A review on Artemisinin Based Combination Therapy for the Treatment of Malaria

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

The project titled "A Review on Artemisinin Based Combination Therapy for the Treatment of Malaria" submitted by Amina Ferdous Choudhury (15346040) of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 30th September 2019.

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

Resistance of malarial parasites to the conventional antimalarial drugs is increasing relentlessly. In response to this situation, artemisinin based combination therapy has been proposed to be the first line defense for treating malaria by the World Health Organization. The artemisinin based combination therapy involves the collaboration of rapid schizontocidal activity of artemisinin or its derivative such as artesunate, artmether and dihydroartemisinin with another drug as partner drug that possess a longer half life. According to a report of International Artemisinin Study Group published in 2004 Artemisinin based combination therapy can cure 90% malaria infected people along with acting against malaria gametocytes as a consequence of which has the ability to potentially reduce the transmission. The efficacy is determined by the drug partnering the artemisinin or its derivative. In addition, most malaria endemic countries are now likely to adopt artemisinin based combination treatments for treating malaria as they are reliably effective.

Keywords: Artemisinin; Combination therapy; Malaria; Antimalarial drugs; Treatment; Efficacy.

Dedication

Dedicated to my parents to whom I owe my achievements

Acknowledgement

While conducting my project, I have been blessed with enormous support from a number of people. However, I never got the opportunity to convey my gratitude towards them. On the verge of accomplishment of this project, I would like to grab the opportunity to acknowledge these people for their heart pledged support and concern about this work.

First of all I would like to thank the Almighty for bestowing me with the ability to accomplish this project with success. I have been privileged to have the opportunityto work under the supervision of **Dr. Md. Abu Bakar**(Associate Professor, Department of Pharmacy, Brac University) whose constant guidance and valuable suggestions showed me the way to shape my project. He has always inspired me to enrich my knowledge and I have had the best learning experience throughout the past few months while working under his supervision. I would like to thank our honorable chairperson **Dr. Eva Rahman Kabir**(Professor and Chairperson, Department of Pharmacy, Brac University) for allowing me to conduct my work and supporting me throughout. I would like to show my gratitude towards **Dr. Hasina Yasmin**(Associate Professor and Academic Coordinator, Department of Pharmacy, Brac University) for her support while conducting my work. Finally, I would acknowledge my family, friends and dear ones for inspiring me to enrich my capability and change my vision.

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List of Acronyms

DHA Dihydroartemisni

FDA Food and Drugs Administration

EMA Europian Drugs Organization

CDC Illness Control and Anticipation

IND Investigational New Medication

API Active Pharmaceutical Ingredient

DHAA Dihydro Artemisinic Acid

THF Tetra Hydrofuran

TMCS Trimethyl Chlorosilane

EC Effective Concentration

IC Inhibitory Concentration

FIC Frictional Inhibitory Concentration

ELISA Enzyme Linked Immuno Sorbent Assay

SD Standard Deviation

AL/ ALN Artemether- Lumefantrine

MAS3 Artesunate- Mefloquine

PCR Polymerase Chain Reaction

DMSO	Dimethyl Sulfoxide
ACPR	Appropriate Clinical and Parasitological Response
PP	Perprotocol
ITT	Intention to Treat
AE	Adverse Events
AUC	Area Under Curve
ASAQ	Artesunate- Amodiaquine
AQ	Amodiaquine
SP	Sulfadoxine Pyrinaridine
PCT	Parasite Clearance Time
FCT	Fever Clearance Time

Chapter 1

Introduction

1.1 Developing antimalarial drug

A vital causeof diseasealong with occurrence of death in many countries around the world and a significantnumber of Australian countries is malaria (Davis, Karunajeewa, & Ilett, 2005). During the Chinese "Cultural Revolution" in the 1970s, there was a periodwhen scientific research was not permitted while malaria induced by *Plasmodium falciparum* became a threatening condition. A number of attempts were taken in order to eradicate malaria which went in vain as available malaria treatments became ineffective for treating malaria as it became resistant which led to an urgent need of antimalarial drug. At the verge of such condition, a new secret project was introduced by the Chinese Government named 523 project appointing Professor Tu You You as the head of the project involving both phytochemical and pharmacological researchers, therefore they started working on the extraction and isolation of antimalarial constituents including search of fever treating medicines(Tu, 2011). The group searched for formulations having antimalarial activity as a consequence of which 640 formulations were shortlisted from over 2000 formulations for further evaluation and a plant named '*Artemisia annua*' had a frequent appearance in the formulations primarily having ~68% inhibition rate with an unstable activity that varied from 12%-40% in subsequent repeats that might have caused due to the geographic origin of the plants, variation in seasons, use of different parts of the plant along with the method used to take out the extraction (According to a report of Professor Tu in 1972 presentations to the scientists in the project). While researching on this, Professor Tu identified from a report ofMr. Ge Hong published about more than 1000 years ago where in one of his suggested formulations, he mentioned about collecting the extract of *Artemisia annua* for having a fever treating activity. In the report, the author mentioned about use of cold water to remove

extraction instead of cookingmedicinal plant in hot water. However, it came on the realization of Professor Tu that the high temperature might have caused the instability for treating fever and using ether instead of ethanol for extracting active ingredients from present in the leaves of the plant sample shows efficacy against rodent malaria. And monkey malaria as. Later on, clinical trial was conducted on 21 patients showing 95%-100% efficacy (Su & Miller, 2015; Tu, 2011).

1.2 Pathogenesis of malaria

Although the greatest suffering due to malaria falls on the people in tropical countries, a potential risk of occurrence of malaria is possessed over the people of almost all continents along with varies in endemicity in different countries and areas as well. However, an area where occurrence of malaria is lower people of various age are affected by the disease as the people living there do not grow the clinical immunity as there is very low exposure to the malaria parasite along with passing on the trend to the next generation such as having an effect on embryo and infant, particularly when a woman is pregnant with her first child and children less than 5 years suffers the most in an area having high endemicity. A more prominent treatment can be developed to cure the disease by understanding the lifecycle of the malaria parasite.The blood vessel vaccination of sporozoites of infected mosquitoes invade the hepatocytes followed my multiplication for a few weeks of the hepatocyte thus producing thousands of merozoites that rupture the cell for invading erythrocyte to run the erythrocytic cycle. In erythrocytic cycle the size of the parasite population is amplified. The Falciperum species has an unique quality of adhering to the venular endothelium of erythrocyte that is infected with the maturing parasites and stay attached until the release of merozoites for invading other erythrocytes as a result in the peripheral circulation, the elevated type appears known as a young form of parasite having a ring infected erythrocyte that gradually develops to cause the disease (M. Philips et. al. 2017).

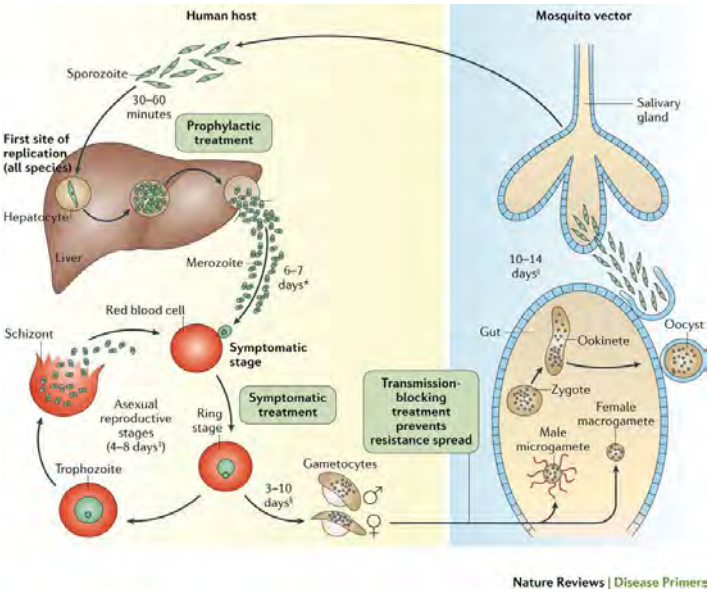


Figure 1: Malaria parasite life cycle (M. Philips et. al. 2017)

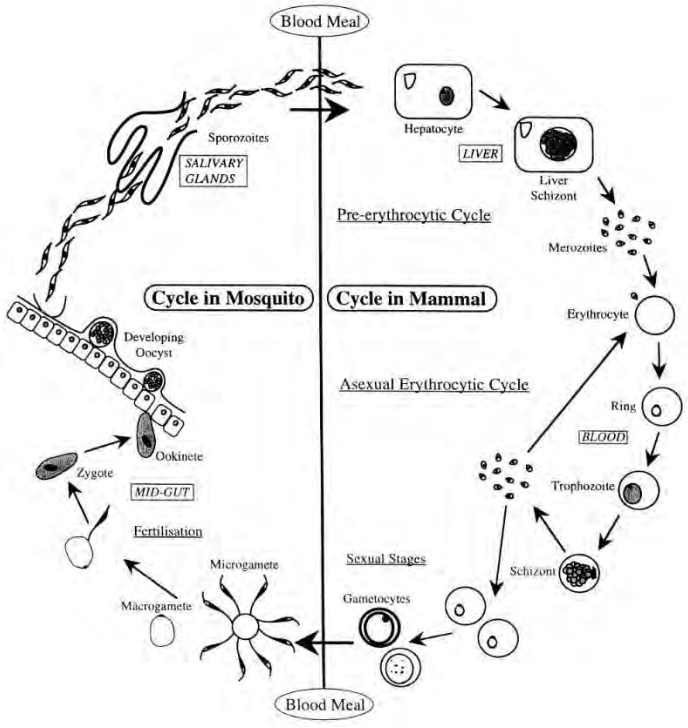


Figure 2: Malaria parasite life cycle in mammals (R.S. Phillips 2001)

Cytokines released by the immune component cells perform a number of functions such as regulating the immunological response, e.g. Initiation, propagation, later effector functions of recirculating immune cells or regulating tissue-residing cells, involved in both qualitative and measurable immune response control and a number of complicated procedures like

hematopoiesis and pregnancy.

Malarial toxins that are soluble products of Plasmodium are produced during the erythrocytic cycle by direct systemic release of inflammatory cytokines. Individuals may exhibit falciparum reactive T cells that may arise from antigenic cross reactivity between parasite derived molecules and environmental organisms. TNF- a may have an effect on cerebral malaria as in cerebral blood vessel endothelium it has intracellular adhesion molecule 1 (ICAM-1). T cells, irrespective to which one has been expressed plays a vital role in disease pathogenesis as the malarial toxins stimulate the mononuclear phagocytes and as a result of activation of the T cells, disease occurs along with killing of the parasites (Miller, Good, & Milon, 2017)**.**

1.3 Symptoms

The symptoms can be categorized according to the two types of malaria.

Table 1: Categorization of malaria

Simple malaria	**Severe/ intensified malaria**
Fever, pain or chills	Fever, pain or chills
Cold & shivering sensation	Reduced Cognizance
Headache	Adoration or adaption of a prone position
Seizure in younger people (Occasionally)	Multiple convulsions
Abnormal sudden sweating and return to regular temperature	Deep breathing and difficulty in respiration
Tiredness	Unusual blood loss and symptoms of anemia
Vomiting (Occasionally)	Jaundice and dysfunctional organ

Although fever, pain or chills are common in both the types, they differ in few aspects. In case of uncomplicated malaria, the disease is passed by anopheles mosquito and if remain untreated in preliminary stage, may arise in severe form. The symptoms for uncomplicated

malaria lasts for maximum 10 hours with a reappearance after some days. The symptoms include a cold and shivering along with rise in body temperature, annoyances and even nausea. In addition, sometimes the young patients are attacked by seizures. Abnormally sweating suddenly also appear with a return to regular temperature in a while alongside a feeling of exhaustion. As the symptoms match with the symptoms of regular flu, uncomplicated malaria often remains undiagnosed hence arising to the severe form. Usually doctors perform diagnosis and treatment according to the symptoms visible. But when there is absence of symptoms, this indicates intensified contagion or some vital organs not functioning properly. In case of intensified malaria, vital organ dysfunction signs are detected through clinical tests. The symptoms for malaria includes a rise in the temperature with cold feeling, reduced cognizance, Adoration or adaption of a prone position, numerous seizures, deep breathing and difficulty in respiration, unusual blood loss and symptoms of anemia, jaundice and dysfunctional organ (Lam, 2018).

1.4 Diagnosis

Patients who exhibit symptoms like irregular fever and chills, headaches, vomiting that arises in some of the patients whereas minor diarrhea in less likely to occur along with anemia with the progression of the disease.

The tests done for diagnosis of malaria includes –

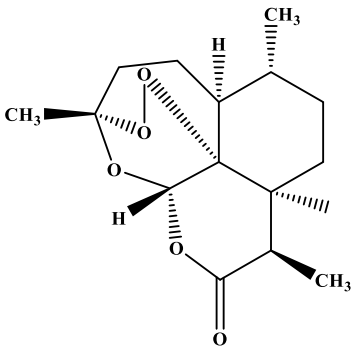
- White blood cell count
- Intra erythrocytic parasite count
- Percentage of neutrophil containing malarial pigment
- Developmental stage of parasite (Severe malaria)

The blood test done for malaria shows the white blood cell count and thrombocytopenia followed by confirming the diagnosis by applying microscopic analysis of blemished tinny and profuse blood films at 1000 magnification. However, intra erythrocytic parasite must be

recognized and counted. Percentage of neutrophil containing malarial pigment and the developmental stage of parasites should be identified in case of severe malaria. For further improbability, repetition of the test should be continued for the next 48 hours the for the confirmation of the disease. Histidine rich protein is used as a monoclonal antibody for diagnosing the disease that denotes the use a test strip for finger pricked blood samples (White, 1996).

1.5 Existing drugs for treating malaria

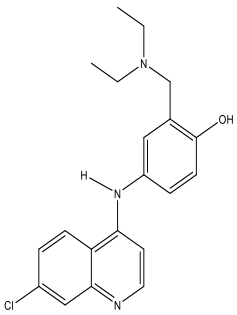
The most effective treatment of malaria caused by *falciparum* malaria lies on artemisinin therapy (https://www.who..int/news-room/fact-sheets/detail/malaria). World Health Organization further suggests treatment of Falciparum malaria by sulfadoxine-pyrimethamine for the pregnant women. Moreover, consistent chemo prevention was also prescribed by WHO for jungle fever anticipation in Africa's Sahel area along with overseeing the dose of amodiaquine per month with the use of pyrimethamine-sulfadoxine in kids (World Health Organization, 2008). Tu You You discovered a drug named artemisinin in 1972 that showed significant efficacy in treating malaria that was improvised to the application of artemisinin based blend medication for treating the disease that was approved by the World Wellbeing Association in mid 2000.



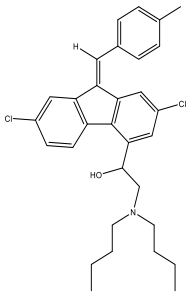
Artemisinin

The discovery of Tu saved a large number of lives and showed its impact in South China, Southeast Asia, Africa and South America. Dihydroartemisinin was further developed by Tu as a bioactive metabolite of artemisisnin and for the discoveries she made, Tu achieved the noble prize in the year 2015 for physiology or drug (https://brittanica.com/biography/Tu-Youyou) . Currently artemisinin based combinations are used for treating malaria. The principle medicines to uncomplicated jungle fever are ACTs: a blend consisting medications, a subordinate of artemisinin and a subsidiary of quinine (Malaria Guideline, World Health Organization). Because of high lipophilicity, artemisinin isn't fundamental decision particle in any exacting administrative blend affirmed by the administrative expert. Rather, semi-engineered subsidiaries are utilized: DHA (reduced hemi-acetal of the principle dynamic metabolite of numerous artemisinin subordinates), artesunate (an exceedingly water-dissolvable sucinate of DHA). Quinine being utilized as drug for the considerable length of time, yet it was distinctly amidst the twentieth century. The favored mix accomplices have been 4-aminoquinolines (like, amodiaquine, piperaquineand pyronaridine) as well as amino alcohols (like mefloquine or lumefantrine). Five ACTs have been affirmed or are being endorsed by FDA, the European Drugs Organization (EMA) and WHO. In critical clinical investigations, these mixes have demonstrated amazingly successful (coming to a sufficient clinical and parasito-sensible reaction (for example the nonappearance of parasitaemia in > 94% of patients at day 28 (Kinfu G. et. al. 2012), are well-endured (as given to > 300 million pediatric patients) and are reasonable (ordinarily not exactly US$ 1 for each portion). After different aftereffects of broad examinations in Asia as well as Africa region, artesunate has been favored as injectable medicament to severe *P. falciparum* malaria (Sinclair et. al. 2012 and Dondrop et. al. 2005). In the US, intravenous artesunate is provided by Illness Control and Anticipation (CDC) intestinal sickness program as an Investigational New Medication (IND) and shows adequacy of > 90% even in oblivious patients (Sinclair et. al.2012).

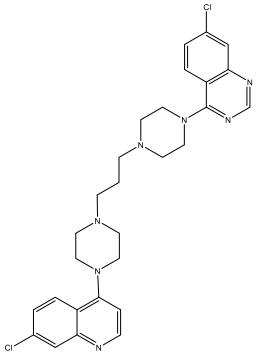
Artesunate suppositories have been in the late phase of item development (Okebe et. al. 2014). For uncomplicated *P. vivax* malaria, chloroquine or ACTs are suggested by the WHO (in spite of the fact that chloroquine isn't utilized in a few nations, for example, Indonesia) (Malaria Guideline, World Health Organization).



