Pharmacological Action and Molecular Mechanism of Formononetin

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Abstract Formononetin is an isoflavone phytoestrogen mainly existing in leguminous plants such as *Trifolium pretense*, *Spatholobus suberectus*, *etc.* Studies have proved that formononetin has good anti-inflammatory, antitumor, antioxidant, antidepressant, anxiolytic and other pharmacological effects. This paper summarizes the pharmacological action and molecular mechanism of formononetin, in order to provide a theoretical basis for the development and clinical application of formononetin.

Key words Formononetin, Anti-inflammatory, Antitumor, Antioxidant, Antidepressant, Anxiolytic

1 Introduction

Formononetin, also known as neochanin, 7-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one, is a natural plant extract. Formononetin is a natural active ingredient extracted from leguminous plants such as the whole grass of *Ononis spinosa*, *Spatholobus suberectus*, *Mucuna sempervirens*, *etc.* [1]. Formononetin, with molecular formula $C_{16}H_{22}O_4$ and molecular weight 268.26, is easily soluble in organic solvents such as methanol, ethyl acetate and ether, but insoluble in water[2]. A large number of studies have shown that formononetin has good anti-inflammatory, antitumor, antioxidant, antidepressant, anxiolytic and other pharmacological effects. This paper summarizes the pharmacological action and molecular mechanism of formononetin, in order to provide a theoretical basis for the further development and clinical application of formononetin.

2 Anti-inflammatory effect of formononetin

Inflammation is a basic pathological process that occurs when biological tissues are stimulated by damage factors, mainly a defensive response. However, excessive inflammation may also lead to the occurrence and deterioration of diseases. Patients with inflammation are often accompanied by symptoms like redness, swelling, heat, pain and dysfunction, and drug therapy is the common treatment of inflammation. It has been reported that formononetin has a good anti-inflammatory effect.

Wei Dongmei *et al.* [3] tested the effect of formononetin on the inflammatory response of septic model mice by Wright staining; the results showed that after treatment with formononetin, the survival rate of septic mice was significantly increased, and the number of leukocytes (total leukocytes, macrophages, neutrophils and lymphocytes) in peritoneal lavage fluid was significantly decreased. The effect of formononetin on the expression level of inflammatory factors in the spleen of mice was detected by enzyme-linked immunosorbent assay (ELISA), and the results revealed that formononetin could significantly reduce the expression levels of myeloperoxidase (MPO), C-reactive protein (CRP), monocyte chemotactic protein-1 (MCP-1), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the spleen of septic mice. Furthermore, the effects of formononetin on the expression levels of related pathway proteins and inflammatory pathways in spleen tissues of septic mice were detected by Western Blot, and the results demonstrated that formononetin significantly decreased the expression levels of phosphorylated mitogen-activated protein kinase (p-p38), phosphorylated extracellular signal-regulated kinase (p-ERK) and p65. These results suggest that formononetin alleviates the inflammatory response of sepsis in mice by inhibiting the activation of MAPK/NF-κB pathway.

Hui Zhang *et al.* [4] detected the effects of formononetin on the viability of rat cardiomyocytes induced by high glucose through Cell Counting Kit-8 (CCK-8) colorimetry, and found that the viability of H9C2 cells induced by high glucose was gradually restored after treated by formononetin. The effect of formononetin on pyroptosis of H9C2 cells was detected by real-time quantitative PCR (RT-qPCR) and *in situ* end labeling staining (TUNEL), and the results manifested that formononetin significantly decreased the mRNA expression levels of pyrolytic factors interleukin-18 (IL-18), Caspase-1 and NOD-like receptor thermal protein domain associated protein 3 (NLRP3) in H9C2 cells. The effect of formononetin on the expression level of inflammatory factors in the supernatant of H9C2 cell culture was detected by ELISA, and the results showed that formononetin significantly reduced the expression levels of TNF-α, IL-6 and IL-1β in the supernatant of H9C2 cell culture. The effect of formononetin on the expression level of related signaling pathway protein in H9C2 cells was detected by Western Blot, and the results revealed that formononetin significantly reduced the expression levels of P2X7R, IL-18,

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Caspase-1 and NLRP3 proteins in H9C2 cells. These results indicate that formononetin alleviates and inflammation and pyroptosis of H9C2 cells by inhibiting P2X7R/NLRP3 pathway.

