

Research Article

Artemisinin: An Evolving Antimalarial-Part One

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Abstract: This review was conceived with the aim of presenting a compact, yet engaging account of the evolution of artemisinin from its humble and ancient origins as an herbal remedy to a modern chemotherapeutic agent, highlighting its unique pharmacological and toxicological profile and the central position it occupies at present in the battle against malaria. Artemisinin is a sesquiterpene lactone endoperoxide with a long and enchanting history. The Chinese had been using concoctions of *Artemisia* for the treatment of various febrile ailments for close to two millennia. The impetus for its extraction in 1972 from *Artemisia annua* came from the battlefields of the Vietnamese war of 1965 to 1973 and the political milieu of the Cultural Revolution that encouraged an inward-looking disposition. Owing to solubility problems with the parent compound, artemisinin other semi-synthetic derivatives are now available and include artesunate, artemether, dihydroartemisinin and arteether. Parasiticidal action resides in the endoperoxide moiety which is also primarily responsible for the toxicity of the artemisinin compounds. As a class, they are the most rapidly acting antimalarial chemotherapeutic agents ever in use, reducing initial parasite burden by a factor of 10^4 per cycle of schizogony. Despite this, high rate of recrudescence occur with monotherapies which necessitates their use in combination with longer acting agents- ACTs. The basis of this high recrudescence is not unrelated to short plasma half-lives, dormancy phenomenon and autoinduction of the metabolizing enzymes. Though safe in humans at recommended dosages, animal studies have continually revealed disturbing side effects most notably, neurotoxicity and, reproductive toxicity manifesting in the twin phenomenon of embryoletality and fetal dysmorphogenesis. In the light of the cautionary tale of thalidomide tragedy, it may not be wise to totally ignore these findings in experimental animals.

Keywords: Artemisinin, chemistry, clinical evolution, derivatives, pharmacology

INTRODUCTION

Malaria is an acute febrile illness caused by hemoprotzoal organisms of the genus *Plasmodium*. It affects predominantly the tropical and semi-tropical regions of the world and is transmitted through the bite of female anopheline mosquito in which the sexual half of its dual life-cycle occurs. The annual rate of clinical illnesses has been estimated at 5 billion, with 3 million associated deaths (Breman *et al.*, 2004). More than 90% of this burden has been borne by Sub-Saharan Africa (UNICEF, 2013) and has further contributed to the economic predicament of this region. The severity of the 'malaria burden' became all the more pronounced as the parasite developed resistance to almost all available antimalarials-chloroquine, sulfadoxine-pyrimethamine and mefloquine from the 1960s to 80s. It was therefore a welcome development that the Chinese research effort was bearing fruits in the isolation and purification of artemisinin, a potent and rapidly acting schizontocide from crude extracts of *Artemisia annua*. With this came revived optimism that

the world-wide efforts at malaria control can transform to eradication. This optimism has been rewarded as the last decade (2000-2010) has seen a drastic downturn in the prevalence and mortality of malaria. Much of this has resulted from the advent and 'judicious' use of artemisinin and its derivatives in the fight against malaria. The story of the discovery and evolution of artemisinin from an ancient Chinese herbal remedy to a modern chemotherapeutic agent of international repute has once again demonstrated the relevance of ancient wisdom to the well-being of the modern man. In this review, the immediate focus is to highlight the sterling qualities of artemisinin and its derivatives- both pharmacological and toxicological that justify the phenomenal rise of artemisinin compounds to the central position in the fight against malaria and, most importantly to caution against complacency in the face of reproductive toxicity in animals.

The past: The history of the discovery of artemisinin is steeped in great antiquity and demonstrates, perhaps more poignantly and succinctly than most other

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accounts of discoveries the saying that, 'war captures the best and the worst trait of mankind' (Bennett, 2011). It is doubtful that the intense effort of an entire segment of the Chinese Scientific community that ultimately resulted in the extraction and purification of Artemisinin would have arisen were it not for the events directly related to and arising from the battlefields of the Vietnamese war of 1965 to 1973: Aside from the casualties sustained in combats, malaria was taking its toll on both sides. The appearance of chloroquine resistant form of malaria further compounded the problem. For the Americans, just as occurred in battling bacterial infection when mass production of penicillin was discovered during World War II (Neushul, 1993), an intense search for chemotherapeutic agents for multidrug-resistant malaria ensued and bore fruit in the discovery of mefloquine (Kitchen *et al.*, 2006), a synthetic 4-quinoline methanol.

Without the kind of massive financial resources that the Americans poured into their antimalarial research program, the Vietnamese had no other alternative but to turn to their fellow Communist, China for help. This coincided with the 'Chinese cultural revolution' (Faurant, 2011) when individuality was discouraged and people were encouraged to look inward for solutions to personal and societal problems (Schaefer and Torre, Year). Communist Party Chairman, Mao Zedong and Prime Minister Zhou Enlai, responded to their Communist allies request by immediately ordering a nation-wide research program code-named project 523 (Miller and Su, 2011; Faurant, 2011) after the date it was inaugurated- 23rd May, 1967. Though the immediate objective was to aid their North Vietnamese allies in their war endeavors, the long-term goal was the discovery of new antimalarial drugs by screening synthetic chemicals and traditional Chinese recipe (Miller and Su, 2011) for antimalarial pharmacological activities.

The project was a collaborative effort that involved Pharmacologists, Pharmacognosists, Phytochemists, Clinicians and Traditional Chinese Medical Practitioners drawn from all over China (Willcox, 2009). According to Youyou Tu, winner of 2011 Lasker DeBakey Clinical Medical Research Award they investigated 'more than 2,000 Chinese herb preparations and identified 640 hits that had possible antimalarial activities. More than 380 extracts obtained from ~200 Chinese herbs were evaluated' (Tu, 2011). However, it was only the crude extract of *Artemisia annua* that yielded significant antiplasmodial activity. The road to the extraction of the active constituent, artemisinin was long and tortuous as it is neither soluble in water nor ether and is temperature-labile (Willcox, 2009). Because of these characteristics, the crude extracts did not yield reproducible results which led to a more intense search of ancient Chinese literature. Through persistence, sweat and toil she came across Ge hong's (284-363 AD) recommendation, 'A

handful of qinghao immersed with 2 L of water, wring out the juice and drink it all' which led her to reflect that, perhaps, the method of extraction they adopted had destroyed the active component (s). A low temperature ethyl ether extraction technique (White, 2008) yielded a product on 4th October, 1971 which neutral, non-toxic portion proved 100% effective against *P. berghei* and *P. cynomolgi* (Tu, 2011). Isolation and purification of the extract in 1972, yielded a colorless, crystalline substance with a molecular weight of 282 Dalton, a molecular formula of C₁₅H₂₂O₅ and a melting point of 156-157°C (Tu, 2011). This was artemisinin (or qinghaosu in Chinese).

