The anticancer Potency of Artemisinin and Its Derivatives

Yimiao Lin1*, Zhixuan Song2, Zhian Xi3

1School of International Education, Beijing University of Chemical Technology, Changping District, Beijing 100029, P.R. China
2Liaoning Province Shiyan High School, Shenyang, Liaoning 110031, China
3Chemistry Department, the College of Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060, United States

*Corresponding author: Yimiao Lin, School of International Education, Beijing University of Chemical Technology, Changping District, Beijing 100029, P.R. China.
Email: 2019090121@mail.buct.edu.cn

ABSTRACT
Artemisinin and its derivatives (Artemisinins) have long been used as antimalaria drugs with considerable safety and efficacy. In recent preclinical researches, artemisinins also exert an anticancer potency via induction of programmed cell death and inhibition of cell growth. Artemisinins can be activated by heme to form Reactive Oxygen Species (ROS), which damage lipids or activate mitochondria-mediated pathway to induce caspase cascades. Artemisinins also get involved in the regulation of protein and gene expression to inhibit the VEGF signaling pathway that is responsible for cell growth and survival. In this review, we focus on current knowledge of the modulation of some detailed pathways that induce apoptosis and inhibit cell growth, including iron-dependent pathway, mitochondrial-mediated pathway, and VEGF signaling pathway. We also collect up-to-date researches to support their efficacy. With future researches and clinical investigation on artemisinins, more detailed molecular mechanisms and anticancer effects will be identified and confirmed.

Keywords: Artemisinins, anticancer, heme, mitochondria-mediated pathway, caspase, NF-κB pathway, PI3K/Akt/mTOR pathway
INTRODUCTION

Artemisinin, with a molecular formula C_{15}H_{22}O_{5}, is a sesquiterpene lactone containing a peroxide bridge. The first record of artemisinin (ARS) should date back to thousands of years ago in China. The treatment of malaria was described in a handbook called Zhouhou Beiji Fang by Ge Hong in Jin Dynasty. In 1967, the Chinese government established ‘National Project 523’ to discover value drugs from traditional Chinese plants. Tu Youyou, who was influenced by Zhouhou Beiji Fang in her childhood, showed interest in the leaves of *Artemesia annua* L. (sweet wormwood), and her group finally extracted ARS under cold temperature. (1)

![Figure 1. The structure of Artesunate (ART) and Dihydroartemisinin (DHA) are designed for better hydrophilic effect (2)](image)

Starting from the test in mouse malaria in 1971, the anti-malaria effect of ARS was gradually recognized by the world. The structure of the 1, 2, 4-trioxane ring in ARS leads to the quick killing of parasites. Then, considering the short half-life and incomplete course treatment of this drug, scientists began to study the combination of ARS with other drugs for the protection and prevention of drug resistance. (1) Researchers also designed new drugs such as DHA (Dihydroartemisinin) and ART (Artesunate), shown in figure 1 to promote metabolism in the human body.

The anticancer effect of ARS and its derivatives is studied by scientists in recent years. They are considered anticancer drugs due to the fact of the higher level of iron in cancer cells. ARS-type drugs can react with Fe (II) to create reactive oxygen species (ROS). These reactive radicals play a role in cell apoptosis, including cleavage of cell membrane and activation of caspases regulating DNA fragmentations. Also, scientists have found that ARS-type drugs, in the form of DHA, can regulate multiple signaling pathways to inhibit the VEGF-induced MAPK pathway which is responsible for tumor cell proliferation. (2) In this review, we will focus on some typical mechanisms of ARS-type drug-induced anticancer effects.

2. ART/DHA-induced apoptosis.

2.1 Formation of ROS.

When DHA enters the human body, it is activated by the free heme derived from the parasites digesting hemoglobin. A recent work done by Melissa with his coworkers shows that only trace amounts of heme within the parasite are enough to stimulate and activate DHA. (3)

As is shown in figure 2, there is an endo-peroxide bridge in DHA. (4) The reactive endoperoxide bridge of DHA will be cleaved when it reacts with heme (Fe^{2+}-FPIX). Then DHA will be transformed into a short-lived alkoxy radical. This alkoxy radical will go through a rearrangement because of thermodynamic induction and will produce a primary carbon-centered free radical. Such carbon-centered free radicals can attack macromolecules and react with other structures such as proteins and lipids, which leads to widespread cellular damage, especially for tumor cells.
Fe (II) is oxidized, and one of the electrons forms a carbon radical \(^{(4)}\)

Also, in the fact that lysosomes are the storage site for Fe\(^{2+}\), they are easily affected by drugs targeting iron, such as artemisinin derivatives. ARTs can release ROS via the Fenton reaction in lysosomes. These ROS can influence the electron transfer chain in the mitochondria and causes damage to lysosomes and mitochondria, as Figure 3 shows. \(^{(5)}\) And it is the damage of mitochondria and lysosomes that causes cell death, including the death of cancer cells. Also, these ROS can lead to protein and DNA damage, thus further causing the death of tumor cells.

