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A Review On Artemisinins: Discovery, Mechanism of Action and Importance In Medicine

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ABSTRACT

Artemisinin is an isoprenoid produced in *Artemisia annua* which is a common type of wormwood that is native to temperate Asia, but adopted throughout the world. Artemisinin was well-known for its antimalarial activity, which is an overwhelming human disease in the tropical areas, which threatens 300–500 million people and kills approximately two million people annually and ranked in the top three of communicable diseases. World Health Organization recommended the Artemisinin-based combination therapies (ACTs) for preventing malaria which augmented the inevitability of artemisinin. Artemisinin limitations like low solubility and low bioavabilty conquer by its derivates collectively known as 'artemisinins'. The focal point of this sharp review explains about the discovery, advanced applications and mechanism of action of artemisinins in treatment of malaria, cancer, viruses and other protozoan parasites.

INTRODUCTION

Artemisinin (fig.1a) is an isoprenoid (also referred as terpenoids) majorly produced in *Artemisia annua*, which is a common type of wormwood that is native to temperate Asia, but adopted throughout the world. Isoprenoids (also referred to as terpenoids) are the oldest class of bio molecules with more than 40,000 identified compounds (Withers et al., 2017).

Isoprenoids universally found in all living organisms and they are more frequent in plants as constituents of essential oils (McCaskill et al., 1997). Its carbon skeleton is derived from farnesyl pyrophosphate (fig.1b), a common precursor in the plant cell, which is synthesized by the condensation of 3 isoprene units (IPP) (fig.1c). Artemisinin is a sesquiterpene lactone containing an endoperoxide linkage in it. Unlike most other antimalarials oxygenated sesquiterpene lactone peroxide, lacks nitrogen containing heterocyclic ring systems and was found to be a better plasmocidal and the blood schizontocidal agent compared to conventional anti-malarial drugs such as chloroquine, quinine etc.

Due to WHO recommendation, demand for artemisinins increased by overwhelming response for ACTs to treat malaria which threatens 300–500 million people and kills approximately two million people annually(WHO, World malaria report 2008). Due to the development of artemisinin derivatives (artelinate, dihydroartemisinin, artemeether, arteether, artesunate, artemisone etc. are collectively known as Artemisinins) (fig.2) with better bioavality and solubility and their target specific action against diverse cancers, viruses, phylogenetically unrelated parasites; use of artemisinins are best alternative chemotherapeutic approach along with other active components to treat dreadful diseases.

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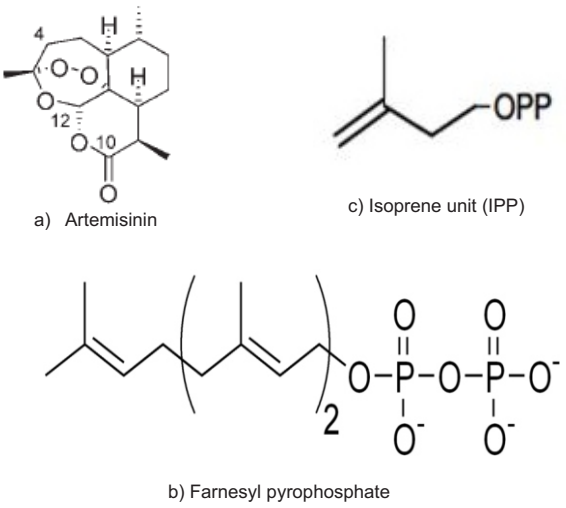

Fig. 1: Artemisinin and its precursor molecules

Discovery of Artemisinin

The project named 523 leading to the discovery of artemisinin was initiated in response to a request from North Vietnamese leaders to stop the deaths of soldiers due to malaria. Short term goal of this project was given relief to army in the form of Sulfadoxine and Piperaquine. The long-term goal of this project is to discover new effective anti malarial drugs. In the intervening time under the guidance of National Steering Group a three a year project plan was settled to survey natural sources to treat malaria, to develop combinational therapies, Chemical synthesis and screening of antimalarial drugs (Jianfang et al., 2013). Artemisinin was discovered in China in the early 1970s by You-You Tu, a phytochemist working in the Institute of Chinese Materia Medica, China Academy of Traditional Chinese Medicine. She led her group in the isolation and extraction of constituents with potential antimalarial activities from Chinese herbs and investigated more than 2,000 Chinese herbs including extracts from *Artemisia annua* L. More than 380 extracts tested against a rodent malaria model. Initially no significant results were obtained with artemisinin and Tu intensely modified the extraction technique to carry out it at low temperature rather than at high temperature.

