

# The Anticancer Effects of Artemisinin and Two Key Derivatives Dihydroartemisinin and Artesunate

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**Abstract.** Compared to non-malignant cells, cancer cells are better suited to oxidative stress. Reactive oxygen species activity is assumed to be increasing, leading to higher oxidative stress in malignancies. The key derivatives of artemisinin are dihydroartemisinin and artesunate. Oxidative stress, activation of apoptosis, blockage of angiogenesis, and iron sagging are the main findings of artemisinin and its derivatives' anticancer actions. It has been established that the endoperoxide content of artemisinin and its derivatives is of crucial pharmacological significance and is the cause of its anticancer properties. The molecular structure further modification could be a possible way to improve the anticancer capabilities. These properties of artemisinin indicate that it is involved in the oxidative lipid damage that leads to cell death. It shows that the cytotoxicity of artemisinin in vivo is affected by many factors such as vitamin E, holotransferrin and C0-Q10. The vitamins are involved in cell metabolism and very often taken by cancer patients. Further study to investigate the possible impacts in vitro and vivo is necessary.

**Keywords:** Artemisinin; dihydroartemisinin; artesunate; anti-cancer.

## 1. Introduction

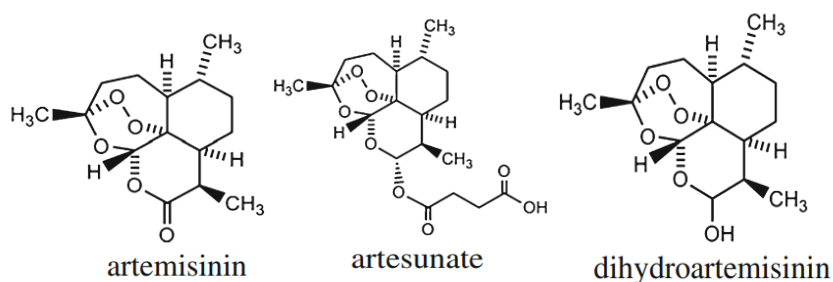
Cancer is the most mortal diseases of humankind for decades. Looking for a better therapeutic methods has been the most urgent and intensive research for scientists. Even many therapeutic methods have been developed, such as immune therapy, surgical therapy, radiotherapy, chemotherapy is still considered as the most useful approach for cancer treatment. However, in many cases, chemotherapy shows limited efficacy and significant side effects [1]. The ideal anticancer drug should be highly potent and specific enough to kill cancer cells, with no obvious or fatal toxicity to normal organs or absent at all.

Many research projects focused on discovering and developing new molecules to improve the current chemotherapy situation. Many natural plants have been investigated and discovered to be potential sources of compounds with significant anticancer properties and, more crucially, little in vivo adverse effects. Natural anti-malarial drug artemisinin, which also has anti-cancer properties, is one potential substance [2]. One of the few medications with a long history of usage as an antimalarial is artemisinin. It will be feasible to identify some potential directions for future research needs after this article summarizes the mechanics, advantages, regulations, and existing and future development of artemisinins.

## 2. Artemisinin and Its Derivatives

Qinghao (*Artemisia annua*), a herb used in Traditional Chinese Medicine, contains a natural plant chemical called artemisinin (ARS), which is typically employed as an antimalarial agent [1]. It has potent anticancer properties, according to recent studies [3]. More further on, it has few side effects and even effective for some tumor cell lines that show drug resistance. Sesquiterpene lactone artemisinin has a 1,2, 4-Tioxane ring structure (Figure 1) [4]. By nature, a short in vivo half-life and poor absorption characterize artemisinin (about 2.5 h), and limited solubility in water or oil [5]. Those pharmacokinetic properties of artemisinin limit the clinical applications. The study results show that the two most attractive derivatives are dihydroartemisinin (DHA) and artesunate (ART). DHA is the one of the major active metabolites obtained from the artemisinin. Now it can be produced through

semi-synthesis. In addition to these semi-synthetic molecules, several fully synthetic ART derivatives have been designed and synthesized, which have strong drug activity [4]. These substances were created with qualities resembling those of artemisinin's semi-synthetic derivatives. These anti-malarial drugs, which have good clinical efficacy and safety, include artesunate, artesunate, artemether, and ART (Figure 1) [6]. Among these synthetic derivatives, the most studied molecule is ART. It is easier to absorb than artemisinin, has a long half-life and low toxicity. The major of this article would be about the anticancer activities of ARS, DHA and ART and their mechanisms as well as regulations.



