Review Article

Anticancer Effect of AntiMalarial Artemisinin Compounds

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Abstract

The anti-malarial drug artemisinin has shown anticancer activity *in vitro* and animal experiments, but experience in human cancer is scarce. However, the ability of artemisinins to kill cancer cells through a variety of molecular mechanisms has been explored. A PubMed search of about 127 papers on anti-cancer effects of antimalarials has revealed that this class of drug, including other antimalarials, have several biological characteristics that include anticancer properties. Experimental evidences suggest that artemisinin compounds may be a therapeutic alternative in highly aggressive cancers with rapid dissemination, without developing drug resistance. They also exhibit synergism with other anticancer drugs with no increased toxicity toward normal cells. It has been found that semisynthetic artemisinin derivatives have much higher antitumor activity than their monomeric counterparts via mechanisms like apoptosis, arrest of cell cycle at G_0/G_1, and oxidative stress. The exact mechanism of activation and molecular basis of these anticancer effects are not fully elucidated. Artemisinins seem to regulate key factors such as nuclear factor-kappa B, survivin, NOXA, hypoxia-inducible factor-1α, and BMI-1, involving multiple pathways that may affect drug response, drug interactions, drug resistance, and associated parameters upon normal cells. Newer synthetic artemisinins have been developed showing substantial antineoplastic activity, but there is still limited information regarding the mode of action of these synthetic compounds. In view of the emerging data, specific interactions with established chemotherapy need to be further investigated in different cancer cells and their phenotypes and validated further using different semisynthetic and synthetic artemisinin derivatives.

Keywords: Anticancer agents, Antimalarials, Antitumor activity, Artemisinins, Novel chemotherapy

Introduction

Cancer therapies consisting chemotherapy, surgery, and radiotherapy are available, but in many cases have limited efficacy.[1] An ideal anticancer drug should have high potency and specificity in killing cancer cells without significant or fatal toxicity on normal cells, which is almost nonexistent. A model useful in predicting which patients are at increased risk of developing severe or fatal toxicity from chemotherapy[2] is used at present in predicting chemotherapy toxicity.

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Modern anti-cancer therapies show better response and survival, but the side-effects and poor quality-of-life, frequently leads to discontinuation, dose reduction, and emergence of drug resistance.

Research has focused on developing safer chemotherapies by (a) exploring the anticancer properties of newer compounds or (b) by assessing drugs used in other nonmalignant diseases. Many plant derivatives are known to be effective against a variety of diseases with broad antibiotic and antimalignant activity.

In this regard, the reports on potential anti-cancerous effects of artemisinins and other antimalarials are a new development in pharmaceutical research as evidenced by nearly 200 papers on this topic published in the last two decades. The following review includes a literature search in PubMed with the key words “antimalarials,” “anti-cancer effects,” “novel chemotherapy,” and “chloroquine and antitumor activity” for

the last two decades. About 127 articles (abstracts and PMC of reviews and experimental data) were examined out of which 94 were finally included.

Artemisinins

Artemisinin is a naturally occurring antimalarial showing anticancer properties.[3,4] It has few side effects[5] and drug resistance, but tolerance can occur.[6] Artemisinin and its derivatives have shown a potent anti-neoplastic activity in both drug-sensitive and resistant cancer cell lines in several studies.[4,7,8] Artemisinin is a sesquiterpene lactone with a 1,2,4-trioxane ring system, extracted from the Chinese herb qinghao. Low water/oil solubility, poor bioavailability, and a short half-life *in vivo* (~2.5h) are the pharmacological shortcomings of artemisinins.[9,10] Hence, three generations of artemisinin-like semisynthetic and fully synthetic endoperoxide compounds were developed to overcome these limitations. Two generations of such semisynthetic compounds-artesunate, arteeter, artemether, and artemisone have good efficacy and tolerability. Of these artesunate is the most commonly used antimalarial although the second-generation artemisinin. Artemisone shows better pharmacokinetic profiles including a longer half-life and lower toxicity.

Antimalarials are also used in conditions like SLE or rheumatoid arthritis. It is known that quinine, chloroquine, primaquine, and quinacrine blocks the uptake and amino acid incorporation *in vivo* but have little effect on cell-free protein synthesis. Hence, the basic action of these drugs is amino acid blockage for uptake by the target cells.[3] Recently, several other targets for anticancer effects of chloroquine/hydroxychloroquine, both in cultured cancer cell lines as well as on human tumors grafted into mice have been reviewed[11] and some suggest that chloroquine synergistically enhanced the effects of conventional chemotherapy through inducing apoptosis in addition to blocking the autophagic but enhanching their anti-angiogenic actions.[12] These molecular targets in cancer cells are not fully identified, but artemisinins may target mitochondria, endoplasmic reticulum (ER), and the lysosome.

