The application of artemisinin and its derivatives as anticancer drug

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Abstract: Artemisinin, a drug originally developed for the treatment of malaria, has been shown to possess properties that cause apoptosis and create reactive oxygen species in carcinogenic cells. It is used, most commonly, in combination with another drug. While the full potential of Artemisinin yet undiscovered, Artemisinin is used mainly to curse and fight against malaria as of now. Studies have demonstrated that in addition to directly killing cancer cells through causing reactive oxygen species (ROS) and apoptosis, certain derivatives of artemisinin have properties that decrease the resistance of cancer cells against other chemotherapeutic drugs such as cisplatin. By using these artemisinin combinations with other drugs, or just it's derivatives, it is possible to prolong the effective treatment period of chemotherapy against many different types of cancers like breast cancer. There is not much information and data dedicated in the possibilities of using Artemisinin and its derivative due to the common usage of ART against malaria. This will be a dedicated summary of the effectiveness of artemisinin against 3 types of cancers. The paper on artemisinin has important value for future anti-cancer applications.

1. Introduction

With the development of society, many diseases have been overcome by humans. However, cancer is still a big problem in the medical profession so far. In medicine, cancer refers to malignant tumors originating from epithelial tissues and is the most common type of malignant tumors. Cancer has biological characteristics such as abnormal cell differentiation and proliferation, loss of growth control, invasiveness and metastasis. The advancement of technology and the emergence of new drugs continue to advance anti-cancer research. Among them, the research of artemisinin in anti-cancer continues to deepen, and more and more experts and scholars are more and more interested in the application of artemisinin in cancer treatment. However, artemisinin was originally used to fight malaria.

As early as in the 16th century, until 1960 before the Vietnam War, antimalarial drugs developed rapidly, forming aromatic and heterocyclic methanol represented by quinine, 4-aminoquinoline represented by chloroquine, and heterocyclic aminophen represented by amodiaquine [1-2]. The good times did not last long, quinine and chloroquine side effects, and by then falciparum plasmodium had gradually developed resistance to them, malaria treatment had done more harm than good to the human body, and the war, intensified in the United States, Vietnam and the spread of the army, so the development of new anti-malaria drugs was considered a priority.

America then invested a great deal of manpower and material resources to study malaria, however Vietnam ask China for help because of limited condition. The participants Tu Youyou and her team had successfully screened annua antimalarial Chinese medicine in October 1971, in 1972 isolated from Chinese traditional medicine artemisinin malaria effective monomer, named artemisinin, and clinical trial success in 1973 [3]. In addition to its remarkable antimalarial effects, artemisinin has been found

to be a remarkable anti-tumor agent, containing a large number of active substances that can be used as new chemotherapeutic agents, and we will discuss its recent anti-tumor mechanism [4].

2. Discovery, Extraction and Synthesis of Artemisinin

2.1 The discovery of artemisinin

What did Tu Youyou and her team do during these six years? Chinese herbal medicine in China can be roughly divided into two categories. One is traditional Chinese medicine recorded in ancient Chinese herbal medicine and medical books, namely traditional Chinese medicine. The other is regional folk herbal medicine. Their team screened a variety of experimental agents for field trials in various provinces, starting with the folk discovery plant Artabotrys uncinatus, which also had high inhibition rates. Its roots are antimalarial, but this plant is scarce and unstable. Later in the group. Subsequently, the research team confirmed the antimalarial effects of artemisia annua after a second screening by Yu Yagang and Gu Guoming [5]. The first prescription of artemisia annua described in the table he listed was Ge Hong's Qiu Bei Ji Fang, which was used as follows: "One hold of artemisia annua, two liters of water, and the decoction was crushed into juice. Compendium of Materia Medica and Shennong Materia Medica Classics have records that artemisia annua can cure fever.

They used to make 95% ethanol extract of artemisia annua, but the malaria inhibition rate in mice was only 40%. Later, they switched to conventional ether extract, and the potency was significantly improved, and the malaria inhibition rate in mice reached 99%-100%, but the toxicity was relatively high. After the acid part was removed, a neutral ether extract with high antimalarial potency and low toxicity was obtained, which made new progress in antimalarial research of artemisia annua. artemisinin was extracted from artemisia annua [6].

The extraction methods of artemisinin include chemical preparation and biological preparation. Artemisinin is a sesquiterpene lactone compound containing peroxide bridge extracted from artemisia annua. There are three extraction methods: chemical synthesis, biosynthesis and extraction and purification [7].

