Bioactivity and Molecular Mechanism of Bilobalide

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Abstract *Ginkgo biloba* is a deciduous tree belonging to *Ginkgo*, Ginkgoaceae. It is often used to treat dizziness, tinnitus, vertigo, hyperlipidemia and diabetic peripheral neuropathy. Bilobalide is a natural active compound derived from the leaves and fruits of *G. biloba*, which exhibits a range of pharmacological effects, including anti-inflammatory properties, obesity treatment, neuroprotection, and hepatoprotection. This paper provides a comprehensive review of the pharmacological actions and molecular mechanisms of bilobalide, with a particular focus on its roles in anti-inflammatory responses, metabolic regulation, neuroprotection, and hepatoprotection. Research has demonstrated that bilobalide exerts its pharmacological effects through the regulation of various signaling pathways, including AMPK/SIRT1/mTOR, STAT3, and Nrf-2/HO-1. This paper aims to establish a theoretical foundation for the further investigation, development, and application of bilobalide.

Key words Bilobalide, Anti-inflammatory, Obesity treatment, Neuroprotection, Hepatoprotection

0 Introduction

Ginkgo biloba is a traditional medicinal material originating from China, classified within the *Ginkgo* genus of the Ginkgoaceae family. As documented in the *Chinese Medicinal Herbal*, *G. biloba* is recognized for its therapeutic effects, which include promoting blood circulation, alleviating blood stasis, clearing meridians, relieving pain, supporting lung function, mitigating asthma, and reducing turbidity and lipid levels. Bilobalide is a sesquiterpene lactone compound derived from the leaves of *G. biloba*[1]. It has a molecular formula of $C_{15}H_{18}O_8$, a molecular weight of 326.3, and is characterized by its appearance as white flaky crystals. In recent years, the ongoing exploration of natural medicines has led to increased scholarly attention, both domestically and internationally, regarding the pharmacological effects of bilobalide, including its anti-inflammatory properties, obesity treatment, neuroprotection, and hepatoprotection[2]. This paper reviews the pharmacological effects and molecular mechanisms of bilobalide in recent years, aiming to provide a theoretical foundation for the comprehensive study and further development and utilization of bilobalide.

1 Anti-inflammatory effects of bilobalide and its molecular mechanism

Inflammation is a fundamental pathological process that arises in response to harmful stimuli and primarily serves as a defensive mechanism. However, excessive and prolonged inflammation can lead to damage to the structure and function of tissues and organs, ultimately resulting in harm to the organism. Research indicates that bilobalide exhibits significant anti-inflammatory effects while presenting fewer side effects on the human body.

Ma *et al.*[3] conducted a study to examine the impact of bilobalide on the progression of osteoarthritis (OA) and to elucidate its mechanism of action. They employed various biological methods, including enzyme-linked immunosorbent assay (ELISA), protein immunoblotting (Western blot) assay, and thiazolyl blue (MTT) assay, to achieve their objectives. The findings indicated that bilobalide markedly suppressed the interleukin-1β (IL-1β)-induced production of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and matrix metalloproteinase 13 (MMP13) in ATDC5 chondrocytes. At the molecular level, bilobalide induced autophagy in chondrocytes by activating the AMPK/SIRT1/mTOR signaling pathway. This process resulted in an increased expression of autophagy-associated *Atg* genes, an up-regulation of LC3 protein expression, and a reduction in the expression of p62 proteins. In living organisms, bilobalide demonstrated beneficial anti-inflammatory properties and protective effects against extracellular matrix (ECM) degradation in a rat model of post-traumatic osteoarthritis (PTOA) induced by anterior cruciate ligament transection (ACLT). Bilobalide inhibited the expression of iNOS and COX-2 proteins in cartilage, while also reducing serum levels of ECM degradation biomarkers through the AMPK/SIRT1/mTOR signaling pathway. This mechanism subsequently alleviated joint pain in PTOA rats.

