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Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication
Artemisinin drugs in the treatment of malaria:
from medicinal herb to registered medication

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Registration in Europe of several artemisinin drugs for the treatment of malaria can soon be expected. Artemisinin is isolated from the herb *Artemisia annua*, in use in China more than 2000 years as a herbal tea against fever. Artemisinin drugs are being used extensively in South-East Asia and increasingly in Africa. Active derivatives have been synthesized – artemether, arteether and artesunate – which are used for oral, intramuscular, rectal and intravenous administration. The origin, mechanism of action, efficacy and safety in patients, the pharmacokinetics and the position of this group of compounds among existing antimalarials are discussed in this review.

Victims are children under the age of five living in Africa below the Sahara; 80–90% of the world malaria burden is carried by this continent.

As the results of trials with malaria vaccines have been disappointing, malaria control still depends on the use of effective antimalarial drugs. However, vaccine research is still proceeding and vaccination remains the most cost-effective strategy for attacking the malaria problem. Malaria control in the past three decades has been dominated by the increasing problem of malaria parasite resistance to the existing antimalarials. In Africa, there is widespread resistance to chloroquine, and resistance to the second-line drug pyrimethamine-sulphadoxine (Fansidar) is increasing.

The problem of drug resistance is greatest in South-East Asia, where there is evidence for resistance or reduced sensitivity against all antimalarial drugs including mefloquine, halofantrine and even quinine. Treatment of severe malaria now relies on the use of the latest antimalarials, the artemisinin-derived drugs. In countries with endemic malaria, these drugs have been available for several years without being properly registered.

Artemisinin, or qinghaosu (Chinese for 'from green herb'), was isolated by Chinese researchers in 1972 from *Artemisia annua* L. (sweet or annual wormwood) and its structure elucidated in 1979 (Ref. 9). The plant, a perennial herb of the family of composite flowers, has been used in traditional Chinese medicine as a remedy for
A characteristic of the artemisinin drugs is the rapid onset of action with clearance of parasites from the blood within 48 h in most cases. The clinical relevance of this rapid action, however, has not been established. A meta-analysis showed a slight survival-benefit with artemisinin drugs compared to quinine in the treatment of severe (complicated or cerebral) malaria. These drugs have surprisingly few adverse effects. Although neurotoxicity can occur in animals, it has never been reported in humans. So far no in vivo resistance has been described, making them also effective in the treatment of multi-resistant malaria. A disadvantage of the artemisinin drugs is the occurrence of recrudescences when given in short course monotherapy regimens. Therefore, combination with a longer-acting antimalarial drug is usually recommended. A striking pharmacokinetic phenomenon in multiple dose studies of some artemisinin analogues is a time-dependent decrease in plasma concentrations probably caused by autoinduction. The artemisinin drugs should be reserved for situations where problems of resistance or unwanted side-effects of the available antimalarial drugs are expected.

Malaria is still one of the major health problems in many tropical countries. Although, in the 1950s, with the use of residual insecticides and effective drugs there was hope of eradication, this hope has vanished by the 1960s with the appearance of DDT resistance. The situation worsened with the emergence of chloroquine-resistant strains of Plasmodium falciparum, the malaria parasite responsible for two million deaths every year. Most chills and fevers for more than 2000 years. Although originally from northern parts of China, the plant now grows wild in many countries. It is easily grown under a wide variety of conditions but the artemisinin yields can vary considerably, depending on plant material and growth conditions. Artemisinin is present in the leaves and the flower of the plant in 0.01–0.8% dry weight. There are no real limitations on the supply of the compound or its derivatives to restrict their future widespread use as antimalarials.

Fig. 1. Structures of artemisinin and its derivatives.
Artemisinin is a sesquiterpene trioxane lactone, containing a peroxide bridge, which is essential for its activity. The lactone can easily be reduced with sodium borohydride, resulting in the formation of dihydroartemisinin, which has even more antimalarial activity in vitro than artemisinin itself. Many derivatives have been synthesized from dihydroartemisinin and out of these arteether, arteether, artesunic and artelinic acid are either currently in use or being evaluated for use (see Fig. 1). The rationale behind the use of these semisynthetic derivatives of artemisinin is related to their physical properties. Artemisinin has poor solubility in water or oil and can thus only be administered orally.

