CHAPTER THREE

Pharmacological action and potential targets of chlorogenic acid

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Contents

Abstract

Chlorogenic acid is a widely distributed natural compound with many important pharmacological effects, which are found in a variety of plants. It is also an important secondary metabolite in plants. As a natural plant extract from a wide range of sources, in vitro and in vivo studies have found that the main pharmacological effects of chlorogenic acid are antioxidant, antiinflammatory, antibacterial, antiviral, hypoglycemic, lipid lowering, anticardiovascular, antimutagenic, anticancer, immunomodulatory, etc. Therefore it may play an important role in promoting human health. For example, it can provide new ideas and new ways for the prevention and treatment of cardiovascular disease, cancer, diabetes, and other chronic diseases, but the specific mechanism of action is unclear. Due to the difficulty of extraction and purification, poor stability, poor solubility, low absolute bioavailability of oral administration, the possibility of

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allergies caused by injection, and so on, there are difficulties in its medicinal research and development. The further study of chlorogenic acid will provide an important theoretical basis for its rational use.

Abbreviation

CGA chlorogenic acid

1. Introduction

Chlorogenic acid has the molecular formula $C_{16}H_{18}O_9$ and molecular weight 354.30. The structure is shown in Fig. 1. When heated and exposed to light, its biological activity can be lost. It is widely found in higher dicotyledonous plants and ferns. Chlorogenic acid (CGA) is a kind of phenylalanine compound produced by the shikimic acid pathway during aerobic respiration. Because CGA has many important biological activities, it has been widely used in many fields, such as medicine, food, health care, and the chemical industry. It is the main active ingredient of many traditional Chinese herbal compound preparations for antibacterial and antiinflammatory purposes, for example, clearing away heat and detoxifying. The content of CGA in honeysuckle and *Eucommia ulmoides* Oliv. is high. It is reported that CGA is absorbed in the stomach of rats by prototype (Lafay et al., 2006). Most CGAs are hydrolyzed to caffeic acid and quinic acid in the intestine through the action of special esterase and then absorbed. After absorption, it is metabolized into glucuronic acid glycosides and sulfate metabolites. The oral absorption rate of CGA is low. It exists mainly in the form of metabolites in plasma and is excreted through the kidneys. In recent years, CGA as has been internationally regarded as plant gold. It has attracted increasing attention, and there are many reports

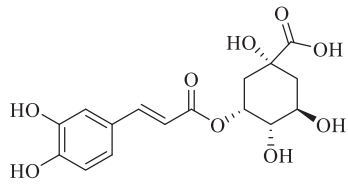

Fig. 1 Structure diagram of chlorogenic acid.

on various aspects of its research. CGA is an important bioactive substance. It has antioxidant, antiinflammatory, antibacterial, antiviral, hypoglycemic, lipid-lowering, anticardiovascular, antimutagenic, anticancer, immune regulation, and other biological functions. However, the specific mechanism of action is unclear.

2. Antioxidant effect of chlorogenic acid

CGA is a new and efficient natural phenolic antioxidant. Its natural antioxidant properties depend on its special molecular structure. It contains five active hydroxyl groups and one carboxyl group. The phenolic hydroxyl group structure reacts easily with free radicals and can form hydrogen radicals with an antioxidant effect to eliminate the activity of hydroxyl radicals and superoxide anions, therefore playing a strong antioxidant role. Antioxidant activity is one of the important activities of CGA, research shows (Hu, Yu, & Zhao, 2006) that CGA has a scavenging effect on three kinds of reactive oxygen species ($^{-}O_2$,·OH and H_2O_2). The scavenging effect and concentration is dose dependent. When the concentration is high, the scavenging effect on these three reactive oxygen species is obvious and stable. When the concentration is low, the scavenging effect on $^{-}O_2$· and ·OH deteriorates, even producing an oxidation-promoting effect.