2.2 Structure and function of artemisinin

The molecular formula is $C_{15}H_{22}O_5$ molecular weight 282.33 component content: C 63.81%, H 7.85%, Or 28.33% [8]. It has colorless acicular crystals, bitter taste and soluble in acetone, ethyl acetate, chloroform, benzene, glacial acetic acid, ethanol, methanol, ether and petroleum ether, almost insoluble in water [9-10]. It is a novel sesquiterpene lactone with a peroxide bond and δ -lactone ring [11]. It has a 1,2, 4-trioxane structural unit including peroxide, which is very rare in nature. Its molecule contains seven chiral centers [12-13]. Amorphane is A amorphane with cross-linked A and B rings, isopropyl groups have A trans-relationship with bridgehead hydrogen, and the A ring carbon frame in artemisinin is interrupted by an oxygen atom [14-15]. Artemisinin acts on its own, mainly by free radicals. For malignant tumor cells to reproduce, they need to continuously synthesize DNA, of which nucleotide reductase is the key, and the synthesis of which requires a large amount of iron to participate in the reaction, which is also necessary, hence the tumor surface has a lot of transferrin receptor iron ions [16-17]. Artemisinin, on the other hand, relies on the peroxide bridge structure in its own structure to react with ferrous ions and break off to produce oxygen free radicals, which can destroy tumor cell membranes and kill cells due to leakage of internal substances. The difference in iron concentration can also distinguish between normal cells and tumor cells, to determine the target of attack [18-19].

3. Anticancer Applications of Artemisinin

3.1 Application of artemisinin and its derivatives in the treatment of breast cancer

Artemisinin (ART) is a sesquiterpene lactone with a peroxy group extracted from the Chinese herb Artemisia annua, and artesunate (ARS) and bisartemisinin (DHA) are synthetic derivatives of artemisinin, which also have the anticancer effect of artemisinin. Artemisinins exert anti-tumor effects

by regulating the cell cycle, inducing apoptosis, inhibiting angiogenesis and suppressing tumor invasion and metastasis. To date, there is a great number of in vitro and in vivo studies, describing anticancer effects of ARTs with encouraging results. Also, several case reports documenting the reducing effect of ARTs on tumour size and growth were published. However, only a few clinical trials with oncological patients were completed and their results published. The most studied are patients with solid tumours: colorectal carcinoma, breast cancer, hepatocellular carcinoma and lung cancer. Different clinical trials mostly Phase 1 and 2 are in progress.

A study found that levels of caspase-8, caspase-9 and caspase-3 were significantly increased in artesunate (ARS)-treated breast cancer MCF-7 cells. The investigators discovered that artemisinin suppressed the proliferation of MCF-7 cells using inverted microscopy, as there was a substantial difference in the survival rate of treated and control cells. MCF-7 cells became spherical and tiny after being exposed to artemisinin, and they were then separated from the flasks. Caspases are a group of proteases with similar structure present in the cytoplasm. caspases are closely associated with eukaryotic apoptosis and are involved in the regulation of cell growth, differentiation and apoptosis. activated executor caspases lead to programmed cell death through the hydrolysis of caspase target proteins. In most cases, different anticancer agents eventually mediate a common apoptotic pathway through the activation of caspases [20]. And in the present study, to investigate the involvement of Capases in artemisinin-induced apoptosis, MCF-7 cells were exposed to different concentrations of artemisinin (5-200 µg. mL^{-1}) for 24h. Furthermore, to assess the time course of caspase activation, cells were treated with a fixed concentration of 25 µg. mL^{-1} and incubated for some time groups (i.e., 4, 8, 12, 18 and 24 h). Enzyme analysis showed that Caspase-8 and -9 activities in artemisinin-treated MCF-7 cells were significantly increased in a dose and time-dependent manner. However, exposure to 25 µg. mL^{-1} artemisinin resulted in the greatest increase in Caspase-8 and -9 activities. Interestingly, a sharp increase in caspase-8 activity was observed after 8 h of exposure to the effective dose of artesunate (25 µg. mL^{-1}), followed by a decrease thereafter. Similar results were obtained in the case of Caspase-9, but after 12 h. Furthermore, cells treated with 25 µg. mL^{-1} artemisinin activated Caspase-3, albeit to a lesser extent at different doses and times, with a significant increase in the level of activation of effector Caspase-3 observed at 50 µg. mL^{-1} and after 24 h of artemisinin exposure [21]. In summary, the study found that artesunate (ARS) can induce apoptosis in breast cancer cells via both the mitochondrial and death receptor pathways.