In patients with severe malaria, oral treatment is often impossible and a parenteral formulation of the drug is required. Therefore, water-soluble artesunate, the hemisuccinate of dihydroartemisinin and the oil soluble arteether have been developed by Chinese scientists for intravenous and intramuscular administration, respectively. Development of oil-soluble arteether has been promoted by the World Health Organization (WHO) and the water soluble artelinic acid by the Walter Reed Army Institute of Research. Artemether and artesunate are now also used as oral formulations and the latter is also available in the form of suppositories. It is WHO policy to promote the use of these drugs intra-rectally as an emergency treatment in primary healthcare situations in developing countries. The delay in treatment, often caused by the long distance to the hospital, is regarded as a significant factor in the high mortality in children. This delay would be substantially reduced if the mother could administer a suppository to her ill child at home. Artemether and artesunate also exert their action through dihydroartemisinin. This metabolite is often present in higher concentrations than the parent drug and exhibits higher activity in vitro. Dihydroartemisinin is also used as an oral drug.

Mechanism of action

The action of artemisinin derivatives is different from that of the other antimalarial drugs, although both the artemisinin drugs and the 4-aminoquinolines interact with haem. Artemisinins have a very fast action and parasite clearance times are much shorter than with other malarial drugs. In Fig. 2 the life-cycle of the malaria parasite is shown and explained. Whereas most of the antimalarials work at the late trophozoite and schizont stage of the malaria parasite, artemisinin derivatives also act already at early trophozoite and ring stages. Artemisinin is only active on blood-stage parasites and does not affect liver-stage parasites or stages within the mosquito. However, it does act on gametocyte development,
Fig. 2. Life cycle of the malaria parasite *Plasmodium falciparum* in humans. After the bite of an infected female *Anopheles* mosquito, sporozoites migrate to the liver and invade hepatocytes within 1 h. After 5–7 days, the infected hepatocytes rupture, releasing thousands of merozoites, which will invade erythrocytes. The parasite will develop and replicate within the erythrocytes. After 24–26 h in the cycle the trophozoite will adhere to the endothelium of small blood vessels. This adherence, named sequestration, is thought to be responsible for the pathophysiology of *falciparum* malaria. In this period (26–48 h) these older stages cannot normally be seen in the blood smear. They will grow, become schizonts and rupture after 48 h, releasing their progeny (16–32 merozoites per schizont) in the blood. With schizont rupture it is thought that a still-unidentified malaria toxin is released causing a cytokine response which leads to the typical malaria symptoms of high fever, chills and malaise.

resulting in decreased transmission in areas where artemisinin compounds are extensively used.

During the blood-stage phase of the parasite, more than 70% of the haemoglobin within the infected erythrocyte is digested. Haem is released, which is toxic for the parasite and therefore neutralized by polymerization into haemazoin. (This polymerization is inhibited by 4-aminoquinolines such as chloroquine.)

It was found that haem or Fe$^{2+}$ catalyses the opening of the peroxide bridge in artemisinin, leading to the formation of free radicals. Malaria parasites are known to be sensitive to free radicals. A mechanistic framework for the Fe$^{2+}$-induced cleavage of artemisinin and its derivatives has been proposed to explain the formation of metabolistic products and the most important pathways (shown in Fig. 3). This mechanism is based on careful analysis of the formed products from reaction of Fe$^{2+}$-salts with artemisinin compounds under different conditions.

The initially formed oxygen radicals rearrange to primary and secondary carbon-centered radicals, intermediates in the formation of known metabolites. These intermediates are involved in the alklylation of proteins. The secondary radical at C$_4$ originates from a 1,5-H shift of C$_4$-H to the oxygen radical. Much effort has gone into investigating the relationship of the stability of the C$_4$ radical and the antimalarial activity. Formation of this radical is crucial for retaining high activity in the artemisinin analogues. Blocking the formation or destruction of the formed radical at C$_4$ reduces the activity significantly. Further support for the formation of carbon radicals has come from trapping experiments of the formed radicals using spin labels.
In parasite cultures treated with artemisinin, adducts of haem and artemisinin have been isolated\textsuperscript{25}. The structure of these adducts has yet not been elucidated. The structure of an adduct of artemisinin and the (probably similar) manganese counterpart of haemin points to reaction of the primary radical product at the meso position of the porphyrin\textsuperscript{26}.

Incubation of the parasite with labeled artemisinin showed incorporation of the label into at least six parasite proteins. This alkylation of proteins is not a random process as it involves specific, and not major, proteins\textsuperscript{27}. It was found that, in particular, a member of the translationally controlled tumour proteins (TCTP) was alkylated\textsuperscript{28}. This could be related to the fact that this protein interacts with haem. The event essential in leading to parasite death is still unknown. Morphological changes in the mitochondria, ribosomes and endoplasmatic reticulum have been described. Since radical-intermediates are involved, processes like DNA cleavage and polyunsaturated fatty acid degradation might also be involved. Free radical scavengers like ascorbic acid, tocopherol, acetylcystein and iron chelators potentially counteract the activity of artemisinin\textsuperscript{29,30}. The increased understanding of the mechanism of action of the artemisinin drugs will facilitate the design of more active compounds\textsuperscript{22,31-32}.