3 Antitumor effect of formononetin

Cancer is a disease caused by excessive proliferation of body cells after losing normal regulation. Cancer cells can invade surrounding tissues and metastasize to other parts of the body through circulatory system and lymphatic system. Common cancer treatment methods include surgical resection, radiation therapy, chemotherapy, targeted therapy, immunotherapy, *etc.* [5]. It has been reported that formononetin has a good antitumor effect.

3.1 Anti-hepatoma effect Jiao Wenpeng *et al.* [6] detected the effect of formononetin on the expression level of cyclooxygenase (COX-2) in the tissues of hepatoma model mice through RT-qPCR assay and immunohistochemical staining, and found that formononetin significantly reduced the expression level of COX-2 in mice. The inhibitory effect of formononetin on the proliferation of hepatoma HepG2 and Bel-7402 cells was determined by CCK-8 colorimetry; after treating HepG2 and Bel-7402 cells, formononetin inhibited the proliferation of HepG2 and Bel-7402 cells in a concentration and time dependent manner. The effects of formononetin on cell cycle of hepatoma HepG2 and Bel-7402 were detected by flow cytometry, RT-qPCR and Western Blot, and the results showed that formononetin up-regulated the cell proportion at G_0/G_1 phase, down-regulated the cell proportion at S and G_2 phase, and significantly inhibited the mRNA expression levels of COX-2 and CyclinD1.

3.2 Anti-cervical cancer effect Wang JY *et al.* [7] detected the effects of formononetin on the expression levels of programmed death receptor-ligand 1 (PD-L1) and related proteins in human cervical cancer HeLa cells through immunofluorescence technology and Western Blot assay; the results proved that formononetin significantly decreased the proportion of PD-L1 positive cells and the phosphorylation of STAT3 in a concentration-dependent manner, and inhibited the expression of MYC, RAF, RAS, p-RAF, p-MEK and p-ERK proteins and the synthesis of PD-L1. Co-immunoprecipitation assay showed that formononetin inhibited the expression of PD-L1 protein by interfering with the interaction between MYC and STAT3. The effect of T cells on the killing activity of Hela cells was investigated by lactate dehydrogenase (LDH) release assay, and the results showed that formononetin significantly enhanced the cytotoxicity of T cells and effectively promoted the specific cleavage of T cells to Hela cells. These results suggest that formononetin can inhibit the expression level of PD-L1 by interfering the synergistic effect of STAT3 and MYC, thus inhibiting the proliferation of cancer cells and angiogenesis, and playing an anti-cervical cancer role.

3.3 Anti-colorectal cancer effect Wang AL *et al.* [8] detected the inhibitory effects of formononetin on the proliferation and invasion of colorectal cancer SW1116 and HCT116 cells through MTT and Transwell assays, and found that formononetin inhibited proliferation and invasion of SW1116 and HCT116 cells in a concentration-dependent manner (0, 20, 50, 100, and 200 μM). The effects of formononetin on the expression level of SW1116 and HCT116 cell cycle related proteins were detected by flow cytometry and Western Blot; the results showed that SW1116 and HCT116 cells treated by formononetin had increased proportion at G_0/G_1 phase, and formononetin inhibited the expression levels of CyclinD1, matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) proteins in a dose-dependent manner (20, 50, and 100 μM). Furthermore, the inhibitory effects of miR-149 and EphB3 on the growth and invasion of SW1116 and HCT116 cells were detected by Western Blot and RT-qPCR assays, and the results demonstrated that formononetin significantly reduced the survival rate of SW1116 and HCT116 cells and the expression level of EphB3 protein. These results indicate that formononetin could block the cell cycle at G_0/G_1 phase by down-regulating the expression level of CyclinD1, and inhibit the proliferation and invasion of colorectal cancer cells by down-regulating the expression level of Mir-149-induced EphB3 protein.

