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Research and development of the anticancer agent AOH1996 and the theoretical rationale for its combination with curcumin

Fangmei Yan

School of Pharmacy, Hunan University of Chinese Medicine, Changsha, 410208, China
1632517431@qq.com

Abstract: *Anti-cancer drug development is a major priority for global healthcare industries, aiming to improve both survival rates and quality of life for cancer patients. AOH1996, a novel anticancer agent, has drawn considerable interest due to its unique chemical structure and promising anticancer activity. This article reviews the progress of AOH1996 from basic research to clinical studies and explores its potential application in the integrative treatment of traditional Chinese medicine (TCM) and Western medicine. Based on a narrative review and analysis of existing literature, this paper first summarizes the basic research and mechanisms of AOH1996, then discusses its efficacy in animal and human trials. AOH1996 has demonstrated significant inhibitory effects across various cancer cell lines, as well as favorable safety and efficacy in animal models. Furthermore, this review discusses the theoretical rationale for combining AOH1996 with curcumin, highlighting mechanistic complementarity while acknowledging that experimental validation remains lacking. Based on current findings, we propose that AOH1996 may serve as an effective monotherapy, and its combination with curcumin—supported by complementary mechanisms—warrants further experimental investigation. Finally, this paper outlines future research directions, including expanding clinical trial scales and optimizing drug administration strategies, so as to provide a scientific basis for the application and development of AOH1996.*

Keywords: *AOH1996, PCNA inhibitor, anticancer drug, curcumin, combination therapy*

1. Introduction

Cancer remains one of the greatest challenges to human health, with rising incidence and mortality rates worldwide, placing a heavy burden on society. Although conventional therapies such as chemotherapy, radiation, and surgery have slowed cancer progression, they are often accompanied by side effects or drug resistance [1,2]. Targeted therapy and immunotherapy have emerged as attractive alternatives due to advances in molecular biology and medicinal chemistry. However, issues related to cost, accessibility, and adverse reactions persist across all treatment modalities, underscoring the critical need for new anticancer drugs.

As a novel antitumor agent, AOH1996 has shown great promise in preclinical trials for treating various cancer types. The drug targets proliferating cell nuclear antigen (PCNA) and effectively inhibits breast cancer, prostate cancer, brain cancer, ovarian cancer, cervical cancer, skin cancer, lung cancer, and other solid tumors. By inhibiting DNA replication and repair, AOH1996 selectively induces apoptosis in cancer cells while exerting minimal effects on normal cells [3]. Its unique chemical structure and mechanism of action open new avenues for anticancer drug development and precision medicine. Moreover, combining AOH1996 with traditional Chinese medicine (TCM) may hold therapeutic potential, although direct experimental evidence is currently lacking.

Given the global relevance of cancer treatment and the unique theoretical framework of TCM, this review summarizes the development of AOH1996 from basic research to clinical trials and discusses the theoretical rationale for combining AOH1996 with curcumin.

2. AOH1996 research and development process

The discovery of AOH1996 is based on detailed study about the process of cancer cells proliferation. Preclinical studies demonstrated that AOH1996 suppresses tumor growth without

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discernable toxicity at the tested doses, whether used alone or in combination [3,4]. Based on the cancer-associated PCNA isoform (caPCNA), researchers used structure-based rational drug design to optimize the lead compound AOH1160, yielding AOH1996 with improved metabolic stability and retained potency. Pre-experiments showed that AOH1996 could selectively bind and inhibit key protein functions, effectively preventing the growth and proliferation of cancer cells [4]. Further study verified the target of AOH1996 by CRISPR-Cas9 gene editing technology, and showed how it works – by interfering with the regulation of the cancer cell cycle via a key pathway [4].

Once the effect on biology was confirmed for AOH1996, a large number of preclinical studies were performed with this compound to assess its safety and effectiveness. AOH1996 has been used in vitro on several human cancer cell lines, such as in the case of lung, breast, and colorectal cancers, amongst others [3].

The results showed that AOH1996 could significantly inhibit the proliferation of cancer cells and showed a high selectivity index [4]. Subsequent tests in mouse xenograft models revealed that the drug not only significantly reduced tumor volume but also showed no obvious side effects, thus forming the premise of the human clinical trials [5].

The report shows that, after the positive results of preclinical research, AOH1996 has entered the stage of human clinical trials (NCT05227326). Findings from the initial stage of the study, which evaluated the drug's safety, tolerability, and pharmacokinetic profile, showed that AOH1996 was well tolerated at every tested dose and that no serious adverse reactions occurred. The trial has since expanded to include several Phase I and Phase Ib studies. Early data indicate that patients treated with AOH1996 often have stable or partial remission, but the actual efficacy still needs to be verified in larger studies.

