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PHARMACOLOGICAL ASPECT OF ARTEMISININ AND ARESUNATE AS POTENT ANTI MALARIAL AGENTS - OVERVIEW

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ABSTRACT

Artemisinin (ARN) is a natural anti-malarial product which can be isolated from the Chinese medicinal herb *Artemisia annua*. It comprises a new generation of antimalarial compounds that feature a nitrogenfree sesquiterpene lactone. Because artemisinin itself has physical properties such as poor bioavailability that limit its effectiveness, semi-synthetic derivatives of artemisinin, including artemether (ARM), artemotil, arteether, artenimol, and artesunate (ARST), have been developed, and are known as more effective than its parent material-artemisinin. Since 2001 the World Health Organization has recommended using artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated malaria in areas experiencing resistance to older medications. In Sudan Artesunate-SP combination is most common. Artesunate is an antimalarial agent. It is a water-soluble hemisuccinate derivative of artemisinin. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate is powerful especially in the treatment of advanced and potentially lethal cases of *P. falciparum* infection. It is a sesquiterpene with an unusual endoperoxide linkage structurally unrelated to other known antimalarials. ARST was developed as a pro-drug for the treatment of both uncomplicated and severe *P. falciparum* malaria. It is available in both enteral and parenteral formulations. It is more potent than artemisinin and is active by virtue of the endoperoxide. Their activity against strains of the parasite that had become resistant to conventional chloroquine therapy and the ability due to its lipophilic structure, to cross the blood brain barrier, it was particularly effective for the deadly cerebral malaria. Oral artesunate is hydrolyzed rapidly back to the metabolite dihydroartemisinin (DHA), which is intrinsically more active as antimalaria agent, as this metabolite is the main contributor to the overall antimalaria activity. Artemisinin and its analogs appear to be better tolerated than most antimalarials. In recent times, emergence resistant of *Plasmodium sp* to many of the cheap and readily available antimalarials has resulted in the continued use and dependence of artemisinins and its based combination. In Sudan, artesunate tablet is the commonest artemisinin product in the market and is available in various strengths from both local and foreign manufacturers. The quality of these antimalarials if not properly safeguarded could lead to therapeutic failure in patients and the development of drug resistance.

KEYWORD: Artemisinin (ARN), *Artemisia annua,* schizonticides.

INTRODUCTION

Artemisinin (ARN) is a natural anti-malarial product which can be isolated from the Chinese medicinal herb *Artemisia annua*. It comprises a new generation of antimalarial compounds that feature a nitrogenfree sesquiterpene lactone.

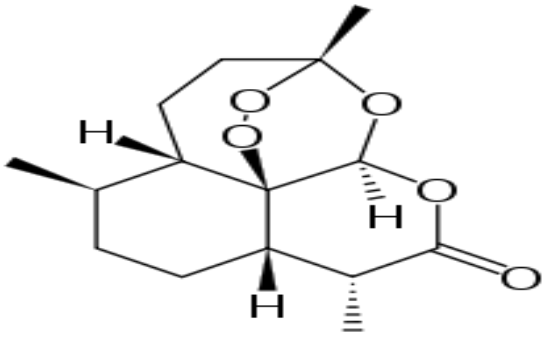


Figure 1: Chemical structure of artemisinin.

