Anticancer Potential of Artemisinin Derivatives Containing Fluorine Atoms

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Abstract. Artemisinin and its derivatives were widely used in treatment of malaria in last decades years. As a natural compound extracted from Chinese herb *Artemisia annua*, artemisinin and its derivatives presented high cytotoxicity to tumor cell and low toxicity to human body. If this great medicine can be used in the treatment of other disease, a new treatment will be found. Many researches were carried out to confirm these compounds can be used as a new anticancer agent. Artemisinin and its derivatives presented cytotoxicity to tumor cells were confirmed by many experiments. Besides some common derivatives which were widely used in the malaria therapy, some new artemisinin derivatives were synthesized and evaluated whether these compounds can become potential anticancer drug. There is research carried out by Shu Li and others synthesized a new type of artemisinin derivatives, artemisinin derivatives containing fluorine atoms, and evaluated these new compound's cytotoxicity to tumor cells. In this review paper, the anticancer activity of artemisinin derivatives containing fluorine atoms were introduced and its cytotoxicity against tumor cells were shown. Then, the anticancer ability of artemisinin containing fluorine atoms and other common artemisinin derivatives: dihydroartemisinin and artesunate were compared. Finally, traditional treatment of cancer, such as chemotherapy and radiotherapy were introduced in this paper. By comparing artemisinin derivatives with traditional treatment of cancer, the big cancer-fighting potential for artemisinin and its derivatives should be see and further investigated. A new series of artemisinin derivatives, compounds containing fluorine atoms have anticancer ability too. Results obtained by literature research and read show artemisinin containing fluorine atoms may be a great potential anticancer drug but still need more exploration and practice like other artemisinin derivatives.

Keywords: Artemisinin Derivatives; Anti-cancer.

1. Introduction

In the 21st century, people all over the world are living longer than before on average, but the population of sick people is increasing. According to the《World Health Statistics 2019》,reported by WHO, average life expectancy in 2019 is 6 years longer than 2000, but only 5 years of the extra life is healthy life. Cancer is one of the serious diseases that takes away people's health. Actually, we recognized cancer for a long time, people who lived in agent Egypt around 1600BC were already had record some cases about 'strange lump' and they attempted to treat this disease by burn the lump. Nowadays, people have more scientific and effective medical intervention on the therapy of cancer, but cancer is still a serious disease which is hard to recovery completely. Patients often have to endure a painful long therapy and the therapy does not always work. This healthy problem has a strong impact on people's lifespan and living quality, scientists around the world have already worked nearly one hundred years to found a cure for cancer. In author's knowledge, doctors began trying to use radiotherapy in the treatment of cancer in 1895-1898 after the discovery of radium and Roentgen rays, and the first case of chemotherapy was happened in 1943 when the chlormethine was be used in the treatment of lymphoma. After constant exploration and practice, these two therapeutic methods are becoming more and more mature. Nowadays, radiotherapy and chemotherapy has become the main pattern of cancer treatment.

However, both of them have strong side effects, radio and antitumor drug will kill the tumor cells and healthy cells at the same time, leading to hair loss, nausea, bleeding, malnutrition and leukopenia. New drugs that can targeted killing of tumor cells and damage to normal tissue as low as possible are

required. Artemisinin and some main derivatives were isolated or synthesized by Chinese scientist Youyou Tu and her team in 1970-1971 as a potential drug for malaria. These compounds extracted from a natural planet, Chinese herb *Artemisia annua*, which is used as Chinese traditional medicine, presented great toxicity to plasmodium and almost no harm to normal tissue. Artemisinin and its derivatives make a lot of contribution in the treatment of malaria, but they can be even more useful. In 1993, Woerdenbag found artemisinin and its derivatives has the ability to fight cancer firstly.[1] Many experiments were carried out subsequently to prove that artemisinin and its main derivatives which were used in the treatment of malaria can be used in cancer therapy too. The results showed these compounds can kill tumor cells with some special mechanism. These compounds have many mechanisms leading to apoptosis of cancer cells and do not hurt normal tissue.

