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Review

Discovery and Development of Artemisinin and Related Compounds

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ABSTRACT

Artemisinin is isolated from the plant *Artemisia annua*, sweet wormwood, an herb employed in traditional Chinese medicine. Prof. You-you Tu discovered artemisinin in the 1960s, so she was awarded the 2015 Nobel Prize in Physiology or Medicine. Artemisinin and its semi-synthetic derivatives are a group of drugs that possess the most rapid action of all current drugs against *Plasmodium falciparum* malaria. In this review, the author investigated history on discovery of artemisinin, ethnopharmacology of *Artemisia* plants, chemistry and pharmacological activities of the relative compounds, and introduced Tu and other Chinese and world scientists' contribution, development of artemisinin and the related compounds and registered and marketed artemisinin drugs in China, UK, and USA. The author also recalled the studies on the mechanism of action of artemisinins and artemisinin combination therapies and summed up the resistance issues. In *Current Recommendations and the Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM)*, that the WHO prevents the development and manages the spread of insecticide resistance is summarized in the technical basis for coordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control. Prof. Tu re-emphasized the artemisinin resistant on five principles to the WHO. She called on the world's scientists to pay attention to the study of drug resistance, and hopes scientists to contribute to break resistance of artemisinins.

Key words

Artemisia; antimalarial drugs; antimalarial resistance; artemisinin; the 2015 nobel prize

1. Introduction

According to the World Malaria Report 2015, released today, more than half of the 106 countries with malaria in 2000 had achieved reductions in new malaria cases of least 75% by 2015. In that same time frame, 18 countries reduced their malaria cases by 50%−75% (WHO, 2015). The decreases in case incidence and mortality rates were the slowest in countries that had the largest numbers of malaria cases and deaths in 2000. Reductions in incidence need to be greatly accelerated in these countries if global progress is to improve. Historically, the emergence of chloroquine resistance in the 1970s and 1980s in Africa was associated with increased hospital admissions and mortality at the community level. Antimalarial resistance has also been associated with increased risk of anaemia and low birth weight, and with

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malaria epidemics and increased transmission. The development and spread of resistance to antimalarial medicines have significantly increased the global cost of controlling malaria over time, and given that new drugs must be continually developed to replace medicines that have become ineffective (WHO, 2015).

Artemisinin is isolated from the plant *Artemisia annua* Linn., sweet wormwood, an herb employed in traditional Chinese medicine (TCM). Prof. You-you Tu discovered artemisinin in the 1960s and was awarded the 2015 Nobel Prize in Physiology or Medicine. Artemisinin and its semi-synthetic derivatives are a group of drugs that possess the most rapid action of all current drugs against *Plasmodium falciparum* malaria. Tu's discovery of a cutting-edge drug developed from an ancient Chinese folk remedy was hardly known beyond China (Tu, 2011). She is best known for discovering artemisinin and dihydroartemisinin which are used to treat malaria. Treatments containing an artemisinin derivative (artemisinin-combination therapies, ACTs) are now the standard treatment worldwide for *P. falciparum* malaria. However, slow parasite clearance in patients treated with ACTs causes more parasites to be exposed to the partner medicine alone, increasing the risk of developing resistance to the partner medicine. If resistance develops to the partner drug, treatment failures with ACTs are likely to increase, as have already been observed in some areas. Malaria control programs in 2015 are deploying tools such as ACT that was not available in 2000. According to the World Malaria Report 2015, it suggested that similar innovation and wide-scale deployment of new tools would be required in the next 15 years, for malaria programs are to advance further and overcome the challenges the current face (WHO, 2015).

2. Ethnopharmacology and chemistry of *Artemisia* plants

2.1 Ethnopharmacology

Artemisia plants were used by Chinese herbalists for thousands of years as a remedy for many illnesses (Figure 1). The earliest record, written on a piece of silk unearthed from the Mawangdui Han Dynasty tombs (168 BC), described the *Artemisia* plants as a treatment for hemorrhoids. Later, in *the Handbook of Prescriptions for Emergency Treatments* by Hong Ge during the Jin Dynasty (Figure 2). In *Compendium of Materia Medica* by Shi-zhen Li (1518–1593) during the Ming Dynasty, sweet wormwood (qinghao) was specifically described as a remedy for fever (Figure 3). Artemisinin (qinghaosu, Figure 4) is the antimalarial principle isolated by Chinese scientists in 1972 from the aerial part of *A. annua*, a plant used in TCM for over 2000 years (Tu, 2011).

2.2 Chemistry studies

In 1971, Chinese scientists demonstrated that the plant extracts had antimalarial activity in primate models. In 1972, the active ingredient, artemisinin (Figure 4), was isolated and its chemical structure was described. Artemisinin is found in the glandular trichomes of the leaves, stems, and inflorescences, and it is concentrated in the upper portions of plants within new growth. Prof. You-you Tu and other researchers firstly isolated artemisinin within the Chinese Project 523.

Figure 1 Plants in genus *Artemisia* Linn. Sensu stricto, excl. Sect. Seriphidium Bess.

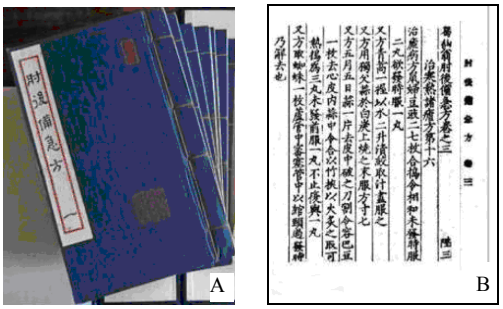

Figure 2 *A Handbook of Prescriptions for Emergencies* by Hong Ge (284–346 CE)

A: Ming dynasty version (1574 CE) of the handbook B: "A handful of qinghao immersed with two liters of water, wring out the juice and drink it all" is printed in the fifth line from the right (From volume 3).

