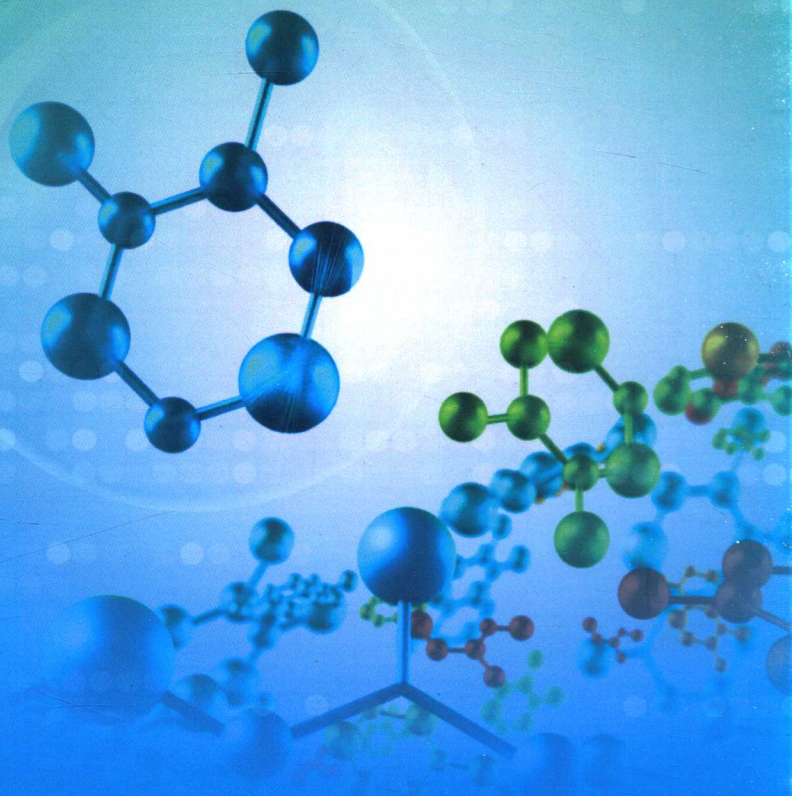
The Biological Function and Molecular Mechanism of Ampelopsin and Exercise Mimetics in Chronic Diseases

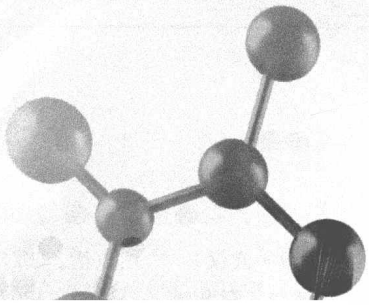
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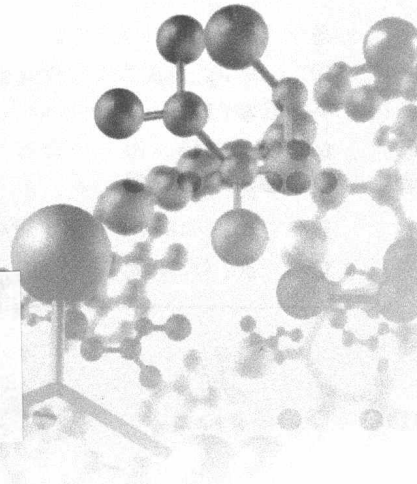


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The Biological Function and Molecular Mechanism of Ampelopsin and Exercise Mimetics in Chronic Diseases

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Abstract

This book mainly discusses the biological function and molecular mechanism of ampelopsin and exercise mimetics in chronic diseases. This book is divided into seven chapters. In chapter 1, I summarize the pharmacological potential of ampelopsin in Rattan tea. In chapters 2-5, I study the anti-inflammatory, antioxidative, anti-tumor, and neuroprotective effects of ampelopsin. In chapters 6-7, I investigate the effects of ampelopsin and exercise (swimming training or voluntary wheel running) on autophagy, brain aging and sarcopenia.

This work was financially supported by the National Natural Science Foundation of China Grants 81601228, Hubei Superior Discipline Groupof Physical Education and Health Promotion, as well as Donghu Scholar Program from Wuhan Sports University.