3.2 Application of artemisinin and its derivatives in the treatment of liver cancer

By constructing a mouse liver cancer model, researchers found that the expression of apoptosis-related proteins Caspase3 and p53 was significantly increased in the liver cancer tissues of mice treated with artemisinin. The p53 gene is a tumor suppressor gene that induces apoptosis in tumor cells [22]. P53 is a transcription factor that is maintained and activated in response to different genotoxic and cellular stress signals, including as DNA damage, hypoxia, oncogene activation, and nutritional deprivation, resulting in cell cycle arrest, apoptosis, senescence, and metabolic adaptation [23].

The researchers used a combination of dimethylnitrosamine (DEN)/carbon tetrachloride (CC14)/ethanol to induce tumor formation in mice, and after 18 weeks, they observed tumor formation in mice. The tumor-bearing mice worked well as a liver cancer model. The tumor-bearing mice were then randomly divided into three groups: the PBS group, the artemisinin group and the artemisinin + Pifithfin-α group, with five mice in each group. The other group of mice was injected with 50 mg/kg body weight of Pifithrin-α intraperitoneally on alternate days for 1 week, followed by 100 mg/kg body weight of artemisinin for 3 weeks. Three groups of mice were executed and the tumor tissues were separated from each group of mice. Five normal mice were taken as the blank group [24].

The results showed that the expression of apoptosis protein Caspase3 was significantly higher in the artemisinin group compared with the blank group and the PBS group. Meanwhile, the expression of p53 in liver cancer tissues under the effect of artemisinin was significantly increased compared with the blank group and PBS group, suggesting that artemisinin up-regulated the expression of p53 in tumor tissues. Researchers employed p53 protein inhibitors in combination with artemisinin in mice to show that the production of the apoptosis protein Caspase3 was considerably reduced in these mice,

further demonstrating the significance of p53 in regulating tumor apoptosis. To summarize, this study confirms that artemisinin regulates apoptosis of hepatocellular carcinoma cells through p53.

3.3 Cytotoxicity of artesunate and dihydroartemisinin against in vitro ovarian cancer cells

Ovarian cancer is a form of malignant cells that rapidly grow in the female ovaries. With around 12.5 cases per 100,000 women per year, it is a rare form of cancer but can be fatal if left untreated and if it's at a later stage [25]. Although rare, this type of cancer affects the female population of age 65 and over the most [26]. The symptoms of ovarian cancer are not clear and often have no symptoms in the early stages. The symptoms of late-stage ovarian cancer can include non-specific symptoms like weight loss and the loss of appetite. Although both chemotherapy and surgical removal work well, having mortality rates decline as much as 1-2% per year, very little has been published about other types of cancer treatments that are not describing one of the both listed previously [27]. It is established in 2016 that only about 20% of women with advanced-stage ovarian cancer can survive up to 12 years after treatment, leaving the rest 80% that failed to get successfully treated [28]. In response to such a malicious disease, the use of artesunate (ART) and dihydroartemisinin (DHA) seems to be a solution due to their effects on the overall growth of malignant cells.

Artesunate (ART) and dihydroartemisinin (DHA), both derivatives of artemisinin, were found to have a greater effect on cell viability in comparison to other artemisinin compounds [29]. As shown in Figure 1A, cell viability in AR780 and OVCAR-3 cells, which are both ovarian cancer cell lines. decrease the most with increasing concentrations of DHA (dihydroartemisinin) and artesunate. Although cell viability of IOSE144, which is a non-malignant cell line, also decreases with increasing concentrations of the ARS compounds, it is at a significantly lesser rate, suggesting that ARS compounds selectively target carcinogenic cells.

Table 1. IC20, 50, and 80 Values for DHA, ART, ARS, and ARM Compounds in AR780 and OVCAR-3 Carcinogenic Cell Lines, in Addition to Non-Carcinogenic IOSE144 Cell line [29].