Figure 3 *Compendium of Materia Medica* by Shi-zhen Li, which records *A. annua* and *A. carvifolia*

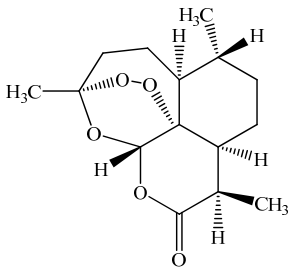

Figure 4 Chemical structure of artemisinin

A part from the active compound artemisinin, recent studies show that *A. annua* possesses the capacity to produce plenty of phenolic compounds, resulting in high anti-oxidant activity. Five major groups (coumarins, flavones, flavonols, phenolic acids, and miscellaneous) containing over 50 different phenolic compounds (Figure 5) were identified from *A. annua* (Jorge et al, 2010).

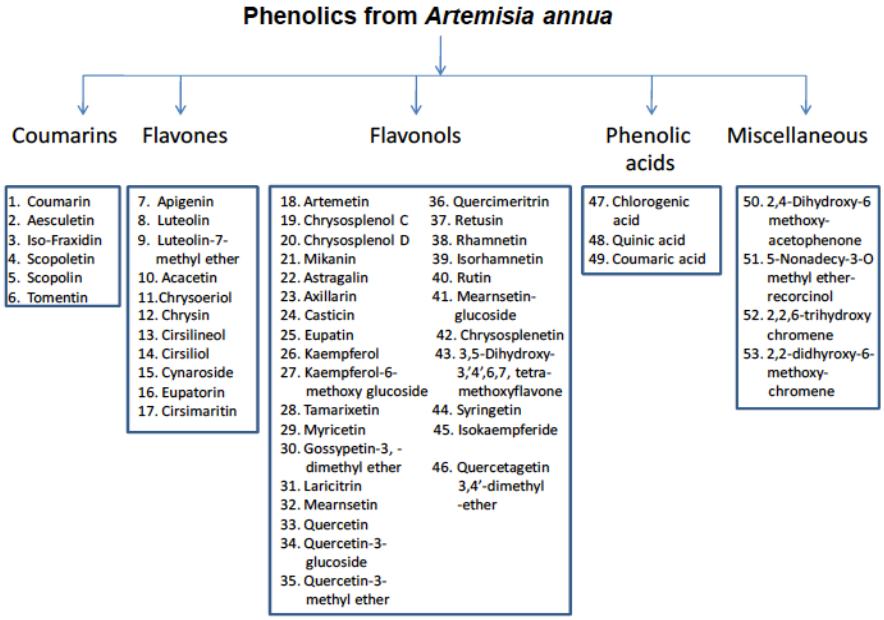

Figure 5 Phenolic compounds from *A. annua*

3. History on discovery of artemisinin

3.1 Project 523 for China Nation program of malaria treatment

In 1967, during the Vietnam War, Ho Chi Minh, the leader of North Vietnam, which was at war against South Vietnam and the United States, asked Chinese Premier En-lai Zhou for help to develop a malaria treatment for his soldiers trooping down the Ho trail, where a majority came down with a form of malaria resistant to chloroquine. As malaria was also a major cause of death in southern provinces of China, under the instructions of Chinese Chairman Ze-dong Mao and Premier En-lai Zhou, a meeting was held on May 23, 1967 in Beijing to discuss the problem of drug-resistant malaria parasites. This led to a secret nationwide program called Project 523, involving over 500 scientists from about 60 different laboratories and institutes (Zhang et al, 2006). Although the short-term goal of the project was to produce antimalarial drugs that could immediately be used in the battlefield, the long-term goal was to search for new antimalarial drugs by screening synthetic chemicals and searching recipes and practices of TCM.

3.2 Contribution of You-you Tu

Upon joining the project unit of Project 523, Tu was initially sent to Hainan province where she studied patients who had been infected with the disease. In 1969, Tu had an idea of screening Chinese herbs. She first investigated the Chinese medical classics in history. She gathered her findings in a notebook called *A Collection of Single Practical Prescriptions for Anti-Malaria*, in which she summarized 640 prescriptions. Her team also screened over 2000 traditional Chinese recipes and made 380 herbal extracts, which were tested on mice. One compound from *A. annua* was effective, which was used for "intermittent fevers", a hallmark of malaria. As Tu also presented at the project seminar, its preparation was described in a 1600-year-old text in a recipe titled as "*The Handbook of Prescriptions for Emergency Treatments*" written in 340 by Hong Ge. This book contained the useful reference to the herb: a handful of *A. annua* immersed with 2 L of water, wring out the juice and drink it all (Tu, 2011). Tu was influenced by Ge and discovered that a low-temperature extraction process could be used to isolate an effective antimalarial substance from the plant. After rereading the recipe, Tu realized that the hot water had already damaged the active ingredient in the plant; therefore she proposed a method using low-temperature ether to extract the effective compound instead. The animal tests showed that it was completely effective in mice and monkeys.

Prof. Tu collected 2000 candidate recipes, ancient texts, and folk remedies for possible leads for the research, had made 380 extracts from 200 herbs, and discovered that the extracts from *A. annua* looked particularly promising in dramatically inhibiting *Plasmodium* growth in animals. Tu found the way to extract it and her innovations boosted potency and slashed toxicity of this extract. In 1972, she and her colleagues obtained the pure substance (artemisinin) that has saved millions of lives. Tu also studied the chemical structure and pharmacology of artemisinin. In 1973, Tu

decided to confirm the carbonyl group in the artemisinin molecule, therefore she accidentally synthesized dihydro-artemisinin (Tu, 2011; Miller and Su, 2011). Tu's work was published anonymously in 1977 and 1979 (Tu, 2011; Qinghaosu Coordinating Research Group, 1977; 1979). Her discovery of artemisinin and its treatment of malaria is a significant breakthrough of tropical medicine in the 20th century and health improvement for people of tropical developing countries in South Asia, Africa, and South America. For her work, Tu received the 2011 Lasker Award in clinical medicine. For her work, she was awarded the 2015 Nobel Prize in Physiology or Medicine on October 5, 2015 (McKenna, 2011; Phillips, 2015).

3.3 Contribution of Chinese scientists

In Project 523, over 500 scientists from about 60 different institutes and laboratories were involved (Zhang, 2006). These scientists joined China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. They studied artemisinin and its derivatives in the chemistry and synthesis, pharmacodynamics, toxicity and clinical tests. After performing further efficacy and safety tests and clinical trials, the isolated active ingredients were assigned to the Institute of Traditional Medicine in Beijing (Zhang, 2006). A clinical trial of the crude extract involving 30 cases of malaria (20 *Plasmodium vivax* cases, nine *P. falciparum* cases, and one mixed infection) was conducted between August and October in 1972.