Anti-cancer activity of artemisinins: The different pathways

The artemisinins' endoperoxide moiety is responsible for its anti-malarial and anti-cancer effects. The endoperoxide bond is probably activated by reduced heme or ferrous iron, leading to cytotoxic carbon-centered radicals which are strong alkylating agents.[13,14] But the absence of the endoperoxide moiety does not completely abolish the anticancer activity but significantly reduces cytotoxicity, suggesting that anticancer activity may involve alternative mechanisms as well.[15] Drug concentrations required to have an effect on cancer cells are usually higher than those to kill malaria parasites.

Iron and heme metabolism have a role in the anticancer activity of artemisinin. Iron, heme or heme-bound proteins are responsible for bioreductive activation of artemisinin.[16-18] It has been found that preloading of cancer cells with iron or iron-saturated holotransferrin (diferric transferrin) triggers artemisinin cytotoxicity,[19-22] with an increase in artemisinin activity up to 100-fold in some cell lines.[23] Furthermore, artemisinins tagged with iron-carrying compounds show greater activity compared with that of artemisinin alone.[23-25] Recently, it was shown that chemical modulation using succinylacetone, a heme synthesis inhibitor, decreases dihydroartemisinin (DHA) cytotoxicity in human promyelocytic leukemia cells (HL-60),[22] which is consistent with previous studies which found that induction of heme oxidase followed by down-regulation of the heme synthesis genes may also inhibit cytotoxicity of artemisinin dimers in the same cancer line.[26] Similarly, treatment with desferroxamine (iron chelator), inactivates these compounds.[27]

Continued multiplication, growth, and survival of malignant cells require higher iron metabolism.[22] Cancer cells demonstrate an increase in transferrin receptors (TfR) that are responsible for uptake and regulation of intracellular iron levels. Expressions of TfR in cancer cells may vary according to cell lines. However, they differ substantially from normal cells resulting in a high selectivity index of artemisinin and its derivatives. For example, leukemia (CCRF-CEM) and astrocytoma (U373) cells express TfR in 95% and 43% of the cell population, whereas normal monocytes express only ~1%.[28,29] Blocking the TfR by pretreatment with specific monoclonal antibodies may abolish artemisinin activity.[29]

High oxidative stress is a common anti-neoplastic property of anticancer drugs.[30] Tumor cells are vulnerable to radical oxygen species (ROS) damage due to lower expression of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase compared to normal cells.[30,31] It is postulated that iron-activated artemisinin releases highly alkylating carbon-centered radicals and ROS.[22,32] ROS generation may contribute to the selective action of artemisinin on cancer cells by DNA damage, enhanced apoptosis, growth arrest and reducing angiogenesis. Several studies have also associated artemisinin cytotoxicity with blocking cytokines, inhibiting tumor invasion, migration, and metastasis.[31] The selectivity of artemisinin may also be enhanced by selective targeting of cancer biomarkers or overexpressed cancer genes and proteins which are not detectable in normal tissues.[32] Artesunate-treated cells demonstrated early oncosis-like microscopic changes at subcellular structures by activation of ROS generation, suggesting that ROS-mediated damage is initiated by some triggering event. Experiments in the HL-60 cell line have revealed that early and rapid generation of ROS is associated with artemisinin-induced apoptosis induction and cell damage.[33] A study has demonstrated that ROS generation in artesunate-treated HeLa cells precedes cytotoxicity implicating it as the trigger. The electron transfer chain (ETC) in the mitochondrion has also been found to play a role in the generation of ROS.[34] However, cytotoxicity still persists in HeLa cells devoid of ETC indicating that other

sources of ROS may be present in these cells.[21] Overall, multiple ROS-independent mechanism of cell damage may operate in artemisinin-induced necrosis.