Under normal conditions of the body, the generation and elimination of free radicals are in equilibrium. When the free radicals are out of balance, various functional obstacles and diseases can occur in the body. For example, when peroxidation is particularly serious, the lipid structure of the cell membrane will be destroyed, and damage to membrane enzymes may lead to cell dysfunction (Bagdas et al., 2015). A recent study has found that CGA has a very strong antioxidant effect and can accelerate wound repair in diabetic rats without affecting the levels of superoxide dismutase and catalase in wounds (Kim, Pan, Kim, Lee, & Park, 2018). A recent research has studied the protective effect of CGA on alcoholic liver injury. It was found that CGA alleviated liver injury by reducing the accumulation of oxidative products such as superoxide, hydrogen peroxide, and hydroxyl radicals, and inhibiting oxidative stress (Zhou et al., 2016). Some researchers studied that CGA ameliorated intestinal mitochondrial injury by increasing antioxidant effects and activity of respiratory complexes. CGA can improve the intestinal mitochondria induced by hydrogen peroxide, increasing the activity of respiratory chain complexes I, IV, and V, and also changing the ultrastructure of mitochondria and mitochondrial damage in rats with colitis induced

by trinitrobenzene sulfonic acid. Therefore it is proved that CGA may be useful for prevention of intestinal diseases, which can lessen and treat mitochondria damage with trinitrobenzene sulfonic acid. It was also found (Luo et al., 2013) that CGA can reduce the apoptotic rate of nucleus pulposus cells induced by hydrogen peroxide, and its mechanism might be related to promoting the expression of Bcl-2 and inhibiting the expression of caspase-3.

CGA can inhibit the activity of xanthine oxidase, reduce the production of oxygen free radicals in the body, and reduce the level of lipid peroxidation in the body by upregulating the activity of antioxidant enzymes (Lou et al., 2016). The possible mechanisms for CGA to exert its antioxidant activity are as follows. It can (1) promote the metabolism of amino acids and glutathione, and improve the level of lipid metabolism; (2) upregulate the signal pathway of nuclear factor E2-related factor 2/antioxidant response element (Nrf2-ARE), inhibiting the degradation of Nrf2 protein mediated by ubiquitin, stabilizing the concentration of Nrf2 protein in cytoplasm, enhancing the transcriptional activity of Nrf2 protein under stress conditions, and protecting and promoting the expression of protective genes such as antioxidant proteins and phase II detoxifying enzymes (Boettler et al., 2011), thereby improving the antioxidant capacity in the body; (3) promote the phosphorylation of protein kinase B and the expression of fork protein transcription factor, antioncogene p53, and antiapoptotic protein B-cell lymphoma-2 by upregulating the signal pathway of phosphatidylinositol-3 kinase/protein kinase B (PI3K-Akt) to inhibit cell apoptosis (Lou et al., 2016); and (4) relate the antioxidant activity of CGA to the mitogen-activated protein kinase (MAPK) signaling pathway. Studies have shown (Ji et al., 2013) that CGA can inhibit phosphorylation of extracellular signal-regulated kinase, c-Jun amino-terminal kinase, and p38 MAPK in the MAPK pathway (Tosovic, Markovic, Dimitric Markovic, Mojovic, & Milenkovic, 2017). A study has found that CGA can produce an antioxidant effect by hydrogen atom transfer or free radical adduct in acidic and neutral media. When in a basic environment (such as physiological pH), sequential proton loss electron transfer has a very high rate, which may be the antioxidant mechanism of CGA.

