Anticancer Potential of Artemisinin Derivative-Dihydroartemisinin

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Abstract. Artemisinin is famous for its effectiveness of treating malaria for years. Potential of artemisinin in treating cancer has been recently recognized. In this study, the anticancer potential of artemisinin and its derivative dihydroartemisinin (DHA) is comprehensively illustrated, including brief introduction of background and clinical applications. Artemisinin derivatives, especially dihydroartemisinin, of which the anticancer mechanism such as induction of apoptosis, inhibition of peripheral blood vessels has also been depicted. Cases of clinical study of cervical cancer and breast cancer are also reported to further proof the anticancer efficiency of dihydroartemisinin. Finally, summary of perspectives and significance of artemisinin and DHA is also provided.

Keywords: Dihydroartemisinin, Artemisinin Derivative, Anticancer, Drug Design

1. Introduction

People have been fighting against cancer for years. From the tetrhumoralism of Hippocrates and Galen, X-ray of Roentgen, to chemotherapy drugs such as paclitaxel, people have made long progress trying to cure cancer. However, even for today, as diversified anti-cancer methods and drugs are provided, people still lack a "perfect" drug that can effectively treat cancer with considerable no side effect. Current methods of treating cancer are not only not very effective but often come with serious side effects. With the development of modern science, cancer has been commonly diagnosed in people. And it's one of the biggest threads for high-quality long line of human beings. It has been reported that lung cancer accounts for 11.6 % of all cancers and 18.4 % of all deaths (as of 2018). [1] In addition, commonly used anticancer drugs, such as docetaxel, are chemically engineered. Therefore, compared with natural derivate drugs, these artificial drugs have stronger side effects, such as strong allergic reactions, bone marrow suppression, and inhibition of the efficacy of gastrointestinal mucosa.

The emergence of artemisinin and its derivative dihydroartemisinin (DHA) may provide new ideas to solve these problems to some extent. Artemisinin and its derivatives are derived from plants and are naturally produced, in contrast to the current mainstream of man-made chemicals. Natural drugs with more complex structures are also less likely to cause side effects than simple man-made chemicals. DHA has special advantages against drug resistance, especially considering that it has a very different mechanism of action from traditional chemical drugs. Because DHA has been shown to have possible anticancer properties in vitro, artemisinin and its derivatives have once again become a hot topic in the medical world.

So far, DHA has been found to have a variety of anticancer mechanism. DHA has been shown to potentially cause apoptosis through PTCT as a target and also to limit energy acquisition by tumor cells inhibiting the expression of PKM2, an important regulator of glycolysis. In addition, scientists have found that DHA, when used in combination with NBV, another major anticancer drug, can also inhibit angiogenesis near tumors. Therefore, one can assume that once the low solubility of DHA in blood can be overcome and can be successfully applied to the clinic, there will be great progress in the anti-cancer field.

1.1. Applications of artemisinin

Artemisinin has been widely used in treatments of variety of illness in all ages, which was regarded as a promising therapeutic in clinical therapy. Within the last few ages, scientist has taken efforts to investigate the effects of artemisinin in inflammatory, some viral, bacterial, fungi and parasite-related infections. Since ancient times, it has been commonly used in Chinese to treat jaundice and hepatitis, also for remitting symptoms like excessive sweating and headache resulted from summer heat. Proverbially, artemisinin has become the noticeable threptic drug for malaria as Chinese pharmacist Tu Youyou discovered that artemisinin could inhibit Plasmodium which cause malaria in 100%, and successfully cured more than one million malaria patients in China. [2]

Recently, research attempted to engage in some anticancer activity of artemisinin and its derivatives, since features of presence of Endoperoxide Bridge and reactive oxygen species in artemisinin might play significant role in anticancer activity. And this initiated a fire-new prospective of cancer treatment. [3]

2. Artemisinin derivatives: dihydroartemisinin (DHA)

In 1973, Tu Youyou and her team accomplished synthesis of the dihydroartemisinin, which become the first-generation derivative of artemisinin. Dihydroartemisinin was obtained by reduction of original artemisinin, hydroxyl groups were added on artemisinin, creating a compound as Figure 1.

