Research Progress on the Antitumor Mechanisms of Nano-Realgar

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Abstract: *Realgar is a sulfide mineral realgar of the realgar family, which is widely used in traditional Chinese medicine. In modern clinical research, it has been applied to leukemia and tumor treatments. However, clinical application of realgar is limited due to its insolubility and low bioavailability. This problem may be solved by using realgar nanoparticles which have shown unique advantages. In this paper, the ancient and modern applications of realgar and the preparation method of realgar nanoparticle are summarized. The antitumor mechanisms of realgar nanoparticles in recent years are explored to provide a reference for the in-depth research of realgar in the field of tumor therapy.*

Keywords: Realgar, As4S4, Antitumor, Mechanism, Nanoparticle.

1. Introduction

Cancer, as one of the leading causes of disease-related mortality in all countries today, imposes a significant burden on global public health and severely hinders the increase in human life expectancy [1]. The search for effective treatments to slow and curb cancer initiation and spread is a pressing matter [2]. Traditional Chinese Medicine (TCM) plays an important role in cancer treatment, with multiple research findings confirming that TCM oncology demonstrates unique advantages under the principles of syndrome differentiation and precision treatment [3]. Realgar is a mineral drug in TCM, primarily composed of arsenic sulfide, and is widely used in the treatment of dermatological diseases, blood disorders, and cancer [4]. In recent years, research into the anticancer mechanisms of realgar and the development of new formulations to reduce toxicity and enhance efficacy has become a focus. This article summarizes research findings on realgar to provide a reference for further investigation and clinical application.

2. Traditional Applications of Realgar and Preparation Methods of Nano-Realgar

Since its first mention in "Shennong bencaojing," realgar frequently appears in ancient TCM texts as a primary component in numerous formulas for both internal and external use, validated through long-standing application.

2.1 Applications of Realgar in Traditional Medicine

Originally used as a pigment, realgar's earliest medicinal use was recorded in "prescriptions of fifty-two diseases" for treating scabies. "Shennong bencaojing" states it: "Counteracts the poison of all creatures, surpasses the five weapons. Refined and taken, it lightens the body and confers immortality." Wu Kun [5] in "Yi Fang Kao" noted: "Realgar's qi is potent; it can ward off evil and malign influences." Li Zhongzi [6] in "Ben Cao Tong Xuan" described it: "Pungent, warm, toxic; a liver meridian agent. It mobilizes liver qi, drains liver wind, eliminates accumulations of fluids, resolves all poisons, wards off all evils, kills all parasites, interrupts malarial disorders, treats snake bites, and

can transform blood into water." In the Eastern Han Dynasty, Zhang Zhongjing's "Jin Gui Yao Lue" included realgar for expelling pathogenic factors, using fumigation externally for genital and anal ulcers, and internally in formulations like "Shengma Biejia Tang" for treating toxic conditions. "Ben Jing Feng Yuan" [7] summarizes earlier uses: "Qian Jin Fang" used realgar powder topically for furuncles and malignant sores; "He Ji Ju Fang" included it in "Jiu Zheng Wan" combined with scorpion tail and croton for alcoholic abdominal masses; "Dou Zhen Zheng Zhi" used realgar and "Zi Cao" for pox-related sores; "Sheng Ji Zong Lu" prescribed realgar mixed with pig bile for tinea capitis. These applications demonstrate realgar's extensive internal and external use in ancient China for treating injuries and diseases. Approximately two thousand years ago, Greeks used realgar for various ailments, including abscesses, sores, epilepsy, and malaria [8]. In ancient Indian Ayurvedic medicine, realgar was used to treat certain chronic diseases [9].

2.2 Preparation Methods of Nano-Realgar

Realgar is a sulfide mineral with the chemical composition As_4S_4. Due to its poor water solubility, traditional preparation methods are cumbersome. Furthermore, upon ingestion, it is poorly absorbed by the gastrointestinal tract, resulting in low bioavailability and necessitating large oral doses, which carries potential toxicity risks [10]. Experimental evidence confirms that nano-realgar exhibits significant antitumor efficacy with no apparent toxic side effects [11]. Several methods exist for preparing nano-realgar:

(1) High-Energy Ball Milling: Wang Xiaobo et al. [12] prepared realgar particles smaller than 100 nm using temperature-controlled, inert atmosphere high-energy ball milling. However, due to their minute size and high surface energy, the particles tend to agglomerate.

(2) Jet Milling: Utilizes high-speed gas streams to cause particle-particle and particle-wall collisions, impact, and friction, achieving size reduction.

