A REVIEW ABOUT MALARIA AND ITS TREATMENT

Swapnil Jadhav* and Neha Shinde

SVKM's Dr. Bhanuben Nanavati College of Pharmacy, India.

1. INTRODUCTION
The issue that is faced by most of the developing and under developed nations which are in tropical and sub-Saharan Africa is malaria, preying almost 250 million people and causing over 800,000 deaths each year. Most of the cases observed are in children under five years and the occurrence is more in pregnant women. (Dondorp A M et al, 2009) Most of the time death is caused due to infection from Plasmodium falciparum. In developed nation such as United States, the cases of malaria is less common affecting only 1300-1500 people per year. 10% of these cases which suffer from life-threatening end-organ damage is treated by IV quinidine gluconate, which has shown better efficacy but its regular use has shown side-effects. (Aregawi M et al, 2009.; Kortepeter M et al, 1998) The three species of malarial parasite-P.falciparum, P.vivax and P.malariae have shown resistance to antimalarial drugs. (World Health Organization. Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000–2010) Resistance to chloroquine (CQ) was first reported in less than twenty years from its introduction in 20th century for treatment against P.falciparum. Then antifolate combination therapy was used which utilised sulfadoxine and pyrimethamine (SP) combination but soon resistance was developed to this combination therapy too. (Young, M.D et al, 1961 ; Harinasuta, T et al, 1962 ; Peters, W. 1987; Roper, C et al, 2004) P. falciparum resistance to the old drug therapy has led to the discovery of new drugs called artemisinin-based combination therapies (ACTs) for malaria control. (Enserink, M, 2007) The basic reason for use of ACTs is to combine any long half-life drug such as mefloquine, amodiaquine, piperaquine, pyrimethamine/sulfadoxine or lumefantrine with fast acting drug artemisinin, which results in achieving effective and rapid eradication of malarial parasite. (World Health Organization, Roll Back Malaria Department, Guidelines for the Treatment of Malaria, 2006.) However, resistance to ACTs is also emerging since parasites started showing resistance towards the long half-life drugs used in combination. (Phyo A.P et
al, 2012) Hence, there is a need of new chemical compounds, which can overcome the mechanism of resistance, in malarial therapy.

2. Life Cycle of Malaria Parasite
Malaria parasite infects and develops both in human as well as in female *Anopheles* mosquito.

Life cycle of malaria can be divided in two stages:
1. Asexual Stage: Human Liver Stage and Human Blood Cell Stage
2. Sexual Stage: Occurs in female *Anopheles* mosquito.

Asexual Stage

Human Liver Stage

- Malaria infection or life cycle begins when a female *Anopheles* mosquito bites a human and suck his blood, in that process mosquito injects the saliva containing sporozoites into the human body.
- These injected sporozoites travel quickly to the liver where they multiply in the liver cells and develop into merozoites.
- These merozoites are then released in the form vesicles (known as schizonts) which travel to the lungs where they burst into the capillaries, spreading merozites into blood for its development in Human Blood Cell Stage/ Erythrocytic stage.
- However, in *P. vivax* and *P. ovale*, a dormant stage of malarial parasite known as hypnozoites persists in the liver cells which can cause relapse, weeks or years later after the treatment, by invading into the erythrocytes.

Human Blood Cell Stage

- The merozoites, when they enter into circulatory system, invade red blood cells where they grow and multiply asexually until the erythrocyte or red blood cell burst resulting in increase in the number of merozoites to attack other red blood cells. Thus, repetition of this cycle, of breaking free of merozoites and attacking fresh red blood cells, causes fever and other clinical manifestations.
- During maturation in the red blood cell, the parasite insert its phospholipid and proteins in the red blood cell membrane which causes host’s hemoglobin to enter into parasite’s food vacuole where it is digested providing source of aminoacids. The free haem which is obtained after digestion of haemoglobin is toxic to the parasite; hence, the parasite
renders it inactive by polymerizing it into haemozoin which is catalyzed by enzyme haem polymerase.

- Some of the merozoites which enter erythrocytes undergo multiplication to form gametocytes, two forms -- male (microgametocytes) and female (macrogametocytes).
- These gametes so formed are then taken up by the female Anopheles mosquito in her blood sucking or blood meal event.

Sexual Stage

- This sporogonic stage occurs in female Anopheles mosquito where parasite develops sexually.
- The microgametes penetrate into macrogametes forming zygotes in the mosquito’s stomach; this zygote later become elongated and motile forming ookinetes which invade through the mosquito’s midgut wall and develops into oocysts.
- The oocysts so formed, matures and develop thousands of sporozoites inside itself which eventually, ruptures, releasing sporozoites in the mosquito’s body.
- These sporozoites then travel to the salivary glands of mosquito which on injection or during blood meal event begins the new malarial cycle by infecting the human host. (Baer K et al, 2007)

![Figure 1: Life Cycle of Malaria Parasite](http://www.cdc.gov/malaria/about/biology/)
3. Possible targets for treatment of malaria

To prevent malaria infection, a drug must be able to act on the following possible clinical targets, in this case, the various forms of malarial parasite which is developing in human host. Thus an antimalarial drug might act on-

