 DOI: 10.19600/j.cnki.issn2152 - 3924.2025.01.016

Anti-tumor, Anti-inflammatory, and Antibacterial Effects of Isobavachalcone and Their Molecular Mechanisms

Hongxue LIANG[1], Wenshuang HOU[2], Xichun HUANG[2], Lei JIANG[1*], Chenghao JIN[2*]

1. College of Pharmacy, Harbin Medical University (Daqing), Daqing 163319, China; 2. College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing 163319, China

Abstract In this paper, the anti-tumor, anti-inflammatory, antibacterial and anti-asthma effects of isobavachalcone and their molecular mechanisms were reviewed to provide theoretical support for further research of isobavachalcone and development of new drugs.

Key words Isobavachalcone, Anti-tumor, Anti-inflammation, Bacteriostasis, Anti-asthma

1 Introduction

Psoralen has the functions of tonifying kidney and strengthening Yang, securing essence and reducing urination, and warming spleen and preventing diarrhea[1]. As a natural active ingredient derived from psoralen[2], isobavachalcone can be dissolved in solvents such as methanol, ethanol and DMSO[3]. Isobavachalcone shows a variety of biological activities in the field of biomedicine, including anti-tumor, anti-inflammatory, antibacterial, anti-asthma and other pharmacological activities. In this paper, the pharmacological action and molecular mechanism of isobavachalcone will be reviewed to provide a theoretical basis for its further research and development.

2 Anti-tumor effect of isobavachalcone and its molecular mechanism

Malignant tumor refers to a disease in which normal cells mutate and proliferate indefinitely under the long-term action of some carcinogenic factors. Malignant tumors have the characteristics of infinite proliferation, strong invasion and strong metastasis. Studies have shown that isobavachalcone can effectively inhibit the proliferation, induce apoptosis and inhibit invasion and migration of various tumor cells.

2.1 Inhibitory effect of isobavachalcone on the proliferation of tumor cells and its molecular mechanism Ren Yali *et al.*[4] tested the inhibitory effect of isobavachalcone on the proliferation of non-small cell lung cancer H460 cells by MTT method. The results show that isobavachalcone inhibited the proliferation of H460 cells in a concentration-dependent manner (1.9, 3.8, 7.5, 15, 30 and 60 μmol/L) and time-dependent manner (24, 48 and 72 h). Besides, the effect of isobavachalcone on the cycle of H460 cells was detected by flow cytometry. The results show that with the increase of isobavachalcone concentration, the number of the cells gradually decreased in the S phase of H460 cell cycle, and gradually increased in the G_0/G_1 phase, indicating that isobavachalcone can block H460 cell cycle in the G_0/G_1 phase. Moreover, the effect of isobavachalcone on the expression level of protein related to H460 cell cycle was detected by western blot assay. The results reveal that the expression level of Cyclin D1 in H460 cells was significantly down-regulated after treatment with isobavachalcone (30 and 60 μmol/L). These results suggest that isobavachalcone can inhibit the proliferation of H460 cells by blocking the cell cycle.

Li Yanxi *et al.*[5] tested the inhibitory effect of isobavachalcone on the proliferation of colorectal cancer HCT116 cells and SW480 cells by CCK-8 method and colony formation experiment. The results show that isobavachalcone could inhibit the proliferation of HCT116 cells and SW480 cells in a dose-dependent (20, 40, 60, 80 and 100 μmol/L) and time-dependent (24, 48 and 72 h) manner. After treatment with isobavachalcone, the number of HCT116 and SW480 cells as well as adhesion rate reduced, and cell contraction and cytoplasm concentration appeared. The effects of isobavachalcone on the expression level of protein related to the proliferation of HCT116 and SW480 cells were detected by Western blot. The results reveal that after treatment with isobavachalcone (50 and 100 μmol/L), the expression level of β-catenin in HCT116 cells and SW480 cells significantly decreased, but its phosphorylation level significantly increased. Moreover, the phosphorylation levels of protein kinase B (AKT) and glycogen synthase kinase-3 (GSK-3β) were significantly declined, suggesting that isobavachalcone could inhibit the proliferation of colorectal cancer HCT116 and SW480 cells by regulating the AKT/GSK-3β/β-catenin signaling pathway.