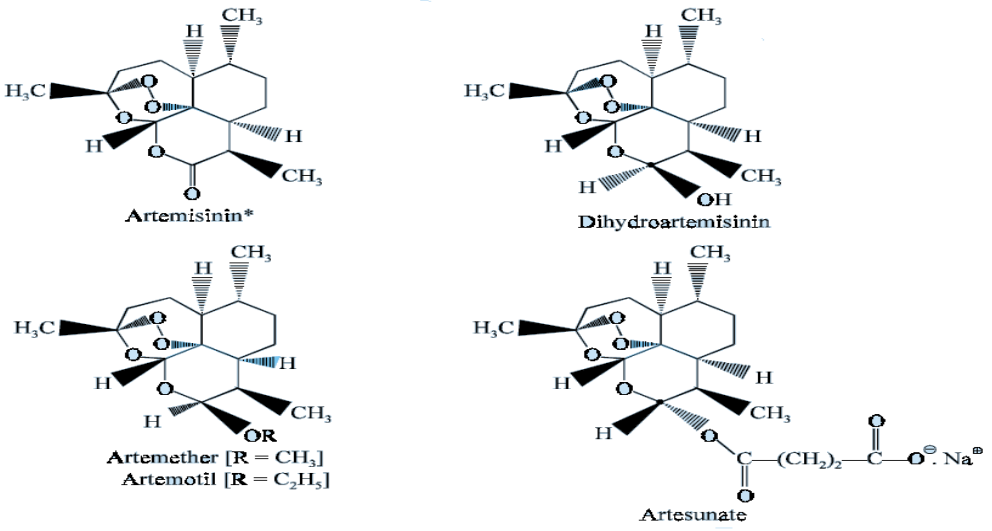
(3R,5aS,6R,8aS,9R,12S,12aR)-octahydro-3,6,9-trimethyl-3,1epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one.

Artemisia has been used by Chinese herbalists for more than a thousand years in the treatment of many illnesses, such as skin diseases and malaria. In the 1960s a research programme was set up by the Chinese army to find an adequate treatment for malaria. In 1972, in the course of this research, Tu- You you discovered artemisinin (ARN) in the leaves of *Artemisia annua*. The drug is named qinghaosu in Chinese. It was one of many candidates then tested by Chinese scientists from a list of nearly 200 traditional Chinese medicines for treating malaria. It was the only one that was effective.[1] It remained largely unknown to the rest of the world for about ten years, until results were published in a Chinese medical journal. The report was met with scepticism at first, because the Chinese had made unsubstantiated claims about having found treatments for malaria before. In addition, the chemical structure of artemisinin, particularly the peroxide, appeared to be too unstable to be a viable drug. Artemisinin is widely used in China, Southeast Asia and Africa for treatment of malaria. It is often used without taking precautions against conditions that might lead to resistance of the malaria parasite to this drug, leading to concern that the effectiveness of artemisinins may be reduced in the near future, as is the case with other classes of anti -malarial drugs.[1] Because artemisinin itself has physical properties such as poor bioavailability that limit its effectiveness, semi-synthetic derivatives of artemisinin, including artemether (ARM), artemotil, arteether, artenimol, and artesunate (ARST), have been developed.[2]

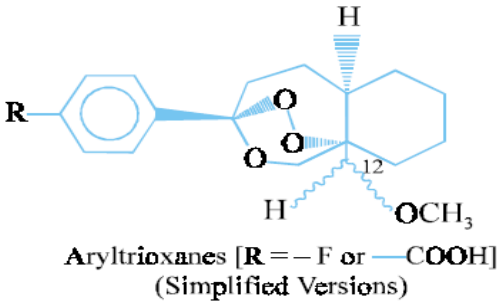
The structure activity relationship of artemisinin and artesunate

Artemisinin derivatives form a group of such new drugs currently under development and already in use in some countries.[3,4] They share a common sesquiterpenoid structure with an endoperoxide function which is essential for the activity. Due to synergism with other oxidant drugs and oxygen and reduction of activity by agents which lower oxidative stress, we and others proposed that the mechanism of their action involves exertion of oxidative stress on the parasite.[5,6] Although the specific nature of the oxidative stress is as yet unclear, the action probably involves desequestration of iron, as the drugs are strongly antagonistic with such iron chelators as desferrioxamine, pyridoxal benzoylhydrazone, and 1, 2-dimethyl-3-hydroxypyrid-4-one.[6] Meshnick et al.[7,8] showed that artemisinin interacts strongly with hemin(ferriprotoporphyrin IX) and its ferrous form,ferroprotoporphyrin IX. In parasite metabolism, hemin is left after digestion of hemoglobin by the parasite. A potentially toxic compound, it is converted to hemozoin (malarial pigment), which has been shown to be essentially beta-hematin, a polymerized form of heme devoid of immediate toxicity.[9] Some antimalarials, such as chloroquine, interfere with the polymerization, probably accounting for the generation of toxicity to the parasite.[10] The interaction of artemisinin with hemin, which leads to adduct formation and presumably interferes with the normal conversion of hemin to hemozoin, could therefore form the basis of its antimalarial action. Hemin or its immediate precursor, ferroprotoporphyrin IX, could therefore be the biological target of artemisinin. Alternatively, the interaction could be similar to, and provide a model for, the interaction of artemisinin with its real, as yet unidentified target within the parasite. It is therefore important to understand the mode of interaction of artemisinin and its derivatives with hemin and ferroprotoporphyrin IX and its relation with the antimalarial action of the drugs. A correlation between a parameter of such interaction and the antimalarial activity would provide strong evidence for its biological significance and could furthermore give a convenient method for primary screening of new artemisinin derivatives.

