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Artemisinins: Pharmacological actions beyond anti-malarial

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ABSTRACT

Artemisinins are a family of sesquiterpene trioxane lactone anti-malarial agents originally derived from *Artemisia annua* L. The anti-malarial action of artemisinins involves the formation of free radicals via cleavage of the endoperoxide bond in its structure, which mediate eradication of the *Plasmodium* species. With its established safety record in millions of malarial patients, artemisinins are also being investigated in diseases like infections, cancers and inflammation. Artemisinins have been reported to possess robust inhibitory effects against viruses (e.g. *Human cytomegalovirus*), protozoa (e.g. *Toxoplasma gondii*), helminths (e.g. *Schistosoma species* and *Fasciola hepatica*) and fungi (e.g. *Cryptococcus neoformans*). Artemisinins have demonstrated cytotoxic effects against a variety of cancer cells by inducing cell cycle arrest, promoting apoptosis, preventing angiogenesis, and abrogating cancer invasion and metastasis. Artemisinins have been evaluated in animal models of autoimmune diseases, allergic disorders and septic inflammation. The anti-inflammatory effects of artemisinins have been attributed to the inhibition of Toll-like receptors, Syk tyrosine kinase, phospholipase Cγ, PI3K/Akt, MAPK, STAT-1/3/5, NF-κB, Sp1 and Nrf2/ARE signaling pathways. This review provides a comprehensive update on non-malarial use of artemisinins, modes of action of artemisinins in different disease conditions, and drug development of artemisinins beyond anti-malarial. With the concerted efforts in the novel synthesis of artemisinin analogs and clinical pharmacology of artemisinins, it is likely that artemisinin drugs will become a major armamentarium combating a variety of human diseases beyond malaria.

Contents

Abbreviations: Bcl-2, B-cell lymphoma 2; CMV, cytomegalovirus; CDK, cyclin-dependent kinase; DHA, dihydroartemisinin; dsDNA, double-stranded DNA; HBV, hepatitis B virus; HIF-1α, hypoxia-inducible factor 1α; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MRSA, methicillin-resistant *Staphylococcus aureus*; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; Nrf-2, Nuclear factor (erythroid-derived 2)-like 2; PI3K, phosphatidylinositide 3-kinase; PZQ, praziquantel; ROS, reactive oxygen species; SERCA, sarcoplasmic, endoplasmic reticulum PfATPase6 calcium pump; SLE, systemic lupus erythematosus; Syk, spleen tyrosine kinase; TfR, transferrin receptor; TIMP, tissue inhibitor of metalloproteinase; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

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1. Introduction

Artemisinins are a family of sesquiterpene trioxane lactone anti-malarial agents, derived from the sweet woodworm *Artemisia annua*, a medicinal herb which has long been used in traditional Chinese medicine to treat fevers. This series of potent anti-malarial derivatives was developed from artemisinin, which is the parent compound isolated in a Chinese drug discovery screen for traditional herbal extracts against *Plasmodium* parasite species in the 1970s (van Agtmael et al., 1999; Li, 2012). There are recent comprehensive reviews on the anti-malarial mechanisms of action of artemisinins (Krishna et al., 2008; Krishna et al., 2010; Ding et al., 2011). The strength of artemisinins in anti-malarial therapy lies in their unique chemical structures which differ much from the standard quinoline (Kremsner & Krishna, 2004), and a significantly rapid clearance of malaria parasites from the blood than other available anti-malarial agents. Artesunate, artemether and arteether are the more widely used derivatives of artemisinin for malaria (Fig. 1), and are universally converted to the active metabolite, dihydroartemisinin (DHA), upon administration into the body (Zhu et al., 1983; Melendez et al., 1991). Among the family of artemisinins, artesunate is the most studied analog due to the addition of a hemisuccinate group which confers substantial water-solubility and high oral bioavailability, resulting in a more favorable pharmacological profile (Newton et al., 2000). Multiple anti-malarial mechanisms of action by artemisinins have been proposed and are generally involved in the formation of free radicals of artemisinin, due to the cleavage of the endoperoxide bond by heme iron in its structure. These free radicals of artemisinin can mediate eradication of *Plasmodium* species by altering biochemical pathways within the parasites (Meshnick et al., 1991; Posner et al., 1995; Jefford et al., 1996), including (1) alkylation of heme molecules and interference with the heme detoxification pathway, (2) inactivation of the sarcoplasmic, endoplasmic reticulum PfATPase6 calcium pump (SERCA), (3) alkylation of cytosolic proteins, such as PfTCTP, a potential tumor protein possibly related to parasite replication, and (4) disruption of mitochondrial functions, as summarized by a recent review (Ding et al., 2011).

There are continuous effort and increasing interest in developing newer derivatives of artemisinins with higher efficacy for anti-malarial treatment, and more efficacious artemisinin-based combination therapies to overcome potential drug resistance (Guo et al., 2012; Singh et al., 2012). In the meantime, with the established record of safety in millions of patients with malaria infection (Efferth & Kaina, 2010; Jelinek, 2013), artemisinins are being investigated in disease conditions beyond malaria, ranging from cancers, inflammatory diseases, to viral and other parasite-related infections. Indeed, this is reflected by the steady growth in publications of artemisinins in non-malarial research

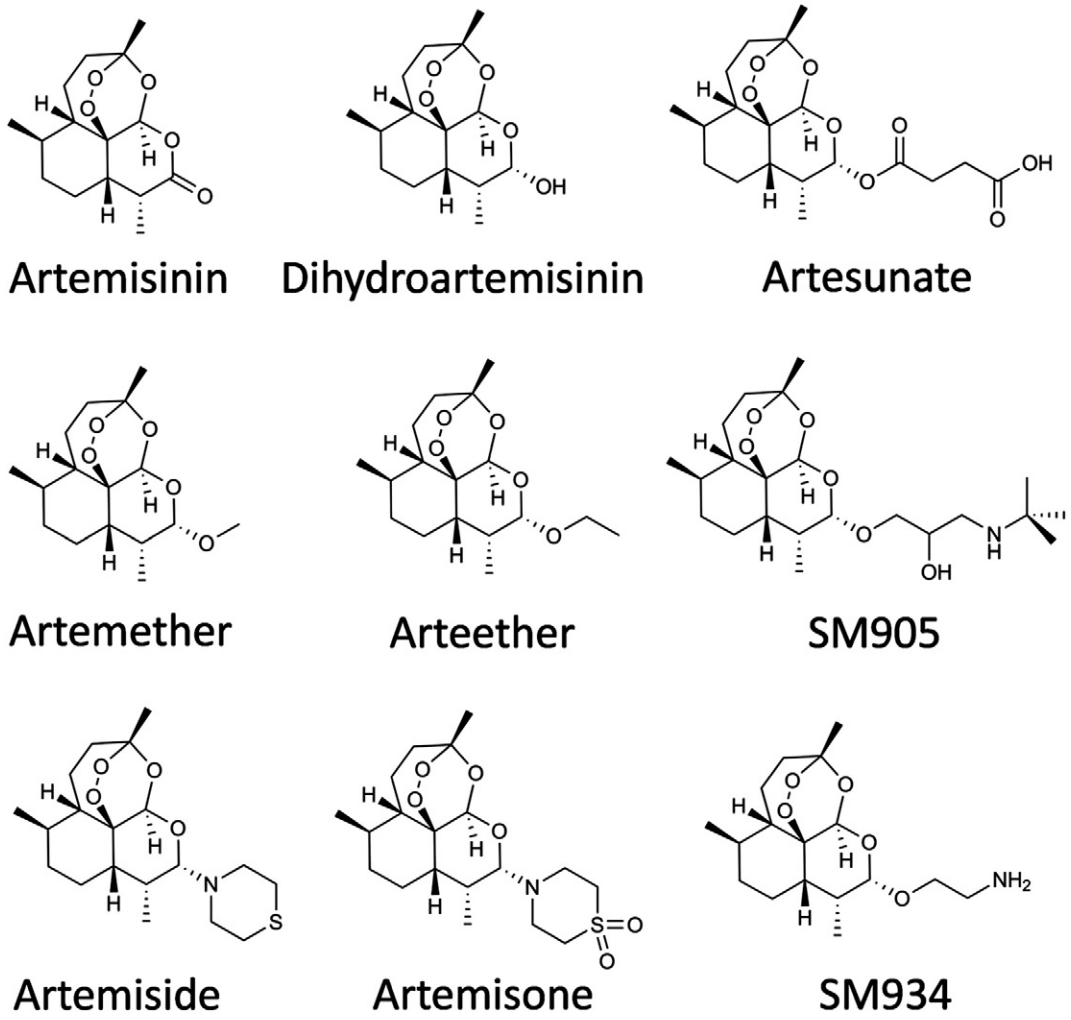

Fig. 1. Chemical structures of artemisinin and its derivatives. Artemisinin (parent drug), dihydroartemisinin (DHA, active metabolite), arteether and artemether (lipid-based derivatives), artesunate (polar derivative), SM905 (1-(12β-dihydroartemisinoxy)-2-hydroxy-3-tert-butylaminopropane maleate, new water-soluble derivative), SM934 (β-aminoarteether maleate, new water-soluble derivative), artemiside (a 10-alkylamino sulfide derivative, lipophilic with limited water-solubility) and artemisone (new 10-alkylamino sulfone derivative with enhanced water-solubility and reduced toxicity) are shown. Most derivatives of artemisinins are semi-synthesized from the parent compound or DHA and are universally metabolized into DHA upon administration into the body.