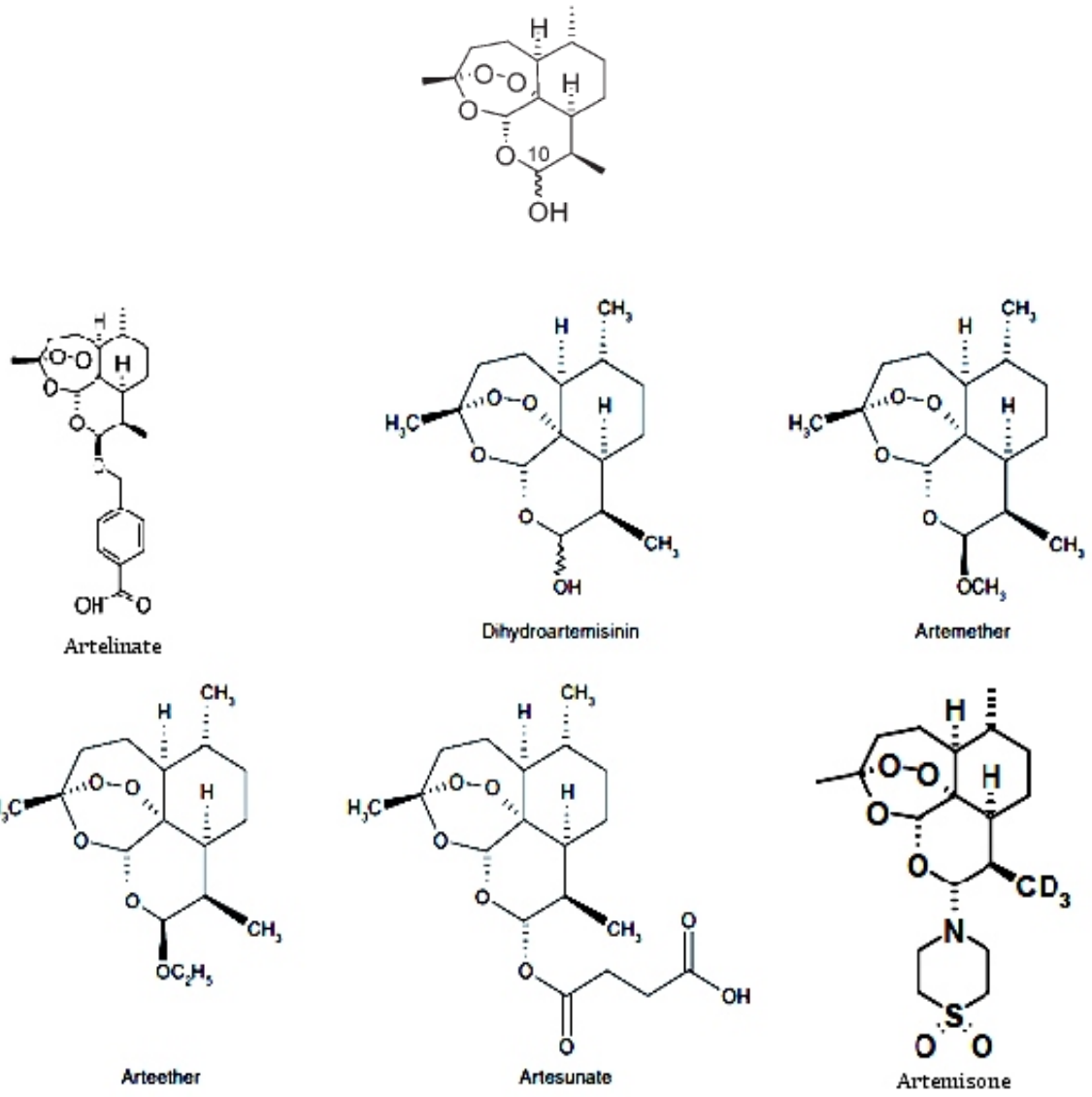

Fig. 2: Artemisinins: derivatives of Artemisinin (Richard et al., .2006)

Much better antimalarial activity was obtained after switching to the lower temperature procedure, and she found that the most effective preparation came from the leaves of *Artemisia annua* L., as evidenced by its significant inhibition of mouse malaria caused by *P. berghei.* There were no facilities for performing clinical trials of new drugs. In order to help malaria sufferers, Tu and her colleagues courageously acted as the first group of volunteers and took the new extract themselves. After their first human experiments Tu and her team went to Hainan (province of China) to verify the efficacy of the extract they carried out antimalarial clinical trials with patients infected by both *P. vivax* and *P. falciparum*. These clinical trials produced encouraging feedback, achieving a rapid disappearance of fever and parasites from the blood. Tu next investigated the isolation and purification of the active component from *Artemisia annua* L. Eventually, in 1972, her team identified a colorless crystalline substance with a molecular weight of 282 Da, a molecular formula of $C_{15}H_{22}O_5$ and a melting point of156-157 °C, as the active compound and named it “Qinghaosu” (“Qinghao” is the Chinese name of *Artemisia annua* L., and “su” means basic element). The stereochemistry and structure of Qinghaosu, was later determined by Tu in 1975 as a sesquiterpene lactone containing an endoperoxide linkage in it (Liao et al., 2009).

Mechanism of action of artemisinins

Mode of anti-malarial activity and Activation of Artemisinin:

The entry of the malaria parasites into their human host is through a mosquito bite. They first enter the liver and replicate there for two weeks, invades red blood cell (RBC), followed by growth, replication and destruction of RBC leads to the symptoms of the disease (fig.3). The artemisinin drugs are known to act specifically during this blood stage. Although the mechanism of action of artemisinins is still not definite, there are strong evidences to suggest that an endoperoxide linkage (1, 2, 4 trioxanes) of artemisinins (fig.4) and a heme iron playcritical roles in their mechanism of action. Iron-mediated cleavage of artemisinin Endoperoxide Bridge was first proposed by the “Meshnick and group”.They suggested that iron might play some role in the action of artemisinin, which could be achieved by activating artemisinin to generate free radicals. Interaction between iron and artemisinin was further revealed by Electronic Paramagnetic resonance (EPR) using the spin trap 5, 5-dimethyl-1-pyrroline-N-oxide (DMPO) (Meshnick et al., 1993). Based on these early and other results, models have been built to elucidate the free radical generating pathways of artemisinin after iron-mediated decomposition (O'Neill et al., 2004). Activation mechanism of artemisinin comprised of two distinct steps. In the first activation step, the heme iron attacks and breaks the endoperoxide linkage of artemisinin to produce oxygen free radical, which is then rearranged to give a carbon free radical. In second step, the carbon free radical produced from the first step will acts on specific targets.Since these initial findings explain different mechanisms of ring opening based on their iron dependency and involvement of carbon centred radicals.

Reductive scission model (homolytic cleavage)

Posner (G.H.Posner et al., 1992,1994,1995) and Jefford (C.W. Jefford et al., 1995) proposed two possible routes 1 and 2 for activation of artemisinin by reductive scission model (fig. 5). In route 1, the heme iron attacks the endoperoxide moiety at the Oxygen -2 position, giving the free radical at the Oxygen-1 position. This process is followed by an intramolecular 1, 5-H shift and the secondary carbon centered (C4 free radical) is obtained. In route 2, the heme iron, on the other hand, attacks the endoperoxide moiety at the Oxygen-1 position, giving the free radical at the Oxygen-2 position. This process is followed by a hemolytic cleavage of the C3–C4 bond, also resulting in the C4 free radical.