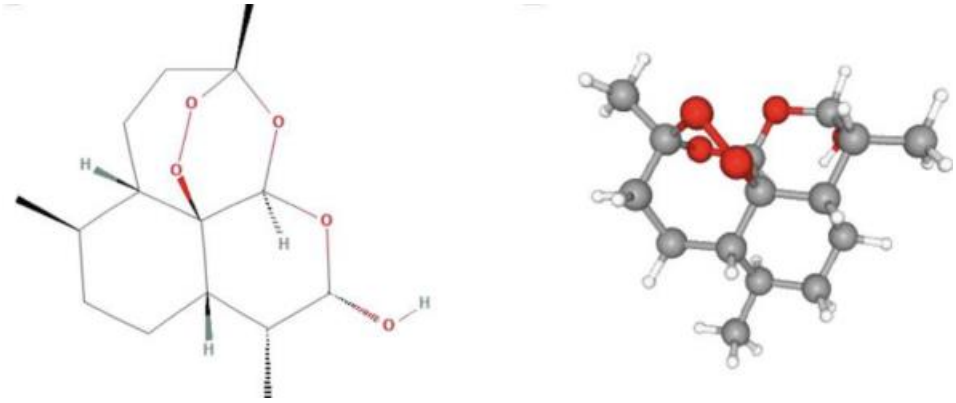

Figure 1. The chemical structure of DHA.

Comparably, DHA has a much larger efficacy than artemisinin in treatment of malaria, and also has a surprisingly low percentage of disease recurrence about 1.95% after taken. In order to maximize the capacity of DHA since it was proved to function remarkably in antimalaria activity, attention of DHA has derived to some other fields such as anticancer. Previous studies demonstrated DHA was the primary metabolite of some other artemisinin derivatives like artesunate and artemether, which meant actions of the derivatives to remedy the disease is determined by production of DHA. In addition, some advantages of DHA like high water solubility, low toxicity and guaranteed safety makes it worth to do deeper research in anticancer activity. Indeed, DHA owns the ability of anticancer, defensing a wide range of cancer like lung cancer, breast cancer, prostate cancer, and ovarian cancer and digestive system tumors and so on. [4]

3. Mechanisms of DHA

3.1. Induction of apoptosis

Apoptosis is a process that a cell receives a signal of ‘suicide’ by biomedical instructions of DNA itself. This occurs commonly within body cells as cells become useless. And apoptosis is crucial for the cell with damaged DNA to die before they become cancerous. However, once cancer onset, proliferation and metabolism of cancerous cells disturb normal mechanism of apoptosis. Tumor can

just escape from the process of apoptosis and continue reproducing and spreading. [5] Apoptosis pathways include exogenous pathways mediated by death receptors and endogenous pathways mediated by mitochondria. (Figure 2.) [3]