2.2 Caspase-dependent anticancer effect.

As is previously described, ROS can be very damaging to mitochondria. Reactive HO\(^{-}\) and ROO\(^{-}\) radicals, transformed from ROS via Fenton reaction, trigger a series of changes, including depolarization of the mitochondrial membrane, peroxidation of membrane lipid and also permeability transition \(^{(6)}\), finally leading to the collapse of the mitochondria membrane. \(^{(7)}\) At this point, cytochrome c, originally stored in the mitochondria, would be released into the cytoplasm.
Figure 4. The mechanism of the mitochondria-mediated pathway is induced by ROS. ART supregulate the level of activated caspase-9, caspase-3 and nucleus AIF. 

As shown in figure 4, released cytochrome c would first bind to APAF1 and induce a conformational change. This change allows it to combine with dATP to form a quaternary protein structure called the apoptosome, which leads to the activation of caspase-9 and further the activation of caspase-3. Handrick and his colleagues observed the release of cytochrome c, activation of caspases, DNA fragmentation, and consequently apoptosis, in T-cell lymphoma Jurkat cells after the dysfunction of mitochondria induced by artemisinin and its active metabolite DHA. In short, the initiation of the caspase-dependent pathway has been shown to be involved with the generation of ROS induced by ARTs, as chelators of Fe3+ significantly alleviate the extent of increased caspase and cytotoxicity of ARTs. Caspases, a large family of proteases, cleave after an aspartate residue in its protein substrate and thus selectively inactivate some important proteins, including ICAD (the inhibitor of caspase-activated DNase) and Poly (ADP-ribose) polymerase (PARP) enzymes, which commits the cells to death. Caspases can promote DNA fragmentation via the cleavage of ICAD. Normally, ICAD combines with CAD to complete its translation and maintains an association with CAD to block its dimerization and inhibit its DNase activity. DNA fragmentation occurs when activated caspase-3 cleaves ICAD and thus activates CAD. This fragmentation appears to be significantly amplified with artemisinin treatment, which causes an increased level of cleaved ICAD.
Figure 5. The level of caspases and cleaved-PARP in cells treated without (negative control group) and with (1, 5, 10 µM) artemisinin: the level of caspase-3, caspase-8, caspase-9 and cleaved-PARP shows a significant increase after artemisinin treatment. 

Caspases also promote DNA fragmentation by blocking the repair mechanism. For example, Poly(ADP-ribose) polymerase (PARP) enzymes, which are the chief responders and contributors to DNA repair, are influenced by caspases, especially caspase-3 and caspase-7. Artemisinin has been observed to promote the cleavage of PARP in a caspase-dependent manner in both HCT116 colon cancer cells and SKM1 cells, as is shown in figure 5. The cleaved, or inactivated PARP would fail to function normally and consequently accelerate the process of apoptosis. ART and its metabolite DHA are responsible for the apoptosis of human lung adenocarcinoma cells, colorectal cancer cells and breast cancer cells via the caspase-dependent pathway, as evidenced by elevated levels of caspase-3, 8 and 9 (Figure 5) in cell lines treated with ART. 

2.3 Caspase-independent anticancer effect

Caspases really assume a pivotal role in artemisinin-induced apoptosis. However, when pretreating the ovarian cancer cells with a pan-caspase inhibitor (Z-VAD-FMK), Green-shields and colleagues still observed the ART-mediated cytotoxicity. This finding indicates the existence of another caspase-independent pathway, which is mediated by Apoptosis-Inducing Factors (AIF). AIF is a mammalian mitochondria protein identified as a flavoprotein oxidoreductase. After ROS damages the mitochondria membrane, AIF will translocate to the nucleus and bring about chromatin condensation and DNA fragmentation without the help of caspase. After ART treatment in cultured cells, significantly increased nAIF (nucleus AIF) detected in western blots in figure 6 verifies the involvement of AIF in ART-induced apoptosis. The translocation and function of AIF can be independent of caspases. Nevertheless, caspase-dependent and independent pathway are not mutually exclusive. Cleaved PARP, the substrate of caspase-3 and caspase-7, also promotes the release of AIF. Furthermore, the combination of caspase-dependent and independent pathway can lead to synergistic effects in apoptosis.
Figure 6. The level of nAIF (nucleus AIF) and tAIF (Total AIF) in cells treated with and without artemisinin: nAIF significantly increased but tAIF remained unchanged after artemisinin treatment (14).