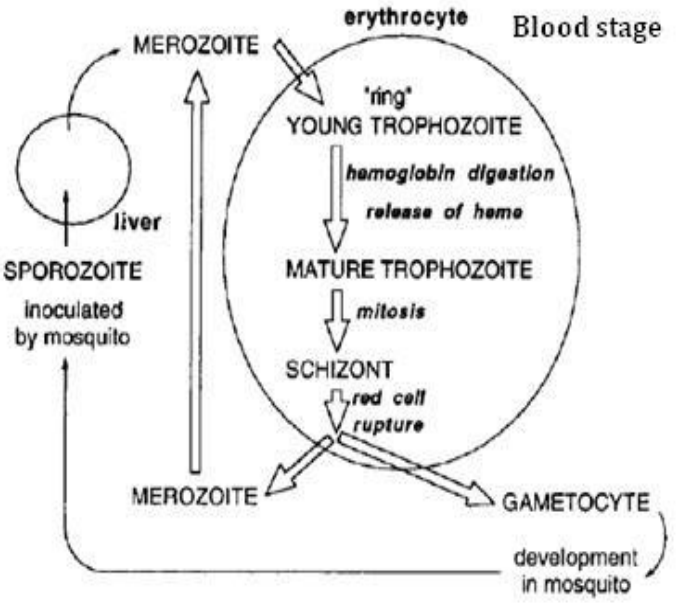

Fig. 3: Life cycle of malaria parasite

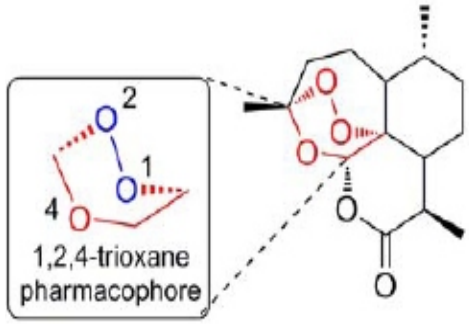

Fig. 4: 124 trioxane of artemisinin

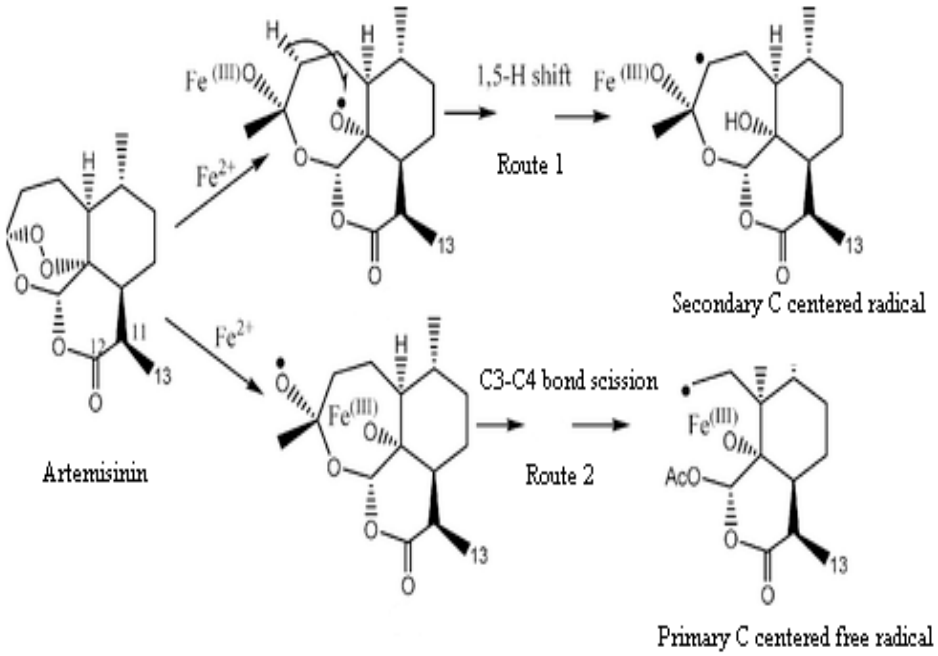

Fig. 5: Reductive scission model

Open peroxide model (heterolytic cleavage)

Open peroxide model suggests that ring opening is driven by protonation of the peroxide or by, complex formation by Fe2+(fig. 6). Haynes and co-workers have proposed that iron acts as a Lewis acid to facilitate ionic bioactivation of the artemisinins (W.M. Wu et al., 1998). 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 hydro peroxide (R.K. Haynes et al., 1996, 2007). Due to heterolytic cleavage of the Endoperoxide Bridge and succeeding capture of water results formation of unsaturated hydro peroxide, which acts on proteins and oxidizes irreversibly. Following Fenton degradation of the unsaturated hydro peroxide produces a hydroxyl radical, a species that can subsequently oxidize target amino acid residues. This theory was supported by artemisinin mediated N-oxidation of tertiary alkylamine derivatives via the intermediacy of such a ring opened peroxide form of artemisinin (R.K. Haynes et al., 1999). This heterolytic cleavage mechanism may have the potential to produce ROS in host that may have inference for the antimalarial activity of these compounds.

Activation of Artemisinin in Tumor cells:

Woerdenbag et al.,(2003) first reported the cytotoxicity of artemisinins in tumor cells. These tumor cells maintain a high intracellular iron concentration to sustain continued proliferation in addition to an increased capacity to synthesize heme. Human cancer cells are known to be richer than normal human cells in receptors for transferrin, an iron transporting protein and have high rates of iron intake via transferring receptors. Artemisinin endo-peroxide could be a trigger for the generation of active oxygen radicals via homolytic cleavage of the weak oxygen peroxide bond accelerated by higher ferrous iron concentration of the cancer cells. This Artemisinin endo-peroxide cause selective and preferential damage to vital cellular structure of active cancer cells. In order to support the above statement, an experiment is carried out by incubation of holotransferrin, which increases the concentration of ferrous iron in cancer cells with dihydroartemisinin (DHA) effectively killed a type of radiation-resistant human breast cancer cell in vitro (Narendra P. Singh et al., 2001). A similar experiment with artemisinin-tagged holotransferrin shows very potent and selective effect in killing a human leukemia cell line (Molt-4) (Henry Laia et al., 2005) proving the newly acquired iron increases cytotoxicity.Stimulation of the synthesis of heme within cancer cells using promoters was shown to increase cytotoxicity of DHA. Whereas inhibition of heme synthesis using inhibitors caused a decrease in cytotoxicity; indirectly showing the iron dependent activation (Zhang et al., 2009). Whilst the anticancer mode of action of artemisinins is comparatively little studied and recognized.