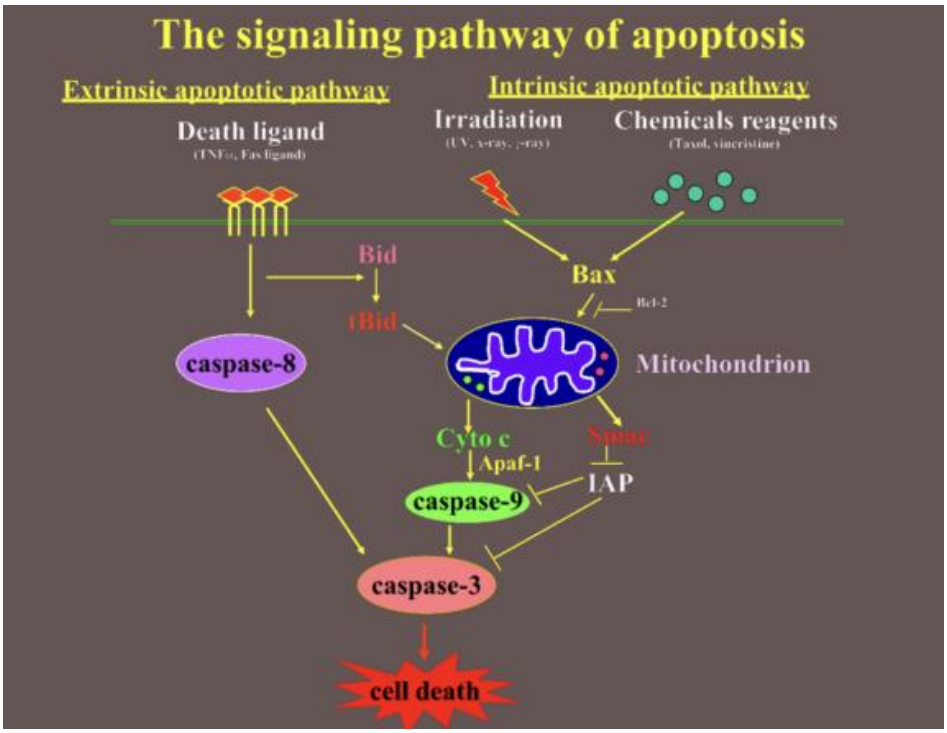

Figure 2. Exogenous pathway and endogenous pathway.

Luckily, scientists claimed that with the aid of DHA, induction of tumor cells apoptosis can be achieved according to studies. Several signaling pathway are closely relevant to apoptosis induced by DHA. For instance, colon cancer cells apoptoses by interactions of DHA and janus kinase 2 (JAK2) or signal transducer activator of transcriptor 3 (STAT3), through the process of targeting of JAK2 or STAT3 signaling. [6] Additionally, apoptosis in BGC823 gastric cancer cells is meditated by induction of c-Jun NH2-terminal kinases (JNK1/2) and p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways by DHA. [7] And DHA has the strongest efficacy to several kinds of cancers compared to other derivatives, this might be attributed to the structure of DHA. The presence of reactive oxygen species (ROS) in DHA is crucial to damage cell organelles, finally leads to cell death. The ROS dependent apoptosis has been observed in cells like Hep3B. With DHA, intrinsic pathway such as increase in activation of caspase 3, ADP-ribose that responsible to destroy the cell structure has been witnessed [8]

DHA targets some molecules to induce apoptosis. In breast cancer, there is a higher-level expression in phosphorylated form of translationally control tumor protein (TCTP) in cancer tissue compared to normal tissue, along with higher histological level and expression of Ki-67. This indicates that TCTP can be a target for DHA to bind thus causes cancerous breast cell apoptosis. [9]

TCTP is highly involved in cell cycle. It is a tubulin-binding protein which interacts with microtubules. Moreover, it is a key target of Polo-like kinase 1(PLK1) that involves in anaphase of mitosis. When TCTP is blocked, PLK1 is not able to perform its function, leading to mitosis catastrophe as disintegrate anaphase and cell apoptosis. DHA is one of TCTP inhibitors whose safety is guaranteed as it has been used to treat malaria. Interaction between DHA and TCTP causes reduction in TCTP levels through ubiquitination and proteasome-mediated degradation. Moreover, DHA can inhibit metabolic pathway of cells to cause apoptosis. Pyruvate kinase M2 (PKM2) is an indispensable regulator in glycolysis of cell respiration. Scientists found that this regulator expresses in a higher extend in cancerous esophageal squamous cells. DHA can inhibit expression of PKM2, leading to inhibition of lactic acid and glucose uptake, so cancerous cells are not able to perform cellular respiration, and facilitates apoptosis of cancerous cells. (Figure 3.)