3. Antiinflammatory effect of chlorogenic acid

Its antiinflammatory effect is another important biological property of CGA. Ohkawara, Takeda, and Nishihira (2017) studied CGA on

inflammatory injury of pancreatic and lung tissues in mice with pancreatitis caused by L-arginine. CGA can reduce pancreatic-related tissues and inhibit the activity of the pancreatic enzyme. At the same time, it can significantly reduce the level of macrophage migration inhibitory factor in the pancreas and serum of mice, indicating that CGA has a strong antiinflammatory effect. Yun, Kang, and Lee (2012) studied the protective effect of CGA on liver ischemia/reperfusion injury. It was found that CGA could significantly improve liver function and pathological injury, and inhibit oxidative stress and tumor necrosis factor-α (TNF-α). The protective effect of CGA protects liver tissue by inhibiting inflammatory reaction and strengthening the antioxidant defense system. When Yu, Zhang, and Wang (2016) studied the effect of CGA on human periodontal ligament cells (hHDLCs), they found that CGA can significantly increase the activity of alkaline phosphatase, promote the proliferation of hHDLCs, promote the formation of mineralized nodules of hHDLCs, inhibit the expression of interleukin (IL)-6, and then inhibit the inflammatory response of human periodontal ligament cells. CGA has a strong antiinflammatory effect on arachidonic acid metabolism by inhibiting the activation of inflammatory factors such as HIF-1a, ICAM-1, VCAM-1, TNF-α, IL-6, and nuclear factor-kappa B (NK-κB) p65, thus protecting cerebral ischemia/reperfusion injury (Miao, Cao, Li, Fang, & Miao, 2017).

Inflammation is the defense response of the body to damaged local tissues. CGA can be involved in the signal pathway of tyrosine kinase/signal transducer and transcriptional activator 3, and inhibit the expression of IL-6 receptor β subunit, tyrosine kinase 1 phosphorylation signal transducer, and transcriptional activator 3 under oxidative stress. It can also reverse regulate the expression and secretion of inflammatory factors (Lou et al., 2016). The inflammatory response is a physiological response to tissue damage caused by exogenous or endogenous factors, and is an important manifestation of autoimmunity. The NK-κB pathway is closely related to the body's autoimmunity, and plays a key regulatory role in the secretion of proinflammatory cytokines, chemokines, and adhesion molecules. The Toll-like receptor (TLR) signaling pathway is an important CGA regulation pathway, and plays the role of an antiinflammatory. CGA inhibits TLR4 signaling pathway activation, downregulates myeloid differentiation factor 88 type, induces nitric oxide synthase (NOS) and an enzyme called cyclooxygenase 2, raises the BMP activin membrane-bound inhibitory factor, thereby inhibiting the TNF-αβ, IL-6 and IL-1 secretion, and relieve inflammation of the liver injury and fibrosis (Ruifeng et al., 2014).

Subsequent studies have found that CGA can also improve the body's inflammatory response by inhibiting TLR2, TLR3, and TLR9 signaling pathways (Lou et al., 2016). A recent research has demonstrated that induced inflammation of fibroblast-like synovial cells (RSC-364) through white cell medium, and found that CGA can inhibit the expression of key molecules in JAK/STAT and the NK-κB signaling pathway and inhibit the activation of these signaling pathways in inflammatory response, thus inhibiting the proliferation of synovial cells. It was found by flow cytometry and Western blot that CGA shows good therapeutic potential in the treatment of rheumatoid arthritis. After pretreatment of diabetic nephropathy model with CGA, the results showed that the expression of nuclear factor red blood cell-derived factor 2, nuclear translocation, and heme oxygenase-1 (HO-1) were significantly increased. The phosphorylation of I and B and NK-κB were significantly decreased. CGA played an antiinflammatory effect by regulating Nrf2/HO-1 and the NK-κB pathway (Bao et al., 2018).