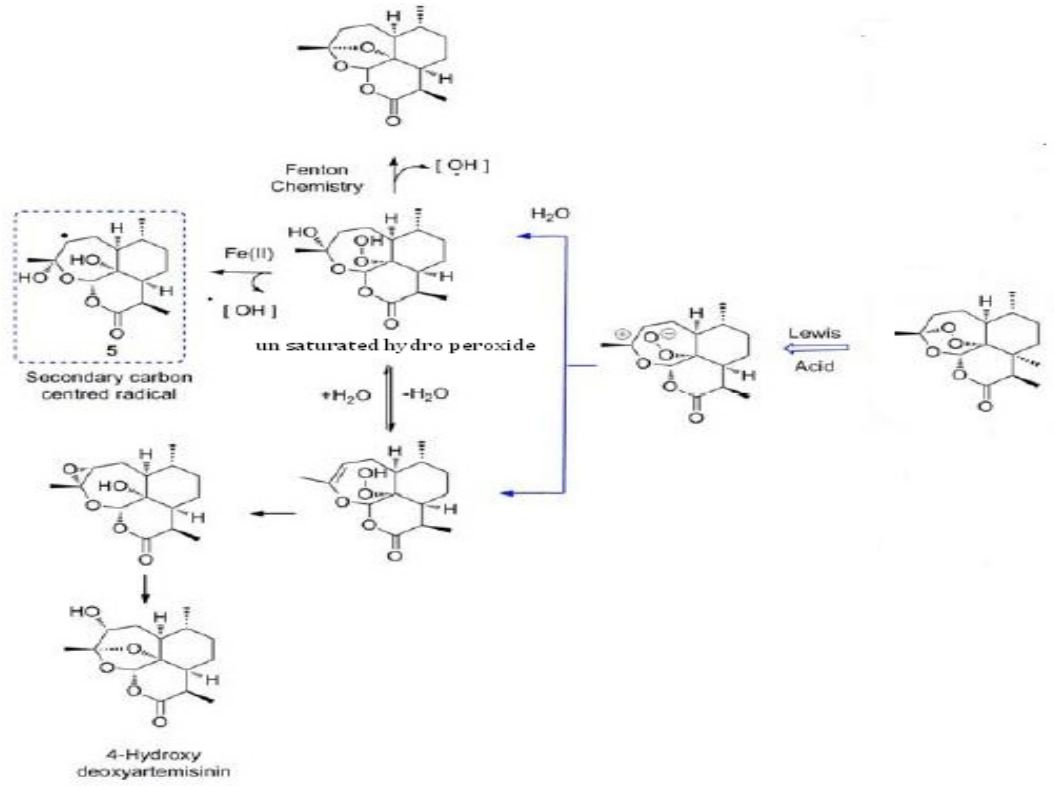

Fig. 6: Open peroxide model (P. G. Bray et al., 2005)

Promising targets of Artemisinin

In malaria parasite:

Heme

The heme model proposes that artemisinin acts inside the vacuoles to inhibit malaria: after cleavage by heme, the resultant free radicals of artemisinin are supposed to randomly alkylate surrounding vacuolar targets such as heme. Alkylation of heme results in formation of heme drug. Adducts which was first reported by Meshnick (Meshnick et al., 1991, 1994) Artemisinin was later shown to be capable of alkylating a heme model at the α, ß and δ carbon atoms (fig. 7). The capability of artemisinin to inhibit the proteolytic activity of digestive vacuoles was shown in both in vitro and ex vivo experiments in which artemisinin could potently inhibit heme polymerization.HPLC, LC-MS experiments also showed the physiological relevance of the interaction between artemisinin and heme (Creek et al., 2008, Robert et al., 2002).

PfATP6

Krishna and colleagues challenged the earlier theory i.e. the specific antimalarial effect of artemisinin was due to its entry into the parasite food vacuole and its interaction with Fe2+-haem resulting free radicals, inhibiting several key parasite components and eventually resulting in parasite death. They conducted experiments with fluorescently labelled artemisinin derivative and powerful high-resolution confocal microscopy showed that the artemisinins do not accumulate in the food vacuole, but are instead spread throughout the parasite. They also gave convincing evidence that the artemisinins act irreversibly inhibiting a metabolic enzyme — the malarial calcium-dependent ATPase (PfATP6) (Eckstein-Ludwig et al., 2003). In the parasite, the Endoplasmic Reticulum (ER) is situated outside the food vacuole (throughout the parasite cytoplasm) .ER is the proposed site of artemisinin action. PfATP6 is a malarial sarcoplasmic endoplasmic reticulum (SERCA) Ca+2-ATPase ortholog of SERCA, which is involved in the influx of calcium in to the ER which is the major source for Ca2+. Ca2+ necessary for host cell invasion, motility (Nagamune et al., 2008) and plasmodium gametocyte differentiation and mosquito transmission and also it is likely to be involved in the regulation and synchronization of parasite cell cycle progression (Oliver Billker et al., 2004). Thapsigargin is a sesquiterpene lactone but lacks endo peroxide bridge and a highly selective inhibitor of a mammalian Ca2+ transporting ATP-ases (SERCA). To prove PfATP6 as artemisnin target , PfATP6 was expressed in frog's eggs (Xenopus laevis oocytes)and it was found that both artemisinin and thapsigargin inhibit the enzyme irreversibly .This mechanism was also highly specific; no other malarial transporters were affected (including the non-SERCA Ca2+ ATPase PfATP4). An iron chelator, desferrioxamine (DFO) used along with thapsigargin and artemisinin; proved the iron –dependent activation of artemisinin by inhibiting the artemisinin antimalarial activity by removing the iron molecules, where as it doesn't show any effect on the inhibitory property of thapsigargin (Alves et al., 2011). Recently the 3D structure of PfATP6 was also modelled and used to predict binding affinities of artemisinin with other antimalarials such as trioxolanes, tetraoxanes, trioxaquines and quinolonesn; no correlation between in silico binding affinity and in vitro antimalarial activity was observed. Alkylation of heme by artemisinin (and other 1, 2, 4-trioxolanes has also been correlated with antimalarial activity, suggesting that heme alkylation could play a significant role in the mechanism of action of these peroxide antimalarial agent (Garah et al., 2009).