The structure activity relationship (SAR) of artemisinin and artesunate



The most important, critical and key structure of the 'drug', artemisinin, is the presence of a 'trioxane' moiety which essentially consists of the endoepoxide and doxepin oxygens that is evidently displayed by a rather simplified versions of 3-aryltrioxanes as shown in the following section, which are responsible for exerting the antimalarial activity against the parasite. It is, however, pertinent to state here that the prevailing stereochemistry at C-12 is not so critical and vital.[11]



Aryltrioxanes [R = – F or —COOH]
(Simplified Versions)

Figure 4: Simplified version of 3-aryltrioxanes.

Interestingly, the reduction of artemisinin to dihydroartemisinin gives rise to a chiral Centre, as shown by a bold black spot in the structure of dihydro-artemisinin that may ultimately lead to the formation of 'prodrugs' which could be either oil soluble or water soluble.

A few characteristic vital features of the above cited 'prodrugs' are enumerated as under:
(i) The two prevailing stereoisomers are found to be active, just as with the simpler aryltrioxanes.
(ii) Only one isomer of the ensuing artemisinin prodrug exhibits predominance exclusively.
(iii) The alpha-isomer predominates in forming the subsequent hemisuccinate ester which is water soluble.
(iv) The beta-isomer predominates in producing the subsequent nonpolar methyl and ethyl ethers.

Other uses of Artemisinin derivatives

Artemisinin derivatives are active against Schistosoma mansoni and Schistosoma japonicum in vitro and in experimental animals.[12,13] This is of mechanistic interest, since schistosomes, like malaria parasites, degrade hemoglobin and produce hemozoin.[14] Activity has also been demonstrated against Leishmania major[15], Toxoplasma gondii[16,17], and Pneumocystis carinii[18] in vitro and against P.carinii in vivo.[19]

Artemisinin derivatives have immunosuppressive activity[20,21] and also, potentially, anticancer activity.[22,23] The activity of artemisinin against cancer cells was potentiated if the cancer cells were first loaded with iron by exposure to transferrin.[22]

The concentrations or doses of artemisinin derivatives which are necessary for these alternate activities in vitro and in vivo are substantially higher than those required for antimalarial activity. Therefore, antimalarial endoperoxides are not likely to be useful for other therapeutic purposes. However, directed- synthesis programs might lead to endoperoxides with useful anti-infective or anticancer activities.[23]

ARTESUNATE

Artemisinin is obtained from the extracts of the plant *Artemisia annua*, with several derivatives; dihydroartemisinin, its methyl ether (artemether), its ethyl ether (arteether) and its hemisuccinate ester (artesunate) are known as more effective than its parent material- artemisinin. These are rapidly gaining grounds as antimalarials that are used for the treatment of severe and uncomplicated multidrug-resistance falciparum malaria. Since 2001 the World Health Organization has recommended using artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated malaria in areas experiencing resistance to older medications.

