Review Articles

Anthelminthic effects of artemisinin and its derivatives

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Abstract

Qinghao (*Artemisia annua,* L.), a famous herb from China, has been used in traditional Chinese medicine. Artemisinin, an active substance is extracted from the leaves and flowers of Qinghao, with a unique sesquiterpene lactone endoperoxide structure showing an antimalarial activity. Derivatives of artemisinin, artesunate and artemether, are the most widely used as antimalarial drugs. Since artemisinin not only possess antimalarial properties, but also active against various parasitic infection. Ongoing research on these compounds has been emphasized on the potential impact for anthelmintic treatment. Artemisinin derivatives exhibited a broad spectrum property against various trematodes, nematode, and cestode both *in vitro* and *in vivo* studies. These helminthocidal properties of artemisinin derivatives presented here provide further data for clinical investigations in the field trial study. However, a better understanding of action of artemisinin derivatives and their biomolecule target may contribute a promising possibility for clinical utility in anthelmintic application. Toxicity of artemisinin derivatives was less and was demonstrated in experimental animals with neurotoxicity and anemia.

Keywords: artemisinin, artesunate, artemether, anthelminthic

ประสิทธิภาพของอาติมิซินินและสารอนุพันธ์ ในการใช้เป็นยาถ่ายพยาธิ

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บทคัดย่อ

ชิงเฮา เป็นสมุนไพรที่เป็นที่รู้จักกันมานานของประเทศจีนและถูกใช้ในตำรายาจีนโบราณเพื่อรักษาโรค สารออกฤทธิ์ที่สำคัญสกัดได้จากส่วนใบและดอกของต้นชิงเฮาคืออาติมิซินิน สารนี้มีโครงสร้างทางเคมีที่เฉพาะคือมีกลุ่ม sesquiterpene lactone endoperoxide อยู่ในโครงสร้างทำให้อาติมิซินินมีคุณสมบัติในการทำลายเชื้อมาลาเรียได้ สารอนุพันธ์ของอาติมิซินิน ได้แก่ อาทีซูเนตและอาทีมีเทอร์ เป็นยารักษาโรคมาลาเรียที่นิยมใช้กันอย่างแพร่หลายในปัจจุบัน นอกจากประสิทธิภาพในการรักษาโรคมาลาเรียแล้ว อาติมิซินินและสารอนุพันธ์ยังมีประสิทธิภาพในการใช้เป็นยาถ่ายพยาธิได้หลายชนิด ผลการศึกษาจากหลอดทดลองและการศึกษาในสัตว์ทดลองพบว่าสารอนุพันธ์ของอาติมิซินินออกฤทธิ์ในวงกว้างในการฆ่าพยาธิ โดยมีฤทธิ์ในการฆ่าพยาธิใบไม้ พยาธิตัวกลม และพยาธิตัวตืดได้ จากผลการศึกษาดังกล่าวเป็นที่น่าสนใจและนำไปสู่การศึกษาต่อในสัตว์ที่ติดพยาธิและการศึกษาในภาคสนามต่อไป การเข้าใจถึงกลไกการออกฤทธิ์และสารชีวโมเลกุลที่เป็นเป้าหมายของสารอาติมิซินินจะช่วยในการพัฒนาปรับปรุงรูปแบบของการให้ยาในผู้ป่วยเพื่อให้มีประสิทธิภาพในการรักษาต่อไป สารอาติมิซินินและอนุพันธ์มีความปลอดภัยสูง ความเป็นพิษที่พบในสัตว์ทดลองคือ ความเป็นพิษต่อระบบประสาทและทำให้เกิดโลหิตจาง

คำสำคัญ : อาติมิซินิน อาทีซูเนต อาทีมิเทอร์ ยาถ่ายพยาธิ

Introduction

For thousands of years, Chinese herbalists treated fever with an extract from the plant called "qinghao" or sweet wormwood (*Artemisia annua* L., Figure 1). In 1971, Chinese scientists isolated and identified the active compound of qinghao that was highly active against *Plasmodium berghei* in infected mice and *P. cynomogi* in infected monkeys (Tu 2011). The active ingredient was structurally elucidated in 1972 named in China "qinghaosu" or "arteannuin" and in the Western "artemisinin" (Klayman 1985; Tu 2011). Not only the antimalarial property, artemisinins also exhibit antihelminthic activity against various trematodes, nematode, and cestode (Utzinger et al. 2001; Jiraung koorskul et al. 2005; Keiser et al. 2006a ; Keiser et al. 2006c; Keiser and Morson 2008; Spicher et al. 2008; Shalaby et al. 2009a)