- Primary schizonts containing asexual forms, merozoites, which are released in the form of vesicles from liver which travel to lung capillaries for its erythrocytic stage, and its eradication in pre-erythrocytic (i.e. in liver) stage as well,
- Hypnozoites which, persists in liver, occurs in malaria caused due to *P. vivax* and *P. ovale*,
- Sexual forms of parasite, microgametocytes and macro gametocytes which are responsible for transmission of malaria in other human host with the help of vector mosquitoes, and
- Ookinetes, which is one of the development stage of malaria parasite inside vector female *Anopheles* mosquito. (L. J. Bruce-Chwatt, 1962)

4. Classification of Anti-malarial Drugs

Anti-malarial drugs can be classified according to its action on different stages of life-cycle malaria parasite, in human as well as in vector host

- Primary tissue schizontocides/ Casual prophylactic drugs: These drugs act on the pre-erythrocytic development stage of malaria parasite i.e. a stage where sporozoites multiply and develop into primary tissue schizonts.
- Anti-relapse Drugs: These drugs act on the persistent hypnozoites or secondary tissue schizonts, which are dormant parasites of *P. vivax* and *P. ovale* organisms.
- Blood schizontocides/ Schizonticidal drugs: Schizonticidal drugs act on the asexual forms of malaria parasite, such as merozoites which develop in erythrocytes, right from its invasion into the erythrocytes till rupturing of red blood cells due to multiplication of these merozoites.
- Gametocytocidal Drugs: These drugs act on the sexual forms of malaria parasite i.e. microgametocytes and macrogametocytes which are developed from the merozoites in red blood cells.
- Sporontocidal Drugs: These drugs have anti-sporogonic action which is effective against parasites developing in vector mosquito.
<table>
<thead>
<tr>
<th>Classification According To The Site of Action</th>
<th>Drugs Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casual Prophylactic Drugs</td>
<td>Biguanide drug (Proguanil) and Diaminopyrimidine drug (Pyrimethamine) is highly active against sporozoites and primary tissue forms of <em>P. falciparum</em> and have little effects on <em>P. vivax</em> parasite.</td>
</tr>
<tr>
<td>Anti-Relapse Drugs</td>
<td>8-Aminoquinolone drugs such as Primaquine, pamaquine and quinocide have high activity against dormant hypnozoites. Primaquine and quinocide are usually used because of their fewer side effects as compared to pamaquine.</td>
</tr>
<tr>
<td>Schizonticidal Drugs</td>
<td>Cinchona alkaloid such as quinine and 4-Aminoquinoline drugs such as chloroquine and amodiaquine have potent and rapid onset of action which are used for suppression of clinical symptoms. Proguanil and Pyrimethamine can produce suppressive cure for blood schizonts of <em>P. vivax</em> parasite</td>
</tr>
<tr>
<td>Gametocytocidal Drugs</td>
<td>Pamaquine, quinocide and primaquine are active on gametocytes of all malaria parasites. Quinine, mepacrine, chloroquine and amodiaquine are active on gametocytes of <em>P. vivax</em> and <em>P. malariae</em>. Pyrimethamine acts long term for two-three weeks after single dose on eradication of sexual forms of <em>P. falciparum</em>.</td>
</tr>
<tr>
<td>Sporontocidal Drugs</td>
<td>Guanides such as Proguanil and Chlorproguanil and 8-Aminoquinolone such as pamaquine and primaquine helps in preventing the development of malaria parasite in vector mosquito.</td>
</tr>
</tbody>
</table>

5. Class of Antimalarial Drugs

4-Amino quinolines: Amodiaquine and Chloroquine

- Chloroquine is effective against erythrocytic forms of all plasmodial species but does not have effect on sporozoites, hypnozoites and gametocytes.
- It is uncharged at neutral pH and hence can diffuse very easily into parasite lysosome, where the drug is converted into protonated, membrane-impermeable form due to acidic pH of the lysosome and this protonated form is trapped inside the parasite where it causes building up of haem and reduction of aminoacids for parasites viability by inhibiting catalytic action of haem polymerase.
- Amodiaquine is similar in action to chloroquine but it was withdrawn because it caused agranulocytosis.

Quinoline-Methanols: Quinine and Mefloquine

- Quinine, an alkaloid derived from cinchona bark, is a blood schizonticidal drug, effective against erythrocytic forms of all plasmodial species.
Mefloquine which is quinolone-methanol compound, similar in action but effective only against *P.falciparum* and *P. vivax*.

Mechanism of action of quinine and mefloquine is similar to that of 4-Aminoquinolines i.e. inhibition of parasites haem polymerase, but these drugs are not as concentrated as chloroquine in parasite’s lysosome.

**Phenantherene-Methanols: Halofantrine**

Halofantrine is a blood schizonticidal drug which is effective against erythrocytic forms of *P.vivax* and strains of *P.falciparum* that are resistant to quinine, chloroquine and pyrimethamine.