Preface

Ampelopsis grossedentata is widely distributed in the South of China. Its tender stems and leaves are exploited in China to prepare a health beverage named as Rattan tea. Ampelopsin is one of the most important flavonoids found in Rattan tea. As a dihydromyricetin (DHM), ampelopsin was first isolated from *Ampelopsis meliaefolia* by Kotake and Kubota in 1940, and was reported as a major bioactive component in *Ampelopsis grossedentata* later. Nowadays, ampelopsin, as a major bioactive component in Rattan tea, has a broad range of biological functions. During the past decade, the bioactivity of ampelopsin in Rattan tea has gained extensive attention and increasing scientific investigations related to its pharmacological functions and underlying mechanisms have been reported. Herein, the current research progress in the pharmacological functions and corresponding action mechanisms as well as potential benefits in chronic diseases have been summarized. This study will start a series of studies of ampelopsin from the following aspects: anti-inflammatory, antioxidative, anti-tumor and neuroprotective effects, and alleviate brain aging and sarcopenia. We mainly focus the effect and molecular mechanism of ampelopsin on inflammation, tumor and neuroprotective effects in different cells and animal models.

Many intervention strategies have been investigated in order to understand their ability for slowing down the aging process of brain and skeletal muscle, and exercise training has been confirmed to inhibit oxidative stress and apoptotic signaling, thus effectively executing neuroprotection and sarcopenia. Aerobic exercise, such as running, has been proven to be a very effective way to improve adult hippocampal neurogenesis and learning. Some reports have also shown that treadmill and voluntary wheel running can improve behavioral performance and accelerate mitochondrial fitness of brain by reducing oxidative damage, rescuing abnormal mitochondrial dynamics, and inhibiting excessive apoptotic signaling. Recent studies have revealed that regular exercise has beneficial effect on structure, metabolism, and function of human and rodent brain. Hence, in the present study, we analyzed whether swimming training or voluntary wheel running could improve learning and memory disorders and the underlying mechanisms in different rat brain aging models.

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Chapter 1 Pharmacological potential of ampelopsin in Rattan tea

Abstract: Rattan tea is made from the leaves of *Ampelopsis grossedentata* and has multiple pharmacological potentials including antibacterial, anti-tumor, antioxidative, hepatoprotective and anti-hypertension functions. These beneficial functions of Rattan tea are highly associated with the bioactivity of ampelopsin, one of the major flavonoid compounds found in Rattan tea. Herein, current research progress in bioactivity and pharmacological mechanisms of ampelopsin has been summarized, providing a better reference for its potential application in the prevention of chronic diseases.

Key words: ampelopsin; Rattan tea; flavonoid; pharmacological potential; chronic disease; Chinese herb

1 Introduction

Ampelopsis grossedentata is widely distributed in the South of China. Its tender stems and leaves are exploited in China to prepare a health beverage named as Rattan tea. Ampelopsin is one of the most important flavonoids found in Rattan tea. As a dihydromyricetin (DHM), ampelopsin was first isolated from *Ampelopsis meliaefolia* by Kotake and Kubota in 1940, and was reported as a major bioactive component in *Ampelopsis grossedentata* later. Nowadays, ampelopsin, as a major bioactive component in Rattan tea, has a broad range of biological functions including hypoglycemic, antioxidative, anti-inflammatory, anti-tumor, hepatoprotective, and neuroprotective effects. During the past decade, the bioactivity of ampelopsin in Rattan tea has gained extensive attention and increasing scientific investigations related to its pharmacological functions and underlying mechanisms have been reported. Herein, the current research progress in the pharmacological functions and corresponding action mechanisms as well as potential benefits for human health have been summarized.

