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Review

Qinghaosu (artemisinin): Chemistry and pharmacology

Ying LI*

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

Qinghaosu and its derivatives are widely used in the world as a new generation of antimalarial drug. Up to now, some important progresses of Qinghaosu research have been made, including synthesis of new Qinghaosu derivatives and analogs, investigation on their bioactivities and mode of actions. The present review briefly describes these efforts made by researchers in China, particularly in this Institute.

Keywords: Qinghaosu; artemisinin; structure modification; antimalarial activity; anticancer activity; immunosuppressive activity; mode of action

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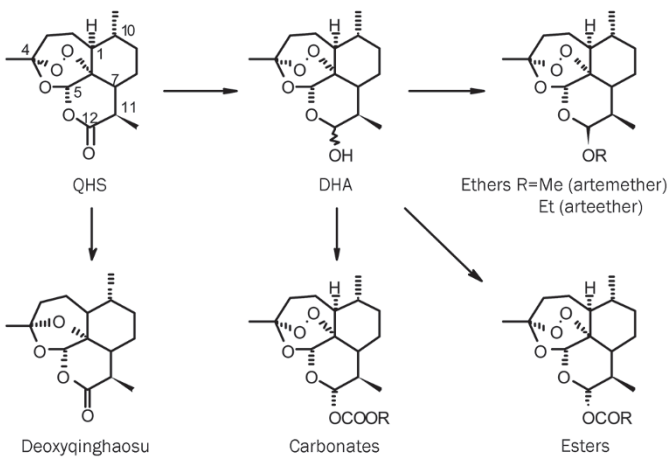
On 23rd May 1967, China established National Steering Group on antimalarial drug research, more than 60 institutes and 500 researchers joined in this project. After screening of over 5000 traditional Chinese medicines, Qinghaosu (QHS), an antimalarial principle, was isolated from *Artemisia annua* L in 1972[1, 2]. At the end of 1975, its unique chemical structure was elucidated, as a sesquiterpene lactone bearing a peroxy group, quite different from that of all known antimalarial drugs[3].

Early pharmacological and clinic studies showed QHS have rapid onset of action, low toxicity and high effect on both drug-resistant and drug-sensitive malaria. However, its shortcomings (poor solubility in water or oil; high rate of parasite recrudescence) needed to be overcome. In 1976, our Institute was charged with this important mission, and a research in relationship between chemical structure and activity immediately started.

First of all, the function of peroxy group for antimalarial activity was examined. The negative result of deoxyqinghaosu (Scheme 1) against *P berghei* in mice demonstrated that the peroxy group was essential. Soon afterwards, it was found that some other simple peroxides including monoterpene ascaridol had no antimalarial activity. These experimental results proved peroxy group to be an essential but not a sufficient factor. At that time, we noted the molecule contained a rare segment -O-C-O-C-O-C=O, and realized that whole molecular skeleton might play an important role for antimalarial activity.



Scheme 1. Synthesis of Qinghaosu derivatives.

When dihydroartemisinin (DHA) was found to be more active than QHS, but was still with poor solubility and lower stability (as a lactol) than QHS, we decided to prepare its derivatives. In 1976–1977, over 50 derivatives of DHA were synthesized (Scheme 1) and evaluated[4, 5].

The first 25 compounds (in oil solution) were tested in mice infected chloroquine-resistant *P berghei* though intramuscular injection[6]. Most of these derivatives showed higher activity than QHS (SD_{50} 6.20 mg/kg) and DHA (SD_{50} 3.65 mg/kg). In the ether series, SM 224 (R=CH_3, SD_{50} 1.02 mg/kg) is more

* To whom correspondence should be addressed.
E-mail yli@mail.shcnc.ac.cn
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active than SM 227 (R=C_2H_5, SD_{50} 1.95 mg/kg) and others. Then, SM 224, SM 108 (ester, R=C_2H_5, SD_{50} 0.66 mg/kg) and SM 242 (carbonate, R=n-C_3H_7, SD_{50} 0.50 mg/kg) were compared with regard to activity, stability, toxicity and cost. Because SM 224 was highly soluble in oil and more stable than others, it was selected as candidate and named as artemether.

At the same period, water-soluble sodium artesunate (ester, R=$COCH_2CH_2COONa$) was developed by the Guilin Pharmaceutical Factory[7]. QHS suppository in 1986; artemether oil injection and sodium artesunate aqueous injection in 1987 were approved as new antimalarial drugs. Since then, other new antimalarial drugs such as dihydroartemisinin, coartem (artemether and benflumetol), co-naphthoquine (naphthoquine phosphate and QHS), compound-dihydroartemisinin were successively developed in China[1, 2].