**Antifolate Drugs: (Type I-Sulfonamides and Sulfones) and (Type II-Pyrimethamine and Proguanil)**

- Type I drugs act by inhibiting the synthesis of folate by competing with p-aminobenzoic acid and type II drugs prevent the utilisation of folate in conversion of dihydrofolate to tetrahydrofolate by inhibiting the enzyme dihydrofolate reductase. Hence, DNA synthesis of the parasite is hampered.
- Antifolate drugs have higher affinity for plasmodal enzyme, to disturb its DNA synthesis, than for mammalian or human enzyme.
- Sulphonamides (drug-sulfadoxine) and sulfones (drug-dapsone) are effective against erythrocytic forms of *P.falciparum*, and are less effective against those of *P.vivax*; but these drugs have no activity against hepatic forms (sporozoites and/or hypnozoites) of plasmodia species.
- Type II drugs- Pyrimethamine and proguanil have slow action against erythrocytic forms of parasite.
- Proguanil has also shown action against initial hepatic stages (i.e. entry and development of sporozoite in liver cell) of parasite.
- Pyrimethamine is used in combination with either dapsone or a sulphonamide (i.e. sulfadoxine).

**8-Amino quinolines: Primaquine**

- Primaquine is the only current drug used from the class of 8-aminoquinolines.
- Tafenoquine and etaquine are more active and slowly metabolised analogues of primaquine.
• These drugs are effective against hypnozoites and hence, they can be used for radical cure of malaria caused by parasites- *P. vivax* and *P. ovale*.

• Also, these drugs have gametocidal action against all forms of plasmodia species, thus preventing transmission of disease.

**Artemisinin and related compounds**

• These compounds were derived from herb, *qing hao* (Chinese), named as *Artemesia annua*.

• Artemisinin, which is a poorly soluble chemical extract obtained from *Artemesia annua*, is an effective blood schizonticide used against acute malaria (cerebral malaria).

• Artesunate which is a water soluble derivative and the synthetic analogues such as artemether and artether has shown higher activity and are better absorbed.

• Mechanism of antimalarial action involves, carbon centred free radical activation in the presence of free ferrous protoporphyrin iron, which is liberated in erythrocytes by parasite’s digestion of haemoglobin.

**Hydroxynaphthoquinone Drug: Atavaquone**

• Atavaquone acts primarily by inhibition of parasite’s mitochondrial electron transport chain, by substituting the natural substrate *ubiquinone*.

• Atavaquone when combined with proguanil is highly effective and well tolerated by the patients.

6. **Drug resistance in malarial plasmodia**

According to WHO, resistance to malaria drug is defined as “the ability of a parasite strain to survive and/or multiply despite the proper administration and absorption of an antimalarial drug in the dose normally recommended” (Roper C et al, 2004)

This is quite worrying for *P. falciparum*, both because of its higher probability to develop resistance and because of its intrinsic higher virulence and morbidity and mortality burden. Lower sensitivity to antimalarial drugs has also been observed in *P. vivax*, while it is extremely rare in the other species of Plasmodia.

**The spread of resistance is a two-step process**

1. A mutant clone suddenly emerges in the replicating parasite population. This clone generally does not fit into the environment than the sensitive ones, unless it is subjected to
selective drug pressure able to kill sensitive parasites but not blood circulating resistant asexual forms (clones).

2. These resistant asexual forms subsequently evolve into gametocytes which possibly spreads in the population.

This event is usually more likely to happen first in low malaria transmission areas where most of the parasite-carrying patients are symptomatic and therefore subject to treatment. Probably, this is the reason why chloroquine and pyrimethamine resistant strains of malaria first appeared in South-East Asia in the early sixties before spreading to the African continent.

The chances of a genetic resistance mutation to occur is dependent factors such as,

- The amount or number of replicating parasites, and
- The drug concentration they are exposed to.

Then it is easy to understand why the therapeutic use of single drugs with long half-life (such as chloroquine or mefloquine) and long decreasing concentration has facilitated the emergence and occurrence resistant plasmodia strains. (WHO. Guidelines for the Treatment of Malaria: Second Edition, 2010)

**Strategies for preventing spread of resistant strains**

Once a drug-resistant mutant or clone has arisen, preventing spread of resistance is difficult. Resistance, as said earlier, is facilitated by the exposure of malaria parasites to sub-therapeutic levels of antimalarial drugs, which kill sensitive parasites but allows resistant mutant parasites to survive and reproduce. Ensuring that the drugs are taken in at a sufficient dose and for a sufficient duration reduces this risk of resistance. When a drug with a long half-life is taken, drug pressure in body is higher because the drug remains in the patient’s blood at low levels for weeks, exposing newly introduced malarial parasites to sub-therapeutic levels. (Hastings IM et al, 2002) This is particularly observed in high transmission areas where people are not only infected more frequently, but also administer themselves with antimalarial drugs frequently whether or not they are have malaria. Drug pressure which occurs in this situation can be reduced by using drugs with a shorter half-life and by restricting the use of anti-malarial drug to patients with confirmed malaria infection.
There is disadvantage to implement both of these strategies

- Using drugs with short half-lives means that if they are used together with other rapidly eliminating drugs they need to be taken for a longer period resulting in less likelihood of cure compared with drugs with longer half-lives, which can be taken over a three-day period or in a single dose.

- Restricting availability of effective drugs to patients who need definitive diagnosis of malaria would reduce access to cure the malaria disease. This may result in an increase in current morbidity and mortality.

Strategies for preventing emergence of resistant strains

It is quite difficult to control drug resistant strain once it has emerged, thus there is a need for strategies to prevent the event of emergence of resistant strains. The effective strategy is to use different combination of drugs which have different molecular targets that delay the emergence of resistance. This led to the emergence of artemisinin-based combination therapy.