2 Antibacterial and anti-inflammatory effects

Ampelopsin in crude plant extracts has been applied to alleviate inflammatory diseases as a broad-spectrum antibacterial drug for several centuries. The ampelopsin extract has strong inhibitory effect on *Staphylococcus aureus* and *Bacillus subtilis*. In addition, ampelopsin is also sensitive to other inhibit pathogenic microorganisms such as *Aspergillus flavus*, penicillium and transport streptavidin. Furthermore, ampelopsin can inhibit the acute or sub-acute inflammation such as croton oil-induced auricular edema or formaldehyde-induced paw edema in mice, and carrageenan-induced paw edema in rats, as

well as abdominal capillary permeability. Similarly, ampelopsin isolated from *Salix sachalinensis* also have strong inhibitory effect on *Cladosporium herbarum*. A structure-activity relationship analysis has indicated that the hydroxyl groups at five positions such as 5,7,3′,4′ and 5′ can significantly enhance the anti-inflammatory activity of ampelopsin.

Inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) can up-regulate the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2), thus stimulating the expressions or activities of inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1) in target cells. Ampelopsin has been reported to inhibit NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and reduce carrageenan-induced acute inflammation *in vivo*, which is consistent with another report that ampelopsin plays an important role not only in reducing the production of proinflammatory mediators such as IL-1β, IL-6 and TNF-α, but also in inhibiting the activity of iNOS. Moreover, ampelopsin can selectively inhibit the production of iNOS in LPS-stimulated macrophages and RAW264.7 cells by suppressing the mRNA and protein expressions of iNOS.

Nuclear factor-kappa B (NF-κB), as a sensitive transcription factor for regulating the gene responsible for both innate and adaptive immune response, has been identified as a major factor involved in the regulation of proinflammatory cytokines such as iNOS and COX-2. Usually, NF-κB resides in the cytoplasm associated with its inhibitor (I-κB). Once the inhibitor is released, NF-κB can be phosphorylated and then translocated to the nuclei. The inhibitory effect of ampelopsin on the induction and expression of iNOS is partially mediated through inhibiting NF-κB activity and reducing the expression of p65 subunit of NF-κB in RAW264.7 cells under the stimulation of LPS. MAPKs and PI3K/Akt are involved in the induction of proinflammatory genes in activated macrophages. However, ampelopsin can exert significant inhibition of the PI3K/Akt signaling pathway without altering the activation of MAP kinases. Moreover, PI3K may act as an upstream regulator of IKK/NF-κB activation, and ampelopsin can inhibit NF-κB-regulated proinflammatory gene expression by blocking the PI3K/Akt/NF-κB signaling cascades.

Another contributor to cellular damage during inflammation is oxidative stress. The administration of ampelopsin can significantly inhibit LPS-induced intracellular ROS levels. On the other hand, as an inhibitor of ROS, N-acetyl-cysteine (NAC) can greatly suppress the phosphorylation of Akt and IKK as well as the downstream I-κB. Due to the association of ampelopsin with these pathways, these studies further confirm the potential anti-inflammatory activity of ampelopsin.

3 Anti-tumor activity

Currently, much attention has been paid to the anti-tumor activity of ampelopsin in cell and animal studies. The anti-metastatic effect of ampelopsin on melanoma and prostate cancer has been confirmed via *in vivo* and *in vitro* studies. In addition, the anti-angiogenesis of ampelopsin by inhibiting the secretion of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) from human hepatocellular carcinoma cells *in vitro*

has been reported. The strong inhibitory effect of ampelopsin on the growth of human hepatocellular carcinoma in mice has also been reported. Ampelopsin exerts its anti-tumor effect by modulating multiple signaling pathways including apoptosis, cell cycle arrest, cell growth inhibition and metastatic inhibition at various cellular levels.