**In vitro and animal studies of artemisinin derivatives**

The artemisinin drugs have potent parasite killing activity *in vitro* with IC\textsubscript{50} values of 0.1–10 ng ml\textsuperscript{-1} (Ref. 33).
Artemether, arteether and dihydroartemisinin are more potent in vitro than artemisinin itself. All the artemisinin derivatives are also active against P. falciparum strains, which are resistant to a broad spectrum of other antimalarials. Combination of artemisinin with halofantrine or quinine had an additive effect with respect to parasiticidal activity. Mefloquine had a potentiating effect whereas chloroquine and pyrimethamine showed antagonism with artemisinin.

Until now, no clinical resistance has been reported although variation in sensitivity for the artemisinin derivatives has been found in isolates of P. falciparum. The least susceptible parasites were seen among mefloquine-resistant isolates. There have been P. falciparum strains cultured in vitro with moderate decreased artemisinin sensitivity and even a resistant P. yoelii strain has been obtained, which showed about 15 times less sensitivity than the parent strain. P. falciparum was found to be less susceptible to artemisinin in thalassemic erythrocytes.

Clinical pharmacology
Pharmacokinetics

For measuring artemisinin and its derivatives in plasma, high-performance liquid chromatography (HPLC) with electrochemical detection is the method of choice with a sensitivity of 1–5 ng ml⁻¹ (Refs 39, 40). As the assay is performed in the reductive mode, strict anaerobic conditions are essential and this technique is therefore only suitable ably extensively hydrolysed to dihydroartemisinin in the gastro-intestinal lumen before first-pass metabolism in the gut wall and liver takes place. In 50% of the cases no artesunate was even detectable in the blood.

After oral intake of artemether, high concentrations of dihydroartemisinin were seen that paralleled artedermether concentrations. The relative bioavailability of oral artemether compared to intramuscular administration was 43% (Ref. 52). Less dihydroartemisinin is seen after intramuscular administration than with the oral route, which suggests that some dihydroartemisinin formation is formed in the gut lumen (as a product of enteric first-pass metabolism of artemether).

Although demethylation of artemether is probably mediated by cytochrome P (CYP) 450 enzymes, no effect was seen from specific CYP 2D6- and CYP 2C19-inhibition on the pharmacokinetics of artemether. It was concluded that artemether metabolism was not subject to the genetic polymorphism for these enzymes to a clinically important degree. From in vitro experiments a major role for CYP 3A4 was identified for the conversion of arteether to dihydroartemisinin. This enzyme is probably also important in the primary metabolism of artemether to dihydroartemisinin. Interaction studies with grapefruit juice, a strong inhibitor of CYP3A4 in the gut mucosa cell, showed more than a twofold increase in artemether bioavailability, which suggests a role for CYP3A4 in the first-pass elimination of artemether.
was responsible for this time-dependent increase in first-pass effect. It is possible that these decreasing plasma concentrations over time contribute to the recrudescences observed in treatment trials with this group of compounds. Time-dependent pharmacokinetics have also been observed in studies with artemether.

**Pharmacodynamics**

An advantage of the artemisinin drugs over most other antimalarial drugs is that they act very rapidly, although the clinical benefit of this rapid action is still under discussion. They achieve considerable shorter parasite clearance times owing to their effect on the ring stages. By eliminating the ring-stage parasites they prevent the development to the more mature pathogenic stages that adhere to the endothelium in the microcirculation and cause complicated or cerebral malaria (Fig. 2). Despite this rapid clearance of parasitaemia and reduction of malaria symptoms, there is frequent recrudescence of infection when the drugs are given as monotherapy or for a short duration. This is not caused by parasite resistance but is probably related to the short half-lives of these compounds, bringing down the parasitaemia below detectable levels, but not eliminating all parasites.

Clinical dose finding studies suggest that the duration of treatment is critical for the cure rate. It is likely that a defined time of drug concentration above the MIC (minimal inhibitory concentration) is necessary for effective treatment. Empirically it is known that for elimination of all parasites effective drug levels during 3–4 life cycles of the parasite are required. This can be achieved by combining the artemisinin with an antimalarial drug having a longer elimination half-life.
There are several factors influencing the pharmacokinetics of the artemisinin drugs. In patients with uncomplicated malaria, higher \( C_{\text{max}} \) were observed than in healthy subjects. A reduced volume of distribution due to malaria or non-linear (saturable) pharmacokinetics of artemether is thought to be involved. Food had no influence on the pharmacokinetics of artemisinin. Although it is thought that this group of drugs is mainly metabolized by hepatic transformation, liver cirrhosis did not influence bioavailability nor delay the elimination. In acute renal failure plasma artemether levels after intramuscular administration were increased, possibly related to the reduced volume of distribution as renal elimination of these drugs is negligible.