Chemistry

Artemisinin presents in the leaves and the flowers of the plant in 0.01-0.8% dry weight (Jain et al. 1996). It is a sesquiterpene lactone with a peroxide bridge linkage which provide a different structure compared to classical antimalarial drugs such as chloroquine, quinine or sulfadoxine (Klayman 1985; Hien and White 1993; Barradell and Filton 1995). The peroxide moiety appears to be responsible for its antimalarial activity. Artemisinin is a potent and rapidly acting blood schizontocide, eliciting shorter parasite clearance times than chloroquine or quinine.

Figure 1 *Artemisia annua* or sweet wormwood plant (A); leaves (B) and flowers (C).(modified from *www.rbgkew.org.uk/plants/artemisinin.html,www.kalyx.com/store/images, www.home.tiscali.be/lpauwels/arteannu.jpg*)

Artemisinin derivatives

A main limitation of artemisinin is poorly soluble in either oil or water and has a poor bioavailability limiting its effectiveness. Therefore, the first generation of semisynthetic artemisinin, artemether, arteether, artesunate, and artelinic acid have been developed and become widely used antimalarials today. Figure 2 shows the chemical structure of artemisinin and derivatives.

All derivatives of artemisinin are metabolized to an active metabolite, dihydroartemisinin, which exhibits the most potent antimalarial property but also the least stable. In most country, artesunate and artemether are the onlytwo derivatives of artemisinin that have been licensed for treatment of *P. falciparum* malaria since 1990 (Kamchonwongpaisan and Meshnick 1996).

Dihydroartemisinin is the product of the first step of chemical synthesis starting with artemisinin. Artesunate and artemether derive from further synthesis steps and are rapidly converted *in vivo* back to dihydroartemisinin.

Artemether is the methyl ether of dihydroartemisinin and is synthesized in a two-step procedure from dihydroartemisinin. Artemetheris dissolved in groundnut oil is marketed in ampoules containing 80 mg of drug for intramuscular injection. This preparation is stable at room temperature for 4 years. An oral (capsule) formulation is also now commercial available (Hien and White 1993).

Artesunate is a water-soluble hemisuccinate derivative which can be administered by intravenous and intramuscular injection. And it is also available as tablets (50 mg). Artesunic acid powder (60 mg per ampoule) is unstable in neutral solutions therefore it has to be freshly dissolved before injection with 5% sodium bicarbonate to produce the salt sodium artesunate. The solution is then diluted with saline or 5 % dextrose before injection (Hien and White 1993).

Artesunate is converted within minutes to dihydroartemisininand elimination half-life is about 45 min after intravenous administration. For oral administration, absorption rate of this drug is rapid reaching the maximum concentration at 45 min and persisting for up to 4 h. The active metabolite, dihydroartemisinin has a plasma elimination half-life of less than 2 h (Davis et al. 2001; Olliaro et al. 2001).