The advantages of artemisinin- based combination therapy (ACT) relate to the unique properties and mode of action of the artemisinin component, which include the following:

- rapid substantial reduction of the parasite biomass
- rapid resolution of clinical symptoms
- effective action against multidrug-resistant P. falciparum
- reduction of gametocyte carriage, which may reduce transmission of resistant alleles (in areas with low or moderate malaria transmission)
- no parasite resistance documented as yet with the use of artemisinin and its derivatives
- few reported adverse clinical effects, however pre-clinical toxicology data on artemisinin derivatives are limited.

Artemisinin (qinghaosu), artesunate, artemether and dihydroartemisinin have all been used in combination with other antimalarial drugs for the treatment of malaria. Of all these drugs Artesunate has the most documented clinical information.

WHO treatment guidelines for malaria recommend different Artesunate ACTs as:

- Artesunate-Sulfadoxine/pyrimethamine
- Artesunate-amodiaquine
- Artesunate-chloroquine
- Artesunate-mefloquine

While numerous countries, including most African nations, have adopted the change in their official malaria treatment policies, cost remains a major barrier to ACT implementation. Because ACTs cost up to ten times as much as older medications, they remain unaffordable in many malaria-endemic countries. In Sudan Artesunate-SP combination is most common[24], which is the subject for discussion in this review.

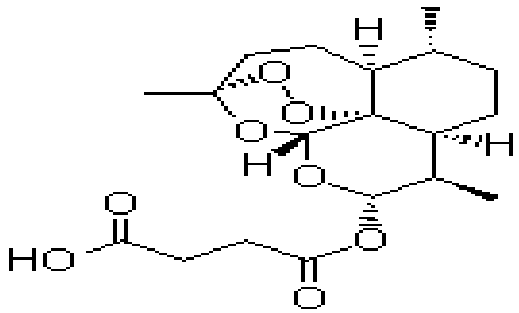


Figure 2: Artesunate chemical structure.

Artesunate (figure 2) is an antimalarial agent. It is a water-soluble hemisuccinate derivative of artemisinin. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate (ARST) is powerful especially in the treatment of advanced and potentially lethal cases of *P. falciparum* infection. It is a sesquiterpene with an unusual endoperoxide linkage structurally unrelated to other known antimalarials. Artesunate is ideal for the treatment of severe malaria, including cerebral malaria. It is also active against chloroquine and mefloquine resistant strains of *P. falciparum.* ARST was developed as a pro-drug for the treatment of both uncomplicated and severe *P. falciparum* malaria. It is available in both enteral and parenteral formulations. It is more potent than artemisinin and is active by virtue of the endoperoxide. Their activity against strains of the parasite that had become resistant to conventional chloroquine therapy and the ability due to its lipophilic structure, to cross the blood brain barrier, it was particularly effective for the deadly cerebral malaria.

Artesunate exist as a fine, white crystalline powder, very slightly soluble in water,freely soluble in methanol. It has chemical designation is (3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10-ol,hydrogen succinate, and its molecular formula is C19H28O8.

Artesunate act as a weak acid with a pH of an aqueous suspension containing 10 mg/g to be 3.5 – 4.5, and a pKa value of 4.6 for the hydroxyl group. ARST is formulated for oral, parenteral, (intravenous and intramuscular) and rectal administration. Combination therapy is only in oral dosage forms.[25] The parenteral formulation is unstable in water, so it must be reconstituted with 5% sodium bicarbonate solution immediately prior to administration. Some attention is paid to mechanistic aspects which clarify stereochemistry. The specific mechanism of action of artesunate and other members has not been extensively. Artesunate in particular is incompatible with basic quinolines by virtue of proton transfer, and has intrinsic chemical instability. At pH 1.2, conversion to dihyroartemisinin (DHA) is rapid, and at pH 7.4, t1/2 is about 10 hours. Because of rapid hydrolysis to dihyroartemisinin (artenimol), artesunate is considered by many as a prodrug of the latter. With a pKa of 4.6, over 99% of artesunate will be ionized at pH 7.4, and thus uptake by passive diffusion from the intestinal tract will be minimal. The functional group responsible for antimalarial activity of artesunate is the endoperoxide bond present. When the parasite that causes malaria infects a red blood cell (RBCs), it consumes haemoglobin(Hb) and liberates free heme, an iron-porphyrin complex. The iron reduces the peroxide bond in artesunate generating high-valent iron-oxo species, resulting in a cascade of reactions that produce reactive oxygen radicals which damage the parasite leading to its death.[12] The remarkable activities of these drugs require assays with sensitivities in the low nanogram-per-millilitre range. Methods used to measure these drugs includes titrimetry (assay only), HPLC with UV detection (HPLC-UV), HPLC with electrochemical detection (HPLC-EC) and HPLC with mass spectrometry (HPLC-MS). The HPLC-EC has been the most widely used for the measurement of artemisinin based drugs and their major metabolite dihydroartemisinin (DHA) in biological fluids.