(3) Microfluidization: Zhan Xiuqin et al. [13], with the aid of dispersants, reduced the aggregation of nano-realgar, effectively improving particle dispersion and stability.

(4) Solvent Transfer Method: Ning Ning et al. [14] prepared a nano-realgar suspension in the dark under a high-purity nitrogen atmosphere. By mixing acetone, 1,2-propanediol, and carbon disulfide, they continuously dissolved and refluxed realgar in a Soxhlet extractor. After evaporating acetone and carbon disulfide from the crude product and concentrating under reduced pressure, they successfully prepared a nano-realgar suspension in 1,2-propanediol with an average particle size of 159.0 nm and high quality.

(5) Chemical Precipitation: During preparation, biomacromolecules serve as templates to precisely control the morphology and particle size of nano-realgar, yielding products with excellent performance.

(6) Thin Film Dispersion-High Pressure Homogenization: Wang Ziyu et al. [15] organically combined chemical precipitation with thin film dispersion-high pressure homogenization to prepare nano-realgar liposomes. These liposomes exhibited good dispersion, a nearly spherical/oval shape, uniform particle size, and an average diameter of 102.3 nm. With the progressive development of nanomedicine technology, nano-realgar, offering reduced toxicity and enhanced efficacy, holds promise for greater application in solid tumors [16].

3. Anticancer Mechanisms of Nano-Realgar

3.1 Treatment of Esophageal Cancer

China has a high incidence of esophageal cancer, accounting for approximately half of the new global cases annually [17]. Research suggests realgar is a potential therapeutic agent for esophageal cancer. Zhang Xuelian et al. [18] found that realgar inhibited the proliferation of esophageal cancer Eca109 and KYSE150 cells, induced ferroptosis, and upon activation of the ferroptosis pathway, effectively suppressed cancer cell migration, invasion, proliferation, and metastasis. Chen Fazhang et al. [19], studying human esophageal cancer cell lines Eca109 and KYSE150, confirmed that realgar inhibited cell proliferation, migration, and invasion in a concentration- and time-dependent manner, inducing ferroptosis, indicating its potential as a therapeutic drug. Zhao Jing et al. [20] revealed that realgar may regulate the Bcl-2/Bax ratio, promote intracellular reactive oxygen species (ROS) generation, decrease mitochondrial membrane potential, and through these combined effects, effectively activate the Caspase-3-mediated apoptotic pathway, inhibiting esophageal cancer cell proliferation.

3.2 Treatment of Lung Cancer

Progress has also been made with nano-realgar in lung cancer. Wang Weiwei et al. [21] found that co-culturing nano-realgar with lung cancer A549 cells inhibited the expression of E-cadherin and integrin β1, significantly inducing apoptosis and suppressing cell invasion and metastasis. Li Huijie et al. [22] demonstrated that nano-realgar targets hypoxia-inducible factor (HIF) effector molecules, regulating the metabolic reprogramming of lung cancer stem cells, thereby inhibiting their invasion and metastasis. Wang Shengmei et al. [23] prepared realgar nanoparticles using a biotemplate method with polyvinylpyrrolidone K30 as a carrier, which reduced

Survivin expression and induced apoptosis in A549 cells. Yang et al. [11] found that nano-realgar may inhibit lung cancer stem cell viability and promote glucose metabolism by downregulating HIF-1α and the PI3K/Akt/mTOR pathway, affecting proteins related to metabolic reprogramming. Lee et al. [24] combined realgar with traditional herbs, finding that the arsenic-herb mixture exhibited significant cytotoxicity in A549 and H460 cells. Its anti-angiogenic and apoptosis-inducing effects may be mediated through inhibition of the STAT3/VEGF/CDK2 signaling pathway, suggesting its potential as an effective anticancer agent.

3.3 Treatment of Hematological Diseases

Studies indicate that realgar demonstrates good safety, low toxicity, and fewer adverse effects like myelosuppression in treating acute promyelocytic leukemia (APL) [8]. Its mechanisms may involve downregulating Bcl-2 and p53 gene expression, degrading the PML-RARα fusion protein, inhibiting cyclooxygenase-2 (COX-2) activity and expression, reducing Bcr-Abl protein levels, and downregulating P-glycoprotein/multidrug resistance-associated protein [25]. Wang Yuan et al. [26] showed that treating NB4-R1 cells with As_4S_4 induced cell cycle arrest, interfered with cell proliferation, exerted cytotoxic effects, and influenced the expression of BCL-2, BAX, and Caspase-3 proteins, inducing apoptosis. Fang et al. [27] successfully prepared epigallocatechin gallate-loaded realgar nanoparticles (EGCG-RNPs) with an average size of 200.30±1.23 nm. Uptake and efflux experiments suggested that EGCG-RNPs enhance the antitumor effects of realgar, possibly by increasing its uptake and prolonging its retention time in HL-60 cells.