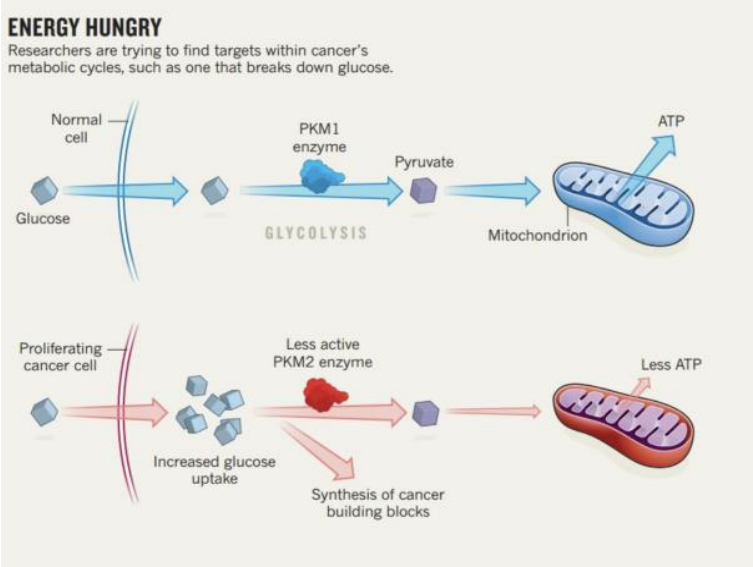

Figure 3. Effects of PKM2 on cell respiration.

Furthermore, it was also found that inhibition of glucose transporter-1 (GLUT-1) activity and glycolytic pathway is accomplished by DHA. The major mechanism pathway includes inhibiting phosphatidyl-inositol-3-kinase thus causing LNCaP apoptosis. [10] (Figure 4.)

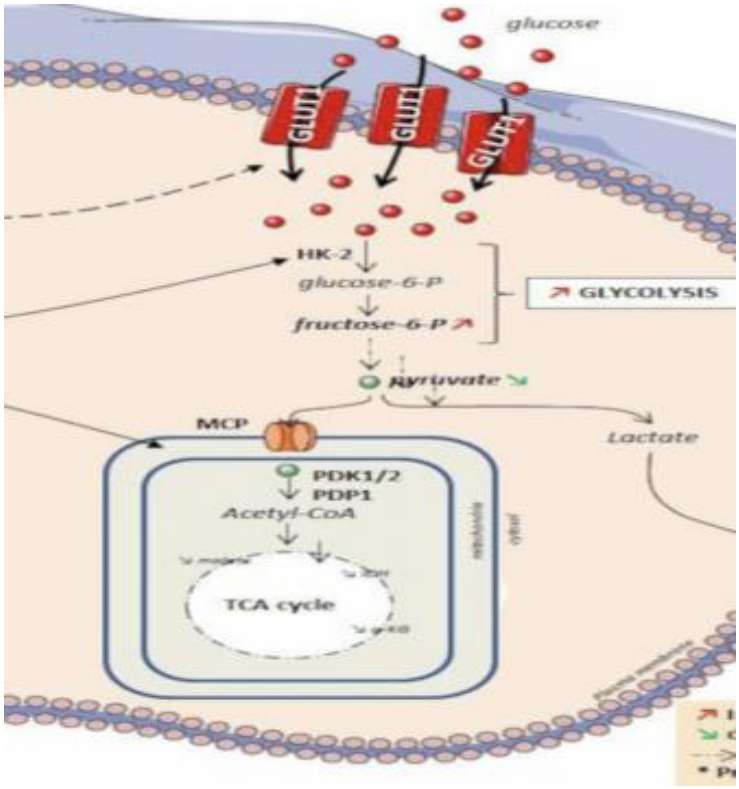

Figure 4. Relationship between GLUT-1 and glycolytic pathway.