In 1973, scientists at Yunnan Institute of Materia Medica, China and Shandong Institute of Traditional Medicine and Materia Medica, China, extracted the antimalarial crystalline principle from *A. annua* and named it soluble artemether and arteether, which also showed greater antimalarial activity. Meanwhile several routes of total chemical synthesis of artemisinin and various attempts to produce the drug using bioengineered microbes have been reported (Li and Wu, 2003; Zeng et al, 2008).

In 1974, Prof. Guo-qiao Li at Guangzhou University of TCM, China, established the clinical trial sites, and the clinical results showed that conducting clinical trials on antimalarial chemotherapy at that time. Clinical trials in Yunnan on 18 malaria cases (14 *P. falciparum* cases including four severe cases and four *P. vivax* cases) produced excellent treatment response. Later in 1975, the relative configuration of artemisinin was solved using X-ray crystal analysis, while the absolute configuration was obtained using anomalous diffraction X-ray crystal analysis in 1976 and was published in 1979 (Zhang, 2006).

Table 1 lists 35 papers published by Chinese researchers from 1977 to 2011. These papers showed value contribution to antimalarial drugs by Prof. Tu and Chinese scientists.

Table 1 Thirty-five papers by Chinese researchers from 1977 to 2011

No.	Authors	Year	Titles	Publication
1	Collaboration research group	1977	A new sesquiterpene lactone−qinghaosu	*Sci Bull*, 3: 142
2	Collaboration research group	1979	Studies on new anti-malarial drug qinghaosu	*Chin Pharmacol Bull*, 14: 49-53
3	Collaboration research group	1979	Antimalarial studies on qinghaosu	*Chin Med J*, 92: 811-816
4	Liu JM et al	1979	Structure and reaction of qinghaosu	*Acta Chim Sin*, 37: 129-143
5	Tu YY	1981	The awarded Chinese invention: Antimalarial drug qinghaosu	*Rev World Invent*, 4: 26
6	Tu YY et al	1981	Studies on the constituents of *Artemisia annua* L	*Acta Pharm Sin*, 16: 366-370
7	Tu YY et al	1981	Studies on the constituents of *Artemisia annua* L. and derivatives of artemisinin	*Chin J Chin Mater Med*, 6: 31
8	Tu YY, et al	1982	Studies on the constituents of *Artemisia annua* L. (II)	*Planta Med*, 44: 143-145
9	Collaboration research group	1982	Chemical studies on qinghaosu	*J Tradit Chin Med*, 2: 3-8
10	XiaoYQ, Tu YY	1984	Isolation and identification of the lipophilic constituents from *Artemisia anomala* S. Moore	*Acta Pharm Sin*, 19: 909-913
11	Tu YY et al	1985	Studies on the constituents of *Artemisia annua* L. (III)	*Chin Tradit Herbal Drugs*, 16: 200-201
12	Wu CM, Tu YY	1985	Studies on the constituents of *Artemisia apiacea* Hance	*Chin Tradit Herbal Drugs*, 6: 2-3
13	Tu YY, Zhu QC	1984	Studies on the constituents of Young *Artemisia annua* L.	*Chin J Chin Mater Med*, 10: 419-420
14	Wu CM, Tu YY	1985	Studies on the constituents of *Artemisia gmelinii* Web. exstechm	*Chin Bull Bot* 3: 34-37
15	Wu CM , Tu YY	1985	Studies on the constituents of *Artemisia argyi* Levl et vant.	*Chin J Chin Mater Med*, 10: 31-32
16	Xiao Q, Tu YY	1986	Isolation and identification of the lipophilic constituents from *Artemisia anomala* S. Moore	*Acta Bot Sin*, 28: 307-310
17	Tu YY	1987	Study on authentic species of Chinese herbal drug qinghao	*Bull Chin Mater Med*, 12: 2-5
19	Yin JP, Tu YY	1989	Studies on the constituents of *Artemisia eriopoda* Bunge.	*Chin Tradit Herbal Drugs*, 20: 149-150

To be continued

Continued Table 1

No.	Authors	Year	Titles	Publication
20	Yang SX et al	1992	Immunologic enhancement and reconstitution by qinghaosu and its derivatives.	*Chin Bull Pharm*, 9: 61-63
21	Gu YC, Tu YY	1993	Studies on chemical constituents of *Artemisia japonica* Thunb.	*Chin Tradit Herbal Drugs*, 24: 122-124
22	Sun XZ et al	1993	Experimental study on the immuno-suppressive effects of qinghaosu and its derivatives	*Chin J Integr Trad West Med*, 11: 37-38
23	Huang L et al	1993	Studies on the antipyretic and anti-inflammatory effects of *Artemisia annua* L.	*Chin J Chin Mater Med*, 18: 44-48
24	Chen PH et al	1998	Effect of dihydroqinghaosu on the development of *Plasmodium yoelii* in *Anopheles stephensi*	*Chin J Parasitol Parastitic Dieases*, 16: 421-424
25	Tu YY	1999	The development of new antimalarial drugs: Qinghaosu and dihydro qinghaosu	*Chin Med J*, 11: 17-18
26	Tu YY	1999	The development of new antimalarial drugs: Qinghaosu and dihydro-qinghaosu	*Chin Med J*, 112: 976-977
27	Xu LM et al	2002	Effect of hydroar-temisinin on lupus BXSB mice	*Chin J Integr Tradit West Med*, 1: 19-20
28	Dong YJ et al	2003	Effect of dihydro-qinghaosu on auto-antibody production, TNFa secretion and pathologic change of lupus nephritis in BXSB mice	*Chin J Integr Tradit West Med*, 23: 676-679
29	Dong YJ et al	2003	The effects of DQHS on the pathologic changes in BXSB mice lupus nephritis and the effect mechanism	*Chin Pharmacol Bull*, 19: 1125-1128
30	Tu YY	2004	The development of the antimalarial drugs with new type of chemical structure—qinghaosu and dihydroqinghaosu	*Southeast Asian J Trop Med Public Health*, 35: 250-251
31	Yang L et al	2006	Determination of scopoletin in qinghao by HPLC	*Chin J Exp Tradit Med Formulae*, 12: 10-11
32	Li WD et al	2006	Dihydroarteannuin ameliorates lupus symptom of BXSB mice by inhibiting production of TNF-alpha and blocking the signaling pathway NF-kappa B translocation	*Int Immunopharmacol*, 6: 1243-1250
33	Zhang D et al	2007	Determination of artemisinin, arteannuin B and artemisinic acid in *Artemisia annua* by HPLC-UV-ELSD	*Acta Pharm Sin*, 42: 978-981
34	Tu YY	2009	*Artemisia annua* L, artemisinin and its derivatives	Beijing: Publisher of Chemical Industry.
35	Tu YY	2011	The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine	*Nature Med*, 17: 1217-1220