The preliminary results with regard to AOH1996 from cellular models, rodent experiments, as well as early clinical trials seem encouraging; however, there are some shortcomings in these studies so far. Detailed information about chronic toxicity and PK profile of AOH1996 is lacking in more advanced animal models. Moreover, the specificity of AOH1996 in vivo remains unknown, including its off-target effects as well as interactions with other signal transduction pathways, which were beyond the scope of this study. Large prospective clinical trials would be necessary for further evaluation of the drug's safety and efficacy in other tumors or groups of patients.

3. Mechanism of action and pharmacological properties of AOH1996

3.1. Mechanism analysis

AOH1996 exerts its anticancer effect through a mechanism distinct from conventional chemotherapy: it selectively kills cancer cells by disrupting the cell cycle. It targets transcription-replication conflicts, which occur when the mechanisms controlling gene expression and genome replication collide. The experimental drug stops cells containing broken DNA from dividing in G2/M, and also inhibits erroneous DNA replication during S-phase. Thus, AOH1996 induces apoptosis in cancerous cells, but it does not affect the proliferation cycle of normal healthy stem cells. Moreover, AOH1996 exerts selective cytotoxicity against malignant cells (with at least 30 fold selectivity ratio in favor of tumor cell sensitivity over normal cell sensitivity) without appreciable toxicity toward nontumor cells, including human PBMCs, small airway epithelial cells (hSEACs) and neural crest stem cells (7SM0032), up to a concentration of 10 μM [3]. By targeting transcription-replication conflicts, AOH1996 induces cancer cell death through a mechanism distinct from conventional chemotherapy, which may reduce the likelihood of cross-resistance with existing agents.

3.2. Pharmacological characteristics

Early animal studies have revealed that by binding to PCNA in cells and interfering with the interaction between PCNA and the transcriptional machinery, AOH1996 can interfere with the transcription-dependent DNA replication process and induce cancer cell death [3]. Second, metabolic stability: compared with some other anti-cancer drugs, AOH1996 has higher metabolic stability in the body and is not readily degraded, which means that both as a single therapy and as a combination therapy, it can inhibit tumor growth without causing detectable side effects [4]. Third, regarding safety and tolerability: In preclinical studies, AOH1996 demonstrated a favorable safety profile, causing no discernable toxicity at up to six times its effective dose in mouse models [3]. Early Phase I data

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indicate that the drug was well tolerated at tested doses, with most adverse events being mild to moderate; however, detailed safety data from larger clinical cohorts remain pending. These results provide strong support for further clinical development. Fourth, Drug interactions: Since cancer patients have to use many drugs simultaneously, it is important for AOH1996 to understand the potential interactions between AOH1996 and other drugs commonly used. Initially, it was found that when used in combination with several common anticancer drugs there is no negative interaction and in some cases, a synergistic effect [3, 6].

3.3. Challenges for monotherapy and the potential of combination therapy for AOH1996 in the treatment of cancer

Despite its promising antitumor activity, AOH1996, like other targeted agents, may face the eventual emergence of acquired resistance during prolonged treatment, and the durability of response in the monotherapy setting remains to be established in larger trials. Preclinical studies have already shown that AOH1996 acts synergistically with several existing agents [3,6], supporting combination strategies as a rational approach to enhance efficacy and delay resistance. Among potential partners, the active constituents of traditional Chinese medicine, such as curcumin (Curcuma longa extract), are attractive candidates because of their multi-targeted, low-toxicity profiles. The anti-tumor mechanisms of curcumin and AOH1996 have great overlap and complementary merits.

