Anti-Cancer, Anti-Oxidant and Anti-Inflammatory Effects of Herbacetin and Its Molecular Mechanisms

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Abstract *Rhodiola rosea*, a perennial herb of the genus *Rhodiola* in the Crassulaceae family, is commonly used to treat depression, fatigue, cancer and cardiovascular diseases. Herbacetin is a natural flavonol compound extracted from *R. rosea* plant, with many pharmacological effects such as anti-cancer effect, anti-oxidant effect and anti-inflammatory effect. In this paper, the pharmacological effects and molecular mechanisms of herbacetin were summarized by consulting domestic and foreign literature, in order to provide a theoretical basis for the development and utilization of herbacetin.

Key words Herbacetin, Molecular mechanisms, Anti-cancer, Anti-oxidant, Anti-inflammatory

1 Introduction

Rhodiola rosea L., also known as Yunduer and Saoluomaerbu, is distributed in China, Russia, Mongolia, North Korea and Japan. *R. rosea* is a perennial herb of the genus *Rhodiola* in the Crassulaceae family. It is known as "Tibetan ginseng" and "Oriental sacred grass". It is often used to treat depression, fatigue, cancer and cardiovascular diseases. Herbacetin (3,4',5,7,8-pentahydroxyflavone, Herbacetin, HBT) is a natural flavonol compound extracted from *R. rosea*, with a molecular formula of $C_{15}H_{10}O_7$ and a molecular weight of 302.236. It is soluble in organic solvents such as methanol, ethanol and dimethyl sulfoxide. Herbacetin has a variety of pharmacological effects, including anti-cancer effect, anti-oxidant effect, anti-inflammatory effect, and hypoglycemic effect[1]. In this paper, the pharmacological activity and molecular mechanism of herbacetin were reviewed, and the theoretical basis for the further development and clinical application of herbacetin was provided.

2 Anti-cancer effect

Cancer, also known as malignant tumor, is a disease caused by abnormal cell proliferation due to mutations in some genes. Cancer cells proliferate rapidly and can spread to other tissues and even the whole body through the blood, with high morbidity and mortality. Under normal circumstances, cancer cells continue to proliferate by absorbing nutrients in the human body, which seriously endangers the life of patients.

Studies have shown that herbacetin can inhibit the proliferation of various cancer cells and reduce the migration ability of cancer cells.

2.1 Anti-hepatocellular carcinoma effect and molecular mechanism Liver cancer, as a malignant tumor caused by carcinoma of hepatocytes or intrahepatic bile duct cells, mainly covers two categories: primary liver cancer and secondary liver cancer. Qiao Yan[2] deeply explored the effect of herbacetin on the proliferation of human hepatocellular carcinoma HepG2 cells through MTT experiment in her study. The experimental results showed that herbacetin significantly inhibited the proliferation of HepG2 cells in a concentration-dependent manner (50, 100, 200 μmol/L). Subsequent AO-EB and DAPI fluorescence staining experiments further revealed the significant effect of herbacetin on the morphology of HepG2 cells, which was manifested by pyknosis of nuclei and decrease of cell number. Flow cytometry experiments revealed that herbacetin also triggered the decrease of mitochondrial membrane potential of HepG2 cells in a concentration-dependent manner (50, 100, 200 μmol/L). Through Western Blot experiment, Qiao Yan's team further confirmed that herbacetin could significantly up-regulate apoptosis-promoting gene (Bax), caspase-3, and peroxisome proliferation-activating receptor in HepG2 cells. The expression levels of peroxisome proliferator-activated receptor coactivator 1α (PGC-1α) and cytochrome C protein, while reducing the phosphorylation level of protein kinase B (AKT), which fully demonstrated that herbacetin induced apoptosis of HepG2 cells by regulating PGC-1α and AKT signaling pathways.

Meng Xu's research focused on the effect of herbacetin on the migration of HepG2 cells[3]. Through the Transwell experiment, he found that after 24 h of treatment with herbacetin at a concentration of 100 μmol/L, the adhesion ability of HepG2 cells decreased significantly, and the migration and invasion of HepG2 cells were inhibited in a concentration-dependent manner (25,

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50, 10 μmol/L). Through Western Blot experiment, he further observed that herbacetin could down-regulate the phosphorylation level of mitogen-activated protein kinase (ERK) in HepG2 cells, and at the same time up-regulate the phosphorylation level of stress-activated protein kinase (JNK) and proliferation protein kinase inhibitor (p38), as well as up-regulate the expression levels of death receptor (Fas), death factor (Fas-L), cysteine proteinase-8 (Caspase 8), tumor suppressor gene (P53), nuclear transcription factor (NF-κB) protein, and down-regulate the expression levels of matrix metalloproteinase 9 (MMP-9), and vascular cell adhesion protein 1 (VCAM-1) protein. These findings reveal that herbacetin induces apoptosis of hepatocellular carcinoma cells by regulating mitogen-activated protein kinase (MAPK) signaling pathway and NF-κB signaling pathway. In addition, herbacetin can also inhibit the migration and invasion of HepG2 cells by up-regulating the expression level of E-cadherin.