The antineoplastic toxicity of artemisinins may also be modulated by other factors like calcium metabolism, ER stress[20,21] and the expression of the translationally controlled tumor protein (TCTP) a binding calcium protein, which also is a parasite target.[4] ER appears to be a plausible site for artemisinin action because in HepG2 cells a fluorescent derivative has been shown to preferentially accumulate there. Although the expression of the gene TCTP was initially correlated with cancer cell response to artemisinins, a definite role for TCTP in this anticancer effect needs confirmation.[35]

Studies of sarcoendoplasmic Calcium ATPase (SERCA) as artemisinin target in cancer has reported that increases calcium concentrations occurred as a result of SERCA inhibition following the treatment with artemisinin.[26,36] Conversely, studies on two artemisinin dimers have shown that ROS-mediated induced ER-stress after treatment was independent of SERCA inhibition.[24] Interestingly, the behavior of a highly potent artemisinin dimer and thapsigargin (an SERCA inhibitor), appears to be similar although mediated at different molecular levels.[26] Thapsigargin lacks the endoperoxide moiety and induces ROS. But, carboxylic acid dimers 8a and 9 of artemisinin are more efficacious orally as antimalarials in rodents than either artelinic acid or sodium artesunate, and are strongly inhibitory but not cytotoxic in several human cancer cell lines.[37]

It has been found that dimeric and trimeric artemisinin derivatives have much higher antitumor activity than their monomeric counterparts. In the last decade, an increased number of reports on artemisinin dimeric compounds have confirmed this. Further studies on four series of C-10 nonacetal dimers, prepared from key trioxane alcohol 10beta-(2-hydroxyethyl) deoxoartemisinin (9b) *in vitro* in HL60 cells show that both phosphate ester dimers (14a and 14b) are more efficacious than doxorubicin. Interestingly, phosphate ester monomers 9c and 9d, active in the low nanomolar region against *Plasmodium falciparum,* are inactive as anticancer agents at higher millimolar dosage, thus emphasizing the importance of two trioxane units for high antimalignant activity, and the nature of the linker in dimers of this type plays an important role in imparting potent anticancer activity.[38]

Artemisinins also have pleiotropic effects. The sensitivity and resistance of tumor cells depend on the mRNA expression of angiogenesis-related genes suggesting artemisinins exert their antitumor effects at least partly by inhibiting tumor angiogenesis. This was validated in 6 out of 30 angiogenesis related genes in one study by microarray data.[39] Artemisinins may also be chemopreventive in addition to being antiproliferative since many chemopreventive drugs have antiangiogenic features.

Anticancer activity of artemisinin has been demonstrated primarily *in vitro* and in animal models. In a study, testing 55 cell lines showed that artesunate showed inhibitory effects against leukemia, colon, melanoma, breast, ovarian, prostate, central nervous system, and renal cancer cells.[39] The semisynthetic derivative DHA showed remarkable antineoplastic activity against pancreatic, leukemic, osteosarcoma, and lung cancer cells.[40] Moreover, artemisone was superior to artemisinin and showed better synergism with other anti-cancer agents.[41]

Artemisinin has also been found to act either directly by causing DNA damage or indirectly by interfering with several signaling pathways involved in carcinogenesis. The indirect DNA damage seems to be commoner than direct damage. In pancreatic cells, artesunate caused DNA fragmentation and membrane damage. Low doses of artesunate were associated with oncosis-like cell death, whereas higher concentrations caused apoptosis. But, extent and type of such damage can depend on the phenotype and the origin of cell line, varying in time- and dose-dependent manner. Notably, higher sensitivity to artesunate was observed in rapidly growing cell lines compared to slow growing cancer cells.[40]

Alternatively, DHA, artesunate, and artemether may possibly modulate genes and proteins coordinating growth signals, apoptosis, proliferative capacity, angiogenesis, tissue invasion, and metastasis. Complex interactions through different pathways may enhance the anticancer effect of these endoperoxide drugs leading to growth control and cell death.

Cyclin-dependent kinases (CDK) are the proteins transmitting signals to complete the cell cycle in normal cells. Normal growth depends on the ability to translate signals for cell division and replication. Proliferation in cancer cells is the result of mutations inducing amplification of growth signals, dysregulation of checkpoints, loss of sensitivity to growth inhibitors and deregulation of apoptosis.[42] Artemisinin compounds have been shown to exert both cytostatic and cytotoxic action on cancer cells at all cell cycle phases, and arrest at G_0/G_1 to S transition is more commonly affected.[7] Artemisinin and its semisynthetic derivatives can interrupt cell growth in cancer lines either by disrupting the cell cycle kinetics or by interfering with proliferation-interacting pathways. DHA and artesunate are very potent growth inhibitors with multiple studies showing DHA as the more potent anticancer artemisinin-like compound than the artesunate, arteeter, and artemether.[7,40,41] Recently, artemisone also has shown such effects in 7 cells lines including melanoma and breast cancer cells with all cell cycle phases being arrested simultaneously by cytostatic effect.[42] Arrest of the cell cycle at G_2/M was also observed after DHA treatment in different malignancies such as osteosarcoma, pancreas, leukemia, and ovarian cancer cells,[43-45] while, artesunate interferes with G_2 in osteosarcoma, ovarian, and other cell lines. The underlying mechanisms of such growth arrest are diverse:[46] (a) alterations in the expression and activity of regulatory enzymes of the cell cycle, such as