AOH1996 may particularly interact with PCNA protein, and interfere in the basic processes of DNA replication and repair. AOH1996 selectively targets caPCNA and disrupts transcription-replication conflicts, leading to DNA damage, cell cycle arrest (S-phase and G2/M-phase), and apoptosis in cancer cells [3]. Curcumin, a multi-targeted natural compound, can induce apoptosis through several pathways independent of AOH1996's mechanism—including ROS production, Ca^{2+} elevation, modulation of cytochrome C and p21, and regulation of Bcl-2 family proteins (up-regulating Bax/inhibiting Bcl-2) [7, 8]. This multi-pathway apoptosis induction, combined with AOH1996's upstream targeting of DNA replication stress, creates a mechanistic complementarity that may enhance antitumor activity. In addition, curcumin's ability to inhibit P-glycoprotein (P-gp) [9] may reduce the efflux of AOH1996, thereby increasing intracellular drug concentration and potency. AOH1996 induces both S-phase and G2/M-phase cell cycle arrest [3, 4] and curcumin can reinforce this mechanism via other pathways inducing apoptosis such as ROS production, Ca^{2+} elevation, and caspase-3 activation, thus achieving the comprehensive inhibition of cancer cell proliferation [8]. Additionally, curcumin has a variety of pharmacological actions such as antioxidant, anti-inflammatory, anticancer, antiproliferative, anti-arthritic and antimicrobial activity [10]. This multi-level synergistic effect, combining AOH1996's precise targeting with curcumin's multi-pathway regulation, may theoretically enhance anti-tumor activity, overcome drug resistance, and reduce toxicity. Although curcumin has a poor bioavailability, it may still be overcome either by the optimization of dosage regimen or via new delivery system (e.g., adjuvants, liposomal curcumin, curcumin nanoparticles) [11] so as to maximize the potential therapeutic application of the combined treatment with AOH1996 and curcumin.

Although the synergistic effect and optimal application mode of curcumin and AOH1996 still require further experimental verification, their highly complementary mechanisms provide a highly promising direction for developing more effective and low toxicity integrated cancer treatment plans.

4. Theoretical foundation and applied research on combining Chinese and Western medicine for cancer treatment

4.1. Comprehension of the causes and developmental mechanisms of malignancies in traditional Chinese medicine

Cancer (malignant tumour) is one of the deadly malignancies which seriously threaten the healthy condition in humans ,and it is known to TCM for a long time, even during the Yin Dynasty or the Shang Dynasty, there is a record of the name of the disease “cancer”, but the name has not been popularly used for a period of time, the Internal Classic of Huangdi named it as “intestine abscess” and “tendon mass” based on the different location of the disease, while in the Classic of Nanjing it was termed “accumulation”, and Zhang Zhongjing referred to it as “mass”. In Treatise on the Sources of Diseases, there are descriptions about “stone abscess”, “stone caries”. During Yuan dynasty, Zhu Danxi elaborated the cause and symptoms of cancer for the first time, and Yang Shiying also described it as

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“Cancer, high and deep, rock cave shape. Poison root deep, perforated through the inside, most on the abdomen in men and most on the breast in women.” In the Ming and Qing dynasty, many doctors had reached a consensus and uniformly called various malignant tumors as "cancer", which has been used ever since.

From the perspective of TCM in tumor occurrence and development: tumors are mainly due to insufficiency of primordial Qi and invasion of evil. On one hand, the emergence of any disease is closely related to the relative weakness of healthy qi, as the classic maxim states: "when healthy qi resides within, evil cannot invade; where evil gathers, its qi is deficient" [12], and cancer is no exception. Due to long-term fatigue and injury, the zangfu function is weak, the healthy qi is weak, and the wei external function is dysfunctional, which cannot resist the external evil normally, thus providing an opportunity for the disease evil. As the "medical school must read" said, "the accumulation of also, the lack of positive qi and then evil to follow it." On the other hand, the six evils of "wind, cold, heat, dampness, dryness and fire" invade the human body when the evil is excessive and are also one of the important reasons for the occurrence of cancer. Furthermore, in recent years the concept of “cancer toxin” has been advanced to interpret the etiology of cancer from a different perspective. This cancer toxin is considered to be internally generated when healthy qi is deficient, resulting in a noxious accumulation within the body that is fierce and hard to restrain, obstructs the flow of qi, gradually consumes Yin and blood, and ultimately causes dysfunction of the zang-fu organs, thereby serving as a crucial factor in cancer development [12].

By means of the preceding study, it is evident that knowledge of Chinese medicine on cancer not only addresses its pathophysiological alterations but also extensively addresses its pathogenesis and therapeutic concepts, so offering important ideas and approaches for modern cancer treatment. Combining old and new knowledge, this method clarifies the biology of cancer and facilitates the investigation of more successful treatments.