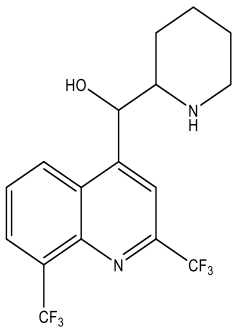
Amodiaquine



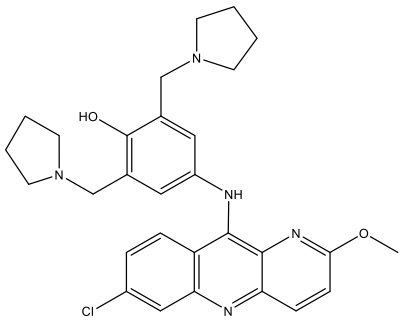
Lumefantrine



Piperaquine



Mefloquine



Pyronaridine

1.6 Need for New Therapeutic Approach

A mass killer of malaria is the Falciparum malaria and is going out of control day after day resulting from increase in resistance to the parasite of malaria parasites towards the drugs available for the particular parasite such as chloroquine, sulfadoxin-pyrimethamine and mefloquine and the degree of resistance is becoming implacable with time (Davis, Karunajeewa, & Ilett, 2005; François Nosten & White, 2007). This consequence lead to the

development of new therapeutic approach for combating against malaria parasite and most prominent approach relies on development of artemisinin based combination therapy (ACT) using multidrug treatment that leads to treatment of successful diseases including HIV and cancer along with providing a longer half life. Artemisinin based combination therapy (ACT) has been endorsed as a standard strategy that is to be followed for all the contagions due to *Plasmodium falciparum* by World Health Organization (WHO) and supported by the Global Fund (GFATM)(Davis et al., 2005; Hutagalung et al., 2005). Although ACT has been considered as an effective treatment for malaria, it is not widely available in the endemic areas. In addition, ACT is now being available at an increasing rate as an over the counter drug in the tropical areas. As ACT is expected to be a good solution for treating malaria and also cost effective reportedly, one of the ACT that is artemether-lumefantrine combination for *Plasmodium falciparum* has been approved for clinical use in Australia (Australian medicines handbook. Richmond, South Australia: Hyde Park Press, 2004: 215-216). Although donor support has increased with better drugs along with better methods of delivery, still there is a gap between the drugs and funds that should be available to meet the global need.Artemisinin drugs are a simple gist of Artemisia annua (qinghao) wormwood plant that has been found in China. At first it was used about 2000 years ago as an antipyretic agent. Thus it was reported to be effective against the malaria fever in the 16th century. Later on, from the extract, its active constituent was identified along with purification of the compound in the 1970 that was named as qinghaosu or artemisinin that was demonstrated to be effective in clinical trial in 1980s. Although it got approval for clinical trial, a number of semisynthetic derivative were developed. This was done with the aim of improving the pharmacological properties along with improving its antimalarial potency (Hien TT, White NJ. Qinghaosu. Lancet 1993; 341: 603-608). It was reported that for fighting in opposition to nonsexual four species of Plasmodium that can affect human, all the artemisinin derivatives

are highly effective (De Vries PJ, Dien TK. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 1996; 52:818-836). These derivatives possess a shorter half life relative to the clearance of parasites. For giving antimalarial effect the presence of endoperoxide moiety from which radicles having damaging ability in the parasite along with altering the major activities of parasite proteins by forming covalent bonds (Meshnick SR. Artemisinin antimalarials: mechanisms of action and resistance. Med Trop 1998; 58(Suppl): 13-17, Eckstein-Ludwig U, Webb RJ, Van Goethem ID, et al. Artemisinins targetthe SERCA of *Plasmodium falciparum*. Nature 2003; 424: 957-961).

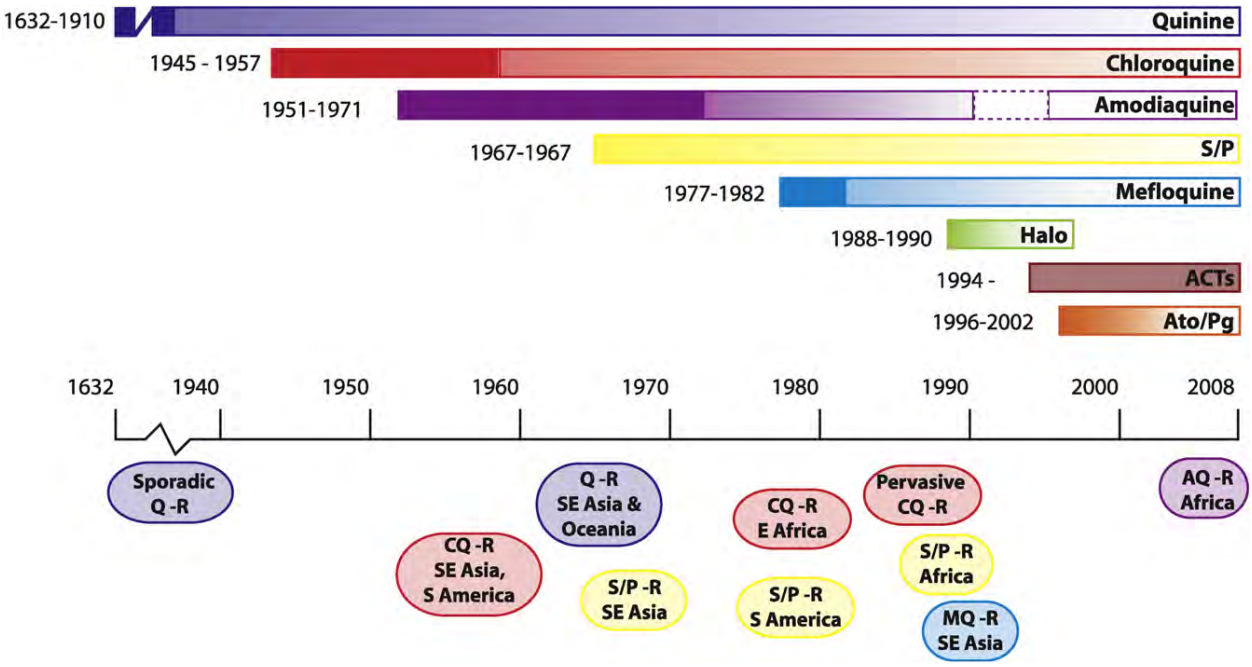


Figure 3: Resistance to the standard antimalarial therapy.

Chapter 2

Method & Materials

2.1 Synthesis of derivatives of artemisinin in a continuous process

Continuous procedures possess a number of advantages including enhanced safety along with purity of the product. Moreover, it has reduced effect on the atmosphere. The study discusses the mixture of a deviating, continuous synthesis with ongoing in-line purification for the preparation ofa number ofactive pharmaceutical ingredients derived from artemisinin that are important elements of Artemisinin Combination Therapies for the treatment of malaria. Artemisinin is mostly obtained from *Artemisia annua* plant along with generating dihydro artemisinic acid 2 as leftover product that can be used for the synthesis of artemisinin by using rearrangement method that is induced by acid through photo-chemistry in a constant manner, leading in rapid and effective conversion (Gilmore et al., 2014)

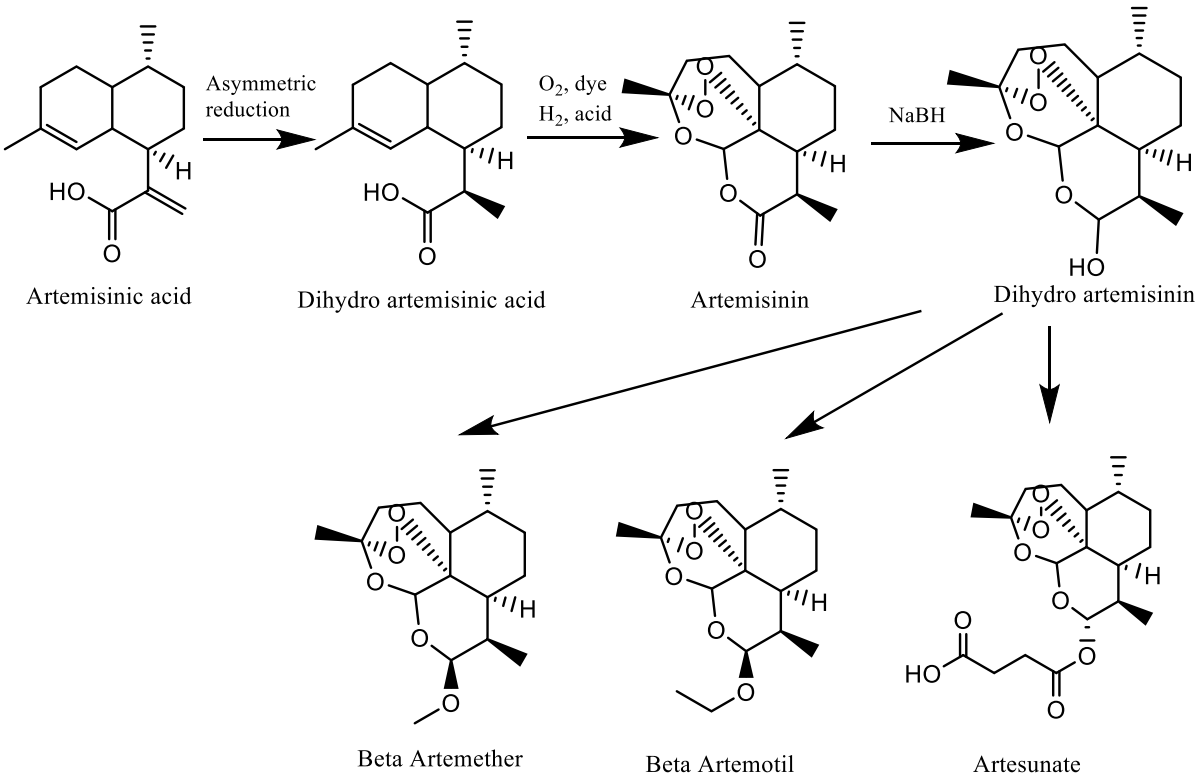


Figure 4: Production of antimalarial APIs from artemisnin

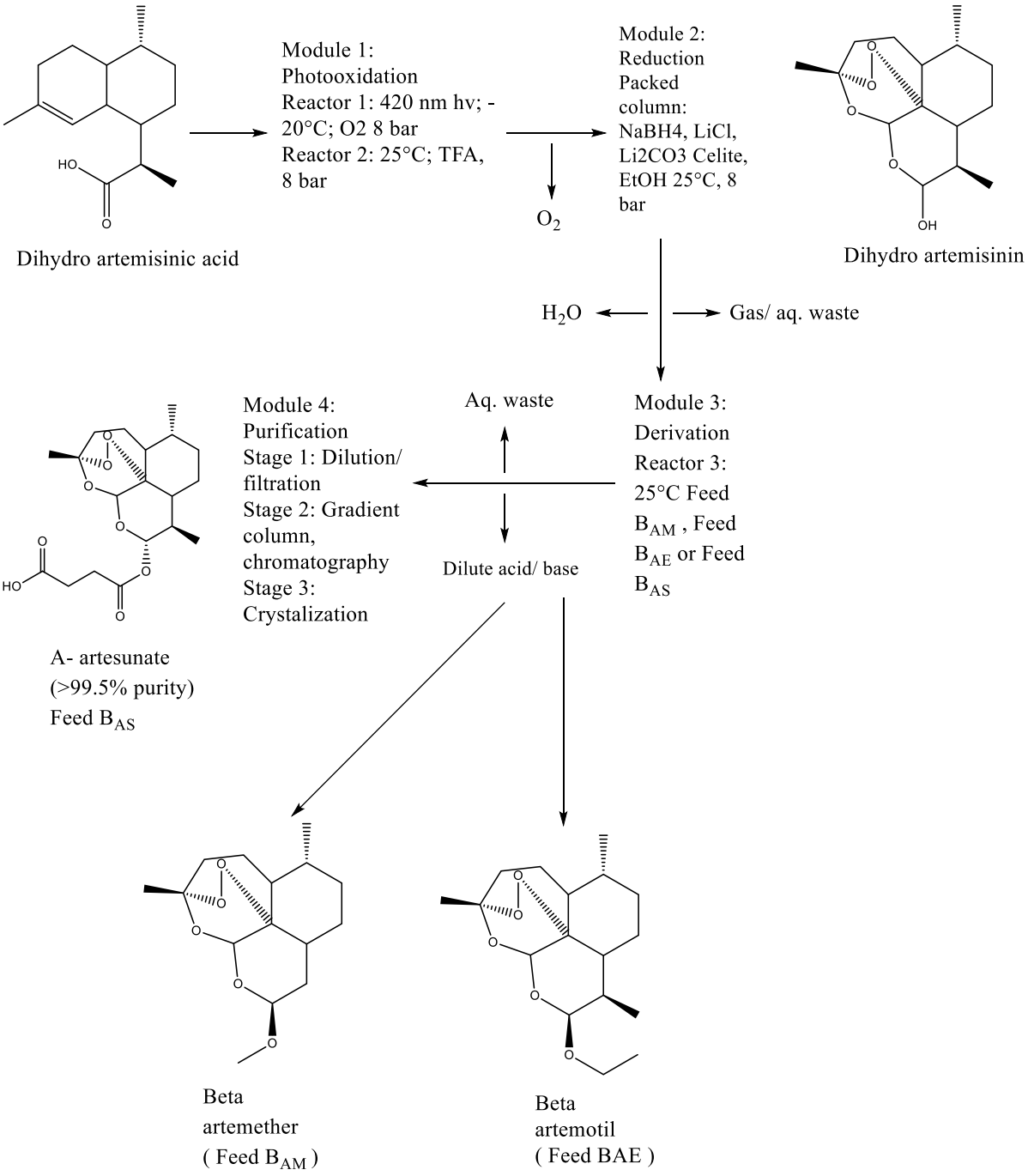


Figure 5: Continuous synthesis of APIs of artemisinin

Here, in this process ongoing synthesis of artemisinin 3 from DHAA 2 is observed for obtaining entirely continuous synthesis and purification of the three primary anti-malarial APIs. While lithium triethylborohydride reduces pure artemisinin 3 to DHA 4 in a constant chemical flow environment. Reduction of $NaBH_4$ is favored by lithium chloride (LiCl) through production of LiBH. By combining the two additives and blending $NaBH_4$, Celites, Li_2CO_3, and LiCl (1:1:1:0.76 (w / w)) as packaged bed products along withadding alcohol as co-solvent like ethanol, full and fresh reduction of crude artemisinin was accomplished. Combination of module 1 and 2 generates the first API from dihydro artemisinic acid. The module 2 represents achieving the effective reduction of carbonyl compounds along with ability to reduce that is stoichiometric with $NaBH_4$.

2.2 Synthesis of mefloquine through fluoride ion catalyzed wittig rearrangement.

Mefloquine is a highly effective antimalarial drug. The research focuses on the prospective implementation of wittig rearrangement to the quinolyl ether that is easily accessible in 90 percent yield from Z-chlocomethyl pyridine and 4-hydcoxy quinolone followed by exposing the ether to phenyl-lithium n-butyllithium or $-1H_2$ in tetra-hydrofuran (THF) or benzene at various temperature between -78°C- 60°C. The carbinol was separated at low yield when ether was handled with NaFI in THF or DMF. Introducing —SiR3 group made the rearrangement easier (Solange, 1989).