Further back in the past: The use of *Artemisia* for medicinal purposes by the Chinese people antedated the extraction and purification of artemisinin in 1972 (Maude *et al.*, 2010). While it is not the intention of this review to undermine the standing of eminent Scientists like Professor Youyou Tu, who was the leader of the team directly linked to the isolation and purification of artemisinin, it must be remarked that, the isolation and purification of artemisinin cannot be separated from the sociocultural milieu spanning the two thousand years of use albeit, in its crude form Brown (2010). For as Hsu (2006) puts it 'Historians and Sociologists...have highlighted the false premises of a historiography that celebrates heroic personalities at the expense of recognizing the importance of the social dynamics that lead to the emergence and validation of new findings.' For artemisinin, this dynamics began more than 2000 years ago as far as documented evidence can show (Maude *et al.*, 2010) and culminated in the Vietnam War of 1965 to 1973. The first documentation of *Artemisia* as an herbal remedy goes back to 168 BC in the book, "Fifty-two Prescriptions" which was discovered during excavation, in Ma Wang Dui tomb in Changsha, Hunan (Hinrichs, 2001). In this book, discovered in 1973 among other herbal remedies, *Artemisia* was recommended as a remedy for hemorrhoids. Prior to this discovery, the Chinese had been relying on another compendium of ancient medical recipes, the *Shennong ben cao jing* (now lost and believed to have been composed in the first century AD) for their ailments (Willcox, 2009).

Celebrated as these ancient texts are, it is the "Handbook of Prescriptions for Emergency Treatment" that assumes the center stage as far as *Artemisia* is concerned, probably because of its role in the extraction of artemisinin. The Author, Ge Hong who lived from 283 to 343 AD during the Jin dynasty compiled it in four volumes and is reputed to be the first person in recorded history to have recommended the use of *Artemisia* for "intermittent fever" (Liao, 2009). In volume 3, prescription No. 2 reads as follows, "Take a bunch of qing hao and two sheng (i.e., 400 mL) of water for soaking it, wring it out to obtain the juice and ingest it in its entirety" (Awofeso, 2011). It was this

instruction that proved crucial in the extraction of artemisinin by Professor Youyou Tu's group. It was like a revelation that redirected their efforts towards techniques using lower temperature after toiling unsuccessfully with the more familiar high-temperature techniques (Tu, 2011).

The transformation to ACT: Having extracted and purified artemisinin, Professor Yuoyou team was faced with the problem of production and formulation which was compounded by outdated equipment. Their patience and persistence was finally rewarded when they obtained 'a capsule of pure artemisinin- that had satisfactory clinical efficacy' (Tu, 2011). With the assistance of Institute of Biophysics, Chinese Academy of Sciences the three dimensional structure of artemisinin was deciphered in 1975 and subsequently published in *Kexue Tongbao*, a Chinese journal in 1977 while the antimalarial properties was published in another Chinese journal, *Yaouxue Tongbao* in 1979 (Maude *et al.*, 2010). However, owing to the then prevailing political culture and the limited availability of the journals outside of China, these facts were not known in the West. Two British malariologists, Nick White and David Warrell, of Wellcome Trust malaria, Bangkok on accessing this information were able to liaise with Professor Li Guoqiao in Guangzhou in further replication of the studies on artemisinin (Awofeso, 2011). This collaboration and the presentation of papers on artemisinin during an International Scientific Conference held in Beijing, 1981 (sponsored by the United Nations Development Programme, the World Bank and the World Health Organization) helped in drawing World attention to this new antimalarial chemotherapeutic agent and its incredible biological potentials. Representatives of WHO requested further insights into their research methods and findings but, were temporarily refused by the Chinese Government.

It was at this point that Scientists at the U.S. Army's Division of Experimental Therapeutics, Walter Reed Army Institute of Research commenced 'artemisinin research' following the chance finding of Artemisia in Virginia and other places in the US. Success was achieved in 1984 and publication was promptly made of the achievement including technique used-petroleum ether extraction and chromatographic separation (Maude *et al.*, 2010). Fully aware of efforts in the West, the Chinese Government approached the WHO for help in the internalization of their 'product' (Dalrymple, 2010). Through collaborative efforts spanning the 1980s and 1990s and involving the WHO, USAID, Kunming Pharmaceutical Corporation in Yunnan Province, London School of Tropical Hygiene and Medicine, Wellcome Trust Funding, among others, artemisinin became fully internationalized with the adoption of Artemisinin Combination Therapy (ACT) as the standard and recommended treatment for uncomplicated malaria in 2001 (WHO, 2010b).

Ethnopharmacology: Artemisia belongs to the family of plants named Asteraceae but previously referred to as Compositae (Faurant, 2011). The origin of the name is uncertain but Bruce-Chwatt (1982) traces the nomenclature to 'Artemisia, wife and sister of Mausolus, King of Halicarnassus in the fourth century BC.' The death of the king was said to have been so bitter to her that she mixes everything she drank thereafter with his ashes in order to make it taste bitter. Another possibility is that it was named after Artemis (or Diana), the Grecian Goddess of the forests and hills (Willcox, 2009). Of the more than 400 species characterized so far (Willcox, 2009), only three-*A. annua*, *A. apiacea* and *A. lanceolata*-and probably more, have been positively known to contain artemisinin (Tan *et al.*, 1998). Non-glandular T-shaped trichomes and 10-celled biseriate glandular trichomes occurring in stems, leaves and flowers are the putative sites of artemisinin biosynthesis and sequestration (Ferreira and Janick, 1996). Although artemisinin can be synthesized *de novo* in the laboratory, this is not economically viable and so, the plant still remains the major source of artemisinin (Haynes, 2006).