2.2 Inducing effect of isobavachalcone on the apoptosis of tumor cells and its molecular mechanism Ren Yali[6] tested the inducing effect of isobavachalcone on the apoptosis of breast cancer cells by Hoechst/PI staining test. The results indicate that isobavachalcone induced the apoptosis of MDA-MB-231 and MCF-7 cells in a concentration-dependent manner (10, 20 and 40 μmol/L). The inducing effect of isobavachalcone on the apoptosis of breast cancer cells was detected by flow cytometry. The results show that the apoptosis rate of MDA-MB-231 and MCF-7 cells rose significantly with the increase of isobavachalcone concentration. The effects of isobavachalcone on the expression level of protein related to the apoptosis of breast cancer cells were detected by western blot assay. The results reveal that after treatment with isobavachalcone (10, 20 and 40 μmol/L), the expression level of B lymphoblastoma-2 gene (Bcl-2) in MDA-MB-231 and

Received: December 1, 2024 Accepted: January 5, 2025
Supported by the Key Research and Development Plan Guidance Project of Heilongjiang Province (GZ20220039); Central Support for Local Universities Reform and Development Fund Talent Training Project (2020GSP16).
Hongxue LIANG, master candidate, research fields: molecular pharmacology of Chinese herbal medicine.
* Corresponding author. Lei JIANG, PhD., associate professor, research fields: preparation of insoluble Chinese medicine nano preparations and their resistance to digestive system diseases. Chenghao JIN, PhD., professor, research fields: cancer pathogenesis and research and development of drugs.

MCF-7 cells significantly dropped, and the expression level of X protein related to Bcl-2 (Bax) significantly increased, indicating that isobavachalcone could induce the apoptosis of MDA-MB-231 and MCF-7 cells by regulating apoptosis-related proteins.

Shi Yi *et al.* [7] detected the inducing effect of isobavachalcone on the apoptosis of tongue cancer cells by flow cytometry. The results show that the apoptosis rate of tongue cancer Tca8113 cells rose with the increasing of isobavachalcone concentration. The effects of isobavachalcone on the expression level of proteins related to the apoptosis of Tca8113 cells were detected by western blot. The results reveal that after treatment with isobavachalcone (20 and 40 μmol/L), the expression level of Bax and Caspase-3 in Tca8113 cells significantly increased, while the expression level of Bcl-2, phosphorylated protein kinase B (p-AKT) and phosphorylated extracellular regulatory protein kinase (p-ERK) significantly declined, suggesting that isobavachalcone could induce the apoptosis of tongue cancer Tca8113 cells by regulating apoptosis-related proteins.

Wang Anhong *et al.* [8] detected the inducing effect of isobavachalcone on the apoptosis of hepatocellular carcinoma cells by flow cytometry. The results show that the apoptosis rate of HepG2 cells rose significantly with the increasing of isobavachalcone concentration. The effects of isobavachalcone on the expression level of proteins related to the apoptosis of HepG2 cells were detected by western blot assay. The results indicate that after treatment with isobavachalcone (3, 4, 5, 6 and 7 mg/mL), the expression level of pro-apoptotic proteins (Bax and Bid) in HepG2 cells significantly increased, while the expression level of Bcl-2 protein significantly decreased, showing that isobavachalcone can induce apoptosis of hepatoma HepG2 cells by regulating apoptosis-related proteins.

2.3 Inhibitory effect of isobavachalcone on the migration and invasion of tumor cells and their molecular mechanisms

Shi Yi *et al.* [9] tested the inhibitory effect of isobavachalcone on the migration and invasion of tongue cancer cells through cell scratch test and Transwell test. The results show that the healing speed and invasiveness of tongue cancer Tca8113 cells decreased with the increasing of psoralen ethyl concentration. The effects of isobavachalcone on the expression level of proteins related to the migration and invasion of Tca8113 cells were detected by Western blot assay. The results reveal that the expression level of p-AKT, matrix metalloproteinase 9 (MMP-9) and matrix metalloproteinase 2 (MMP-2) in Tca8113 cells significantly decreased after treatment with isobavachalcone (10, 20 and 40 μmol/L). These results indicate that isobavachalcone could inhibit the migration and invasion of tongue cancer Tca8113 cells by regulating AKT signaling pathway.