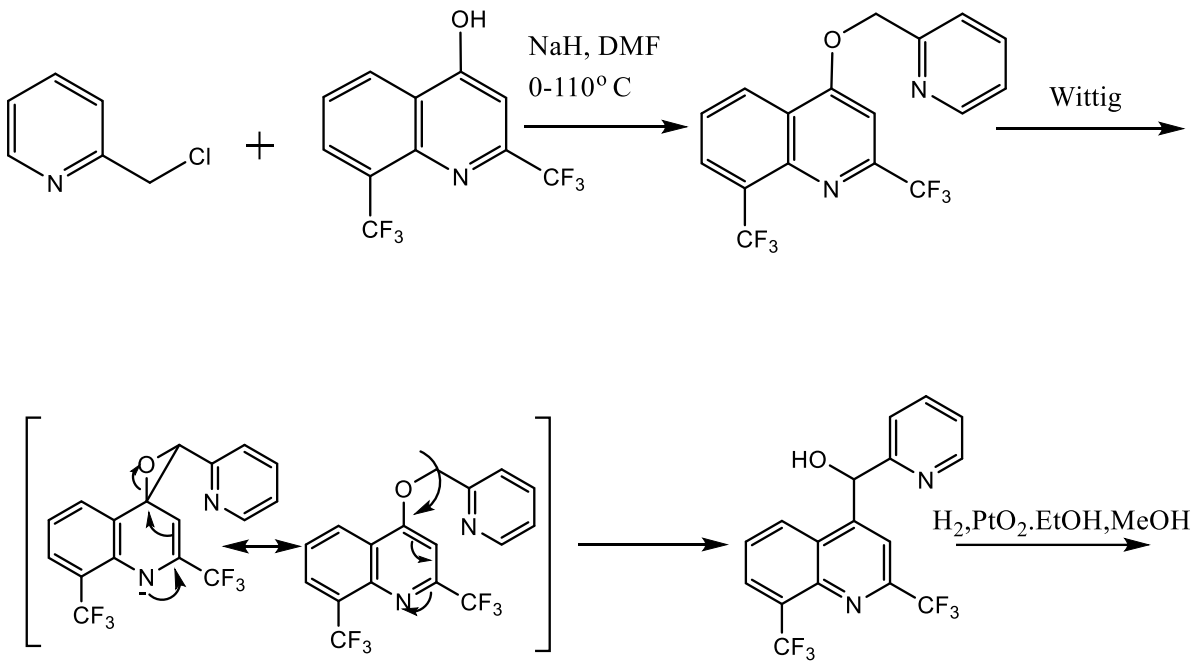


Figure 6: 1st Scheme

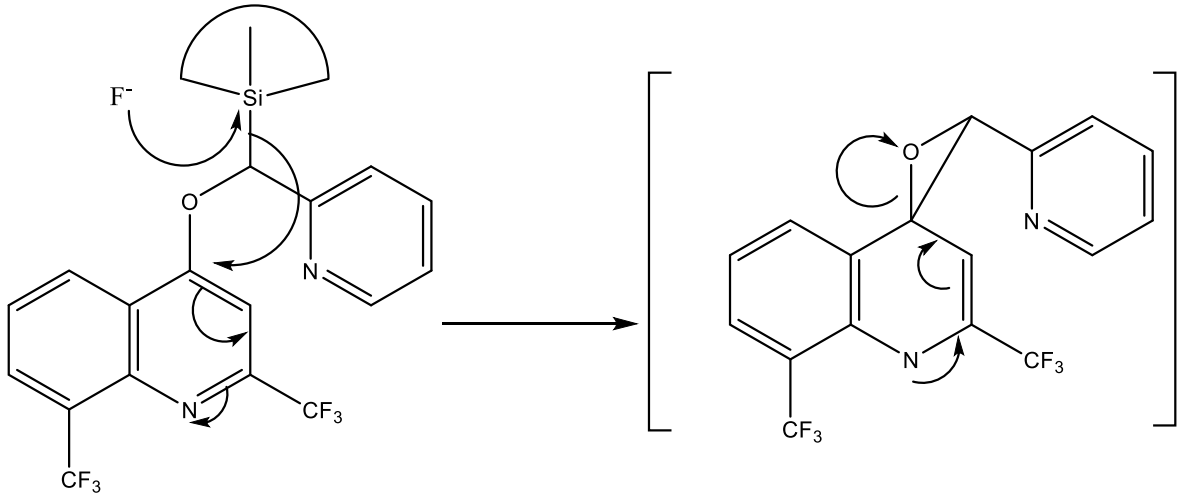


Figure 7: 2nd Scheme

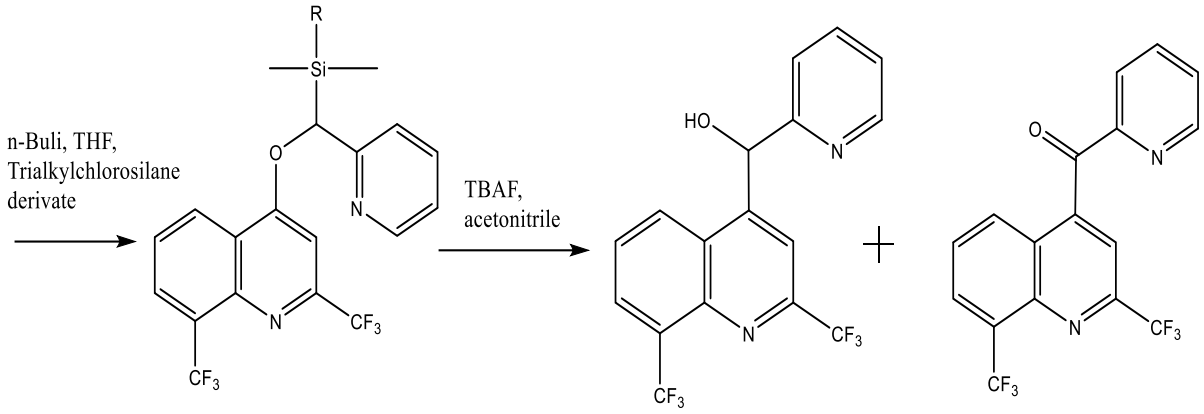


Figure 8: 3rd Scheme

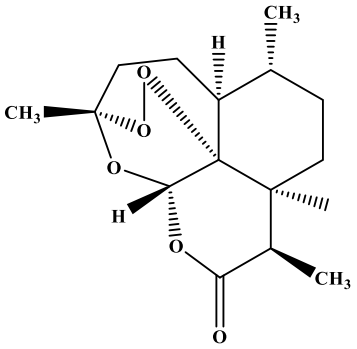
As shown in Scheme 3, the silylated derivatives were synthesized. N-butyllithium treatment at -76OC. THF produced a profound green anion that was silylated with either tri methyl chlorosilane(TMCS) or t-butyldimethyl chlocosilane (Solange, 1989).

Chapter 3

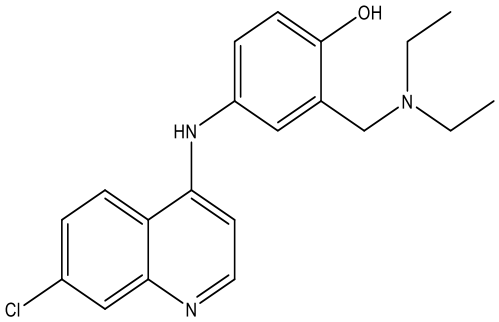
Results and Discussion

3.1 *Plasmodium falciparum*: artemisinin combination with Amodiaquine, Pyronaridine and chloroquine; an in vitro study.

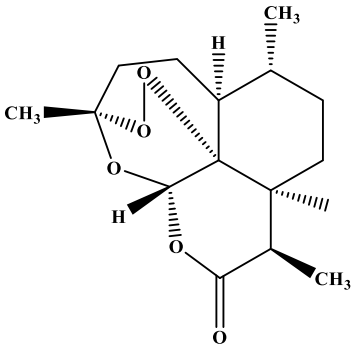
Artemisinin, amodiaquine base, chloroquine phosphate and pyronaridine tetra phosphate were collected from different sources for conducting the study (Falade et al. 2005; Makanga et al. 2006).



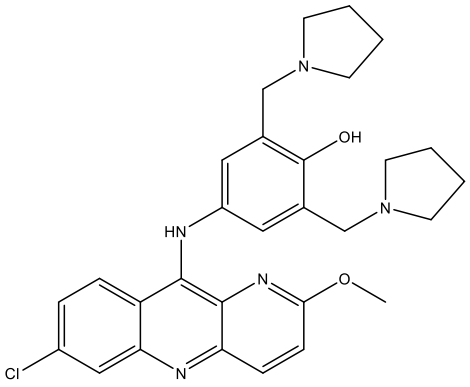
Artemisinin



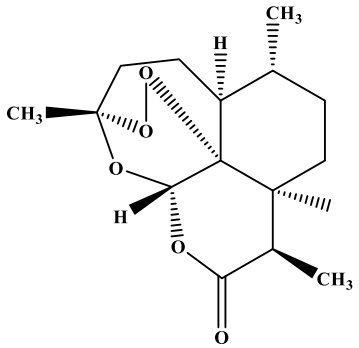
Amodiaquine



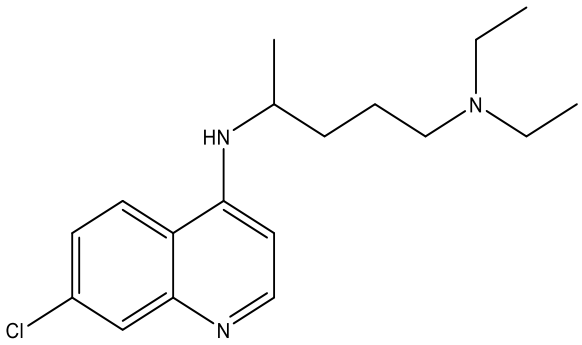
Artemisinin



Pyronaridine



Artemisinin



Chloroquine

Assay Method:

The mixture of artemisinin-amodiaquine was liquefied in a solution of 95 percent alcohol (v / v) with a peak concentration of 0.0001 percent ethanol, and the mixture of pyronaridine-chloroquine was dissolved in water, followed by the preparing of a stock solution of 10-3 M by diluting distilled water alternatives(Gupta, Thapar, Mariga, Wernsdorfer, & Björkman, 2002). The research was carried out using three strains of *Plasmodium falciparum* using the technique of candle jar method (Trager and Jensen 1976). The standard RPMI 1640 medium (GibcoBRL, Life Technologies AB, Sweden) was supplemented by 25 mM Hepes buffer, 2 mg / ml sodium bicarbonate, 0.5 μg / ml gentamicin (50 mg / ml) amd 10% humamn AB+ serum accompanied by the use of non-affected human O+ erythrocytes after twice washing with Tris-Hanks buffer (SBL Vaccine AB, Sweden).For all medicines from inventory alternatives, 10 times dilution in water was performed in a sequential manner. For single drugs in duplicates, in vitro studies were conducted to evaluate individual drug sensitivities against all three strains and then the values of EC50 were obtained.Next, solutions of drug were formulated for the test of interaction with drug based on EC50 values acquired where the concentration ranged from 10-2 -102 for each drug.Aliquots of 100 μl of these drug alternatives were implemented into 96 microtiter plates having a flat bottom accompanied by one-by-one evaporation of all solvents by adding 100 μl of culture medium with an original

0.2-0.5 percent parasitemia and 5 percent hematocrit. After that, the plates were incubated in the candle jars at 37ºC for 48 hours.Lastly, the parasites were also counted for determination of the increase of parasite and to which extent they are inhibited. The outcome acquired was further evaluated statistically using the log-concentration / response prohibit technique from the Litchfield and Wilcoxon hypothesis.

Table 2: Inhibition of the growth of 3 strains of Plasmodium falciparum due to Artemisinin, Amodiaquine, Chloroquine and pyronaridine.

			Effective Concentrations (10^{-9} M)	
Antimalarial Dugs	Degree of efficacy	Lab strain 1	FCR3	F32
Artemisinin	EC_{50}[a]	13.9	13.2	5.1
	EC_{90}[b]	54.7	55.0	10.2
	EC_{99}[c]	137.6	140.6	15.8
Amodiaquine	EC_{50}	0.2	0.2	0.8
	EC_{90}	21.4	27.7	50.0
	EC_{99}	1500.7	1675.4	2598.4
Chloroquine	EC_{50}	98.0	40.3	36.5
	EC_{90}	309.5	142.5	102.5
	EC_{99}	587.9	383.6	320.5
Pyronaridine	EC_{50}	23.6	33.5	37.5
	EC_{90}	54.6	47.5	75.6
	EC_{99}	112.0	65.0	134.5

Table 3: Range and type of interaction between artemisinin with other drug molecules at EC50, EC90 and EC99, expressed as molar proportions of partner drug/ Artemisinin (Obtaining mean value from 3 strains of Plasmodium falciparum)

Partner drug	Drug/artemisinin proportion range at EC_{50}[a]	Drug/artemisinin proportion range at EC_{90}[b]	Drug/artemisinin proportion range at EC_{99}[c]
Amodiaquine	*	12-46 additive <12 synergistic	>521 additive <521 synergistic
Chloroquine	48-150 additive <48 synergistic	24-51 additive <24 synergistic	<1.4 additive >1.4 synergistic
Pyronaridine	1-8 additive ,1 synergistic	0.4-8 additive <0.4 synergistic	0.2-8 additive <0.2 synergistic

Table 4: Collaboration of artemisinin with other drug molecules at a molar concentration ratios that is relevant clinically.

Partnering drug	Range of molar ratio	Observed/expected EC_{50}[a]	Observed/expected EC_{90}[b]	Observed/expected EC_{99}[c]
Amodiaquine	2.2-40.0	* 0.75-0.96	0.70-1.83 Synergistic-additive 0.50-1.72	0.19-0.50 Synergistic 0.09-0.50
Chloroquine	2.2-40.0	Synergistic 0.90-1.72	Synergistic-additive 0.86-1.95	Synergistic 1.48-1.98
Pyronaridine	0.3-6.0	Synergistic-additive	Synergistic-additive	Additive

From the result, a greater EC_{50}, EC_{90} and EC_{99} values were observed with lab strain 1 than FCR3 and F32 strains. However, pyronaridine showed similar response for all the three

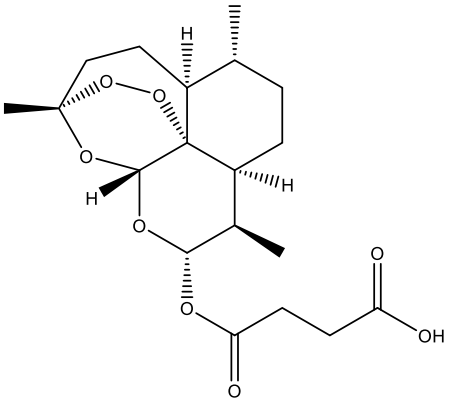
strains. A higher sensitivity was observed in case of the strain F32 in response to artemisinin with a similar profile for strain 1 and FCR3.

Artemisinin-amodiaquine combination shows synergistic effect and with combination of chloroquine, a weak synergism was observed. Addition of pyronaridine showed a moderate to marked synergistic effect.

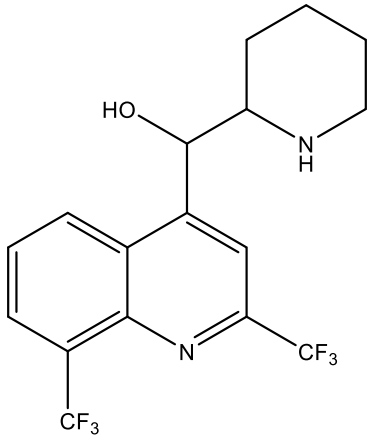
P. falciparum strains F32 and FCR3 were chloroquine susceptible, while lab strain 1 had a profile that was partly chloroquine resistant. Despite the elevated sensitivity of all three strains to artemisinin, F32 was the most susceptible, the EC99 being nearly one order of magnitude smaller than those of Lab strain 1 and FCR3. In comparison, with respect to amodiaquine, strain F32 was less susceptible.

3.2 A propective study on the effects of artesunate-mefloquine mixture in Western Thailand on the occurrence of *Plasmodium falciparum* disease including mefloquine opposition.

A research was done for evaluating the variables affecting malaria incidence onthe Karen people above 13-year span including the impacts of mixture of artesunate with mefloquine on evaluating the resistance of mefloquine. The research evaluated the incidence of malaria in Shoklo and Maela, that are ethnic Karen individual villages situated on Thailand's northeastern frontier(F. Nosten et al., 2000). Shoklo's population grew rapidly from 3000 in 1984 to 7000 in 1992, after which it stabilized.The region is one of small and fragile for transmitting malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*, with detectingmalaria caused by *Plasmodium ovale* occasionally. All of the diseases caused due to*Plasmodium falciparum* and most *Plasmodium vivax* were indicative (Falade, Ogundele, Yusuf, Ademowo, & Ladipo, 2008).



Artesunate



Mefloquine

Administration of to efficient antimalarial drugs like mefloquine and artesunate was firmly regulated in camps in Shoklo and Maela, and therapy was only provided after microscopic confirmation of infection.On the beginning of the program, mefloquine with sulphadoxine pyrimethamine, which supplied 15 mg / kg of mefloquine, was the first consideration for treating falciparum malaria. However, this showed resistance that was elevated in the five years after 1985, and in 1990, mefloquine monotherapy was altered to an elevated dose (25 mg / kg). Additionally, this treatment was initially effective at more than 90 percent, but there was a drastic increase in resistance. Moreover, rate of cure in in-vivo investigation had dropped to 60 percent by mid-1994, with 10 percent of patients failing to clear parasitaemia. Following a sequence of studies, the mixture of elevated-dose (25 mg / kg) mefloquine and artesunate (4 mg / kg daily) for three days was usually implemented in the selected colonies for providing therapy of *Plasmodium falciparum* malaria that is not complicated in 1994 ; chloroquine (25 mg / kg) was still handled for *P. vivax* diseases.

Cohort Studies:

In Shoklo, a prospective research of two teams was done for providing accurate estimation of the occurrence of malaria. The first were random cohorts, chosen from the camp population in 1992 and 1997. Every moment, the selection method was comparable and explained

elsewhere.To guarantee that the sample was representative, a comparison was done between the demographic features along with geographic distribution of sample with population of the entire camp. Every week (or daily for kids in 1992) respondents were visited at home. Additionally, peripheral smear of malaria from blood were collected when respondents testified indicative signs of malaria.In the 1997 cohort, preliminary along with recrudescent infections were differentiated using genotyping of parasite with PCR, which enabled accurate dimension of incidence densities. The technique enabled for monitoring recrudescent instances of *Plasmodium falciparum* and movement of the people.The next group had females who were expecting. From 1986, all of the expecting females in both camps were encouraged for attending antenatal-clinic consultations every week that was directed for reducing malaria-related death and occurrence of the disease through early identification and therapy of all parasitaemic episodes during pregnancy. A blood film for malaria parasites was acquired and screened at each visit by microscopy. Estimation of the incidence of malaria by only counting the amount of first instances in pregnancy of *P. falciparum* or *P. vivax* diseases.

Determining efficacy of drug by in- vivo research:

Patients infected with uncomplicated *P. falciparum* malaria were given treatment and observed on a daily basis until symptoms and parasitaemia were resolved. Weekly follow-up was done for assessing the clinical and parasitological effectiveness of the drug moieties. Analysis of the cumulative percentages of patients staying aparasitaemic throughout 28 days after therapy was done to compare information from previous and later research. As respondents stayed for the length of follow-up in the transmission region, the further appearance of the parasites due to failure of therapy or else a fresh contagion.This difference was made by adjusting the rate of for fresh contagion rate in the entire population earlier

1996 and by genotyping parasites after that date. Estimation of the rate of cure was accessible for each 6 months from 1990 to the end of 1999.

In-vitro and entomological studies:

Studies of in-vitro vulnerability began during 1995. Earlier information was accessible from peers working in the same area on in-vitro vulnerability of the isolates that are local. Standard radio-labeled assay for uptake and inhibition of hypoxanthine was used to test *P. falciparum* isolates.The isolates that were evaluated was mostly gathered from the patients residing in Maela. Entomological surveys were conducted between 1994 and 1999 during each rainy season. Men's team gathered landing of mosquitoes with torchlight and tubes on their arms and legs that were bare. Indoor and outdoor pairs of males were gathered in shifts from 1800 hours till midnight, from midnight till 600 hours indoors and from 500 hours till 700 hours outdoors in every station. Trained engineers recognized Anopheline mosquitoes on site. Analysis of sporozoite carriage was done by ELISAs from 28 of the 44 entomological studies by dissectingthe heads and thoraces of anopheline mosquito.

Statistical Analysis:

For Windows, the report analyzed SPSS information (version 8.0). Proportions are provided at a CI of 95%. The research analyzed the relationship among rate of occurrence in expecting females along with treatment rates from 1990 till 1997 through linear regression. The time pattern of the outcomes of in-vitro antimalarial drug vulnerability (IC50) was analyzed with Pearson's correlation coefficient on log transformed information per month. Interpreting the variations in occurrence of malaria and drug resistance is often confused due to a number of variables such as demographic movements, intensity of transmission heterogeneity, changes in using drug along with problems while assessing real occurrence and acquiring precise reaction information.