Besides some common derivatives used in malaria therapy, scientists also synthesized some new artemisinin derivatives as potential anticancer drug. Experiment carried out by Shu Li was synthesized ten artemisinin containing fluorine atoms and named them from 9a to 9j. Compound 9j is the most potent anticancer drug against cancer cell line in vitro experiment. [2] Because of compound 9j presented the most cytotoxicity against cancer cell line MCF-7, only compound 9j were further investigated to confirm the antitumor mechanism.

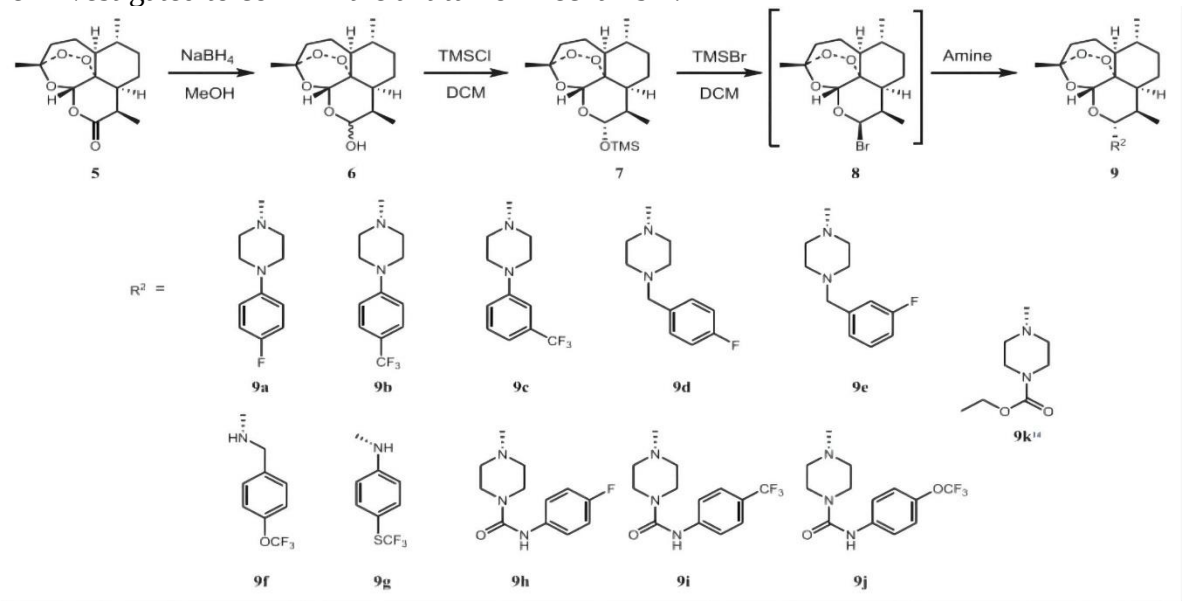


Figure 1. The synthetic routes of artemisinin derivatives containing fluorine atoms and the structure of compounds 9a-9j referenced from paper written by Shu Li and other researchers.

2. Artemisinin derivatives containing fluorine atoms

2.1. Cytotoxicity of artemisinin derivatives containing fluorine atoms 9a-9j

In the experiment carried out by Shu Li and other researchers, they begin with the design and synthesize compounds containing fluorine atoms, finally they get ten compounds and confirmed each one target compounds' structure. (Figure 1)