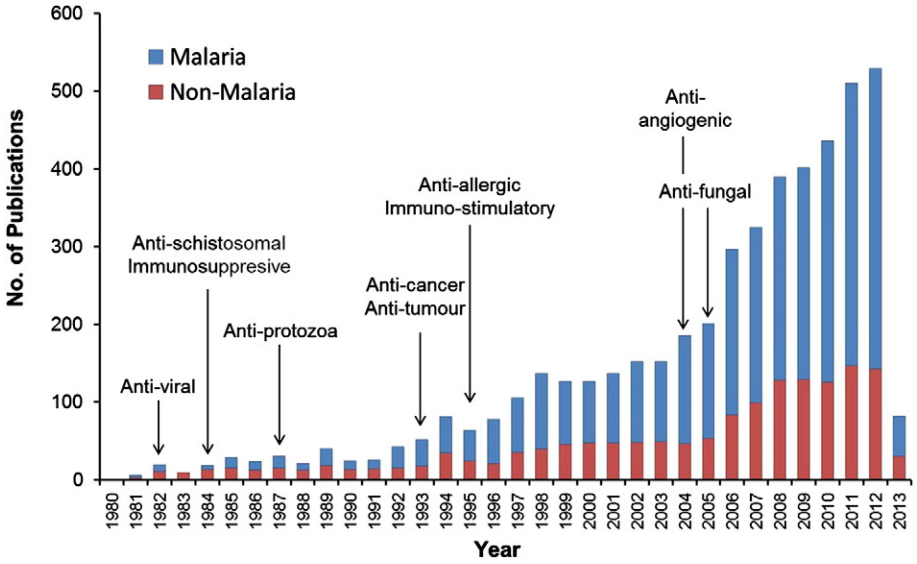

Fig. 2. Increasing trend of artemisinin-related publications. Number of artemisinin-related malaria (blue bars) and non-malaria (red bars) publications per year identified in PubMed was tabulated. The respective arrows indicate the year in which novel biological activities other than anti-malarial actions have been ascribed to artemisinins. Information was accessed on 20th February 2013.

over the past decade (Fig. 2). This review provides a comprehensive overview of non-malarial use of artemisinins, update on proposed pharmacological mechanisms of action of artemisinins in different disease conditions, and discussion on drug development of artemisinins beyond anti-malarial.

2. Anti-viral activities of artemisinins

2.1. Herpes viruses

Artemisinins, especially artesunate, possess strong inhibitory effects against double-stranded DNA herpes viruses including cytomegalovirus (CMV), herpes simplex virus 1 (HSV-1), human herpesvirus 6A (HHC-6a) and also Epstein-Barr virus (Efferth et al., 2008; Milbradt et al., 2009). The human CMV (HCMV) is a major cause of infections in neonates, patients with AIDS and transplant recipients (Schreiber et al., 2009). Current mainstream anti-CMV agents such as ganciclovir, foscarnet and cidofovir mainly target at the viral DNA polymerase activity preventing the elongation of viral DNA, but they are hampered by considerable dose-dependent side effects including bone marrow suppression and emergence of drug resistance (Harter & Michel, 2012). Hence, there is a strong demand for alternative anti-viral agents with mechanisms of action different from the current anti-CMV therapies.

Artesunate has IC_{50} values of 1 μM for Towne strain HCMV and 5 μM for AD169-GFP strain HCMV in vitro, and at 5–15 μM, artesunate is capable of inhibiting the growth of Towne strain HCMV in vitro by up to 99% (Efferth et al., 2002; Kaptein et al., 2006). It has been shown that HCMV can induce DNA binding activities of both nuclear factor-κB (NF-κB) and Sp1 transcription factors in the host, which is closely linked to the efficiency of HCMV replication. Artesunate was found to exert its anti-CMV action via inhibition of the transactivation of NF-κB and Sp1 (Schreiber et al., 2009). It is unclear at this moment what plasma artesunate or DHA level is required to achieve similar inhibitory effect in human. In an experimental rat CMV infection model, artesunate given orally at 50 mg/kg with ferrous (II) salt significant lowered CMV DNA loads in the spleen of immunosuppressed rats, indicating that the anti-viral effects of artesunate might require formation of free radicals via the cleavage of the endoperoxide bond by ferrous iron (Kaptein et al., 2006).

In patients suffering from CMV infection complications after hematopoietic stem cell transplantation, oral artesunate (100 mg/d) afforded a rapid reduction of virus load (1.7–2.1 log reduction) in whole blood and improved hematopoiesis within 10 days, as compared to patients who did not show improvement after treatment with ganciclovir or cidofovir (Shapira et al., 2008). Notably, drug resistance was not observed in patients receiving artesunate treatment, probably because the anti-viral mechanisms of artesunate involve cellular responses rather than directing at viral targets. Indeed, in malaria patients with human immunodeficiency virus (HIV) co-infection, there were delays in parasite clearance after treatment of artemisinin (Birku et al., 2002), implicating a mechanism of action for artemisinins dependent on host immune competency. Hence, artemisinins may possess unique therapeutic advantage against drug-resistant HCMV infection.

2.2. Hepatitis B and C viruses

Artesunate has also been shown to be effective against hepatitis B virus (HBV) replication (Cui et al., 2010). Artesunate was found to suppress HBV surface antigen (HBsAg) secretion with an IC_{50} value of 2.3 μM, and to reduce HBV-DNA levels with an IC_{50} value of 0.5 μM (Romero et al., 2005) in vitro, at a concentration range below the plasma drug concentration required in anti-malarial treatment (~7 μM) (Batty et al., 1996). Combination of artesunate with lamivudine, a first-line nucleoside analog reverse-transcriptase inhibitor for chronic hepatitis B, has been shown to produce synergistic anti-HBV effects (Romero et al., 2005), which further enhances the therapeutic potential for artesunate especially in combating lamivudine-resistant HBV strains. Nevertheless, the molecular mechanisms underlying the potent anti-HBV activity of artemisinins remain to be determined.

As for HCV, a member of the single-stranded RNA flaviviridae family, artemisinin was found to inhibit HCV replicon replication in Huh5-2 cells with an IC_{50} of about 78 μM. More importantly, when combined with iron-containing metalloporphyrin, the anti-viral activity of artemisinin was enhanced by 5 folds, with no cytotoxic activities observed (Paeshuyse et al., 2006). Therefore, anti-HCV activity of artemisinin may be linked to the production of reactive oxygen species and alkylation of HCV protein. These findings suggest a therapeutic value of combined artemisinin with iron-containing compound for HCV infection, especially for patients with cirrhosis who do not response well to interferon therapy.

3. Anti-parasitic activities of artemisinins

3.1. Trypanosoma

Protozoan parasite of the genus *Trypanosoma* is the cause of a fatal disease called the African trypanosomiases or sleeping sickness (Donelson, 2003). Artemisinins were found to inhibit the in vitro growth of the species *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense* with an IC_{50} range of 13–23 μM, demonstrating better efficacy against *T. brucei* (Mishina et al., 2007; Nibret & Wink, 2010). The anti-protozoan action might be associated with the inhibition of calcium-dependent ATPase activity in *T. cruzi* membranes, or mitochondrial damage, similar to its anti-malarial mechanisms (Mishina et al., 2007).

