PART-II

Pharmacokinetics of an Antimalarial
Trioxane 99-411
Chapter-1

Review of Literature
1.1 Malaria

Half of the world's population is at risk of malaria, and an estimated 243 million cases led to nearly 863,000 deaths in 2008, of which 89% were in the African Region, followed by the Eastern Mediterranean (6%) and the South-East Asia Regions (5%). Malaria is especially a serious problem in Africa, where one in every five (20%) childhood deaths is due to the effects of the disease [1-2]. South East Asia and South America are of interest because of the early development of drug resistance in malaria parasites in these regions. India’s official figures show that every year, malaria infects 1.5-2 million peoples, killing 500 to 800 of them. Over the time, human immune system adjusts to combating the malaria parasite and the adult mortality in endemic areas is fairly low. The higher rate of mortality is concentrated on children below five years of age, travelers, migrants from non-malaria regions to endemic areas and in population with compromised immunity, including pregnant women and patients with HIV.

The world malaria report 2009 summarizes information received from 108 malaria endemic countries and other sources and updates the analysis presented in the report 2008. It highlights progress made in meeting the World Health Assembly (WHA) targets for malaria to be achieved by 2010 and 2015 and new goals on malaria elimination contained in the Global Malaria Action Plan (2008).

To accelerate the progress in malaria control, the 2005 World Health Assembly advanced the Roll Back Malaria (RBM) targets defined in 2000 by African Heads of State, set a coverage target of 80% or more for four key interventions: insecticide-treated nets for people at risk, appropriate antimalarial drugs for patients with probable or confirmed malaria, indoor residual spraying for households at risk and intermittent preventive treatment in pregnancy (in high-transmission areas) [3]. The Health Assembly specified that, as a result of these interventions, the numbers of malaria cases and deaths per capita should be reduced by 75% or more between 2000 and 2015. These goals were affirmed in the Global Malaria Action Plan [4].

In 1998, malariologists celebrated the centenary of the demonstration that the female mosquito transmits malaria. It was reported in early 1897 itself by Ronald Ross that the
The malarial parasite could infect the female mosquito and was shown in the succeeding years that the parasite completes its developmental cycle in mosquito and, when that mosquito feeds on a blood meal, it can pass on the parasite [5, 6]. This discovery allowed a line of attack on the disease other than using quinine, the only known antimalarial then. However, even with the huge strides in biology and medicine during the last 100 years, the malaria still exists as a serious health problem affecting the life of millions of people worldwide [7].

The human malaria is caused by four different species of malaria parasites, belonging to the genus *Plasmodium*. These are: *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. *Plasmodium falciparum* is by far the most prevalent species and causes most problems as a result of its virulence and drug resistance. It has a rapid rate of asexual reproduction in the host and an ability to sequester to small blood vessels, with a high risk for development of cerebral malaria.

### 1.2 Drug Resistance: Challenge in Malaria Control

Drug resistance has been a serious obstacle to malaria control. Worldwide Antimalarial Resistance Network (WARN) database is to improve the treatment of malaria, by informing drug selection and use and providing a prompt warning of when treatment policies need changing [8]. This database should include data on the clinical pharmacology of antimalarial drugs in addition to their therapeutic efficacy as well as the molecular and *in-vitro* markers of resistance. The clinical pharmacology component of the database is essential to ensure optimal dosing with currently available and newly introduced antimalarial drugs by defining the correlation between drug concentration and clinical and parasitological response. This requires three key questions to be answered: firstly, what is the profile of "therapeutic" drug concentrations that needs to be sustained to eradicate the plasmodial biomass? Secondly, are these drug concentrations over time being achieved in the majority of all target groups, including infants, pregnant women and those with prevalent co-morbid diseases (especially HIV/AIDS, malnutrition) or are there important sub-groups being under (or over) dosed? For example inadequate drug levels have been repeatedly found in pregnant women and young children [9-10].

The widespread and in some cases rapid development of drug resistance requires that
measures to be taken to prevent such being the fate of the remaining effective compounds and any new compounds which might be produced in the future. A number of factors have been identified which play a role in the emergence of drug resistance. The pharmacodynamic and pharmacokinetic properties of the antimalarials are very important. Drugs which have longer elimination half life ($t_{1/2}$) are potentially more likely to be selected for drug resistance than those which are rapidly eliminated [11]. In the former situation, some parasites are likely to be exposed to the drug at sub-therapeutic or sub-optimal levels for extended periods of time. These sub-optimal levels are sufficient to inhibit, but not to kill the parasite, thereby providing conditions which may promote selection of resistant parasites. Ironically, drugs with longer half lives were seen as desirable because they could be used prophylactically and taken on a weekly basis rather than daily and when used for treatment could be given as a single curative dose. Most of the commonly used drugs like chloroquine, mefloquine, pyrimethamine and sulfur compounds fall into this category. Drugs that are rapidly eliminated will exert minimal selection pressures, as they at remain sub-therapeutic level for shorter periods of time in the body [11].