3.2. Inhibition of peripheral blood vessels

Studies have shown that combination of Artemisinin with NVB, which is a popular synthetic chemical anticancer drug primarily used to treat breast cancer, effectively inhibits the autocrine (which is the process by which pheromones are discharged into the interstitial fluid outside the cell and then fed back to the cell itself) and paracrine (which is a way for hormones to affect surrounding cells by diffusion) effects of VEGF by simultaneously targeting tumor blood vessels, and thereby limit its nourishing effects on cancer cells. Artemisinin not only directly inhibited the metastasis of MDA-MB-231 cells, known as breast cancer cells, but also indirectly affected the tube formation of

HUVECs (HUVECs are vascular cells whose proliferation to some extent represents the formation of vessels in the vicinity of cancer cells). Then artemisinin inhibits cancer cells from secreting VEGF. VEGF is A special glycoprotein, especially VEGF-A, which is considered to be an important hormone to promote angiogenesis near tumor cells. VEGF can stimulate the proliferation, migration and formation of blood vessels. In 1971, Professor Judah Folkman and collogues at Harvard Children's Hospital proposed that it would be impossible to break 3 cubic centimeters without angiogenesis (vessel formation), which is an important basis for cancer cells to grow. This hypothesis was confirmed in the 1990s, as VEGF is one of the most critical factors in tumor growth. Artemisinin together with NVB work by preventing the connection between the receptor KDR on the surface of tumor cells with VEGF. This reduces the production of blood vessels around cancer cells, indirectly depriving them of nutrients and causing them to die. By influencing autocrine and paracrine mechanisms, artemisinin combined with NVB regulates tumor growth and peritumor vascular synthesis. Therefore, it can be concluded that artemisinin and its derivatives, when combined with NVB, may significantly reduce the angiogenesis around tumors and greatly inhibit tumor proliferation. [11]

4. Auxiliary drugs

Although artemisinin and its derivatives have high anticancer activity, their low solubility is a major factor limiting their anticancer activity. To address these problems, some researchers hope to use FCA@mSiO2 as an important transporter.

FCA@mSiO2 is a nanocarrier that has the ability to transport hydrophobic ART and iron ions into cancer cells. FCA@mSiO2 is characterized by the release of ART and iron ions by sensing changes in pH. After FCA@sSiO2 is endocytosed, it gradually undergoes a lower pH, and when the pH is lower, it is more likely to release the contained ART and iron ions. However, the released iron ions and ART will interact with each other in cancer cells to form free radicals, which can cause a large number of important cell structures to undergo alkylation, thus greatly disrupting cell activity. [12]

5. Clinical study

5.1. Anti-cancer effects of DHA in nude mice [13]

Nude mice, a variant of a congenital thymus defect, are born with defective T-lymphocytes but normal B-cell function. Therefore, nude mice are ideal hosts for human tumor xenotransplantation.

In nude mice, study has been carried out to compare performances of high dose DHA, medium dose DHA, low dose DHA and 5-FU (a chemical drug commonly used in cancer treatment). The 5-FU group showed the highest inhibition rate as 50.74%. The high-dose DHA group ranked second with 36.54%. The medium-dose DHA group ranked third with 26.55%. And the low-dose DHA group ranked last with 12.43%. The statistical P values of tumor weight among pairwise comparison of the high-dose group with the medium-dose group and the control group were all less than 0.05, indicating statistically significant difference caused by high level DHA dosage. The effect of low-dose DHA group was not obvious, while 5-FU group showed a strong inhibitory effect on tumor.

Although 5-FU has a remarkable effect on tumor suppression, its side effects are also obvious. Not only does it make the patient sick, but it also temporarily destroys the immune system. Compared with 5-FU, the side effects of DHA were relatively small. At present, there is no obvious in vitro or clinical evidence shown that DHA has serious toxic and side effects. In the previous study, it has been found that 5-FU treated nude mice generally showed decreased appetite, decreased body temperature, decreased activity, decreased responsiveness and weight loss, which have not been observed from DHA treated mice. In this way, DHA is more likely to serve a relatively safe anticancer drug. In addition, the surviving gene, a gene expressed in almost all tumor tissues but only in a few normal tissues, is widely recognized by the academic community to inhibit apoptosis. It can be used as one of the screening and diagnostic criteria for some tumors. In control group, the mean expression

positive rate of surviving gene was 0.5930, 5-FU was 0.4581, and that of DHA high concentration group was only 0.3260. In conclusion, compared with conventional chemical anticancer drugs, DHA functions in varied mechanism, from affecting vascular receptor VEGF to directly releasing oxygen free radicals to destroy the cell structure of cancer cells, and even affecting the expression of surviving gene through unknown pathways. A variety of ways to fight cancer can not only make therapy more effective, but also better prevent cancer cells from developing drug resistance.