Although Ge Hong was the first to associate Artemisia with "intermittent fever," it was Li Shizhen living from 1518 to 1593 who associated Artemisia with a clinical condition closest to the modern description of malaria (Brown, 2010) in his famous Classified Materia Medica (*Ben cao gang mu*), published posthumously in 1596 (Willcox, 2009). Li Shizhen, drawing from the work of a previous Chinese Sage, Shen Gua asserted that, of the two species of Artemisia native to China- *Artemisia annua* (*huang hua hao*) and *Artemisia apiacea* (*qinghao*)- it was the later that was the more effective antimalarial (Hsu, 2006). This contradicts the modern fact of phytochemistry that *Artemisia annua* is the species with higher content of artemisinin (Awofeso, 2011). It is possible to reconcile these two points of medical records belonging to two eras centuries apart, by considering the possibility that, ancient Chinese extraction techniques (now lost) may have yielded higher contents of antimalarial moieties from *Artemisia apiacea* (Hsu, 2006). Moreover, it is worth noting the fact that, though *A. apiacea* contains lesser quantity of artemisinin, its content of flavonoids (which are known to exert synergistic antimalarial effect with artemisinin) is comparable with that of *A. annua* (Ferreira *et al.*, 2010).

Artemisia as a group is rich in several bioactive substances beside artemisinin. These include terpenoids, flavonoids, coumarins, glycosides, sterols and polyacetylenes (Ferreira *et al.*, 2010). Some of these constituents especially, those from *Artemisia annua* are said to exert specific antimalarial activity in addition to anti-cancer, anti-inflammatory, anti-leishmanial, anti-schistosomial, anti-viral, anti-fungal and hepato-protective effects, among others (Tan *et al.*, 1998). In addition, among common medicinal plants,

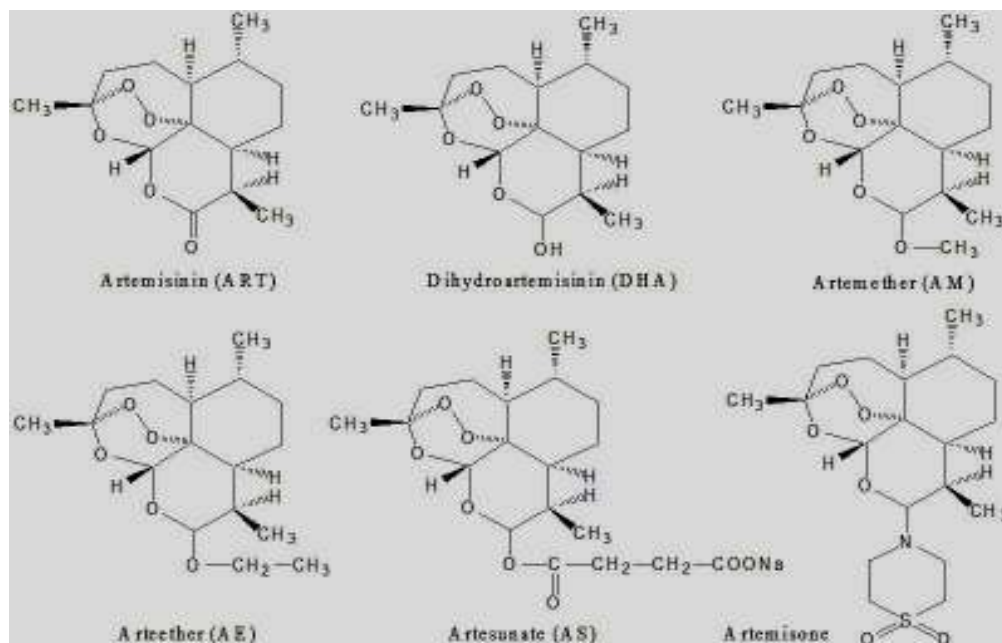


Fig. 1: Chemical structures of artemisinin and its derivatives (Available at www.google.com.ng/search?q=image+of+artemisinin&client=firefox-a&rls=org.mozilla:enUS:official&channel=np&tbm=isch&ei=G0KuUfFCuqm0AX75oCQDg&start=80&sa=N)

crude extracts of *A. annua* is said to have one of the highest anti-oxidant capacity as adjudged by its ORAC (oxygen radical absorbance capacity) value (Zheng and Wang, 2001) which is a property directly related to the total phenolic content of a plant (Ferreira *et al.*, 2010). Of the constituents with antimalarial activity, the flavonoids artemetin, casticin, chrysoplenetin, chrysoplenol-D, cirsilineol and eupatorin have received considerable attention (Brisibe *et al.*, 2009; Nijveldt *et al.*, 2001; Zheng and Wang, 2001; Brown, 1992); they have *in vitro* antiplasmodial activity (assessed by inhibition of the incorporation of hypoxanthine by *Plasmodium*) at concentration of 23 μ M and higher and are reputed to synergize the antiplasmodial activity of artemisinin when administered orally (Ferreira *et al.*, 2010). Another pointer to the *in vivo* antimalarial activities of these non-artemisinin constituents of *Artemisia* is the fact that, *A. abrotanum* which contains no artemisinin is used as an effective antimalarial in Turkey (Willcox, 2009). This is an indirect pointer to the fact that, these non-artemisinin constituents exert antimalarial effects *in vivo*. In addition, since the modulation of P-glycoprotein and the cytochrome P-450 drug metabolizing enzymes by certain herbal remedies have been attributed to their flavonoid contents (Zhou *et al.*, 2004), the possible positive affectation of the pharmacokinetic parameters of artemisinin by these natural flavonoids present in *Artemisia annua* may add to the antimalarial effects of the crude extract independent of artemisinin content (Weathers *et al.*, 2011). The antimalarial effect of these various constituents forms the basis for the agitation by

some that artemisinin tea or infusion be adopted in poor settings for the treatment of malaria (Weathers *et al.*, 2011; Willcox, 2009). However, the WHO (World Health Organization) (2012) in a position statement warns against herbal remedies with *Artemisia* based on the variability of constituents in different chemotypes of the plant and the underdosage that may occur with all the possible consequences on long-term sensitivity of plasmodial organisms to artemisinin and its derivatives.

Chemistry: Artemisinin, a sesquiterpene lactone with internal peroxide bridge is structurally different from all previous antimalarials in use. It has the chemical designation 3R, 5aS, 6R, 8aS, 9R, 12S, 12aR)-octahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4.3-j]-1, 2-benzodioxepin-10(3H) one (Chemical and Artemisinin, Retrieved from: <http://www.chemicaland21.com/lifescience/phar/ARTEMISININ.htm>). The chemical structures of artemisinin and some of its very commonly used derivatives are shown in Fig. 1.