The cytotoxicity of these compounds confirmed by vitro experiment against seven different cancer cell lines: U87MG, SH-SY5Y, MCF-7, MDA-MB-231, A549, A375. Each of them was generally more effective on antitumor than the original artemisinin. However, the condition for each cancer cell line is a little different. For U87MG cells, compounds 9a, 9f, 9g, 9h, 9i and 9j have moderate antiproliferation activity. For SH-SY5Y cells, compounds 9f, 9h, 9i and 9j have potent cytotoxic activities. For MCF-7 cells, compounds 9a–j has significant potent antiproliferation activity. Compounds 9j presented strongest anticancer activity in this test. For MDA-MB-231cells, compounds 9i and 9j have significant potent antiproliferation activity. For A549cells, compounds 9c–j has moderate antiproliferation activity. For A375 cells, compounds 9h and 9j have significant potent

antiproliferation activity. What's more, human normal liver cell line L02 were used to evaluate whether these compounds will hurt healthy cells. Artemisinin, dihydroartemisinin, doxorubicin and temozolomide were used as positive controls. (Table 1)

Table 1. Cytotoxic activity of 9a–j and control drugs against cell lines, referenced from the paper written by Shu Li and other researchers.

compound	IC_{50} Values[a]						
	U87	SH-SY5Y	MCF-7	MDA-MB-231	A549	A375	L02
9a	25.5±2.7	>50	28.8±0.1	>50	>50	>50	11.2±1.6
9b	>50	11.2±0.9	27.0±5.7	>50	>50	40.9±3.1	20.5±0.5
9c	>50	15.0±2.7	7.5±0.5	12.5±0.3	12.9±0.1	20.0±4.6	9.1±1.0
9d	>50	>50	3.5±0.2	12.0±0.7	35.9±0.3	12.8±0.1	17.1±1.6
9e	>50	5.0±0.4	6.8±1.4	32.9±2.9	44.4±4.0	46.5±3.0	23.2±4.0
9f	17.7±0.5	20.3±1.9	10.2±0.8	21.8±3.0	17.9±0.7	10.9±0.1	35.7±2.8
9g	28.0±1.1	5.0±0.2	5.3±0.3	22.9±3.4	41.8±2.4	26.5±1.5	9.3±2.1
9h	28.7±0.3	3.2±1.7	5.5±0.1	10.9±2.5	17.6±3.2	9.1±0.5	15.6±2.2
9i	15.2±2.4	3.6±2.3	2.7±0.1	5.8±0.7	21.1±3.3	15.0±1.1	4.0±0.2
9j	19.7±2.6	>50	2.1±0.2	8.4±1.6	19.9±2.6	3.4±0.7	6.7±0.2
artemisinin	>50	>50	>50	>50	>50	>50	>50
dihydroartemisinin	>50	>50	>50	45.7±2.7	46.8±0.7	38.5±2.6	34.0±1.6
doxorubicin	NT[b]	NT[b]	1.0±0.1	2.8±0.5	2.3±0.2	0.4±0.1	0.9±0.1
temozolomide	>50	>50	NT[b]	NT[b]	NT[b]	NT[b]	NT[b]

[a].IC_{50} values are indicated as the mean ± SD(standard error)of at least three independent experiments. The cells were continuously treated with compounds for 72 h.
[b]. NT means not tested.

From the results shown above, compound 9j presented the potential to be a broad anticancer drug. According to the data, compound 9j also presented the strongest cytotoxicity against MCF-7 cells. Shu Li and other researchers only further investigated the mechanism of cytotoxicity of compound 9j, that is also why the author only introduce the mechanism of compound 9j in the above part.

2.2. Anticancer mechanism of compound 9j

In the experiment design and synthesize artemisinin containing fluorine atoms, they design and synthesized ten artemisinin containing fluorine atoms and named them from 9a to 9j. The structure of these ten compounds were confirmed. (Figure 1) The cytotoxicity against U87MG, SHSY5Y, MCF-7, MDA-MB-231, A549 and A375 cancer cell lines of these compounds are confirmed in vitro by MTT assay, a way usually used to test the viability condition. (Table 1) Compound 9j presented the most anticancer potent ability against the human breast cancer MCF-7 cells and low cytotoxicity against human normal liver cell line L02. Shu Li and other researchers in this experiment believe this compound most likely to be broad anticancer drug, so they decided to further investigated cytotoxicity mechanism of compound 9j against MCF-7 cell line.