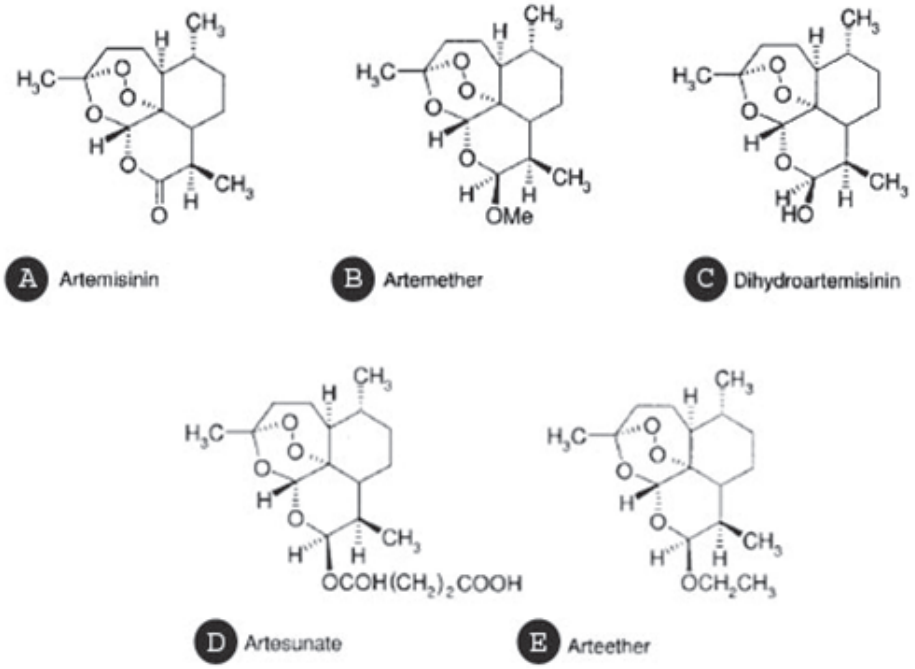

Figure 2 Chemical structures of A. artemisinin, B. artemether, C. dihydroartemisinin, D. artesunate, and E. arteether (Keiser et al. 2006a)

Proposed targets of artemisinins

It is well established that the mechanism of action of artemisininderivatives appears to involve the intraparasitic iron (Fe^{2+}), which catalyzed the cleavage of the endoperoxide bridge to produce the carbon-center free radicals (Meshnick 2002). The actions of these free radicals in malaria parasite are though to be composed of the following that mediate the killing action of the drugs (O'Neill et al. 2010).

1. Alkylation of heme

Alkylation of heme by artemisinins was present in the form of heme-artemisinin adducts which were found both *in vitro* and *in vivo* experiments (Meshnick et al.1993b). The heme-artemisinin adducts were demonstrated in red cell membranes which resulted in protein thiols oxidation *in vitro* (Meshnicket al.1993b). Heme-drug adducts were present in the spleen and urine of mice harboured *Plasmodium vinckei* after artemisinin administration (Robert et al. 2005).

2. Alkylation of parasite protein

The covalent reaction of artemisinin and parasite proteins were demonstrated in situ and *in vitro* studies (Meshnick et al. 1993a). Six malarial proteins were radiolabelled by endoperoxides of arteether, dihydroartemisinin and arteflene *in vitro* (Meshnick et al. 1993a).

3. Inhibition of a Ca^{2+} transportation ATP-ase (SERCA)

The sesquiterpene lactone within the structure of artemisininsis highly selective inhibitor of mammalian Ca^{2+} transportation ATP-ase (SERCA) resulted in reduction of cytosolic free calcium concentration.

4. Oxidative damage to parasite membrane

The study of Hartwig et al. (2009) showed that artemisinins accumulated within neutral lipid of *P. falciparumand* caused parasite membrane damage.

5. Disruption of the electron transport chain in mitochondria

Li et al. (2005) demonstrated that mitochondria could be the primary target of artemisinin, since mitochondria are a rich source of transition metals, including iron and copper (Kispal et al. 1999), and the mitochondria could consequently be damaged by the locally generated free radicals (Li et al. 2005).

Pharmacological action

1. Antimalarial activity

In 2005, The World Health Organization (WHO) recommended Artemisinin Combination Therapies (ACTs) as a standard treatment of uncomplicated malaria caused by *P. falciparum* and *P. vivax* (WHO 2006).

2. Anthelminthic activity

There are still controversial to limit use of artemisinins in malarial treatment. However, the researches concerning anthelminthic action have been reported. There are many evidences showing the potential of artemisinin and derivatives in anthelminthic effects *in vitro* and *in vivo* as well as in clinical studies.

2.1 Trematocidal activity

Due to the feeding habits of trematode sand malaria parasites are similar in which trematodes must consume host hemoglobin for their nutrition and generate ferrous-heme degradation products. Artemisinins might act against fasciolas and schistosomes via multiple mechanisms which have been proposed in plasmodium parasites including: alkylation of heme, alkylation of parasite protein, inhibition of SERCA, oxidative damage to parasite membrane, and disruption to the electron transport chain in mitochondria.

2.1.1 Schistosomicidal activity

In early 1980s, the schistosomicidal property of artemisinin derivatives was investigated (Le et al. 1982). Presently, it is well established that artemisinin and its derivatives also display potent antischistosomal activities as demonstrated in laboratory animal model and field trial studies (Utzinger et al. 2007).