Results & Discussion:

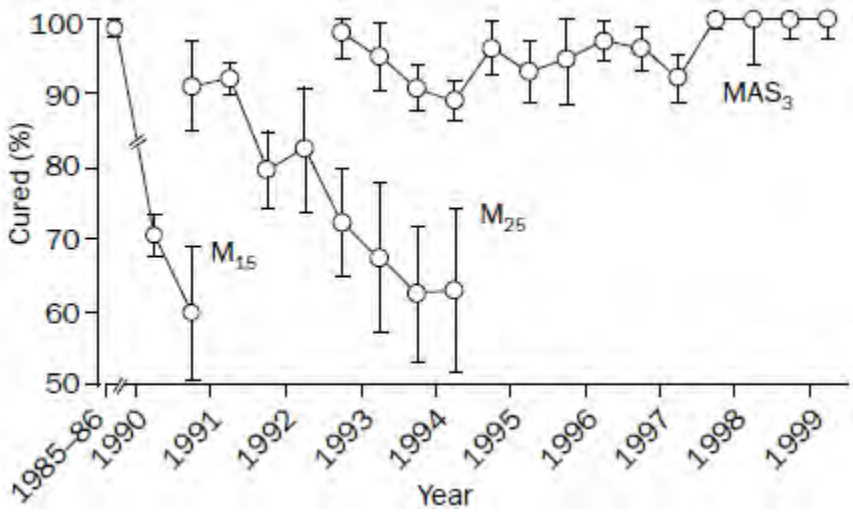


Figure 9: Increasing rates of cure (95% CI) for distinct schemes by potential research evaluated at day 28 M15 is denoted for mefloquine 15 mg/kg; M25 is denoted for mefloquinue 25 mg/kg; MAS3is denoted for mefloquine and artesunate combination.

Mefloquine efficacy (15 mg / kg) along with sulphadoxine pyrimethamine were originally 98 percent (95 percent CI 97–100) during 1985.However, by 1990, the rate of cure dropped to 71 percent (67–77). Mefloquine alone in a higher dose (25 mg / kg) was originally efficient at 90 percent, but between 1990 and 1994, efficacy decreased quickly.This decrease was followed by an increase in the percentage of people having gametocytaemia. Percentage of entire instances of *Plasmodium falciparum* detected in Shoklo who received treatment from the derivatives of artemisnin increased from 12·5% (235 of 1873) in 1992 to 56·0% (925 of 1638)by the middle of 1994.The rate of cure, estimated during 63 days after mefloquine therapy that was uncombined at an elevated dose, had dropped to 49 percent at that moment. In 16 percent of kids, life-threatening high-grade therapy errors happened. Monotherapy for Mefloqujne was then halted.Implementation of mefloquine-artesunate mixturefrom mid-1994 onwards, resulted in 100 percent efficacy until Shoklo was closed in early 1998 and no additional decrease in in-vivo efficacy was seen in Maela.

In- vitro susceptibility:

Table 5: In- vitro analysis of Plasmodium falciparum isolates susceptibility

Year	**Geometric mean (95% Cl)** **mefloquine IC_{50} (ng/mL)**
1995	
• Shloko (n=24)	47.2 (34.4-64.8)
• Maela (n=9)	56.4 (25.8-123.3)
1996 (n=46)	34.5 (27.0-44.2)
1997 (n=45)	31.3 (23.9-40.9)
1998 (n=54)	24.2 (19.7-29.7)
1999 (n=58)	25.4 (19.7-32.9)

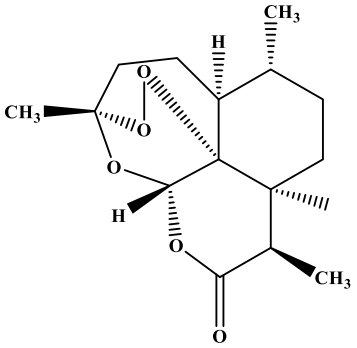
Children and colleagues used a morphology method in 1985 for assessment of the in-vitro vulnerability of *Plasmodium falciparum* isolates in this region, estimating that mefloquine IC50 was 2·86 ng / mL. During 1991 till 1994, mefloquine's geometric mean IC50 was 27·6 ng / mL (95% CI 21·0–36·0) for 44 Shoklo isolates evaluated by microdilution radioisotope method. Therefore, rise in the rate of inactivity of mefloquine had been connected with the growth of in vitro resistance. In 1995, a geometric mean mefloquine IC50 of 47·2 ng / mL (34·0–65·0) reported maximum values. Later 1995, mostly isolates were taken from near of Maela camp, where in mid-1994, combination of mefloquine with artesunate was familiarized and an identical kind of in-vitro vulnerability had been observed. Between 1995 and 1999, in-vitro resistance to mefloquine was dropped. In this research, early diagnosis and efficient mefloquine therapy were connected with the original decrease in occurrence of malaria caused by *Plasmodium falciparum* (1986–88). Subsequent increase in caseload was likely due to enhanced counteraction with an enhanced amount of recrudescing diseases along with diffusion from these diseases. Patients with recrudescent diseases in this society

were three times more probable than those whose main infections were healed to create patent gametocytaemia. This led in an adverse association among the occurrence of *Plasmodium falciparum* malaria and the rate of cure with resistant malaria parasite conduction preferential. For all instances of *Plasmodium falciparum* malaria, the overall implementation of the mixed artesunate with elevated dose mefloquine for three days had several implications. Cure rates have risen to over 90% and have since stayed even greater. Since 1998, there has been a 100 percent cure rate with this regime (95 percent CI 97–100).The very elevated rate of cure led in fewer recruiting diseases. The transmission benefit of resistant to mefloquine was reduced by the impact of artesunate on the bearing of gametocytes— a 9 times decrease in the concentration of gametocytes in main contagions comparing to an 18 times decrease in recrudescent mefloquine diseases.Since 1995, the in-vitro vulnerability of isolates of *Plasmodium falciparum* acquired in that region to mefloquine had risen considerably. The other diseases had been further triggered by parasites that were sensitive to mefloquine introduced into nearby camps.*Plasmodium vivax* had turned into the most prevalent class of malaria. Because emigration from the camps can not explain this shift, and anopheline mosquito vector figures had not changed, the impacts are ascribed to fresh and extremely efficient combination drug therapy for malaria. Fascinating decrease in occurrence of *Plasmodium falciparum* malaria were observed in other areas as well where approximately 120,000 individuals reside, where the mixture of mefloquine-artesunate was implemented.The mixture of artesunate and mefloquine is likely to be useful for controlling several epidemics of *Plasmodium falciparum* malaria in identical region is crucial in the spread of drug resistance. If early detection of the disease along with providing multidrug therapy was indulgent, malaria caused by *Plasmodium falciparum* would have reappeared quickly in the communities of the camp.Not all factors that contribute to the changes in *Plasmodium falciparum* contagion incidence had been recognized. Decreased occurrence of

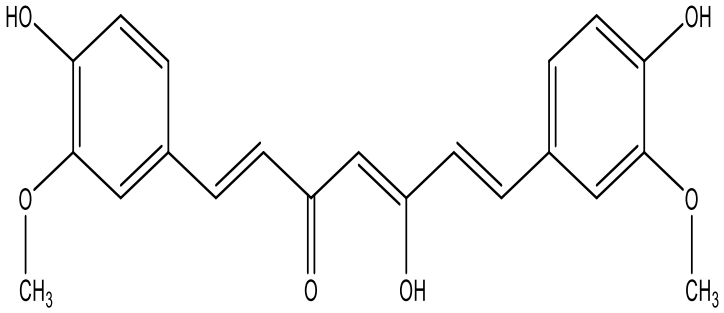
Plasmodium vivax malaria observed in Shoklo afterwards 1995 cannot be credited with usage of artesunate. Along with this, a number of unknown environmental variables had a chance of leading towards decline since then in *Plasmodium falciparum* and *Plasmodium vivax* malaria diseases, while using insecticide-impregnated bed nets accompanied the significant decline in malaria incidence rather than preceding it. Earlier evaluations in this region proposed only a peripheral advantage out of this technique of inhibition without any impact on occurrence of *Plasmodium vivax* malaria in kids.If this inference is correct, for this region of Thailand, these drugs are the mostly efficient controlling method. Additional research on these drugs are required for controlling the disease. However, positive impacts we describe on the incidence of malaria may not be generalizable to other fields of malaria. Using derivatives of artemisinin may possess slight or no impact on the occurrence of malaria in fields of elevated malaria transmission.Immune people, in spite of harboring the parasite's sexual and asexual phases, can stay symptom-free and untreated. Therefore, gametocytes reservoir is big and may be adequate to offset the decrease in transmissibility in patients who have received the treatment. However, combination therapies can relax the development of drug being resistant in these fields.While artemisinin derivatives can not safeguard the drugs for treating malaria with which these have been combined afterward the elimination of artemisinin in specific patient, they will decrease the chances of choosing mutants that are resistant to drug in original contagion along with decreasing the transfer of parasites that are resistant by increasing effectiveness against malaria and reducing the carriage of gametocytes. Most importantly, the mixture will give artemisinin derivative mutual protection from resistance choice. The researchsuggests that drugs for treating malaria should not be used on their own, rather should be used in conjunction with other molecules, to provide a safeguard to them from opposition emerging.

3.3 Artemisinin and curcumin combination as antimalarial therapy.

Curcumin is a natural product having a number of therapeutic properties. Curcumin possess less toxicity than many other drugs as it is obtained from natural source. A research was done for analyzing the efficacy of curcumin that was combined with artemisinin as an anti malarial therapy. For conducting this research,artemisinin and curcumin were bought from Sigma Chemicals, Bangalore, India (98 percent of curcuminoid content). Alpha, beta-Arteether (EMAL) created by the Central Drug Research Institute, Lucknow, India, was a kind donation from IPCA Laboratories Ltd, Mumbai, India (Nandakumar, Nagaraj, Vathsala, Rangarajan, & Padmanaban, 2006).



Artemisinin



Curcumin

Assay Method & Result:

In human O-positive washed erythrocytes, the *P. falciparum* FCK strain, a local chloroquine-resistant isolate, was grown using conventional protocols. Parasites were synchronized with 5% (wt / vol) D-sorbitol and crops were pooled and 0.15% (wt / vol) saponin produced from the erythrocyte for further processing.The 50% inhibitory levels (IC 50s) of artemisinin and curcumin had been detected by mea-surging [3 H] hypoxanthine intake into the fungus as a measure of feasibility in *P. falciparum* cultures.The formula used to calculate fractional inhibitory levels (FIC) is –

$$A_C / A_E + B_C / B_E$$

Where A_C and $B_C$denotes concentrations of A and B in a combination along with a specific extent of its consequence, e.g. IC 60 ; A E and B E are concentrations of A and B when used individually for providing the effect to the same extent, based on the interaction with an additive should result in 1 as value. GraphPad Prism 4 had been used for the study. FIC was obtained from graphs. Means ±. Using Microsoft Excel, normal deviations along with Student's t test were analyzed.

For in- vitro research, on day 0 Swiss mice (25 to 28 g) were given injection with *P. berghei*-infected mouse blood (60-70 percent parasitemia) intraperitonally, causing the animals to develop elevated parasitemia consequently die within 5-8 days. Alpha, beta-arteether artemisinin derivative was given as intramuscular injection at various doses during the first day. Up next, curcumin was given orally during first, second and third day (100 mg / kg body weight) in dimethyl sulfoxide. Notes were taken when the drugs were administered alone or in conjunction for external symptoms and mortality. On various days for parasitemia, tail vein blood had been evaluated using Giemsa for staining slides.Comprehensive trials show that IC 50 s are 45- 50 nM for artemisinin and 15- 18 µM, correspondingly. According to this, a number of artemisinin along with curcumin combinations had been used for generating dose-response curves, along with using data calculating the FIC (Adjuik et al. 2002; Sowunmi et al. 2005).

Table 6: calculation of FIC

Inhibition level	**FIC (mean ± SD)[a]**	***P* value**
IC_{60}	0.81 ± 0.06	<0.05
IC_{75}	0.79 ± 0.05	<0.05
IC_{80}	0.83 ± 0.06	<0.05
IC_{90}	0.78 ± 0.07	<0.01

The findings shown in the table show that the FIC is less than 1 at all ICs tested, and the findings are statistically important. The observed values were higher than 0.5, and in *P. falciparum* society the interaction between curcumin and artemisinin would be called non-synergistic.

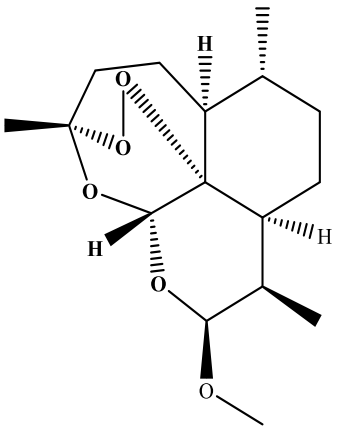
The effectiveness of the mixture of curcumin-artemisinin in the culture of *P. falciparum* resulted us to assess its in vivo effectiveness. The research shows that alpha, beta-arteether or curcumin monotherapy in the doses stated enhance the persistence of the mice infected with *Plasmodium berghei* (25 to 28 g). However, it did not provide full safeguard.So, while the animals infected with *P. berghei* die between 5 and 7 days, at 500, a single injection of alpha, beta- arteether at 750 µg and 1.5 mg results in animal deaths ranging from 9 to 11, 16 to 18 and 32 to 34 days respectively. Therapy with a mixture of alpha, beta-arteether, and curcumin, however, showed a good rate of survival. Three-days oral curcumin regimen with injecting one alpha injection, beta-arteether at 750µg or 1.5 mg per infected mouse resulted in full animal protection against revival with 100% survival.

The mixture of curcumin-artemisinin may be superior from multiple views. These are from natural long-standing use sources. Furthermore, curcumin current in a dietary supplement is not known to have any resistance. Artemisinin is at danger of developing resistance when commonly used as single therapy.Curcumin is tolerablein elevated doses and has been provided to cancer patients for up to 8 g / day for 3 months without toxic side effects of arteether in a combination with curcumin that provides complete protection in mice infected with *P. berghei*.If inferred to the human dose, the dose suggested for the mixture of artemisinin-lumefantrine would be almost one-third. Thus, the dose of artemisinin may reduce and the treatment price may reduce.Moreover, the efficacy of curcumin in conjunction with alpha, beta-arteether should be considered though curcumin possesses a decreased bioavailability in rodents and humans and fast metabolism. However, the fast clearance of

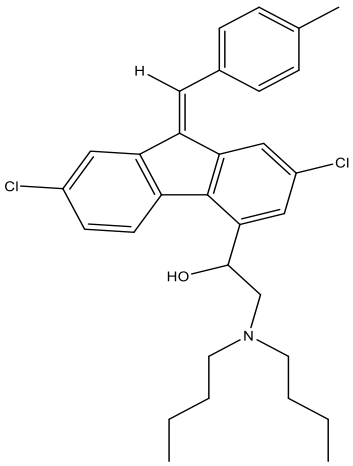
artemisinin and curcumin compensate the pharmacokinetic incompatibility issue. Bioavailability may, however, be improved. A small dose of pepper piperine can increase curcumin intake in hu- mans by 2,000 percent, and the mixture is well tolerated. Considering all these aspects, the artemisinin and curcumin combination is worthy of a thoughtful therapy for human trial for treating malaria.

3.4 Pharmacological & molecular bases of combination of artemether and lumefantrine and their therapeutic response in *Plasmodium falciparum* malaria that is resistant to multiple drugs.

Combining Artemether withlumefantrine is presently a commonly accessible treatment produced in a set dose preparing with 20 mg of artemether and 120 mg of lumefantrine per tablet. The fixed-dose scheme guarantees that parasites of malaria meet lumefantrine along with artemisnin and their metabolites thus offering a safeguard to prevent resistance against these two. However, lumefantrine is lipophilic in nature and this drug is absorbed in an erratic manner resulting in administration of the drug two times in a day. The WHO is now advocating a greater 6-dose scheme for treating malaria that is caused by*Plasmodium falciparum*in all fields, regardless of host immunity concentrations or multidrug resistance prevalence (Byakika-Kibwika et al., 2010).



Artemether



Lumefantrine

On Thailand's western boundary, resistance of *Plasmodium falciparum* malaria to various antimalarial drugs that include chloroquine, sulfadoxine-pyrimethamine, mefloquine, and halofantrine is observed.However, resistance to artemisinin derivatives has not been reported yet. The study identifies key function of pfmdr1 amplification in for the determination of the molecular foundation of resistance of mefloquine. Cross-resistance between lumefantrine, mefloquine, and halofantrine was noted in vitro, and a prevalent resistance mechanism was suggested by subsequent molecular research. The study gives a hypothesis that says, polymorphisms of pfmdr1 is likely to contribute to therapy failure after administration of artemisinin and lumefantrine combination therapy. This study evaluated the cumulative artemisinin- lumefantrine therapy experience at the Malaria Research Unit in Shoklo to explore the comparative effects of host, pharmacokinetic, and parasitological variables for the determination of therapeutic result after therapy with the combination of artemisnin and lumefantrine (Price et al., 2015).

Methods & Materials:

Study sites:

Studied patients were registered in three comparative studies at Malaria Research Unit in Shoklo. Patients came from four Karen groups residing on the northeastern frontier of Thailand in a malarious mountain forest. Malaria transmission in the region is very little and almost every infections with *Plasmodium falciparum* and *Plasmodium vivax* are identical. From 2000 till 2002, an additional patient cohort was also recruited at SMRU for monitoring the effectiveness of the 6-dose artemisinin and lumefantrine combination regimen clinically.

Patients:

From 1995 till 2002, patients infected with *Plasmodium falciparum* malaria had been hired for engaging in potential research chemotherapeutically. A complete clinical examination was finished upon admission, and blood was drawn for malaria parasitemia

quantitation.Combination of artemisinin and lumefantrine was given as tablet with a fixed dose. This contained artemether 20 mg and lumefantrine 120 mg per tablet. People whose weight ranged between 10–14 kg had received a single tablet in each dose and people whose weight rangedfrom 15 to 24 kg got two tablets in a dose. Besides, Three tablets per dose were given to people whose weight ranged from 25kg to 34 kg and 5 tablets in a dose was given to patients whose weight was above 35 kg.Three dosing regimens were selected for administration-

1. Artemisinin and lumefantrine 48 or AL48, a regimen having four doses given at 8, 24, and 48 hours along with admission.
2. Artemisinin and lumefantrine 60 or AL60, a regimen having six doses given at admission for a duration of three days and 8, 24, 36, 48, and 60 hours.
3. Artemisinin and lumefantrine 96 or AL96, a regimen having six doses given at entry for five days and 8, 24, 48, 72, and 96 hours.