CDK2-4 and -6 and D type cyclins (G_1-to-S-phase transition) or CDK1, and A-type cyclin (G_2/M), (b) down-regulation of CDK transcription, inhibition of CDK promoters or increase of p21, p27, and CDK inhibitor and (c) down-regulation of interacting proteins targeting multiple pathways. These have been documented in pancreatic, pulmonary, prostate and bone cancers. One study has identified artesunate with topoisomerase II, an inhibitor which inhibits the growth by interaction through multiple pathways.[47] Hence, artemisinins may interfere with several pathways that are common to different cancers.

Apoptosis is a process is mediated by a balance between the proapoptotic Bax, and the antiapoptotic Bcl2 Bcl2 family genes and their effects on the mitochondria are important for cancer control. An increase in the Bax/Bcl2 ratio leads to release of cytochrome c followed by stepwise activation of caspases leading to cell death.[33,48] Artemisinin sensitivity depends upon the level of expression of antiapoptotic (Bcl2) and proapoptotic genes (Bax) in a cancer cell line.[49] Apoptosis is rapidly induced by artemisinin in many cancer cell lines where mitochondrial membrane damage plays a central role in this process of cell death. Treatment with DHA in leukemia cells induced apoptosis after 1 h. In general, the apoptotic effects of artemisinin have been attributed to activation of the intrinsic pathway. Many studies have revealed that artemisinin-like compounds induce apoptosis by modulating the Bax/Bcl2 ratio.[20,46,50-53] Validating these observations, DHA and artesunate caused cytochrome c release, Bax overexpression, increase in Bax/Bcl2 ratio 31, and activation of caspases 3 and 9 in osteosarcoma cells. DHA also activates caspase 8 and decreases the levels of CDC25B, cyclin B1, and nuclear factor-kappa B (NF-kB). In the same circumstances, artesunate exposure decreases survivin which has also been involved in the apoptotic DHA response in lung cancer cells.[33] Similar findings have been reported in hepatoma cancer lines treated with DHA, where DHA and artemisinin seem to have similar potency.[54]

Some studies describe alterations on molecules acting on the extrinsic apoptotic pathway.[49] DHA may increase the transcription of the cell death receptor 5 (DR5) promoter and induces DR5 in different prostate cancer lines. In a study, combination treatment with TRAIL (a DR5 ligand) significantly increased the DHA proapoptotic effect by up to 35%.[55]

Artemisinins usually promote apoptosis rather than necrosis in most cases although both have been reported. Induction of apoptosis is a major advantage of artemisinins' anti-malignancy action since there is no associated inflammation or cell damage due to necrosis. Artemisinin-induced necrosis is associated with low levels of ATP and defective apoptotic mechanisms.

Artemisinins in cancer metastasis

Distant metastasis is a process in which malignant cells invade and spread through the extracellular matrix, associated with high mortality and morbidity. Artemisinin has anti-invasive effect in highly aggressive and invasive cancers, that is correlated with altered expression of the matrix metalloproteinases (MMP) genes and their effects on $\alpha v\beta 3$ integrins.[56] During metastasis, the cancer cell loses the of expression of E-cadherin, a calcium-binding transmembrane molecule involved in cell to cell adhesion. Several genes encoding extracellular matrix processing proteases, motility factors, and adhesion proteins also act at different steps during metastasis. Inhibition of metastasis is achieved by artemisinin by increasing cell-cell adhesion by enhancing E-cadherin activity and Cdc42 activation. Artemisinin augments cell to cell adhesion by increasing E-cadherin activity and Cdc42 activation which inhibits metastasis.[57] It has been observed that some cancer cells can have specific proteins interacting in different pathways. For example, in non-small cell lung cancer and fibrosarcoma, DHA treatment induced low levels of MMP2, MMP7, or MMP9 driven by AP-1 and NF-kB modulation or inactivation[58] suggesting DHA affects different pathways. Animal investigations have shown that in lung cancer, lymph node metastasis and lymphangiogenesis were resisted by artemisinin-mediated inhibition of vascular endothelial growth factor C (VEGF-C).[59] Artesunate generally has been found to be more effective in less differentiated cell lines.