7. Artemisinin-based Combination Therapy

Rationale

The probability of a resistant parasite arising simultaneously from two drugs with different modes of action which is the product of per parasite mutation frequencies multiplied by the total number of parasites exposed to drugs. (White N, 1999) Thus, if the probability of a parasite being resistant to drug A is one in $10^9$ and that to drug B is one in $10^9$ then, the probability that a parasite to be resistant to both the drugs is one in $10^{18}$, resulting in a billion-fold reduction in probability. Resistance to artemisinins has never been reported and are therefore much less likely to occur than in some other drugs such as SP (sulfadoxine-pyrimethamine).

Artemisinins are effective partner drug because they are more effective than any other antimalarial, decreasing the burden of parasites by approximately $10^4$ per asexual cycle (White N, 1999) and therefore reducing the number of parasites that are exposed to the partner drug alone. They also inhibit the production of gametocytes and therefore have a potential to reduce transmission of parasite. (Price RN et al, 1996)
Artemisinins administered as monotherapy must be taken for seven days for radical cure. But even though, following up of seven-day regimens is extremely low and a three-day regimen is generally regarded as the maximum because most people discontinue treatment when they feel better usually after a couple of days, and this usually results in late recrudescences with monotherapy. These artemisinins, when used in combination with another effective drug, a three-day course is sufficient. (Nosten F et al, 1994.)

**The argument against using ACTs**

There are a number of concerns about use of ACT, the chief one being cost. (Bloland PB et al, 2000) These artemisinin combination therapies cost more than US$1 for an adult course, so, to use these ACTs as first-line therapy, subsidy will be required to make sure that these combination therapy are available to everyone, even those who cannot afford the market price of these therapies. Secondly, the main concern is that, by utilising the artemisinin derivatives now, we are taking risk to lose our most valuable antimalarial. This is particularly a concern in many tropical countries because the capacity of the government to deliver health care to the population is often inadequate due to a chronic lack of resources. Under these conditions, implementing a change in drug policy without taking into considerations the above stated problems is likely to result in low rates of coverage and the inappropriate use of the drugs.

If artemisinin-based combination such as (artesunate and mefloquine) or (artesunate and SP) is used today, then it must be formulated and packed in combination since there is a risk that patients will take only the artemisinin derivative responsible for rapid symptom resolution and that too for a few days. This will not only result in failure of treatment, but it also theoretically increases the risk of drug resistance emerging in the future. (Shwe T et al, 1998)

8. **Need of New Anti-malarials**

The latest gold standard medicine for treatment of malaria is the artemisinin combination therapy which consists of fixed dose of chemical derivatives of artemisinin, and a longer acting parter drug which ultimately traces itself back to the natural product quinine. This combination therapy is extraordinary effective, curing more than 98% of patients and also relatively safe, often with no serious adverse effects seen in phase III trials on several thousand patients. However, in the long run, there will always be a need for new drug molecules because no matter how efficiently the existing medicines are used there will be a constant threat of emergence of resistance. The signs of resistance to artemisinins are emerging in some parts of Cambodia where patients are taking longer duration to clear their
fever. (Dondorp AM et al, 2009) Thus, this showed that there is an early need and urgent priority for development of new chemical entities against new resistant strains of malarial parasite.

**Desired and Essential Characteristics**

- New antimalarials should be active against all known malaria parasites that infect humans (*P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi*). (Baird, J.K. 2010)
- New drugs should have selectivity towards Plasmodium species while exhibiting zero toxicity or tolerable toxicity profiles in humans.
- These new antimalarials should target Plasmodium species via only parasite-specific modes of action involving molecular targets and organelles found exclusively in these protozoans. (Schrader, F.C et al 2012)
- New antimalarial drug leads should be active against blood stages of various Plasmodium species and block transmission of gametocytes of Plasmodium species from humans to vector female Anopheles spp. mosquitoes during blood meal event.
- New antimalarials should be desirable to inhibit Plasmodium spp. development in its initial sporozoite stage (during its injection in human host) or in the liver stage.

**Why Natural Products?**

Natural products and their derivatives have been a powerful and efficient part of the fight against malaria in the past. The history of anti-malarial chemotherapy is intimately linked with the serendipitous discovery of quinine which is an original natural product identified from barks of cinchona tree, and purified in 1820. The attempt to synthesize quinine led to the development classical 4-aminoquinolines and amino-alcohols such as amodiaquine, chloroquine and mefloquine, which have been used for malarial treatment over the last century. The herbal product naphthoquinone-lapichol, also was identified as an active ingredient against malaria, found from the same cinchona tree bark. This indeed helped to direct the selection of lapinone, which in turn resulted in the discovery of atovaquone, a component of Malar-one®, a mainstay of malaria prophylaxis for travellers. (Hudson AT 1993)

**Probable Advantages of Natural Products**

(a) Natural products are source of potential new pharmacophores which would be useful for killing the malarial parasite. These new pharmacophores would give a new chemical insight
and represent a novel mechanism of action, such as the artemisinins which showed wide cellular selectivity and therapeutic window in preclinical animal models.