In all of these cases, administration of the drug was detected and in case of vomiting before half an hour prior to the ingestion of dose, full dose administration had been read. If the patient vomits after ingestion during half an hour to one hour, repetition of half of the dose was done. Observation of each patient was done carefullyevery day after registration till resolution of symptoms along with parasitemia were resolved. Next, follow up was performed every week until 42 days for assessing the clinical and parasitological efficacy of the given drugs.

P. falciparum isolation along with drug susceptibility test:

P. falciparum fresh isolates were regularly obtained and evaluated for in vitro drug susceptibility, for the patients engaged in AL studies clinicallywithadditional chemotherapy research undertaken from 2001.Evaluations were conducted during 4 to 8 hours of gathering,

with no previous cryopreservation, inhibition of ^{3}H-hypoxanthine with measurement of quality outlined somewhere else.

Collecting the samples:

Samples of blood were gathered from all patients on Whatman filter paper (3MM) during starting of the survey. However, the day of parasite reappearance for patients experiencing therapy failure was observed as well. Patients enrolled in 2 of the research also gathered venous blood samples to identify the lumefantrine pharmacokinetic profile.Blood was taken into heparinized tubes by venepunture and centrifuged for 15 min at 1000 g. Plasma had been transferred to polypropylene tubes immediately afterwards along with storing at -70 ° C till the shipment. Lumefantrine was evaluated with a detection limit of 40 ng / mL in plasma using high-performance liquid chromatography with UV detection.DNA was available on a filter spot from whole blood samples (in heparinized tubes storing at -70 ° C) or from 50 μL capillary blood, and was extracted as elsewhere mentioned.

Statistical Analysis:

Evaluation of data was done using Windows (SPSS) software. Proportions were examined using the correction of Yates Š2 test or the precise test of Fisher. Evaluation of suboptimal lumefantrine concentrations on the seventh day was done. The connection between genotypic information and clinical reaction to therapy had been assessed through survival analysis including the patients missed to follow up, They had reappearance of the infection but had not been considered as failures to the treatment. Results were adapted for individuals for whom PCR was not feasible or indeterminant based on the temporal likelihoods of recrudescence and reinfection determined from values for patients having full information. Failure levels were calculated using the cumulative incidence rate at day 42 and contrasted using the log rank test of Mantel-Haenszel. For multi variable analysis, modeling of Cox proportional risks was used. Because age (a host immunity surrogate marker) and baseline

parasitemia in this research site are reliable determinants of this therapeutic reaction, both have always been included in the model.

Result:

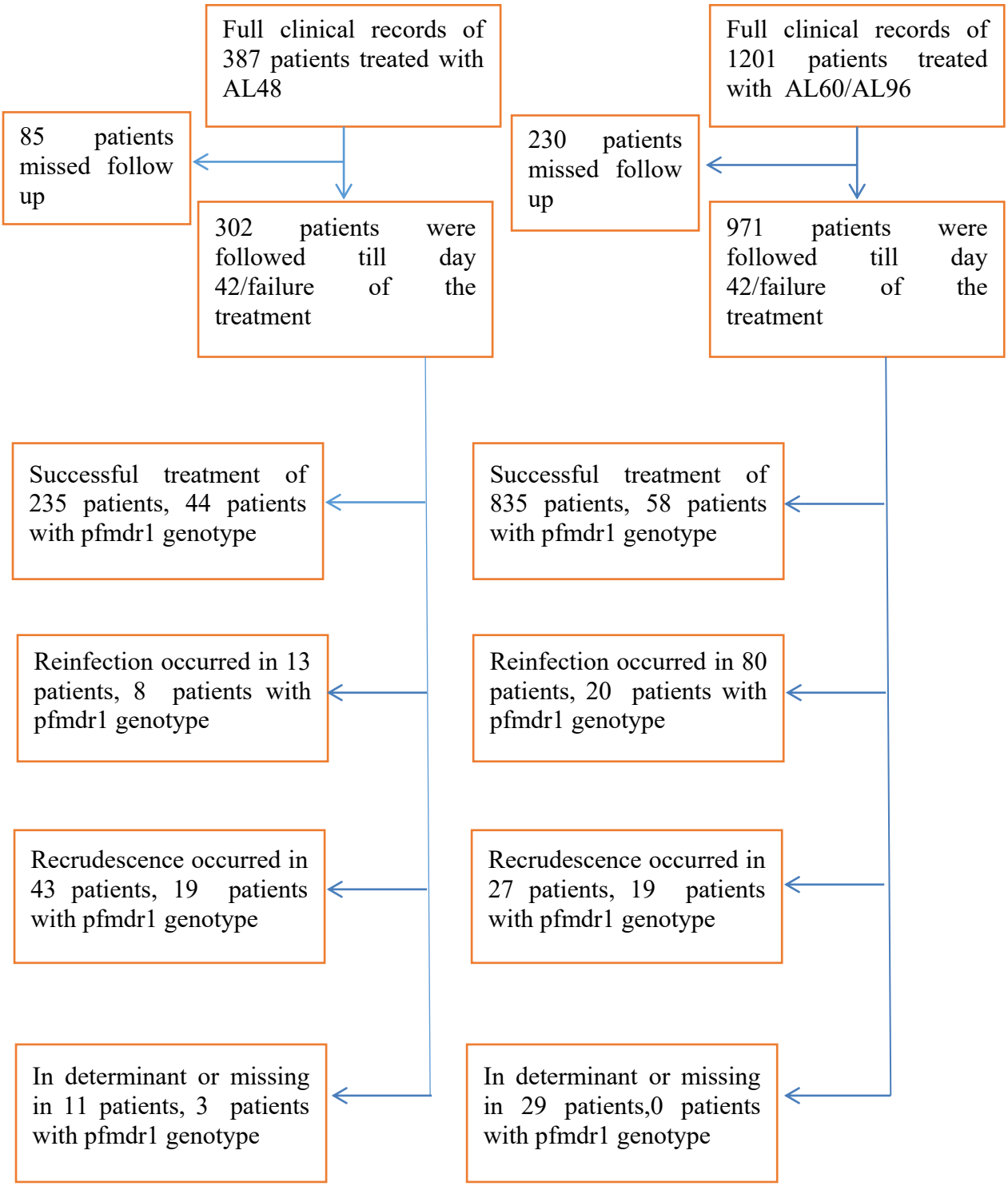


Figure10: Schematic representation of Artemether- lumefantrine 4 doses regimen above 48 hours, AL60, 6 doses regimen of AL above 60 hours; AL96, 6 doses regimen of AL above 96 hours

1588 patients in total suffering from uncomplicated *Plasmodium falciparum* malaria were registered for the research between December 1995 and June 2002 and were given a full course of artemether- lumefantrine therapy. Among them, 43% patients had been hired for comparison research and some of the remaining 901 patients were registered in routine monitoring of AL's therapy effectiveness. A total of 387 patients were given a four doses regimen above 48 hours (Artemether- lumefantrine 48), 1114 patients were given a six doses regimen above 60 hours (Artemether- lumefantrine 60), and 87 patients were given a 96 hours (Artemether- lumefantrine 96) six doses regimen. Overall, of 1,588 patients, 1273 (80%) finished at least 42 days of follow-up.

All of these three treatment schemes were endured in satisfactory manner. Alongside, 1406 (91%) of 1551 patients had parasitemia clearance within 48 hours. Defervescence was acquired by 554 (95%) of 584 patients. Besides, among 1587 patients, 18 patients had vomiting within one hour afterward the first dose of artemether- lumefantrine on the day of admission.In kids < 5 years of age (4 [9.1%] of 44 kids), the incidence of vomiting during one hour after absorption of the first dose had been greater compared to kids whose age ranged between 5 years to 14 years (19 [4.3%] of 441 kids) and grownups (8 [0.7%] of 1103 adolescents ; P = .001).Early vomiting was also 4.1 times greater in patients with fever (95 percent CI, 1.7–9.9-fold) and 5 times greater in patients who have experienced vomiting earlier (95 percent CI, 2.5–10.2-fold).

Observation of the treatment :

Overall, within 7 days of therapy, no patients encountered early therapy failure or parasitemia reappearance. The cumulative threat of failure by day 42 was 20% in AL48 group (95% CI, 15 percent –24 percent), 13.1 percent in the AL60 group (95% CI, 11 percent –15 percent) and 3.9% in AL96 group (95 percent CI, 0 percent –9.6 percent) (P=.001).On 163 (80

percent) of 203 combined isolates, three loci genotypes were effectively conducted. PCR-corrected failure levels with 42nd day were 13.3 percent (95 percent CI, 9.6 percent –17 percent) after completion of the AL48 scheme, 3.2% (95% CI, 1.8 percent –4.6 percent) after completion of the AL60 scheme, and 0 percent after completing the AL96 scheme (whole P<.001). Average recrudescence period was 21 days after completing the four-dose regimen and 27 days after completing the six-dose regimen. The cumulative rate of reinfection within 42 days of completion of a regimen was 10.5 percent (95 percent CI, 8.7 percent –12.3 percent).However, no significant changes could be seen among the two treatment groups.

Artemisinin based combination therapy attains its antimalarial impact by an original fast decrease in parasite biomass that is attributive to short-acting but extremely powerful artemether, along with subsequent elimination of the residual parasites by lumefantrine which is naturally less active but more slowly removed.The originally suggested four doses regimen had association with excessively elevated rate of recrudescence; consequently, dosage recommendations were altered to six doses above 60 hours, a regimen that cures disease in greater than 95% patients.The region under thetime curve of concentration of plasma lumefantrine (AUC) is the most significant determinant of therapeutic reaction in these research, and the plasma lumefantrine level on the seventh day has been shown as an useful AUC surrogate. An experience of vomiting was a single clinical factor associated with later failure, that has an association with a fourteen percent decrease in level of lumefantrine in plasma in day 7. Though a six doses regimen had better accuracy in reaching appropriate concentrations of lumefantrine, rate of recrudescence risen from 1.2 percent to 13 percent in the seventh day levels had been found to be less than 175 ng/mL.

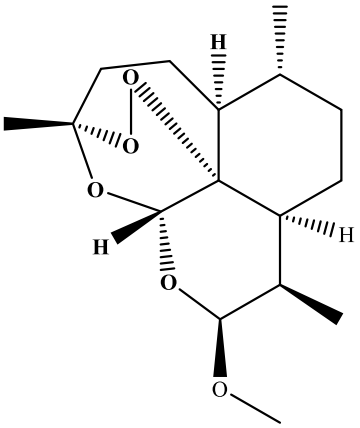
Artemether's longer course probably decreased the original biomass of the parasite to adequate concentrations to eliminate the infection through reliably greater concentrations of lumefantrine.The 6-dose scheme therefore offers an adequate amount of drug for overcoming

even parasites that have partial resistance, while the four doses scheme does not provide adequate concentrations of lumefantrine for overcoming parasites that are sensitives.

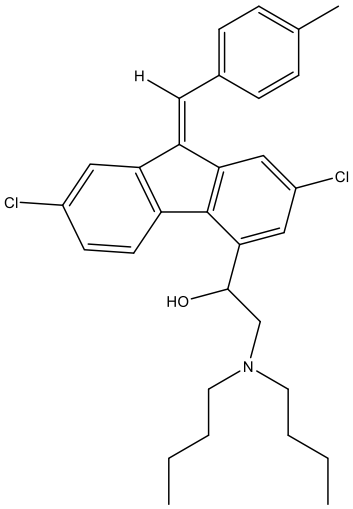
To conclude, it can be said that, the 6-dose coartemether regimen is a secure and efficient therapy of *Plasmodium falciparum* malaria, even in fields with predominant resistant to a number of drug isolates. Though lower concentrations of lumefantrine were associated with an enhanced danger of therapy failure at day 7, these concentrations happened in less than 10 percent patients who received a six doses regimen, that ensures the rate of cure surpassed 96 percent.

3.5 Comparison of clinical effectiveness of combination of artemether with lumefantrine and chloroquine for treating *Plasmodium vivax* in Thailand.

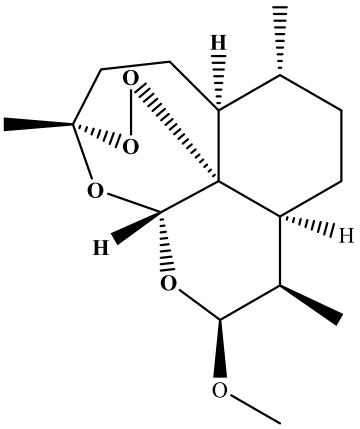
The aim of this research was to discover alternative schemes to treat the two species of malaria using falciparum antimalarial drugs.Patients infected with *Plasmodium vivax* malaria, in case of female, they were not pregnant before enrollment in this research, positive only asexual forms of *Plasmodium vivax* in blood smears, weight = 40 kg and age = 15 years, were registered in the research that was conducted for 28 days. The research omitted patients with serious lack of nutrition, pregnancy and lactation, concurrent febrile disease that would affect follow-up (Krudsood et al., 2007).



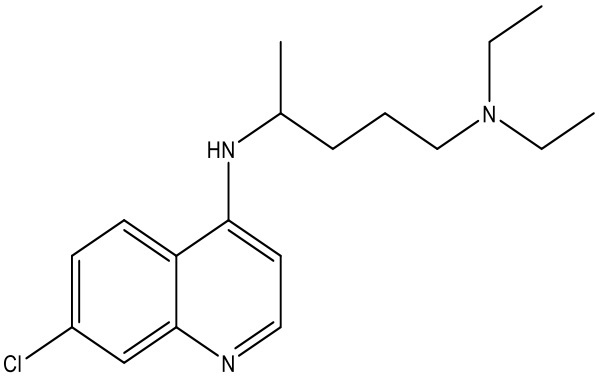
Artemether



Lumefantrine



Artemether



Chloroquine

Selected patients were subjected to 1 of these treatments as follows-

- Group I artemether and lumefantrine four tablets, each tablet that contains artemether 20 mg with lumefantrine 120 mg administered by oral route at 0, 8, 24, 32, 48 and 60 hours so 24 tablets in total, next 15 mg of primaquine daily in adults for 14 days,
- Group II chloroquine 25 mg base per kg administered above3 days followed by primaquine as group I.

In addition, primaquine was not provided to patients with G6PD deficiency. Demographic baseline information including full blood count, status of G6PD and biochemistry were acquired on admission.

Clinical evaluation was conducted on a daily basis and axillary temperature was registered and blood smears were examined on entry and every 12 hours till parasite clearance and then on days 3, 7, 14, 21 and 28 respectively.Treatment failure was described as -

I. Clinical worsening after therapy because of *Plasmodium vivax* disease requiring hospitalization in the presence of parasitemia,

II. Presence of parasitemia and axillary temperature at any moment from day three to day twenty eight.

III. Existence of parasitemia on any day from day seven to the twenty eighth day, regardless of clinical circumstances.

Results:

Table 7: Interpretation of therapeutic responses

	1st Group Artemether- lumefantrine9 (n= 47)	2nd Group Chloroquine (n= 51)
Number of patients who missed follow- up	9 (19.1%)	9 (18%)
Number of patients with 28 days follow up	38 (80.9%)	42 (82%)
Number (percentage) at day 28	37 (97.4%)	42 (100%)
Fever clearance time (hour) Mean (SD)	21.8 (11.2)	25.3 (10.8)
Range	4-70	4-90
Parasite clearance time (hour) Mean (SD)	41.6 (7.2)	55.8 (7.8)
Range	14-71	23-106

Of the 98 patients, 47 were assigned for the treatment with artemether and lumefantrine and 51 were treated with chloroquine. Eighteen patients did not participate in follow-up for the group treated with artemether-lumefantrine on days 15, 16, 16, 21, 21, 23, 25, 26 and 27 and for the group treated with chloroquine on days 13, 16, 16, 23, 23, 23, 26, 26 and 27 that is shown in the above table. Only one therapy failure was reported on day 26 in the group receiving artemether-lumefantrine therapy. Another patient in the group receiving artemether-lumefantrine combination was diagnosed with Day 1 infection with *Plasmodium falciparum* and was effectively cured for both species on Day 28. In the chloroquine-treated group, the fever clearance time and the parasite clearance time were mildly longer (21.8 hours versus 25.3 hours, $P = 0.12$; and 41.6 hours versus 55.8 hours, $P < 0.01$).