Shu Li and other researchers investigated the mechanism of cytotoxicity against cancer cell line of compound 9j. This research was separated in two parts. First, Shu Li and other researchers tested whether cytotoxicity of compound 9j is apoptosis relative. They choose 0,1,2.5,5,10,20 six different concentrations of 9j between 0μm to 20μm and separately treat MCF-7 cells for 24hours. Results show the higher the concentration, the higher the percentages of apoptosis cells. (Figure 2)

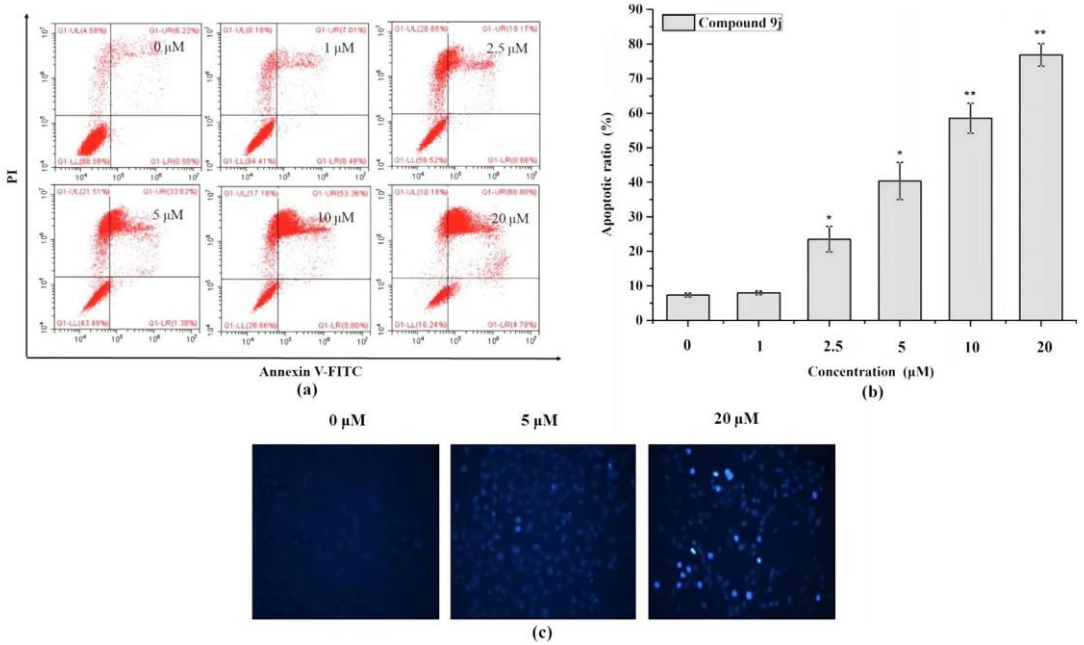


Figure 2. (a) Result of MCF-7 treated with or without 9j by flow cytometric analysis. (b) The total apoptosis ratio in treated cells and control cells (c) Hoechst33258 staining of MCF-7 cells treated or untreated with 9j. Referenced from the paper written by Shu Li and other researchers.

Then, they wanted to confirm if there is the arrest effect of compound 9j on cell cycle distribution. They treated MCF-7 cells with increased concentrations between 0 to 10μM for 48 hours, flow cytometry analysis were used to determine the arrest effect of compound 9j. Results show compound 9j significantly arrested cells at G1phase. The percentage of cancer cells at G1 phase were increased from 45.21% to 47.95%, 56.57%, 64.46%, 76.68%, 83.50% (concentration of 9j is 0.5μM, 1μM, 2.5μM, 5μM, 10μM, respectively). So, the results show cytotoxicity mechanism of compound 9j is arrested tumor cells at G1 phase. (Figure 3)