(b) Natural medicinal products are often perceived as being safe by the patient community. In United States, over 19% of the adult population use herbal medicinal product (Kennedy J, 2005) and the regulatory authorities had legally mandate to develop a quality position on such herbal products. Long-term medicinal use is currently accepted by some regulatory authorities as evidence of an adequate safety profile. (European Medicines Agency: Guideline on non-clinical documentation for herbal medicinal products in application for marketing authorisation, 2006) Lack of well-documented safety data is concerning in case of vulnerable populations such as small infants and expectant mothers, in context with any disease. Even though malaria is an acute disease, it is important to determine and demonstrate the safety of drug after repeated dosing since in high endemic areas, a child can have more than ten episodes of malaria per year. This safety data is essentially needed for the study of genotoxicity. The main objective in anti-malarial therapy is to identify compounds which are selectively toxic against or inhibit the action of parasite rather than the host. (WHO: General guidelines for methodologies on research and evaluation of traditional medicine, 2000)

(c) There is a strong chance from the malaria endemic countries to be more active in using their ethno-pharmacological heritage to identify new medicines to combat malaria. Recently, African Network for Drugs and Diagnostics Innovation (ANDi) has highlighted the role of African countries which can play in discovering new medicinal products. (Mboya-Okeyo T et al, 2009) In recent meeting of ANDi, half of the projects presented described the activities of un-purified natural products. Support for such research activities by governments of disease endemic countries via organizations such as ANDi would be helpful to develop new tools and improve existing ones. The development of natural medicinal products is one of the area where researchers from disease endemic countries can be benefited.

(d) Most of the communities use herbal medicinal products. This is evident that the natural products used by the community had shown efficacy or betterment for the respective disease state. This is actually the key success factor. If these crude drugs which are safe and effective in humans can be standardized, and if the clinical activity is defined, and plasma samples from patients can be shown to have ex-vivo activity, then, decisions can be made early on which natural products to work on and purify further. This would be helpful on substantial focusing of natural product related drug discovery. The pattern for drug discovery is same
and consistent i.e. initial identification of natural product scaffolds, then their modification for clinical use by a combination of pharmaceutical chemistry, formulation development and combination therapy. The continual threat of emergence of drug resistance means that there will always be a need for new classes of molecules to combat malaria.

**Identification of active ingredients**

The history of malaria treatment has shown that, if the active ingredient is identified then, more potent and long acting medicines can be discovered. Purification and isolation permits the separation of the active moiety from non-active and toxic molecules. (Jiménez-Díaz MB et al, 2009) It is essential to have an assay which is robust and which reflects the clinical condition. If the process of purification and isolation begins with extracts known to be clinically active in man, then the process is much easier. But first, it is important to verify and confirm the clinically observed activities in vivo i.e. by studying the pharmacokinetics and pharmacodynamics of crude drug on mice infected with *P. berghei* and *P. yoelii* to understand better metabolic issues before progressing on to in-vitro assays. (Krishnan A et al, 2007) If the murine model is not able to depict the human clinical situation and describe anti-parasitic activity of crude drug, then the problem is either with the murine models, or else, it might be because of the different parasite species or the compound might be less potent against the murine parasite than against *P. falciparum*. This difference in potency of drug against *P.berghei* infected mouse model can be solved by utilising humanized or SCID (severe combined immuno-deficiency) mice models, which can be infected with parasite *P. falciparum*. (Druihle P et al, 1988.) Presently, the finest information would be obtained by assessing LC-MS/MS data and bioactivity between human and murine plasma samples and also, by examining the impact of liver microsomes on the molecules in the decoctions. Once the activity is confirmed in murine (rats or mice), then either the complete extract can be tested on cellular models or the extract can be fractioned for testing in cell-based assays in vitro. But, if it is known that the patient plasma samples have ex-vivo anti-parasitic activity, and if the final active moiety does not have activity at a concentration which is achieved in plasma during the clinical study, then it is unlikely to be the really active. By resetting the thresholds to focus on more active molecules, and insisting on observational clinical data and ex vivo analysis, the entire process of identification could be streamlined, and this failure of obtaining inactive molecule can be largely avoided. Once the active moiety is identified, it still needs some optimization. This optimization will most probably be needed with all new
herbal based active anti-malarials where the perception is that the ‘Nature’ has designed the magic drug, which just needs to be identified, formulated and marketed.

In reality, if therapeutic activity of drug, under test, is lost on fractionation then it results from inactivation of the active molecule. It is demonstrated that, if two active ingredients are absolutely required for activity, then it means that two of the inactive fractions have to be recombined to give the activity. Also, it is important to differentiate synergistic activity of two moieties from the presence of solubilizing or stabilizing factors. For example, in case of artemisinin; its potency and solubility can be improved by the chemical modification to artemesin. However, in plant, the partial solubility of artemisinin is overcome by solubility potentiator i.e. flavone casticin. (White NJ et al, 2009) In reality, it is seen that synergy requires two active ingredients which when combined have a resultant activity greater than the sum of the two ingredients on their own. The main objective of combination therapy from the viewpoint of the WHO is that one drug should protect the other drug against resistance. Thus, both of the active moieties need to have intrinsic anti-parasitic activities and different - modes of action and mechanisms of resistance. Until and unless a natural product extract has two or more such active moieties, it is acting as a mono-therapy but, there is a probability that such mono-therapy decoctions may facilitate the generation of resistance, however this might be contradicted with the observation that the Chinese used this decoction for centuries without generating resistance. (Jarcho S et al, 1993) Of the thousands natural medicinal products which have been suggested to be active against malaria, only a few of them have been demonstrated to be active in patients and animal models prior to fractionation. These are summarized in the following Table:-Natural Medicinal Products which can be Potential Anti-malarial.