Cell Line	Inhibitory Concentration	DHA (Concentration)	ART (Concentration)	ARS (Concentration)	ARM (Concentration)
A2780	IC20	0.83	0.73	7.68	2.08
	IC50	16.45	17.60	>500	53.90
	IC80	327.63	426.16	>500	>500
OVCAR-3	IC20	0.56	0.46	3.93	2.08
	IC50	6.58	6.86	342.51	38.79
	IC80	77.38	103.21	>500	>500
IOSE144 (Immortalized non-tumourigenic OSE cells)	IC20	1.84	5.60	>500	97.95
	IC50	106.03	>500	>500	>500
	IC80	>500	>500	>500	>500

In addition, as shown in the table in Table 1, the IC20, IC50, and IC80 values (the concentrations for 20, 50, and 80% inhibition of growth) of DHA and ART are lower in comparison to ARS and

ARM. This means that DHA and ART are the most cytotoxic artemisinins against these carcinogenic cells. However, while the IC values for DHA and ART are relatively similar for the carcinogenic cell lines, for the non-tumorigenic IOSE144 cell line, the inhibitory concentration values are significantly lower for DHA compared to ART. This suggests that DHA is actually the most effective at inhibiting carcinogenic cell growth while having a lesser impact on non-malignant cell growth. In the plots in Figure 1B, it can be seen that the higher doses of DHA have an increasing effect on cell inhibition, as they deviate the most from the control. This difference is lesser in the IOSE144 cell line, again suggesting that DHA selectively affects carcinogenic cells.

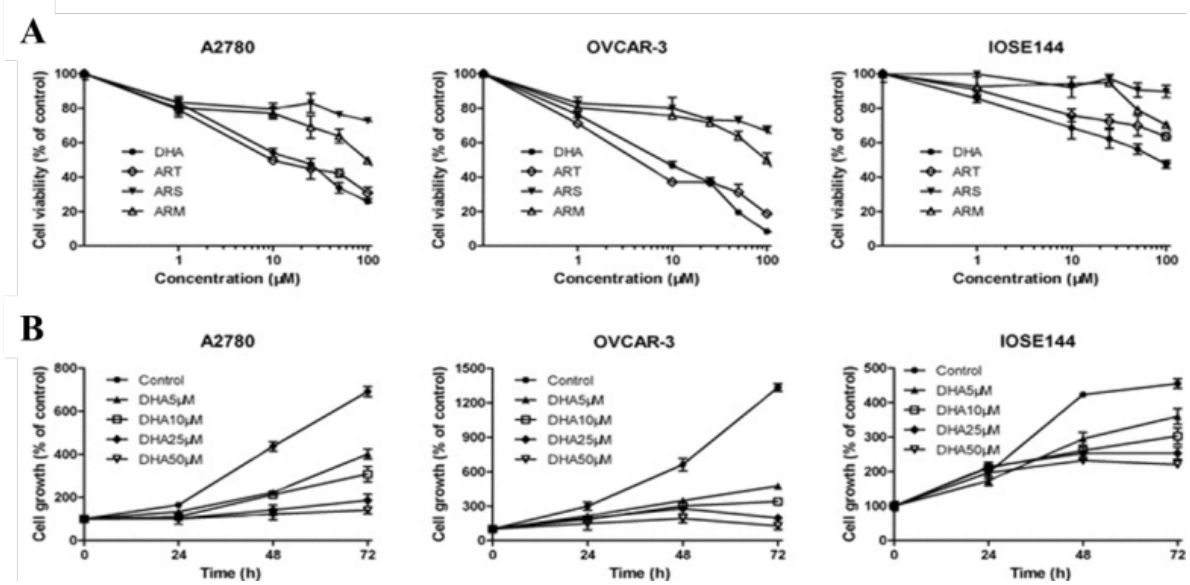

Figure 1. A: Plots of cell viability (% of control) for the compounds in the 3 cell lines. B: Cell growth in the 3 cell lines when treated with DHA of 5, 10, 25, and 50 μM concentrations in comparison to a control (non-DHA treated) [30].

4. Conclusion

Artemisinins have been shown to have a variety of functions, including induction of apoptosis, formation of reactive oxygen species in carcinogenic cells, and inhibition of tumor growth and metastasis, since their discovery. Artemisinin and its derivatives exhibit less hazardous side effects, a broad anti-tumor range, excellent safety, and enhanced efficacy when combined with other anti-tumor medications when compared to other anti-tumor drugs with similar efficacy. Artemisinin and its derivatives have a complex anti-tumor mechanism, but the number of relevant clinical trials and treatments is currently increasing, with promising outcomes. As a result, natural resource-derived medications have the potential to become novel anticancer drugs after years of study and testing.

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