Qin *et al.*[4] examined the effects of bilobalide on neuroinflammation in mouse microglial cells (BV-2). The investigation employed various biological methods, including ELISA, Western blot analysis, and real-time quantitative polymerase chain reaction (RT-qPCR), to elucidate the underlying mechanisms of action. The treatment of microglial cells with bilobalide at concentrations of 100 and 200 nM resulted in a significant reduction in the expression levels of interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and IL-1β. Concurrently, bilobalide markedly inhibited the production of iNOS and COX-2, as well as decreased the

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expression of the p65 protein. Furthermore, bilobalide was found to up-regulate the expression of the *lincRNA-p21* gene and the beclin-1 protein, while down-regulating the expression of the p62 protein and p-STAT3 protein. Notably, the knockdown of the *lincRNA-p21* gene resulted in the reversal of all aforementioned effects. The aforementioned results indicate that bilobalide exerts an inhibitory effect on neuroinflammation and facilitates autophagy by up-regulating *lincRNA-p21* and inhibiting the STAT3 signaling pathway.

2 Therapeutic effects of bilobalide on obesity and its molecular mechanism

Obesity is a prevalent chronic metabolic disorder primarily characterized by an excessive accumulation of body fat and an abnormal distribution of adipose tissue. Severe obesity not only predisposes individuals to a range of multi-system diseases, including those affecting the cardiovascular, respiratory, and digestive systems, but also adversely impacts mental health and overall quality of life. Several studies have indicated that bilobalide may possess beneficial therapeutic effects in the treatment of obesity.

Bu *et al.* [5] investigated the effects of bilobalide on the apoptotic processes in mature 3T3-L1 adipocytes, as well as its underlying mechanisms of action. This study employed a variety of biological methods, including flow cytometry, TUNEL staining assay, mitochondrial membrane potential assay, Western blot analysis, RT-qPCR, *etc.* The findings indicated a significant increase in the early apoptosis rate of adipocytes, a notable enhancement in the intracellular levels of reactive oxygen species (ROS), and a substantial decrease in mitochondrial membrane potential following treatment with bilobalide at concentrations of 25, 50, and 100 μM. At the molecular level, bilobalide up-regulated the expression of the pro-apoptotic protein Bax, as well as caspase-3 and caspase-9, while simultaneously down-regulating the expression of the anti-apoptotic protein Bcl-2 in 3T3-L1 cells. These findings suggest that bilobalide induces caspase-3-dependent apoptosis in mature adipocytes derived from 3T3-L1 cells.

Priyanka *et al.* [6] investigated the effects of bilobalide on hypoxic adipocytes (3T3-L1) and elucidated its mechanism of action utilizing various biological methods, including MTT assay, flow cytometry, and ELISA. The findings indicated that treatment with bilobalide at concentrations of 10, 20, and 50 μM significantly enhanced the viability of 3T3-L1 cells. Additionally, there was a notable reduction in ROS levels, an increase in mitochondrial membrane potential, and a significant decrease in the expression of the hypoxia marker HIF-1α, as well as a reduction in the release of lactic acid and glycerol. At the molecular level, bilobalide down-regulated the expression of intracellular TNF-α, IL-6, IL-1β, and interferon-γ (IFN-γ). This suggests that bilobalide may reduce the expression of inflammatory factors by mitigating oxidative stress, thereby enhancing mitochondrial function and protecting 3T3-L1 cells from alterations induced by hypoxia.

3 Neuroprotective effects of bilobalide and its molecular mechanism

Neurodegenerative diseases (NDs) are characterized by the degeneration of neurons and their associated myelin sheaths, frequently accompanied by an inflammatory response. The onset of degenerative changes within the nervous system activates immune response mechanisms that facilitate the differentiation of inflammatory cells. This process can lead to a vicious cycle of neuroinflammation. Several studies have indicated that bilobalide may function as a potential neuroprotective agent to inhibit inflammatory responses and safeguard the nervous system.

Chen Yangyang *et al.* [7] investigated the effects of bilobalide on the nervous system, as well as its mechanism of action, utilizing various biological methods including cytotoxicity assay, ELISA and lactate dehydrogenase (LDH) assay. The findings indicated that the expression levels of TNF-α and IFN-γ in mouse macrophages (RAW264.7) and mouse microglial cells (BV2) were significantly reduced following treatment with bilobalide at a concentration of 50 μg/mL. Meanwhile, bilobalide was found to down-regulate the expression of inflammatory factors, including TNF-α, IFN-γ, IL-6, and interleukin-23 (IL-23), in macrophages (BMDMs). Additionally, it promoted the release of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). Furthermore, bilobalide decreased the LDH content in PC12 cells, resulting in elongated synapses and an increased number of cells. The findings indicate that bilobalide has the potential to enhance the microenvironment of the central nervous system and mitigate neuronal damage by down-regulating inflammatory factor expression in macrophages and microglial cells and inducing their secretion of various neurotrophic factors.