**Natural Medicinal Products which can be Potential Anti-malarial**
(Timothy NC Wells, 2011) (Pohlit A M et al; 2013)

<table>
<thead>
<tr>
<th>Natural Product/Active Moiety</th>
<th>Natural Source</th>
<th>Mechanism Of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yingzhaosu A</td>
<td>Artabotrys uncinatus (Ying Zhao)</td>
<td>Presumed to be free radical activation in the presence of free ferrous iron – liberated in erythrocytes by parasite digestion of haemoglobin.</td>
</tr>
<tr>
<td>Cryptolepine</td>
<td>Cryptolepis sanguinolenta</td>
<td>DNA intercalation</td>
</tr>
<tr>
<td>Strictosamide</td>
<td>Nauclea pobequinii</td>
<td>Not known</td>
</tr>
<tr>
<td>Protopine Allocryptopine Berberine</td>
<td>Argemone Mexicana</td>
<td>Not known</td>
</tr>
<tr>
<td>Vernodalin</td>
<td>Vernonia amygdalina</td>
<td>Not known</td>
</tr>
<tr>
<td></td>
<td>Plant/Herb</td>
<td>Action/Effect</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Febrifugine</td>
<td>Dichroa febrifuga</td>
<td>Prevents haemoglobin formation.</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Curcuma longa</td>
<td>Inhibits cytochrome P450 enzymes and so may prolong the plasma half-life of anti-malarial drugs.</td>
</tr>
<tr>
<td>Aspidocarpine</td>
<td>Aspidosperma desmanthum Benth. ex Müll. Arg.</td>
<td>Not known</td>
</tr>
<tr>
<td>Uleine, Olivacine</td>
<td>Aspidosperma ulei Markgr.</td>
<td>Interacts with DNA with a high binding affinity. Also, inhibition of DNA topoisomerase II activity. Recently, formation of covalent DNA adducts mediated by ellipticine oxidation with cytochromes P450 and peroxidases was proposed as one of its mode of action.</td>
</tr>
<tr>
<td>Ellipticine</td>
<td>Aspidosperma varagasi A. DC.</td>
<td>Not known</td>
</tr>
<tr>
<td>Polyrenylated acylphloroglucinols, Isogarcinol, Cycloxanthochymol, Garcinol</td>
<td>Moronobea coccinea Aubl.</td>
<td>Not known</td>
</tr>
<tr>
<td>Isobrucein B, Neosergeolide</td>
<td>Picrolemma sprucei Hook. f.</td>
<td>Not known</td>
</tr>
<tr>
<td>4-nerolidylcatechol</td>
<td>Piper peltatum L</td>
<td>Not known</td>
</tr>
<tr>
<td>SimalikalactoneD, Simalikalactone E</td>
<td>Quassia amara L</td>
<td>Not known</td>
</tr>
<tr>
<td>Prenylated xanthones, Polyrenylated acylphloroglucinols</td>
<td>Rheedia acuminata</td>
<td>Not known</td>
</tr>
<tr>
<td>Orinocinolide, simalikalactone D</td>
<td>Simaba orinocensis Kunth</td>
<td>Not known</td>
</tr>
<tr>
<td>5 &amp; 8-hydroxy hydroxyethyl naphtho[2,3-b]furan-4,9-diones</td>
<td>Tabebuia incana</td>
<td>Not known</td>
</tr>
<tr>
<td>Amplexine (djalonenol)</td>
<td>Tachia grandiflora</td>
<td>Not known</td>
</tr>
<tr>
<td>Cyclic alkyl polyols</td>
<td>Tapirira guianensis Aubl.</td>
<td>Not known</td>
</tr>
<tr>
<td>Avicine hydroxide, Nitidine hydroxide, Fagaridine</td>
<td>Zanthoxylum. rhoifolium Lam</td>
<td>Not known</td>
</tr>
</tbody>
</table>

**Not known:** Active moieties isolated from following plants and/or herbs which has shown anti-plasmodial activity but the mechanism of action is not known.

9. **Other Approaches towards Anti-malarial Therapy**

**Enhancing Immunity of Host:** Instead of focusing mainly on killing the parasites one should also consider other mechanisms which may not only directly affect the pathogen but also stimulate natural and adaptive defence system of the host. The immune system of the host plays a major role in complete suppression or elimination of the pathogens. (Stevenson MM et al, 2004.) Extracts or single compounds that can stimulate innate and/or adaptive immunity may be able to contribute to prophylaxis and treatment of not only for malaria, but...
also for viral, bacterial, parasitic and fungal diseases. (Masihi KN et al, 2000) (Muniz-Junqueira MI, 2007) Sadly, only little work has been devoted to the use of these drugs for the prophylaxis and treatment of malaria. Picroliv, which is a standard fraction isolated from ethanol extract of the roots and the rhizomes of Picrorhiza kurroa (family-Scrophulariaceae) has been reported to activate the host immune system. Simultaneous administration of Picroliv was found to enhance efficacy of chloroquine against malaria in experimental murine mouse. (Dwivedi V et al, 2008) The occurrence of cerebral malaria is generally considered to be primarily of immunological origin. (Hunt NH et al, 2006) Several compounds with known effects on the immune system were tested in a murine model of cerebral malaria. Of the compounds tested, it was found that only curcumin and the synthetic Rhokinase inhibitor fasudil had significant effects on the progression of the disease. Although neither drug caused a reduction in parasitaemia, survival of the treated mice was significantly increased, and the development of cerebral malaria was either delayed or prevented. The investigators concluded that an immunomodulator efficient in preventing cerebral malaria should be administered together with anti-malarial drugs, rather than alone, to prevent severe malaria disease. (Waknine-Grinberg JH, 2010) (Mimche PN et al, 2011.)