3.2. Toxoplasma gondii

T. gondii is an obligate, intracellular, apicomplexan protozoan that is capable of causing a chronic asymptomatic infection. Extensive reports have shown *T. gondii* infection, like malaria, is sensitive to inhibition by artemisinin and its derivatives, such as DHA and artemether (Ke et al., 1990; Berens et al., 1998; Jones-Brando et al., 2006; D'Angelo et al., 2009; Hencken et al., 2010). Artemether has an IC_{50} of 0.31 μM against *T. gondii*, which is 100 times more potent than the current front-line drug trimethoprim (Hencken et al., 2010). Other artemisinin derivatives have also demonstrated varying degrees of efficacy (IC_{50} = 1–40 μM) in inhibiting the growth of *Toxoplasma* as shown by D'Angelo et al. (2009). Calcium homeostasis has been one of the key mechanisms of action of artemisinin against apicomplexan parasites. Artemisinin can inhibit the parasite SERCA leading to Ca^{+2} overload and growth inhibition (Nagamune et al., 2007). In in vivo murine models of toxoplasmosis and reactivated toxoplasmosis, new derivatives artemiside and artemisone (10 mg/kg/d for 8 days) markedly suppressed *T. gondii* replication and reduced mortality (Dunay et al., 2009). Artemisinins have displayed selective activity at different steps of the tachyzoite life cycle by inhibiting *T. gondii* replication, growth, and attachment to and invasion of host cells (D'Angelo et al., 2009), demonstrating its therapeutic potential for the prevention and treatment of human *Toxoplasmosis*.

3.3. Schistosoma

Helminths are metazoans which are classified into cestodes (tapeworms), nematodes (roundworms) and trematodes (flukes). From a public health perspective, the most important helminth is the trematodes including blood flukes of the genus *Schistosoma*, and the foodborne trematodes, such as the liver flukes (*Clonorchis sinensis*, *Fasciola gigantica*, *Fasciola hepatica* and *Opisthorchis* spp.). Artemisinin and its derivatives have shown efficacy against both the schistosomal infection and foodborne *F. hepatica* infection (Keiser & Utzinger, 2007).

Schistosomiasis is a chronic and debilitating parasite disease caused by trematodes from the *Schistosoma* family (Liu et al., 2011). This disease is ranked second only to malaria in terms of socioeconomics and the importance for public health, and affects more than 200 million individuals worldwide. There are five known *Schistosoma* species affecting humans: *S. japonicum*, *S. mansoni*, *S. haematobium*, *S. mekongi* and *S. intercalatum* (Liu et al., 2011). Praziquantel (PZQ) is the first-line drug in controlling the morbidity caused by *Schistosoma* infection (Prevention and control of schistosomiasis and soil-transmitted helminthiasis, 2002). However, PZQ is unable to prevent reinfections in humans and does not have prophylactic effects. Hence, there is an urgent demand for a potent therapeutic compound capable of preventing schistosomiasis, as well as related reinfections.

Artemisinin was first discovered to possess anti-schistosomal properties in 1980 (Chen, 1980), followed by artemether, artesunate and DHA being reported to be effective against *S. japonicum*, *S. haematobium* and *S. mansoni* in schistosomiasis models in mice, rabbits, dogs and hamsters, resulting in significant worm burden reduction of at least 86% (Xiao et al., 1995; Shuhua et al., 2000a,b,c; Yang et al., 2001; Xiao, 2005; Utzinger et al., 2007; Li et al., 2011; Li, H.J. et al., 2012; Li et al., 2012a,b). Artemisinins in combination with PZQ have demonstrated highest efficacy against juvenile and adult worms in schistosomiasis models in rabbits and hamsters (Shuhua et al., 2000c; Utzinger et al., 2001). Moreover, the therapeutic potential of artemether against adult schistosomes in mice was found to be enhanced by N-acetylcysteine (NAC) by down-regulating oxidative stress induced by *S. mansoni* (Seif el-Din et al., 2011).

The anti-schistosomal actions of artemisinins have also been reported in humans. Artesunate, at doses usually given in malaria infection (8 tablets of 50 mg over 5 days), achieved a dramatic 65% reduction in worm egg counts in *S. haematobium*-infected schoolchildren (De Clercq et al., 2002). In combination with PZQ, artesunate and artemether could achieved even higher efficacies against schistosomiasis infections (Borrmann et al., 2001; Hou et al., 2008; Inyang-Etoh et al., 2009; Liu et al., 2011). On the other hand, a meta-analysis showed that multiple prophylactic doses of artemether or artesunate over 1- or 2-week intervals prevented schistosomiasis with a protection rate of 65–97% (Liu et al., 2011). That study also revealed that increased dosages of artemisinins with shorter treatment intervals could provide even higher protection rate of 78–99%, particularly in combination therapy with PZQ. Other than PZQ, artesunate has been combined with sulfamethoxypyrazine/pyrimethamine (SMP), which showed comparable efficacy of 93% in egg reduction in *S. haematobium*-infected children (Sissoko et al., 2009).

It has been demonstrated in *S. mansoni* that oxidative killing is an anti-schistosomal mechanism of action for artemisinins by depleting glutathione level and increasing lipid peroxidation in the parasitic worms (Zhai et al., 2002; El-Bassiouni et al., 2007). Besides, artemether was found to reduce parasite glycogen and protein contents, and inhibit ATPase activity in *S. japonicum* (Xiao et al., 1997). Artemether can also cause damage to the tegument and musculature of schistosomulae, and exert its helminthotoxic effect through synergy with heme-containing compounds (Shuhua et al., 2000b). Notably, the anti-schistosomal activity of artemisinins does not require T cell immunity and was found to be equally effective against *S. mansoni* in both athymic and immunocompetent mice (Keiser et al., 2010).

3.4. Foodborne trematodes

Foodborne trematodes, especially the liver flukes such as *F. hepatica*, cause liver and biliary tract infections in millions of people worldwide, upon ingestion of infected raw vegetation, water and/or raw livers. Fascioliasis is routinely treated with triclabendazole (Keiser & Utzinger, 2009). Recently, an in vitro study confirmed the fasciocidal effect of a pure form of artemisinin at the dosage of 2 mg/ml, and all worms were killed as early as 23 h after incubation (Ferreira et al., 2011). In *F. hepatica*-infected rat models, artemether or artesunate at 200 mg/kg was found to disrupt the tegument and gut of the liver fluke in a time-dependent manner (Keiser & Morson, 2008; O'Neill et al., 2009). In another *F. hepatica*-infected sheep model, artemether given a single dose of 40, 80 or 160 mg/kg intramuscularly showed significant egg count reductions (Keiser et al., 2008). The fasciocidal activity of artemisinins may require the formation of free radicals of artemisinin via the cleavage of the endoperoxide bond by heme iron, as it has been reported that incubation of *F. hepatica* with artemether or artesunate at 10 μg/ml for 48 h together with hemin (iron-containing porphyrin) results in killing of all worms in the medium (Keiser & Morson, 2008).

4. Anti-fungal activities of artemisinins

It has been demonstrated that artemisinin, DHA, artemether, and arteether displayed four to ten times higher activities against *Cryptococcus neoformans* as compared to amphotericin B, a positive control drug

against fungal infection, with IC_{50} values ranging from 0.045 to 2.0 μg/ml (Galal et al., 2005). *C. neoformans* infection results in cryptococcosis, a very common life-threatening opportunistic infection especially in immunocompromised patients such as HIV/AIDS patients (Brizendine et al., 2011). Besides, *Aspergillus fumigatus* is another most frequent cause of invasive fungal infection in immunosuppressed individuals. Artemisinin was able to inhibit the growth of *A. fumigatus* after a 24-hour incubation, but with a higher IC_{50} value of 125 μg/ml (~0.4 μM) (Gautam et al., 2011). Their anti-fungal activities are highly linked to their tetracyclic trioxane ring system and the production of free radicals, which is also important for their anti-malarial activities (Galal et al., 2005). In addition, artemisinin may disrupt mitochondrial function by inhibiting NADH dehydrogenase (Gautam et al., 2011).

5. Anti-inflammatory actions of artemisinins

Artemisinins have been investigated in a variety of inflammatory disease models, owing to their favorable safety profiles as well as a potent pharmacological action on multiple signaling pathways (Shakir et al., 2011). Artesunate was first discovered to possess immunosuppressive effects in the 1980s, as shown by inhibition of mitogen-stimulated mouse spleen cells and human peripheral lymphocytes in-vitro (Shen et al., 1984). Subsequently, artemisinin, DHA and arteether were reported to possess potent immunosuppressive effects in vivo, as demonstrated by the robust suppressions of antigen-specific IgM- and IgG-mediated antibody responses and other humoral responses in mice (Tawfik et al., 1990; Sun et al., 1991). Despite the discovery of potent immunosuppressive activities of artemisinins in these reports, there was a lack of disease-specific therapeutic investigation for artemisinins in the 1980s to 1990s.