3.4 The 1981 Beijing WHO meeting in China

In 1981, the fourth meeting of the Scientific Working Group on the Chemotherapy of Malaria, sponsored by the United Nations Development Programme, the World Bank, and the World Health Organization (WHO), took place in Beijing (Figure 6).

Prof. Tu presented the findings related to artemisinin at the WHO meeting in China. During a special program for research and training in tropical diseases, a series of presentations on artemisinin and its antimalarial properties elicited enthusiastic response. Prof. Tu, the first speaker of the meeting, presented the report "Studies on the Chemistry of Qinghaosu". The efficacy of artemisinin and its derivatives in treating thousands of patients infected with malaria in China attracted worldwide attention in the 1980s. Prof. Jia-xiang Shen is the member of organizers from China Pharmaceutical Administration (CPA) for the meeting. Shen finished meeting files in English. Prof. Shu-yuan Song, a member of the Project 523 program, was presented at the meeting (Tu, 1981). The studies disclosed on this presentation were then published in 1982 (China Cooperative Research Group, 1982a; 1982b; 1982c; 1982d; 1982e).

While several routes of total chemical synthesis of artemisinin and various attempts to produce the drug using bioengineered microbes have been reported, the commercial source of artemisinin is still from the *Artemisia* plants (Li and Wu, 2003; Zeng et al, 2008).

4. Drug metabolism and pharmacokinetics

Drug metabolism and pharmacokinetics of artemisinin and its derivatives were carried out by many researchers. The rapid elimination of artemisinin in humans is advantageous in preventing the selection of resistant parasites by residual concentration of the drugs. On the other hand, the short half-life of artemisinin is also attributed to poor cure rates and high rates of recrudescence (> 25%) for short courses of artemisinin treatment (3–5 d). Even 7-d regimens of artemisinin monotherapy only cure 80%–90% of uncomplicated

Figure 6 Delegates at the fourth meeting of Scientific Working Group on Chemotherapy of Malaria in Beijing in 1981

Prof. Zhong-pu Ji (center, first row), president of the Academy of TCM, delivered the opening remarks to the meeting. Prof. You-you Tu is in the second row (fourth from the left). Prof. Shen is in the first row (the first from the left. Prof. Song is in the last row (the sixth from the left).

falciparum malaria (Nosten and White, 2007; Kalilani et al, 2007). Despite being the fastest drugs against all erythrocytic stages of malaria parasites, artemisinin and derivatives also have a very short elimination half-life (1 h), which precludes their use for malaria prophylaxis.

Artemisinin is mediated by the liver cytochrome P450 enzyme CYP2B6 (Svensson et al, 2003). The drug can also auto-induce P450 metabolizing enzymes, resulting in lower serum concentration of the drugs in subsequent administration (Ashton et al, 1998; Gordi et al, 1998; Gordi et al, 2002; Simonsson et al, 2003; Asimus et al, 2007). In humans, artemisinin derivatives are rapidly biotransformed into their bioactive metabolite DHA, which is later eliminated by glucuronidation (Lee and Hufford, 1990; Ilett et al, 2002). Depending on the derivatives, the extent of conversion varies: artesunate is converted to DHA within minutes, while conversion of artemether and arteether is slower (Li et al, 1998; Olliaro et al, 2001).

Some preparations are available, including artesunate, artemisinin, artemether, and dihydroartemisinin in clinical therapy. However each may have different pharmacokinetic properties and more information is needed to determine optimal dose and comparative efficacy with each other and with conventional parenteral treatments for severe malaria. In a clinical study carried out by Gomes et al, individual patient data from 1167 patients in 15 clinical trials of rectal artemisinin derivative therapy (artesunate, artemisinin, and artemether) were pooled in order to compare the rapidity of clearance of *P. falciparum* parasitaemia and the incidence of reported adverse events with each treatment (Gomes et al, 2008; Dondorp et al, 2009). Data from patients who received comparator treatment (parenteral artemisinin derivative or quinine) were also included. Primary endpoints included percentage reductions in parasitaemia at 12 and 24 h. (1) A parasite reduction of > 90% at 24 h was defined as parasitological success. (2) Artemisinin and artesunate treatment cleared parasites more rapidly than parenteral quinine during the first 24 h treatment. (3) A single higher dose of rectal artesunate treatment was five times more likely to achieve >90% parasite reductions at 24 h than that were multiple lower doses of rectal artesunate, or a single lower dose administration of rectal artemether. They suggested that artemisinin and artesunate suppositories rapidly eliminate parasites and appear to be safe. There are less data on artemether and dihydroartemisinin suppositories. They also suggested that more rapid parasite clearance of single high-dose regimens achieving immediate high drug concentration may be the optimal strategy.

5. Pharmacological activities of artemisinin

5.1 Antimalarial activity

A better extract was obtained by a low-temperature ether-based extraction method. Purification processes were used to isolate the active molecule. And, clinical trials showed the active ingredient to be an effective drug (Miller and Su, 2011). Artemisinin is a sesquiterpene lactone with an endoperoxide bridge and has been produced semisynthetically as an antimalarial drug. The efficacy of tea made from *A. annua* in the treatment of malaria is dubious. Several studies have found that artemisinin was not soluble in water and the concentration in these infusions was considered insufficient to treat malaria (Mueller et al, 2004; Räth et al, 2004; Jansen, 2006). A review indicated that artemisinin-based remedies were the most effective drugs for the treatment of malaria in 2012 (Fairhurst et al, 2012). Despite global efforts were combating malaria, it remains a large burden for the population, particularly in tropical and subtropical regions (Chrubasik and Jacobson, 2010). The WHO recommends ACT based on artemisinin resistance. In 2013, a review suggested that although *A. annua* might not cause hepatotoxicity, haematotoxicity, or hyperlipidemia, it should be used cautiously during pregnancy due to a potential risk of embryotoxicity at a high dose (Miller and Su, 2011).