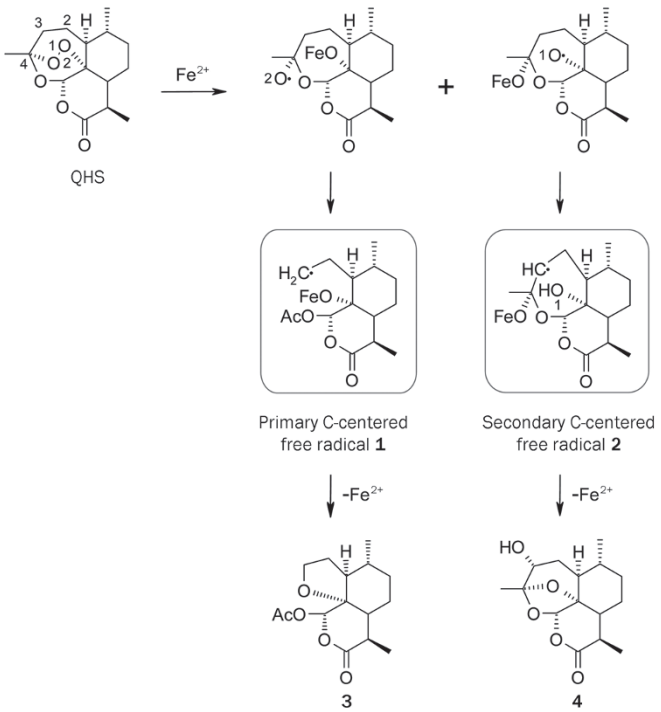
Since 1980s, millions malaria patients in the world (mainly in China, Southeast Asia and Africa) were saved by administration of QHS, QHS derivatives and their combinations. Artemether, artesunate and coartem have been enrolled in WHO's "List of Essential Medicines" .

Artemisinins can kill drug-resistant *P falciparum*, its antimalarial mechanism must be different from that of the previously-used antimalarial drugs. Elucidation of its mode of action is really an interesting project. Up to now, many research papers have been published[8–14]. Chinese researchers first reported in 1979 that artemisinin drugs had a direct parasiticidal action against *P falciparum* in the erythrocytic stage both *in vitro* and *in vivo*, and observed their morphologic changes under the electron microscope[15, 16] . The main pathological ultrastructural changes caused by QHS were quick damage of the membrane system of the asexual forms of parasites, the swelling and spiral deformation of the membrane of food vacuoles, limiting membrane and the membrane of mitochondria, followed by swelling of the nuclear membrane and endoplasmic reticulum. Among the latter results made in China[17–21], a series of reaction of artemisinins and Fe^{2+}, which involes the intermediate of oxygen-centered or carbon-centered free radicals, is noteworthly.

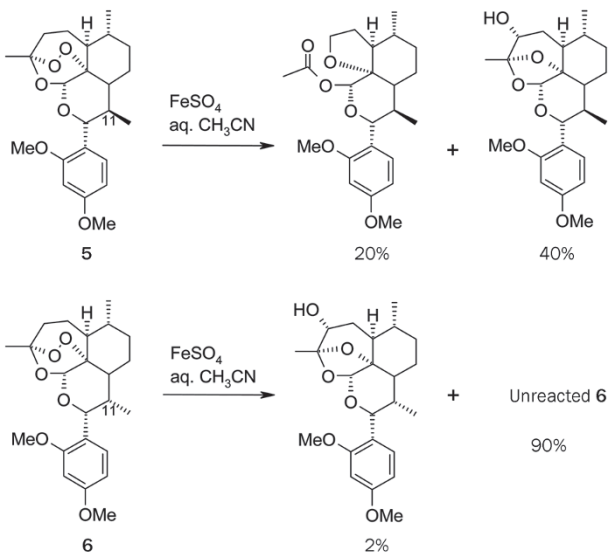
For exploring the mode of action, reaction of QHS and Fe^{2+} was carefully studied by Chinese chemists[22, 23]. The major products tetrahydrofuran **3** and 3-hydroxy deoxyqinghaosu **4** were proposed to be derived from primary C-centered free radical **1** and secondary C-centered free radical **2,** respectively, as shown in Scheme 2. In addition, the electron spin resonance (ESR) signals of the C-centered free radicals were detected[23, 24].

