Anticancer, Anti-inflammatory and Antibacterial Effects of Aloin and Its Molecular Mechanism

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Abstract Aloin is the main medicinal component extracted from *Aloe vera*. It is a natural anthraquinone compound, with anticancer, anti-inflammatory and antibacterial and other biological functions. This paper reviews the pharmacological action and related mechanisms of aloin, in order to provide a theoretical basis for the development and utilization of aloin.

Key words Aloin, Anticancer, Anti-inflammatory, Antibacterial

1 Introduction

Aloe vera is a plant that integrates medicine, food, health care, beauty and ornamental effects. It has been used in traditional medicine to treat constipation, dermatitis, ulcers, *etc.*, with a long history of medicinal use[1]. Aloin, also known as aloe emodin, is an anthraquinone substance extracted from the endepidermis of aloe leaves. Aloin is a yellow needle-like crystal, with molecular formula $C_{21}H_{22}O_9$, molecular weight 418.39 and melting point 148 - 149 ℃. It is easily soluble in organic solvents such as pyridine, glacial acetic acid, formic acid, acetone, methyl acetate and ethanol[2]. In recent years, a large number of studies have revealed that aloin has antitumor, anti-inflammatory, antibacterial and many other pharmacological effects. This paper summarizes the pharmacological effects of aloin, in order to provide a theoretical basis for the development and utilization of aloin.

2 Antitumor effect

Cancer is a general term for a large group of diseases in which abnormal cells grow and spread uncontrollably beyond normal limits and spread to other parts of the body, causing death. It is one of the most serious diseases endangering global human health.

2.1 Inhibiting cancer cell proliferation Infinite cell proliferation is an important feature of cancer cells. In the process of cell proliferation, it will rob the nutrients of normal cells, consume the energy of the body, and lead to the death of the body in severe case. Cai Huarong *et al.*[3] treated KESY70 esophageal cancer cells with different concentrations (10, 20, 40, 80, 120 μmol/L) of aloin for 24 h, and tested the effects of aloin on the viability and proliferation of KESY70 cells by CCK-8 assay. The results showed that with the increase of aloin concentration and the extension of treatment time, the survival rate and proliferation ability of KESY70 cells decreased gradually. Western Blot assay was further employed to detect the effect of aloin on proliferation-related protein expression in KESY70 cells, and the results suggested that aloin significantly down-regulated the expression level of proliferation marker protein PCNA in KESY70 cells, indicating that aloin can inhibit cell proliferation by regulating the expression level of proliferation-related proteins in KESY70 cells. Liu Ping *et al.*[4] treated A549 non-small cell lung cancer cells with different concentrations (0, 10, 50, 100 μmol/L) of aloin for 24 h, and tested the effect of aloin on the viability of A549 cells by MTT assay. The results demonstrated that aloin significantly inhibited the vitality of A549 cells. Flow cytometry was further conducted to detect the effect of aloin on A549 cell cycle. Compared with the control group, aloin significantly increased the proportion of A549 cells at G_2 phase from 20.9% to 42.3%, and finally blocked A549 cells at G_2/M phase, indicating that aloin can inhibit A549 cell proliferation and induce A549 cell cycle arrest. Zhou Tianhong[5] treated CMT1211 and CMT7364 canine breast cancer cells with different concentrations (10, 20, 40, 80, 160 μmol/L) of aloin for 24 h, and detected the effects of aloin on the growth of CMT1211 and CMT7364 cells by CCK-8 assay. The results showed that aloin significantly inhibited the growth of CMT1211 and CMT7364 cells. Immunofluorescence assay was performed to test the effects of aloin on the cell cycle of CMT1211 and CMT7364. After treatment with aloin, the expression level of p-ERK1/2 in CMT1211 and CMT7364 cells was significantly decreased, indicating that aloin reduced the phosphorylation level of ERK1/2 by regulating the MAPKs signaling pathway, and blocked CMT1211 and CMT7364 cells at S phase, finally inhibiting cell proliferation. These results indicate that aloin can effectively inhibit the proliferation of various cancer cells such as esophageal cancer, non-small cell lung cancer and canine breast cancer.

2.2 Inducing apoptosis of cancer cells Apoptosis is a non-inflammatory form of programmed cell death, which is a molecular cascade with complex mechanism and is closely related to the occurrence and development of cancer. Inducing apoptosis of cancer cells is an effective way to treat cancer. Wang Ziqian *et al.*[6] treated HGC-27 and MKN-28 gastric cancer cells with different concentrations of aloin (100, 200, 400 μg/mL) for 24 h, and