4. Antibacterial and antiviral effects of chlorogenic acid

In terms of antibacterial activity, CGA has broad-spectrum antibacterial activity, and has certain inhibitory on *Escherichia coli*, *Staphylococcus aureus*, yeast, *Aspergillus niger*, and *Bacillus subtilis*, as well as good resistance activity against *Staphylococcus aureus* and *Escherichia coli*. CGA has a stronger effect on fungi than bacteria, accompanied by a certain dose effect (Zhu, Zhang, & Lo, 2004). It is clinically used for treating acute bacterial infection. Studies have found that CGA can destroy the biofilm of *Pseudomonas aeruginosa* and *Aspergillus fumigatus* and affect the normal growth of these strains to achieve a bacteriostatic effect (Yan, Xiao, Zhou, & Yang, 2017). The bacteriostatic effect of CGA is mainly affected by temperature and increases with the increases in temperature, but when the temperature exceeds 60 °C, the bacteriostatic effect obviously decreases (Karunanidhi, Thomas, van Belkum, & Neela, 2013). The early research has found that CGA has a significant inhibitory effect on the envelope synthesis of *Stenotrophomonas maltophilia* organisms. It is believed that CGA can be used as a safety antibacterial drug or a combination of antibacterial drugs to treat *Stenotrophomonas maltophilia* infection.

At present, the antibacterial mechanism of CGA is unclear. According to existing literature, the antibacterial mechanism of CGA may be related to the following aspects: (1) The bacteriostatic mechanism of CGA to *E. coli* may be caused by destroying the structure of the bacterial cell wall

and membrane, which leads to an increase in cell permeability, thus causing cell electrolyte, enzymes, DNA, and RNA to leak out, thus affecting cell structure stability and gradually causing cell death (Zhou, Luo, Xiong, & Tang, 2014). (2) CGA contains a phenolic hydroxyl group, which is the most suitable reaction substrate for phenolic enzyme catalysis. It can affect the activity of related metabolic enzymes, reduce the level of substance and energy metabolism, and cause the metabolic process to be blocked, thus inhibiting the activity of bacteria (Feng et al., 2005). Studies have found that the antibacterial mechanism of CGA may be related to noncompetitive inhibition of arylamine acetyltransferase in bacteria. CGA can change the permeability of cell membranes by inhibiting a change in β-galactosidase to affect a content change in protein. It does so by reducing sugar and acetone in the metabolic process of bacteria and hindering the material and energy metabolism and protein synthesis of the strain. It can also inhibit the metabolism of sugar by bacteria, resulting in insufficient energy of bacteria and further affecting their growth and reproduction (Luo et al., 2016). (3) CGA has strong polarity and high affinity for macromolecular substances such as lipids. It can bind to the surface of bacteria and change their membrane structure, increasing the permeability of the bacterial membrane, causing partial leakage of DNA, RNA, electrolyte, enzymes, and nutrients in bacteria, and affecting protein synthesis (Francisco et al., 2013). (4) CGA may reduce the number of bacterial flagella by inhibiting their synthesis, thus reducing the colony effect of bacteria, which is another possible mechanism for CGA to play an antibacterial role (Ren et al., 2015).

Regarding antivirus, there are few related studies, and its mechanism of action is unclear. Studies have found that CGA has significant blocking and inhibiting effects on influenza A virus FM1 strain, herpes simplex virus, porcine parvovirus in vitro, and porcine reproductive and respiratory syndrome virus (Ojha et al., 2013). CGA has obvious inhibitory effects on respiratory syncytial virus, a synergistic effect when combined with licorice, and enhances an in vitro antirespiratory syncytial virus effect (Liao et al., 2017). CGA is the main antiviral active ingredient of honeysuckle flower. After purification, CGA has certain therapeutic effects on influenza virus, respiratory syncytial virus, viral myocarditis, herpes virus, and adenovirus. The mechanism of action of CGA possibly plays an effective antiviral role by appropriately downregulating interferon (IFN)-β and regulating the TLR3 signaling pathway through the key signaling molecules TLR3, TBK1, and IRF3 (Du, 2017). Some enzyme inhibition experiments proved

that CGA and its derivatives can inhibit ceramidase activity and thus have antiviral activity (Gamaleldin Elsadig Karar, Matei, Jaiswal, Illenberger, & Kuhnert, 2016; Ma, Bolraa, Ji, He, & Ma, 2016).