5.1. Artemisinins in autoimmune diseases

DHA, artesunate, artemether and a new water-soluble derivative SM905 have been reported to possess promising protective effects against experimental models of rheumatoid arthritis (Cuzzocrea et al., 2005; Mirshafiey et al., 2006; J.X. Wang et al., 2008; Wang et al., 2012; Li et al., 2013). Among these artemisinin derivatives, artesunate (3–5 mg/kg/d, i.p.) has been consistently shown to attenuate inflammatory symptoms and prevent subsequent development of tissue edema, cartilage and bone destruction in experimental rheumatoid arthritis, mediated by the suppression of proinflammatory cytokines like TNF-α, GM-CSF, IL-1β, IL-6, IL-8 and IL-17α via inhibition of the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt and NF-κB signaling pathways (Mirshafiey et al., 2006; Xu et al., 2007; Mo et al., 2012; Li et al., 2013). Artemisinins were also found to exert beneficial anti-angiogenic actions in rheumatoid arthritis by suppressing angiogenesis-related factors such as matrix metalloproteinase-2 (MMP-2) and MMP-9, vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1α (HIF-1α) (Mirshafiey et al., 2006; Y. He et al., 2011). Artemisinins have demonstrated effective anti-arthritic properties, with comparable efficacy but significantly reduced side effect profile as compared to the standard anti-arthritic drug, methotrexate.

In an experimental murine model of systemic lupus erythematosus (SLE), oral artesunate at 125 mg/kg/d over 16 weeks exhibited comparable immunosuppressive effects to cyclophosphamide, by repressing MCP-1 and B cell-activating factor (BAFF) levels, leading to a significant reduction in anti-nuclear antibody and anti-double-strand (dsDNA) antibody production, proteinuria, serum creatinine as well as related renal pathology (Jin et al., 2009). Studies have also revealed that a 3–8 week regime of oral water-soluble artemisinin analog SM934 (2.5 and 10 mg/kg/d) exhibited pronounced suppression of proteinuria, glomerulonephritis development, Th-1 and Th-17 immune responses, and increases in anti-dsDNA, IgG2a and IgG3 antibodies, while promoted increases in Th-2 responses, and serum IL-10 and IL-4 levels in experimental murine models of SLE (Hou et al., 2012, 2011). SM934 demonstrated mixed actions on different subsets of T cells, suppressing the memory/effector T cells, while promoting regulatory T cell development. Notably, these studies have revealed that SM934 can exhibit extensive protective effects in a chronic systemic inflammatory condition, comparable to clinically effective corticosteroid drug like prednisolone (Hou et al., 2012) or immunosuppressant like rapamycin (Hou et al., 2011).

In lupus nephritis, a severe and frequently-occurring secondary kidney-specific inflammation following SLE, oral DHA (5–125 mg/kg/d) was found to suppress serum levels of anti-dsDNA antibody and TNF-α and abrogate renal pathology in mice via blockade of NF-κB p65 subunit nuclear translocation (Li et al., 2006). Besides, oral artesunate (150 mg/kg/d) has demonstrated stronger protective effects than prednisone in experimental lupus nephritis, by lowering serum levels of TNF-α and IL-6, and NF-κB p65 subunit and TGF-1β expressions in renal tissues (Wu et al., 2010). Furthermore, artesunate was found to induce higher expression of glucocorticoid receptor α (GRα) in peripheral blood mononuclear cells and of transcriptional coactivator P300/CBP protein than prednisone in lupus nephritis mice (X.L. Wu et al., 2012).

Artesunate has been shown to possess therapeutic actions against inflammatory bowel disease (IBD) (Yang et al., 2012). Artesunate (150 mg/kg/d) dramatically mitigated colon pathology and inflammatory damage in experimental colitis induced by dextran sulfate sodium salt (DSS) or trinitrobenzene sulfonic acid (TNBS). These anti-inflammatory effects of artesunate corroborated well with the suppression of Th-1 and Th-17 cytokines, IFN-γ and TNF-α via inhibition of NF-κB activities.

5.2. Artemisinins in allergic inflammation

Artemisinins were first reported to possess anti-allergic properties in 1994 in an experimental guinea pig model of allergic contact dermatitis, where topical artesunate (0.85–5%) markedly inhibited allergic inflammatory skin reactions (Chen & Maibach, 1994). The study also uncovered that the anti-inflammatory effects of artesunate were specific against allergic reactions but not toxin (irritant)-induced contact dermatitis. A recent investigation using topical artesunate in experimental allergic contact dermatitis concurred with the findings, and further linked the immunosuppressive effects to down-regulation of NF-κB p65 subunit, T-bet and IFN-γ expressions (H.J. Li et al., 2012; Li et al., 2012a). In an uncontrolled trial in a Chinese hospital conducted on 90 subjects with allergic skin disorders, topical artesunate demonstrated potent efficacy against eczema, erythema multiforme, polymorphous sunlight eruption and hydroa aestivale, and moderate effectiveness against atopic dermatitis, psoriasis vulgaris and dermatomyositis (Yu & Jin, 1997).

The anti-allergic mechanisms of artesunate have been further explored in experimental models of allergic anaphylaxis (Cheng et al., 2013). Artesunate (3–30 mg/kg), given intraperitoneally, prevented IgE-mediated vascular permeability in a passive cutaneous anaphylaxis mouse model, and blocked IgE-induced mast cell degranulation in the lungs, increase in plasma histamine level, and subsequent hypothermia in a passive systemic anaphylaxis mouse model. In RBL-2H3 cells and mature human mast cells, artesunate was found to directly inhibit IgE-induced mast cell degranulation, by blocking Syk tyrosine kinase phosphorylation, the downstream phospholipase Cγ (PLCγ) activation, and elevation in inositol trisphosphate (IP3) and intracellular Ca^{+2} levels. These findings strongly support a therapeutic role for artemisinins in the treatment of mast cell-mediated allergic responses.

Artesunate has recently been shown to protect against experimental allergic asthma. At 3–30 mg/kg/d, artesunate given intraperitoneally markedly inhibited both ovalbumin- and house dust mite-induced total and eosinophil counts in bronchoalveolar lavage fluid, anti-inflammatory effects comparable to dexamethasone (Cheng et al., 2011; Ho et al., 2012). Notably, artesunate was also found to inhibit aeroallergen-neutrophil infiltration, which has not been achievable by

corticosteroid drugs (Maneechotesuwan et al., 2007; Ito et al., 2008). Indeed, a slight depression of neutrophils has also been reported in malaria patients receiving a five-day regime of oral artesunate (600–1200 mg) or intramuscular artemether (480 mg) (Bunnag et al., 1991). In addition, artesunate drastically suppressed aeroallergen-induced increases in Th-2 cytokines and chemokines, IL-17, IL-33, MUC5AC, and adhesion molecules in the airways (Cheng et al., 2011). These protective effects by artesunate in allergic asthma have been associated with its pronounced inhibition of the PI3K/Akt signalling cascade and NF-κB activation.

In contrast to the formation of free radical oxygen species (ROS) via cleavage of the endoperoxide bond by heme iron in its structure as a mechanism to kill *Plasmodium* parasites and to induce cytotoxic effects in cancer cells, in allergic asthma, artesunate was found to decrease the levels of oxidative and nitrosative damage markers including 8-hydroxy-2-deoxyguanosine, 8-isoprostane and 3-nitrotyrosine, in inflamed airways. These antioxidative effects of artesunate were correlated with the inhibition of expression of NADPH oxidases and inducible nitric oxide synthase (iNOS), and elevation of superoxide dismutases and catalase, probably via induction of nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) by artesunate in allergic airways (Ho et al., 2012).

Although artemisinins are recognized as a family of exceptionally safe drugs and have been used by millions of malaria patients worldwide, there have been four cases of IgE-mediated anaphylactic reactions to oral and intravenous artesunate (Leonardi et al., 2001; Mohapatra et al., 2009; Dube et al., 2012). These allergic reactions to artemisinins are considerably rare and somewhat paradoxical to the potent anti-allergic actions of artemisinins reported. However, these allergic reactions to artemisinins have not been well understood and further investigations are required to identify the mechanisms involved and the susceptible populations.