Artemisinin is the parent compound of all artemisinin compounds and is poorly soluble in water and oil, though it is soluble in many aprotic solvents like chloroform, acetone and alcohols (Li and Zhou, 2010). This solubility problems makes the parenteral preparation difficult to compound. It has a crystalline appearance with a melting temperature of 156-157°C (2011). For an endoperoxide, it is remarkably stable as only temperatures of 190°C and above can lead to its decomposition (Li and Zhou, 2010). It is combustible and incompatible with strong oxidizing agents, acids,

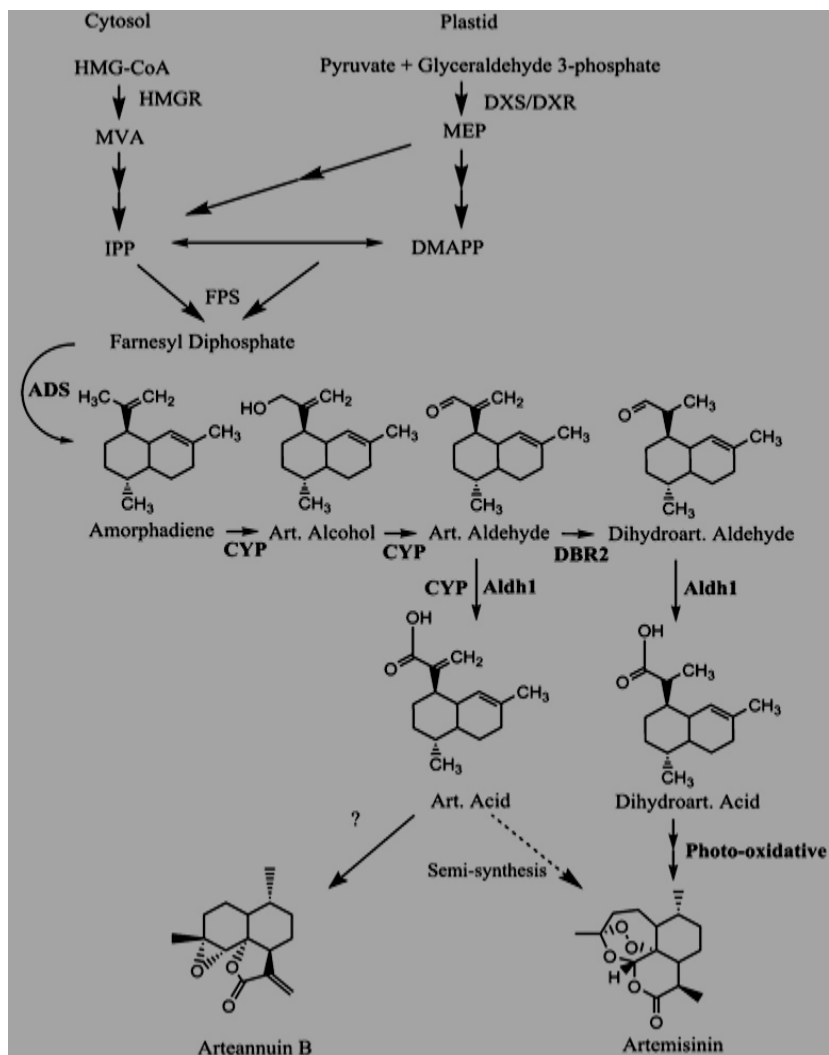


Fig. 2: Pathway of artemisinin biosynthesis in *A. annua*; *ADS* amorphadiene synthase; *Aldh1* aldehyde dehydrogenase 1; *CYP* CYP71AV1; *DBR2* double bond reductase 2; *DMAPP* dimethylallyl diphosphate; *DXS* 1-deoxyxylulose 5-phosphate synthase; *DXR* 1-deoxyxylulose 5-phosphate reductoisomerase; *HMGR* 3-hydroxy-3-methylglutaryl-CoA reductase; *IPP* isopentenyl diphosphate; *MEP* methyl erythritol phosphate; *MVA* mevalonic acid (Available from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3106422/figure/F1/>)

acid chlorides, acid anhydrides and, may absorb and react with carbon dioxide from the air (Meryer Chemical Company, 2010). Biological activity resides in the endoperoxide moiety while reduction reaction at the lactone group on C-10 aids in synthesis of its derivatives (Li and Zhou, 2010)- oil-soluble methyl (artemether) and ethyl (arteether) esters; dihydroartemisinin, a lactol reduction product with low water-solubility and artesunate, a water-soluble hemisuccinate salt of dihydroartemisinin. The pathway of artemisinin biosynthesis in *Artemisia annua* (which is concentrated in the trichomes in stems, leaves and flowers) involves the mevalonate (in cytosol) and dimethylallyl diphosphate (plastid) tributaries leading up to farnesyl diphosphate as the central focus. The reaction continues through amorphadiene, alcohols and aldehydes to the

final products, Arteannuin B and Artemisinin (Weathers *et al.*, 2011). This is depicted in Fig. 2.

Pharmacodynamics and antimalarial actions: The mode of action of the artemisinin compounds have been a point of much debate and controversy (Saunders *et al.*, 2012; Krishna *et al.*, 2008). What is incontestable is that, as a group, they are the most rapidly acting antimalarial drugs ever in use (White, 1999). The artemisinins inhibit plasmodial growth at nanogram concentrations. The IC₅₀ in one study was 1.2 ng/mL for dihydroartemisinin, 1.6 ng/mL for artesunate and 4.8 ng/mL for artemether compared to 0.4 ng/mL, 32 ng/mL, 149 ng/mL, 354 ng/mL, 27 ng/mL, 4.1 ng/mL for atovaquone, lumefantrine, chloroquine, quinine, mefloquine and halofantrine respectively (Brockman *et al.*, 2000). A reduction of initial parasitaemia by a

factor of 10^4 /cycle of schizogony has been reported (Woodrow *et al.*, 2005) which translates to a cure of a parasite burden of 10^{12} in 6 days of treatment. The mean fever clearance time for artemisinin in one of the early therapeutic trials was 32 h compared to 2-3 days for older agents (German and Aweeka, 2008). Alin *et al.* (1996) demonstrated time to Parasite Clearance (PCT) of 31 ± 3.6 h and 26.4 ± 3.6 h after oral artemisinin and artesunate, respectively.