5. Hypoglycemic and lipid-lowering effects of chlorogenic acid

CGA can maintain blood glucose concentration and prevent diabetes to a certain extent. Peng et al. used a high-fat diet to cause small intestinal glucose metabolism disorder and disrupted blood glucose balance in rats (Peng, Zhu, Zhong, Xu, & Wang, 2015). By adding CGA to a high-fat diet, the expression of glucagon proglucagon mRNA in different intestinal segments (duodenum, jejunum, ileum, and colon) was analyzed by real-time quantitative polymerase chain reaction. It was concluded that CGA could affect glucose metabolism by regulating the expression of glucagon transporter and glucagon in the intestine. Thus CGA controls blood glucose and insulin to maintain blood glucose balance. Ma et al. performed mouse experiments and found that CGA could prevent obesity and obesity-related metabolic syndrome in mice induced by diet. CGA increases the sensitivity of fatty liver and insulin and reduces chronic inflammation in obese mice (Ma, Gao, & Liu, 2015). These findings suggest that CGA has potential health benefits in managing obesity and obesity-related metabolic disorders.

The hypoglycemic mechanism of CGA may be related to the inhibition of the glucose-6-phosphate-shifting enzyme and glucose absorption. CGA has been identified as a novel specific inhibitor of glucose-6-phosphate-shifting enzyme in rat liver microsomes. Because glucose-6-phosphate-shifting enzyme plays an important role in the regulation of blood glucose balance in vivo, it is essential to produce endogenous glucose from glycogen isogenesis and glycogen decomposition. Intake of CGA will help to reduce hepatic glycogen excretion in type 2 diabetes mellitus. As a result, CGA has become a potential choice for the treatment of diabetes. It was also found that CGA could stimulate insulin secretion mediated by glucagon-like peptide 1 and affect blood glucose content. Studies have shown that CGA can activate AMP-activated protein kinase (AMPK), upregulate the gene expression of glucose transporter 4 (GLUT4) to stimulate the absorption of glucose in skeletal muscle, downregulate the gene expression of glucose-6-phosphate-shifting enzyme, inhibit gluconeogenesis, and reduce the synthesis of fatty acids. CGA promotes GLUT4 migration to cell membranes by activating

AMPK, thus promoting glucose uptake (Jung, Lee, Park, Jeon, & Choi, 2006; Ong, Hsu, & Tan, 2012). Beam and others believe that CGA can regulate the transport mechanism of glucose to skeletal muscle by influencing insulin content to a certain extent (Beam et al., 2015).

In terms of lipid lowering, Ma et al. showed that CGA can effectively prevent dietary obesity and related metabolic syndrome (Ma et al., 2015). Balzan et al. pointed out that an extract of Paraguay tea could significantly reduce the contents of triacylglycerol and cholesterol in rat plasma, and CGA played an important role in this process (Balzan et al., 2013). Previous study also pointed out that CGA can regulate cholesterol metabolism by inhibiting the activity of hydroxymethyl glutaric coenzyme A reductase (Hao et al., 2016). Liu and other studies proved that a long-term high-fat diet can produce a large accumulation of fat in liver cells, and CGA has a significant effect on reducing the accumulation of fat in liver caused by high-sugar and high-fat diets (Liu et al., 2015).

Rodriguez de Sotillo found that intravenous administration to rats of CGA at 5 mg/mL for 3 weeks reduced plasma cholesterol and triglyceride by 44% and 58%, respectively, and also significantly reduced liver triglyceride levels (Rodriguez de Sotillo & Hadley, 2002). Frank et al. also showed that the contents of vitamin E and cholesterol in the lung and liver of Sprague–Dawley rats decreased when CGA and caffeic acid were added to their food (Frank, Kamal-Eldin, Razdan, Lundh, & Vessby, 2003).

CGA has also been shown to reduce fatty acid synthesis and fat deposition by downregulating the gene expression of aconitase catalase, fatty acid synthetase, and nuclear transcription factor peroxisome proliferator-activated receptor-gamma 2 (Zheng, Qiu, Zhang, & Li, 2014). Pancreatic lipase is a kind of lipase synthesized and secreted by the pancreas, which is responsible for the decomposition of 50–70% dietary fat. It is reported that plant extracts such as CGA are natural inhibitors of pancreatic lipase and can be used in the intervention of obesity and diabetes (De la Garza, Milagro, Boque, Campion, & Martinez, 2011).