**Fig. 1** The chemical structure of ART and its derivatives [6]

### 3. Artemisinin's Anti-cancer Properties and Their Mechanisms

The main findings of anti-cancer capabilities of ART and its derivatives include (1) oxidative stress, (2) apoptosis induction, (3) angiogenesis inhibition, and (4) iron sagging [7]. It is more typical for artemisinin and its derivatives to play a significant part in the treatment of cancer by causing iron sagging in cancer cells and controlling cellular iron metabolism [8].

Compared to non-malignant cells, cancer cells may be more able to adapt to oxidative stress. Reactive oxygen species (ROS)-producing enzymes such as cyclooxygenase, lipoxygenase, and NADPH oxidase are expected to be active more frequently in tumors, which increases oxidative stress [9]. The main findings of anti-cancer capabilities of artemisinin and its derivatives include (1) oxidative stress, (2) apoptosis induction, (3) angiogenesis inhibition, and (4) iron sagging [5]. It is more typical for artemisinin and its derivatives to play a significant part in the treatment of cancer by causing iron sagging in cancer cells and controlling cellular iron metabolism [7].

In actuality, it has been established that the endoperoxide present in artemisinin and its derivatives plays a crucial pharmacological role in determining both their antimalarial and anticancer effects [3]. The features of artemisinin suggest that it contributes to the oxidative lipid damage that results in ferroptosis, which causes cell death. Most intriguingly, both initial cancer cells and drug-resistant cancer cells exhibit this potential. The endoperoxide bond might be activated through the reduction of heme (FPFeII) or ferrous iron(Fe(II)). Because of the higher iron content in tumor cells, the higher anticancer activity was observed. A major area of focus for the treatment and prevention of tumors is the management of iron and oxygen metabolism [9].

According to some research, ART with iron activation and its derivatives cause harm by releasing radical oxygen species with a carbon core that are strongly alkylating. Cell alterations in cancer cells treated with artemisinin, such as increased apoptosis, growth arrest, angiogenesis inhibition, and DNA, may be caused by free radicals. According to certain research, the toxicity of ART and its derivatives is also linked to abnormal cytokinesis, elevated levels of oxidative stress, and repressed tumor invasion, migration, and metastasis [10]. Artemisinin and its derivatives may have a selective effect on cancer cells as a result of ROS production. Because cancer cells contain less antioxidant enzymes, they are more susceptible to ROS damage. Hence, raising oxidative stress is a typical mechanical anticancer route. Translationally controlled tumor protein expression (TCTP), which is also believed to be a target for parasites, as well as endoplasmic reticulum stress and calcium metabolism appear to have an impact on the antitumor toxicity of ART and its derivatives [11]. Several malignancies and cell lines exhibit the common artemisinin-induced impact of apoptosis.

Apoptosis can be induced by DHA and ART both quickly [9]. The levels of anti-apoptosis (Bcl2) and pro-apoptosis (Bax) gene expression in cancer cell lines are correlated with this apoptosis. The cascade of events leading to cell death is thought to be significantly influenced by mitochondrial membrane damage. Several investigations have demonstrated how ART and its derivatives control the Bax/Bcl2 ratio to trigger apoptosis [10].

#### 4. Case Study of ART Performance in Breast Cancer

For selectively causing lysosomal programmed cell death in breast cancer cells, artesunate is a unique technique; nevertheless, it has limited impact on non-transformed mammary epithelial cells. The interruption of endolysosome transport, inhibition of autophagy, and increased generation of reactive oxygen species ahead of mitochondrial apoptosis are all components of artesunate-induced planned lysosome cell death. By conducting *in vivo* tests, it will be possible to assess whether artesunate increases endogenous innate immune responses as well as the effectiveness of iron supplementation in improving the specificity of ART in cancer cells, such as TNF-mediated mitochondrial apoptosis, and apoptotic effects of atypical, programmed cell death reported separately, as well as the antiangiogenic, signaling [11].