Another factor that determines the rate at which resistance may develop is the nature of the mechanism of resistance. Resistance may develop suddenly, where it is conferred by a single mutation, as in the case of anti-folates [12]. Further, frequency of infection, i.e. the intensity of transmission can influence the rate at which resistance emerges. Thus, if a patient is re-infected, after recently being successfully treated, but when sub-therapeutic levels of the drug remain in blood, conditions prevail which can exert selective pressure on the re-infection of parasites. Mutations resulting in resistance are at low frequency and therefore the chance that a resistant parasite will emerge from the re-infection will generally be higher where the parasite load is higher [13]. Thus it is possible to extend the life-span of existing antimalarial drugs by its careful and judicial use. In the choice of drug combinations, the drugs should have similar pharmacokinetics and pharmacodynamics and not interact in any adverse way such that additional problems of toxicity might occur. It is preferable that known drug resistance mechanisms in each of the drugs being combined are not linked.
1.3 Emerging Antimalarial Drugs

In general, malaria is a curable disease and if everyone has access to early treatment, nobody should die of it [14-16]. For the past 50 years, there have been two main classes of antimalarial agents in use, the anti-folates and the cinchona alkaloids or the quinoline containing drugs. In most cases, these drugs are targeted at the asexual erythrocytic stage of the parasite. The anti-folates include the diaminopyramidines, such as pyrimethamine and trimethoprim; biguanides represented by the proguanil or cycloguanil and chloroproguanil; and the sulfonamides, including sulfonamides and the sulfones. It was discovered that the latter two types of drugs have synergistic activity with pyrimethamine or proguanil, the most frequently used combination being pyrimethamine-sulfadoxine and pyrimethamine-dapsone [16].

The quinoline containing drugs include the cinchona alkaloids, quinine and quinidine, and the amino-alcohol quinine analogues mefloquine [17] and halofantrine. There are also 8-amino quinoline, primaquine and the 4-amino quinolines and its relative amodiaquine which are used for their gametocidal effect and their action on the liver stages of Plasmodium. In addition to these, newer therapies in development include antibiotics such as tetracycline, doxycycline and azithromycin; atovaquine-a hydroxyl-naphthoquinone [18], pyronaridine-a derivative of mepacrine and derivatives and analogues of the Chinese traditional herbal drug artemisinin [19].

Artemisinin, or qinghaosu (Chinese for ‘from green herb’), was isolated by Chinese researchers in 1972 from Artemisia annua L. (sweet or annual wormwood) and its structure was elucidated in 1979. The plant, a perennial herb of the family of composite flowers, has been used in traditional Chinese medicine as a remedy for chills and fevers for more than 2000 years. Although originally from northern parts of China, the plant now grows wild in many countries [20]. It is easily grown under a wide variety of conditions but the artemisinin yields can vary considerably, depending on plant material and growth conditions. Artemisinin is present in the leaves and the flowers of the plant in 0.01-0.8% dry weight [21]. Artemisinin and its derivatives (Artemether, Arteether, Artesunate) are another promising antimalarials used increasingly in Asia and Africa where multidrug resistant P. falciparum is
prevented. They are rapidly effective and well tolerated, but its total synthesis is too complex to exploit and the yield of extraction from plant is still low, despite of the attempts toward enhancement of artemisinin or one of its precursor production in *A. annua* [20]. As a result, they remain expensive treatments that are hardly accessible to people in endemic areas [22].

In the early 1970s, Chinese chemists reported isolation and structure elucidation of the sesquiterpene 1,2,4-trioxane artemisinin (qinghaosu) the highly active antimalarial component of the ancient *Artemisia annua* (sweet wormwood) Chinese herbal remedy for fevers [23]. This important discovery represented a breakthrough in finding an effective antimalarial that was not quinoline-based and, therefore, that was effective against multidrug-resistant malaria parasites. Chemical synthesis of a mono-deoxygenated version of artemisinin established that the peroxide unit in this natural trioxane was essential for its high antimalarial potency [24]. Sodium artesunate is a succinic acid half ester of the reduced lactol form of artemisinin that, although prone to hydrolysis, is fast-acting, water-soluble, effective, and widely used in areas of the world where malaria is endemic [25]. No resistance to such trioxanes has been seen in the field or in the research laboratory. In combination with other antimalarial drugs, sodium artesunate is rapidly becoming the drug of choice in most third-world cases of malaria [26]. Understanding the chemical mechanism of action and the metabolism of artemisinin and related endoperoxides is essential to guide rational design of new antimalarial trioxanes.