6. Anticardiovascular effects of chlorogenic acid

Oxygen free radicals are one of the important factors causing endothelial injury. Vascular endothelial cell injury is the basis of pathological conditions such as platelet aggregation, coagulation, apoptosis and proliferation of vascular smooth muscle cells, and disorder of vascular tension regulation. As a free radical scavenger and antioxidant, CGA has been widely

recognized. By scavenging oxygen free radicals and antilipid peroxidation, it can increase the concentration of potassium ions in blood, reduce the levels of triacylglycerol and cholesterol in blood, protect vascular endothelial cells, and thus have an effective protective effect on the cardiovascular system. Isochlorogenic acid B has a strong inhibitory effect on the biosynthesis of thrombin and damage to endothelin cells induced by hydrogen peroxide, and it can promote the release of prostaglandin and antiplatelet agglutination in rats.

It has been found that CGA can reduce cerebral infarction and blood–brain barrier and brain edema by inhibiting lipid peroxidation and matrix metalloproteinase activity, and significantly inhibiting brain injury (Lee et al., 2012). Kim et al. showed that the apoptotic nucleus agglutination of neurons induced by hydrogen peroxide was strongly inhibited and the activity of antioxidant enzymes was increased after treatment with CGA (Kim et al., 2012). Inflammatory factors such as IL-1, IL-6, and TNF-α can lead to cardiomyocyte hypertrophy, abnormal myocardial contractility, and cardiomyocyte apoptosis. It has been found that CGA can protect myocardium by inhibiting the expression of inflammatory factors in myocardial cells. The MEK/ERK signaling pathway is the main pathway for many cytokines to regulate apoptosis and proliferation. During the process of cardiomyocyte injury, the MEK1/ERK1/2 signaling pathway can be abnormally activated by G-protein-coupled receptors, receptor encephaline kinase, cardiotrophin-1, and stress stimulation, which play an important regulatory role in the growth, proliferation, and apoptosis of cardiomyocytes. Restoration of perfusion of myocardial tissue after ischemia can induce and accelerate cell apoptosis. Geng et al. confirmed that CGA can inhibit the activation of the MEK/ERK signaling pathway and reduce myocardial ischemia/reperfusion injury in rats (Geng, Zhang, & Li, 2019). Anchor protein-B is expressed in many tissues and cells, and plays an important role in the correct localization of membrane proteins and intracellular proteins. Deficiency of anchor protein-B leads to arrhythmia, and its level is significantly decreased in myocardial tissue with failure. The decrease in expression is related to the increase in reactive oxygen species (Kashef et al., 2012). He conducted experiments in suckling mice to prove that CGA can regulate the expression of anchoring protein-B in myocardial cells after hypoxia-reoxygenation through the reactive oxygen species pathway, thus playing a protective role in myocardial cells (He, Shen, Liu, Cao, & Hong, 2014).

7. Antimutagenic and anticancer effects of chlorogenic acid

CGA has a strong ability to inhibit mutation. It can inhibit the mutation induced by aflatoxin B and nitrosation, and effectively reduce the mutation of bone marrow red blood cells caused by radiation. The study found that CGA has radiation protection. The results of comet assay show that CGA can significantly reduce DNA damage caused by X-rays in human lymphocytes, and the damage is reduced by 5.99–53.57% (Cinkilic et al., 2013). Li et al. irradiated human skin fibroblast ESF-1 with 40 J/cm^2 ultraviolet A and 70 mJ/cm^2 ultraviolet B, then treated it with different concentrations of CGA. After 24 h, it was found that CGA could significantly enhance the photoaging cell activity induced by ultraviolet A and damaged cell activity induced by ultraviolet B. In addition, CGA can increase the activity of superoxide dismutase and decrease the content of malondialdehyde and the activity of lactate dehydrogenase in cell culture medium (Li, Song, Chen, & Xu, 2013).