To sum up, Meng Xu's research showed that herbacetin induced apoptosis of HepG2 cancer cells by regulating the MAPK signaling pathway and inhibited their migration, thus playing a significant role in anti-liver cancer.

2.2 Anti-skin cancer effect and molecular mechanism Skin cancer, a skin disease induced by ultraviolet irradiation, chronic irritation and other factors, mainly includes basal cell carcinoma, squamous cell carcinoma and melanoma. In studies, Dong Joon Kim *et al.* [4] examined the effect of herbacetin on the proliferation of various skin cells by MTT assay, including normal skin N/TERT cells, mouse epidermal JB6 cells, human epidermal carcinoma A431 cells, human skin melanoma SK-MEL-5 cells, and malignant melanoma SK-MEL-28 cells. The experimental results showed that herbacetin inhibited the growth and proliferation of JB6, A431, SK-MEL-5 and SK-MEL-28 cells in a concentration-dependent manner (10, 20 μmol/L), but had no obvious effect on the normal cell activity. Further Western Blot experiments and ornithine decarboxylase (ODC) assay experiments revealed that herbacetin could down-regulate the expression level of glycogen synthase kinase-3β (GSK-3β) protein in these cells, and at the same time reduce the phosphorylation level and activity of ERK, Golgi peripheral membrane protein (p65) and ODC. Reporter gene activity assay experiments further showed that herbacetin could down-regulate the expression levels of activator protein-1 (AP1) and NF-κB protein. Through animal experiments, Dong Joon Kim's team observed that herbacetin could significantly reduce the number and volume of tumors in skin cancer mice, reduce the activity of ODC in mice, and reduce the expression level of ERK, ribosomal protein S6 kinase (RSK), and AKT proteins and the phosphorylation level of GSK3β and ODC, which fully demonstrated that herbacetin inhibited the proliferation of skin cancer cells by regulating AKT and NF-κB signaling pathways.

Li *et al.* [5] focused on the effects of herbacetin on melanoma cell proliferation and neovascularization. Through CCK-8 experiments, they found that herbacetin could inhibit the proliferation of melanoma A375 cells and Hs294T cells in a concentration-dependent manner (15 – 30 μmol/L), but had no obvious toxic side effects on human umbilical vein endothelial cell (HUVEC).

2.3 Effect on other cancers In addition to the above cancers, the mortality rate of many other cancers is also increasing year by year, including breast cancer, colon cancer, gastric cancer, pancreatic cancer, *etc.*, which deserves attention. Sumiko Hyuga *et al.* [6] examined the effect of herbacetin on the proliferation and migration of human breast cancer MDA-MB-231 cells by cell counting experiment and Transwell experiment. It was found that herbacetin inhibited the proliferation and migration ability of MDA-MB-231 cells in a concentration-dependent manner (0.66, 3.3, 16.5 μmol/L). The effect of herbacetin on the expression of migration-related proteins in MDA-MB-231 cells was further examined by Western Blot assay. The results showed that herbacetin could down-regulate the phosphorylation levels of intermediate epithelial converting factor (c-Met) and AKT in MDA-MB-23 cells. After c-Met bound with hepatocyte growth factor (HGF), c-Met was activated by dimer formation and phosphorylation of several sites in the membrane-proximal region, thereby activating the PI3K/AKT pathway, indicating that herbacetin could inhibit the migration of human breast cancer cells by regulating the P13K/AKT signaling pathway.

Dong Joon Kim *et al.* [7] examined the effect of herbacetin on the proliferation of HCT116 cells by MTS assay. The results showed that herbacetin had a good ability to inhibit the proliferation of HCT116 cells. Further, animal experiments were conducted to detect the effects of herbacetin on physiological and biochemical indicators of colon cancer model mice. The results showed that herbacetin could reduce the number and volume of intestinal polyps in APCMin + mice with colon cancer, down-regulate the activity of ODC and the content of putrescine and spermidine *in vivo*, and inhibit tumor growth *in vivo*, without obvious toxic side effects and weight loss in mice. The effect of herbacetin on the expression of related proteins in colon cancer model mice was further detected by Western Blot experiment. The results showed that herbacetin could down-regulate the phosphorylation levels of ERK and RSK proteins, indicating that herbacetin could down-regulate the phosphorylation levels of ERK and RSK by inhibiting the activity of ODC, thus inhibiting the proliferation of colon cancer cells. In summary, the results indicated that herbacetin could inhibit the proliferation and migration of various cancer cells by regulating P13K/AKT signaling pathway.

3 Anti-oxidant effect

Free radicals are normal products of metabolism, which have the characteristics of strong oxidation, high activity and non-selectivity. When the body is subjected to harmful stimulation, the level of free radicals surges, and excessive free radicals will attack cells and tissues, leading to tissue damage, which will seriously cause the body to suffer from pathological changes. Studies have shown that herbacetin can effectively scavenge free radicals produced by the body under harmful stimuli, and has good anti-oxidant capacity.