4 Antioxidant effect of formononetin

The human body continuously produces a large number of free radicals in the process of metabolism, but excessive free radicals will lead to the occurrence of diseases. Common antioxidant methods mainly use antioxidant substances to remove free radicals and indirectly remove substances that are prone to produce free radicals. Studies have shown that formononetin has good antioxidant effects.

Xie Na *et al.* [9] detected the inhibitory effect of formononetin on the proliferation of rat hepatic stellate cells HST-T6 by CCK-8 colorimetry, and found that formononetin significantly inhibited the proliferation of HST-T6 cells in a concentration-dependent manner (5, 10, 20, 40, 80, 160 and 320 μmol/L). The effect of formononetin on the expression level of reactive oxygen species (ROS) in HST-T6 cells was detected by DFA-DA fluorescent probe; after treatment with formononetin, the activity of superoxide dismutase (SOD) in HST-T6 cells was significantly increased, while the expression levels of malondialdehyde (MDA) and ROS were significantly decreased. The effect of formononetin on the expression level of inflammatory factors in HST-T6 cells was detected by ELISA, and the results demonstrated that formononetin significantly decreased the expression levels of IL-1β and TNF-α in HST-T6 cells. Furthermore, the effects of formononetin on the expression level of fibrogenic factor type I collagen, NADPH oxidase 4 (NOX4) protein and recombinant and synthetic protein (Nrf2) in HST-T6 cells were detected by immunofluorescence and Western Blot assay; the results displayed that formononetin significantly down-regulated the expression level of type I collagen and

NOX4 protein in HST-T6 cells, and up-regulated the expression level of Nrf2 protein. The above results indicate that formononetin can reduce the expression level of reactive oxygen species and inflammatory factors by inhibiting Nrf2/NOX4 pathway, thus playing an antioxidant role.

Zhang Xinyun *et al.* [10] detected the effects of formononetin on renal function, blood glucose and blood lipid in diabetic nephropathy model rats using glucose meter, creatinine (Scr) kit and urea nitrogen (BUN) kit, and the results showed that the expression levels of urine MAER, serum Scr and BUN, fasting blood glucose and serum TG, TC, LDL and HDL in rats were significantly reduced after treatment with 40 mg/kg formononetin. The effects of formononetin on renal tissue oxidation indexes in diabetic rats were detected by MDA, SOD and glutathione peroxidase (GSH-Px) kits; after treatment with formononetin, the expression levels of MDA and ROS in renal tissue of model rats were significantly decreased, while the activities of GSH-Px and SOD were significantly increased. RT-qPCR and Western Blot were further used to detect the effect of formononetin on the expression level of related proteins in renal tissue of model rats; after treatment with formononetin, Nrf2/ heme oxygenase-1 (HO-1) signaling pathway was activated, and the mRNA and protein expression levels of Nrf2 and HO-1 in renal tissues were increased. These results suggest that formononetin inhibits oxidative stress in renal tissue by activating Nrf2/HO-1 signaling pathway, thereby exerting antioxidant effects and alleviating renal injury in diabetic nephropathy model rats.

5 Antidepressant effect of formononetin

Depression, a form of manic depression, is a common mental illness with symptoms such as slow thinking, cognitive impairment and low mood. Medication is the main treatment for depression. Some studies have showed that formononetin has a good antidepressant effect.