They are active against all stages of the erythrocytic parasite (Cui and Su, 2009) though less so for early ring forms and schizonts than for late ring forms and trophozoites (Meshnick *et al.*, 1996). In addition, among all antimalarials they are reputed to have the highest effect on the ring stages of the parasite (Saralamba *et al.*, 2011). Just like quinine, mefloquine, primaquine and chloroquine (Laurence *et al.*, 1997), they are gametocytocidal (Woodrow *et al.*, 2005) which translates to lower transmission rate in low transmission areas, though such benefit has not been demonstrated for high transmission areas (Krishna *et al.*, 2004). Additionally, their gametocytocidal effect is restricted to the early form (stages 1-4) (White *et al.*, 2012; Chotivanich *et al.*, 2006) in contrast with primaquine action which is specific to older gametocytes (WHO, 2011a). However, hepatic stages are not affected and this rules out their use for radical cure or causal prophylaxis (Woodrow *et al.*, 2005). Like previous antimalarials in use, they are not active against merozoites (Wilson *et al.*, 2013). The spectrum of their biological actions extends to the twin pathophysiological mechanisms of microvascular ischemia in malaria infection- cytoadherence and rosetting. These two phenomena are said to be markedly addressed by the artemisinins than by older antimalarials like quinine and chloroquine (Udomsangpetch *et al.*, 1996). Despite these laudable properties, the artemisinins as a class suffer from the singular feature of high rate of recrudescence when used as monotherapies (Alin *et al.*, 1996; Ittarat *et al.*, 2003; Codd *et al.*, 2011; Miller *et al.*, 2013). Recrudescence is the reappearance of asexual erythrocytic stages of malaria parasite traceable as the original infection for which a correct therapeutic course of drug was administered (Rouse *et al.*, 2008). In one study the rate of recrudescence was as high as 50% of those receiving 5 days or less of Artemisinin for *P. falciparum* or *P. vivax* infection (Nguyen *et al.*, 1993). However, recrudescence fell to 10-23% (when therapy was extended to 5-10 days) and 9.5% (when additional agent was added). Another study reported a recrudescence rate of 48, 10, 2% for 3-, 5- and 7-day regimens respectively (Li *et al.* (1994) as cited by WHO, 2010b). The basis of this high rate of recrudescence with an otherwise excellent class of antimalarials has not been completely elucidated. An early line of thinking was that for a group of drugs with very short residence time in the blood, daily dosing

schedule was inadequate to maintain the minimum parasiticidal concentration long enough to eliminate all the blood parasites (Mordi *et al.*, 1997; White, 1997) with the consequential selection of resistant strains or, the establishment of parasite residuum that propagates the blood stages. According to Woodrow *et al.* (2005), for a group of drugs that reduces the initial parasite biomass by a factor of 10^4 /cycle of schizogony, (total biomass even in severe infection is $\sim 10^8 - 10^{12}$) and can theoretically eliminate all infection with monotherapy of 7 days (White, 1997), recrudescence should not exist after this duration of therapy; in fact, contrary to this, recrudescence beyond this duration of treatment has been reported (Nguyen *et al.*, 1993; Giao *et al.*, 2001) and even as late as 20 days from start of treatment (Teuscher *et al.*, 2010). This explanation is therefore insufficient to account for the high rate of recrudescence observed with these drugs. Additional facts worth noting are that, though the degree of recrudescence is positively correlated with baseline parasitaemia (Ittarat *et al.*, 2003), decreases with prolongation of treatment duration (Nguyen *et al.*, 1993) and escalating dosages (Nosten, 2010) sensitivity of the original clone to the artemisinins is still maintained by the recrudescence progeny (De Vries and Dien, 1996) - facts, marking out recrudescence as different from the usual pattern of resistance (Ittarat *et al.*, 2003).

An alternative explanation backed by experimental findings is the dormancy theory. Nosten (2010), citing Kyle and Webster (1996) described it as a state 'in which some of the parasites exposed at ring stage in vitro become metabolically inactive and resumed growth after removal of the drug.' This phenomenon is not unique to plasmodial species as it has been noticed in bacteria (Dworkin and Shah, 2010) and other microbes (Jones and Lennon, 2010) and with non-artemisinin agents-atovaquone/proguanil (Thapar *et al.*, 2005), pyrimethamine/mefloquine (Nakazawa *et al.*, 1995). With regards to plasmodial dormancy on exposure to the artemisinins, one of the earliest descriptions of this phenomenon was by Kyle and Webster (1996) as cited by Nosten (2010). It was confirmed by Teuscher and colleagues (2010), who, using an in vitro technique that synchronized the growth from the ring stage of five laboratory strains of *P. falciparum*, were able to monitor metabolic quiescence on exposure to dihydroartemisinin and subsequent recovery from this state. In another experiment, although the terms 'dormancy' or 'quiescence' were not used specifically, Nakazawa *et al.* (2002) demonstrated the same phenomenon in vitro. Others like LaCrue *et al.* (2011) using *P. vinckei* have documented results suggesting the existence of this phenomenon in vivo. To further underline the relevance of this phenomenon to recrudescence and, possibly artemisinin-tolerance, Witkowski *et al.* (2010) documented that, parasite undergoing dormancy

showed aberrant transcription involving 'overexpression of heat shock and erythrocyte surface proteins and the downexpression of a cell cycle regulator and a DNA biosynthesis protein.' Furthermore, clinical isolates of resistant *P. falciparum* from Western Cambodia were observed to exhibit evidence of reduced metabolic activity in the first half of erythrocytic cycle (corresponding to early ring stages). This was reversed at the schizonts stage (Mok *et al.*, 2011). It is not far-fetched to surmise that this phenomenon may act, not in isolation but in concert with short plasma half-life and autoinduction (as may occur in a prolonged course of therapy that may be needed in clinical situations like cerebral malaria) of artemisinin metabolism to bring about recrudescence.