Realgar also shows prominent therapeutic effects in chronic myeloid leukemia (CML) [28]. Wang et al. [29] applied nano-realgar to a refractory AML mouse model, finding that it inhibited HDAC activity, induced multilineage differentiation and apoptosis of AML cells, and significantly prolonged the survival time of model mice. Wang et al. [30] treated CML cell lines with nano-realgar, showing significant inhibition of proliferation in K562, K562/AO2 cells, and primary bone marrow cells from CML patients. The mechanism involved downregulation of intracellular ROS levels, decreased expression of HIF-1α, induction of autophagy, degradation of the anti-apoptotic BCR-ABL gene, and induction of CML cell differentiation. Concurrently, apoptosis and cell cycle arrest induced by nano-realgar were significantly higher than with conventional realgar, with the former showing approximately 178 times the cytotoxicity. This research suggests nano-realgar is a potential drug for leukemia treatment.

3.4 Treatment of Breast Cancer

Breast cancer is a common malignancy in women. Nano-realgar, as an effective antitumor agent, is widely studied for breast cancer treatment. Li Xiurong et al. [31] found that nano-realgar effectively inhibited the migration and invasion of breast cancer MCF-7 cells, demonstrating significant anti-metastatic effects. Further research by Li Xiurong et al. [32] revealed that treating MCF-7 cells with nano-realgar reversed epithelial-mesenchymal transition (EMT), effectively counteracting metastasis in a concentration-dependent manner. The mechanism involved

upregulation of E-cadherin (E-cad) expression, downregulation of HIF-1α, and reversal of Snail mRNA expression. Wang Haimei et al. [33] applied nano-realgar clinically to treat ulcerated wounds from breast cancer, achieving significant efficacy by promoting wound healing and improving patient outcomes and long-term survival rates. Ming Xiaofang et al. [34] demonstrated that acid-processed nano-realgar (water milling) effectively inhibited the proliferation of three breast cancer cell lines in a time- and dose-dependent manner. The autophagic cell death observed in MDA-MB-435S cells might occur via a non-PI3K-Akt-mTOR pathway. Xi et al. [35] discovered that nano-realgar significantly inhibited the migration and invasion of mouse mammary cancer cells in vitro and suppressed lung and liver metastases in vivo, potentially linked to decreased MMP-2 and MMP-9 expression and inhibition of tumor angiogenesis. Wu Jun et al. [16] found that nano-realgar significantly inhibited the proliferation of breast cancer stem cells and effectively reduced the proportion of the CD44+/CD24- cell subpopulation.

3.5 Treatment of Gynecological Tumors

Realgar demonstrates considerable efficacy in gynecological oncology. Qin Yan et al. [36] showed that nano-realgar induces apoptosis in human ovarian cancer SKOV3 cells, potentially by regulating apoptosis-related factors like the Bcl-2/Bax ratio. Ma Shuyun et al. [37] confirmed that nano-realgar inhibits Skov3 cell proliferation and promotes apoptosis, possibly by inhibiting intracellular Bcl-2 protein expression and upregulating Bax expression. Further exploration by Ma Shuyun et al. [38] suggested that nano-realgar may exert significant pro-apoptotic effects on COC1 cells by enhancing Bax and Caspase-3 mRNA levels while reducing the expression of the anti-apoptotic protein Bcl-2. In cervical cancer research, Liu Rong et al. [39] treated human cervical cancer Siha cells with a nano-realgar suspension, finding that it inhibited cell proliferation and enhanced apoptosis by suppressing the expression of human papillomavirus type 16 E6 and E7 oncogenes, achieving antitumor effects. Li Lijie [40] reported that nano-realgar exhibited significant inhibitory effects on Caski cells, moderate inhibition on Hela cells, and relatively weaker effects on C33A cells. Li Lijie et al. [41] treated three cervical cancer cell lines with varying concentrations of nano-realgar, showing effective inhibition of proliferation and induction of apoptosis. High concentrations notably induced G2/M phase arrest in HPV-positive cervical cancer cells.