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tested the effect of aloin on the nuclear morphology of HGC-27 and MKN-28 cells by DAPI staining test. After treatment with aloin, the nuclear morphology of HGC-27 and MKN-28 cells were concentrated and broken in different degrees. Annexin V/PI double staining assay was utilized to figure out the effect of aloin on the apoptosis rate of HGC-27 and MKN-28 cells. When the concentration of aloin was 400 μg/mL, the apoptosis rates of HGC-27 and MKN-28 cells were significantly increased by 20% and 30%, respectively, indicating that aloin could induce apoptosis of HGC-27 and MKN-28 cells. Liu Haiyun *et al.* [7] treated U251 glioma cells with different concentrations of aloin (100, 120, 140, 160, 180, 200 μg/mL) for 24 h, and tested the effect of aloin on apoptosis of U251 cells by Hoechst 33342 staining test. After treatment with aloin, the number of U251 cells decreased significantly, and some of the nuclei were broken to form apoptotic bodies. Flow cytometry was applied to monitor the effect of aloin on apoptosis rate of U251 cells. Compared with the control group, the apoptosis rate of U251 cells significantly increased from 7.2% to 25.4% after being treated with aloin for 48 h, and the expression level of pro-apoptotic protein Bax gradually increased with the increase of aloin concentration, indicating that aloin can induce apoptosis of U251 cells by inducing karyopyknosis of U251 cells and up-regulating the expression level of Bax protein. He Jiming[8] treated HOS and MG-63 osteosarcoma cells with different concentrations (10, 20, 40, 80, 120 μmol/L) of aloin for 24 h, and detected the effects of aloin on the expression levels of pro-apoptotic protein and autophagy marker protein in HOS and MG-63 cells by Western Blot assay. Compared with the control group, aloin treatment group increased the expression levels of LC3BII and LC3BI proapoptotic protein in HOS and MG-63 cells. Meantime, it was also found that aloin could up-regulate the expression levels of autophagy marker proteins such as ATG-5 and Beclin-1. Flow cytometry was employed to test the effects of aloin on apoptosis rate and autophagic flux of HOS and MG-63 cells. After treatment with aloin, the apoptosis rate and autophagic flux of HOS and MG-63 cells were significantly increased, suggesting that aloin could mediate the apoptosis and autophagy of HOS and MG-63 human osteosarcoma cells by up-regulating the expression levels of pro-apoptotic protein and autophagy marker protein. Cheng Zhenyu *et al.* [9] tested the effect of combined treatment of aloin and cisplatin on apoptosis of HGC-27 cells by flow cytometry, and found that the apoptosis rate of HGC-27 cells increased to 33.74% after treatment with aloin and cisplatin. Western Blot assay was performed to test the effect of combined treatment of aloin and cisplatin on apoptosis-related protein expression in HGC-27 gastric cancer cells. The expression levels of PARP, caspase-3 and caspase-7 proteins in HGC-27 cells were significantly increased after combined treatment of aloin and cisplatin, suggesting that aloin could induce apoptosis of HGC-27 cells by regulating the expression levels of apoptosis-related proteins. These results indicate that aloin can effectively induce apoptosis of cancer cells such as esophageal cancer, gastric cancer, glioma and osteosarcoma.

2.3 Inhibiting the migration and invasion of cancer cells The migration and invasion of cancer cells is the main characteristic of cancer and the primary cause of death of cancer patients. The migration and invasion of cancer cells is a complex and multi-factor regulated dynamic process. Liu Ping *et al.* [4] tested the effects of aloin on the migration and invasion ability of A549 non-small cell lung cancer cells through scratch assay and Transwell test, and found that aloin could significantly inhibit the migration and invasion ability of A549 non-small cell lung cancer cells, with an inhibition rate of 50%. Liu Haiyun *et al.* [7] tested the effect of aloin on the mobility of U251 glioma cells through scratch assay, and found that the cell scratch spacing in the aloin treatment group was significantly greater than that in the control group. Western Blot assay was performed to detect the effect of aloin on the expression of migration-related proteins in U251 cells. The results indicated that aloin could inhibit the migration of glioma U251 cells by down-regulating the expression level of migration-related protein MMP2 in U251 cells. Du Yichao *et al.* [10] treated MHCC97H human liver cancer cells with different concentrations (100 and 200 μmol/L) of aloin for 24 h, and tested the effects of aloin on migration and invasion ability of MHCC97H cells through scratch assay and Transwell test. Compared with the control group, the aloin treatment group significantly inhibited the migration and invasion of MHCC97H cells, and the high concentration aloin group had a stronger inhibition on the migration and invasion of MHCC97H cells, indicating that aloin had a concentration-dependent effect on the migration and invasion of MHCC97H cells. Cai Tianyu *et al.* [11] treated BGC-823 gastric cancer cells with aloin for 24 h, and tested the effect of aloin on the mobility of BGC-823 cells by scratch assay and Transwell test. Compared with the control group, the aloin group significantly reduced the cell mobility from 50% to 10%. Western Blot assay was carried out to detect the effect of aloin on the expression of migration-related proteins in BGC-823 cells. The results showed that aloin significantly up-regulated the expression level of N-cadherin protein and down-regulated the expression level of E-cadherin protein, indicating that aloin can inhibit the migration ability of BGC-823 cells by regulating the expression level of intracellular migration-related proteins. Liu Weibin *et al.* [12] treated A549 non-small cell lung cancer cells with different concentrations of aloin (10, 20, 40, 80, 120 μmol/L) for 48 h, and tested the effects of aloin on the migration and invasion ability of A549 cells by scratch assay and Transwell test. Compared with the control group, the invasion number of A549 cells in the aloin treatment group decreased significantly and the migration rate decreased from (21.36 ± 2.32) % to (18.42 ± 3.69) %, indicating that aloin can effectively inhibit the migration and invasion ability of A549 cells. The above results suggest that aloin can effectively inhibit the migration and invasion of non-small cell lung cancer, liver cancer, gastric cancer and glioma.