PHARMACOLOGICAL PROPERTIES

Pharmacokinetic properties

To date, the pharmacokinetic data of Artemisinin (ARN) and its derivatives has generally been difficult to acquire due to the complexity involved in measuring their concentrations in biological fluids.

Oral artesunate is hydrolyzed rapidly back to the metabolite dihydroartemisinin (DHA), which is intrinsically more active as antimalaria agent, as this metabolite is the main contributor to the overall antimalaria activity.[26,27] Because of rapid hydrolysis to dihydroartemisinin (also referred to as artenimol), artesunate is considered by many as a prodrug of the latter.[28] Current knowledge of the absorption phase after oral administration is incomplete. We have established that artesunate stability varies as a function of pH and temperature.Once absorbed, ARS is rapidly metabolized to dihydroartemisinin (DHA) which then accounts for most of the antimalarial activity.[26,27] Thus, the pharmacokinetic parameters of both the parent drug and its active metabolite are essential in monitoring the activity of ARST, and to permit the optimization of drug dosage regimens used in treating malaria.

The enzymatic hydrolysis of ARST to DHA is catalyzed by an esterase enzyme in the blood and also by cytochrome P450 3A4 in the liver. This transformation of artesunate to dihydroartemisinin follows pseudo-first order kinetics at constant temperature; as a result, in the stomach at pH 1.2 artesunate is short-lived (tl/2 = 10.3 minutes), whilst at neutral pH its half life is significantly longer (tl/2 = 7.3 hours in plasma).

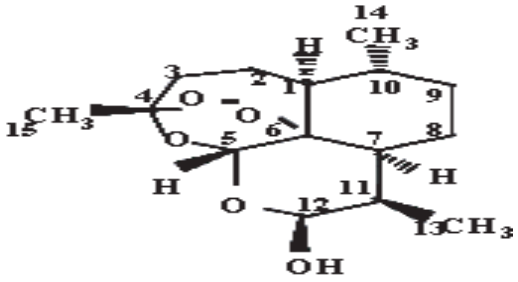


Figure 3: Chemical structure of dihydroartemisinin.

Esterases too play a role in the oxidation of artesunate.[29] It is therefore not surprising that pharmacokinetic studies that start sampling too late after dosing may fail to detect artesunate. In another study in the rat[30] in which oral, intramuscular and intravenous administration of artesunate in 0.9% saline were compared, the Tmax after oral administration of artesunate at 10 mg/kg was 30 minutes. Bioavailability after oral administration was 29.5 ± 4.6%. The Tmax of artesunate following oral administration in published studies in humans varies between an average of 0.25 hr (15 min)[31,32] and 0.66 hr (39.6 min)[33] in healthy volunteers, and 1.7 hr (ca. 1 hr 42 min) in children with falciparum hyperparasitaemia.[34] The calculated absorption tl/2 of parent compound for two different formulations of oral artesunate were 0.18+/- 0.16 and 0.16+/-0.16 hr in one study (10.8 and 9.6 min respectively) in healthy volunteers.[35] In another study, the Tmax was longer in convalescent than acute phase patients (0.5 and 1 hr (30–60 min), following the administration of 200 and 100 mg of artesunate, respectively). First sampling point was reportedly 0.25 hr (15 min) in two studies[36,37] and 1 hr in one.[38] Overall, published animal and healthy volunteers data are in general agreement, and correspond to the Tmax of dihydroartemisinin in this study. Parameters are modified during malaria infection.