In terms of anticancer, CGA has significant inhibitory effects on colorectal cancer, liver cancer, laryngeal cancer, lung cancer, colon cancer, etc., and is considered to be an effective chemical protective agent for cancer. CGA can also reduce the utilization rate of carcinogens and transport in the liver to achieve anticancer effects. Because of invasion, infinite proliferation and metastasis of tumor hepatocytes are the most critical steps in the formation and diffusion of malignant tumor cells, and CGA has a certain inhibitory effect on tumor hepatocytes. CGA can inhibit the proliferation of human lung cancer A549 cells to some extent. The effect of CGA on the proliferation of lung cancer cells was detected by the tetrazolium method. The apoptotic rate was observed by flow cytometry. The results showed that CGA can inhibit the proliferation and metastasis of A549 cells and promote cell apoptosis (Tian et al., 2016). CT26 colon cancer cells in logarithmic growth phase were inoculated into mice, and CGA was used for intervention. From the tumor inhibition rate and pathological sections, it was found that CGA significantly inhibited CT26 colon cancer xenografts (Xiao et al., 2012). In addition, CGA inhibited the growth of HCT116 and HT29 colon cancer cells in vitro in a dose-dependent manner, induced cell cycle arrest in the S phase, and inhibited the activation of extracellular signal-related kinases in both cell types (Hou, Liu, Han, Yan, & Li, 2017).

Excessive growth of human acute promyelocytic cells can trigger leukemia. Liu et al. divided human acute promyelocytic cells into an experimental group and a control group, and treated the experimental group with 1.0, 5.0, and 10.0 μmol/L CGA, respectively. After 48 h, the growth of human acute promyelocytes was significantly inhibited in a dose-dependent manner; after 72 h, the growth inhibition rate and apoptosis rate of human acute promyelocytic cells were significantly increased in a dose-dependent manner compared with the control group. Its mechanism of action is to inhibit the growth of the G_0 and G_1 phases of the cell cycle, thereby inhibiting the growth of human acute promyelocytic cells and inducing apoptosis (Liu, Zhou, Qiu, Lu, & Wang, 2013). In the study of the effect of CGA on the retinoblastoma cell line HXO-RB44, we found that CGA may inhibit the proliferation of the retinoblastoma cell line by increasing the expression of HXO-RB44 oncogene and reducing the expression of cyclin to inhibit the proliferation of this cell line (Zhao, He, Yao, Liu, & Zheng, 2016).

CGA can produce anticancer effects by blocking the cell growth cycle, inducing apoptosis, and inhibiting the proliferation of cancer cells. The main molecular mechanisms may be related to the following aspects: (1) Induction of tumor cell apoptosis may be related to increased intracellular reactive oxygen species associated with decreased aminopeptidase N, epidermal growth factor receptor tyrosine kinase, and matrix metalloproteinase activity (Tian et al., 2016). (2) The intensity of signal factors in signal transduction pathway of cell growth and reproduction should be reduced, as should the viability of cancer cells. (3) The activity of quinone oxidoreductase-glutathione transferase and nicotinamide adenine dinucleotide phosphate should be increased, and the induction of the carcinogenic effect of oxidants resisted. (4) The activity of carcinogenic kinases such as MAPK to prevent cell carcinogenesis should be reduced. (5) The expression of tumor suppressor genes such as Nrf2 should be stimulated and the growth of cancer cells inhibited. CGA can also regulate the expression of cholesterol metabolism-related genes and proteins by inhibiting lipid accumulation in hepatocellular carcinoma cells, thus promoting the metabolic disorder of hepatocellular carcinoma cells and inhibiting their growth (Lai et al., 2017).