Huang Rongrong *et al.* [8] investigated the effects of bilobalide on neuronal cell damage in HT22 cells, as well as its underlying mechanisms of action, utilizing various biological methods including CCK-8 assay, ELISA, and Western blot analysis. The findings demonstrated that treatment with bilobalide (at concentrations of 12 and 24 μmol/L) significantly enhanced the survival rate of HT22 cells induced by $A\beta_{25-35}$, while concurrently reducing intracellular levels of ROS. At the molecular level, bilobalide was found to down-regulate the secretion of inflammatory factors, including IL-1β, IL-6, and TNF-α, as well as the expression of proteins, such as NLRP-1, ASC, and caspase-1 in $A\beta_{25-35}$-treated HT22 cells. These results suggest that bilobalide may mitigate neuronal damage induced by $A\beta_{25-35}$ through the inhibition of ROS generation and the activation of NLRP-1 inflammasome.

Xiang *et al.* [9] examined the effects of bilobalide on astrocytes within the central nervous system, as well as to elucidate its mechanism of action through various biological methods, including

ELISA, RT-qPCR, and Western blot assays. The findings indicated that treatment with bilobalide at concentrations of 1, 5, and 10 μM resulted in a significant reduction in the expression levels of TNF-α, IL-6, and IL-1β in astrocyte lysates. At the molecular level, bilobalide up-regulated the protein expression of Aβ-degrading enzymes, specifically NEP, IDE, and MMP2 in astrocytes. Concurrently, it down-regulated the expression of prominent proteins such as PSD-95 and synapsin-1 in astrocyte-APP/PS1 neuronal lysates. These findings suggest that bilobalide may mitigate neuronal deficits by inhibiting the expression of inflammatory factors while simultaneously promoting the expression of Aβ-degrading enzymes in astrocytes.

4 Hepatoprotective effects of bilobalide and its molecular mechanism

Liver injury is a pathological condition characterized by the damage of hepatocytes, and it can result from a multitude of factors, consequently impairing the liver's normal physiological functions. Common etiological factors contributing to liver injury include viral infections, drugs, chemicals, autoimmune factors, as well as genetic and metabolic diseases. In recent years, numerous studies have provided evidence for the beneficial hepatoprotective effects of bilobalide.

Wu Jianqi *et al.* [10] examined the effects of bilobalide on hepatic injury and its underlying mechanisms in a septic mouse model through various biological methods, including serum biochemical index detection, ELISA, and immunohistochemical staining assays. The findings indicated that bilobalide significantly reduced the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the expression of pro-inflammatory factors such as TNF-α, IL-6, and IL-1β in the liver tissues of septic mice. Conversely, bilobalide was found to up-regulate the expression of the anti-inflammatory factor IL-10 and to decrease neutrophil infiltration in the livers of these mice. At the molecular level, bilobalide down-regulated the expression of p-P65, p-STAT3, p-JNK, p-ERK, and p-P38 proteins, suggesting that bilobalide could attenuate sepsis-induced hepatic injury by inhibiting the activation of the MAPK/NF-κB pathway.

Yao Ning *et al.* [11] investigated the effects of bilobalide on liver injury and its mechanism of action in non-alcoholic fatty liver disease (NAFLD) model mice via biological methods such as serum biochemical index experiments, histopathological observations, and Western blot analysis. The findings indicated that bilobalide significantly reduced the levels of ALT, AST, MDA, total cholesterol (TC), and triglycerides (TG) in both serum and liver tissues. Additionally, bilobalide mitigated the extent of steatosis and the degree of inflammatory cell infiltration in the livers of NAFLD model mice. At the molecular level, bilobalide was found to up-regulate the expression of nuclear factor E_2-related factor 2 (Nrf-2) and heme oxygenase 1 (HO-1) proteins, while down-regulating the expression of high mobility group protein B1 (HMGB1), p-P65, and nuclear factor-κB inhibitory factor α (IκBα) proteins in the livers of NAFLD mice. These findings suggest that bilobalide may enhance lipid metabolism, reduce oxidative stress, and mitigate inflammation in NAFLD-affected mice by regulating the Nrf-2/HO-1/HMGB1/NF-κB signaling pathway, ultimately leading to the alleviation of liver injury.