Angiogenesis, needed for metastatic cancer cell survival, occurs by proliferation of endothelial cells through induction of VEGF, fibroblast growth factor (FGF), its receptors, and cytokines.[60] It involves several mechanisms including hypoxia-driven activation of expression of hypoxia-inducible factor (HIF)-1α and the aryl hydrocarbon receptor nuclear translocator and is controlled by angiostatin, endostatin, thrombospondin, TIMPs, PAI-1, and others.[59,60] Artemisinins, artesunate, and other derivatives inhibit neovascularization by modulating gene expression of angiogenic factors,[39] through downregulating growth factors (VEGF, FGF), HIF-1α, new vessel mediator angiogenin (ANG), the cysteine-rich angiogenic inducer (CYR61), some metalloproteinases (MMP9, MMP11, and BMP1), and collagens along with upregulation of angiogenesis inhibitors.[39,61] This is supported by experimental studies in different systems, proposing other molecular interactions too. NF-kB is crucial in regulating these multiple processes playing a key role in the anticancer effects. It is activated by DNA damage and other sources of cell stress and it is a mediator of apoptosis resistance[62] in response to drug exposure and studies show that a cytokine called Scatter factor (a hepatocyte growth factor) mediated cell protection involves antiapoptotic signaling from its receptor (c-Met) to PI3 kinase → c-Akt → p21-activated kinase-1 (Pak1) → NF-kB[63] Reduced levels of NF-kB have been previously associated with proliferation and metastasis inhibition suggesting that NF-kB regulation may be a key role in the multimodal action of DHA in this system.[64,65] In addition, DHA prevents angiogenesis by depleting the levels of the VEGF flt-1 and KDR/flk-1-receptors. Similar effects were experimentally validated in lymphatic endothelial cells and

Lewis lung carcinoma.[66] In pancreatic cells (BxPc-3) and BalB/c nude mice, DHA induced inhibition of NF-kB DNA binding and down-regulation of angiogenic-related targets like MMP9, VEGF, COX2, and IL-8.

Artemisinins in drug resistant cancer cells

Many tumors develop drug resistance over time. A leading cause is a drug efflux generated by overexpression of membrane protein pumps resulting in ineffective/low drug concentrations.[67] Cytotoxicity of artemisinins has shown to be unaffected in otherwise resistant/multiresistant cancer cells. One study revealed that genes related with resistance to the established anticancer drugs such as MDR1 P-glycoprotein (Pgp), MRP1, and BCRP had no impact on artemisinins effect, and confirmed later on that the antitumor activity of artemisinin remains intact when resistance to other agents is present.[40] Artemisinins are effective in doxorubicin, metrotexate, and hydroxyurea-resistant cancer lines without cross-resistance. Furthermore, artesunate proapoptotic effect is not affected in a doxorubicin-resistant leukemia cell line; rather it potentiates doxorubicin's apoptotic effects.[8] In another study, intact anticancer potency of artesunate was found equally in chemoresistant and chemosensitive neuroblastoma cell lines and primary neuroblastoma cultures.[68] Here, sensitivity to artesunate was intact in vincristine, doxorubicin, cisplatin, topotecan, mephalan, and etoposide-resistant cells. Only one cell line showed low sensitivity to artesunate which was related to low ROS formation and high expression of glutathione cysteine ligase (GCL), where depletion of glutathione mediated by a GCL inhibitor improved artesunate sensitivity. Pgp or p53 attenuation did not affect the sensitivity to artesunate. DHA has shown better efficacy in cell lines such as cholangiocarcinoma and hepatocarcinoma compared to other drugs; moreover, upregulation of MDR1, MRP1-2, or MRP3 had no effect on its potency.[69]