5.3. Artemisinins in septic inflammation

Oral artesunate (50–200 mg/kg) was revealed to synergize with antibiotics such as ampicillin to prevent up to 90% mortality in sepsis mouse model induced by lethal doses of *Escherichia coli* challenges, by blocking the release of pro-inflammatory cytokines TNF-α and IL-6 (Wang et al., 2006). These potent anti-inflammatory effects of artesunate could also suppress inflammation produced by CpG-containing oligodeoxynucleotides (CpG ODN), lipopolysaccharide (LPS), heat-killed or live *E. coli*, and were attributed to the suppression of NF-κB activation and Toll-like receptor 4 (TLR4) and TLR9 expressions (Wang et al., 2006; B. Li et al., 2009).

Besides *E. coli*, artesunate (7.5–30 mg/kg) given intramuscularly helped protect against lethal doses of gram-positive pathogen *Staphylococcus aureus*-induced mouse sepsis by reducing TNF-α and IL-6 release via blockade of TLR2, nucleotide-binding oligomerization domain 2 (Nod-2) and NF-κB activation. In combination with antibiotics ampicillin sodium–sulbactam sodium (AMPS), artesunate further enhanced survival rates against lethal doses of live *S. aureus* challenge as compared to AMPS treatment alone (Li et al., 2010). Moreover, artesunate in combination with antibiotic oxacillin was found to be highly effective against mouse sepsis induced by methicillin-resistant *S. aureus* (MRSA), a major pathogenic strain resistant to multiple antibiotics (Jiang et al., 2011). Notably, the study also revealed that oxacillin or artesunate alone was ineffective in protecting the MRSA-infected mice, indicating that it is an invaluable strategy by synergistic combination of artemisinins with antibiotics as an effective therapeutic arsenal against bacteria-induced sepsis.

In an experimental model of LPS-induced uveitis which mimics infections of the iris and ciliary body by gram negative bacteria such as *Shigella*, *Salmonella*, or *Yersini*, resulting in a pronounced inflammation of the aqueous humor, artesunate (10 and 100 mg/kg) given intravenously was as effective as prednisolone in suppressing infiltrating inflammatory cell counts, and the levels of TNF-α, MCP-1, nitric oxide and prostaglandin E_2 in the aqueous humor. These findings support a therapeutic potential for artemisinins in the treatment of human uveitis (X.Q. Wang et al., 2011).

5.4. Artemisinins in other inflammatory conditions

Artemisinin was demonstrated recently to be capable of extenuating amyloidogenesis and neuroinflammation in a model of Alzheimer's disease (AD) in APPswe/PS1dE9 double transgenic mice (Shi et al., 2013). Artemisinin (40 mg/kg) given intraperitoneally daily for 30 days abrogated β-secretase activity and decreased neuritic plaque burden in AD mouse model. These anti-inflammatory effects of artesunate have been ascribed to the inhibition of NF-κB activity and the activation of NALP3 inflammasome. Another therapeutic prospect for artemisinins has been investigated in an experimental rat model of endometriosis. Artesunate at 150 and 300 mg/kg daily, given intragastrically, for 4 weeks increased apoptosis index and significantly reduced Bcl-2 and microvascular density of the implanted ectopic endometrium, with the protective effects comparable to a modified progestogen danazol (Wang, S.J. et al., 2011; Wang, X.Q. et al., 2011; Wang, X. et al., 2011).

6. Anti-cancer actions of artemisinins

Cancer is a leading cause of death in industrialized countries. It is characterized by self-sufficiency in growth signals, insensitivity to growth inhibitory signals, enabling replicative immortality, tissue invasion and metastasis, sustained angiogenesis, and evasion of apoptosis (Hanahan & Weinberg, 2011). There is a renewed interest in the discovery of new and innovative anti-cancer drugs from medicinal herbs, and artemisinins stand out as a family of bioactive molecules with high potency against cancer cells. Together with its established safety record in anti-malarial treatment, artemisinins possess promising drug profiles in the crossover usage as anti-cancer agents. Artesunate has demonstrated cytotoxic effects against breast, central nervous system, colon, leukemia, melanoma, ovarian, prostate and renal cancer cell lines obtained from the Developmental Therapeutics Program of the National Cancer Institute (NCI) of the USA (Efferth et al., 2001, 2003). DHA also displayed anti-cancer effects on glioma, breast, colon, lung, ovarian and pancreatic cancer cells (Singh & Lai, 2001; Kim et al., 2006; Mu et al., 2008; H. Chen et al., 2009; T. Chen et al., 2009; Lu et al., 2011).

Artemisinins are hydrophobic molecules capable of permeating through the cellular membrane to elicit anti-cancer functions. Like anti-malarial strategy, artemisinins act via the endoperoxide component present in its structure, and formation of ROS such as superoxide anion and hydroxyl radicals, to induce cellular damage. In the presence of free iron, artemisinins can convert itself into cytotoxic carbon-centered radical, a highly potent alkylating agent, to induce direct oxidative damage to cancer cells (Efferth et al., 2003, 2004; Firestone & Sundar, 2009; O'Neill et al., 2010). The role of heme or iron in artemisinin-mediated anti-cancer activity was further confirmed by the use of cobalt protoporphyrin, a heme oxygenase (HMOX1) activator, resulting in the degradation of heme and weakening of artemisinin anti-cancer activity. The declining trend was reversed by the introduction of tin protoporphyrin, a HMOX1 inhibitor, leading to the promotion of heme level and a resultant increase in artemisinin anti-cancer activity (Stockwin et al., 2009).

Artemisinins have been shown to decrease cell proliferation, reduce angiogenesis and trigger apoptosis in cancer cells (Efferth et al., 2001, 2003; Krishna et al., 2008; Sertel et al., 2013). On a side note, recent studies revealed higher stability and better efficacy of artemisinin-derived synthetic dimers in the treatment of cancer (Paik et al., 2006; Stockwin et al., 2009; Gong et al., 2013).

6.1. Artemisinins abate cancer cell proliferation

Cell division is required for growth and repair, and cell cycle progression requires cyclins and cyclin-dependent kinases (CDK), where the formation of cyclin–CDK complexes during the G1 phase helps prepare the cells to enter the S phase (Rhind & Russell, 2012). To keep cell division in check, inhibitors of cell division comprising of the CDK-interacting protein/kinase-inhibitory protein (cip/kip) family members such as p21, p27 and p57 (Yoon et al., 2012), play a role modulatory role. Recent studies have revealed that artemisinins have gained a foothold in cancer drug development by inducing growth arrest at various stages of cell division cycle. In prostate cancer cells (LNCaP), artemisinin was able to inhibit cell division by inducing G_1 cell cycle arrest via the ablation of phosphorylated retinoblastoma protein (pRb), a mediator cooperating with E2F transcription factors and CDKs to push forward the cell cycle progression through G1 into S phase. Artemisinin was also shown to disrupt specificity protein 1 (Sp1) transcription factor from binding to CDK4 promoter (Willoughby et al., 2009). Likewise, artemisinin was able to induce G1 cell cycle arrest in human breast cancer cell line (MCF7) (Tin et al., 2012) and in human nasopharyngeal cancer cells (CNE-1 and CNE-2). Artemisinin suppressed the level of cyclin D1, cyclin E, CDK2, CDK4 and CDK6, but up-regulated p16 and p27 (inhibitors of cell cycle division) levels in nasopharyngeal carcinoma cell lines (Wu et al., 2011).

Besides, artesunate could markedly impede the growth of leukemia (J-Jhan), small cell lung carcinoma (H69), colon carcinoma (HCT 116) and glioma (U251) cell lines by inducing cell cycle arrest at G2/M phase (Steinbruck et al., 2010). DHA displayed similar anti-cancer mechanism of action against ovarian cancer cell line (OVCA-420) (Jiao et al., 2007). In contrast, in pancreatic cancer cell lines (BxPC-3 and AsPC-1), DHA inhibited cell cycle progression at the G_0/G_1 into S phase instead. In that study, DHA reduced the level of cyclin E, CDK2, CDK4 and CDK6, and the activity of NF-κB; but amplified the p27 level (Chen et al., 2010).

6.2. Artemisinins augment apoptosis in cancer

Programmed cell death is maintained by the balance between the B-cell lymphoma 2 (Bcl-2) family of pro-apoptotic proteins including Bcl-2 associated X protein (BAX), Bcl-2 homologous antagonist killer (BAK) and Bcl-2-associated death promoter (BAD), and anti-apoptotic proteins such as Bcl-2 and B-cell lymphoma-extra-large (Bcl-xl) (Thomas et al., 2013). Detection of damaged DNA up-regulates tumor suppressor protein p53, which in turn increases the level of BAX, elevates the BAX/Bcl-2 ratio, and enhances permeabilization of the mitochondria membrane, leading to the release of cytochrome c (caspase activator) and eventually apoptosis (Chipuk et al., 2004). Another pathway of apoptosis lies in caspase-dependent process whereby inactive procaspases are cleaved to effector caspases. Activated caspase can in turn cleave other downstream substrates in the cell to execute programmed cell death (Hengartner, 2000). In cancer, the tumor cells adapt to evade apoptosis by down-regulating pro-apoptotic factors and up-regulating anti-apoptotic factors leading to survival and growth (Fiandalo & Kyprianou, 2012).