5 Anticancer effects of bilobalide and its molecular mechanism

Cancer is a pathological condition characterized by the uncontrolled proliferation of cells within the body. Malignant cells possess the ability to infiltrate adjacent tissues and disseminate to distant sites via the circulatory and lymphatic systems, thereby presenting a significant threat to human health and life. Several studies have indicated that bilobalide exerts a beneficial inhibitory effect on the proliferation of cancer cells.

Liu *et al.* [12] investigated the effects of bilobalide on AGS gastric cancer cells, employing various biological methods, including MTT assay, DAPI staining, and flow cytometry, to elucidate its mechanism of action. The results indicated that treatment with bilobalide at concentrations of 25 and 30 μM significantly reduced the viability of AGS cells. Additionally, there was a marked increase in ROS levels. Observations revealed that AGS cells exhibited signs of cellular crumpling, fragmentation of the cellular nucleus, and aggregation of nuclear chromatin. Concurrently, the number of AGS cells at the G_2/M phase progressively increased. At the molecular level, bilobalide was observed to down-regulate the expression of p-PI3K1/2, p-AKT, and other associated proteins, while simultaneously up-regulating the expression of AKT, p38, and p-JNK1/2 proteins in AGS cells. In rat studies, bilobalide demonstrated a significant capacity to mitigate gastric mucosal damage in rats with MNU-induced gastric cancer, leading to a down-regulation of proteins such as TNF-α, NF-κB, prostaglandin E_2 (PGE_2), and IL-6.

6 Antidepressant effects of bilobalide and its molecular mechanism

Depression is a prevalent chronic disorder of the central nervous system, characterized by a high incidence, low treatment rates, elevated suicide rates, and significant relapse rates. This condition poses substantial risks to both the physical and mental well-being of affected individuals. Several studies have indicated that bilobalide may have a beneficial effect in alleviating symptoms of depression.

Yang Chengying[13] conducted an investigation into the effects of bilobalide on depression-like behavior and its underlying mechanisms in mice subjected to chronic unpredictable mild stress (CUMS). This study employed various biological methods, inclu-

ding behavioral experiments, ELISA, Western blotting, and quantitative mRNA analysis. The findings indicated a significant increase in the sucrose preference index, alongside a notable reduction in immobilization time during both the tail suspension test and the forced swimming test in mice administered bilobalide at a dosage of 20 mg/kg. Furthermore, bilobalide was observed to downregulate the secretion of inflammatory factors, including TNF-α and IL-6. At the molecular level, the expression of BDNF protein, as well as the mRNA levels of AKT and β-catenin, exhibited a significant increase in model mice following treatment with bilobalide. Conversely, the mRNA content of GSK-3β demonstrated a significant decrease. These findings suggest that bilobalide may facilitate the activation of the downstream AKT/GSK-3β/β-catenin signaling pathway by enhancing BDNF expression in the brains of CUMS mice, thereby effectively alleviating depressive symptoms.

7 Conclusions and prospects

Bilobalide, a natural active ingredient derived from the traditional Chinese medicine *G. biloba*, exhibits a range of pharmacological activities, including anti-inflammatory effects, obesity treatment, neuroprotection, and hepatoprotection. Despite its significant potential for development and utilization, the specific molecular mechanisms underlying its pharmacological actions and clinical effects remain inadequately understood. Therefore, it is imperative to conduct more systematic and in-depth investigations at the molecular, cellular, and animal levels, in addition to further clinical trials. Such research is essential for a comprehensive assessment of the efficacy, safety, and applicability of bilobalide, thereby providing a theoretical foundation for examining the pharmacological effects of bilobalide, as well as for its further development and application.

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