Regulation of proliferation and cell cycle arrest Ampelopsin-containing serum can inhibit the proliferation of B16 cells by interrupting DNA synthesis. Similarly, ampelopsin produces an obvious inhibition on the proliferation of Bel-7402, HL-60, and K562 cells in a dose-dependent manner. Interestingly, ampelopsin can also inhibit the growth of H22 cells with the IC_{50} being 18. 65 μg/mL, but the inhibitory rate is negatively correlated with ampelopsin. Previous studies have demonstrated that the pharmacological activity of ampelopsin is cancer specific. Ampelopsin can inhibit the growth of androgen-sensitive (LNCaP) and androgen-insensitive (PC-3) human prostate cancer cell lines *in vitro*. On the contrary, these anti-proliferative effects of ampelopsin are not observed in normal prostate epithelial cells even at higher doses. Ampelopsin exhibits antiproliferative effects on both androgen-sensitive and androgen-insensitive human prostate cancer cell lines *in vitro*, which are mediated by down-regulating cyclin-dependent kinase (CDK2) in LNCaP cells and cell cycle 2 (CDC2) protein level in PC-3 cells. Since CDC2 is considered as an essential molecular target for designing anti-cancer drugs, ampelopsin should be a promising molecule for the development of anti-cancer drugs. Moreover, ampelopsin also exhibits anti-tumor effect on bladder cancer in orthotopic xenograft models, which is mediated by cell cycle arrest.

Inhibition of apoptosis The capacity to induce apoptosis in tumor cells is a primary pharmacological mechanism of certain anti-tumor drugs. The overloading of calcium ions can result in the opening of mitochondrial permeability transition pores, thereby inducing changes in osmotic pressure, ATP depletion and mitochondrial shrinkage, and finally apoptosis. Lung cancer is a serious threat to human health and ampelopsin can induce the apoptosis of lung adenocarcinoma cell line AGZY-83-a cells by promoting intracellular calcium level and caspase-3 activity. However, the mechanism of the ampelopsin-induced increase in intracellular calcium level and apoptosis is still elusive. Bcl-2 is an important regulator of apoptosis that exhibits increased expression in both metastatic tumors and non-metastatic tumors. Ampelopsin induces apoptosis in prostate cancer mainly through down-regulation of Bcl-2. Furthermore, ampelopsin in the concentration range of 2. 2-14. 84 mg/L can exert inhibitory effects on the growth of gastric cancer cell line SGC-7901. The chemotherapeutic drug mitomycin (MMC) combined with ampelopsin produces an obvious synergistic inhibition on the growth of SGC-7901 cells, which is also correlated with the inhibitory expression of apoptotic proteins such as Bcl-2 and survivin. Therefore, the down-regulation of Bcl-2 can be an important molecular mechanism of ampelopsin-induced apoptosis. The application of anti-cancer drugs combined with ampelopsin will be a novel therapeutic strategy for cancers.

Inhibition of invasion and migration Metastasis is the major cause of death associated

with cancer. Ampelopsin can significantly attenuate the metastatic process of cancers by inhibiting the degradation of the basement membrane. Ampelopsin can also inhibit the adhesion of B16 mouse melanoma cells to fibronectin, lamin, or matrigel, thus attenuating invasion into the reconstituted basement membrane as demonstrated by *in vitro* experiments. Matrix metalloproteinases (MMPs), a family of secreted or membrane-associated proteins capable of digesting extracellular matrix components, are thought to play an essential role in cancer metastasis. Moreover, many factors can affect the invasion and metastasis of cancers. The up-regulation of MMP-2/-9 expression is the critical step of invasion and metastasis of cancer cells. Ampelopsin can inhibit the invasion of human breast cancer (MDA-MB-231) cells in a dose-dependent manner, and the mechanism may be associated with the down-regulated expression of MMP-2/-9 in both the extracellular matrix and the intracellular space. Additionally, ampelopsin may inhibit the expression of CXCR4, a protein associated with prostate cancer, resulting in the inhibition of invasion and migration of prostate cancer cells. Adriamycin (ADM) is a potent anti-tumor agent in clinical cancer treatments. Unfortunately, the application of ADM is limited due to its cytotoxicity to skeletal muscle, heart and liver. In 4T1 breast cancer cells, ampelopsin combined with ADM can inhibit tumor growth of S180- and 4T1-transplanted mice. Ampelopsin can sensitize the anti-tumor efficacy of ADM, and attenuate ADM-induced toxicity in heart and liver. All of these investigations further confirm the anti-invasion potential of ampelopsin during cancer treatments. In addition, ampelopsin can increase intracellular accumulation of chemotherapeutic drugs in multi-drug resistance (MDR)-associated tumor cells and correspondingly reduce the cytotoxicity of chemotherapeutic drugs by inhibiting the efflux of drugs by permeability glycoprotein (P-gp), suggesting that ampelopsin may be also a promising MDR modulator.