In human colon cancer cell line (HT29), artemisinins were found to activate BAX to induce the release of cytochrome c, leading to apoptosis in cancer cells (Riganti et al., 2009). In human prostate cancer cell line (DU145), artesunate induced the release of cytochrome c and promoted caspase-dependent apoptosis by the cleavage of procaspases 3 and 9 (Nakase et al., 2009). In human lung adenocarcinoma cells (ASTC-a-1), DHA was able to activate procaspase-3 and induce apoptosis (Lu et al., 2009).

Artesunate proved itself to be a more potent anti-cancer agent with an IC50 of 2.69 μM than artemisinin with an IC_{50} of >50 μM, against neuroblastoma cell line. It activated caspase-3, which led to apoptosis in both chemosensitive (UKF-NB-3) and chemoresistant neuroblastoma cells (Michaelis et al., 2010). It has also been observed that artesunate induced apoptosis of breast cancer cell lines (MCF-7, T47D and MDA-MB-231) and leukemic T cells (CEM, J16 and Molt-4) via iron-dependent ROS formation, and subsequent cytochrome c release and cleavage of procaspases-2, 3, 8 and 9 (Efferth et al., 2007; Hamacher-Brady et al., 2011). However, there are reports revealing that in addition to apoptosis, artemether induced swelling necrosis in gastric cancer cell line (PG100) (Alcântara et al., 2013), and artesunate mediated ROS-induced swelling necrosis in pancreatic cancer cell lines (Panc-1, BxPC-3 and CFPAC-1) (Du et al., 2010).

6.3. Artemisinins impede angiogenesis in cancer

Angiogenesis, when controlled with a fair balance between stimuli and repressors, plays a critical role in wound-healing, reproduction, growth and development (Chung & Ferrara, 2011). In tumor cells, there is a growth restriction of about 1–2 mm^3 on its own, due to the lack of oxygen and nutrients (McDougall et al., 2006). Thus, tumor cells will tip the angiogenesis balance favoring the angiogenic stimuli (e.g. MMPs and VEGF) and inhibiting angiogenic repressors (e.g. thrombospondin (TSP) and tissue inhibitor of metalloproteinase (TIMP)) to expand its blood supply for growth (Weis & Cheresh, 2011). The lack of oxygen and nutrients places the tumor in hypoxic condition, which activates HIF-1α and nuclear factor-κB (NF-κB), leading to transcriptional expression of VEGF (Rius et al., 2008). It has been revealed that the cytotoxic effects of artemisinin and its derivatives (e.g. artesunate, arteether and artemether) correlated well with 30 differentially regulated angiogenesis-related genes in 60 human tumor cell lines (Anfosso et al., 2006).

Artemisinin was able to reduce the levels of HIF-1α and VEGF in mouse ES cells, implicating the use of artemisinin as an anti-angiogenic agent (Wartenberg et al., 2003). In C57BL/6 mouse Lewis lung carcinoma model, oral artemisinin given at 50 mg/kg/d significantly reduced lymphangiogenesis through decreased expression of VEGF-C (J.X. Wang et al., 2008). In BALB/c nude mice implanted with human ovarian cancer cells (HO-8910) with augmented expression of VEGF and its receptor KDR/flk-1, subcutaneous artesunate at 50 mg/kg/d suppressed the levels of VEGF and KDR/flk-1 and tumor growth (Chen et al., 2004). In a similar manner, artemisinins were found to reduce VEGF and angiogenesis in Kaposi's sarcoma (KS-IMM) xenografted mice and in a rat glioma model (Dell'Eva et al., 2004; Wu et al., 2009).

6.4. Artemisinins abrogate tissue invasion and metastasis of cancer

Metastasis occurs when malignant cancer cells are detached from the primary tumor and degrade the extracellular matrix, allowing its escape beyond the primary tumor site (Nguyen & Massague, 2007). The detachment of cancer cells from the primary tumor is correlated with the down-regulation of E-cadherin, a transmembrane molecule involved in cell-to-cell adhesion (Canel et al., 2013). Proteolytic enzymes such as MMPs play a critical role in extracellular matrix degradation, favoring tumor migration, invasion and metastasis. The activities of MMPs can be counterbalanced by TIMPs to minimize matrix degradation and metastatic potential (Bourboulia & Stetler-Stevenson, 2010).

In human melanoma cells (A375M), artemisinin was able to reduce MMP2 level by over 3 folds and block cell migration (Buommino et al., 2009). On the other hand, DHA suppressed the levels of MMP2 and/or MMP9, and metastasis in human ovarian cancer cells (HO8910PM) and in human pancreatic cancer cells (BxPC-3 and PANC-1) via NF-κB inhibition (S.J. Wang et al., 2011; B. Wu et al., 2012). Likewise, in non-small cell lung cancer (H460 and H1299), artesunate was able to inhibit metastasis by abrogating both MMPs and NF-κB activity (Rasheed et al., 2010). When tested in hepatocarcinoma cells (HepG2 and SMMC-7221), artemisinin significantly up-regulated E-cadherin and down-regulated the levels of both MMP2 and TIMP-2. In BALB/c nude mice grafted with HepG2 tumor, artemisinin at 50 mg/kg/d blocked tumor

metastasis by 50% (Weifeng et al., 2011). In a poorly-differentiated human colorectal carcinoma cell line (CLY), artesunate increased expression of E-cadherin and altered the subcellular localization of β-catenin from the nucleus to the plasma membrane, indicating an inhibition of the hyperactive Wnt signaling pathway, and leading to apoptosis and reduction in tumor growth (L.N. Li et al., 2008).

6.5. Targeted delivery of artemisinins against cancer

The specificity of targeting cancer cells over normal mammalian cells partly lies in the over-expression of transferrin receptors (TfR). Cancer cells are highly replicative, thus it requires a heavy load of iron, which serves as a cofactor in the synthesis of deoxyriboses before cell division (Daniels et al., 2012). Transferrin, a glycoprotein with iron bounded to its protein moiety, binds to cell surface TfR leading to endocytosis into the cells. In the endosome, a sharp drop of pH to 5.5 or lower triggers the release of iron from transferrin, whereby iron is then pump out into the cytoplasm while transferrin (without iron) and transferrin receptors are recycled back to the cell membrane.

Recent studies have shown that artemisinin–transferrin conjugates displayed higher anti-cancer efficacy than artemisinins alone (Lai et al., 2009; Nakase et al., 2009, 2008). In breast cancer cells (MCF-7), DHA–transferrin conjugate demonstrated at least 280 times more potent anti-cancer activity than in human normal breast cells (Xie et al., 2009). In this way, artemisinin acts as a pro-drug, and the drug–transferrin conjugate is internalized into cancer cells via TfR-mediated endocytosis and gets activated inside the low pH endosome. When the transferrin receptors are blocked by pre-incubation with anti-TfR monoclonal antibodies, artemisinin activity was significantly down-regulated (Efferth et al., 2004; Daniels et al., 2012). Artemisinin–transferrin helps to increase its specificity towards cancer cells and minimize potential side effects on normal mammalian cells.

6.6. Clinical experience with artemisinins against cancers

Taken together, artemisinins have been shown to be able to inhibit tumor growth, induce growth cycle arrest, promote apoptosis, negate angiogenesis and tissue invasion of the tumor, as well as cancer metastasis (Fig. 3). The dosages required for cancer treatment are largely similar to the doses used in anti-malarial therapy; and from numerous clinical toxicity studies of artemisinin and its derivatives in malaria, some patients were spotted to develop slight neurotoxicity (Efferth & Kaina, 2010), otherwise, minimal side effects and resistance were observed (Liang & Albrecht, 2003; Dondorp et al., 2010; McGready et al., 2012).