##### 4.1. Activation of Mitochondrial Apoptosis Using Artesunate

Conditions under which ART caused MCF-7 cells to die significantly as a function of concentration and duration in an experiment (Figure 2A). Krebs-Henseleit solution (KHS), an alkaline salt solution with a pronounced glucose content that has been proven to be cytotoxic to cancer cells, was used to cultivate MCF-7 cells at doses of 1, 10, and 20 g/ml with or without ART [11]. During the time points of 24 and 48 hours, the early and late apoptotic cells as well as the necrotic cells were labeled with the elimination dye YO-PRO-1, and the late apoptotic cells as well as the necrotic cells were labeled with PI [11]. Cell viability was then determined. Positive control for inducing apoptosis was the inflammatory cytokine TNF (43 ng/ml), and at 24 and 48 hours, glaring cell death was found. Apoptosis was induced using the inflammatory cytokine TNF (43 ng/ml) as a positive control, and at 24 and 48 hours, glaring cell death was found. During 24 and 48 hours after treatment, ART concentrations of 10 and 20 g/ml dramatically and dose-dependently induced cell death. Only after 48 hours did 1 g/ml ART significantly increase cell death.

Cell death brought on by ART is brought on by ROS. Early research has demonstrated the importance of ROS as a mediator and factor in the induction of cancer cell death by art. Hence, using H2DCF-DA (a living cell ROS indicator), other researchers assessed the effect of ART on MCF-7 cells' ROS levels during the first (18 h) phase of cell death signaling [11].

Cell death was assessed with two dyes when artesunate was 1, 10, and 20 ug/ml. Iron-catalyzed synthesis of artesunate Breast cancer cells experienced programmed cell death brought on by lysosomal reactive oxygen. Iron is closely related to the transport and utilization of oxygen in breast cancer cells, which further indicates that artemisinin-induced oxidative destruction of some functional structures in breast cancer cells programmed cell death is closely related. This was further confirmed in a second study that transferrin is also an important factor involved in cellular oxygen utilization and energy metabolism. The results showed that the artesunate induced breast cancer cell death is significantly correlated with the concentration.

Compared to cells treated with TNF, those treated with art showed a considerable increase in ROS levels. Cells were co-treated with trolox (TX), a water-soluble vitamin E derivative and effective hydroxyl radical scavenger, to ascertain the involvement of ROS in death signaling. TX stopped the formation of ROS brought on by art while having no effect on the ROS concentrations in cells treated with TNF [11]. Additionally, TX had no impact on TNF-induced cell death, but it greatly decreased Art-induced cell death at both 24 and 48 hours (Figure 2). These findings imply that TNF-caused cell death is not the result of ROS, but rather of Art-induced cell death. These findings imply that the

chemical makeup of the endoperoxide components of artemisinin and its derivatives is related to the anticancer action of antiretroviral therapy.

*Artesunate Triggers Lysosomal Cell Death*

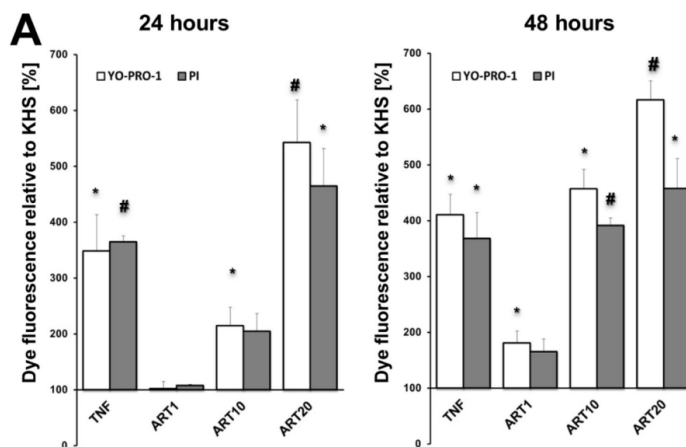


Fig. 2 Effects of ART concentration on lysosomal through iron catalysation [11]

**4.2. Induction of Molt-4 Cell Apoptosis Using Dihydroartemisinin**

Previous study demonstrates that artemisinin causes human cancer cells to undergo apoptosis. HA causes the death of Molt-4 cells and may have an effect on transferrin. Dihydroartemisinin's protracted impact on Molt-4 cells was investigated in this experiment.