4 Hepatoprotective function

Ampelopsin F and G isolated from *Ampelopsis brevipedunculata* Var. Hancei can suppress carbon tetrachloride (CCl_4)- or galactosamine-induced liver injury in rats. Ampelopsin E and cis-ampelopsin E have obvious inhibitory effects on hepatitis virus. In galactosamine-, D-galactosamine- and alpha-naphthyl isothiocyanate (ANIT)-induced acute liver injury models, ampelopsin can inhibit the activity of serum transaminases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and alleviate pathological damage of liver tissues. Ampelopsin can also reduce the production of malondialdehyde (MDA) in CCl_4-induced liver injury and increase superoxide dismutase (SOD) activity. A diet containing ethanolic extract (1%) or ampelopsin (0.1%) can significantly suppress the increase of serum lactate dehydrogenase (LDH), ALT, AST and alpha-tocopherol levels in rats with D-galactosamine-induced liver injury. In addition, ampelopsin also has a protective effect against CCl_4-, D-galactosamine- and LPS-induced liver cell damage in mice. Ampelopsin mainly inhibits the collagen formation of hepatic M cells. After hepatic M cell transplantation in cultured rat liver tissues, collagen fibers are easily produced. However, ampelopsin can inhibit the formation of new collagen fibers, and

further disperse old collagen fibers into small micro-blocks. The hepatoprotective function of ampelopsin can provide a promising therapeutic strategy for hepatitis B.

5 Anti-hypertension effect

In animal experiments, ampelopsin can antagonize norepinephrine (NE) and high K^+-induced contraction of rabbit aorta, suggesting that ampelopsin plays a blockage role in intestinal smooth muscle and aortic α-receptor. Further studies have confirmed that the contraction associated with Ca^{2+} release caused by NE can be significantly inhibited by ampelopsin. However, ampelopsin has obvious inhibitory effect on extracellular Ca^{2+} release only at higher concentrations. These findings suggest that ampelopsin may be an anti-hypertension drug by selectively blocking voltage-dependent calcium channel (PDC).

6 Regulation of plasma lipids and blood glucose

An anti-atherosclerotic effect of ampelopsin has also been reported. The intragastric administration of *Ampelopsis grossedentata* reduces the levels of serum total cholesterol (TC) and triglyceride (TG), and increases serum high-density lipoprotein cholesterol (HDL-C) level in rats, indicating a hypocholesterolemic effect of ampelopsin. In accordance with these findings, a drink containing *Ampelopsis grossedentata* at a dose of 9 g/d was administered to 100 patients with primary hyperlipidemia, resulting in obvious reductions in TC, TG and plasma lipid by 42%, 72% and 28% after 45 days administration, respectively. In addition, ampelopsin can significantly increase the content of serum SOD, and reduce the content of MDA. Therefore, ampelopsin can prevent the invasion of vascular smooth muscle cells and matrix degradation, which correspondingly contributes to protective effects against atherosclerosis and cancer.

During the investigation into the relationship between green tea and obesity-related insulin resistance syndrome, green tea was found of enhance insulin activity *in vitro* and reduce hypertriacylglycerolaemia in mice. Currently, the effective drugs for treating diabetes at home and abroad are metformin and glibenclamide, but clinical practice has proved that both metformin and glibenclamide have obvious side effects. In alloxan-induced diabetic or hyperglycemic mice, ampelopsin can reduce blood glucose level. Interestingly, ampelopsin has no impact on blood glucose level in normal mice. In contrast, glibenclamide significantly reduces blood glucose level in normal mice. One of the characteristics of diabetes is the inability of insulin to inhibit the production of hepatic glucose. In D-galactose-induced impaired glucose tolerance (IGT) animal models, intragastric injection of ampelopsin can significantly reduce the concentrations of fasting blood glucose and 2 h postprandial blood glucose and insulin levels. Moreover, ampelopsin has a certain protective effect against early renal damage by inhibiting the content of microalbuminuria and the activity of LDH in kidney. These findings suggest that ampelopsin can effectively improve the health status of IGT rats and provide a protective function against early kidney damage.