A case report has described a 70% reduction in tumor size of laryngeal squamous cell carcinoma in patients treated with artesunate for two months (Singh & Verma, 2002). Another case study revealed a reduction of disease progression in a pituitary macroadenoma patient treated with artemether for 12 months (Singh & Panwar, 2006). Furthermore, artesunate was found to control the tumor growth and improved survival of the patients with metastatic uveal melanoma (Berger et al., 2005). Although these case reports were built on very small number of patients, the observed promising efficacies of artesunate laid the foundation for subsequent clinical trials. In a randomized controlled trial conducted in 120 advanced non-small cell lung cancer patients, 60 patients in control group were treated with standard chemotherapy (vinorelbine and cisplatin) and another 60 patients were treated intravenously with 120 mg artesunate together with standard chemotherapy. The artesunate-treated patients showed improved short-term and one-year survival rates, and prolonged time to cancer progression as compared to the control group, with negligible side effects observed (Zhang et al., 2008).

7. Current developments and limitations of artemisinins

Apart from chemical modifications of the monomeric artemisinin to produce more potent anti-malarial analogs such as artemiside, artemisone (Dunay et al., 2009; Guo et al., 2012), SM905 and SM934 (Wang et al., 2008a; Hou et al., 2009, 2011, 2012), other strategies include the development of multimeric artemisinin conjugates with improved clinical efficacies and less adverse effects. Some of these artemisinin dimers, trimers and even tetramers (Beekman et al., 1997; Ekthawatchai et al., 2001; Mott et al., 2013) have demonstrated promising therapeutic advantages not only in malaria (Lombard et al., 2012), but also in anti-cancer (Paik et al., 2006; Singh et al., 2011) and anti-viral (Arav-Boger et al., 2010; R. He et al., 2011) therapies. These dimers and trimers have shown greater structural and chemical stabilities leading to improved potency, bioavailability and safety, as compared to artemisinin monomer. However, it remains to be determined if these novel synthetic artemisinin analogs, and artemisinin dimers and trimers will be more efficacious in other disease models such as fungal infections, autoimmune disorders and allergic inflammation, and by which mechanisms of actions to achieve the expected therapeutic outcome.

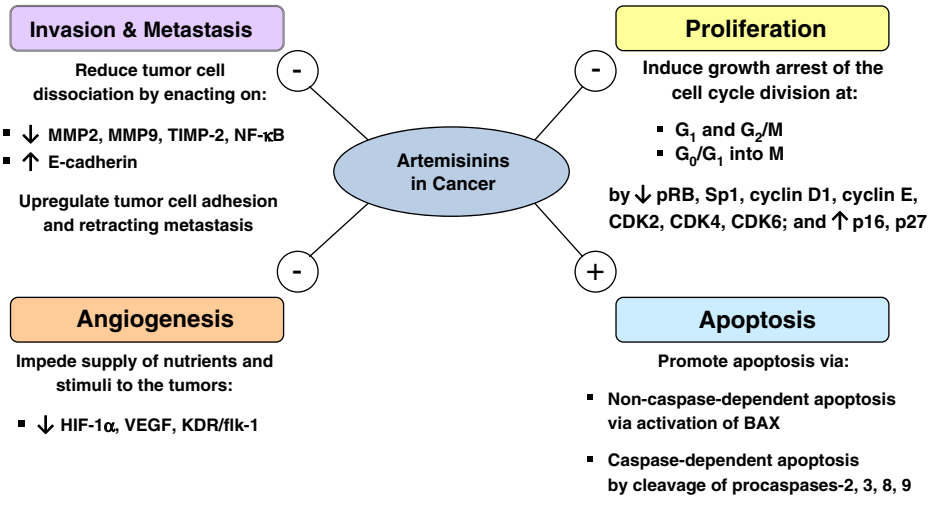

Fig. 3. Overview of the anti-cancer mechanisms of action for artemisinins. + indicates activation and − indicates inhibition.

Table 1
Comparison of effective doses of artemisinins in various disease models.

Therapeutics	Diseases	Effective doses[a]	Drugs	Routes	Remarks	Ref
Anti-malarial	*Plasmodium*	2–4 mg/kg	ARTS	i.v.	Clinical	Dondorp et al., 2005
Anti-viral	Herpes virus	1–15 μM	ARTS	–	In-vitro	Efferth et al., 2002
		50 mg/kg	ARTS	p.o.	In-vivo	Kaptein et al., 2006
		100 mg/d	ARTS	p.o.	Clinical	Shapira et al., 2008
	Hepatitis B virus	0.5–2.3 μM	ARTS	–	In-vitro	Romero et al., 2005
	Hepatitis C virus	78 μM	ATM	–	In-vitro	Paeshuyse et al., 2006
Anti-parasitic	*Trypanosoma*	13–23 μM	ATM	–	In-vitro	Mishina et al., 2007
	Toxoplasma	10 mg/kg	AMSO	s.c.	In-vivo	Dunay et al., 2009
	Schistosoma	80 mg/d	ARTS	p.o.	Clinical	De Clercq et al., 2002
	Trematodes	2 mg/ml	ATM	–	In-vitro	Keiser & Morson, 2008 ; Ferreira et al., 2011
		200 mg/kg	ARTS	p.o.	In-vivo	
Anti-fungal	*Cryptococcus*	0.045–2 μg/ml	AETH	–	In-vitro	Galal et al., 2005
	Aspergillus	125 μg/ml	ATM	–	In-vitro	Gautam et al., 2011
Anti-inflammatory	RA	3–5 mg/kg	ARTS	i.p.	In-vivo	Mirshafiey et al., 2006
		0.5 mg/kg	SM905			J.X. Wang et al., 2008
	SLE	10–125 mg/kg	ARTS	p.o.	In-vivo	Jin et al., 2009
			SM934			Hou et al., 2011
	Lupus nephritis	5–125 mg/kg	DHA	p.o.	In-vivo	Li et al., 2006
	IBD	150 mg/kg	ARTS	p.o.	In-vivo	Yang et al., 2012
	Sepsis	7.5–30 mg/kg	ARTS	i.m.	In-vivo	Li et al., 2010
		50–200 mg/kg	ATM	p.o.	In-vivo	Wang et al., 2006
	Uveitis	10–100 mg/kg	ARTS	i.v.	In-vivo	X.Q. Wang et al., 2011
	Alzheimer's Disease	40 mg/kg	ATM	i.p.	In-vivo	Shi et al., 2013
	Endometriosis	150–300 mg/kg	ATM	i.g.	In-vivo	X. Wang et al., 2011
Anti-allergic	Dermatitis	0.85–5%	ARTS	Topical	In-vivo	Chen & Maibach, 1994
	Asthma	30 μM	ARTS	–	In vitro	Ho et al., 2012
		3–30 mg/kg	ARTS	i.p.	In-vivo	Cheng et al., 2011
	Anaphylaxis	3–30 mg/kg	ARTS	i.p.	In-vivo	Cheng et al., 2013
Anti-cancer	Lung carcinoma	5.54–20 μM	DHA	–	In-vitro	Lu et al., 200
		50 mg/kg	ATM	p.o.	In-vivo	J. Wang et al., 2008
	Ovarian cancer	50 mg/kg	ARTS	s.c.	In-vivo	Chen et al., 2004
	Kaposi's sarcoma	1–15 μM	ARTS	–	In-vitro	Dell'Eva et al., 2004
		100 mg/kg	ARTS	p.o.	In-vivo	
	Glioma	5–25 μM	DHA	–	In-vitro	Huang et al., 2007
		33–66 mg/kg	AMET	p.o.	In-vivo	Wu et al., 2009
	Colorectal carcinoma	20–100 μM	ARTS	–	In-vitro	L.N. Li et al., 2008
		300 mg/kg		i.v.	In-vivo	
	Melanoma	10–200 μmol/l	ARTS	–	In-vitro	Ramacher et al., 2009
		1 mg/d		i.p.	In-vivo	
	Breast cancer	100–300 μM	ATM	–	In-vitro	Sundar et al., 2008
	Leukemia	0.11–3.76 μM	DHA	–	In-vitro	Lu et al., 2008
	Gastric cancer	1.08–13.61 μM	DHA	–	In-vitro	Lu et al., 2008
	Pancreatic cancer	12.5–100 μM	DHA	–	In-vitro	S.J. Wang et al., 2011
		2–50 mg/kg		i.p.	In-vivo	

ATM—artemisinin; DHA—dihydroartemisinin; ARTS—artesunate; AMET—artemether; AETH—arteether; AMSO—artemisone.
[a] Effective doses are drug dosage/concentration ranges in which intended pharmacological effects were observed.