Pharmacodynamic properties

Mechanism of action

Artesunate together with other derivatives of artemisinin are known for their potent antimalarial activity. In addition, anecdotal reports suggest that systemic artesunate has immunoregulatory effect.[39] There is no consensus as to how artemisinin derivatives kill parasites. Even their organellar site of action within the parasite remains controversial.

At the chemical level, one theory states that when the parasite that causes malaria infects a red blood cell, it consumes hemoglobin and liberates free heme, an iron-porphyrin complex. The iron reduces the peroxide bond in artemisinin generating high-valent iron-oxo species, resulting in a cascade of reactions that produce reactive oxygen radicals which damage the parasite leading to its death.[40] Numerous studies have investigated the type of damage that oxygen radicals may induce. For example, Pandey et al. have observed inhibition of digestive vacuole cysteine protease activity of malarial parasite by artemisinin.[41] These observations were supported by ex vivo experiments showing accumulation of hemoglobin in the parasites treated with artemisinin and inhibition of hemozoin formation by malaria parasites. Artemisinins have also been shown to inhibit PfATP6, the parasite's SERCA-type enzyme (calcium transporter), expressed in Xenopus oocytes. In this isolated system, resistance to artemisinin is reported to be conferred by a single mutation in PfATP6.[41] A study from French Guiana in field isolates of malaria parasites identified a different mutation in PfATP6 that was associated with resistance to artemether, lending some support to the idea that PfATP6 represents the main target for artemisinin derivatives. However this series of studies does not constitute convincing evidence that PfATP6 is the site of action of artemisinins, or that mutations in PfATP6 cause reduced artemisinin susceptibility. Robust evidence in this context can only be obtained by a transfection study, and it is notable that data from such a study were presented at the Molecular Approaches to Malaria Conference (Lorne, Australia) in February, 2008[42] recently; the parasite's digestive vacuole has again been implicated in artemisinin action.[43] A 2005 study investigating the mode of action of artemisinin using a yeast model demonstrated that the drug acts on the electron transport chain, generates local reactive oxygen species, and causes the depolarization of the mitochondrial membrane.[44]

Draw backs

Artemisinin and its analogs appear to be better tolerated than most antimalarials. In fact, artemisinins have been studied more extensively than many other antimalarials,[45] and it is estimated that about 2 million people have so far been treated with ACT, with little report of gross toxicity. The most commonly reported adverse effects have been nausea, vomiting, and diarrhea. Irreversible neurotoxicity has been seen in animals, but only after doses much higher than those used to treat malaria. Artemisinins should be avoided in pregnancy if possible because teratogenicity has been seen in animal studies.[26]

There are currently no documented information on antidotes for the management of artesunate poisoning, Activated charcoal (AC) was evaluated as possible antidotes for the management of artesunate overdose and /or poisoning.

Synthesis[46,47]

Artemisinin (Qinghaosu) has also been produced by semisynthesis. Some phenotypes of *A. annula* have been found to produce as much as 1% Artemisinin, but the yield is normally very much less, typically 0.05–0.2%. The more abundant sesquiterpene in the plant is artemisinic acid (qinghao acid, typically 0.2–0.8%).

Fortunately, artemisinic acid can be converted chemically into Artemisinin by a relatively simple and efficient process. At the same time, Artemisinin can be reduced to the lactol (hemiacetal), and this can be used

for the semi-synthesis of a range of analogs, of which artemether, arteether, and the water-soluble sodium salts of artelinic acid and artesunic acid are promising second-generation antimalarial drugs. The total synthesis of Artemisinin was completed in 1983, and the Peroxy Bridge was confirmed to be its unique feature conferring antimalarial activity.