As noted above, the mechanism of action is not fully understood and is an area of intense and continuing research. The widely-held view is that ferrous Iron (Fe^{2+}) in the food vacuole facilitates a reductive cleavage of the endoperoxide bridge into Oxygen-centered free radicals: these are then converted into Carbon-centered radicals by 'intramolecular hydrogen abstraction from CH₂ groups on the periphery of artemisinin by the Oxygen-centered radicals' (Woodrow *et al.*, 2005). It is these oxygen-centered radicals that damage vital plasmodial macromolecules leading to its death. If this explanation really obtains in nature, the question arises as to why this group of compounds should exhibit such a high level of target specificity, while free radicals (hydrogen peroxide, peroxyxynitrite, hydroxyl radical, singleton oxygen) are known to be rather non-specific in target destruction. An alternative branch-off of this theoretical proposition is that, it is the carbon-centered alkyl radicals that actually damages parasite vital molecules. The source of Iron needed for activation is contentious but, Klonis *et al.* (2011) insist on haemoglobin as the source as they have successfully arrested haemoglobin digestion with the attendant abrogation of artemisinin action. An alternative mode of action was provided by Eckstein-Ludwig *et al.* (2003). In an elegant experiment that expressed PfATP6 (*P. falciparum* orthologue of SERCA- endoplasmic-sarcoplasmic reticulum Calcium ATPase) in *Xenopus* oocytes, they provided evidence that inhibition of this pump by activated artemisinin is their mode of action. Additionally, their data was in conformity with the generally held view that Fe^{2+} is a requisite factor in the activation of artemisinins, without which parasite inhibition failed to occur. Furthermore, imaging studies by same group dispels the notion that activation of artemisinin is an intravacuolar event as label (BODIPY-thapsigargin) concentrated in the cytosol of the parasite and not in the food vacuole. A third mechanism of action has been proposed similar to the action of chloroquine; it has been proposed that activated artemisinin compounds act by forming adducts with haeme and rendering haeme in various alkylated states- 'haemarts'. These adducts bind to

Plasmodium falciparum histidine-rich protein II (PfHRP II) inhibiting haemozoin formation, a process that leads to accumulation of haeme that finally kills the parasite. Not only so, the 'haemarts' create a milieu that further impede the formation of haemozoin from haeme- a vicious cycle (Kannan *et al.*, 2005).

Pharmacokinetics: The route of administration of the artemisinin derivatives differ depending on the physical characteristics of the derivative and the nature of the formulation. Artemisinin itself is available as oral and rectal preparations; artesunate, as the most versatile is available as oral, rectal, intramuscular and intravenous preparations; artemether is marketed as oral and intramuscular preparation while arteether is administered intramuscularly (Krishna *et al.*, 2004; Woodrow *et al.*, 2005). Dihydroartemisinin (DHA), an antimalarial by its own right is the chief metabolite of the artemisinin compounds through which their biological actions are accomplished (Woodrow *et al.*, 2005). The only exception to this proxy Pharmacodynamics is artemisinin itself which is not metabolized into DHA and exerts its antimalarial effect all its own (Zhang *et al.*, 2001; Woodrow *et al.*, 2005). The *in vivo* conversion rate to dihydroartemisinin is said to be highest for artesunate, followed by arteether, artemether and artelinate (Li *et al.*, 1998). They are regarded as intermediate-extraction (De Vries *et al.*, 1997) compounds after oral administration and do contribute to their own metabolism after repeated dosing (Asimus and Gordi, 2007; Ashton *et al.*, 1998) due to autoinduction of CYP2B6 with minor contribution from CYP3A4 in individuals with low expression of CYP2B6 (Svensson and Ashton, 1999). Their oral bioavailability ranges from 19 to 35% in rats (Navaratnam *et al.*, 2000). Food does not seem to significantly affect their pharmacokinetic parameters (Dien *et al.*, 1997) when orally administered but may increase time to maximum plasma concentration (Tracy and Webster, 2004). Protein-binding for the artemisinins is said to range from 43 to 81.5% (Medhi *et al.*, 2009) with that of artemether being 77% (Li *et al.*, as cited by Batty *et al.* (2004). Colussi *et al.* (1999) estimated that 33% of bound artemether was to α_1 -acid glycoprotein, 17% to albumin, 12% to High Density Lipoproteins (HDL), 9.3% to Low Density Lipoproteins (LDL) and 12% to very low density lipoproteins (VLDL). Artemisinin itself is metabolized into >24 products-hydroxylated, deoxyhydroxylated, dihydroxylated metabolite and deoxyartemisinin (Liu *et al.*, 2011a) -all more polar entities and are excreted via bile and urine. The fate of the artemisinin derivatives may follow same pattern as artemether metabolism is not markedly different from the parent compound (Liu *et al.*, 2011b).

The pharmacokinetic parameters of artemether vary depending on the routes of administration (Tracy and Webster, 2004). In one estimate, the oral

bioavailability of artemether (using bioassay that measures serum concentration of dihydroartemisinin) was said to be higher than that obtained via the intramuscular route; serum concentrations during acute phase of illness were also found to be higher than during convalescence (Silamut *et al.*, 2003). Absorption through the intramuscular and per rectal routes is notably erratic (Teja-Isavadharm *et al.*, 1996) and incomplete (Karbwang *et al.*, 1997). After oral dosing, artemether undergoes extensive first-pass metabolism to its active metabolite, dihydroartemisinin (Silamut *et al.*, 2003). In a group of healthy Pakistani male subjects, the maximum plasma concentration, time to maximum plasma concentration, terminal elimination half-life, volume of distribution and clearance after oral dosing were estimated at 184 ± 100 ng/mL, 1.56 ± 0.68 h, 2.00 ± 0.71 h and 666 ± 220 L and 257 ± 140 L/h, respectively (Ali *et al.*, 2010). This volume of distribution of 666 ± 220 L argues for extensive tissue distribution and, animal studies by Jiang *et al.* (1989) utilizing intravenous fat emulsion of artemether, revealed extensive tissue partitioning with concentrations highest in the brain. Lower concentrations were obtained in the heart, lungs, skeletal muscles and the kidneys. In another animal study (utilizing radioactively-labeled DHA) brain concentration of DHA was two-fold higher than plasma concentration with the highest tissue concentration found in intestines and spleen (Xie *et al.*, 2009). In the same study, it was also reported that brain concentration of artemether decayed at a slower rate than serum concentration. The last point is doubly significant in view of the singular characteristics of neurotoxicity shared by all the artemisinin compounds. Similar pattern of concentration decay was observed with artesunate by another group (Zhao and Song, 1989). In a study involving patients with cerebral malaria, Davis *et al.* (2002) demonstrated a more prolonged decay of DHA concentration (after artesunate administration) in the cerebrospinal fluid than the plasma, indicating perhaps, CNS retention of DHA following multiple dosing regimens. This report, if replicated should establish the selective accumulation of artemisinin and derivatives in the brain as a predisposing mechanism of neurotoxicity.