For artemisinin drug development beyond anti-malarial therapy, it is apparent that different laboratories have reported different effective dose ranges for similar animal models, lacking a concerted effort to validate the efficacies of any artemisinin lead compounds in different animal models, and hampering the progress for any non-malarial clinical trial. Table 1 lists the reported effective dose ranges for different artemisinin derivatives used in both in vitro and in vivo studies of a variety of non-malarial disease models, which provides easy reference and comparison of relative potencies of different artemisinin derivatives in different disease models. Another limitation with the development of artemisinins beyond malarial is the lack of toxicological data for chronic exposure to artemisinin. Acute exposure to artemisinins for anti-malarial therapy has resulted in infrequent neurotoxicity and hypersensitivity reaction (Leonardi et al., 2001; Mohapatra et al., 2009; Dube et al., 2012). It is essential to conduct chronic toxicological studies and drug-interaction studies for artemisinins in preparation for long-term use of artemisinins in autoimmune and inflammatory diseases.

8. Summary and future outlook

Artemisinins are a class of potent bioactive molecules that possess a rich assortment of biological activities beyond anti-malarial, which includes anti-viral, anti-parasitic, anti-schistosomal, anti-protozoal, anti-fungal, anti-inflammatory, anti-allergic, and anti-cancer (Fig. 4). In addition to the formation of free radicals via the cleavage of the endoperoxide bond by heme iron in its structure to execute anti-malarial action and cytotoxic effects against cancers, artemisinins have also been shown to modulate a wide panel of signaling molecules or pathways including TLRs, Syk tyrosine kinase, PLCγ, PI3K/Akt, MAPK, β-catenin/Wnt, STAT-1/3/5, NF-κB, Sp1 and Nrf2/ARE (Fig. 5) (Mirshafiey et al., 2006; Xu et al., 2007; B. Li et al., 2008; L.N. Li et al., 2008; Hou et al., 2009; Li et al., 2010; Cheng et al., 2011; Ho et al., 2012; Cheng et al., 2013; Li et al., 2013). However, the exact signaling molecules or receptive substances which directly interact with artemisinins remain unclear. It is likely to involve several key signaling molecules, considering that artemisinins can elicit broad-spectrum inhibitory effects on several major signaling pathways. Identification of these artemisinin binding targets is imperative to further understand which chemical functional groups in artemisinins are responsible for the modes of action in different disease conditions. Synthetic chemistry of artemisinin and screening for novel artemisinin analogs for potent anti-malarial action has been the central strategy for artemisinin drug development for improved efficacies and safety (Li, 2012; Njuguna et al., 2012). The next major milestone in artemisinin

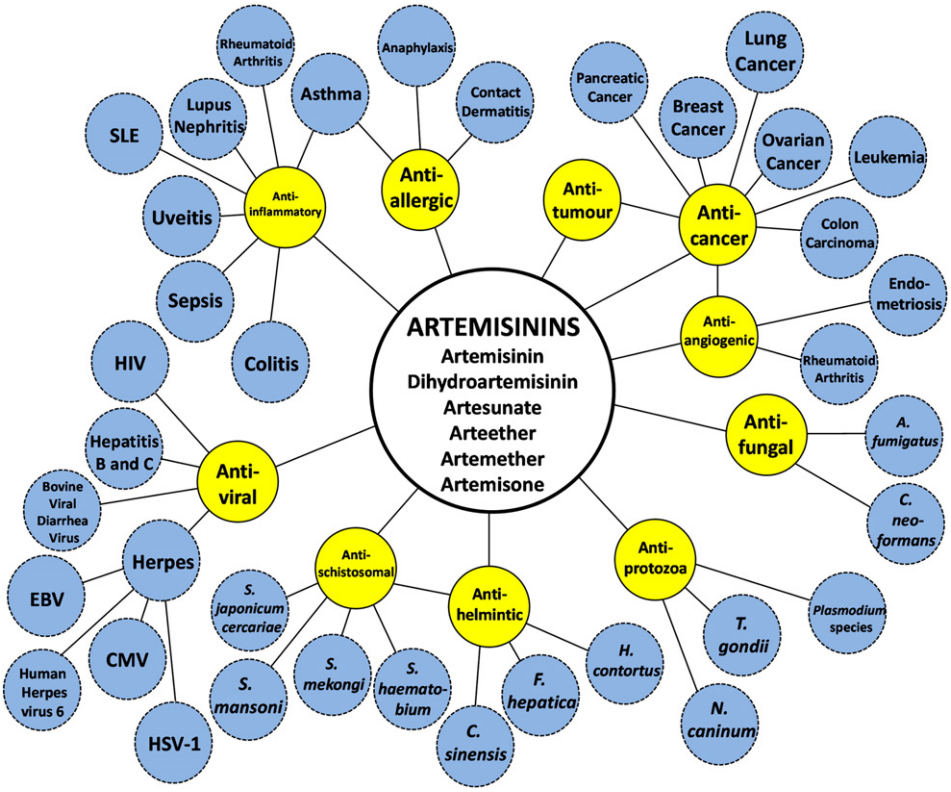

Fig. 4. Bubble map outlining various biological activities of artemisinins and their potential clinical applications in different diseases. Yellow circles with solid lines indicate different biological activities, and blue circles with dotted lines indicate potential applications of respective biological activities to various human diseases.

research will be to explore therapeutic potential of these novel analogs in disease models beyond malaria, including infectious diseases, cancers and immune disorders.

Presently, we have extensive in vitro and in vivo preclinical data in the literatures in support of therapeutic application for artemisinins, especially artesunate, in a variety of human disease conditions. However, despite the established safety record for artemisinins, clinical studies of artesunate on non-malaria-related diseases are still very limited. In the clinical trial registry portal hosted by the National Institute of Health, there are only 6 clinical trials investigating artemisinins beyond malaria posted as shown in Table 2. Another frontier for artemisinin drug research for the next decade shall be translational medicine putting artemisinin derivatives, especially artesunate, into patients for the development of novel anti-viral, anti-fungal, anti-cancer, anti-allergic and immunosuppressive agents. With the concerted efforts in the novel synthesis of artemisinin analogs and clinical pharmacology of artemisinins, it is likely that artemisinin drugs will become a major armamentarium combating a variety of human diseases beyond malaria.

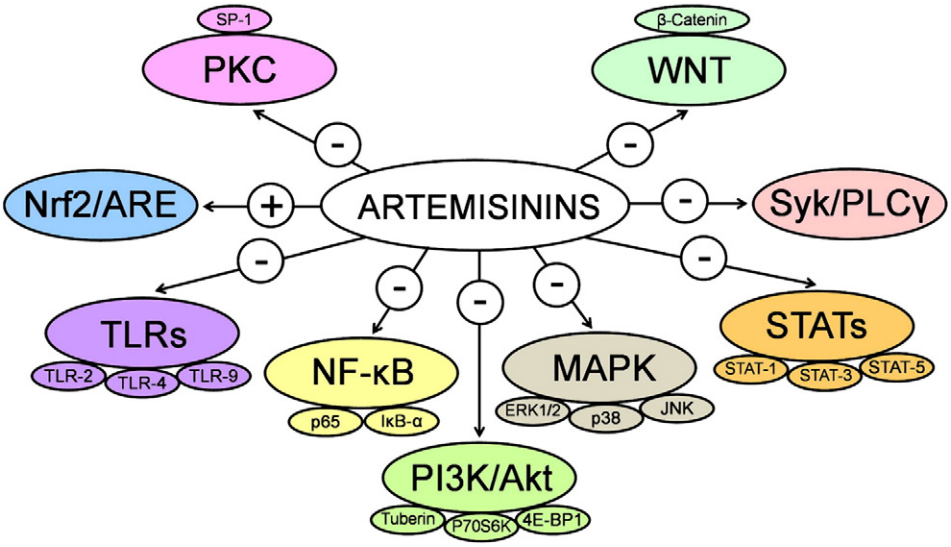

Fig. 5. Overview of the proposed modes of action of artemisinins on major signaling pathways that may contribute to its anti-inflammatory, anti-allergic and anti-cancer activities. + indicates activation and − indicates inhibition.

Table 2
Registered clinical trials on artemisinins for therapeutics beyond anti-malarial.

Derivative	Disease conditions	Phase	Status (last updated)	Clinical trial
Artemisinin	Schizophrenia	III	Completed (2012)	NCT00753506
			Completed (2011)	NCT01391403
Artemether	Anemia, helminthiasis, malaria, schistosomiasis	IV	On-going (2011)	NCT01459146
Artesunate	*Schistosoma mansoni*	III	Completed (N.A.)	NCT01054651
Artesunate	*Schistosoma haematobium*	III	Completed (2008)	NCT00510159
Artesunate	Cytomegalovirus	III	Completed (2010)	NCT00284687
Artesunate	Metastatic breast cancer	I	On-going (2011)	NCT00764036

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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