5.2 Anticancer activities

Artemisinin was tested for the treatment of cancer (Hou et al, 2008; Cabello et al, 2011; Lai et al, 2013; Sanjeev et al, 2014; Bhisutthibhan et al, 1998). In general it has been shown that specific flavonoid compounds could inhibit specific cancer cell growth as well as cell proliferation. Artemisinin has anticancer activity as well, because it contains an endoperoxide group. Many studies show anticancer results analyzing different flavonoids, such as flavones and flavonols. Synthetic artemisinin derivatives are being investigated for their potential use as anticancer drugs.

5.3 Asthma

Animal experiments show that artesunate, a synthetic derivative of artemisinin, has anti-allergic properties by effecting mast cell degranulation. This makes artesunate a candidate for treatment of allergic asthma. The mechanism of action of artemisinin involves cleavage of endoperoxide bridges by iron, producing free radicals (hypervalentiron-oxo species, epoxides, aldehydes, and dicarbonyl compounds) which damage biological macromolecules causing oxidative stress in the cells of the parasite (Cumming et al, 1997). Malaria is caused by apicomplexans, primarily *P. falciparum*, which largely resides in red blood cells and itself contains iron-rich heme-group (Posner and O'Neil, 2004).

6. Development of artemisinin and related compounds

The chemical structure of artemisinin provided further foundation for improvement of the drug. Several derivatives were subsequently produced in China to treat malaria, including artemether, artesunate and dihydroartemisinin. Extension of Project 523 also led to the discovery of several synthetic antimalarial drugs including pyronaridine, lumefantrine (benflumetol), naphthoquine, and so on. It is a sesqui-terpene with a peroxide bridge linkage, the peroxide moiety appearing to be responsible for the antimalarial activity. Artemisinin was formulated in China in both oil and water for intramuscular injections and as tablets and suppositories. Antimalarial drugs under development were reviewed by Krishna, Olliaro and Trigg (Krishna et al, 2008; Olliaro and Trigg, 1995). Artemisinin and related compounds are listed in Figure 7.

6.1 Registered and marketed artemisinin drugs in China

The drug's poor solubility stimulated Chinese scientists to synthesize more soluble derivatives by the formation of dihydroartemisinin and its esterification or etherification to artesunate and artemether. All these derivatives have a more potent antimalarial activity than the parent compound and appear to be the most rapid acting of all antimalarial compounds developed so far. The following formulations are produced in China: oral tablets of artemether and artesunate, more recently, of dihydroartemisinin; injectable formulations of artemether in groundnut oil for intra-muscular administration and of sodium artesunate for iv administration; and suppositories of artemisinin. An injectable formulation of artemether has been produced and registered by Rhone-Poulenc Rorer in conjunction with the Kunming Pharmaceutical Factory in China and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). In China, the registered and marketed artemisinin drugs are listed in Table 2.

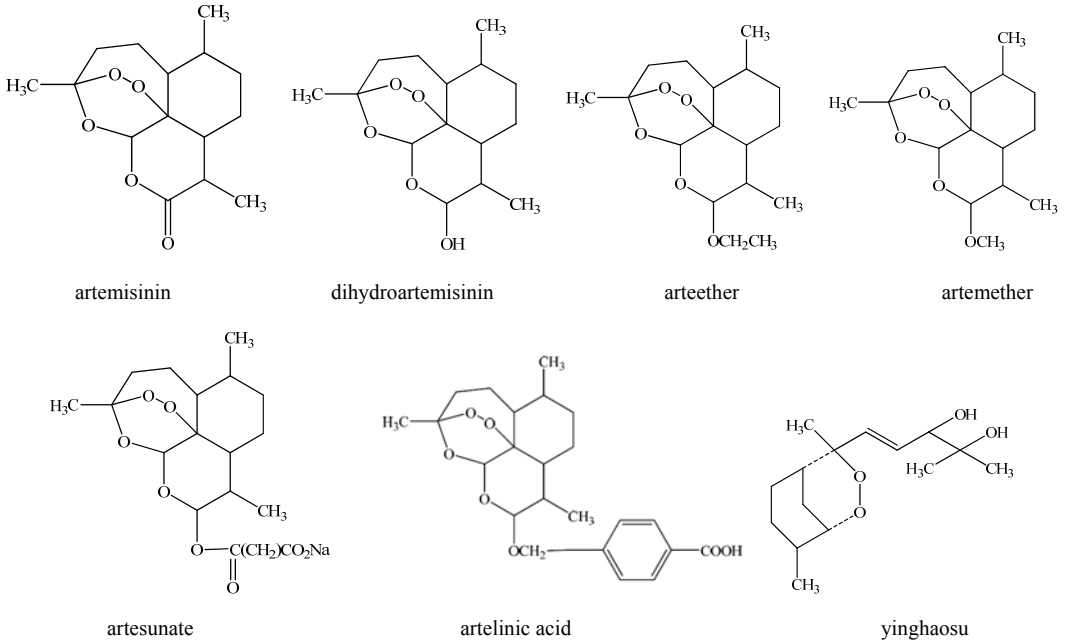

Figure 7 Artemisinin and related compounds

Table 2 Registered and marketed artemisinin drugs

No.	Active compounds	Formulations	Registered year
1	artemisinin	Chemical raw materials	1986 (China)
2	artemisinin	Tablet	1986 (China)
3	artemisinin	Suppositories	1986 (China)
4	artesunate	Chemical raw materials	1987 (China)
5	sodium artesunate	Injection	1987 (China)
6	dihydroartemisinin	Chemical raw materials	1993 (China)
7	dihydroartemisinin	Tablet	1993 (China)
8	dihydroartemisinin	Injection	1993 (China)
9	dihydroartemisinin	Complex tablet	1997 (China)
10	artemether	Tablet (Coartem)	2009 (USA, FDA)
11	arteether	Injection (Artecef)	2000c (UK, MCA)
12	artesunate	Tablet	2008 (China)