4.2. The synergistic effect of Western medicine and Chinese medicine

Designing comprehensive plans by grouping the diagnosis and treatment team in multiple specialties including oncology, surgery, radiotherapy, Chinese medicine, rehabilitation, nutrition, and psychology—multidisciplinary tumor rehabilitation is a therapeutic model. With an eye on improving prognosis, with the goals of promoting healing, and improving patients’ quality of life, the model focuses on addressing functional disorders caused by malignancies as well as treatment itself; it emphasizes individual rehabilitation needs and is more patient-centred than the traditional disease-centric idea of an MDT meeting. While local and global guidelines provide standard Western medical treatment for malignant tumours, the application of traditional Chinese medicine (TCM), through syndrome differentiation, has a great significance for alleviating clinical symptoms and preventing recurrence and metastasis. We believe that the treatment methods between TCM and western medicine need to be complementary and integrate their advantages so as to achieve the best effect on rehabilitation. Curcumin combined with cisplatin can produce a synergistic antitumor activity. In cell experiments, in human lung cancer cell line A549, after curcumin(5~40μmol/L) was used alone or cisplatin(1~4mg/L) was used alone, the inhibitory rate of cell growth were respectively 6.5%~27.93% and 7.12%~26.88%; while when they are used together (at 20μmol/L curcumin+1mg/L cisplatin), the inhibition rate reached 28.37%, significantly higher than that in either single-drug group [13].

It provides a fresh route for tumor rehabilitation and acts as a useful guide for managing other major disorders. Developing a tumor rehabilitation model suited for the unique qualities of Chinese medicine depends on this combination of modern medicine with TCM, therefore fostering both therapeutic and social advantages.

4.3. Practical case analysis

In order to have a more thorough understanding about how to apply integrated Chinese and western medicines for treating cancers, here there are some concrete practical examples as follows. Example 1 is an integration between tradition and modernity, and case 2 is a possibility for technological innovation, both show that there is need to do “thorough treatment” in different aspects:

Actual Case 1: Clinical observation of patients with cancer and depression

Li Liling made clinical observation on cancer patients with depression in "Study on the Quality of Life of Cancer Patients Treated with Integrated Chinese and Western Medicine and Antidepressant

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Treatment" [14]. Seventy-six patients were enrolled and randomly assigned to either a treatment group or a control group. Both groups received the standard first-line chemotherapy regimen for cancer. On this foundation, the treatment group was additionally given antidepressant medication, specifically fluoxetine hydrochloride dispersible tablets (Lilly Suzhou Pharmaceutical Co.)., LTD.), orally once a day, the dose was between 20 and 40 mg, and oral Chinese medicine decoction and adjuvant psychotherapy were given according to syndrome differentiation. The entire observation period was 6 months until the patient died or reached the end point of observation at 6 months. The effectiveness and tolerability of fluoxetine hydrochloride in individuals with cancer-related depression were assessed using the Hamilton Depression Scale (HAMD), the Treatment Emergent Symptom Scale (TESS), and relevant laboratory examinations at 2 weeks, 1 month, 2 months, and 6 months following initiation of therapy. Patients' quality of life before and after drug administration was evaluated with the World Health Organization Quality of Life Scale (WHOQOL) and the Karnofsky Performance Status (KPS).

The results indicated that, after treatment, the HAMD score in the treatment group was significantly lower than that in the control group ($p<0.01$). Furthermore, the statistical analysis showed a significant difference in the overall effective rate between the two groups ($p<0.05$), with the treatment group achieving an effective rate of 78.12% compared to 26.63% in the control group. Furthermore, after treatment ($P<0.05$), the scores of World Health Organization Quality of Life Rating Scale (WHOQOL) and KPS were considerably raised, so indicating that integrated Chinese and western antidepressant therapy not only improved the remission rate of depressed symptoms of patients but also greatly enhanced their quality of life.

At half-year after intervention, the authors reported that participants from the experimental group showed a significant increase in the rate of symptoms alleviation compared to controls, such as lower tiredness, higher appetite and recovery of physical force; also, QOL score revealed significant improvement in general well-being, decrease of depression symptomatology and anxiety. The pathological examination result showed that more patients in experimental groups obtained PR or SD, suggesting that the Chinese medicine adjuvant therapy might be helpful in improving the effect of chemotherapy. This study has shown that integration of TCM into western therapies exerted synergic benefits on alleviating chemotherapy-related depression, as well as increasing QOL, thus providing guidelines for later integrative TCM treatments of AOH1996.

Actual case 2: Safety outcomes of Immune cell therapy in the treatment of malignant tumors

In November 2023, a group from Japan published an expert paper on the safety evaluation of immune cell therapy against malignant tumor disease in the prestigious journal "Cytotherapy" [15], which was led by collaboration between 39 medical institutions and discussed in detail about the AEs related to immune cell therapy using big data as real-world evidence, proving the good safety performance of tumor immune cell therapy without serious adverse effects;

As of September 30, 2022, more than 7 years, 3,839 patients with malignant neoplasm have been treated with immune cells and were between 1 year old to 95 years old (median: 64 years). At initial diagnosis,86.8% had an Eastern Cooperative Oncology Group performance status of 0 or 1; and 3,234 patients (84.2%) had advanced-stage or relapsed disease. Also,484 patients (12.6%) receive immune cell therapy for the prevention of disease recurrence, 507patients (21.3%) receive immune cell therapy without any combinations of therapies, while 873 patients (36.7%) were treated with immune cell therapy in combination with chemotherapy.