The *in vitro* finding, adult *Schistosoma japonicum*, *S.mansoni*, and *S. haematobium* worms were incubated with artemether (0.5-20 μg/ml) and hemin

(50-100 μg/ml) showing rapidly decreased motility. Then, gradually increased in the vesiculation of tegument was observed leading up to parasite death within 24-72 h (Xiao et al. 2001a). Artemether, administered to various animals experimentally infected with *S. japonicum*, resulted in marked reductions of the schistosome worm burden (Utzinger et al. 2001). The schistosomulae are more susceptible to artemether than the adult stage. Up to now, a number of *in vivo* studies confirmed antischistosomal properties for artemether, artesunate and also dihydroartemisinin (Utzinger et al. 2001; Jiraungkoorskul et al. 2005).

The possible mechanisms of artemisinin and its derivatives in helminthotoxic effects were purposed including alteration of schistosome tegument and biochemical pathways in parasites (Utzinger et al. 2001). Tegumental alterations were carried out following drug administration to juvenile *S. japonicum, S.mansoni* and *S.haematobium*, by means of scanning electron microscopy (SEM; Xiao et al. 1996; Xiao et al. 2000c; Xiao et al.2000d; Xiao et al. 2001b). Mild or moderate swelling of the tegumental ridges became apparent 8 h after artemether treatment. Increasing alterations were related with severity at 3-7 d post treatment. Morphological alterations included swelling and fusion of tegumental ridges. The syncytium showed vesiculation, peeling and erosion. SEM observations in adult stage of *S. japonicum, S. mekongi,* and *S. mansoni* revealed similar features of tegumental alterations as seen in schistosomula (Xiao et al. 1996; Xiao et al. 2000a; Jiraungkoorskul et al. 2005). Studies with transmissible electron microscope (TEM) in juvenile *S. mansoni* showed that artemether induced morphological alterations in the tegument, subtegumental musculature, parenchymal tissues, and gastrodermis (Xiao et al. 2000c). The most notable biochemical change in adult schistosomes after *in vivo* treatment with artemether was the reduction in their glycogen content. In addition, the enzyme activities of the major glycolytic enzyme tested, hexokinase, glucose phosphate isomerase, phosphofruktokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglyceral kinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, and the enzymes alkaline phosphatase, acid phosphatase and adenosine triphosphatase were reduced in schistosomes recovered from artemether-treated mice relative to untreated control mice (Xiao et al. 2000b). These reductions were related to an inhibition of glycolysis rather than to an interference with glucose uptake (Utzinger et al. 2001).

2.1.2 Fasciocidal activity

An *in vitro* incubation in 10 μg/ml of artemether or artesunate caused severe tegumental damage in adult *F. hepatica* (Keiser and Morson 2008). Similarly, the alterations of surface were observed in adult *F. gigantica* when incubated with 10-30 μg/ml of artesunate (Shalaby et al. 2009b). In addition, artesunate at the concentrations between 40-80 μg/ml was effective against juvenile *F. gigantica* (Tansatit et al. 2012). Initially, swollen tegumental folds and ridges was appeared as a small patch with scattered in multiple loci. Subsequently, blebs were formed at surface then turned to be patches of lesions (Keiser and Morson 2008; Shalaby et al. 2009b; Tansatit et al. 2012). Morever, TEM observations also convinced that tegumentalmitochondria of fluke could be the primary target of artemisinin. After treatment with artesunate, these mitochondria exhibited severe swelling, rupturing of the outer membrane and contained flocculent densities (Tansatit et al. 2012).

Recently, the fasciocidal property of artemisinins against both juvenile and adult stages of *Fasciola hepatica* and *F. gigantica* has been reported (Keiser et al. 2006b; Shalaby et al. 2009b; Duthaler et al. 2010; Keiser et al. 2010; Tansatit et al. 2012). A randomized controlled study showed a high efficacy of artesunate in the treatment of symptomatic human fascioliasis in Vietnam (Hien et al. 2008). Artesunate at a single dose of 40 mg/kg intravenously and intramuscularly exhibited 77.4% and 91.9% worm burden reductions

respectively in naturally *F. hepatica* infected sheep (Keiser et al. 2010). An oral administration of artesunate at a dose of 200 mg/kg resulted in 95% and 56.4% reduction of worm burden in rats harbouring adult and juvenile *F. hepatica*, respectively (Duthaler et al. 2010). A high oral dosage of 400 mg/kg artesunate could completely eradicate adult *F. hepatica* harboured in rats (Keiser et al. 2006b). Likewise, 72 h *in vitro* incubations in 50 and 100 μg/ml artesunate resulted in 75% and 100% mortality of adult and juvenile *F. hepatica*, respectively (Duthaler et al. 2010).