Chen Sheng *et al.* [10] tested the inhibitory effect of isobavachalcone on the migration and invasion of prostate cancer PC3 cells by cell scratch test and modified Matrigel Boyden chamber assay. The results show that with the increase of isobavachalcone concentration, the mobility rate and invasion number of PC3 cells decreased. Furthermore, the effects of isobavachalcone on the expression level of proteins related to the migration and invasion of PC3 cells were detected by western blot. The results reveal that after treatment with isobavachalcone (10, 20 and 40 μmol/L), the expression level of nuclear factor erythroid 2 related factor (Nrf2) and superoxide dismutase 1 (SOD1) protein in PC3 cells significantly increased. These results indicate that isobavachalcone could inhibit the migration and invasion of PC3 cells by activating Nrf2/ARE signaling pathway.

Xiao Yi *et al.* [11] tested the inhibitory effect of isobavachalcone on the migration and invasion of nasopharyngeal carcinoma cells through cell scratch test and Transwell test. The results show that with the increase of isobavachalcone concentration, the mobility rate and invasion number of nasopharyngeal carcinoma CNE1 cells decreased gradually. The effects of isobavachalcone on the expression level of proteins related to the migration and invasion of CNE1 cells were detected by western blot. The results reveal that after treatment with isobavachalcone (10, 20 and 40 μmol/L), the expression level of epidermal growth factor receptor (EGFR), phosphorylated phosphatidylinositol 3-kinase (p-PI3K), mammalian target of rapamycin (mTOR) and AKT phosphorylation level of CNE1 cells significantly dropped. The results indicate that isobavachalcone could inhibit the migration and invasion of nasopharyngeal carcinoma CNE1 cells by regulating PI3K/AKT/mTOR signaling pathway.

3 Anti-inflammatory effect of isobavachalcone and its molecular mechanism

Inflammation is the body's defensive response to harmful external stimuli. Inflammation is characterized by redness, swelling, heat, pain, and dysfunction. Studies have shown that isobavachalcone can improve inflammatory symptoms by inhibiting the expression of related inflammatory factors. Gao Dehong *et al.* [12] detected the effect of isobavachalcone on the expression level of pro-inflammatory factors in rats with lung injury model through enzyme-linked immunosorbent assay. The results show that after treatment with isobavachalcone (25 and 50 mg/kg), the secretion of bronchoalveolar lavage fluid (BAL) and tumor necrosis factor (TNF-α) in the serum of lung injury model rats significantly declined, indicating that isobavachalcone could improve inflammatory symptoms of lung injury model rats by reducing the expression of pro-inflammatory factors. Dong Wenhong *et al.* [13] tested the effects of isobavachalcone on the kidney tissue of diabetic model rats through HE staining experiment. The results show that the mesangial expansion, glomerular hypertrophy and inflammatory cell infiltration of diabetic model rats were significantly improved after treatment with isobavachalcone (12.5, 25 and 50 mg/kg). Furthermore, the effects of isobavachalcone on the expression level of proinflammatory factors in diabetic rats were detected by enzyme-linked immunosorbent assay. The results reveal that after treatment with isobavachalcone (12.5, 25 and 50 mg/kg), the expression level of IL-1β, TNF-α and interleukin-6 (IL-6) in the serum of diabetic model rats significantly dropped, suggesting that isobavachalcone could improve the inflammatory symptoms of diabetic model rats by reducing the secretion of inflammatory factors in the serum.