Lack of cross-resistance between anticancer agents and artemisinins may depend on different mechanisms. Most of the conventional anticancer agents are nucleoside analogs, whereas artemisinin's action is primarily mediated by an ROS-dependent mechanism. In leukemias and human small cell lung cancer, artemisinins show no significant inhibition toward Pgp or MRP1,[8] suggesting overexpression of protein pump may not affect artemisinin's potency. In another system, however, artemisinin increases doxorubicin resistance by upregulating mdrp through a different mechanism.[70] Malignancy, especially hematological, can have a high potential to modulate their intrinsic systems leading to activation alternative rescue pathways which needs addition of multiple agents. Studies indicate convergence of diverse genetic signal pathways to a limited number of key downstream regulators of apoptosis. Convergence of pathways can be targeting these pathways is one way to address the problem of genetic heterogeneity in cancers like acute leukemia. This would imply treating multiple molecular aberrations with fewer drugs and enhanced therapeutic benefit.[71]

Artemisinins in Combination Chemotherapy for Cancer

In anti-cancer combination therapy, the antineoplastic action of artemisinin may add independent antitumor activity with no added side effects. The reason may be that diverse action of artemisinin in different pathways may improve overall synergism.[70]

A strategy to overcome multidrug resistance in cancer involves the use of a combination of the antineoplastic agent and a chemomodulator which inhibits resistance-causing proteins. The effects of antimalarial drugs on human recombinant glutathione S-transferase (GSTs) activity in searching for clinically effective inhibitors of these enzymes need investigations as they are reduced significantly by antimalarials and thereby can increase the efficacy of alkylating agents to overcome drug resistance.

It has been reported that resistant cancer cells become sensitive by adding artemisinin to the conventional treatment called "chemosensitization." DHA and artesunate have exhibited the strongest chemosensitizing/synergistic effects.[8,72] while artemisinin showed only additive and antagonistic actions. DHA significantly improves the efficacy of gemcitabine in pancreatic cancer which commonly develops resistance. *In vitro* and *in vivo* analysis in pancreatic cells demonstrated a DHA-induced increase in 4 fold growth-inhibition and 2-fold apoptosis respectively, compared to gemcitabine alone.[65] A dual action of DHA in potentiating gemcitabine activity and the inhibiting resistance has been attributed to DHA inhibition of gemcitabine-induced NF-kB activation and subsequent action on its targets.[65] A similar effect has been shown in hepatoma cancer cells irrespective of their p53 status.[54] DHA improves tumor growth inhibition synergistically by 45% when in combination with gemcitabine, while artemisinin only induces additive effects.[69]

Similarly, greater antitumor activity was observed in animal models when DHA in combination with cyclophosphamide and cisplatin was used in lung cancer and temozolomide in glioma cells. DHA promotes apoptotic and necrotic activity of temozolomide through ROS generation.[73] Increased artesunate anticancer activity has been observed in different combination regimens. Significant synergism was achieved with artesunate and the immunomodulator drug, lenalidomide combination. These evidences suggest that DHA and artesunate can potentiate antitumor agents and counter tumor resistance. However, the benefits of such combination therapy need to be validated further because therapeutic effects are influenced by the mode of action of the drugs and multiple interactions in particular systems and schedules. Artemisinins also improve radiation therapies. DHA treatment inhibits the radiation-induced expression of GST with associated ROS generation in glioma cells, where combination treatment is more effective than radiation or DHA alone.[74] The adjuvant

effect of artemisinin in other cancer treatments including hyperbaric oxygen has also been reported.[75]

Resistance to Artemisinins

Artemisinin resistant malaria has been reported from the Greater Mekong sub-region of the Asia Pacific (Cambodia, China, Lao People's Democratic Republic, Myanmar, Thailand and Vietnam) and in sub-Saharan Africa, specifically Angola.[76] In addition, unstable tolerance has been reported in patients with therapeutic failure. However, these tolerant strains are unstable and develop after several years of continuous drug exposure.[77] The multiple modes of action of artemisinins at different carcinogenic pathways may explain this. Some cell lines have shown low or no response to artemisinin or its derivatives. Examples include breast cancer cells, gastric cancer, and metastatic nasopharyngeal cancer cell lines.[56] In breast cancer, artemisinin response may be modified by estrogen receptors (ERα and ERb) responsible for cell proliferation. A recent study found some levels of cross-resistance to artesunate and DHA in a cisplatin-resistant neuroblastoma cell line, which was partially overcome by L-buthionine-S, R-sulfoximine, an inhibitor of the antioxidant GLC.[68]