Among the artemisinins, artesunate is the most widely used perhaps, because of its solubility in water and availability for oral, intramuscular, intravenous and intra-rectal administration. Its conversion to dihydroartemisinin is fastest among this class of drugs and is said to occur with a $t_{1/2}$ of 2-3 min (Batty *et al.*, 1998) producing antimalarial effect approximately 4-5 times that of artemisinin (De Vries and Dien, 1996). Its biological effect is therefore largely attributed to dihydroartemisinin generated in vivo (Woodrow *et al.*, 2005).

Dihydroartemisinin (DHA) exists in solution (and possibly the plasma) as two anomers- α -DHA and β -DHA [artenimol] (Xiaonan, 2010) with equilibrium reached after 18 h of reconstitution at 4°C. The β -

anomer is the immediate product of the artemisinins transformation in serum (Navaratnam *et al.*, 2000). In a study to define the pharmacokinetic and pharmacodynamic parameters of artesunate and its active metabolite, DHA among severe *P. falciparum* malaria patients in Uganda, Byakika-Kibwika *et al.* (2012) estimated the median maximum plasma concentration of DHA after intravenous bolus administration of 2.4 mg/kg artesunate to be 3140 (1670-9530) ng/mL. This was achieved after (T_{max}) a lapse of 0.14 (0.6-6.07) hours and disappeared from the plasma with a $T_{1/2}$ of 1.31 (0.8-2.8) hours. In a similar design among a group of Thai patients with severe malaria, Newton *et al.* (2006) estimated Median (range) DHA C_{max} to be 2128 (513-5789) nmol/L with elimination half-life of 0.34 (0.14-0.87) hrs and extreme inter-patient variability. This variability may explain the difference in estimates between the Thai and Ugandan groups in addition to racial factor since both groups used the same analytical methods- hplc/spectroscopy.

Toxicology: As antimalarial chemotherapeutic agents, artemisinin and its derivatives are remarkably safe and well tolerated in humans. Though some tissue lesions together with their functional correlates still occur in animal studies using doses of artemisinin and derivatives that mimic clinical usage without same being detected in humans (Gordi and Lepist, 2004) caution is advised as large-scale clinical data needed to detect rare events are still lacking (Efferth and Kaina, 2010). At supraclinical doses, toxicological and toxicokinetic studies in animals have established pathological lesions in almost all the tissues of the body traditionally examined. These studies include single-dose acute toxicity and multiple-dose subacute toxicity studies. Though a lot of inter-species variability exist in terms of dosage threshold for a particular lesion, types and extent of lesion, maximum tolerated doses and reversibility of signs of toxicity on withdrawal of agents, the one common characteristic in the great majority of studies is the excessively high doses needed to induce such lesion; for example, 320 mg/kg of artesunate was administered before observation of cardiovascular effects (bradycardia, hypotension and ECG changes) in one dog and there were no CNS effects at doses up to 200 mg/kg (WHO, 2011b). The same source quoted a single-dose LD_{50} from an earlier study for oral and intramuscular artemisinin in mice as 4228 mg/kg and 3840 mg/kg, respectively.

Neurotoxicity has been one of the most examined issues in animal studies involving the artemisinin compounds. In fact, on the basis of it interest was temporarily halted in the development and marketing of artemotil (β -arteether) by Scientists at the Walter Reed Army Institute of Research (Maude *et al.*, 2010): Arteether (and artemether) was demonstrated to cause fatal neurological complications with progressive cardiopulmonary involvement in rats and dogs as a

result of pathological lesions in the pons and madulla (Brewer *et al.*, 1994). The demonstration of lack of such effects in humans at clinical doses (Nagelschmitz *et al.*, 2008; Gordi and Lepist, 2004; Hien *et al.*, 2003; Kissinger *et al.*, 2000; Van Vugt *et al.*, 2000) helped revive the research and development of these two fat soluble esters of artemisinin to the extent that, artemether features prominently in artemisinin-based combinational therapy at present. Reports of adverse effects in humans exist. In as much as it can be considered academic pomposity to disregard clinical reports that seem to establish causal relationship between certain neurological manifestations in human (Panossian *et al.*, 2005; Franco-Paredes *et al.*, 2005; Elias *et al.*, 1999) and artemisinin compounds it should be noted that, these reports are in the minority and some Writers (Li and Hickman, 2012) question the basis of the Authors' conclusions.

Comparison of the stereo electronic properties of individual artemisinin compound with their in vitro neurotoxicity has revealed a positive correlation between intrinsic lipophilicity and neurotoxicity (Bhattacharjee and Karle, 1999). The fact that artesunate, a water-soluble derivative is least associated with neurotoxicity among the artemisinin compounds (Nontprasert *et al.*, 2000) is in support of the immediately foregoing. Still in support of lipophilicity being essential for neurotoxicity, Sanjana *et al.* (2012) demonstrated that, in consonance with its lipophobicity profile artesunate effectively suppressed convulsive episodes in the maximal electro-shock seizure model of epilepsy in albino mice. In another angle to the neurotoxicity potentials of artemisinin compounds, Li and Hickman (2012) examined the role of:

- The rate of and extent of conversion of individual artemisinin compounds to dihydroartemisinin-the active metabolite
- The brain/CSF concentration of each compound at onset of neurotoxicity
- Total drug exposure- AUC/C_{max}
- Plasma half-life, in the aetiopathogenesis of neurotoxicity of the artemisinin compounds in animals. Based on data pooled from numerous studies they argued that the total drug exposure time (and to some extent, the plasma half-life which can increase exposure time under certain conditions) was the single most important factor and that the vehicle of formulation- sesame oil or peanut oil- confers a depot effect on intramuscular artemether and arteether, thereby increasing their total exposure time and in consequence, their neurotoxic potential. This conclusion has been supported by findings that neurotoxicity and lethality of artesunate and artemether significantly decreases with once daily oral administration (as opposed to intramuscular injection of a sesame oil formulation with its depot effect) (Nontprasert *et al.*, 2000). Further support comes from a report

of reduction in incidence and severity of neuronal lesion when cremophore was used as formulation vehicle(reducing the depot effect and consequently, total drug exposure time) for artemotil (β -arteether) as opposed to sesame oil (Li *et al.*, 2002).