4 Bacteriostatic effect of isobavachalcone and its molecular mechanism

Microbes are a general term for tiny organisms that are difficult to see with naked eyes, such as bacteria, fungi, and viruses. They are mainly characterized by small size, variety, rapid reproduction and strong ability to adapt to the environment. Studies have shown that isobavachalcone can achieve bacteriostatic effect by destroying the structure of bacteria, fungi and other microorganisms and interfering with metabolism. Guan Lijie *et al.* [14] detected the effect

of isobavachalcone on the change of mycelium morphology of *Valsa mall* mycelium by scanning electron microscopy. The results show that after treatment with isobavachalcone (2 mg/L), the mycelium tips atrophied, and the mycelium thickness was not uniform; the melting part of the cell wall had holes. Besides, the effects of isobavachalcone on the structure of the mycelial cells were detected by transmission electron microscopy. The results reveal that after treatment with isobavachalcone (10 mg/L), the cell wall was seriously damaged, and the boundaries of cell membrane, mitochondrial membrane and cell nuclear membrane were blurred, while the number of intracellular vacuoles increased, indicating that isobavachalcone could achieve antibacterial effect by destroying the cell wall and membrane system of *V. mall* mycelium.

Liu Xiwang *et al.* [15] tested the antibacterial activity of isobavachalcone against *Clostridium difficile* ATCC 43255, BAA-1803 and CICC 22951 by microdilution method. The results show that the minimum bactericidal concentration of isobavachalcone against ATCC 43255, BAA-1803 and CICC 22951 was 8 μg/mL, showing significant antibacterial activity. The effects of isobavachalcone on the morphological changes of *C. difficile* was detected by scanning electron microscopy. The results suggest that the cells of *C. difficile* was rapidly wrinkled after treatment with isobavachalcone (8 μg/mL). The effect of isobavachalcone on the cell membrane morphology of *C. difficile* was detected by PI staining. The results reveal that after treatment with isobavachalcone (8 μg/mL), the fluorescence intensity of *C. difficile* infected was as high as 1 500 RFU, indicating that isobavachalcone could play a antibacterial role by destroying the cell membrane of *C. difficile*.

5 Anti-asthma effect of isobavachalcone and its molecular mechanism

Asthma is a chronic respiratory inflammatory disease mainly characterized by eosinophilic and mast cell responses[16-17]. The interleukin family is associated with chronic inflammation and hyperreactivity of the airways, and plays an important role in the occurrence and development of bronchial asthma. Studies have shown that isobavachalcone can play an anti-asthmatic role by regulating the expression of interleukin family. Liang Zheng *et al.* [18] detected the effect of isobavachalcone on the expression of inflammatory factors in spleen cells of asthmatic mice by flow cytometry and enzyme-linked immunoassay. The results show that after treatment with isobavachalcone (25 mg/kg), the number of $CD4^+$ cells in the spleen and the expression level of interleukin-4 (IL-4) in the serum dropped significantly, and the expression level of interferong (IFN-g) increased significantly. These results indicate that isobavachalcone could improve asthma symptoms of model mice by regulating cytokine expression.

6 Prospects

Isobavachalcone, as a small molecular compound of chalcone in psoralen fruit, has a good therapeutic effect in anti-tumor, anti-inflammation, bacteriostasis and anti-asthma, and has good medicinal value and development potential. Although there are many reports on isobavachalcone at present, most of them are limited to the superficial study of pharmacological effects of isobavachalcone, and there is a lack of systematic and detailed reports on the molecular mechanisms related to activity and clinical applications. Based on the theories of molecular biology, cell biology, structural biology, physiology, molecular pharmacology and biopharmaceutical science, further basic studies should be carried out at the molecular, cellular and animal levels to provide theoretical support for further development and utilization of isobavachalcone.

References

[1] YAN YX, WANG HL, HUANG JX, *et al.* Textual research on the nature, taste, efficacy and medication taboos of *Psoralea corylifolia* [J/OL]. Journal of Liaoning University of Traditional Chinese Medicine, 1-18[2024-12-02]. (in Chinese).

[2] SONG LL, WANG WW, SUN Y, *et al.* Apoptosis of imatinib-sensitive and imatinib-resistant chronic myelocytic leukemia cells induced by isobavachalcone[J]. Journal of Shanghai Jiao tong University: Medical Science, 2014, 34(9): 1309-1314. (in Chinese).

[3] LUAN LJ, SHEN XY, LIU XS, *et al.* Qualitative analysis of Psoraleae Fructus by HPLC-DAD/TOF-MS fingerprint and quantitative analysis of multiple components by single marker[J]. Biomedical Chromatography, 2018, 32(2): e4059.