However there is a great difference between murine and human malaria, and immunomodulatory effects have not yet been tested clinically.

**Multidrug Resistance Inhibitors**

Multidrug resistance is a phenomenon which usually involves a protein molecule or “pump” that straddles (connecting inside to outside) the cell membrane and which captures from the cytosol a foreign substance such as the anti-malarial agent with the help of lipophilicity. The pump then undergoes a conformational change which throws the entering substance out of the cell, an energy consuming step powered by ATP. “P-glycoproteins” are one such class of pumps. Some herbal anti-malarials such as Artemesia annua have been used for several thousand years, but resistance does not seem to have appeared at a significant level. In contrast, most isolated, purified and synthetic anti-malarials have suffered from the appearance of resistant strains of *Plasmodium*. Resistance to isolated artemisinin in *Plasmodium yoelii* was detected at a very early stage and the first signs of artemisinin resistance are appearing in *P. falciparum* from patients in Cambodia. (Chawira AN et al, 1986) (Dondorp AM et al, 2010)
Two of the A. annua flavones, chrysosplenol-D and chrysosplenetin, have been shown to inhibit MDR in a multi-drug resistant Staphylococcus aureus microbes. (Stermitz FR et al, 2002)

Bidens pilosa also contains a flavone (quercetin- 3, 3’-dimethylether rhamnoglucoside) that contributes to the anti-malarial activity and its extract reverses resistance to chloroquine. (Mbacham W et al, 2005)

Epigallocatechin-3-gallate (EGCG), the most abundant tannin in green tea, is reported to be an MDR inhibitor and has an anti-malarial activity which is additive when combined with artemisinin. (Hong J et al, 2003) (Sannella AR et al, 2007)

Alkaloids are also involved in reversal of resistance. For example, cinchonine is a P-glycoprotein inhibitor and a very potent MDR blocker and to a lesser extent quinine also has the same activity. Cinchonine has shown favourable physico-chemical properties for entry into the Plasmodium cell and also it has low toxicity in mice and rats. Chinchonine has been observed to reverse resistance to quinine in vitro. Since similar mechanisms are involved in malaria drug resistance, cinchonine should be tested as a multidrug inhibitor to use in the treatment of malaria with any proven active, natural or synthetic drug, whose activity has declined due to resistance. (Genne P et al, 1992). (Furusawa S et al, 2001) (Warhurst DC et al, 2003. ) (Solary E et al, 2000) (Frappier F et al, 1996) (Druilhe P et al, 1988)

Interestingly some traditional healers have started using medicinal plants in combination with chloroquine to enhance its effect. Investigation of some such plants in Madagascar has shown that they reverse chloroquine resistance in vitro and in mouse models. These include bisbenzylisoquinoline alkaloids of Strychnopsis thouarsii and Spirospermum penduliflorum, alkaloids of Hernandia voyronii, and strychnobrasiline and malagashanine from Strychnos myrtoides. Three indole alkaloids, icajine, strychnobrasiline and isoreticuline from Strychnos icaja, S. myrtoides and S. variabilis respectively, which have no intrinsic anti-malarial activity, have been shown to reverse chloroquine resistance in in-vitro tests with P. falciparum. (Ratsimamanga-Urverg S et al, 1992) (Ratsimamanga-Urverg S et al, 1994). (Rasoanaivo P et al, 1994) (Frederich M et al, 2001)

Very few MDR inhibitors have been tested clinically. The concept was proven in the case of chloropheniramine reversing chloroquine resistance, but because of differing
pharmacokinetics, it needed to be given for seven days. More recently it has been shown that azithromycin and chloroquine act synergistically in the treatment of chloroquine-resistant falciparum malaria. Azithromycin also acts synergistically with arteether in the treatment of multidrug-resistant rodent malaria. A standardized extract of Strychnos myrtoides (containing strychnobrasiline and malagashanine) has been tested clinically in combination with chloroquine but was only given for three days, and this was not enough to reverse clinical resistance to chloroquine. (Sowunmi A et al, 1998) (Dunne Michael W et al, 2005) (Tripathi R et al, 2005) (Willcox ML et al, 2008)

Because of the short half-life of these natural products, a longer duration of treatment, at least seven days, is probably necessary to produce a clinical effect. Compounds which inhibit multidrug resistance can probably exist in many more plants. But, these compounds often have little or no direct antimicrobial effect, so would be discarded in the process of screening and fractionation. However, when combined with compounds which have moderate antimicrobial activity, they may reveal a much higher level of activity. Although many plant extracts have been screened in-vitro for anti-malarial activity, few have been tested for their ability to alter resistance mechanisms.