In multiple dose studies with artemisinin, in both healthy subjects and patients, peak plasma artemisinin concentrations on day 6 were reduced to only 20% of those on day one, whereas the half-life was unchanged. It was suggested that autoinduction of enzymes in the liver or gut

Another argument for combination therapy, as pointed out by White, is that effective reduction in parasite biomass by the artemisinins may slow the development of resistance to the co-administered drugs. For example in a artemether + mefloquine treatment regimen, the effective reduction of parasite load by artemether will reduce the change that a mefloquine-resistant parasite will survive, replicate and cause treatment failure. The necessity of a combined therapy to prevent recrudescence is also illustrated with in-vitro studies that have shown a static effect on early ring stages of the parasite after exposure to low concentrations of dihydroartemisinin (20 ng ml\(^{-1}\)) for 24 h. These forms remained in a metabolic resting state up to six days followed by renewed growth. Finally, the combination of an artemisinin drug with a longer acting agent has not only the advantage of fast action and prevention of recrudescence but the treatment period will be shortened and thus improved patient compliance can also be obtained.

Clinical data on efficacy

Studies in uncomplicated malaria

Numerous studies with various compounds in more than 10 000 patients have been performed in China, Vietnam and Thailand, some in African countries and a
few in South America\textsuperscript{72-77}. No significant differences were found in efficacy or toxicity profiles between the different compounds. They were effective against all human malaria parasites. In once or twice daily regimens these drugs mostly showed rapid clinical improvement, very well tolerability by children and adults and clearance of parasites from the blood within 48 h. However, in three-day courses with artemisinin, artesunate, artemether or dihydroartemisinin administered either orally, intramuscularly, intravenously or intrarectally recrudescence rates varied from 44-54\% (Ref. 66). With five-to-seven-day regimens the 28-day cure rate increased to >90\%. To improve both efficacy and patients' compliance in short course regimens of two to three days, artemisinin preparations were combined with long-acting drugs such as mefloquine, doxycycline or lumefantrine (formerly called benflumetol) and this way cure rates of 95–100\% were reached\textsuperscript{78-82}. Soon a fixed combination tablet of artemether and lumefantrine (Riamet\textsuperscript{TM}) will be registered in Europe as a three-day regimen for the treatment of travellers returning from the tropics and as standby-treatment while on vacation.

**Studies in severe malaria**

The rapid action of artemisinin derivatives led to high expectations for the treatment of severe complicated malaria. Unfortunately, these expectations have so far not convincingly been fulfilled\textsuperscript{89,93-96}. Despite many interventions, complicated or cerebral malaria still has a mortality rate of 15–25\%. In a study of 576 Gambian children with cerebral malaria, intramuscular artemether was as haematopoiesis and had toxic effects on the heart resulting in bradycardia and QT\textsubscript{c} prolongation\textsuperscript{92,93}. Neurotoxicity with a high fatality rate, with lesions in the pons and medulla in the brain, were seen in rats and dogs after treatment for a week with five to six times the usual dose of artemether or arteether\textsuperscript{92-94}. Rats and dogs seemed more vulnerable to these drugs than primates. This neurotoxicity usually occurred in a pre-terminal time period. There is anecdotal information that the neurotoxicity would be reversible. The auditory and vestibular nuclei were most sensitive to the toxicity demonstrated in the neuropathology and in testing the auditory discrimination task in rats\textsuperscript{95,96}. Depending on the species, the doses related to NOAEL (No Observed Adverse Effect Level) of arteether and probably artemether ranged from about 6.25 mg kg\textsuperscript{-1} day\textsuperscript{-1} in the dog to 100 mg kg\textsuperscript{-1} day\textsuperscript{-1} (total or divided daily doses over seven days) in the monkey. In vitro toxicity occurred in neuronal cells as well as neuroblastoma cells in concentration ranges well within the limits achieved in vivo (10\textsuperscript{-6}-10\textsuperscript{-4} M)\textsuperscript{96}. The most toxic compound tested was dihydroartemisinin and the least toxic were artelinic acid and artemisinin. In rats toxicity was greater after intramuscular than after intravenous or oral administration which is probably related to different pharmacokinetics.