7 Neuroprotective effect

Oxidative stress is believed to be a primary factor in neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease and Huntington's disease, as well as

amyotrophic lateral sclerosis. ROS-associated with oxidative stress can induce cell apoptosis through mitochondrial dysfunction and lipids, proteins and DNA damages. Therefore, antioxidants have gained tremendous attention as promising therapeutics for neurodegenerative diseases. Rattan tea is a popular beverage worldwide, especially in China or other Asian countries. Ampelopsin in Rattan tea is an antioxidant flavonoid that can enhance cellular antioxidative defense by activating ERK and Akt signaling pathways, induce heme oxygenase-1 (HO-1) expression and protect PC12 cells from H_2O_2-induced apoptosis. In pentobarbital sodium-induced mouse hypnosis models, ampelopsin can significantly extend the incubation period caused by sodium pentobarbital, shorten the time of the hypnotic effect of sodium pentobarbital, and significantly reduce ethanol-induced inebriation reaction. The mechanism may be highly associated with increased activity of liver microsomal enzyme and accelerated metabolism of alcohol or sodium pentobarbital. Therefore, ampelopsin may have a novel function as a neuroprotectant in neurodegenerative conditions.

8 Antioxidant effect

ROS is implicated in many pathogenic processes including inflammation and cancer. Detoxification of ROS by antioxidants provides a protective measure against these diseases. Natural plant components, especially antioxidants, have attracted great interest due to their safety properties. Ampelopsin, as a kind of flavonoids from *Ampelopsis grossedentata*, has excellent antioxidative capacity, which is confirmed in trials and quantum chemical experiments. Ampelopsin has excellent antioxidative activity due to its similarity with tertiary butylhydroquinone (TBHQ). It is well known that free radicals can induce lipid oxidation of biomembranes and damage of cell membrane is the basis for tissue damage and for pathological processes of many diseases. Similarly, exogenous damage is also strongly correlated with free radical reactions. Ampelopsin has a strong inhibitory effect on H_2O_2-induced oxidative damage in cell-free systems including red blood cells and *in vitro* cell cultures, which is due to the scavenging of free radicals by ampelopsin. On the basis of mechanistic analysis, ampelopsin exerts strong scavenging capacity for free radicals, reduces radical-induced lipid peroxidation of cell membranes and inhibits MDA generation. Similarly, ampelopsin has been reported to significantly inhibit superoxide anion radical activity and lipid peroxidation of liver homogenate and mitochondria under the stimulation of auto-oxidation and induced-oxidation. The inhibitory effects of ampelopsin on free radicals and lipid peroxidation are concentration-dependent. Therefore, ampelopsin has favorable antioxidative activities to mitigate cellular oxidative stress and stimulate the potential of cellular vitality, thus accomplishing the prevention of neurodegenerative diseases, cardiovascular diseases, cancer and other diseases.

9 Conclusion

Naturally occurring substances derived from diets or dietary supplements such as Rattan tea provide a new approach for the prevention and therapy for chronic diseases. Ampelopsin, as the predominant compound in Rattan tea, possesses potent anti-

inflammatory activity by inhibiting the activation of NF-κB and PI3K/Akt pathways, and exerts inhibitory effect on oxidative stress by scavenging free radicals and attenuating the lipid oxidation as well as reducing ROS levels. Ampelopsin can reduce the risk of cancer and regulate blood glucose and lipids, but the underlying mechanisms remain to be further explored. Rattan tea provides the contribution to human health promotion. More convincing evidence is still need to be confirmed by *in vitro* experimental studies and *in vivo* clinical studies in the future. Future studies should focus on not only the understanding of the mechanisms, but also the development of ampelopsin as a chemopreventive agents in the treatment of diseases.