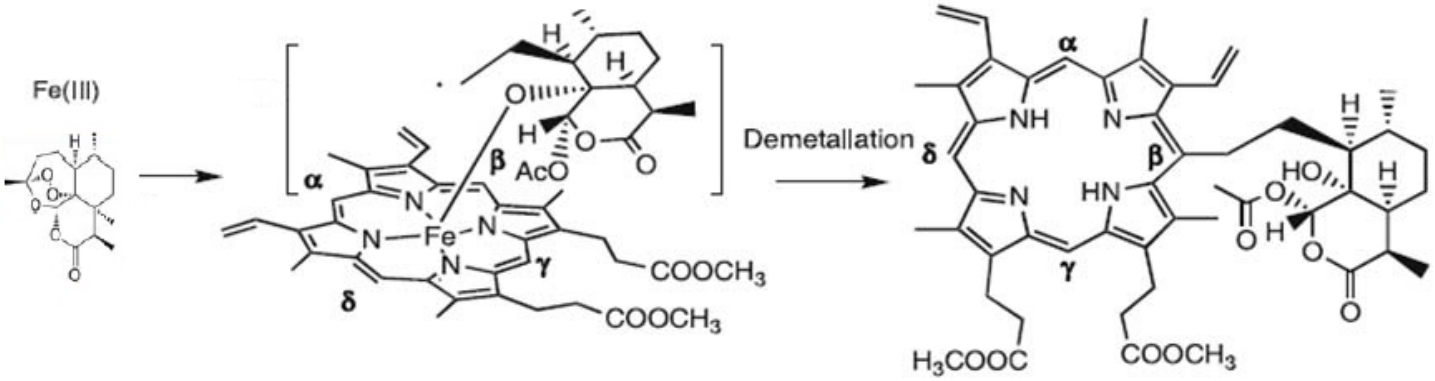

Fig. 7: Alkylation of a heme model by the action of primary carbon centered radical (Robert et.al 2002).

Mitochondria

Artemisinins inhibitory effect is mediated by disrupting the normal function of mitochondria through depolarizing their membrane potential in yeast. Genetic studies of the mitochondrial NADH dehydrogenases encoded by (NDE1 or NDI1), revealed the electron transport chain importance in artemisinin activation. Deletion of these genes gives resistance to artemisinin; whereas overexpression severely increases sensitivity to artemisinin. In yeast, artemisinins inhibitory effect is mediated by reactive oxygen species. Dual role of mitochondria is observed during the action of artemisinin: the electron transport chain stimulates artemisinin's effect, by activating it, and the mitochondria are subsequently damaged by the locally generated free radicals (Li et al., 2005).

Recently experiments demonstrated that the artemisinin directly acts on mitochondria and it inhibits malaria in similar way as in yeast .Indirect immunofluorescence assay revealed that artemisinins are distributed to malarial mitochondria and directly damage their functions. Also interference of mitochondrial electron transport chain (ETC) can alter the sensitivity of the parasite towards artemisinin. It remains unknown how precisely artemisinin is rapidly potentiated by malaria or yeast. This ETC activation is not observed in human and rodent, conceivably, structures of ETCs or properties of mitochondrial components in these organisms vary much. For example, yeast and malaria both possess similar NADH dehydrogenases which are one-polypeptide proteins; whereas NADH dehydrogenases of mammalian cells are large complexes composed of many different subunits. Addition of iron chelator desferrioxamine drastically reduces ETC activity and correspondingly assuages artemisinin induced ROS production in isolated malarial mitochondria; the effect of iron and ETC might be the same thing. Conceivably, the iron (iron-sulfur or haem) in the ETC participates in the action of artemisinins. (Wang et al., 2010).

Protein alkylation

Protein alkylation was first reported by Meshnick in vitro with human serum albumin using [14C] artemisinin and [3H] dihydroartemisinin. Mass spectrometry study revealed that two molecules of artemisinin were covalently attached to the albumin (Ying-Zi et al., 1993). This concludes that the binding between artemisinin and albumin probably involves thiol and amino groups (Asawamahasakda et al., 1994). Later, the same group showed that when P. falciparum-infected erythrocytes are incubated with [3H] dihydroartemisinin (DHA), [3H] arteether and [14C] arteflene, in addition to haem, several malarial proteins were covalently labeled. One of the major alkylated proteins was the malarial translationally controlled tumor protein (PfTCTP), a 25 kDa parasitic protein that binds calcium like other TCTP. In P. falciparum, this protein of which the function is unknown appears to be associated with food vacuolar membranes (Bhisutthibhan et al., 1998,1999).The covalent linkage between DHA and the Pf TCTP has been confirmed in vitro, with recombinant P. falciparum TCTP. Pf TCTP binding to artemisinin depends on the presence of haem and a single cysteine residue in the TCTP sequence. Like haem alkylation, alkylation of cysteine residues is an intramolecular reaction, given by Wu and co-workers where the artemisinin was activated with an iron-glutathione complex (□-Glu-Cys-Gly). In these conditions the generated free radicals also reacted in an intramolecular reaction manner with the thiyl group of amino acids. Artemisinin-glutathione adducts were then identified (Liu et al., 2003).Recently docking study of TCTP, Fe of heme and artemisinin suggesting the possibility of alkylation occurred through Cys14 residue Thus alkylation of a protein via formation of a thio ether bond is a conceivable reaction for the artemisinin (Chae et al., 2006).