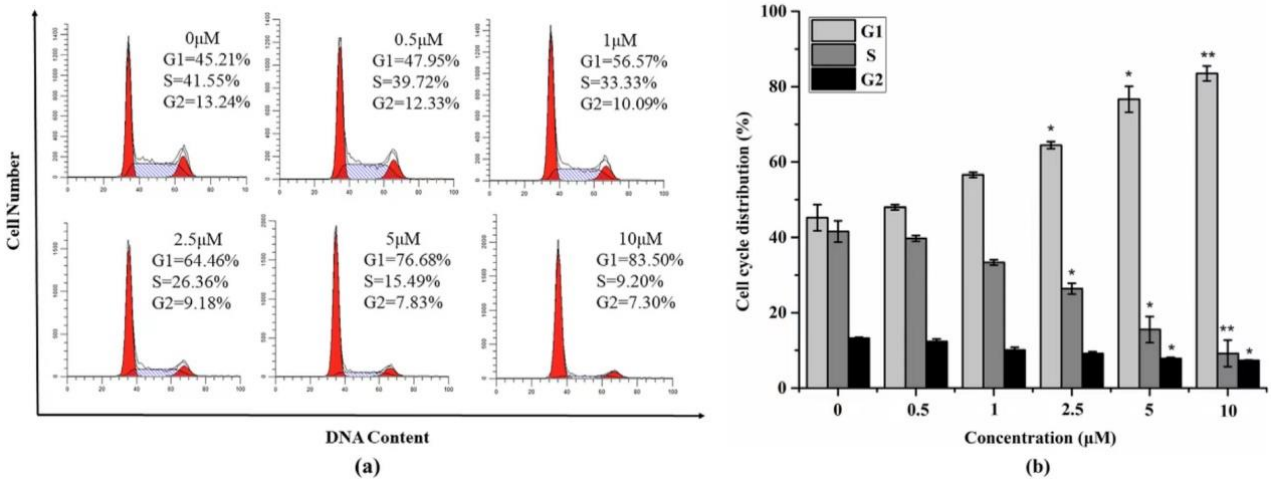


Figure 3. Condition of MCF-7 cells cycle. Referenced from the paper written by Shu Li and other researchers.

3. Other artemisinin derivatives: Dihydroartemisinin and Artesunate

There are so many artemisinin derivatives synthesized as potential or licensed drug. There is no doubt that all the artemisinin derivatives have significant anticancer activity, this phenomenon was confirmed by a lot of experiment in vitro or vivo. The most active two compounds of the licensed artemisinin compounds are dihydroatemisinin (DHA) and artesunate. DHA actually is the metabolite

of artemisinin, artesunate is the derivatives of artemisinin. Most of the experiment were focused on these two compounds due to they have the strongest anticancer activity. [3] DHA and artesunate are inhibitory to so many kinds of cancers such as melanoma, breast, ovarian, prostate, lung cancer cells as potential broad anticancer drugs. [4],[5] These two compounds were most expected to become anticancer drug.

3.1. Anticancer mechanism of common artemisinin derivatives

The mechanism of anticancer activity of artemisinin and its derivatives are diverse. Some experiment results presented this mechanism is iron-related. [6],[7] Some experiments found this mechanism is also regulated by calcium metabolism [8],[9],[10], endoplasmic reticulum stress [11], or the expression of the translationally controlled tumor protein. [12] What is more, artemisinin has been found to kill tumor cells by inducing DNA damage or indirectly interfering with a range of signaling pathways involved in several marks of tumor tissue. [3]

3.2. Anticancer mechanism of DHA and artesunate

DHA and artesunate are very effective at arrest antiproliferation tumor cell. [13] Some research presented the DHA is better than artesunate at the aspect of arrest tumor cells. [14] Besides this way to kill cancer cells, artemisinin and its derivatives are confirmed can leading to apoptosis by modulating the Bax/Bcl2 ratio. [15-17] DHA and artesunate caused cytochromec release, Bax overexpression, increase in Bax/Bcl2 ratio in an experiment against osteosarcoma cells. [18],[19] Except the cytotoxicity of DHA and artesunate to tumor cells, these two compounds also can inhibit the metastasis of cancer cell. [20-22]