3. Inhibition of VEGF signaling by DHA.

The elevated level of VEGF expression is found in most types of cancer. The binding of VEGF-A to VEGFR-2 activates the mitogen-activated protein kinase (MAPK) cascades and PI3K pathway that controls the cell proliferation and survival for tumor angiogenesis. (26) Scientists have found that DHA can inhibit the process of VEGF binding VEGFR-2. One of the examples is through the inhibition of the NF-κB pathway. Fengyun Dong and his colleagues have observed in the experiment that DHA can decrease the binding of p65 protein to VEGFR-2 promotor by changing the motif of NF-κB instead of changing the DNA sequences, which leads to the suppression of the VEGFR-2 production in human umbilical vein endothelial cells. (27) Inhibition of this transcription also causes the decrease level of IL-8, a pro-angiogenic cytokine acting as an autocrine growth factor in colorectal cancer. (28), (29)

Figure 7. VEGFR-2 production is reduced by changing the binding capacity of p65, which leads to inhibition of transcription (27)
DHA can also regulate PI3-kinase/Akt/mTOR signaling pathway to decrease the formation of HIF-1α, which is a transcription factor responsible for VEGF expression. Compared with the role in the NF-κB pathway, DHA does not directly block the transcription process. Instead, it reduces the formation of HIF-1α through down-regulation of the translation factor. As shown in Figure 8, the 4E-BP1/eIF-4E complex plays a significant role in inhibiting HIF-1α translation. (30) Phosphorylation of 4E-BP1 can reduce its affinity to eIF-4E, which leads to upregulation of protein. (30) DHA can inhibit all the phosphorylation processes in the PI3-kinase/Akt/mTOR signaling pathway. Min Kong has compared the effect of drugs between ART and AKT inhibitor VII on ccRCC cells in his experiment and observed the inactivation of AKT activity to mTOR. (31) Studies have also shown that DHA directly blocks the phosphorylation of 4E-BP1 by mTOR. (30), (32) Since the formation of HIF-1α requires degradation of the 4E-BP1/eIF-4E complex, translation of HIF-1α will be suppressed if 4E-BP1 cannot be phosphorylated.

Figure 8. PI3-kinase/Akt/mTOR signaling pathway, activated by VEGF, has positive feedback on VEGF signaling. DHA can block the phosphorylation of 4E-BP1 to inhibit the translation of HIF-1α, thus reducing VEGF expression (33)

**Conclusion and Future Experiment**

Artemisinin and its derivatives, known as effective antimalarial drugs, may have transposable mechanisms between induction of parasite death and cancer cell apoptosis and exert a potential anticancer activity. Owing to the generation of ROS from the break of the end peroxide bridge, ARTs theoretically exhibit high cytotoxicity specific to most cancer cells. Studies have suggested that ARTs induce cancer cell death and inhibit cell proliferation via various mechanism, including iron-dependent, caspase-dependent and - independent pathway, and down-regulation of VEGF signaling. Besides, the combination of artemisinin and some anticancer pharmacophores contributes to additive or
synergistic effects in mitochondria-mediated pathway and anti-angiogenesis. (22), (34)

However, there is still a long way to go before ARTs can be safely and effectively applied to cancer therapy. In absence of clinical trials and research, current insight into ARTs remains insufficient to evidence their potency and practicability in cancer treatment. Some molecular mechanisms of the ARTs-induced cytotoxicity are still unclear or unknown to us. (35) Apart from that, several clinical cases reported on the underlying embryotoxicity (36) and neurotoxicity (37) and raised doubt on the safety of ARTs, which led the World Health Organization to set a restriction on the usage of ARTs. (38) In general, more research should focus on the molecular mechanisms and the clinical treatment of artemisinin derivatives. Artemisinin and its derivatives can be a promising candidate in fighting against the global cancer pandemic.

REFERENCE


1295-1304.