6.2 Registered and marketed artemisinin drugs

A growing number of pharmaceutical companies now produce and market artemisinin and its derivatives, some of which have been registered for use in several countries of south-east Asia and in many other parts of the world. Recommendations for their use were made by the WHO in 1993 and these will be reviewed again shortly. Although oral formulations of artemether and dihydroartemisinin have been registered in China, only limited studies on oral artemether and none on dihydroartemisinin have been carried out in other countries. Most data on these formulations are either at present unavailable or are published in Chinese. A recent study in Viet Nam suggests that artemisinin suppositories may have a comparable efficacy to injectable parenteral artemether or artesunate but this has to be confirmed (Hien et al, 1992). Effective suppository formulations would have particular use at the periphery of the health care system, particularly in patients who are unable to swallow oral medications. The development of an injectable formulation of arteether has been supported by the UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases (TDR). Its spectrum of activity is similar to artemether. Phase I clinical studies have shown the drug to be safe and well tolerated; the resorption from the intramuscular depot appears slow with a long elimination half-life. It is currently in Phase II trials (UNDP/World Bank/WHO, 1993). In addition, oral artesunate has been produced and registered by Mepha AG and Viet Nam produces artemisinin tablets, capsules and suppositories as well as artesunate tablets and capsules for local use.

6.3 Other effective derivatives with antimalarial activity

Attempts to synthesize more soluble, stable, and effective derivatives are continuing. Artelinic acid is being developed by WRAIR as a more water-soluble iv formulation than artesunate for the treatment of severe and complicated malaria (Lin et al, 1987). In Phase I trials in human volunteers, the demonstration of antimalarial activity related to both the trioxane ring of the artemisinin molecule and the peroxide group of yinghaosu, the antimalarial principle of *Atrobotrys uncinatus*, has led to the synthesis of a variety of peroxide, trioxane, and tetraoxane analogues (Peters et al, 1993a; 1993b; Posner et al, 1995). Although several compounds have shown antimalarial activity in *in vitro* and *in vivo* models, only arteflene, as an oral formulation, has so far reached human Phase I clinical trials, and Phase II clinical trials of artelinic acid were carried out in 1994 (Posner et al, 1994; Weidekamm et al, 1994; Salako et al, 1994). Although a longer duration of treatment and/or its combination with other antimalarials such as mefloquine might enhance the efficacy, the projected cost of such treatment was judged too high (Cumming et al, 1997).

7. Mechanism of action of artemisinins

Artemisinin derivatives are the most recent single drugs approved and introduced for public antimalarial treatment. The mechanisms of action attributed to artemisinin include interference with parasite transport proteins, disruption of parasite mitochondrial function, and modulation of host immune function and inhibition of angiogenesis.

Artemisinin is a sesquiterpene trioxane lactone whose endoperoxide bridge is essential for antimalarial activity. The development raises serious concerns for the future of artemsinins and this is not helped by controversy related to the mode of action. Although a number of potential targets have been proposed, the actual mechanism of action remains ambiguous. The mechanism of action of artemisinin involves cleavage of endoperoxide bridges by iron, producing free radicals (hypervalentiron-oxo species, epoxides, aldehydes, and dicarbonyl compounds) which damage biological macromolecules causing oxidative stress in the cells of the parasite (Cumming et al, 1997; Meshnich et al, 1996). Malaria is caused by apicomplexans, primarily *P. falciparum*, which largely resides in red blood cells and by itself contains iron-rich heme-groups (in the form of hemozoin) (Posner and O'Neil, 2004). The precise mechanism of action is still highly controversial; The endoperoxide pharmacophore alone has stimulated the development of several different classes of totally synthetic endoperoxides. Understanding the mechanism of action of this class of drugs will allow the prediction of potential resistance mechanisms and aid targeted

design of future antimalarial agents. In the review by O'Neill et al, the authors analyzed and discussed the recent evidence explaining bioactivation and potential molecular targets in the chemotherapy of malaria (O'Neill et al, 2010). To study the mechanism of the action of the artemisinins, many hypotheses have been proposed, such as iron and heme model, mitochondria model, PfATP6 model, and so forth. Studies suggest that the mechanism of the artemisinins mainly involves two aspects: the activation of artemisinin and targeting response (Sun et al, 2010).

7.1 Reductive scission model

Early work by Posner (Posner et al, 1995; Posner and Oh, 1992; 1994) and Jefford (Jeffors et al, 1995; 1996) proposed that these oxygen-centered radicals subsequently rearrange to form carbon-centered radicals, although the nature of the proposed radical and the mechanistic pathways giving rise to their formation were different in each case. Low valent transition ions (ferrous heme or non heme exogenous Fe^{2+}) were found to bind to artemisinin and after subsequent electron transfer induce reductive scission of the peroxide bridge to produce oxygen-centered radicals which rearrange to give carbon-centered radicals.

7.2 Open peroxide model

The alternative model suggests that ring opening is driven by protonation of the peroxide or by complexation by Fe^{2+}. Haynes and co-workers have proposed that iron acted as Lewis acid to facilitate ionic, rather than radical bioactivation of the artemisinins (Haynes et al, 2007). In addition, it has also been suggested that non-peroxidic oxygen plays a role in facilitating ring opening of the peroxide to generate the open hydroperoxide (Haynes and Vomwiller, 1996; Haynes et al, 1999). Artemisinin is first activated by intraparasitic heme-iron which catalyzes the cleavage of this endoperoxide. The free radical intermediate may then kill the parasite by alkylating and poisoning one or more essential malarial proteins (Haynes and Vomwiller, 1996; Mesnick, 1998).

7.3 Iron-dependent bioactivation vs heme-dependent bioactivation in parasites

The species originally thought to be responsible for bioactivation was heme iron, as this form of iron is in abundance within the parasite due to the degradation of hemoglobin releasing soluble heme. However, intracellular Fe^{2+} and Fe^{3+} occur also in equilibrium inside the food vacuole of the parasite, where digestion of hemoglobin takes place. In the presence of desferrioxamine, the drug is completely washed out, suggesting that iron is required for the drug to become covalently bound to parasite macro-molecules. The observations are suggested that the labeled compounds are first being accumulated by the parasite and then activated by a non-heme chelatable iron source (Haynes et al, 1996a; 1996b; 2007; Stocks et al, 2007). During the asexual blood stage of its lifecycle, the malaria parasite *P. falciparum* grows and multiplies in the hemoglobin-rich environment of the human erythrocyte. The interaction with iron represents an Achilles' heel that is exploited by many antimalarial drugs. The parasite deals with hemoglobin breakdown products and on the role of iron as a mediator of the action of the drugs (Klonis et al, 2013).

