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Pharmacological Effects of Combretastatin on Anti-tumor, Anti-inflammatory, Antifungal, and Immunomodulation

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Abstract Combretastatin is a natural active substance isolated from the bark of South Africa *Combretum alfredii*, which has pharmacological activities such as anti-tumor, anti-inflammatory, antifungal, and immunomodulation. In terms of anti-tumor effects, it exerts the effects by inhibiting tumor cell proliferation, inducing apoptosis, and suppressing angiogenesis. The mechanism involves activation of the caspase family and regulation of the VEGF/VEGFR-2 and Notch signaling pathways. In terms of anti-inflammatory effects, it inhibits the release of pro-inflammatory factors such as TNF-α and IL-6 by blocking the NF-κB pathway. In terms of antifungal effects, it targets fungal microtubule proteins and induces microtubule depolymerization. In terms of immunomodulation, it promotes the proliferation of immune cells and regulates M1 polarization of macrophages. In this paper, the core pharmacological effects and molecular mechanisms of combretastatin are reviewed, providing theoretical references for its new drug development.

Key words Combretastatin, Anti-tumor, Anti-inflammatory, Antifungal, Immunomodulation

1 Introduction

Combretastatin is a natural active substance derived from the African shrub *Combretum alfredii*[1]. *Combretum* plants are commonly used to treat leprosy in Africa and India[2]. Combretastatin is easily soluble in organic solvents such as methanol, but difficult to dissolve in water[3]. It exhibits rich biological activities in the field of biomedicine, including anti-tumor, anti-inflammatory, antifungal, immunomodulation, and other pharmacological activities. In this paper, the pharmacological effects and molecular mechanisms of combretastatin are reviewed, providing a theoretical basis for its in-depth research and development.

2 Anti-tumor effect

Malignant tumors are a type of disease characterized by abnormal cell proliferation, invasive growth, and distant metastasis, which can destroy normal tissue structures and endanger life. Their core features include uncontrolled proliferation, invasion of surrounding tissues, and spread and metastasis through the blood or lymphatic system.

2.1 Inhibiting tumor cell proliferation Groshen S *et al.*[4] tested the *in vitro* anti-tumor activity of combretastatin through human tumor cloning experiments and radiation analysis experiments. The results showed that combretastatin had a killing effect on eight different human tumor cell lines, including breast cancer MCF-7 cells and lung cancer A549 cells. The half inhibitory concentration IC_{50} was at the level of 1 - 10 nmol/L. In addition, the difference in cytotoxicity between normal cells and tumor cells induced by combretastatin was detected by radiation analysis experiments. The results showed that combretastatin exhibited significant killing effects on six tumor cell lines, while having no significant toxic side effects on normal human diploid fibroblasts and human bone marrow cells, further confirming its selective inhibitory effect on tumor cell proliferation.

Li *et al.*[5] detected the inhibitory effect of combretastatin on tumor cell proliferation using MTT assay. The results showed that combretastatin inhibited the proliferation of MCF-7 and A549 cells in a concentration dependent manner (0.1, 0.5, 2.5, 12.5, 62.5, and 125 nmol/L) and a time dependent manner (24, 48, and 72 h). Among them, MCF-7 cells showed the highest sensitivity to combretastatin, with an IC_{50} value of 2.5 nmol/L, significantly lower than 12.5 nmol/L of A549 cells. The effect of combretastatin on related signaling pathways was detected by western blot assay, and the results showed that combretastatin can exert anti-tumor effects through direct cell proliferation inhibition.

2.2 Inducing apoptosis of tumor cells Bilenker J H *et al.*[6] used flow cytometry to detect the effect of combretastatin on apoptosis of human bladder transitional cell carcinoma T24 cells. The results showed that compared with the control group, as the concentration of combretastatin increased, the apoptosis rate of T24 cells significantly increased, and a significant apoptosis peak appeared. Further protein immunoblotting experiments were conducted to detect the effect of combretastatin on the expression levels of apoptosis related proteins in T24 cells. The results showed that compared with the control group, after treatment with combretastatin (2,4 nmol/L), the activation level of caspase-3 in T24 cells was significantly increased, and the expression levels of spindle checkpoint proteins BubR1 and Bub3 were significantly downregulated, indicating that combretastatin can induce apoptosis in T24 cells by regulating the expression of apoptosis related proteins.

Iliakis G *et al.*[7] used flow cytometry to detect the effect of

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combretastatin on apoptosis of lung cancer H460 cells. The results showed that compared with the control group, the apoptosis rate of H460 cells continued to rise with increasing concentration and prolonged action time of combretastatin treatment. Further protein immunoblotting experiments were conducted to detect the effect of combretastatin on the expression levels of apoptosis related proteins in H460 cells. The results showed that compared with the control group, after treatment with combretastatin (30, 60 nmol/L), the activation levels of caspase-3 and caspase-9 in H460 cells were significantly increased, specific cleavage of polyadenosine diphosphate ribose polymerase (PARP) occurred, and the expression of spindle checkpoint proteins Bub1 and Mad1 was abnormal. It indicated that combretastatin can induce apoptosis in H460 cells through the mitochondrial apoptosis pathway.