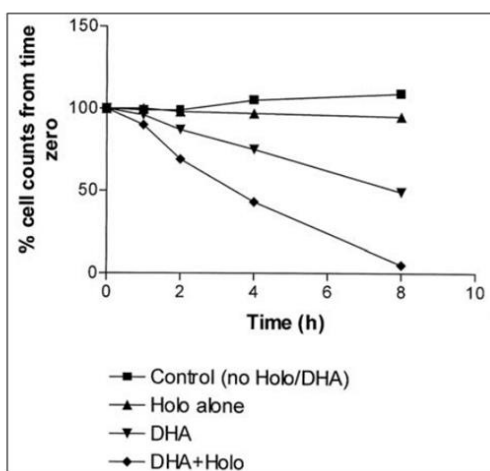


Fig. 3 Averaged percentage of Molt-4 cells counted from zero [12]

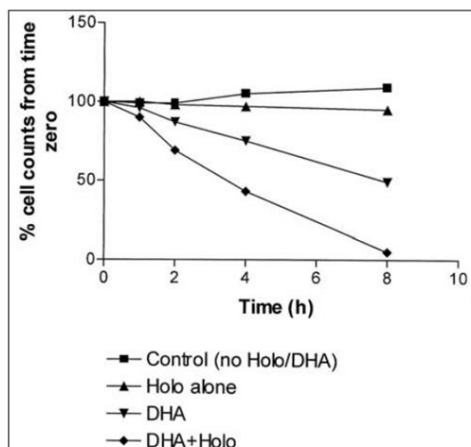


Fig. 4 Averaged percentage of Molt-4 cells showing apoptosis in 4 treatment groups [12]

The results are shown in Figure 3 and Figure 4. DHA treatment significantly reduced cell count and increased apoptosis of cancer cells. The addition of holotransferrin further enhanced cell apoptosis induced by dha. Changes in cell count and apoptosis scores were significantly different between total transferrin treatment alone and the control group. In both cases, total transferrin caused a slight decrease in cell count and an increase in apoptosis compared to untreated controls. The artemisinin derivative used in this study, DHA, is a more water-soluble and potent artemisinin analogue in cell culture. These results are consistent with earlier findings that DHA and other analogues of artemisinin carry an endoperoxide group and produce free radicals in the presence of iron [12,13]. These carbon-based free radicals may damage large molecules of cells, including DNA, leading to apoptosis. Total transferrin pretreatment significantly enhanced the apoptosis-inducing effect of artemisinin. This finding supports the idea that iron-induced free radical formation plays a major role in artemisinin cytotoxicity. This result also indicates that the anti cancer activities of ART is correlated with the chemical properties of the portion of endoperoxide in the artemisinin and its derivatives.

## 5. Conclusion

A naturally occurring chemical called artemisinin has a poor bioavailability and a low solubility in water or oil. Several new artemisinin derivatives have been discovered and synthesized. The results show that the most attractive two derivatives are DHA and ART. DHA is water soluble and easier to absorb than artemisinin. Some studies have shown that it has strong anticancer effects in against breast cells and Molt-4 cells.

Studies have shown that the cytotoxicity of different artemisinin derivatives varies greatly. These derivatives show different degrees and functions of anti-tumor activity. The conclusion is that different chemical structures have great influence on anti-tumor properties, and the modification of chemical structures may be a good direction to design artemisinin derivatives with stronger anti-tumor properties.

It has been established that the endoperoxide content of artemisinin and its derivatives is crucial pharmacologically and is the cause of its anticancer properties. The properties of artemisinin indicate that it contributes to the oxidative lipid damage that causes cell death. ROS production may be related to the selective effects of artemisinin and its derivatives on cancer cells. Cancer cells are more susceptible to damage from ROS. Heme (FPFell) or ferrous iron may help to initiate the endoperoxide link (Fell). Surprisingly, the artemisinin derivative dihydroartemisinin caused breast cancer cells to undergo apoptosis. transferrin had an effect on artemisinin derivatives, while transferrin itself was not cytotoxic, which greatly enhanced dihydroartemisinin cytotoxic play. It shows that the cytotoxicity of artemisinin in vivo is affected by many factors. Many vitamins are involved in cell metabolism and, such as vitamin B1, B2 promote sugar metabolism and vitamin C have antioxidant function, while vitamins D and E enhance immunity. These common nutrition products are often taken as supplements by tumor and cancer patients. It is worth exploring in the future.

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