Qiao Yan *et al.* [8] examined the scavenging ability of herbacetin on free radicals (DPPH) and hydroxyl radicals (·OH) through reactive oxygen species detection experiments. The results showed that herbacetin could scavenge DPPH and ·OH, and the ability to scavenge ·OH was stronger than that of vitamin C, and down-regulated the oxidation and carbonylation levels of bovine serum albumin (BSA) treated with Cu^{2+}/H_2O_2 or AAPH, indicating that herbacetin could achieve anti-oxidant effects by scavenging free radicals. Wang Yu *et al.* [9] examined the effect of herbacetin on the activity of related enzymes in serum of SD rats with non-alcoholic fatty liver through liver homogenate biochemical index experiments. The results showed that herbacetin could reduce the content of free fatty acids (FFA) in rat serum and up-regulate the activity of superoxide dismutase (SOD), peroxidase (CAT), and glutathione peroxidase in liver homogenate (GSH-Px), heme oxidase (HO-1). Further, Western Blot experiment was used to detect the effect of herbacetin on the expression of related proteins in liver tissue of non-alcoholic fatty liver model rats. The results showed that herbacetin could up-regulate the expression level of nuclear factor E2-related factor 2 (Nrf2) protein in rat liver tissue, down-regulate the expression levels of cytochrome P450 enzyme 2E1 (CYP2E1) and cytochrome P450 enzyme 4A (CYP4A) protein, indicating that herbacetin exerted anti-oxidant effect by up-regulating the expression level of Nrf2 protein and down-regulating the expression level of CYP2E1 and CYP4A protein.

4 Anti-inflammatory effect

Inflammation refers to the body's defense response under the stimulation of inflammatory factors. Inflammation mainly occurs locally, but in severe or chronic conditions, the inflammatory process may spread to other sites, showing systemic symptoms. If the initial inflammation is not controlled, it will lead to rheumatoid arthritis, diabetes, cancer and other diseases. Studies have shown that herbacetin can reduce the inflammatory response and reduce the damage caused by inflammation to the body.

Li *et al.* [10] examined the effect of herbacetin on the proliferation of mouse macrophage RAW264.7 cells by MTT assay. It was found that herbacetin did not affect the activity of RAW264.7 cells at concentrations of 10, 25 and 50 μmol/L. The effects of herbacetin on the secretion of pro-inflammatory factors and related gene expression in RAW264.7 cells induced by lipopolysaccharide (LPS) were detected by RT-PCR, ELISA and reporter gene activity assay. The results showed that herbacetin could down-regulate the expression level of inducible nitric oxide synthase (iNOS) in LPS-induced RAW264.7 cells, down-regulate tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) secretion and mRNA expression level. The effect of herbacetin on the expression level of inflammation-related proteins in LPS-induced RAW264.7 cells was further examined by Western Blot experiment. The results showed that herbacetin could down-regulate the expression levels of iNOS, JNK, NF-κB and p65 phosphorylation in cells, and promote the nuclear translocation of p65, indicating that herbacetin could achieve anti-inflammatory effect by regulating JNK and NF-κB signaling pathways.

Muhammad Umar Ijaz *et al.* [11] tested the effect of herbacetin on related enzyme activity in cyclophosphamide (CYC)-induced rat kidney tissue by anti-oxidant enzyme activity determination experiment, and found that herbacetin could up-regulate the activity of CAT, SOD, GSH-Px and glutathione reductase (GSR) in rat kidney tissue, while down-regulating the content of reactive oxygen species (ROS) and malondialdehyde (MDA). The effect of herbacetin on renal function markers in rat kidney tissue induced by CYC was examined by ELISA. It was found that herbacetin could up-regulate the creatinine clearance rate in rat kidney tissue and reduce the levels of inflammatory markers including urea, creatinine, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), NF-κB, TNF-α, IL-1β, interleukin-6 (IL-6), iNOS, cyclooxygenase-2 (COX-2), *etc.* The effect of herbacetin on the expression of related genes in rat kidney tissue induced by CYC was examined by RT-qPCR. The results showed that herbacetin could up-regulate the expression levels of anti-apoptotic markers (Bcl-2), Bax and Caspase-3 in rat kidney tissue. The effects of herbacetin on CYC-induced rat kidney tissue were further examined by immunohistochemistry. The results showed that herbacetin could improve epithelial cell degeneration, leukocyte infiltration and renal tubular and glomerular atrophy in rat kidney tissue, and had no obvious toxic side effects on mouse kidney, indicating that herbacetin could exert anti-inflammatory effect by regulating NF-κB signaling pathway, thereby improving CYC-induced renal toxicity in rats. In summary, the results indicated that herbacetin could regulate JNK and NF-κB signaling pathways to exert anti-inflammatory effect.

5 Conclusion

As a natural organic compound in *R. rosea* plant, herbacetin is commonly used to treat depression, fatigue, cardiovascular diseases and other diseases, and has many pharmacological effects such as anti-tumor effect, anti-oxidation effect and anti-inflammatory effect. However, the research on its specific mechanism of action is still in the primary stage, and it is necessary to combine modern biotechnologies such as molecular biology, cell biology, immunology and experimental zoology to carry out more in-depth, more comprehensive and more systematic research at the cellular and molecular level, so as to provide a theoretical basis for the further development and utilization of herbacetin.

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