4. Artemisinin derivatives containing fluorine atoms and DHA/artesunate

Artemisinin derivatives containing fluorine atoms are new series of derivative compounds that be designed and synthesized as a potential anticancer drug. This opinion combines artemisinin and its derivatives and compounds containing fluorine atoms, all of the target compounds in this experiment presented higher anticancer ability than artemisinin, which is confirmed this opinion is feasible. Unfortunately, the author only found one paper about these compounds, there is still long way to make these compounds mature enough to be anticancer drug.

Some other traditional and licensed artemisinin derivatives, mainly DHA and artesunate, were introduction too. It more clear about what the anticancer mechanism of traditional artemisinin and its derivatives is. As the only one compound that be further investigated the anticancer mechanism of ten compounds containing fluorine atoms, compound 9j anticancer through arrest the tumor at G1 phase. This is same to one of the anticancer mechanisms of traditional artemisinin and its derivatives. Both of compound 9j and DHA, artesunate have unique mechanism to identify the tumor cell, they are effectively at kill tumor cells and causes very little damage to the healthy tissue. Through the comparison, the author believe compound 9j is a potent compound as new anticancer drug like common artemisinin derivatives DHA and artesunate. However, sometimes the anticancer ability of artemisinin in animal experiment is not ideal, there still need more research to make sure these drugs is safe for cancer patient

5. Traditional treatment of cancer

5.1. Chemotherapy and Radiotherapy

Chemotherapy is traditional treatment of cancer. In author's knowledge, this treatment was first used to treat cancer in 1943. There are three stages in the history of chemotherapy. In 1943, the chlormethine was used in the treatment of lymphoma became the beginning of modern cancer chemotherapy, this case becoming the first milestone. In 1970, doxorubicin and cis-platinum were used in the clinic treatment, became second generation chemotherapy drugs. Since 1966, gemcitabine,

docetaxel, irinotecan, oxaliplatin enter the clinic successively. For over a hundred years, numerous experiments were carried out attempt to relieve the suffering of cancer patients who receive chemotherapy. Chemotherapeutic drugs were constantly being updated, new transfusion devices were invented and applied, implantable venous access port and peripherally inserted central catheter were widely used in chemotherapy nowadays. Even though the drugs and chemotherapy are getting better constantly, chemotherapy is still painful for patients, new chemotherapy drug still need to be developed.

Radiotherapy is another common treatment of cancer that is usually used. In the beginning, radiotherapy is an adjunct to cancer treatment due to low accuracy and low energy intensity. Precise radiotherapy has become increasingly mature nowadays, over a hundred years of development. But radiation in used still damage normal tissue and leading to terrible side effects.

Artemisinin and its derivatives are still not used in clinical treatment, but these compounds are expected to become a better anticancer drug than before. Traditional treatment of cancer has many deficiencies, new drug and treatment means are still required. More attention should be pay in the area of artemisinin and its derivatives anticancer.

6. Conclusion

Artemisinin and its derivatives were useful medicine in the treatment of malaria, more and more experiments proved these compounds also can be a great anticancer drug. Even though there are already have many experiments proved artemisinin and its derivatives have the potential to be a new effective anticancer drug, them cannot be applied directly in the cancer therapy directly. Artemisinin and its derivatives are great and safe drugs in the treatment of malaria, but not a mature drug for anticancer. Even though artemisinin and its derivatives still can't be used in clinical treatment of cancer, the more focuses are required. Artemisinin derivatives containing fluorine atoms is a new line of research. These compounds were proved have anticancer ability and minimal side effects like other traditional artemisinin derivatives. All of the artemisinin derivatives have potential to be a better anticancer drug in the future. These compounds are expected to be more effective and lower side effect anticancer drug than traditional treatment. However, more research and practice are still required to make sure this drug will be effective and safe for cancer patient.

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