[4] REN YL, ZHAO GY, RAO HN, *et al.* Isobavachalcone inhibits the progression of non-small cell lung cancer via regulating Sirtuins-related signaling pathways[J]. Pharmacy and Clinics of Chinese Materia Medica, 2022, 13(4): 33-37, 53. (in Chinese).

[5] LI Y, QIN X, LI P, *et al.* Isobavachalcone isolated from *Psoralea corylifolia* inhibits cell proliferation and induces apoptosis via inhibiting the AKT/GSK-3β/β-catenin pathway in colorectal cancer cells[J]. Drug Design, Development and Therapy, 2019, 13: 1449-1460.

[6] REN YL. Isobavachalcone exhibits anti-TNBC efficacy via targeting SIRT2 and triggering ROS-mediated pathway[D]. Chengdu: Chengdu University of Traditional Chinese Medicine, 2022. (in Chinese).

[7] SHI Y, JIN XH, WU WZ, *et al.* Effects of isobavachalcone on proliferation and apoptosis of tongue squamous cell carcinoma Tca-8113 cells and their mechanisms[J]. Journal of Jilin University (Medicine Edition), 2016, 42(4): 725-730. (in Chinese).

[8] WANG AH, LU GY, ZHOU K, *et al.* Corylifolinin induced Bcl-2 family-dependent apoptosis in HepG2 cel[J]. Pharmacology and Clinics of Chinese Materia Medica, 2012, 28(5): 23-25. (in Chinese).

[9] SHI Y, WU WZ, HUO A, *et al.* Inhibitory effect of isobavachalcone on migration and invasion of Tca8113 cells and its mechanism[J]. Chinese Pharmacological Bulletin, 2015, 31(12): 1741-1745. (in Chinese).

[10] CHEN S, LIU Y, WANG TB, *et al.* Effect of isopsoraleachalcone on proliferation, migration, apoptosis, and invasion of prostate cancer PC3 cells[J]. China Pharmaceuticals, 2023, 32(8): 39-43. (in Chinese).

[11] XIAO Y, LI PP, ZHOU J, *et al.* Effects and mechanism of isobavachalcone on the proliferation and metastasis of nasopharyngeal carcinoma CNE1 cells[J]. Chinese Pharmacist, 2021, 24(9): 1623-1629. (in Chinese).

[12] GAO D, LIU F, LI Z, *et al.* Isobavachalcone attenuates Sephadex-induced lung injury via activation of A20 and NRF2/HO-1 in rats[J]. European Journal of Pharmacology, 2019, 848: 49-54.

[13] DONG WH, CHU QQ, LIU SQ, *et al.* Isobavachalcone ameliorates diabetic nephropathy in rats by inhibiting the NF-κB pathway[J]. Food Biochemistry, 2020, 44(9): e13405.

[14] GUAN LJ, ZHAO LH, DONG RF. The influence of isobavachalcone to cellular structure and mycelial morphology of Valsa mali Miygabe et Yamada.[J]. Agrochemicals, 2014, 53(4): 290-292. (in Chinese).

[15] LIU XW, YANG YJ, QIN Z, *et al.* Isobavachalcone from *Cullen corylifolium* presents significant antibacterial activity against *Clostridium difficile* through disruption of the cell membrane[J]. Frontiers in Pharmacology, 2022, 13: 914188.

[16] MAHAJAN SG, MEHTA AA. Suppression of ovalbumin-induced Th2-driven airway inflammation by β-sitosterol in a guinea pig model of asthma[J]. European Journal of Pharmacology, 2011, 650(1): 458-464.

[17] BOUSQUET J, JEFFERY PK, BUSSE WW, *et al.* Asthma. From bronchoconstriction to airways inflammation and remodeling[J]. American Journal of Respiratory and Critical Care Medicine, 2000, 161(5): 1720-1745.

[18] LIANG Z, HAO WL, SUN XY, *et al.* The anti-asthmatic effect of isobavachalcone: An experimental study[J]. Hunan Journal of Traditional Chinese Medicine, 2016, 32(5): 177-179. (in Chinese).