2.1.3 Opisthorchicidal activity

The trematocidalactivity of artemisinins against *Opisthorchis* sp. was investigated in hamster model (Keiser et al. 2006c). Worm burden reductions of 77.6% and 65.5% were demonstrated in hamsters harboured adult *O. viverrini* following a single dose of 400 mg/kg artesunate and artemether, respectively (Keiser et al. 2006c).

2.1.4 Clonorchicidal activity

The effect of artemisinin and its derivatives against the Chinese liver fluke, *Clonorchis sinensis* was initially investigated in 1983 (Chen et al. 1983). Administration of 30-60 mg/kg artemether for 5 d resulted in 83-100% worm reduction in rats harboured-*C. sinensis* (Chen et al. 1983). In 2008, the action of artesunate and artemether against adult *C. sinensis* in *vivo* was also demonstrated to support the previous observation (Xiao et al. 2008). A single administration of 150 mg/kg of artesunate or artemether resulted in total mortality of the adult *C. sinensisin* rat model. Whereas the control group that received oral dose of 150 mg/kg praziquantel showed 80.7% worm burden reduction (Xiao et al. 2008).

2.1.5 Echinostomacidal activity

The effects of artemisinin derivatives against adult Echinostoma caproni were examined both *in vitro* and *in vivo* (Keiser et al. 2006a). Exposure of adult *E. caproni* for 72 h to 1 μg/ml artesunate resulted in 100% mortality of the flukes. With increasing concentration to 100 μg/ml, all of the adult flukes died within 24 h. The treatment with 100 μg/ml dihydroartemisinin and artemether resulted in 100% mortality following 6 and 72 h, respectively (Keiser et al. 2006a). For the *in vivo* examination, administration of artemether at 800 and 1,100 mg/kg to mice harboured adult *E. caproni* resulted in 27% and 100% worm reduction, respectively (Keiser et al. 2006a).

2.2 Nematocidal activity

The effects of artemisinins against nematode parasites have been demonstrated by Shalaby et al. (2009a). *In vitro* treatment with 10 μg/ml artemether caused morphological alterations to the cuticle of adults *Toxocara canis* following 24-48 h. Surface alterations were characterized by distortion of sensory papillae, swollen and lesions of the lips which later lost of the cuticle of this part. These alterations are similar to those observed in adult worms after incubated with albendazole sulfoxide (Shalaby et al. 2009a).

2.3 Cestocidal activity

Protoscolicidal activity of artemisinin derivatives was investigated by Spicher et al. (2008). Artsunateat the concentrations of 10-40 μM caused 100% mortality of the larva of *Echinococcus granulosus* following 6 d of *in vitro* treatment (Spicher et al. 2008).

Resistance

Artemisinins have been recommended by The World Health Organization (WHO) as the first-line therapy for *P. falciparum* infection since 2005 (WHO 2006). The first case of resistance of *P. falciparum* infection against artemisinin was reported from the area near Thai-Cambodia border in the early 2000s. Subsequently, Noedl et al. (2008) reported the evidence of artesunate resistance in malaria patients from western Cambodia (Noedl et al. 2008). Recently, there was an evidence of falciparum malaria resist against combination on artesunate-mefloquine which exhibited in Pailin, Western Cambodia, and Wang Pha, Norwestern Thailand (WHO 2012).

Toxicity

The toxicity of artemisinin therapy in human was found to be very low, artemisinins are also safe to be administered in pregnant women (McGready et al 1998). However, toxicity of artesunate injection has been reported in experimental animals (Zhao 1985; Xie et al. 2005). Artesunate intravenous injection at the doses of 480 and 640 mg/kg produced neurotoxic in Guinea pigs and rabbits, respectively (Zhao 1985). Intravenous injection of 240 mg/kg artesunate in rats for three consecutive days resulted in anemia and reduced reticulocyte numbers of the rats (Xie et al. 2005).

Conclusion

Artemether and artesunate, derivatives of artemisinin are widely used for the treatment of malaria, including highly drug resistant strains. They also exhibit the promising anthelminthic effects to other trematodes, such as *F. hepatica, F. gigantica, O. viverrini, C. sinensis,* and *E. caproni*. Their efficacy also extends to nematode: *T. canis* and cestode: *Echinococcusgranulosus*.

In addition to helminthes parasites, activity of artemisinins in medicine have also been reported. Further activities of artemisinins include anti-viral and anti-cancer properties, implicating its possible application in virus, bacteria and cancer chemotherapy.

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