Parasite membranes

Recently artemisinin was shown to accumulate within neutral lipids and cause parasite membrane damage. In the parasite digestive vacuole, neutral lipids closely associate with haem and promote hemozoin formation. Artemisinin and its derivatives are activated by haem-iron within the neutral lipid environment where they begin oxidation reactions that damage parasite membranes. The cellular accumulation prototype and effects on lipids were entirely endoperoxide dependent, because analogs lacking the endoperoxide moiety failed to label neutral lipid bodies or induce oxidative membrane damage (Hartwig et al., 2009).

Targets in Tumour cell:

Nitric oxide synthase (NOS)

Several studies have shown different mechanistic pathways that can selectively induce apoptosis and inhibit angiogenesis. Recently shown that artesunate may exert a potent anti-tumor activity through simply alkylating hemoenzymes and subsequently inhibiting the activity of haem containing nitric oxide synthase (NOS), which catalyzes the production of Nitric oxide (NO). NO presents in a higher level in cancerous tissues compared to their normal cells. It also recognized that NO can protect against cellular damage and cytotoxicity from reactive oxygen species (ROS) and organic peroxides; and also NO regulates cancer growth, migration, invasion, survival, angiogenesis, and metastasis in a concentration-dependent manner. ART inhibiting the NOS by binding to the heme of NOS, alleviates NO generation, thus eliminates protection from anti-tumor genesis (Zeng et al., 2011).

DNA damage

Recent study revealed in human leukemia cell lines (HL-60) cells that Artemisinin endoperoxides induce concentration and time-dependent apoptosis via mitochondrial membrane depolarization, and activation of caspases led to DNA degradation (fig. 8) (Amy E. Mercer et al., 2007). Recently investigated that artesunate induced high-level oxidative DNA damage (DNA double-strand break (DSB)) in CHO-9 tumor cell line compared with normal cells. DNA damage is not caused by direct binding of artesunate to DNA but because of induction of oxidative stress leads to apoptosis. But it occurs at higher dose level in mammalian cells than in Plasmodia. This is specifically acting on tumor cells because tumor cells are less efficient in repairing artesunate induced DNA damages (Paul et al., 2008).

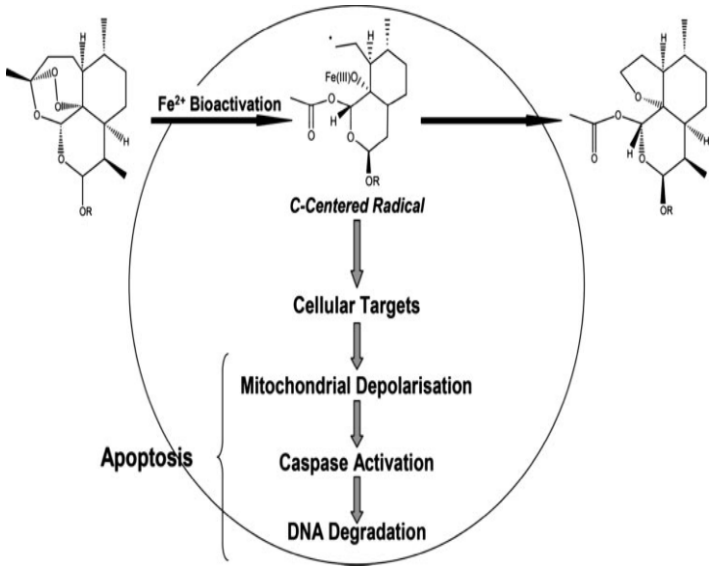

Fig.8: Chemical and molecular pathways of endoperoxide-induced HL-60 cell death (Amy E. Mercer et.al 2007)

Cell cycle arrest

Another promising target of artemisinins is Cell Cycle of tumors cell. There is evidence for antiproliferative effect of artemisnin deriavatives (containing cyano and aryl groups) in vitro against P388 murine leukemia by inhibiting the G1phase of cell cycle (Y.Shan et al., 2001). Similar type of effect observed by DHA on ovarian cancer cells potentially inhibiting cell growth by apoptosis induction and G2 phase arrest. Here it is observed that P53 mutant tumor cells are less sensitive to DHA than wild type cells; they need more amount of DHA than wild type cells for cell death. Engage of p53 with DHA is still unknown (Jiao et al., 2007).

Bax translocation and Apoptosis

DHA targets the Bcl-2 gene family, most prominent regulators of apoptosis.DHA down regulates the Bcl-xL and Bcl-2 which are known as anti-apoptotic proteins and up regulates the pro-apototicBax and Bad genes at m-RNA and protein levels. Experimental evidences revealed that silencing of Bcl-2 or Bax increased or reduced sensitivity to DHA induced apoptosis. This demonstrates that the DHA carries both cell cycle arrest as well as Bcl-2 gene family mediated apoptosis. Recent report demonstrates and intense the Bcl-2 gene family regulation by DHA (Tsuruta et al., 2004, Jiao et al., 2007). Generally C-Jun N-terminal Kinase (JNK) (JNK (MAPK family) promotes Bax translocation to mitochondria leads to cell apoptosis. Competitive inhibitor of JNK, SP600125 synergistically enhanced the dihydroartemisinin (DHA) induced cell apoptosis by accelerating Bax translocation to mitochondria and succeeding intrinsic pathways involving mitochondrial membrane depolarization, cytochrome c release, and caspase-9 and caspase-3 activation leads to apoptosis (Lu et al., 2010).

Canonical or Wnt/ β -catenin pathway

Wnts are a family of 19 secreted glycoproteins that accumulate in the extracellular matrix to activate pathways in adjacent cells. Wnt ligands trigger these pathways by binding the appropriate frizzled receptors. The accumulation of β -catenin in the cytoplasm of normal cells is prevented by gene products that promote its destruction by phosphorylation and ubiquitin-mediated proteosomal degradation (Fig. 9a).Due to Mutations in (Wnt/ β-catenin pathway) the tumor suppressor gene APC and mutations in β-catenin or Axin leads to increased levels of β -catenin in cytoplasm and translocates into the nucleus and it forms a complex with TCF/LEF family protein and activates several transcription factors which are involved in continues cell proliferation (cancer) ,cellular Invasion and angiogenesis (fig. 9b) (Peter et al., 2007, Marielba et al., 2008).