3.6 Treatment of Skin Cancer

Realgar exhibits particularly significant efficacy in external use. Lin Yanyan [42] found that nano-realgar significantly inhibited the proliferation of human skin squamous cell carcinoma A431 cells in a dose-dependent manner, simultaneously suppressing tumor cell growth by inhibiting angiogenesis. Synergistic effects were observed when combined with cisplatin, enhancing antitumor activity. Qi Yuanfu et al. [43] discovered that nano-realgar inhibits proliferation and induces apoptosis in A431 cells, potentially by promoting Caspase-3 expression and inhibiting Survivin expression. Further studies by Qi Yuanfu et al. [44] indicated that nano-realgar induces apoptosis in A431 cells, possibly

through upregulating p53 expression and downregulating Bcl-2 expression, with a synergistic effect when combined with cisplatin. Liu Zhaidong et al. [45] clinically used nano-realgar to treat ulcerated tumor wounds, achieving good results with reduced ulcer size.

3.7 Treatment of Gastric Cancer

In gastric cancer research, Zhang et al. [46] found that treating human gastric cancer cell lines AGS and MGC803 with realgar upregulated KLF4 and E-cadherin expression, while downregulating β-catenin, VEGF, CD34, and Sp1 expression. This revealed that As4S4 treatment reduces the cells' ability to adhere and disrupt the basement membrane, effectively inhibiting tumor cell migration and angiogenesis. Zhang et al. [47] combined realgar with JQ1, cisplatin, irinotecan, or celecoxib in gastric and colon cancer cells, observing potent antitumor effects. Notably, As4S4 and JQ1 showed synergistic activation of p53 expression and inhibition of c-Myc expression, while combinations with cisplatin and celecoxib activated multiple apoptotic pathways. Zhang Lian [48] proposed that arsenic sulfide may enhance adhesion and reduce distant metastasis by upregulating KLF4, an upstream target gene of MMP-2/E-cadherin, and inhibiting angiogenesis, thereby effectively suppressing gastric cancer cell invasion and metastasis. Zhang et al. [49] discovered that realgar inhibits cancer cell proliferation via the NFATc3/c-Myc pathway, offering a new strategy for gastric cancer treatment. Tan et al. [50] combined realgar with the BRD4 inhibitor JQ1 in gastric and colon cancer cells, finding synergistic downregulation of nuclear factor of activated T-cells (NFATc3), upregulation of apoptotic proteins, and substantial induction of apoptosis via the mitochondrial pathway, enhancing the cytotoxicity of BET inhibitors and showing potential therapeutic advantages. Hu et al. [51] found that realgar downregulates the circRNA_ASAP2 / Wnt / β-catenin pathway, exerting antitumor effects and inhibiting gastric cancer cell proliferation and metastasis.

4. Discussion and Prospects

Traditional Chinese Medicine has a millennia-long history in preventing and treating tumors with proven efficacy [52]. In ancient China, realgar was used early on for conditions resembling abdominal masses and accumulations. The reviewed applications and mechanistic studies indicate that realgar achieves anticancer effects by inhibiting tumor cell proliferation and migration, promoting apoptosis, and suppressing tumor angiogenesis. This multi-system, multi-target mechanism grants realgar an important position in cancer therapy and underlies the TCM principle of "treating different diseases with the same method." However, due to current experimental limitations, studies are often based on 2D cell culture systems, and mechanistic explorations remain relatively narrow, typically reflecting anticancer effects from a single perspective, hindering a comprehensive understanding of realgar's anticancer mechanisms. Therefore, a primary future research direction is the comprehensive characterization of these mechanisms. The key to solving this may lie in constructing 3D tumor models that better simulate the tumor microenvironment.

Furthermore, realgar's poor solubility, low bioavailability,

and concerns regarding teratogenicity, mutagenicity, and carcinogenicity have historically limited its application in oncology [10]. With the development of nanomedicine in TCM, reducing realgar particle size to below 100 nm yields a product with low toxicity, high efficacy, and improved solubility, demonstrating superior antitumor effects. Moreover, synergistic use with other anticancer drugs significantly enhances antitumor activity. However, more in-depth research is needed on how to utilize realgar materials more effectively. The research focus is on developing novel realgar formulations that enable high concentrations of realgar within tumor tissues while minimizing systemic effects.

Achieving safer and more effective clinical application of realgar requires the organic integration of characteristic TCM theories with modern technology to advance the modernization of TCM. With the support of modern science and technology, realgar is poised to demonstrate even more remarkable efficacy in the field of oncology.

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