2.3 Inhibiting tumor angiogenesis Li Y *et al.* [8] used MTT assay, Transwell assay, and tube formation assay to detect the effect of combretastatin on angiogenesis related functions of human umbilical vein endothelial cells (HUVECs). The results showed that combretastatin inhibited VEGF induced proliferation, migration, and capillary like tube formation of HUVECs in a concentration dependent manner (0.1, 0.5, 1, and 5 μmol/L). The effect of combretastatin on angiogenesis *in vivo* was detected using a chick chorioallantoic membrane (CAM) model. The results showed that compared with the control group, the number and density of neovascularization branches in the CAM model were significantly reduced after treatment with combretastatin, indicating that combretastatin can effectively inhibit angiogenesis *in vivo*. Further detection was conducted through protein immunoblotting and enzyme-linked immunosorbent assay (ELISA), and the results showed that combretastatin can exert anti-tumor angiogenesis by inhibiting the VEGF/VEGFR-2 signaling pathway.

Shi Y W *et al.* [9] conducted *in vivo* imaging observation experiments to detect the effect of combretastatin on angiogenesis in transgenic zebrafish embryos. The results showed that combretastatin inhibited the formation and branching of interstitial blood vessels in zebrafish embryos in a concentration dependent manner (7, 8, and 9 ng/mL). The effect of combretastatin on angiogenesis in adult zebrafish pectoral fin regeneration model was detected through histomorphological observation experiments. The results showed that after intraperitoneal injection of combretastatin (5, 10, and 20 mg/kg), compared with the control group, the number of vascular plexuses formed in zebrafish pectoral fin regeneration gradually decreased with increasing drug concentration, and angiogenesis was significantly inhibited. Further real-time fluorescence quantitative PCR experiments were conducted to detect the effect of combretastatin on the expression of genes related to angiogenesis in zebrafish. The results showed that combretastatin can inhibit angiogenesis in zebrafish by regulating the Notch signaling pathway.

3 Anti-inflammatory effect

Inflammation is a defensive response of the body to pathogenic factors, but excessive or sustained inflammation can lead to various diseases such as rheumatoid arthritis and neuroinflammation. In recent years, studies have found that combretastatin has good anti-inflammatory activity, and its mechanism of action is mainly related to regulating inflammatory signaling pathways and inhibiting the release of pro-inflammatory cytokines.

Davydova M P *et al.* [10] tested the anti-inflammatory activity of combretastatin in mice through foot swelling experiments. The results showed that combretastatin inhibited histamine induced foot swelling in mice in a concentration dependent manner (10, 20, and 40 mg/kg). The swelling inhibition rates were 28.5%, 42.3%, and 56.7% at 1 h after administration, and increased to 35.2%, 51.8%, and 68.4% at 3 h after administration, respectively. The anti-inflammatory effect of combretastatin was detected through mouse skin vascular permeability experiments, and the results showed that the abnormal increase in vascular permeability was significantly inhibited. Further inflammatory mediator detection experiments were conducted to detect the effect of combretastatin on the synthesis of inflammatory factors. The results showed that compared with the control group, the synthesis level of prostaglandin E2 (PGE2) in mice treated with combretastatin (40 mg/kg) decreased by 62.1%, indicating that combretastatin can exert anti-inflammatory effects *in vivo* by reducing the synthesis and release of inflammatory mediators.

Kadioglu O *et al.* [11] used enzyme-linked immunosorbent assay (ELISA) to detect the effect of combretastatin on the secretion of inflammatory factors in RAW264.7 macrophages. The results showed that combretastatin inhibited the release of inflammatory factors in a concentration dependent manner (1, 5, and 10 μmol/L). At a concentration of 10 μmol/L, the secretion levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were reduced by 72.3% and 68.5% respectively when compared to the control group. Through cell immunofluorescence experiments, the effect of combretastatin on the localization of key molecules in the inflammatory signaling pathway was detected. The results showed that compared with the control group, LPS induced p65 protein nuclear translocation was significantly inhibited after combretastatin treatment. Furthermore, the effect of combretastatin on the expression of NF-κB signaling pathway related proteins was detected by protein immunoblotting experiments. The results showed that combretastatin could inhibit inflammatory response of macrophages by blocking the NF-κB signaling pathway.

4 Antifungal effect

Fungi are a type of heterotrophic microorganism with eukaryotic cell structure, with a wide variety of species and strong reproductive ability. The infection of their hyphae and spores can easily cause fungal diseases in animals, plants, and humans. Research has shown that combretastatin can exert antifungal effects by targeting fungal microtubule cytoskeleton, disrupting fungal cell structure, and interfering with their normal life activities.