The research encompassed numerous cancer types, such as pancreatic, colorectal, lung, gastric, breast, cervical, ovarian, liver, and esophageal cancers. A summary of adverse events associated with cell therapy at a Japanese clinical establishment. The total number of all administered doses was 31,890 and the incidences of adverse events were 3.0% (960 cases). The incidences of adverse events were 1.8% (363 cases) in 19,661 doses (3,470 patients) of αβT cell therapy, 1.1% (9 cases) in 845 doses (175 patients) of γδT cell therapy and 1.6% (10 cases) in 626 doses (164 patients) of natural killer cell therapy.

The incidence of adverse events in 10,748 doses (1,416 patients) of dendritic cell (DC) vaccine treatment was 5.4% (578 cases), significantly higher than other treatments.

Further analysis found that adverse events associated with immune cell therapy were limited, not serious, and safe to use. Injection site reactions were the most commonly reported adverse events, with a total of 449 events, most of which were related to DC vaccine treatment. Among all other adverse events, fever (228 cases), fatigue (141 cases), and itching (131 cases) were frequently reported. In contrast, three patients experienced serious adverse events requiring hospitalization (fever, abdominal

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pain, and interstitial pneumonia), but these were weakly related to immune cell therapy and were considered to be an effect of treating the primary disease. The safety data of immune cell therapy in this study provided important reference for the design of the clinical study of AOH1996, especially in terms of the toxicity management of combination therapy.

For tumor therapy, we always hope that there will be more effective and safer anticancer drugs to help most cancer patients. AOH1996 has progressed to clinical trials, but its long-term efficacy and safety in humans remain to be confirmed in larger, controlled studies. Despite promising preliminary findings, the drug still needs to complete Phase II and Phase III trials before regulatory approval.

While relying on Western medical therapies, traditional Chinese medicine offers distinctive benefits in the treatment of cancer. Chinese medicine emphasizes syndrome differentiation and treatment, combines the patient's constitution and condition, and uses herbal medicine, acupuncture and other means for comprehensive conditioning. In recent years, integrated approaches that utilize both traditional Chinese medicine and Western medicine have been increasingly emphasized and applied in cancer therapy. Numerous studies indicate that the proper use of Chinese medicine can significantly reduce chemotherapy-related adverse reactions, boost immune function, and enhance patients' quality of life.

In addition to traditional chemo-therapeutic drugs, radiation therapy (radiotherapy) and surgery, there are new methods such as immunotherapy by using immune cells for the treatment of cancers, a fact which is backed up with abundant clinic trials and many case reports. The immune cell therapy targets the tumour cells through stimulating and amplifying the host immunity of patients, which mostly avoids accidental damage to healthy cells, minimizes the toxic side effects of treatment, and has large anticancer effects, which will benefit even more cancer patients.

Looking to the future, AOH1996 is not only expected to become a rising star in the field of anti-cancer but may also play an important role in the treatment of combining Chinese and Western medicine.

5. Conclusion and prospect

AOH1996 has shown significant anticancer activity by selectively targeting caPCNA and disrupting transcription-replication conflicts in cancer cells. Its unique mechanism may help delay drug resistance when used in combination with other agents. AOH1996 has fewer side effects compared to traditional chemotherapy drugs, making it suitable for adjuvant therapy in patients who cannot tolerate conventional chemotherapy. Its unique chemical structure, mechanism of action, and excellent pharmacological properties suggest that AOH1996 may become a more effective and safer anti-cancer agent. However, barriers and limitations remain. Current clinical evidence is sparse, and large-scale controlled trials are needed. Its exact mechanism of action requires further identification, and preclinical work on combination therapy has just begun. Drug resistance may limit long-term effectiveness, and individual patient differences (e.g., genetic polymorphisms or health status) may affect treatment response. Long-term safety and resistance profiles need further investigation through clinical trials. Future studies should explore combinatorial therapies with other anti-tumor drugs or standard therapy, develop personalized therapy approaches, and conduct detailed mechanistic studies to identify potential drug targets and pathways. In summary, AOH1996 has shown gratifying efficacy in preliminary experiments but requires more research and practical verification before widespread clinical use. This paper is expected to provide meaningful guidance to researchers, encourage greater participation in anti-cancer drug development, and offer additional concepts and approaches for future cancer therapies.

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