Cheng Yao *et al.* [11] tested the effects of formononetin on the behavior of depression model rats through sugar-water preference test (SPT) and forced swimming test (FST); the results showed that after treatment with formononetin, the sugar water preference coefficient of rats in SPT decreased significantly, and the immobility time of rats in FST increased significantly. ELISA, MDA detection kit, SOD detection kit and CAT detection kit were adopted to detect the effects of formononetin on the expression levels of ACTH, CORT and oxidative stress in the serum of depression model rats; after treatment with formononetin, the expression levels of ACTH and CORT and the content of MDA in the serum of depression model rats were significantly increased, while the activities of SOD and CAT were significantly decreased. The effect of formononetin on the expression level of Nrf2/ARE signaling pathway in hippocampus was detected by Western Blot, and the results showed that formononetin could significantly reduce the expression levels of Nrf2, quinone oxidoreductase 1 (NQO-1) and HO-1 protein in rat hippocampus. These results demonstrate that formononetin can inhibit oxidative stress by activating Nrf2/ARE signaling pathway, thus playing an antidepressant role.

6 Anxiolytic effect of formononetin

Anxiety disorder is a kind of emotional disorder mainly characterized by anxiety. Patients experience physical symptoms such as tinnitus, blurred vision, and overall discomfort. Drug therapy, psychotherapy and cognitive behavioral therapy are major methods for the treatment of anxiety disorder[12]. Studies have shown that formononetin has a good anxiolytic effect.

Wang XS *et al.* [13] tested the effect of formononetin on anxiety-like behavior of chronic inflammatory pain model mice through open field (OF) and elevated plus maze (EPM) tests; continuous administration of 25 mg/kg formononetin for 8 h significantly extended the distance the mice moved, the time they moved in the central area, and the time they extended their arms, and reduced the time they spent closing their arms. The effects of formononetin on the expression levels of N-methyl-D-aspartate receptor (NMDAR), AMPA receptor subunit GluA1 and NF-κB signaling pathway related protein were detected by Western Blot; after treatment with formononetin, CAMP reaction elemen-binding protein (CREB) was activated, and the expression levels of phosphorylated CREB and total CREB protein were significantly increased, while the expression levels of p-GluA1-S831, p-GluA1-S845, GluA1 and Iba-1 protein were significantly decreased. These results make clear that formononetin can inhibit the inflammation and overexcitation of nerve cells by inhibiting CREB signaling pathway, thus playing an anti-anxiety role.

7 Prospects

As a natural active substance extracted from leguminous plants such as *T. pretense* and *S. suberectus*, formononetin has a very high development value and application prospect. A large number of studies have shown that formononetin has good therapeutic effects in anti-inflammatory, antitumor, antioxidant and neuroprotective aspects. Although there are many reports on formononetin, most of them stay in in the apparent study of pharmacological actopm, and there are few studies on the specific pharmacological molecular mechanism, animal and clinical test of formononetin. Therefore, it is necessary to strengthen the research on molecular level, cellular level, animal level and clinical test of formononetin, in order to provide a theoretical basis and data support for further development and clinical application of formononetin.

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terprises in Fushun is limited, and the annual output of organic fertilizer is low. The government should increase investment in organic fertilizer processing, provide correct guidance and support, develop organic fertilizer processing industry, efficiently develop organic fertilizer commercialization, and increase research and development of organic fertilizer processing technology to make it simple and routine.

6.3 Strengthening technical research, promotion, publicity and training on soil, fertilizer and water in organic agriculture It is recommended to strengthen the research and application of organic agriculture theory and technology. The organic production method needs to adopt a large number of new technologies, especially biotechnology and ecological technology. Besides, it is recommended to set up special agencies in agricultural authorities or governments at all levels to be responsible for comprehensive supporting services such as technical promotion, training and guidance in organic agriculture in terms of soil, fertilizer and water. In addition, it is recommended that governments at all levels use various publicity tools and methods to raise the awareness of leaders and business managers at all levels while conducting education and training. It is feasible to use television, newspapers, the Internet and other means to build a national organic agriculture information exchange platform.

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