Li Y *et al.* [12] tested the antifungal effect of combretastatin on

plant pathogenic fungi through mycelial growth inhibition experiments. The results showed that combretastatin had a half effective inhibitory concentration EC_{50} of 12.5 and 18.3 μg/mL respectively against *Rhizoctonia solani* and *Magnaporthe oryzae*, and had significant inhibitory effects on the mycelial growth of both fungi. The effect of combretastatin on the spore germination of *M. oryzae* was detected through spore germination experiments. The results showed that combretastatin inhibited the spore germination of *M. oryzae* in a concentration dependent manner (10, 20, and 40 μg/mL). Compared with the control group, the spore germination rate decreased by 78.6% at a concentration of 40 μg/mL. Further *in vitro* microtubule polymerization experiments were conducted to investigate the antifungal mechanism of combretastatin. The results showed that combretastatin can specifically bind to fungal microtubule proteins, with a binding energy of −8.2 kcal/mol. This indicated that combretastatin can exert antifungal effects by targeting fungal microtubule proteins and inhibiting microtubule polymerization.

Roberson R W *et al.* [13] tested the antifungal effect of combretastatin on *Uromyces appendiculatus* through spore germination rate experiments. The results showed that combretastatin inhibited the summer spore germination of *U. appendiculatus* in a concentration dependent manner (0.65×10^{-5}, 1.3×10^{-5}, and 2.6×10^{-5} mol/L), and the spore germination rate at a concentration of 2.6×10^{-5} mol/L was only 18.7% of the control group. Through indirect immunofluorescence microscopy observation experiments, the effect of combretastatin on the microtubule skeleton of *U. appendiculatus* was detected. The results showed that compared with the control group, combretastatin (1.3×10^{-5} mol/L) treatment resulted in significant depolymerization of microtubule skeleton. The effect of combretastatin on the hyphae of *U. appendiculatus* was tested through hyphal growth experiments. The results showed that compared with the control group, the hyphal growth rate of *U. appendiculatus* was reduced by 68.2% after treatment with combretastatin. It indicated that combretastatin can inhibit spore germination and hyphal growth of fungi by inducing fungal microtubule depolymerization and disrupting the cell skeleton structure.

5 Immunomodulation effect

Immunity is a physiological function of the human body that allows the body to recognize its own and non self components, thereby destroying and repelling antigenic substances that enter the body, as well as damaged cells and tumor cells produced by the body itself, in order to maintain human health. Immunity involves both specific and non-specific components.

Through spleen cell proliferation experiments, Pettit G R *et al.* [14] found that combretastatin promoted mouse spleen cell proliferation in a concentration dependent manner (0.1, 0.5, 1, and 5 μmol/L), with a 42.3% of increase in spleen cell proliferation rate compared to the control group at a concentration of 5 μmol/L. The ELISA results showed that compared with the control group, the secretion levels of interferon-γ (IFN-γ) and interleukin-2 (IL-2) in mouse spleen cells were significantly increased after treatment with combretastatin (1, 5 μmol/L). The mixed lymphocyte reaction experiment showed that compared with the control group, the proliferation rate of T cells stimulated by allogeneic antigens was increased by 56.7% after treatment with combretastatin (5 μmol/L). It indicated that combretastatin can positively regulate the immune function of the body by promoting the proliferation and activation of immune cells, upregulating the secretion of immune related cytokines.

Smith A B *et al.* [15] used flow cytometry to detect the regulatory effect of combretastatin on macrophage polarization in mice. The results showed that combretastatin promoted macrophage polarization towards M1 type in a concentration dependent manner (0.2, 1, 5 μmol/L), with a significant increase in the expression rate of M1 type marker CD86 and a significant decrease in the expression rate of M2 type marker CD206. The results of real-time fluorescence quantitative PCR experiments showed that compared with the control group, the mRNA expression of M1 type gene was significantly upregulated and the mRNA expression of M2 type gene was significantly downregulated in mouse bone marrow macrophages treated with combretastatin. Its immunomodulation effect was further tested through *in vivo* tumor loading experiments. The results showed that compared with the control group, the infiltration of M1 macrophages and CD8 + T cells in tumor tissues of tumor bearing mice significantly increased after treatment with combretastatin. It indicated that combretastatin can enhance the body's anti-tumor immune response by regulating macrophage polarization, and reshaping the tumor immune microenvironment.

6 Summary and prospects

As an active substance from natural sources, combretastatin has shown good biological activity and high potential for drug development in anti-tumor, anti-inflammatory, antifungal, immunomodulation and other aspects. As a typical microtubule inhibitor and tumor vascular blocker, combretastatin can significantly inhibit tumor angiogenesis[16]. After optimization with nano formulations such as liposomes and polymer bonds[17], it has outstanding research value and broad clinical translation prospects in targeted therapy for solid tumors. At present, the research on combretastatin has clarified some of its action mechanisms, but there are still many areas that need to be explored. In the future, further research can be conducted on its dose-effect relationship and targeted delivery strategies in different disease models to enhance bioavailability and tissue selectivity. Simultaneously, it could explore the molecular mechanisms underlying the synergistic effects of multiple activities and conduct research on combination therapy[18], to broaden the scope of clinical applications.

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developed. The method is stable and reliable, and can provide a scientific basis for the quality control and further development and utilization of pomelo peel.

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