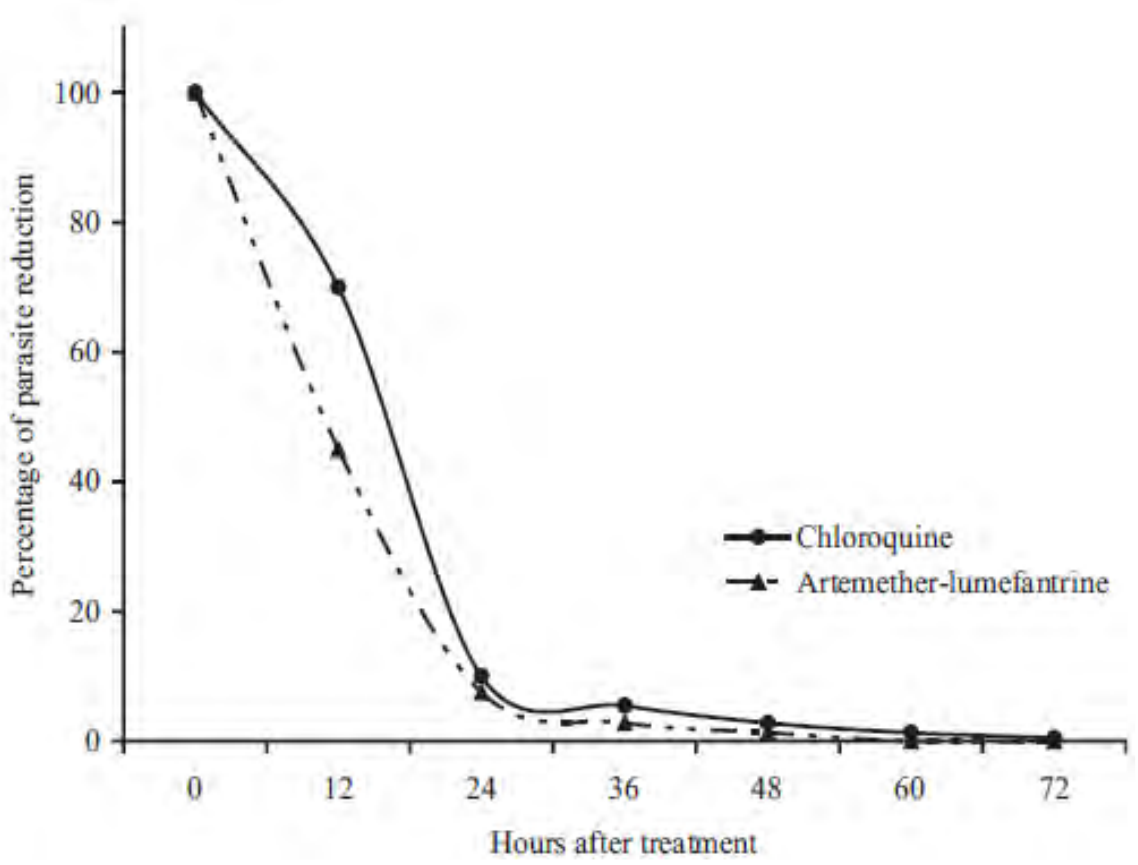
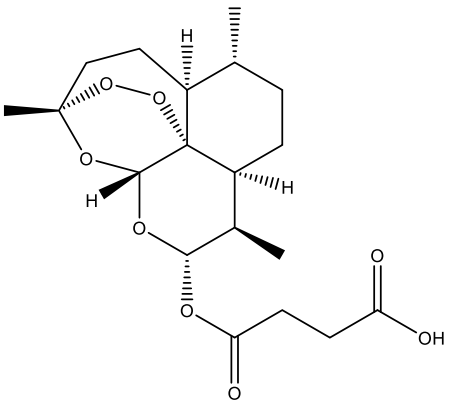


Figure 11: Parasite reduction percentage for chloroquine and artemether- lumefantrine combination

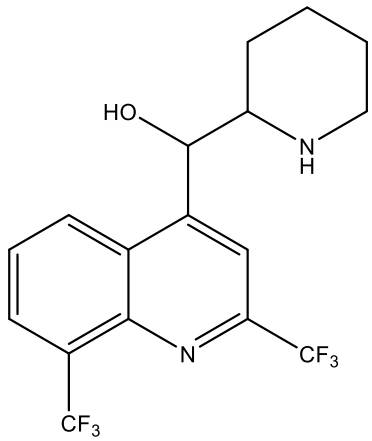
In the above figure, parasite reduction percentage for chloroquine and combination of artemether- lumefantrine is shown and it can be interpreted that the parasite clearance time for artemether- lumefantrine combination is less than the parasite clearance time taken by chloroquine. Moreover, both of these treatments were tolerable for the selected patient groups. In addition, some side effects were observed in the artemether-lumefantrine group such as headache that was found in 4.3% patients, dizziness in 4.3%, and vomiting was observed in 2.1% patients. This observation failed to distinguish clinical signs along with symptoms of malaria and symptoms that are related to drugs. Nevertheless, the severity of these side effects were very slight to reasonable. There was no severe adverse event discovered.

3.6 Comparing Artesunate and mefloquine combination with artemether and lumefantrine combination for treating multidrug resistant malaria caused by *Plasmodium falciparum* on Thailand's western frontier.

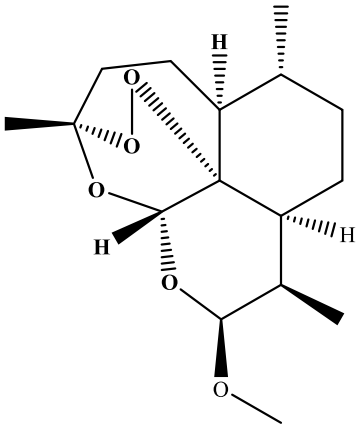
The study is an open label, two armed study that was performed for assessment of the effectiveness of the six-dose artemether-lumefantrine mixture provided above 3 days to treat uncomplicated infections of *P. falciparum* in adults and kids on Thailand's western frontier (Hutagalung et al., 2005).



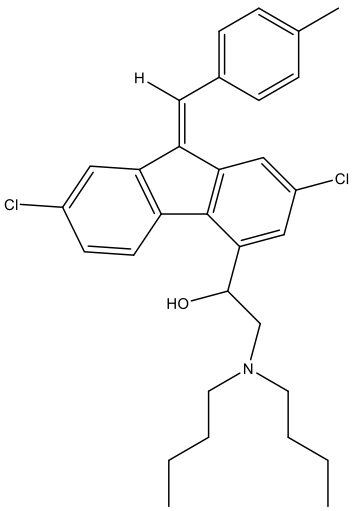
Artesunate



Mefloquine



Artemether



Lumefantrine

Assay Methods & patients:

Patients were hired from two communities: Karen ethnic minority displaced persons residing on Thailand's western boundary. This is an environment where *Plasmodium vivax* and multidrug-resistant *Plasmodium falciparum* transmission is small and unsteady. Among the selected patients, none were pregnant and did not receive mefloquine over past 63 days, and any serious disease, serious or complex malaria were not reported.They were given either artemether-lumefantrine or mefloquine- artesunate six-dose regimen. Patients had been observed and blood smears had been withdrawn regularly until they were aparasitaemic, followed by a weekly collection that continued up to six weeks. Then on Giemsa-stained dense and thin blood movies weekly count of parasite were determined. After excluding the episodes on entry and during therapy, the person-gametocyte weeks were calculated per 1,000 person- weeks.

Drug regimens:

Tablets were obtained at 0 and 8 hours and two times in a day for the following two days by patients assigned to the artemether-lumefantrine group (ALN). Artemether along with lumefantrine was given as tablet where each of the tablets had artemether 20 mg and lumefantrine 120 mg. Doses were given according to the weight of the body. The least dosage was a single tablet in each dose for patients whose weight was below 15 kg; patients between 15 and 24 kg were given 2 tablets, patients having a weight that ranged within 25 and 34 kg were given 3 tablets patients with weight that is over 35 kg were given four tablets in a dose.

Measured outcome:

The main therapeutic result measure in this research was the incidence of day 42 in both treatment groups of microscopically and genotypically confirmed recrudescent of diseases. The instant therapy reactions such as clearance of parasites, clearance

of fever, occurrence of adverse events, and severity of anaemia were secondary considerations. Calculation of size of the sample had been done to identify a 7% difference in rate of failure between the two regimens with 90% CI and 80% authority assuming a 20% decrease.

Statistical analysis:

For Windows, version 11, data were analyzed using SPSS. Categorical data were compared, as appropriate, using the Chi-square test with the correction of Yates or the exact test of Fisher. Continuous variables corresponding to a normal distribution were compared using the Student t test.Log-transformed or contrasted data not normally distributed using the Mann-Whitney U test. Cross-tabulations were used to calculate the relative risks. Treatment organizations compared the rates of negative occurrences at three distinct phases (days 1–2, days 3–7, and days 14–42). The events were counted only once for each of the three periods.Survival analysis assessed the PCR-adjusted treatment rates and compared them using the log-rank test. The meaning level (p) was set at 0.05 for all statistical tests.

Clinical & parasitological findings:

The original reactions were comparable in both the groups. No patient had serious malaria. Upon admission, 55.0% (133/242) of ALN patients and 57.9% (140/242) of MAS3 patients had a tympanic temperature of approximately 37.5 ° C. During3rd day (2 in ALN and 1 in MAS3), all but three patients had a standard temperature.

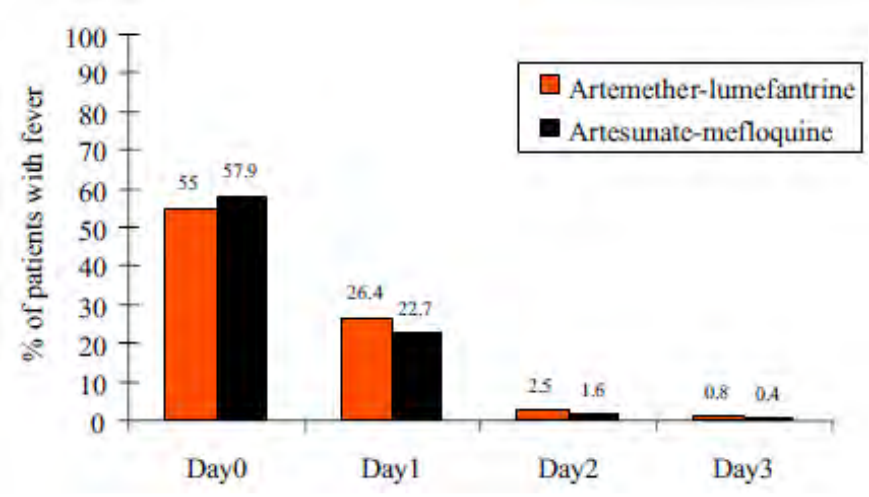


Figure 12: (%) of patients having fever

There was no distinction among the two groups shown in figure 12 in the time of fever clearance. Parasite clearance times were short and by the second day most patients cleared their parasitaemia shown in the above figure.

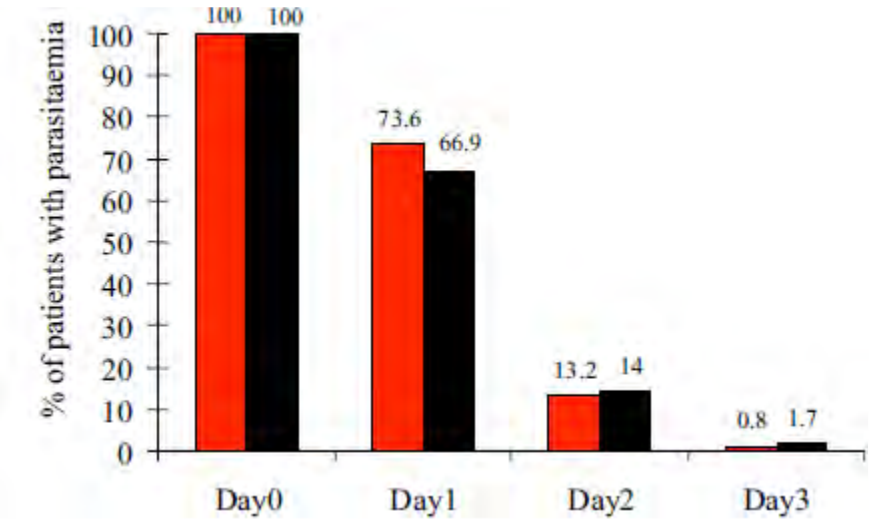


Figure 13: Patients having positive slide for asexual Plasmodium falciparum shown in (%)

The above figure indicates the proportion of asexual *P. falciparum* patients in the two groups receiving the treatment with favorable slide. Four (1.8%) of 227 patients in the recipients of artemether-lumefantrine and three (1.3%) of 238 recipients of artesunate-mefloquine had a favorable blood film ($P > 0.05$) by day three. Here, 12.3% patients on admission were anaemic (haematocrit < 30%), 10.8% in the ALN group and 13.8% in the MAS3 group ($P= 0.33$). In the group getting MAS3, the average (SD) reduction in the haematocrit value at day

7 from baseline was higher than in the ALN group: 9.3% (SD,11.5%; 95% CI, 7.7% to 10.9%) compared to 6.7% (SD, 11.4%; 95% CI, 5.1 to 8.3%) respectively (P= 0.023)

Table 8: Patients Response to the treatment; ALN= Artemether- lumefantrine combination, MAS3= Artesunate-mefloquine combination

Treatment Group	**ALN (n= 245)**	**MAS3 (n= 245)**
Compliance no. (%)		
Completed day 7	241 (99.6%)	240 (99.2%)
Completed day 28	232 (95.9%)	233 (96.3%)
Completed day 42	225 (93%)	227 (93.4%)
Cumulative proportion of patients with clinical failure, no (%)		
Day 7	0 (0)	0 (0)
Day 28	13 (5.6)	14 (6)
Day 42	27 (12)	24 (10.6)
PCR no.		
Novel	23	14
Recrudescent	2	8
Novel+ recrudescent	1	1
Indeterminate/ missing	1	1
PCR/ adjusted cure rates no. (%)		
Day 7	0 (100)	0 (100)
Day 28	2 (99.1)	9 (96.1)
Day 42	3 (98.8)	9 (96.3)

There were 27 fresh infections of *P. falciparum* among artemether-lumefantrine and 24 among artesunate-mefloquine recipients (P > 0.05) during the 42-day follow-up period shown

in the above table. The mean age was 13.6 years in patients with therapy failure (n = 12 ; SD = 8.5) and 23.7 years in patients with successful therapy failure (n = 438 ; SD = 15.2).

Other parasitological findings:

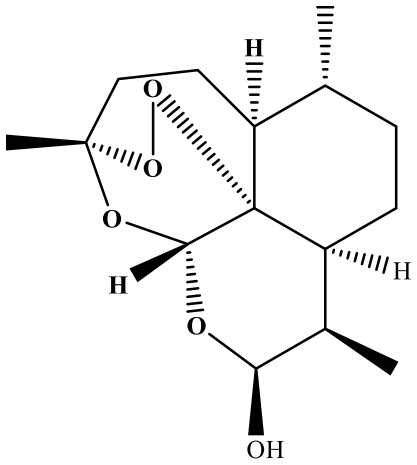
During follow-up, 119 (26.3%) of the 452 patients had identified parasitemia caused by *Plasmodium vivax*. In the MAS3 group (29 out of 227) there were considerably less instances of vivax malaria than in the ALN group (90 out of 225) ($P < 0.001$). In the MAS3 group (40 days; range, 13–43 days) the average time for *P. vivax* parasitaemia to appear was longer than in the ALN group (28 days; range, 14–43 days; $P<0.001$).In the artemether-lumefantrine group, twenty patients (8.3 percent) and 19 (7.9 percent) in the artesunate-mefloquine group had gametocytes identified in the first three days. With the exception of one patient in the ALN group, gametocytes were removed within the first week of therapy. Gametocytes created in 1.2 percent (3/241) of the ALN group and 1.3 (3/240) of the MAS3 group (between day 7 and 42) after exclusion of these. The levels of the person-gametocyte were small and comparable for both groups: 2.7 (95% CI, 0.6–7.8) per 1000 person-weeks.

Both treatments in this research quickly and reliably cleansed fever and parasitaemia. Both treatments have been well tolerated and have been extremely efficient. Importantly 2/3 fewer *Plasmodium vivax* infections and 12 days longer average time for *Plasmodium vivax*parasitaemia to appear in the MAS3 group were most likely due to the longer mefloquine terminal half-life compared to lumefantrine.

3.7 Research on the impacts of antimalarial drug dihydroartemisinin (DHA) on embryos of rat; in vivo and in vitro.

The impacts of dihydroartemisinin (DHA) on whole rat embryo crops (WEC) exposed in vitro on GD9.5–11.5 to DHA at 0.01–2 µg / mL for the whole 48 h culture or 1.5 h at the start or end of the crop were revealed in a research. DHA has been researched as the prevalent bio active metabolite of all artemisinin derivatives in use and due to its increased inherent

antimalarial activity hence DHA mainly impacted red blood cells (RBCs) with greater levels and longer exposure inhibiting angiogenesis during yolk sac hematopoiesis. An in vitro research was performed in order to evaluate the effects of RBC harm on mortality and dysmorphogenesis throughout gestation.Pregnant rats were subjected to DHA on GD9.5 and 10.5 and then evaluated embryos during gestation on separate days (Longo et al., 2006). Doses of 15 mg / kg / day and 7.5 mg / day were chosen to be embryolethal and 50% embryolethal. The choice of doses was based on WHO / TDR information.



Dihydroartemisinin

The studies showed a high dose-response with artesunate between 10 (15% resorptions) and 15–17 mg / kg (100% resorptions) when directed on GD10; comparable findings were obtained by 15 mg / kg artesunate (38μmol/kg) to 11.1 mg / kg DHA (38 μmol/kg). Studies of DHA provided from GD6 to 16 to pregnant rats showed a comparable dose-response between doses of approximately ≤10 and approximately ≥20 mg / kg / day.

Blood samples were taken half an hour after dosing in rats at the expected peak concentration (Tmax) time for evaluating drug exposure. Absorption and removal are very fast; after giving either artesunate or DHA, $t_{1/2}$ for DHA is 50–80 min for artesunate / DHA in malaria patients.The in vivo research was supplemented by a parallel study using the WECmodel to evaluate the potential role of reactive oxygen species in DHA-induced developmental toxicity

by evaluating glutathione (GSH) in visceral yolk sac, appropriate embryo and embryonic RBCs.During the 48 h culture, embryos and embryonic blood were also gathered at separate time points to observe potential DHA impacts by electron microscopy on Wolffian blood islands and embryonic RBCs. Using the in vitro model prevented maternal blood cells from interfering with or contaminating embryonic RBCs.

Methods & Materials:

DHA (artenimol, Holleykin, Guanzhou, China) was administered at 7.5 and 15 mg / kg / day suspended in 0.5% Methocel + 1% between 80 mg / day in vivo research. Suspension concentrations were respectively 0.75 and 1.5 mg / mL. Administration quantity was 10 mL / kg.DHA was mixed in DMSO (Sigma) in the in vivo research at levels of 0.05 and 0.1 mg / mL immediately prior to use, and then diluted 1:1000 in the crop medium (added to the crop medium in 5 μL DHA solution quantity in 5 mL culture medium) to achieve final levels of 0.05 and 0.1 μg / mL.