Recently it is observed that Artesunate inhibitory effect on the hyperactive Wnt/b-catenin pathway in colorectal cancer cell line CLY. Genes c-myc and survivin are over expressed in colorectal cancers. Artesunate treatment decreased mRNA levels of these genes, consequently effected the tumor cell survival by promoting the cell apoptosis and the inhibition of cell division. Artesunate treatment also increases the cell adherence property by translocation of β-catenin from nucleus to adherent junctions of membrane where it promotes cell attachments (Li et al., 2007).

Angiogenesis regulators

From the m RNA expression profile of 30 out of 90 angiogenesis-related genes correlated significantly with the cellular response to artemisinins. Fundamental angiogenic regulators encoded by genes such as vascular endothelial growth factor (VEGFC), fibroblast growth factor-2 (FGF2), matrix met al.,loproteinase-9 (MMP9), thrombospondin-1 (THBS1) and hypoxia-inducing factor α (HIF1A) are down regulated by artemisinin derivatives leads to repression of tumor (Anfosso et al., 2006). Alike effect of atremisinins (ARTs) was observed in the mouse embryonic stem cell-derived embryoid bodies by down regulating the hypoxia-inducing factor α (HIF1A), vascular endothelial growth factor (VEGF) via generating the Reactive Oxygen Species (ROS). Anti-angiogenesis can be reversed by upon co treatment with free radical scavengers like mannitol and Vitamin E signifying the mechanism of action of ARTs by ROS generations (Wartenberg et al., 2003).

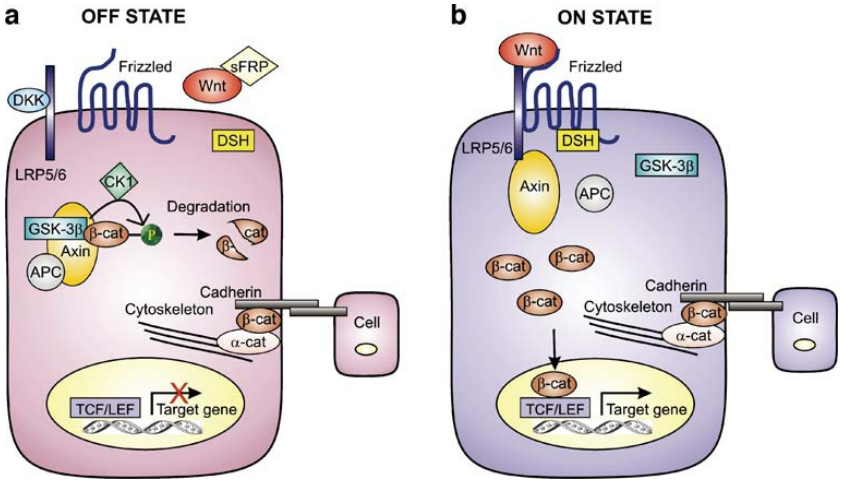

Fig.9: The canonical Wnt signaling pathway; a) In the absence of active Wnt ligands ("off state"), β-catenin is held in a destruction complex composed of Axin, APC, and GSK-3β. Subsequently, β-catenin is phosphorylated and after ubiquitination, it is degraded by the proteasome. Under these conditions, Wnt target genes are kept in a repressed state. β-catenin also exists in a cadherin-bound form regulating cellular adhesion via α-catenin. b) Upon binding of Wnt ligands to the Frizzled receptor(s) ("on state"), β-catenin is uncoupled from the degradation complex and translocates into the nucleus where it binds to TCF/LEF-family transcription factors and activates Wnt target gene transcription. APC, adenomatous polyposis coli; α-cat, α-catenin; β-cat, β-catenin; CK1,casein kinase 1; DKK, Dickkopf; DSH, Dishevelled; GSK-3β,glycogen synthase kinase-3β; LEF, lymphoid enhancer-binding protein;LRP, low-density lipoprotein receptor-related protein; P, phosphorylation; sFRP, secreted Frizzled-related protein; TCF, T-cell factor (Peter Neth et al., 2007).

Endoplasmic reticulum stress

Anti-cancer activity was also found to be associated with Endo plasmic Reticulum (ER) stress, which was demonstrated by induction of glucose-regulated protein 78 (GRP78) as well as growth arrest and DNA-damage-inducible gene 153 (GADD153) which are involved in ER stress. After DHA treatment; DHA-induced ER stress activated the transcription of GRP78 and GADD153 in an iron dependent manner, which resulted in accumulation of GRP78 and GADD153 at protein levels. GADD153 is an important pro-apoptotic molecule which triggers a signal transduction network to induce cell cycle arrest or apoptosis. DHA also promotes translocation of GADD153 to cell nucleus (Jin-Jian Lu et al., 2011).

Antiviral Activity

Human cytomegalovirus (HCMV)

HCMV is a major cause of disease in immune compromised individuals, including patients with AIDS and transplant recipients, and it is a common cause of inborn infection leading to developmental abnormalities and hearing loss (Mocarski et al., 2007). Currently available anti cytomegalovirus drugs such as ganciclovir, foscarnet, and cidofovir, target the viral DNA polymerase, and usage is limited by its toxicity, low oral bioavailability and drug resistance by virus. Artemisinin derivative artesunate suppress the HCMV growth by inhibiting the replication of HCMV which is firmly co regulated with cellular activation pathways arbitrated by the direct or indirect interaction with cellular DNA-binding factors, such as NF-kB and Sp1whicha are activated by Phosphoinositol 3–kinase. After treatment with artesunate a reduction in HCMV-induced protein synthesis and a reduction in the DNA binding activity of NF-kB and Sp1 were observed Protein kinases Akt and p70S6K, is also inhibited by artesunate which are downstream effectors of phosphoinositol 3–kinase (Efferth et al., 2002). Consequently reduction of immediate early proteins IE2p86 (fig. 10) expression critically limits viral replication, which is essential for the initiation of subsequent regulatory steps.