Chapter 2 Ampelopsin reduces endotoxic inflammation via repressing ROS-mediated activation of PI3K/Akt/NF-κB signaling pathways

Abstract: Ampelopsin (AMP), a plant flavonoid, has potent anti-inflammatory properties *in vitro* and *in vivo*. The molecular mechanisms of ampelopsin on pharmacological and biochemical actions of RAW264.7 macrophages in inflammation have not been clearly elucidated yet. In the present study, non-cytotoxic level of ampelopsin significantly inhibited the release of nitric oxide (NO) and proinflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in a dose-dependent manner. Consistent with NO inhibition, ampelopsin suppressed lipopolysaccharide (LPS)-induced expression of inducible NO synthase (iNOS) by inhibiting nuclear factor κB (NF-κB) activation, which highly correlated with its inhibitory effect on IκB kinase (IKK) phosphorylation, IκB phosphorylation and NF-κB nuclear translocation. Further study demonstrated that ampelopsin suppressed LPS-induced activation of Akt without effecting mitogen-activated protein kinase (MAPK) phosphorylation. A pharmacological inhibitor of the phosphoinositide 3-kinase (PI3K)/Akt pathway, LY294002, abrogated IKK/IκB/NF-κB-mediated iNOS gene expression. Finally, we certificated that ampelopsin reduced reactive oxygen species (ROS) accumulation, and an antioxidant *N*-acetyl-L-cysteine (NAC) significantly repressed LPS-induced PI3K/Akt phosphorylation and the downstream IKK/IκB activation. NAC thereby inhibited LPS-induced iNOS expression and NO production. The present results suggested that the anti-inflammatory effect of ampelopsin was due to inhibiting the interconnected ROS/Akt/IKK/NF-κB signaling pathways.

Key words: ampelopsin; ROS; Akt; NF-κB; RAW264.7 cells

1 Introduction

Inflammation has long been recognized as a localized protective response to many pathological conditions including tissue injury and microbial invasion that is characterized by pain, redness and swelling. A key feature of the inflammatory response is the activation of proinflammatory cells, mainly macrophages and monocytes. Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria. During infection, LPS

is released in the circulation to trigger a number of host cells, especially mononuclear phagocytes, to produce and release a wide variety of proinflammatory cytokines and mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, prostaglandin E_2 (PGE_2) and nitric oxide (NO). Among these mediators, PGE_2 and NO are the products of the inducible isoforms of cyclooxygenase (COX)-2 and inducible NO synthase (iNOS) enzymes, respectively. Although these proinflammatory cytokines and mediators are required for efficient control of growth and host-defense response, dysregulation of their production can lead to pathological conditions such as bacterial sepsis, rheumatoid arthritis, septic shock and other chronic inflammatory diseases.

Mechanistically, LPS activates toll-like receptor (TLR)-4, which is expressed on proinflammatory cells, to build signaling complexes including CD14, LPS-binding protein (LBP) and myeloid differentiation protein (MD)-2. Activation of TLR-4 leads to the activation of two different signaling pathways, the myeloid differentiation factor-88 (MyD88)-dependent and the TIR domain-containing adaptor inducing interferon-β (TRIF)-dependent pathways. MyD88-dependent pathway mediates a number of intracellular signaling proteins activation, including extracellular signal-related kinase (ERK)-1/2, p38 mitogen-activated protein kinase (MAPK) or c-Jun NH_2-terminal kinase (JNK) and phosphoinositide 3-kinase (PI3K)/Akt. Each of these signaling proteins regulates expression of inflammatory genes via activation of several transcription factors, including nuclear factor kappa B (NF-κB). Moreover, it has been also known that LPS initially promotes the production of reactive oxygen species (ROS), which elicits a wide spectrum of responses by activating transcription factor NF-κB through MAPK and PI3K/Akt pathways. In previous studies, anti-inflammatory effects of pharmacological agents targeted at ROS, MAPK, PI3K, or NF-κB have been reported in animal models of inflammatory diseases. Therefore, development of inhibitors targeted at different enzyme molecules of the LPS signaling cascades is an attractive strategy for the therapy of inflammatory diseases.