Artemisinin is first activated by intraparasitic heme-iron which catalyzes the cleavage of this endoperoxide. The parasite deals with hemoglobin breakdown products and on the role of iron as a mediator of the action of the antimalarial drugs. During the asexual blood stage of its lifecycle, the malaria parasite *P. falciparum* grows and multiplies in the hemoglobin-rich environment of the human erythrocyte. Porsche believes that artemisinin has two ways to generate intermediate compound, and Fe^{2+} played an important role in the catalytic reaction. The reaction can be alkylating protein, and causing cell death. Therefore, the reaction is likely to be an important step in reaction produce of the drug. Two intermediate products may be a potential final form caused by alkylating agents in malarial treatment (Cumming et al, 1997).

7.4 Study on artemisinin drug target

When plasmodium falciparum-infected erythrocytes are incubated with 10-^{3}H-dihydroartemisinin, several malaria-specific proteins become labeled. One of these proteins is the *P. falciparum* translationally controlled tumor protein (TCTP) homolog. *In vitro*, dihydroartemisinin reacts covalently with recombinant TCTP in the presence of hemin. The association between drug and protein increases with increasing drug concentration, plateauing at approximately 1 drug/TCTP molecule. By Scatchard analysis, there appear to be 2 hemin binding sites on TCTP with dissociation constants of 18 μmol/L. When the single cysteine moiety is blocked by pretreatment with iodoacetamide, hemin binding is not affected, whereas drug binding is reduced by two-thirds. Thus, TCTP reacts with artemisinin *in situ* and *in vitro* in the presence of hemin and appears to bind to hemin. The function of the malarial TCTP and the role of this reaction in the mechanism of action of artemisinin await elucidation. Authors suggested that although it is possible that TCTP is the artemisinin drug target, the observed reaction may also be unrelated to the killing effect of the drug. In the observed interactions between TCTP, hemin, and artemisinin, the study provided clues to the true target as well as insights into the physiological role of TCTP. Meshinick suggested that the resulting free radical intermediate may then kill the parasite by alkylating and poisoning one or more essential malarial proteins (Meshnich, 1998).

7.5 Action mechanism of artemisinin combination therapies

After a major change in treatment policies, artemisinins are now the frontline treatment to aid rapid clearance of parasitaemia and quick resolution of symptoms. Since

artemisinin and its derivatives are eliminated rapidly, artemisinin combination therapies (ACTs) are now recommended to delay resistance mechanisms. In spite of these precautionary measures, reduced susceptibility of parasites to the artemisinin-based component of ACTs has developed at the Thai-Cambodian border, a historical 'hot spot' for MDR parasite evolution and emergence. This development raises serious concerns for the future of the artemsinins and this is not helped by controversy related to the mode of action. Although a number of potential targets have been proposed the actual mechanism of action remains ambiguous. Authors discussed the recent evidence explaining bioactivation and potential molecular targets in the chemotherapy of malaria (Waknine et al, 2006). ACTs are currently the preferred treatment for malaria. These combinations may prevent the induction of parasite drug resistance. This development raises serious concerns for the future of the artemsinins and this is not helped by controversy related to the mode of action.

8. Resistance of artemisinins

8.1 Development of resistance of artemisinins

Artemisinin and its derivatives are often paired with longer-acting partner drugs as ACTs, which are now the first-line treatment for *P. falciparum* throughout the malaria-endemic world. Artemisinin resistance has emerged in Southeast Asia, manifesting itself as delayed clearance of parasitemia following treatment with artemisinin derivatives (Dondorp et al, 2009; Klonis et al, 2013; Noedl et al, 2008).

Recently, the resistance has been shown to be associated with mutations within a kelch protein located on *P. falciparum* chromosome 13 (K13 propeller) (Kyaw et al, 2013; Ariey et al, 2014), and the secondary loci may also be involved. K13 propeller mutations have been associated with delayed parasite clearance in several Southeast Asian countries, and have been shown to have spread between countries as well as to have emerged independently in different countries (Straimer et al, 2015). Despite rapid clearance of parasites in all African studies conducted thus far, K13 propeller mutations have been observed at low levels in parasites from African study sites (Takala-Harrison and Laufer, 2015; Ashley et al, 2014).

The Global Plan for Artemisinin Resistance Containment (GPARC) established by the WHO, it is being implemented. Drug-resistant parasites repeatedly arise as a result of widespread use of antimalarial drugs and have contributed significantly to the failure to control and eradicate malaria throughout the world (WHO, 2011a; 2011b). Therefore, future development of resistance may be associated with overproduction or mutations of this transporter. However, a general mechanism, such as alterations in general drug transport pathways, is feasible.

Malaria treatment is based on guidelines that can be slow to change in response to resistance patterns and are not tailored to specific infection in the individual patient. When an antimalarial drug is failing, the clinical results are not immediately obvious. Drug-resistant parasites repeatedly arise as a result of widespread use of antimalarial drugs and have contributed significantly to the failure to control and eradicate malaria throughout the world. In 2015, Takala-Harrison studied artemisinin resistance mutations among *P. falciparum* (Takala-Harrison et al, 2015). Takala-Harrison and Laufer suggested that the spread of resistance to chloroquine and sulfadoxine-pyrimethamine, and examined the effect of the removal of drug pressure on the survival of resistant parasites (Takala-Harrison and Laufer, 2015). Artemisinin-resistant malaria is now emerging in Southeast Asia in a unique and unexpected pattern. They analyzed the most recent genomic and clinical data to help predict the behavior of resistance to new antimalarial medications and inform strategies to prevent the spread of drug-resistant malaria in Africa in the future.