In vivo study:

On Days 9.5 and 10.5 of pregnancy, DHA was given orally one time everyday to pregnant rats.The controlled ones received the vehicle alone. The control group included 14, 16 and 12 pregnant women, 7.5 mg/ kg/ day and 15 mg / kg / day, respectively. The clinical indications were noted regularly. For each animal, body weight was registered. During gestation and at term, animals were cesarean-sectioned at distinct times. After 30 minutes of giving the first dose (consistent with the highest plasma concentration moment for DHA in rats), blood samples of the first five animals discovered to be sperm-positive were gathered from tail veins into heparinized tubes in each therapy group.Plasma was disconnected from the entire blood right after by centrifugation at 10 000 g for 2 minutes, at 4 degree centigrade, moved to adequately marked plastic cryotubes and stored at −80 degree centigrade until thawed for DHA analysis. For each animal, a single sample was taken to prevent interference with the

pregnancy course.DHA plasma levels were evaluated using a high-performance liquid chromatography (HPLC) technique using a reduction mode electrochemical detector. The difference between day and day was 2.6% and 8.3% at 30 ng / mL and 4.9% and 5.9% at 60 ng / mL. DHA plasma levels were evaluated using a high-performance liquid chromatography (HPLC) technique using a reduction mode electrochemical detector. The difference between day and day was 2.6% and 8.3% at 30 ng / mL and 4.9% and 5.9% at 60 ng / mL.The quantification threshold for spiked plasma samples was 4.0 ng / mL, corresponding to a three-fold baseline noise peak at 0.005 full-scale absorbance unit.

Statistical analysis:

Statistical analysis was conducted using one-way ANOVA with multiple comparisons or chi-square testing of Tukey on litter information gathered at GD20. The mean level was set at $P < 0.05$.

In vitro investigation:

Embryos were collected from pregnant female rats (9.5 days, one to three somites) and grown in 25mLglass bottles (five embryos per bottle) comprising 5mL of heat-inactivated sterile rat serum and antibiotics (penicillin 100 IU / mL, streptomycin 100µg / mL).On GD9.5 DHA has been added to the culture medium. During 48 hours culture, the medium was not renewed. Culture was conducted for 48 hours and the medium was the same throughout.

Results & Discussion:

In control livestock and dams treated with either dose of DHA, no signs of maternal toxicity were noted in the in vivo study. No obvious changes in uterine blood flow were noted in livestock treated with DHA at cesareansection. As in vitro and in the lack of maternal anemia, the aim of DHA in vivo was primitive RBCs generated during yolk sac hematopoiesis; all embryos exposed in utero lived to GD13. The results show that the resulting anemia results in tissue hypoxia and cell damage, which may be either diffuse or

focal, depending on its length and severity, and may result in embryonic death.Some embryos / fetuses, however, survive with or without sequelae. Morphological changes and/or congenital malformations may happen at the moment of harm as a consequence of the most delicate organs.When it affects embryonic cells that are either not yet engaged or can overcome the incident, it is possible to repair the cell damage and the concept grows usually. On the contrary, a series of dysmorphogenetic occurrences may result in focal harm affecting cells that are engaged at that moment or with little plasticity.Successful proliferation and migration of cells are critical to the growth of embryos. The study shows that after exposure to DHA, at least two types of cells can not overcome huge cell death. These are

1. Pulmonary neural crest cells
2. Mesenchyme cell condensations that form pre-cartilage cells.

For ordinary limb development, there are two main stages such as-

- Relocation of undistinguishable mesenchymal cells to the site of upcoming skeletogenesis in adequate numbers
- Collaboration with epithelial cells that leads toward mesenchyma condensation.

Macroscopic changes of limb and girdle bones may occur from the absence of a critical concentration of "condensed mesenchyme" at future locations of skeletogenesis, that can be result from surplus death of cell owing to anemia and hypoxia in a critical period of development.

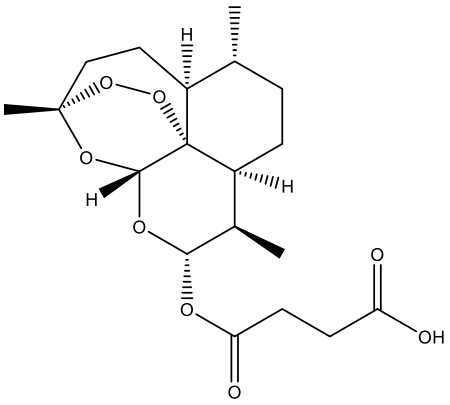
The research confirms DHA's fast initiation of action on primitive RBCs once they join circulation. There is no distinct elucidation of the primitive RBC's susceptibility to DHA. Adult erythrocytes are metabolically inactive, cannot be divided, anti-oxidant defense packed cells with lower attraction to artemisinin that focus and affect parasites of intra-erythrocytic malaria.Falciparum malaria contracted during pregnancy is a severe danger to the mother's and her child's life, thus requiring efficient treatment.The embryotoxic impacts obtained in

animal research have not been reported in humans to date, despite proof of embryolethality and dysmorphogenesis in animals. Data from a restricted amount of pregnant females exposed to artemisinin derivatives, including a tiny amount in the first trimester, indicate no proof of defects or delays in development.

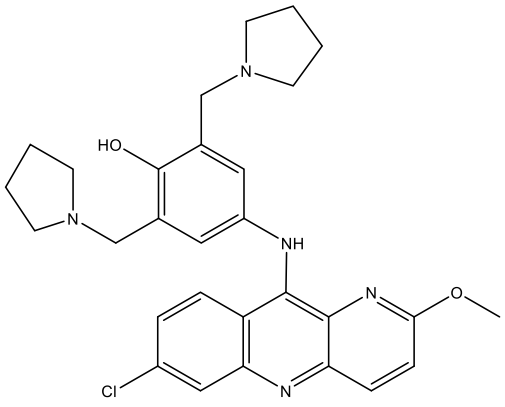
To conclude, it can be said that, artemisinin mixtures are extremely effective antimalarials and are essential for malaria therapy, yet cannot be referred for initial pregnant females. Together with other information, these in vitro and in vivo information inform the choice of appropriate animal models and the design of research to elucidate the human condition well.

3.8 Combining Pyronaridine-Artesunate in a fixed dose to treat malaria in Gabon's pediatric patients.

Pyronaridine is effectively used in Eastern Asia as antimalarial monotherapy and is presently being marketed in China as oral and injectable formulations. Consequently, the rationale for therapy with a mixture of fixed-dose pyronaridine-artesunate is to obtain both fast symptomatic relief and elevated cure rates and delay drug resistance growth. As kids are mainly impacted by malaria, this research explored pediatric patients to determine the most suitable dose of pyronaridine-artesunate in 3 distinct tablet preparations and in a new preparation of granules(Ramharter et al., 2008).



Artesunate



Pyronaridine

The main goals are the determination of-

1. Pharmacokinetics aspect of the drug of the

2. Bioavailability of the co- formulation of granules

3. How much safe and tolerable all of these doses are.

Materials & Methods:

The open labeled research was intended for tablet co-formulations of combination of three distinct fixed-dose pyronaridine-artesunate — having 6:2, 9:3, and 12:4 mg / kg ratios of pyronaridine tetraphosphate: artesunate.At day 28, the polymerase chain reaction (PCR)–modified appropriate clinical and parasitological response (ACPR) rate aided as the main concluding idea of effectiveness in perprotocol (PP) assessment and as the secondary effectiveness result in changes in the intention-to-treat (ITT) assessment.15 patients were included from the four cohorts each. The sample size was selected in this research to achieve minimum 12 patients for every dose rate that can be evaluatedfor main result measures.

Next, to assess the co- formulation of granules, a protocol extension was introduced. This drug formulation was created precisely for the therapy of kids who are younger in order to boost the drug's tolerability; the dose of 9:3-mg / kg pyronaridine: artesunate had been assessed in 15 individuals based on prior outcomes. This was done for comparing bioavailability of the tablet and granule co-formulations. The patients participated in this study were infected with *Plasmodium falciparum* malaria, between the age group of 2 to 14 years and with a body weight of 10 to 40 kilogram and were tolerable to oral therapy. Pregnancy and malnutrition are the factors that were absent in all these patients. The first Pyronaridine- artesunate combination given was 6:2 mg/kg followed by increasing the dose to 9:3 mg/ kg and then 12:4 mg/ kg sequentially. Three groups named group A, B and C were designed where the co-formulations having pyronaridine tetraphosphate 48 mg and artesunate 16 mg, pyronaridine tetraphosphate 72 mg and artesunate 24 mg and pyronaridine

tetraphosphate 96 mg and artesunate 32 mg were given respectively to the three groups. In another group named group D, a sachet containing pyronaridine tetraphosphate 160 mg and artesunate 20 mg was given after masking with granules. All the above preparations were given till three days on a regular basis with liquid along with follow up till 42nd day.

Pharmacokinetic study was done for pyronaridine, artesunate and the main active metabolite dihydroartemisinin. For this study, 1 mL blood was withdrawn earlier administering the regimen along with 0.25, 0.5, 1, 1.5, 2.5, 4, 8 and 12 hours after administering the 1st drug followed by before administering the 2nd and 3rd ones, after 24 hours of in taking the 3rdregimen that is for pyronaridine, after 168, 336, 504 hours of starting treatment. Liquid chromatography was used for assessment of the plasma concentration of artesunate, dihydroartemisinin and pyronaridine. Mass spectrometric technique was used alongside for assessing the plasma concentration of artesunate and dihydroartemisinin. Recrudescent and freshly acquired infections were compared with merozoite surface antigen (MSA). DNA was withdrawn from the spots of blood.

Statistical Analysis:

On the grounds of a predefined data validation scheme, data precision was verified. For quantitative factors, standard descriptive statistics were used and rates and ratios were used for categorical factors (SAS version 8.2). Using precise 95 percent Pearson-Clopper confidence intervals, ACPR was summarized. Analysis of survival of Kaplan-Meier analyzed parasite clearance times.

Results & Discussion:

Among the sixty patients, fifteen were allocated to each level of dose along with distinguishable baseline features and PP analysis eliminated 11 patients.

Table 9: Adverse Drug Event (AE) experienced by the patients

	Group A: 6:2 mg/kg tablets (n=14)	Group B: 9:3 mg/kg tablets (n=15)	Group C: 12:4 mg/kg tablets (n=15)	Group D: 9:3 mg/kg granules (n=15)
Total Data				
Any at least possibly AE	13 (93)	11 (73)	13 (87)	12 (80)
Drug related AE	5 (36)	4 (27)	5 (33)	3 (20)
Serious AE	2 (14)	0 (0)	0 (0)	0 (0)
Any definite AE				
Infection	9 (64)	8 (53)	10 (67)	11 (73)
GI Disorder	7 (50)	3 (20)	3 (20)	1 (7)
Headache	5 (36)	1 (7)	2 (13)	1 (7)
Fatigue/ pyrexia	2 (14)	2 (23)	1 (7)	2 (13)
Cough	2 (14)	1 (7)	2 (13)	1 (7)
Splenomegaly or hepatomegaly	1 (7)	2 (23)	3 (20)	1 (7)
Anorexia	2 (14)	0 (0)	1 (7)	1 (7)
Any possibly drug related AE				
GI Disorder	3 (21)	2 (13)	2 (13)	1 (7)
Fatigue /pyrexia Splenomegaly/ hepatomegaly	1 (7) 0 (0)	1 (7) 1 (7)	0 (0) 2 (13)	1 (7) 0 (0)
Anemia	1 (7)	0 (0)	0 (0)	0 (0)
Anorexia	1 (7)	0 (0)	0 (0)	1 (7)
Headache	1 (7)	0 (0)	0 (0)	1 (7)
Hyperhidrosis	0 (0)	0 (0)	1 (7)	0 (0)

Here, between 73 to 93 percent patients suffered from minimum one adverse event while running the course. However, all of these adverse events were slight to reasonably intensified. Parasitemia was not found while following up of the patients in the 3rd week. In the hematological analysis, the 1st 72 hours of treatment induced a decline in median hemoglobin. Moreover, an increase in the count of platelet along with eosinophil had been observed throughout whereas biochemical factors remain unchanged.

Pharmacokinetic assay:

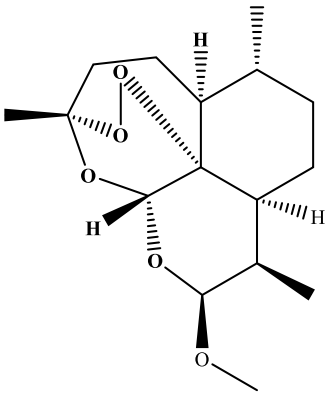
For artesunate, the mean maximum plasma concentration (Cmax) as well as the mean $AUC_{0-\infty}$ either increased in a dose-dependent manner from 93 ng / mL to 287 ng / mL / h and from 104 to 232 ng / mL / h, respectively ; the time for Cmax was 0.5–1.0 h, and the end half-life was 0.5–1.2 h.Dihydroartemisinine, the main active metabolite of artesunate, showed a slight rise in both Cmax, from 479 to 1186 ng / mL, and AUC, from 1055 to 2961 ng / mL / h; after administration, the time for Cmax was 1.3–1.7 h. The pharmacokinetic parameters of the co-formulation of granules were contrasted with those of the same dose tablet formulation.There were no statistically significant variations in patients receiving granulated co-formulation except for a greater Cmax of pyronaridine.

Efficacy:

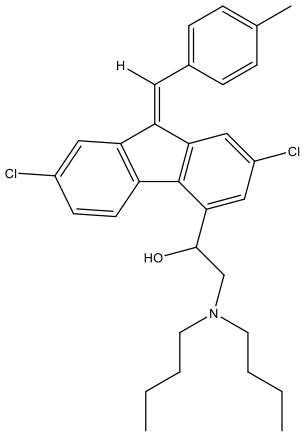
Initially, none of the patients faced failure of the treatment. In 14 patients *Plasmodium falciparum* malaria reappeared along with reappearance of *Plasmodium ovale* malaria in 1 patient in the 39th day. Efficacy corrected by polymerase chain reaction at 42nd day was found to be in between 89 to 100 percent in all the four groups with a lesser time for clearing the parasite.

3.9 High effectiveness of two combinations of artemether-lumefantrine along with artesunate- amodiaquine in Ibadan, Nigeria.

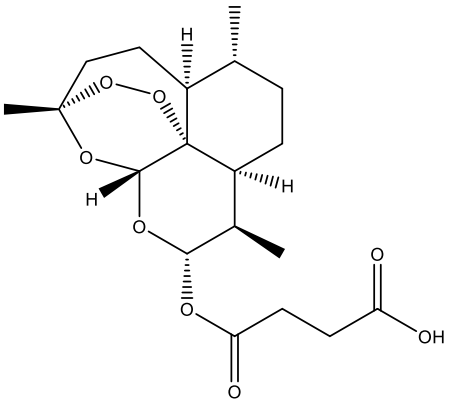
Lumafantrine can be categorized as an aryl amino alcohol with high lipophilicity. The combined product of artemether- lumefantrine provides a slower but enhanced antimalarial effect(Lefevre et al. 2001). If this combination is given for 2 to 3 days to the patient, results with an increased rate of cure with a decreased fever and parasite reduction time. However, the combination of AL was first intended to be registered as a four doses regimen, six doses regimen has been preferred over it.Along with this, combination of artesunate and amodiaquine that is a 4 aminoquinoline has shown essicacy against malaria.The research assessed the comparative effectiveness and safety of artemether- lumefantrine and artesunate-amodiaquine combination. (Falade et al., 2008).



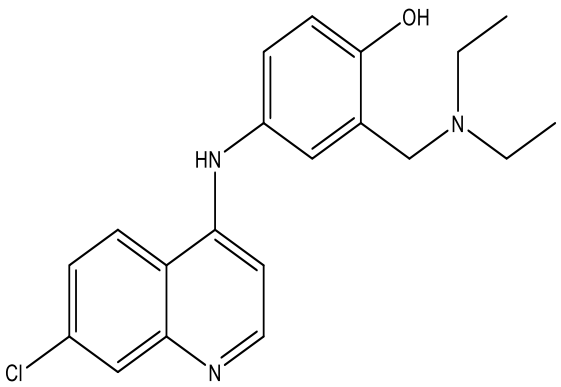
Artemether



Lumefantrine



Artesunate



Amodiaquine

Assay method & patients:

The research was designed as an open labeled and well-ordered clinical trial enrolling boys and girls of 6 months to 10 years having symptoms of acute malaria that is not complicated with a temperature of ≥37.5 degree centigrade and confirming the presence of parasite.

Size of sample:

Calculation of the size of the sample was done using the latest antimalarial drug evaluation rules of the WHO (WHO 2003) that calculated size of sample of 100 patients with 50 patients in each of the treatment groups. In order to create a minimum sample size of 120, an extra 20% of the calculated figure was introduced to compensate for patients who missed follow-up or removed from the research.The calculation was based on the assumption of a clinical cure rate of 94 percent for AL and 85 percent for ASAQ. This provides 9 percent of the therapy effect size. At 95 percent CI, the accuracy level was taken as 10 percent.

Allocation of treatment with case management:

Selected children were subjected to one of the two treatments. Artesunate was given one time in a day for 3 days at a dose of 4 mg ⁄ kg body weight, and amodiaquine was administered once in a day for 3 days at a dose of 10 mg amodiaquine base ⁄ kg body weight.Group 2 randomized patients got AL for 3 days twice daily. There were artemether 20 mg and

lumefantrine 120 mg in each AL tablet. Kids whose weight ranged from 5 to < 15 kg were given 1 tablet, whose weight ranged from 15 to < 25 kg were given 2 tablets, while whose weight ranged from 25 to < 35 kg were given 3 tablets two times in a day for 3 days.Patients were noted for 30 min after drug administration. Re-administration of the drugs was done in case of vomiting within the timeframe. Furthermore, febrile kids with temperature ranging from ≥38 degree centigradewere given paracetamol orally.

Follow up of patients:

During zero to seven days and on days 14, 21 and 28, respondents of the study were followed up on a daily basis. The vital signs of each patients were noted. Additionally, dense blood films had been prepared from blood samples followed by staining with 10% giesmsa stain. Then this was examined with light microscope having magnification of ×1000. Molecular analysis was done at day 28. On days 7 and 28, venepuncture took 5 ml venous blood for analyzing full blood count and blood chemistry.