3 Anti-inflammatory effect

Inflammation is the body's response to tissue damage, and it is a

pathological change in local inflammation caused by physical injury, ischemic injury (insufficient blood supply to the organ), infection, exposure to toxins, or other types of trauma, manifesting as symptoms such as redness, swelling, heat, and pain. Shu Kegang[13] treated chondrocytes with different concentrations (0, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400, 800 μmol/L) of aloin for 24 h, and tested the effect of aloin on chondrocyte activity in osteoarthritis rats model by Calcin-AM/PI staining test. The activity of chondrocytes in the aloin treatment group was significantly increased, and the increase of chondrocytes activity was most obvious when the concentration of aloin was 50 μmol/L. ELISA was further carried out to detect the effect of aloin on the expression level of inflammatory factors in chondrocytes, and it was found that the expression levels of inflammatory factors IL-1β, TNF-α, IL-6 and NO in chondrocytes were significantly reduced. Wei Liangxin *et al.* [14] tested the effect of aloin on the mRNA expression of inflammation-related genes in subchronic AFB1 immune damage model rats through real-time fluorescent quantitative PCR assay. Compared with the control group, aloin significantly reduced the gene expression levels of inflammatory factors IL-1β, IL-6, TNF-α and IFN-γ in rats, and showed a concentration-dependent inhibition effects on IL-1β and TNF-α. Zhang Xiaoni[15] tested the effect of aloin on the infiltration of inflammatory cells in sepsis model mice by RT-PCR, and found that aloin inhibited the infiltration of inflammatory cells in sepsis model mice. ELISA was employed to detect the effect of aloin on the expression level of inflammatory factors in the serum of mice, and the results showed that the expression levels of IL-6, IL-1β and TNF-α in the serum of mice were decreased after being treated with aloin. These results indicate that aloin can effectively relieve inflammation by regulating the level of inflammatory cytokines.

4 Antibacterial effect

Bacterium is a kind of pathogenic microorganism widely existing in nature, and often causes greater harms to the environment, humans and animals. It is a pathogen of many diseases, such as tetanus, typhoid, pneumonia, syphilis, cholera and tuberculosis, *etc.*, with strong infectiousness. Bai Le *et al.* [16] detected the antibacterial ability of aloin by MIC test after treatment of aloin at different concentrations (0.25, 0.5, 1, 2, 4, 8, 16 mg/mL) for 24 h. The results showed that aloin effectively inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Trichophyton rubrum* when the concentration was 1 mg/mL, and had a good inhibitory effect on the proliferation of the four kinds of bacteria, with the strongest inhibitory effect on *E. coli*. Wang Ye *et al.* [17] tested the antibacterial effect of aloin through drilling test and MIC test. When the concentration of aloin was 12.5 mg/mL, the mean inhibitory ring diameter of *S. aureus* ATCC25923 was (21.50 ± 1.29) mm; when the concentration of aloin was 15 mg/mL, the mean inhibitory ring diameter of clinical strain SA1.5 was (17.00 ± 0.91) mm. Aloin had a good inhibitory effect on the growth of *S. aureus* ATCC25923 and clinical strain SA1.5. Zhang Shasha *et al.* [18] tested the antibacterial ability of aloe extract by filter paper method, and found that the average antibacterial diameter in the high concentration aloin treatment group was 1.24 cm, and that in the low concentration aloin treatment group was 3.10 cm, which were all significantly increased compared with that in the blank control group. These results showed that aloin had a good inhibitory effect on the growth of *E. coli*, *S. aureus*, *C. albicans* and *T. rubrum*.

5 Prospects

As a common natural active substance, aloin has many biological activities, such as enhancing immunity, antitumor, anti-inflammatory and antibacterial, and has no toxic side effects on human body. Hence, it has a broad prospect of drug development and clinical application. However, most of the researches on aloin remain in the aspects of pharmacology, function and nutritional composition, while few efforts have been dedicated to its specific molecular mechanism, and there is a lack of detailed and reliable basic research and experimental data as the theoretical basis for drug development. Therefore, it is necessary to study the molecular mechanism of aloin at the cellular, molecular and animal levels in a more comprehensive and in-depth way, in order to provide a theoretical basis for further research and development and clinical application of aloin.

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