However, *in vitro* resistance has been reported in experimental studies. These studies indicate that upregulation of the tumor suppressor p16^{I}NK4A and the antioxidant protein, catalase, may induce artesunate resistance irrespective of p53 status.[78] Further, MDA-MB-231 metastatic cells showed complete artesunate resistance, whereas a similar treatment in non-metastatic cell line MDA-MB-468 exhibited lesser resistance. Further investigation on the mechanism of artesunate resistance indicates that upregulation of NF-κB, AP-1, and NMP-1 overcome artesunate apoptotic and antimetastatic action, thus allowing tumor progression.[79] It is presently unclear, whether artesunate-induced resistance are permanent, if other semisynthetic endoperoxides may induce a similar effect and whether a combinational therapy may delay or reverse the effect on cell lines bearing this phenotype. Another issue to consider is whether cancer cells may develop DHA resistance after repeated administration thereby limiting its antitumor effects. But experiments in DHA-resistant Molt-4 human lymphoblastoid cell line (RTN) showed no significant cross-resistance to two synthetic artemisinin compounds.[80]

Artemisinins Toxicity

Dose-dependent toxicity is a major problem of anticancer therapy that can be overcome by increasing its efficacy with lower toxic drug concentrations. In spite of a wide use of artemisinin derivatives, toxicity in humans is negligible. The toxicity of artemisinin-like compounds can occur with long-term use, but treatments up to one year have shown no adverse effects.[17] DHA is the most neurotoxic artemisinin derivative. This has been reported in animal studies in a dose- and time-dependent manner (≥7 days).[81] Hence, rapid elimination of artemisinin in oral form is safer than slow-release/oil-based intramuscular formulations.[5] Clinical doses for malaria is 3 times higher than its anticancer activity.[40] Thus, artemisinin may have benefits as an anticancer agent, as it can be used in combination without increased side effects while efficacy and dose-reduction of more toxic anticancer agents can be possible. Brainstem neurotoxic encephalopathy has been reported in animal studies usually associated with long-term high-dose treatments.[82] Fatal overdose, especially in children may occur.

Artemether and artesunate have been used with good tolerability and lack of significant side effects in a variety of cancers such as laryngeal squamous cell carcinoma, malignant skin cancer, pituitary macroadenoma and advanced non-small cell lung.[17,83,84] They showed a substantial reduction of tumor size, increased survival with a significant improvement in disease control and metastasis reduction, especially with combination chemotherapy.[17] No new artesunate-related side effects were reported.[85]

New semisynthetic and fully synthetic derivatives of artemisinins with antineoplastic action

Limitations of artemisinins are short half-life, limited availability, and solubility; but they can be partly overcome by potent compounds with enhanced pharmacological properties. Novel semisynthetic endoperoxide compounds with selective anti-cancer activity have been developed. It has been found that dimeric and trimeric artemisinin derivatives display much higher antitumor activity than their monomeric counterparts. There has been an emphasis on the nature and stereochemistry of the dimer linker which may influence anticancer activity. The linker itself is inactive. Showing IC_{50} ranging from 0.014 to 6 μM, these compounds demonstrated 3–60 fold more anticancer toxicity according to the nature of the linker, and with lipophilicity or electrophilic substitutions[85] in different cancer lines. An increased anticancer activity seems to be conferred by the stereoisomery of the linker and an amide terminus.[86] Homodimers of artesunic acid also have nanomolar inhibitory values when tested in chemo-resistant and sensitive leukemia cells. Although these new semisynthetic artemisinins have shown promise, further research is needed.

The different stereochemistry of the ether linkage of the 5 dimers of artemisinins and some of its derivatives influence their anticancer activity. As an example, DHA (with a non-symmetrical dimer called dimer-3) and dihydrodeoxyartemisinin (with the corresponding endoperoxide lacking dimer or dimer-5) as an endoperoxide moiety had a stronger cytotoxicity.[13] They showed potent antiangiogenic properties, chemical stability, and greater cytotoxicity than the artesunate. This emphasizes the importance of two trioxane units for high antiproliferative activity, and also that the nature of the linker in dimers of this type plays a crucial role in imparting potent anticancer activity.[87]

Fully synthetic Artemisinins such as some trioxolanes and ozonides with improved pharmacokinetics are under clinical development[86] as artemisinins are potentially effective drugs with enhanced pharmacokinetics and targeted anticancer properties. A series of tetraoxacyclohexanes have been shown to exhibit anticancer properties. A triol substituted compound has displayed prominent antitumor action *in vivo* toward melanoma and ovarian cancer in low concentrations.[88] Other have synthesized compounds with dual action (antimalarial/anticancer effect). These deoxycholic acid and cholic acid derived mixed tetraoxanes are cytotoxic at very low concentrations and particularly effective against melanoma cancer.[89]