Statistical analysis:

Data gathered were registered for evaluation in Case Record Forms and entered the database for evaluation in Epi-info version 6.04 along with using SPSS version 10. Data efficacy assessment was performed for the purpose of treating populations and by protocol. Based on Kaplan – Meier product-limit estimates of failure, cumulative study of existence among the these groups was contrasted. Means and normal deviations (±SD) were contrasted by comparing sizes with chi- square. Using the combined t-test, results of haematology and liver enzymes were analyzed as well. Arithmetical values had been provided as ± SD, p-values < 0.05.

Results:

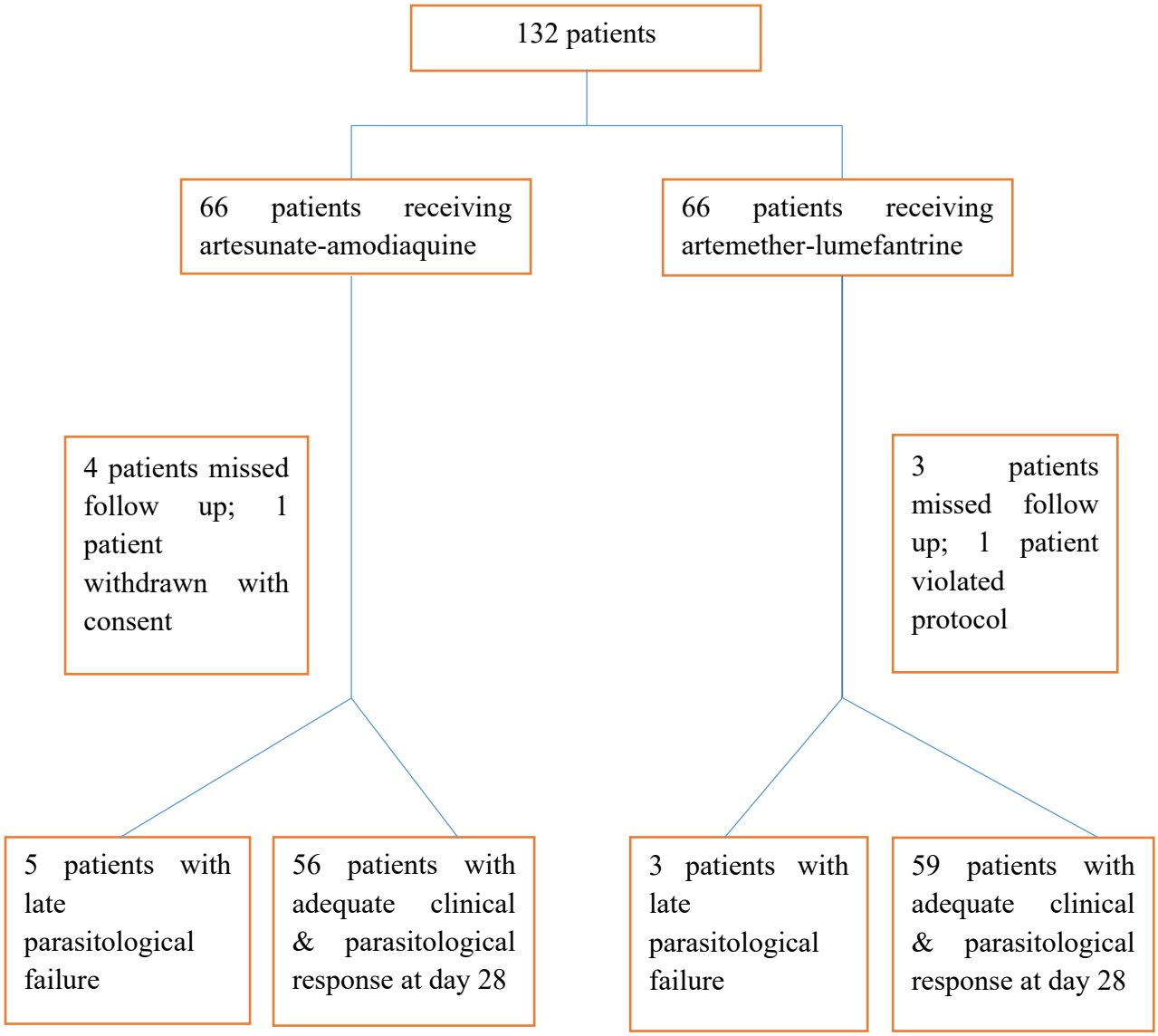


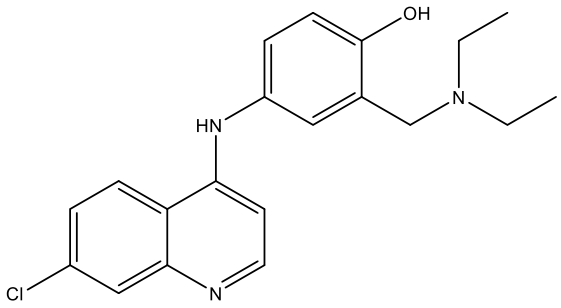
Figure14: Study profile

The research included a total of 132 patients where 61 males and 71 female patients.Artemeher- lumefantrine (AL) and artesunate- amodiaquine (ASAQ) was given to 66 kids. Nine patients were removed from the research along with seven patients in total who lost to follow-up. Fever (100%), vomiting (51%) and abdominal pain (35%) were prevalent complaints. All of these drugs were tolerable. 5 of 66 (7.6%) ASAQ-treated kids and 4/66 (6.1%) AL-treated kids had vomiting the medicine within 30 minutes as a result, were re-dose. None of them had vomiting after re-dose. 93 percent parasite was reduced after

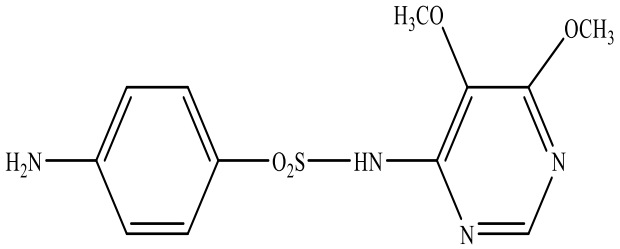
induction of ASAQ treatment and 95% reduction of parasite was observed for AL therapy within 24 hours. However, anemia, cough and abdominal pain was observed as adverse effect during the treatment. The two artemisinin based combination demostrated a good and well tolerated safety profile for treating uncomplicated malaria.

3.10 Efficacy of amodiaquine, sulphadoxine- pyrimethamine and their mixture to treat uncomplicated malaria caused by *Plasmodium falciparum* in kids in Cameroon at the moment of policy change in combination therapy based on artemisinin.

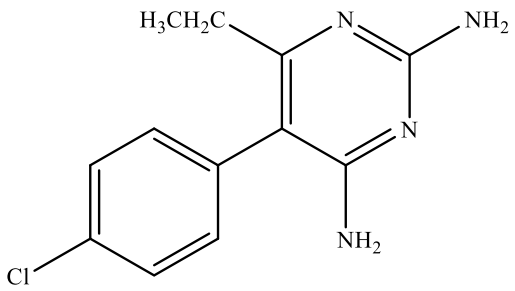
In 2004-2006, a tri-arm, double-blind, study for determining effectiveness and safety of AQ, SP, and AQ+SP for the therapy of *P. falciparum* malaria that is not complicated was performed in selected kids of Cameroon to assess the output of SP, AQ, and mixture in Cameroon and to establish a standard for tracking resistance evolution (Mbacham et al., 2010).



Amodiaquine



Sulphardoxine



Pyrimethamine

Assay method:

Doses were given according to the schedule of a dose/ weight. To control fever, all kids were provided a dose of paracetamol, 250 mg, half an hour before the trial drug was provided. Amodiaquine- SP was given either as a monotherapy or as a mixture of 10 mg / kg / day amodiaquine for 3 days and/or half a 250 mg sulphadoxine and 12.5 mg pyrimethamine tablet for 10 kg SP body weight and assessment was done on 3, 7, 14 and 28th day and was categorized as early treatment failure, late clinical failure, late parasitological failure or adequate clinical and parasitological response.

Statistical analysis:

Microsoft access was used as database. An Exploratory Data Analysis (EDA) was performed for providing key trend and dispersion measurements. In some cases, the Mantel Haenszel test had been performed as well. The Kruskal Wallis test had been performed in case of continuous variables (e.g. parasitaemia) that are not distributed in a normal manner. MicroSoft Excel 2003 had been used for creating graphs.

Result:

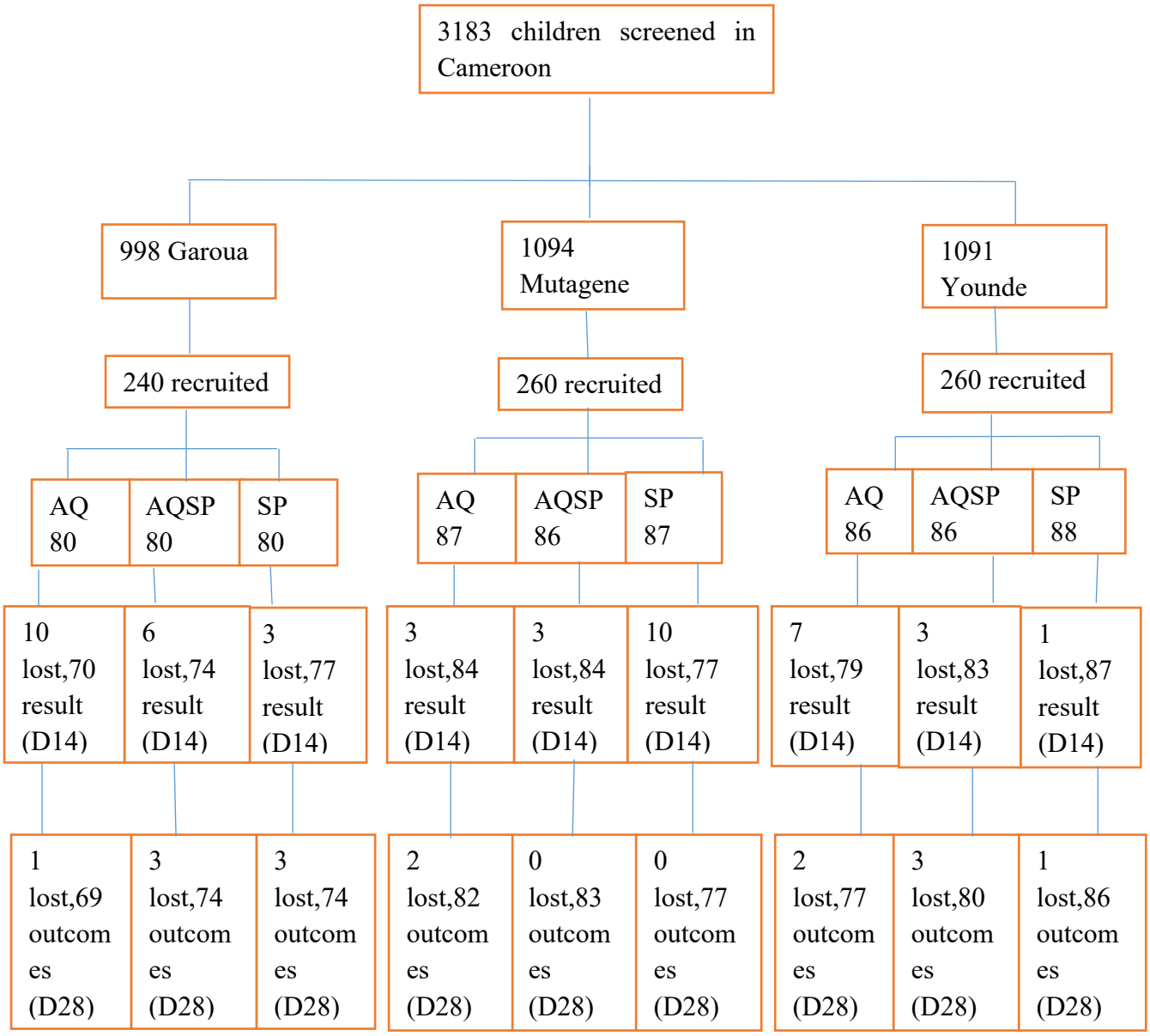


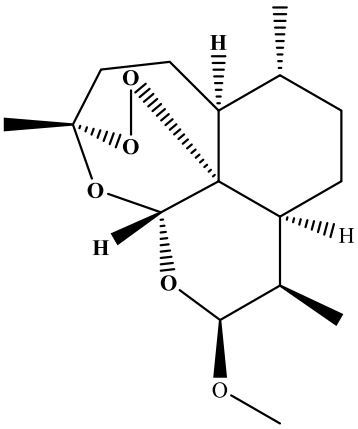
Figure 15: Study design

This study showed that SP, AQ and AQ+SP were not sufficiently efficient in treating uncomplicated falciparum malaria in Cameroon between 2004 and 2006; SP failed more often than AQ or AQ+SP. In the research, SP, AQ and the mixture of these two had shown good tolerability with the primary side effects being fatigue was reported in kids receiving AQ resolving by day 14 and Day 28.The study showed that artesunate-

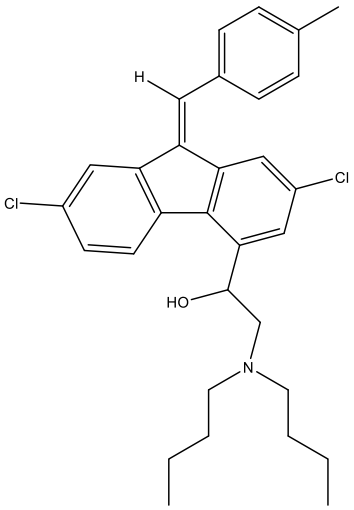
amodiaquinecombination is highly effective and tolerable in Cameroon and elsewhere, either as a co-blister or as a fixed dose mixture (ASAQ) and caused no adverse events.

3.11 Oral combination therapy of artemether- benflumetol (CGP 56697) with mefloquine for treating acute *Plasmodium falciparum* malaria in Thailand.

This study has been designed as a double-blind test that includes 252 adult patients. The patients received treatment with either CGP 56697 or mefloquine, CGP 56697, a fresh oral fixed mixture of artemether and benflumetol. This research disclosed that in Thailand, CGP 56697 is efficient against*Plasmodium falciparum* malaria that is resistant against multiple drugs. However, greater doses are likely to be required to enhance the rate of cure (Looareesuwan et al., 1999).



Artemether



Benflumetol

Patients & assay methods:

Each patient was examined prior to the beginning of therapy. Routine hematology and biochemical studies, including glucose-6-phosphate dehydrogenase have been conducted.

Complete count of blood, serum electrolytes, complete and direct bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, globulin, aspartate and alanine aminotransferases, and urinalysis included pretreatment studies. On days 1, 3, 7, and 28, the analysis were repeated.On days 1, 3, 7 and 28, thorough daily tests and repeated electrocardiograms were performed for determining adverse effects. After beginning therapy during the first 72 hours, then for the first seven days and weekly for four weeks, parasitological examination of dense blood movies was performed every 6 hours. Malaria parasite counts/microliter was determined using Giemsa-stained thick and thin films to calculate the RBC for a thin film or WBC for a thick film.

Dosage and administration:

Each tablet had been comprised of artemether 20 mg along with benflumetol 120 mg and a four dosage regimen was given to each of the participant. The first dose was given at 0 hours, followed by 8, 24 and 48 hours. Consequently, individual doses comprised of artemether 80 mg along with benflumetol 480 mg. Therefore, artemether 320 mg and benflumetol 1920 mg was provided above 48 hours of the treatment period in a whole. Additionally, mefloquine was administered in a normal course of therapy: each tablet contained 250 mg base, five tablets with three (750 mg) administered at 0 hour and two (500 mg) administered for next 8 hours therefore 1250 mg therapy course as a whole.

Outcome measure:

Treatment reaction was marked by

1) Cure frequency of 28 days

2) Clearance time for parasites (PCT)

3) Clearance time for fever (FCT)

4) Clearance time for gametocytes.

At the beginning of therapy till the initial moment blood movies were negative, the time of clearance of the parasites stayed negative for the following 48 hours.Time for clearance of fever was the moment after the beginning of the therapy until the temperature dropped to 37.58C and stayed below that temperature for the next 48 hours.The rate of cure at 28-day was described in the 28-day follow-up as the lack of recrudescence

The dosage of CGP 56697 tested in the trial has great tolerability, particularly with respect to vomiting, safety profile, and fast intervention to clear blood parasites, indicates a very promising fresh malaria combination therapy.

Chapter 4

Conclusion

This review emphasizes on need for new therapeutic approach for the treatment of malaria. As artemisinin is the only available drug for treating malaria, this study focused on combining some other molecules with artemisinin that can enhance the efficacy of the drug. Moreover, artemisinin is likely to become resistant in some of the regions of the world. However, resistance to artemisinin is spreading all over the world rapidly as a result of which, a new therapeutic approach for treating malaria has become compulsory. Since no other medication for treating malaria is not available, artemisinin based combination has become the noble approach for treating malaria. This review includes several in- vivo and in-vitro analysis along with the outcomes of some clinical trials done with artemisinin based combination therapy. This will facilitate the researchers as well as the students in finding the authentic data from the clinical outcomes and interpret a possible conclusion from the results found. We further plan to conduct some experiment by combining drug molecules with artemisinin and its derivatives as antimalarial therapy for determination of the efficacy and safety profile of the drug.

Chapter 5

Future Prospects

Artemisinin based combination therapy has been proved to be an effective treatment against malaria. However, very few investigations have been performed so far for analyzing the efficacy of artemisinin based combination therapy. Various effective combinations have been developed that has not drawn much attention and very few research has been conducted. Moreover, combination of artemisinin derivatives with other molecules has also shown positive outcome in some cases. The future prospect of this study involves detailed investigation of those effective combination through in- vivo and in- vitro assay and providing more evidence of the efficacy of artemisinin based combination therapy so that the therapy can reach to clinical trial and thus providing an effective means of avoiding artemisinin resistance.

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