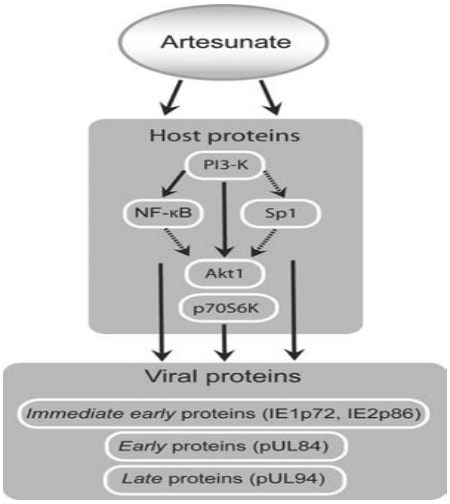

Fig.10: Mechanism of action of artesunate against human cytomegalovirus. (Thomas Efferth et al., 2008)

Apart from activity against HCMV artemisinins also inhibit Hepatitis B, C and partially inhibit HIV-1 strains (NL4-3 and Ba-L) replication (Paeshuyse et al., 2006, Romero et al., 2005). Human herpes virus 6A (HHV-6A) , Herpes simplex virus-1(HSV-1), Epstein–Barr Virus (EBV) and Bovine viral diarrhea virus (BVDV) replication also in habited by the action of artesunate (Efferth et al., 2008).

Anti protozoan and apicomplexan parasites activity

Toxoplasma gondii is a species of parasitic protozoan causes the disease Toxoplasmosis. Artemisinin acts on TgSERCA (PfATP6 orthologue) and disturbs the calcium homeostasis in T. gondii in vitro and in vivo; which is necessary for protein secretion, motility, and invasion into and exit from host cells (Kisaburo Nagamune et al., 2007). Artemisinin also inhibits calcium-dependent ATPase activity in Trypanosoma cruzi and Trypanosoma brucei membranes, suggesting a mode of action via membrane pumps (Yuliya V. Mishina et al., 2007). Artemisinins are also active against phylogenetically unrelated parasites, like Schistosoma spp and Leishmania spp.

CONCLUSIONS

Artemisinin is definitely one of the most promising natural products investigated in the past two decades; fortunately, this compound activity not only limited to malaria rather than it have activity against cancer as well as a variety of viral and protozoan diseases. Significance of artemisinin is increasing because of its multifunctionality, increased specificity to their molecular and cellular targets; this is important to treat different diseases.May be it is having variety of cellular targets. Artemisinin acts inside the food vacuole of parasite to inhibit the growth ;after activation by heme free radicals of artemisinin are supposed to randomly alkylating a heme at the α, ß and δ carbon atoms. Alkylation of heme results in formation of heme drug adducts shows the physiological relevance of haem and atrmisinin by HPLC, LC-MS studies. Later on fluorescently labelled artemisinin experiments revealed that the artemisinin do not restricted to food vacuole rather than it distributed throughout the cytoplasm of parasite. Same experiment proved that the Atrtemisinin specifically inhibits the PfATP6 enzyme irreversibly in an iron dependent manner. But recently the 3D structure of PfATP6 was modelled and used to predict binding affinities of artemisinin with other antimalarials no correlation between in silico binding affinity and in vitro antimalarial activity was observed. Apart from the alkylation of above proteins, it also alkylates Ca+2 binding malarial translationally controlled tumor protein (PfTCTP) at Cys14 residue via formation of thio-ether bond.

Artemisinins also targets the malaria parasite mitochondria by depolarization of membrane leads to the apoptosis. NADH dehydrogenases genes (NDE1 or NDI1) of electron transport chain also affect the artemsinin activity by involving in the ROS generation process. Deletions of these genes increase the resistance of strain towards artemisinin where as over expression of same increases the sensitivity of parasite to artemisinins. Some evidences also there for the parasite membrane degradation by artemisinin by affecting the neutral lipids.

Targets in tumour cell somehow different from malaria parasite and has variety of targets which increases treatment efficacy and to decrease unwanted side effects moderately with usual cancer therapies. Tumor cells are rich in iron transporting transferrin protein and it specifically acts on cancerous cells than normal cells. Alkylation of NOS (haem containing) enzyme inhibits the NO generation which is involved in regulation of most of the cancer cell poperties. Another study revealed concentration and time-dependent apoptosis via mitochondrial membrane depolarization, and activation of caspases led to DNA degradation. Artemisinin also targets the cell cycle of the cancer cells and also involved in the Bax gene translocation form cytoplasm to mitochondria leads to apoptosis of cancer cell. Recent studies revealed that DHA involved in the ER stress by inducing the GRP78 and GADD153 genes and translocation of GADD153 to the nucleus leads to cell cycle arrest and apoptosis.

One more important target in cancer cell is hyper active Wnt/ β -catenin pathway. Artesunate treatment inhibited target genes (c-myc and surviving) of this pathway which are over expressed in colorectal cancer also increases the cell adherence property by translocation of β-catenin from nucleus to adherent junctions of membrane where it promotes cell attachments. One more important characteristic of tumour cell is angiogenesis; also inhibited by the artemisinins by inhibiting the gene products which are involved in angiogenesis, surprisingly it is having different cellular targets which affects the existence of cancer cells and also effectively used along with other natural anti cancer agents. In in vitro studies, several groups have reported that artemisinins have antiviral properties. Artemisinins reduce replication rates of hepatitis B, C viruses, herpes viruses and HCMV and HIV-1 strains.

From all cases there is strong evidence that artemisinins having multi cellular targets, which is obliging in inhibiting the multi dug resistance malarial as well as tumor cells and other protozoans. This superiority makes artemisinin and its derivatives as a novel agent in various diseases intervention. Artemisinins productivity is very low in Artemisia annua L compared to its requirement; combitorial biosynthesis of artemisnin precursors is in progress in order to reach the provisions of artemisinin. Consequently they will be most affordable to the patients to treat various diseases.

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