Artemisinins and genetics in cancers

Recent research has been focusing on the determination of the mechanism of bioactivation and molecular events underlying the artemisinin effects. mRNA expression profiles associated with tumor cell response to artesunate, arteether, and artemether have shown that 208 out of 464 genes (45%) correlating significantly with IC_{50} values of at least one artemisinin derivative.[90] These genes were from different classes like drug resistance genes, DNA damage and repair genes, apoptosis-regulating genes, proliferation-associated genes, oncogenes, tumor suppressor genes, and also cytokines. Two different gene clusters were identified. One contained predominately genes which correlated significantly to all three artemisinin derivatives. This overlapping set of genes indicates common molecular mechanisms of tumor inhibition by all three drugs in which genes affecting cellular proliferation may play a central role. The second cluster contained genes differentially associated with the response of artemisinin derivatives to cancer cells. The number of correlating drug resistance genes in this cluster increased in the order Artesunate < Arteether < Artemether, paralleled by similarly increasing IC_{50} values of these three drugs. At present, the precise molecular events involved in initial triggering of ROS production in cancer cells remain elusive. Other aspects like ROS-independent mechanisms, direct DNA damage, and the role of p53 status in genotoxicity need to be further studied.

Since immunological markers are also involved in tumor formation by their ability to induce chronic inflammatory response, evasion of tumor recognition, and induce tolerance,[91] whether artemisinin may affect in these events also remains to be determined. Recently, 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.[92]

The potential advantage of antimalarials over conventional chemotherapy is that they cause less toxicity, have good synergism with other anti-cancer agents and radiotherapy, easy to administer, cheap and easily available. Their targeted anti-cancer activity is a great advantage, as demonstrated in retinoblastoma cells;[93] along with their lesser potential for developing drug resistance, and have a chemo/radio-sensitizing property.[17,82,86] The normal cells are generally unaffected.

Although human trials are unavailable, a safety and efficacy study with artesunate was conducted by Rutteman *et al*.[94] in 23 dogs with non-resectable tumors for 7–385 days at a dosage of 651–1178 (median 922) mg/m^2. No neurological or cardiac toxicity was observed. Seven dogs exhibited no adverse effects at all. Transient fever and hematological/gastrointestinal toxicity occurred in 16 dogs. One dog died from pneumonia. Plasma artesunate and DHA levels fell below the limit of detection within 8–12 h after artesunate administration while levels after 2 h were close to 1 μM. Artesunate produced a long-lasting complete remission in one case of cancer and short-term stabilization of another seven cases.

Future efforts should concentrate on improving the selectivity of artemisinins by selectively targeting cancer biomarkers or overexpressed cancer genes and proteins which are not detectable in normal tissues in order to improve the toxicity profile of present day anti-cancer therapy.

Conclusion

Widely used as antimalarials for long, this drug class has diverse biological properties including strong anticancer activity; but how the antitumor activity is exerted following artemisinin activation is still not well-understood but is associated with multiple mechanisms, including reactive oxygen species (ROS), oxidative DNA damage, sustained DNA double-strand breaks, and apoptosis. A better understanding of common mechanisms under similar conditions in different cell systems will greatly help developing targeted artemisinin derivatives. Their ability to kill cancer cells through multiple and heterogeneous molecular events is documented, although the exact molecular basis of artemisinin-induced cell damage is not fully known. Apart from NF-κB, survivin, NOXA, HIF-1α, and BMI-1, other molecules need investigation that may influence drug response, drug interactions, mechanisms of resistance, and associated effects in normal cells. Experimental evidences, mostly animal studies indicate that artemisinins and its derivatives may be a therapeutic alternative in the future, particularly in highly metastatic and aggressive cancers without developing drug resistance. Synthetic endoperoxides may act synergistically with other anticancer drugs without additional side effects. However, the benefits of artemisinins and DHA with specific and established chemotherapy in the clinical setting need to be further explored in different cancers by co-targeting multiple pathways to minimize shifting of cancer biomarkers and drug toxicity. Simultaneously, long-term therapy with artemisinins will require close monitoring. Artemisinin antagonistic reactions and resistance must be cautiously validated using different semisynthetic derivatives. DHA, artesunate, and artemether are the endoperoxides

currently licensed for therapeutic use. Overall, discovery of artemisinin compounds' antitumor effect has opened new vistas and the need for further large scale studies in this regard including candidate genes and cancer biomarkers.

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