-2 (Bcl-2), and up-regulated the expression levels of the pro-apoptotic protein B-cell lymphoma/leukemia-2-related protein (Bax) and cleaved-caspase-3. These results suggest that EsA can induce apoptosis in breast cancer cells through a mitochondria-dependent pathway.

3 Prospects

EsA is a compound extracted from Phytolaccae Radix, a traditional Chinese medicine. It has many pharmacological effects, such as anti-tumor, anti-inflammatory and so on, and has broad development and application prospects. However, most of the studies on EsA remain in its efficacy, and the specific pharmacological mechanism of EsA is still in its infancy. Therefore, it is necessary to comprehensively explore the mechanism of action of EsA at the molecular, cellular and animal levels by combining the theoretical knowledge and experimental techniques of molecular biology, cell biology, toxicology, pharmacology, clinical medicine and other disciplines, so as to obtain more comprehensive and in-depth research results and provide a scientific basis for the development and application of EsA.

References

[1] ZHOU KL, LU WQ, TANG SL. Variation patterns of contents of water-soluble extractives and esculentoside A and selection of superior germplasms in *Phytolacca acinosa*[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2018, 24(4): 44-50. (in Chinese).

[2] LI B, LIN H, TANG ZQ, *et al.* Study on mechanism of Phytolaccae Radix and its split components based on network pharmacology[J]. China Journal of Chinese Materia Medica, 2021, 46(10): 2434-2442. (in Chinese).

[3] ZHANG XG, TANG JY. Effects of esculentoside A on the proliferation of glomerular mesangial cells[J]. Shaanxi Journal of Traditional Chinese Medicine, 2013, 34(8): 1075-1077. (in Chinese).

[4] ZHANG XG, TANG JY. Influence of esculentoside A on proliferation and CDK2 of IL-1β induced glomerular mesangial cell[J]. Chongqing Medicine, 2013, 42(21): 2496-2499. (in Chinese).

[5] TANG JY, DONG Y, ZHANG XG, *et al.* Effect of esculentoside A on IL-1β-induced activation of ERK pathway in glomerular mesangial cells [J]. Guangdong Medical Journal, 2016, 37(11): 1613-1617. (in Chinese).

[6] TANG JY, DONG Y, ZHANG XG, *et al.* Influence of esculentoside A on activation of ERK1/2-AP-1 pathway of glomerular mesangial cell induced by IL-1β[J]. Chongqing Medicine, 2017, 46(16): 2183-2186. (in Chinese).

[7] WENG XX. Intervention effect of Esculentoside A on MAIT cells and their inflammatory factors in renal tissue of MRL/lpr mice[D]. Zunyi: Zunyi Medical University, 2022. (in Chinese).

[8] ZHANG F, WANG X, QIU X, *et al.* The protective effect of Esculentoside A on experimental acute liver injury in mice[J]. PLoS One, 2014, 9(11): e113107.

[9] WANG LD, ZHANG SL, CHENG H, *et al.* Nrf2-mediated liver protection by esculentoside A against acetaminophen toxicity through the AMPK/Akt/GSK3β pathway[J]. Free Radical Biology and Medicine, 2016, Dec(101): 401-412.

[10] SHAO Q, XUE S, JIANG Y, *et al.* Esculentoside A protects against osteoarthritis by ameliorating inflammation and repressing osteoclastogenesis [J]. International Immunopharmacology, 2020, Mar 9 (82): 106376.

[11] ZHENG QY, WANG HF, ZHENG XM, *et al.* Effects of esculentoside A on production of IL-1 and TNF by rabbit synovial cells[J]. Academic Journal of Second Military Medical University, 2001(5): 425-426. (in Chinese).

[12] HE Z, LI X, WANG Z, *et al.* Esculentoside A alleviates cognitive deficits and amyloid pathology through peroxisome proliferator-activated receptor γ-dependent mechanism in an Alzheimer's disease model[J]. Phytomedicine, 2022, Jan 29(98): 153956.

[13] HE Z, ZHANG H, LI X, *et al.* The protective effects of Esculentoside A through AMPK in the triple transgenic mouse model of Alzheimer's disease[J]. Phytomedicine, 2023, Jan(109): 154555.

[14] MOMENAH MA, ALMUTAIRI LA, ALQHTANI HA, *et al.* Esculentoside A Inhibits Proliferation, Colony Formation, Migration, and Invasion of Human Colorectal Cancer Cells[J]. Evidence-Based Complementary and Alternative Medicine, 2023, Feb 10(2023): 7530725.