It is worthy to note that compound **3** and **4** are also the major metabolites of QHS *in vivo* or in human[25, 26].

With the clarification on the C-centered free radicals participation mechanism, it is then interested whether this free radical mechanism is related to its antimalarial activity. Thus, the stable and UV-detectable C-12 aromatic substituted derivatives of QHS were synthezied[27]. Using the usual Lewis acid as the catalyst, the Friedel-Crafts alkylation gave the desired product 2′,4′-dimethoxyphenyl deoxoartemisinin **5** and also 11α-methyl epimer **6** as the by-product (Scheme 3). These



Scheme 2. Formation of Carbon-centered free radicals.



Scheme 3. Reaction of QHS derivatives and $FeSO_4$.

products were separated and subjected to both bioassay and chemical reaction with ferrous ion. It is interesting to find that the derivative with normal configuration at C-11 showed higher antimalarial activity and also higher chemical reactivity in the reaction with ferrous ion. On the contrary, the 11α-methyl epimer **6** was obviously less active and almost inert to the reaction with ferrous ion (Scheme 3, Table 1).

In the case of 11α and 11β-epimers of α-hydroxy naphthyl deoxoqinghaosu, similar result was also obtained[28]. Their

Table 1. The ED_{50}- and ED_{90}-values against *P berghei* K173 strain (administered orally to mice as suspensions in Tween 80).

Compound	ED_{50} (mg/kg)	ED_{90} (mg/kg)
Artemether	1	3.1
5	1.27	5.27
6	4.18	76.27

lower activity may be attributed to the steric hindrance around O-1 atom in 11α-epimer, which blocks the way for Fe^{2+} to attack O-1 (Figure 1).

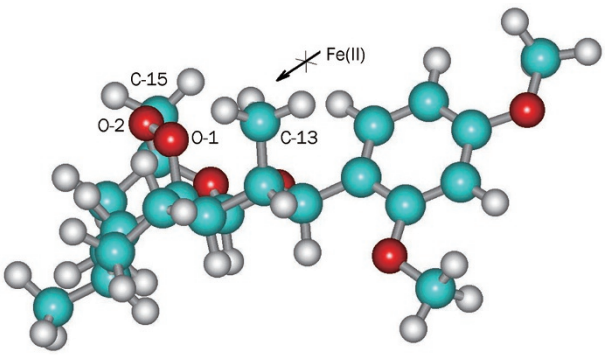


Figure 1. 11α-methyl group blocks the attack of Fe^{2+} on O-1.

The close relationship between antimalarial activity and the primary C-centered free radicals strongly suggests that the Fe^{2+}-induced cleavage of peroxide bond in artemisinins leads to C-centered free radicals, a highly potent alkylating species. Then, what targets would be alkylated? Up to now, a growing list shows that heme, heme-containing protein, translationally controlled tumor-associated protein (TCTP), sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA)-type protein encoded by PfATP6 might be the targets[14]. Recently, Chinese researchers reported that artemisinins are distributed to malarial mitochondria and directly impair their functions[29].

As for process of alkylation of targets, there was another interesting topic. Considering that malaria-parasite-infected red blood cells have a high concentration of the reducing glutathione (GSH) and excess GSH might be responsible for protecting the parasite from the toxicity of heme, Chinese chemists have performed some reactions, such as artemisinins reacted with cysteine or GSH in the present of a catalytic amount of ferrous ion[27, 30–33]. Successful isolation and identification of the cysteine-artemisinins or GSH-artemisinins adducts proved the formation of covalent bond in these adducts. It is instructive that the C-centered radicals derived from QHS and their derivatives could attack free cysteine and cysteine rasidue not only in peptide but also probably in protein.

The outstanding characteristics of QHS, including high antimalarial action , less significant side effects and less clinical resistance, impelled researchers to extend its medical application. As a result, hundreds of artemisinin derivatives and analogs were synthesized and widely assayed in China. So far, the successful examples are artemether and artesunate, which were found to be effective in the treatment of Schistosomiasis[34, 35]. From 1993, artemether and artesunate were studied in randomized, double-blind, placebo-controlled trials in China, and approved as the prophylactic drugs for schistosomiasis japonica in 1996[2]. Afterwards, they were found to have similar activity against *S mansoni* and *S haematobium* in other countries[36].