Ampelopsin (3,5,7,3′,4′,5′-hexahydroxyl-2,3-dihydrogen flavonol, AMP, Fig. 2-1) is one of the most common flavonoids, isolated from the tender stems and leaves of the plant species *Ampelopsis grossedentata* (*Hand-Mazz*) *W. T. Wang*, known as Rattan tea, a health beverage in China. There is more than 27% of ampelopsin in the tender stems and leaves of the species, especially, more than 40% in the cataphyll. Ampelopsin is reported to possess numerous pharmacological activities, such as anti-inflammatory and antimicrobial activity, relieving cough, antioxidation, anti-hypertension, hepatoprotective effect and anti-carcinogenic effect. It was recently reported that ampelopsin could inhibit NO production in LPS-challenged RAW264.7 macrophages and Rattan tea extraction could reduce carrageenan-induced acute inflammation *in vivo*. Moreover, ampelopsin acted to prevent the oxidative stress *in vivo* that may have been due to ROS formed by macrophages by the action of D-galactosamine. Nevertheless, the detailed molecular/signaling mechanisms underlying the anti-inflammatory effects of ampelopsin have not been clarified.

In the present study, we examined the potential anti-inflammatory properties of

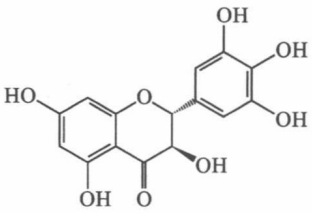


Fig. 2-1 Chemical structure of ampelopsin

ampelopsin in LPS-stimulated peripheral macrophage RAW264.7 cell line. To investigate the molecular mechanisms responsible for the LPS-induced inflammatory responses, the roles of signaling molecules including ROS, NF-κB, MAPK and PI3K/Akt were evaluated. Our results showed that ampelopsin blocks NF-κB nuclear translocation by interfering with ROS-mediated PI3K/Akt phosphorylation and associated IκB kinase (IKK)/IκB signaling pathway to suppress LPS-induced acute inflammatory responses.

2 Materials and methods

Antibodies and reagents Ampelopsin was a generous gift from Jishou University (Hunan, China). Polyclonal antibodies against JNK/SAPK, phospho-JNK/SAPK (Thr183/Tyr185), p38 MAPK, phospho-p38 MAPK (Thr180/Tyr182), p42/p44 MAPK, phospho-p42/p44 MAPK (Thr202/Tyr204), phospho-Akt (Ser473), Akt, phospho-IKKα/β (Ser176/177), IKKβ, NF-κB p65 and phospho-IκBα (Ser32) were purchased from Cell Signaling Technology. Antibody to COX-2 (C20) was obtained from Santa Cruz Biotechnology. Monoclonal antibody to iNOS was purchased from BD Pharmingen. All secondary antibodies used for Western blotting were purchased from Rockland Immunochemical. LPS (from *Escherichia coli* 0111:B4), *N*-acetyl-L-cysteine (NAC), MTT and PI3K inhibitor LY294002 were obtained from Sigma. CM-H2DCFDA was obtained from Invitrogen.

Cell culture and transfection RAW264.7 murine macrophage-like cells, purchased from the CBCAS (Cell Bank of the Chinese Academic of Sciences, Shanghai, China), were cultured in DMEM (Invitrogen) containing 10% fetal bovine serum (HyClone) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) (HyClone) at 37 ℃ in an atmosphere of 5% CO_2. Transient transfection was carried out using the FuGENE HD transfection reagent (Roche) according to the manufacturer's instructions. The total amount of DNA was normalized by empty control plasmids.

Cell viability assay RAW264.7 cells were seeded into 96-well plates at 5×10^4 cells per well 24 h before treatment. Following treatment with ampelopsin and/or LPS for the indicated time periods, cell viability was determined using the MTT assay. In brief, 15 μL (5 mg/mL) MTT working solution was added to each well and after incubation at 37 ℃ for 4 h, the MTT solution was removed and 200 μL of DMSO was added to dissolve the crystals. The absorbance of each well at 570 nm was measured using the ELx 800 Universal Microplate Reader (Bio-Tek, Inc.).

Western blotting Cells were rinsed twice with ice-cold PBS, and solubilized in lysis