Artesunate is prepared from dihydroartemisinin by reacting it with succinic acid anhydride in basic medium. Pyridine as base/solvent, sodium bicarbonate in chloroform and catalyst DMAP (N,N-dimethylaminopyridine)and triethylamine in 1,2-dichloroethane have been used, with yields of up to 100%. A large scale process involves treatment of DHA in dichloromethane with a mixture of pyridine, a catalytic amount of DMAP and succinic anhydride. The dichloromethane mixture is stirred for 6–9 h to get artesunate in quantitative yield. The product is further re-crystallized from dichloromethane. alpha-Artesunate is exclusively formed (m.p 135–137˚C).

Toxicity of Artesunate

Neurotoxicity of Artesunate

Although there is no clinical evidence for neurotoxicity in humans, high doses of artemisinin derivatives are neurotoxic in vitro and in experimental animals. Artemisinin derivatives are also toxic to neuronal cells in vitro. Wesche et al.[48] demonstrated that a series of artemisinin derivatives inhibited protein synthesis by two neuroblastoma cell lines and by primary fetal rat neuronal cells. Artesunate and dihydroartemisinin were the most toxic derivatives. Fishwick et al; also observed toxicity to neuroblastoma cells in vitro and found toxicity to glioma cells in vitro as well.[49] Surprisingly, these authors found artemether to be more toxic than dihydroartemisinin in inhibiting neuroblastoma cell proliferation. Several artemisinin derivatives significantly inhibited neurite formation in neuroblastoma cells. The toxicity of artemisinin derivatives to neuronal cells may also be iron dependent, since heme potentiates the toxicity of artemisinin derivatives to neuroblastoma cells in vitro.[50] An iron chelator also inhibits the acute toxicity of artemisinin to mice[51], although no effect of this chelator could be demonstrated on arteether-induced neuropathology in rats. The toxic effect of artemisinin derivatives on neurons appears to involve protein alkylation.[51] Thus, the mechanism of neurotoxicity is similar in many respects to the mechanism of antimalarial action. Therefore appropriate measures need to be taken to maintain proper and safe use of this medicine. However, since the drug has been used safely in millions of people, this neurotoxic effect is unlikely to be clinically relevant.

Osteo-toxicity

Artesunate has demonstrated the fastest clearance of all anti-malarials currently used and acts primarily on the trophozite phase, thus preventing progression of the malaria disease. It is converted to active metabolite dihydroartemesinin that then inhibits the sarcoplasmic/endoplasmic reticulum Calcium ATPase encoded by P. falciparum.[52,53] The mechanisms of its osteo-toxicity could not yet be scertain presently; but it is possible that, like ethanol, artesunate could facilitate the action of the calcification inhibitors[54], that is, a family of inorganic pyrophosphatases, phosphonates and diphosphonates; these act normally to prevent calcium deposits from forming on soft tissues.[55]

CONCLUSION

In recent times, emergence resistant of Plasmodium sp to many of the cheap and readily available antimalarials has resulted in the continued use and dependence of artemisinins and its based combination. This situation is more pronounced in the tropics where the incidence of malaria remains a serious burden with high infant and maternal mortality. Moreover, the World Health Organization (WHO) has warned that the artemisinins must be jealously guarded through combination with other known antimalarials of diverse classes. Following the WHO's adoption of the new malaria policy, advocating the use of artemisinin-combination therapy (ACT), many drug manufacturing companies have embarked on the production of artemisinin-based combination regimens, a situation that led to proliferation of diverse brands in the markets. Many African countries lack the resources to employ advanced techniques such as high performance liquid chromatography (HPLC), mass spectrometry (MS), and Raman spectroscopy which can be used to ascertain the authenticity of artemisinin products in the market and are therefore vulnerable to counterfeiting and substandard drugs. Also, artesunate and other artemisinins, being generally expensive drugs, are potential targets of counterfeiting. As a result of the above factors, Africa has been cited as the next possible destination for counterfeit and substandard artemisinins. In Sudan, artesunate tablet is the commonest artemisinin product in the market and is available in various strengths from both local and foreign manufacturers. The quality of these antimalarials if not properly safeguarded could lead to therapeutic failure in patients and the development of drug resistance.

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