**Clinical studies**

Hundreds of thousands of patients have been treated with artemisinin derivatives and so far no major side effects have been reported, which is very different from experiences with most other antimalarials. No neuro-
efficacious as intramuscular quinine. The mortality rates in both groups were 21%. Three and 5% of the children respectively showed neurological sequelae after recovery. Although the mean parasite clearance time was 48 h compared to 60 h with quinine, fever clearance time was not shorter. There was an increase in the rate of convulsions and a longer duration of coma in the artemether treated group. In another study among 560 adults with severe malaria in Vietnam, the mortality rate in the intramuscular artemether group was 13% and 17% in the intramuscular quinine group. Again the parasites cleared faster from the blood on artemether but the fever lasted a little longer as did the duration of coma in the artemether group. A recent systematic review/meta-analysis of the Cochrane Library evaluated 18 studies in severe malaria. It was concluded that across all studies survival was somewhat better in a total of 1147 patients treated with artemisinin drugs than 1070 treated with quinine. (Odds Ratio 0.73, 95% CI 0.58–0.91). In other words, 24 patients are needed to treat with an artemisinin drug instead of quinine to prevent one more death from severe malaria. Overall, no significant difference in neurological sequelae has been demonstrated.

**Adverse effects**

**Preclinical studies**

Most toxicity studies in animals have been done with arteether. High doses in monkeys inhibited the logical abnormalities in patients have been seen until now. It is unknown if cumulative neurotoxicity is of concern but subclinical injury could occur with each treatment course for separate episodes of malaria. This possible risk prohibits the use of artemisinin drugs for malaria prophylaxis. Tests for early detection of neurotoxicity are not available but are under development (sound localization test, brainstem auditory evoked potential). As in animals, QTc-prolongation without any arrhythmias was also observed in malaria patients treated with artemether. This phenomenon is likely related to malaria and not to artemether, as it was also seen during treatment with other antimalarials.

Little is known about the effect on foetal development in humans. A toxic effect on embryos, but no teratogenicity, was described in mice, rats and rabbits after large doses of arteether. Use of artemisinin derivatives during pregnancy has only been reported anecdotally. No abnormalities were found in children of mothers who had been treated with artemisinin or arteether during the second or third trimester of pregnancy. In view of the severe course of malaria during pregnancy, artemisinin derivatives should be considered in case of quinine resistance.

**Future prospects and concluding remarks**

It is expected that the introduction of the artemisinin drugs in countries in South-East Asia and Africa will
further increase. In case of (multi)-drug resistant malaria, the need for these new compounds is clear. However, introducing them as the ‘magic bullet’ for a higher price in countries where the usual drugs are still effective will cause serious constraints on the local health budgets. Furthermore, overuse of these new promising drugs will most likely lead to resistance, as we have seen for all the other introduced antimalarials until now. We have discussed earlier in this review that there are theoretical arguments to use artemisinin drugs in combination with other antimalarials to delay development of resistance.

After artemisinin and its derivatives (first generation), work is in progress on completely synthetic (second generation) trioxane compounds. Future studies will have to find out if even more active compounds can reduce the mortality rate in severe malaria although reducing the time between symptoms and treatment is probably more crucial to improve survival.

The artemisinin drugs are also suitable in the initial treatment of vivax malaria, followed by primaquine for elimination of the liver stage forms. Other indications than malaria for the artemisinin drugs are being investigated. There is conflicting evidence for antiprotozoal activity against *Toxoplasma gondii* and *Pneumocystis carinii* in vitro and in rats. *Babesia* species, also living inside erythrocytes but not degrading haemoglobin, appeared not susceptible to artesunate. Artemether and artesunate were registered in China in 1996 as prophylactics of *Schistosoma japonicum*, as activity was demonstrated against the young forms of *Schistosoma* species. Artemether has been successfully used in oral...
Candida albicans infections in AIDS-patients in Africa\textsuperscript{106}. Some favourable effect on cutaneous leishmaniasis has been demonstrated in mice\textsuperscript{107}. In addition, cytotoxicity for certain tumour cells has been described\textsuperscript{108}.

The artemisinin derivatives have proved their efficacy in the treatment of malaria, including severe and cerebral malaria and multiresistant malaria. They deserve an important place in the treatment of malaria owing to their efficacy, absence of major side effects and relatively low costs of manufacturing and distribution. The high frequency of recrudescences necessitates protracted treatment in monotherapy (five to seven days) or preferably combination with some other longer-acting agent such as mefloquine or benflumetol. The artemisinin derivatives should be reserved for those situations where problems of resistance or unwanted side-effects of the available antimalarial drugs are to be expected.

Selected references