5.2. DHA efficacy in cervical cancer [14]

Cervical cancer is the second common cancer occur within female population, which perplexed women for a long time and inserted many side effects. In many developing countries, there was no access to massive application of vaccine. So cervical cancer cases were frequently presented in later stage, with symptoms of pain and bleeding, leading to death eventually only after a few months, as the primary tumor is invasive which progresses towards uterus and blocking urinary flow. Studies have been carried out to investigate if oral artenimol-R (succinate ester of DHA) has an effect on cervical cancer and its safety.

Method In total, ten patients were recruited in the trial. A 28-day trial was initiated at first stage. The symptoms of patients after a month were recorded. The time of symptom remission in successful cases was also evaluated, which was the primary endpoint. The secondary endpoint was to establish the adverse effect profile. Analysis of tumor by immunohistochemistry, which can characterize gynecological tumor to provide additional specific information, was regarded as the exploratory endpoint. After these, if symptoms of pain or vaginal discharge recurred, the second treatment was given with another 28 days. In order to prevent tumor lysis phenomenon, dose of drug would be carefully increased from 100mg/day in the first week to 200mg/day in later period of treatment. (Figure 5.)

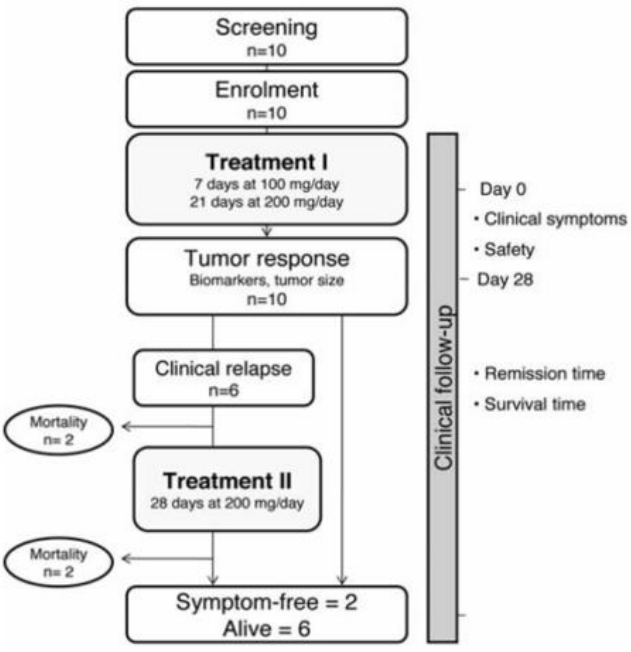

Figure 5. Overview process of the experiment.

Results Following the initial treatment period, almost all patients were monitored for clinical symptoms, pain and vaginal discharge, included nine patients suffered from vaginal discharge and six suffered from pain. Clinical symptoms were followed up and recorded daily during the 28-day treatment. At the end of the first stage treatment, disease in all nine symptomatic patients was in a state of clinical remission, the symptoms disappeared. The median time was 7 days, which was reduced compared to control group. Four of the patients relapsed, but after receiving second stage treatment, the symptoms remitted to a large extend. However, two of the patients died after twelve months.