The novel trioxanes being developed by Central Drug Research Institute, Lucknow, India., have shown promising antimalarial activity and are under development for clinical use. These trioxane antimalarials are synthetic analogues similar to artemisinin containing 1,2,4-trioxane nucleus responsible for their pharmacological activity [27-30].

1.4 Research Investigated

The increasing resistance of the malarial parasite against the contemporary drugs has further compounded the malaria problem. Against this background, isolation of artemisinin as the active principle of *Artemisia annua*, is a major breakthrough in malaria chemotherapy [31]. Artemisinin-type antimalarials seem to act by releasing a cascade of potentially cytotoxic
intermediates after activation of the crucial oxygen-oxygen bond by ferrous species [32] found in the parasite and/or in the erythrocytes they infect [33]. These peroxides cause gross morphological changes in malaria parasites [34] and biochemical evidence points to protein alkylation as part of the way in which damage is exacted on the invaders [35]. In addition, various studies indicate that artemisinin may covalently bond to the porphyrin portion of heme [36, 37] and a recent report characterizes the first artemisinin-porphyrin adduct formed during a heme model degradation.

Although natural artemisinin shows promising antimalarial activity, its poor bioavailability prompted the search for better analogues. Chemical insight into artemisinin’s biological mechanism of action has allowed rational design of some new trioxane and endoperoxide antimalarial drug candidate that are efficacious and safe.

Artemisinin and its semisynthetic derivatives such as artemether, arteether and artesunic acid are effective against both the chloroquine-sensitive and chloroquine-resistant malaria and because of their rapid action are finding increasing use for the treatment of malaria caused by multidrug resistant \textit{P. falciparum} [38]. The peroxide group, present in the form of 1,2,4-trioxane, is essential for the antimalarial activity of these compounds. Several synthetic trioxanes have shown promising antimalarial activity both \textit{in-vitro} and \textit{in-vivo} [39].

Many synthetic antimalarials with peroxide-bridges have been developed, but most suffer from low bioavailability, short half-lives and toxicity, a defect shared in part by semi-synthetic artemisinin and analogues. Therefore, the need exists to identify novel peroxide antimalarial agents with high oral activity, devoid of toxicity and moreover affordable. The development of cheap antimalarial drugs having similar mode of action to that of artemisinin is the main task of several research groups [31, 40-43].

In the quest to develop compounds with improved oral bioavailability, low toxicity and more affordable for the treatment of complicated and severe malaria, CDRI developed a promising trioxane antimalarial compounds in its drug discovery program [29, 30, 44-46]. These antimalarials developed by CDRI are considered as a new cost alternative to the existing class of drugs. The 99-411 is one of the promising antimalarial trioxane (Figure 1.1).
Pharmacokinetic (PK) studies play an important role at all stages of drug development. PK studies on one hand provide information to the chemists for structural modification in terms of appropriate PK parameters such as bioavailability, $t_{1/2}$ and clearance. On the other hand at the later phases of development it unfolds the PK parameters of candidate drugs, which help in optimization of therapy. Pharmacokinetic studies also play a vital role in the initial selection of candidate drug, which is to be put under development thus reducing the attrition rate. Pharmacokinetic study data of 99-411 in rats after oral and intravenous doses has been reported previously (R.P. Singh et al.). The information regarding the elimination of the drug as unchanged form or as its metabolites can be derived from excretion studies. The extent of tissue storage is, in turn, known to influence the pharmacokinetics and tissue distribution plays a major part in the pharmacological profile of a compound with respect to its duration of action, its potency in-vivo, and its toxicology. There is no information on the distribution and excretion of 99-411 in rats. Tissue distribution and excretion study was performed in rats after single oral administration in rats.

Monkeys, as non-human primates, are often used to develop pharmacokinetic data to assist in understanding potential pharmacokinetics in humans [47]. Single, multiple dose pharmacokinetic studies of 99-411 were performed in Rhesus monkeys after oral administration.

Toxicokinetic (TK) study is generally required for toxicological evaluation and safety assessment, particularly in the pivotal toxicological studies which are requested on the basis of drug registration. The estimation points of TK data are: (1) determination of TK profile during toxicity study, (2) selection of dose, dosing form, alternative dosing route, (3) help for
the evaluation of toxicity (non-effect/toxic dose, toxicological mechanism), (4) comparative evaluation between animal and human cases, (5) recommendation of the starting dose in the first human clinical trial. On the other hand, as a very recent trend, TK data are practically used for the purpose of drug discovery such as lead-optimization and candidate-selection. The toxicokinetic study of 99-411 was performed in male and female *Rhesus* monkeys after oral administration [48]. The study samples were analysed using validated LC-MS/MS method.
References


45. Singh C, Puri SK. Substituted 1,2,4-trioxanes as antimalarial agents and a process of producing the substituted 1,2,4-trioxanes. United State Patent 6316493 B1. (2001).