For *P. falciparum* parasites that are resistant to artemisinins in Southeast Asia, the resistance is associated with several polymorphisms in the parasite's K13-propeller gene. But the molecular epidemiology of the artemisinin resistance genotypes in African parasite populations is unknown. Taylor et al developed an assay to quantify rare polymorphisms in parasite populations that uses a pooled deep-sequencing approach to score allele frequencies, validated it by evaluating mixtures of laboratory parasite strains, and then used it to screen *P. falciparum* parasites from >1100 African infections collected since 2002 from 14 sites across sub-Saharan Africa. They found no mutations in African parasite populations that are associated with artemisinin resistance in Southeast Asian parasites. However, they observed 15 coding mutations, including 12 novel mutations, and limited allele sharing between parasite populations, consistent with a large reservoir of naturally occurring K13-propeller variation. Nevertheless polymorphisms associated with artemisinin resistance in *P. falciparum* in Southeast Asia are not prevalent in sub-Saharan Africa. Therefore, the rapid, scalable molecular surveillance offers a useful adjunct in tracking and containing artemisinin resistance (Taylor et al, 2015).

Mutations in the *P. falciparum* K13-propeller domain have recently been shown to be important determinants of artemisinin resistance in Southeast Asia. Kamau et al investigated the prevalence of K13-propeller polymorphisms across sub-Saharan Africa. A total of 1212 *P. falciparum* samples collected from 12 countries were sequenced. None of the K13-propeller mutations previously reported in Southeast Asia were found. The study provides the baseline prevalence of K13-propeller mutations in sub-Saharan Africa (Kamau et al, 2015).

8.2 Mechanisms of insecticide resistance

The spread of insecticide resistance, especially pyrethroid resistance in Africa, is a major threat for vector control programmes. Insecticide resistance management has to be considered as important as epidemiological cost-effectiveness in all programmatic decisions about vector

control (WHO, 2011a; 2011b). In "Current recommendations and the Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM)", that the WHO prevents the development and manages the spread of insecticide resistance is summarized in the technical basis for coordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control (WHO, 2010).

8.2.1 Mechanisms of insecticide resistance

Two main mechanisms of insecticide resistance have been identified: (1) Target site resistance and metabolic resistance. Target site resistance occurs when the site of action of an insecticide is modified in resistant mosquito populations so that the insecticide no longer binds effectively and the insect is therefore unaffected, or less affected, by the insecticide. (2) Target site resistant mutations can affect acetylcholinesterase, which is the molecular target, carbamates, or voltage-gated sodium channels, which is known as knock-down resistance. Metabolic resistance occurs when increased levels or modified activities of a detoxifying enzyme system prevent the insecticide from reaching its intended site of action.

Ariey et al suggested that drug resistance to several antimalarials is sometimes either due to changes in drug accumulation or efflux mechanisms (chloroquine, amodiaquine, quinine, halofantrine, and mefloquine resistance) or due to decreased affinity of the drug target which may result from point mutations in the respective genes that encode these targets (pyrimethamine, cycloguanil, sulphonamide, atovaquone, and artemisinin resistance) (Ariey et al, 2014; Foote et al, 1994; Ward et al, 1995; Miotto et al, 2013).

8.2.2 The GPARC plan

The GPARC plan recommends that in areas with evidence of artemisinin resistance, an immediate, multifaceted response should be launched with the aim of containing and, if feasible, eliminating the resistant parasites. The selection of insecticides for IRS. In particular, resistance management measures should be part of every vector control programme, and deployed preemptively, without waiting for signs of the presence of resistance or of control failure. For contain or eliminate artemisinin resistance where it already exists prevent artemisinin resistance where it has not yet appeared.

The WHO GPARC plan (WHO, 2011a; 2011b) included consultation with almost 150 stakeholders. The key of the plan is: (1) define what is known, what is assumed and what remains unknown with regard to insecticide resistance among malaria vectors, its spread and operational impact, and options for managing the problem; (2) estimate the potential impact of insecticide resistance on malaria burden, and the financial cost of monitoring and managing insecticide resistance; and (3) based on these elements, define the plan for managing insecticide resistance and the way forward in the action plan with clear responsibilities, and ongoing research and development requirements. The GPARC goals and reconmmendstions are: (1) stop the spread of resistant perasites; (2) increase monitoring and surveillance to evaluate the artemisinin resistance threat; (3) improve access to rational treatment with ACTs; (4) invest in artemisinin resistance-related research, and (5) motivate action and mobilize resources (WHO, 2010; 2011a; 2011b). In the report on the Nobel award ceremony in 2015, Prof. Tu re-emphasized the WHO recommended the artemisinin resistant on five principles. She called on the world's scientists to pay attention to the study drug resistance, and hopes scientists to contribute to break resistance of the artemisinins.

8.2.3 Combating drug resistance

Artemisinin and its derivatives should not be used as oral monotherapies for the treatment of uncomplicated malaria as poor adherence to the required 7 d of treatment results in partial clearance of malaria parasites which will promote resistance to this critically important class of antimalarials. The spread of resistance to antimalarial drugs over the past few decades has led to an intensification of efficacy monitoring to allow early detection of resistance in order to revise national malaria treatment policies and ensure proper management of clinical cases. A key component of overall containment plans to halt the spread of resistant parasites. Despite the observed changes in parasite sensitivity to the artemisinin, the clinical and parasitological efficacy of ACTs has not yet been compromised. In other areas in this region, the efficacy of both components of the combination is put at risk.

8.2.4 ACTs recommended for using drugs

Using an ACT containing a partner drug to which there is already resistance (and is therefore not effective) can increase the risk of development or spread of artemisinin resistance. Severe malaria should be treated with a parenteral artesunate and followed by a complete course of an effective ACT as soon as the patient can take oral formulations. Where complete parenteral treatment of severe malaria is not possible, the patients should be given pre-referral treatment and referred immediately to an appropriate facility for further treatment. Options available for pre-referral treatment are: rectal artesunate, quinine, artesunate or artemether. The based on the efficacy of the combination in the country or area of intended use, the five ACTs recommended for use are: (1) artemether plus lumefantrine, (2) artesunate plus amodiaquine, (3) artesunate plus mefloquine, (4) artesunate plus sulfadoxine-pyrimethamine, and (5) dihydroartemisinin plus piperaquine.

Conflict of interest statement

The authors declare no conflict of interest.

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