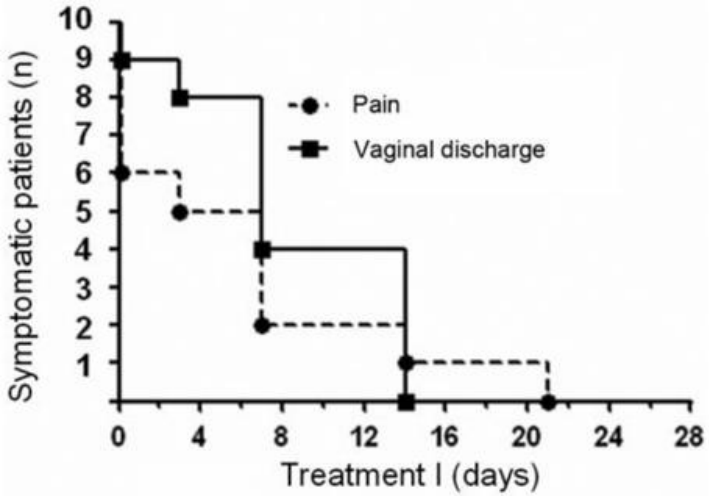

Figure 6. Changes of number of symptomatic patients once they received days of treatment.

During the followed-up period after the treatment period, the safety of DHA to patients was also proved. During the first treatment period, half of the patients did not suffer from any adverse effects, others only be affected by 'flu-like syndrome', as getting headache and fever.

5.3. DHA efficacy in breast cancer [9]

Expression level of phospho-TCTP in breast cancer cells compared to normal breast cells is analyzed using immunohistochemistry, observing TCTP expression level in 85 human breast cancer cell specimens. Results have shown that TCTP has positive correlations with cell proliferations. Most of the samples have the TCTP expression level higher than 4%, as the increase in lymph node, HER2 and Ki-67 levels indicates cell proliferations.

Attention has been focused on the impact of DHA on post-translational modifications of TCTP. As PLK-1 can localize TCTP and phosphorylate it during mitosis of MDA and SKBR3 cells, with the addition of PKL-1 inhibitor significantly reduce TCTP level. TCTP expression level is positively related to cell viability, this explains why apoptosis of cancer cells is caused by reduction of TCTP level. The caspase 3 or caspase 7 activity is relevant to cell apoptosis, and for the cells which received DHA treatment have shown the caspase 3/7 activity experienced an increase, which inducing caspase-dependent apoptosis.MCF7 and BT-474, which are two kinds of breast cancer cell lines, correspondingly derived from primary invasive ductal carcinoma and metastatic invasive ductal carcinoma, have experienced a dramatically decrease in TCTP level after 72 hours of DHA treatment shown in Western Blot analysis. (Figure 7.)

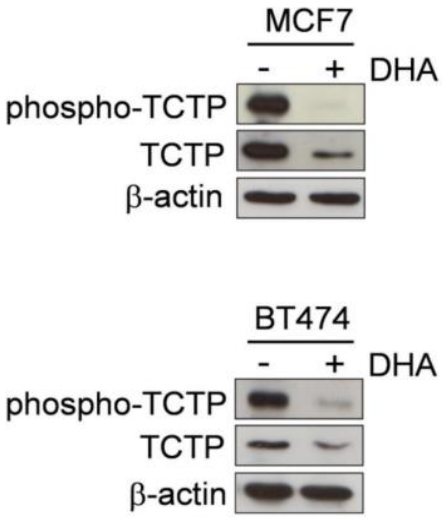

Figure 7. Comparison of TCTP levels in MCF7 and BT474 before and after DHA treatment.

6. Conclusion

Artemisinin and its derivatives cannot only treat malaria, but also play a significant role in anticancer activity. DHA is cytotoxic to cancer due to its unique structure, which treats severity cancers such as cervical cancer and breast cancer in considerable extend through a variety of mechanisms such as cell apoptosis induction, peripheral blood vessel induction. With cooperation of auxiliary drugs, efficacy of DHA can be enhanced. Whereas there are still some limitations on applications which need more evidence to prove the feasibility in the future. Overall, atermisinin and derivatives has a large potential and bright perspective of anticancer. Further studies are necessary to be conducted to comprehensively understand mechanism details of DHA, as well as more and larger scale clinical trials. These studies will lay the foundation for the safety and efficacy clinical use of DHA as an anticancer drug.

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