Since 1992, when this Institute first reported that some components of *Artemisia annua* L (such as artemisinic acid, artemisinin B) have antitumor activity *in vitro*[37, 38], hundreds of papers have described the cytotoxicity of QHS and related compounds against tumor cells. Artesunate and DHA are most favorable molecules. In 2001 Efferth *et al* reported that artesunate showed antitumor activity against 55 tumor cell lines, and it was most active against leukemia and colon cancer cell lines. It is notable that none of CEM leukemia sublines, resistant to doxorubicin, vincristin, methotrexate or hydroxyurea showed cross-resistance to artesunate[39]. Since then, more new artemisinin derivatives and analogs were synthesized and tested against human cancer cells. Some compounds showed high activity at the nano- to micromolar range[40]. For example, this Institute in collaboration with French cooperator found that a dihydroartemisinin ether containing cyanoarylmethyl group **7** (Figure 2) was very active against P388 and A549 cell lines, as comparable to VCR, whereas its deoxy-analog **8** (Figure 2) was inactive. Thus peroxy group was proved to be essential for antitumor activity, like for antimalarial activity[41, 42]. In addition, the type of derivative was proved to target G_1 phase of the cell cycle[41]. Thus far, antitumor activity of artemisinins and the underlying mechanisms have been

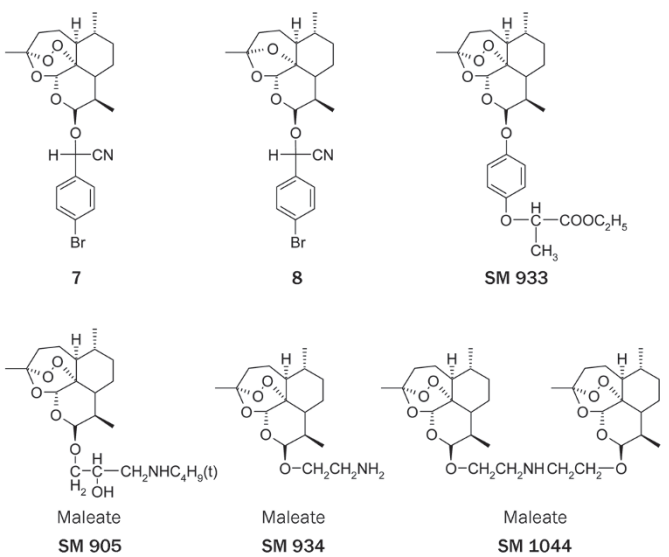


Figure 2. Some compounds with anticancer or immunosuppressive activity.

widely studied[43–49], however, few clinical trials were reported.

Antimalarial chloroquine was used as immunosuppressive agent. Therefore, the research on immunological activity of QHS and its derivatives became an attractive program in China in the 1980′. At the begining, mainly new antimalarial drugs (QHS, artemether and artesunate) were studied in laboratories in China. Afterwards, the clinical trials of artesunate for the treatment of DLE, SLE, rheumatoid arthritis, polymorphous light eruption and chronic actinic dermatitis were conducted. Some clinical data were promising, for example, 56 patients with lupus erythematosus (DLE 16, SCLE 10, and SLE 30) were treated by sodium artesunate (iv 60 mg, once a day, 15 d a course, 2–4 courses), with effective rate at 94%, 90%, and 80%, respectively[50]. To search for highly potent, low toxic immunosuppressive agents, more artemisinin derivatives were synthesized and screened in this Institute since 2001[51–54]. Compounds with various structures were tested in respect to their cytotoxicity to lymphocyte, inhibition activity on ConA-induced T cell proliferation and LPS-induced B cell proliferation, in comparison with QHS, artesunate, artemether and cyclosporin A (CsA). At last, SM 735, SM 905, SM 933, and SM 934 (Figure 2) were selected and tested in the animal models for 2,4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity (DTH) reaction, sheep red blood cell (SRBC)-induced antibody production, and experimental autoimmune encephalomyelitis (EAE). Up to date, a number of papers related their immuno-suppressive activity and possible mechanisms have been published mainly from this Institute[55–64]. The preclinical research of SM 934 is in progress.

During the latter period of the program of the relationship between chemical structure and activity of artemisinins, SM 1044 was isolated as a by-product during the preparation of SM 934, and interestingly showed excellent antileukemia activity *in vitro* and *in vivo* in the Ruijin Hospital, Shanghai Jiao Tong University School of Medicine[65–69]. These alkaline artemisinins may be a new kind of promising candidates. I sincerely hope they will be successfully developed for treatment of cancer, auto-immune or other diseases, like artemether as antimalarial drug 30 years ago.

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