The importance of reproductive and developmental toxicity is reflected in the volume of published work and is of paramount importance if adverse fetal effect is to be avoided in clinical situations. Though it is not always possible to perfectly extrapolate results of studies in animals to humans, it costs nothing to err on the side of caution bearing in mind the sad tale of thalidomide (Kim and Scialli, 2011). What is clear from this cautionary tale is that cognizance must be taken of the tiniest thread of evidence of embryopathy in preclinical toxicity studies. Though animal testing was not as rigorously pursued in the 1950s and 60s as it is today, there was enough evidence that could have forestalled the disaster had we not ignored the evidence that did not fit our theories, mindset and beliefs- that the placenta 'is impervious to toxic substances except in such large doses that they killed the mother.' (Dally, 1998).

Evidence abounds in Medical Literature alluding to the toxicity of artemisinin compounds to animal fetuses of several species. The earliest studies were undertaken by the Chinese- individuals or corporations-and their findings have been summarized by the WHO (2011b); all the artemisinin derivatives exhibited embryoletality with unusually steep dose-response curves without necessarily affecting the mother and, at dose similar to clinical dosages. These effects were noted in early pregnancy with occasional pre-implantation loss and fetal dysmorphogenesis involving the cardiovascular system, axial skeleton and the limbs. Late fetal absorption was also noted. Fertility was not affected even with some reduction in sperm count. More recent studies have produced results similar to these (Clark *et al.*, 2011, 2010, 2008; Li *et al.*, 2008; White and Clark, 2008). However, the mechanism of embryoletality in animals and the reasons for the absence of fetal toxicity in humans have been elusive until recently; Clark *et al.* (2011) demonstrated severe reticulocytopenia in pregnant rats on 21 days post-conception after administration of oral doses of artesunate and artelinate on day 12 post-conception. This was attributed to the destruction of fetal erythroblast which induced severe anemia and consequently, fetal death.

This has been supported by a more recent study showing that artemisinin compounds with the endoperoxide bridge arrest pro-erythroblast and basophil erythroblast differentiation towards erythroid lineage while substitution at the C-10 position prevents further proliferation of differentiated erythroid cells (Finaurini *et al.*, 2012). Furthermore, DHA was shown to downregulate glycophorin A (GpA) receptors expression and to cause the under-expression of globin

gene in erythroid cell lines in addition to inducing apoptosis in erythroblasts-one of the established mechanisms of its anti-cancer effect. The ability of the artemisinin compounds to inhibit angiogenesis and vasculogenesis has also been noted in some cell lines (Crespo-Ortiz and Wei, 2012). White and Clark (2008) demonstrated that the most sensitive period for embryoletality of artemisinin compounds in rats is between 10 and 14 days post-conception which also coincide with the beginning of cardiac activity (Li and Weina, 2010). This period is the fixed duration for the production of fetal erythroblasts. Outside of this period, very little or no toxic effect is noticed. This is a correlation linking artemisinin embryotoxicity to its effect on fetal erythroblasts. The immediately foregoing also serves to explain why embryotoxic effect of the artemisinin compounds has not been observed in humans. In humans, fetal erythroblast formation occurs over a wider period of time- day 16 to day 60 post-conception (Li and Weina, 2010). For drugs that are given over 3 days, the emphatic fetohematologic effects observed in rats are very unlikely since replenishment of destroyed erythroblasts would likely obviate such effect. That aside, toxicokinetic study in pregnant rat has revealed selective partitioning of the active metabolite, dihydroartemisinin in fetal tissues with fetal blood and hepatic concentrations of DHA 3.8 to 8.8 times higher than maternal blood concentration at all time of measurement (Clark *et al.*, 2010). In the fetuses, most of this was concentrated in the marrow and liver. The rate of conversion to DHA in pregnancy is also said to be enhanced with values 2.2 and 4.5 times that of a non-pregnant rat on days 1 and 3, respectively of artesunate administration (Li and Weina, 2010). In summary therefore, cell-cycle arrest of fetal pre-erythroblasts, antiproliferative effect on differentiated fetal erythroid cells, inhibition of angiogenesis and vasculogenesis and, apoptosis induction in fetal erythroid cells aided by the selective partitioning of the artemisinin compounds into fetal marrow and liver, at a developmentally critical period of gestation are all factors contributing to the embryotoxicity of artemisinin and its derivatives, at least in animals.

Apart from periphery and central neuronal lesions, pathological lesions have been established in other tissues with administration of the artemisinin compounds. Izunya *et al.* (2010) reported 'mild sloughing of germ cells from the basement membrane and varying degrees of reduced population of germcells' after the administration of oral doses of artesunate (2-8 mg/kg/day) to four groups of male Wistar rats. A more extensive reproductive toxicological study in male rats with 2.9 mg/kg of oral artesunate (Olumide and Raji, 2011) over 6 weeks revealed no adverse effects on spermatozoa characteristics, serum concentrations of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) testosterone or, testicular and epididymal

histology in the short term (5 days). However, when the therapy was extended to 6 weeks, there were adverse effects that disappeared with time on all indices. In a study designed to assess the effect of oral artemether (25-75 mg/kg/day over 3 days) on hematological profile of male Wistar rats, Osonuga *et al.* (2012) demonstrated dose-dependent but reversible reduction in mean hemoglobin concentration and total white blood cells count.

This review has highlighted the wisdom that, history is not for our forbears long gone but for us in the present. For as Winston Churchill once said 'the farther backward we look, the farther forward we can see' (Frater, 2007), it is not intellectually tasking to surmise that, were it not for Ge Hong's instructive documentation, the search for the active principle (s) in *Artemisia annua* may have continued for a longer period of time and may be, lost to ensuing frustrations or be overtaken altogether by unforeseen intervening circumstances. Furthermore, animal toxicity studies had hinted at possible embryotoxic effect of thalidomide yet, partly on the assumption that the placenta is impervious to toxic substances except in such large doses that they killed the mother (Dally, 1998), these warnings were discountenanced. In as much as the unique spectrum of artemisinin pharmacological profile must be harnessed for humanity, the warnings from the past must have a continually engaging presence in our collective psyche.

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