8. Immunomodulatory effects of chlorogenic acid

CGA has certain immunomodulatory ability, but there is little research on its immunomodulatory effect at home and abroad. The activation of immune cells is the first link of the inflammatory response. CGA plays

an immunomodulatory role by inhibiting the production of antiinflammatory cytokines by macrophages. In vitro studies showed that CGA could significantly enhance the proliferation of T-cells induced by influenza virus antigen and induce the production of IFN-γ and IFN-α from human lymphocytes and human peripheral blood leukocytes (Jin et al., 2006). In addition, Dai Yi found that CGA can improve the metabolism and phagocytosis of peritoneal macrophages in mice, promote the release of NO, proinflammatory cytokines, IL-1β, and TNF-α, and inhibit the release of antiinflammatory cytokines IL-10 in a dose-dependent manner, playing an immunomodulatory role under normal and lipolipid-stimulated conditions (Dai, Xu, Shangguan, & Zhao, 2015). Studies have shown that for allergic rhinitis in mice, CGA can significantly reduce the spleen index of mice, increase the thymus index and the content of IFN-γ in mouse nasal lavage, and reduce the IL-4 of mouse nasal lavage fluid. CGA significantly reduced the levels of histamine, IgE, IL-4, IL-5, and IL-10 in the serum of allergic rhinitis mice, increased the content of IFN-γ, and increased the value of IFN-γ/IL-4. It also downregulated the expression of IL-4, IL-5, and IL-10 mRNA in nasal mucosa of allergic rhinitis mice, upregulated the expression of IFN-γ mRNA, and exerted immunomodulatory effects through various immune cytokines to slow down allergic rhinitis in mice (Li, Jiang, Liu, Di, & Hong, 2015).

In addition, CGA can induce autoimmunity to remove malignant tumor cells. Normally, the body can recognize tumor cells by autoimmune reaction and remove them by cellular immunity. However, malignant tumors can inhibit cellular immunity and escape the recognition of immune cells, thus multiplying in large numbers in the body. Studies by Yang and others have shown that CGA can induce the expression of NOS, TNF-α, and macrophage inflammatory protein-2, regulate the immune system to recognize cancer cells correctly, and eliminate them through its own cellular immune function to prevent further canceration of cells (Yang, Zhang, Wang, Li, & Lv, 2018). Kang et al. studied the effect of CGA on BALB/cEMT-6 female mice by expression microarray. It was found that CaN and NFAT genes related to the immune system regulatory pathway were activated, and the CaN/NFAT signaling pathway was widely used. This can increase the expression of Th1 cytokines (IFN-γ and IL-2), inhibit the expression of Th2 cytokines (IL-4 and IL-10), and activate T-cells, macrophages, natural killer cells and B-cells to regulate the immune function (Kang et al., 2015). Recent studies have shown that CGA can inhibit the growth of glioma cells by promoting STAT1 activation, increasing lipopolysaccharide/IFN-λ-mediated M1 macrophage-related markers such as inducible NOS, major

histocompatibility complex II, and CD11c, and reducing the expression of IL-4-mediated M2 macrophage markers Arg and CD206 by inhibiting STAT6 (Yang, Yang, Kang, Kang, & Li, 2018). In addition, the proportion of CD11c-positive macrophages in G422 mice treated with CGA increased and M2-positive macrophages decreased, which was consistent with the weight loss of tumors in G422 mice (Xue et al., 2017).

9. Conclusion

CGA is a kind of natural compound that is widely distributed and has many pharmacological activities: it has antioxidant, antiinflammatory, antibacterial, antiviral, hypoglycemic, lipid lowering, anticardiovascular, antimutagenic, antitumor effects, and can regulate the immune system. It has high clinical application value too. Its biological activity and function have been given increased attention and its application is now becoming more and more extensive. At present, research into CGA is gradually improving, for example, the production efficiency of CGA is showing signs of progress. However, the biosynthesis and regulation, pharmacological activity and development, and utilization of CGA are not systematic and are far from perfect. Its pharmacological activity, action mechanism, structure–activity relationship, toxicology, and clinical research still need further study to fully tap into its potential medicinal value, and make it play a greater role in medicine and the chemical, cosmetics, and food industries.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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