10. Vaccines: Need of the Hour

Vaccines are the most powerful health tools mankind has created, which are now required for complete or possibly worldwide eradication of malaria. Since there is still not an ideal vaccine for malaria, thus even a modestly effective vaccine could substantially reduce the continuing heavy burden of malaria disease. (Plowe CV et al, 2009) *P. falciparum* is responsible for most severe malaria disease and deaths, hence it has been the target of most vaccine development efforts. Clinical falciparum malaria originates when parasite effectively hijack the host red blood cell expressing on the erythrocyte surface highly variant *P. falciparum* erythrocyte membrane proteins (PfEMP1s), which are encoded by a large, diverse family of up to 60 var genes in each parasite genome (Su X, Heatwole VM, Wertheimer SP, et al. 1995). PfEMP1s are expressed on the red blood cell surface in cytoadherent clumps known as knobs. Infected red cells sequester in the microcirculatory compartments of organs, most notably in the brain and placenta, leading to disease. The slowly acquired immune protection against uncomplicated malaria over years of repeated exposure to malaria is thought to represent the accumulation of protective immune responses to a repertoire of
diverse antigens, probably including both PfEMP1s and the surface proteins that are the targets of most vaccine candidates.

**Types of Vaccines**

- **Pre-erythrocytic vaccine**: Vaccine which would completely block infection, preventing the parasites from reaching the blood and causing disease, and thus preventing transmission.

- **Blood-Stage Vaccine**: These vaccine candidates are based on antigens that coat the surface of the invasive merozoites and/or are involved in the process of erythrocyte invasion. The hope is that immunization with these antigens will generate antibodies that block invasion and curtail parasite replication in the blood, reducing the risk or severity of clinical illness.

- **Vaccine targeting gametocytes**: These vaccines would block the transmission of gametocytes but would not prevent infection or disease in vaccinated individual. Thus, highly efficacious pre-erythrocytic or blood-stage vaccines that prevent sexual reproduction would also block transmission, so the term “transmission-blocking” vaccines rightly applies to them. (malERA Consultative Group on Vaccines. A research agenda for malaria eradication: vaccines. PLoS Med. 2011; 8:e1000398)

**RESEARCH ON VACCINES TILL NOW**

- **RTS, S** was the first malaria vaccine to demonstrate meaningful levels of clinical protection in field trials. RTS, S is composed of the central repeat region (R) and T cell epitopes (T) of CSP using the hepatitis B surface antigen (S) as a carrier matrix. It is co-expressed in *Saccharomyces cerevisiae* with an additional S, hence “RTS, S.” (Ballou WR, Rothbard J, Wirtz RA, et al. 1985)

- **CSP** (circumsporozoite protein) contains a central repeat region that elicits antibody responses, covered on each side by non-repetitive regions containing T cell epitopes. Antibodies directed against the central repeat region cause the protein coat (i.e. CSP) to slough off and block invasion into hepatocytes, suggesting that vaccine-induced antibodies might prevent infection. (Gordon DM, McGovern TW, Krzych U, et al. 1995) (Stoute JA, Slaoui M, Heppner DG, et al. 1997)

- **Trials in children and infants who are naturally exposed to malaria** have demonstrated efficacy in the range of 30%–56% against clinical disease and up to 66% against
infection, and a good record of safety and tolerability. A phase II trial showed 26% efficacy against all malaria episodes over nearly four years, 32% against a first or only episode of clinical malaria, and 38% in preventing severe clinical episodes. After 45 months, the prevalence of parasitemia was significantly lower in vaccinees than in the control group (12% versus 19%). (Alonso PL, Sacarlal J, Aponte JJ, et al. 2004) (Bejon P, Lusingu J, Olotu A, et al. 2008)

- RTS,S/AS01, a modification of RTS,S prepared by adding antigens to create a multi-stage, multi-antigen RTS,S-based vaccine, targets the pre-erythrocytic circumsporozoite protein (CSP) of *P. falciparum*. RTS, S/AS01 is currently being evaluated in a large phase III trial of 16,000 children and infants in seven African countries. Early results of the phase III trial suggest similar efficacy to that seen in phase II trials. (Bojang KA, Milligan PJ, Pinder M, et al. 2001) (Heppner DG Jr, Kester KE, Ockenhouse CF, et al 2005)

Obstacles for Vaccine Development:

- The size and plasticity of the *P. falciparum* genome, which has about 23 million bases of DNA organized into 14 chromosomes and about 5,000 genes. (Gardner MJ, Hall N, Fung E, et al. 2002) This order of magnitude larger is than the genomes of most of the viruses and bacteria for which vaccines have been successfully developed.

- Mutation during mitotic reproduction in the haploid liver and blood stages and genetic recombination during the diploid sexual reproductive stages in the mosquito result in extensive genetic diversity which greatly complicates the choice of candidate antigens for vaccine development. (Takala SL, Plowe CV. 2009)

- Immunization with stage-specific vaccines typically protects against only that life-cycle stage.

11. CONCLUSIONS

- Due to occurrence of resistance with various existing anti-malarial therapies, there will always be a need of new drug moiety for treatment of malaria. However combination therapies are effective but only with the use of new drug molecules, further as time passes, resistance to these drugs will also be observed as today with the existing combination therapy (ACT’s).

- With the research in drug resistance inhibitors, this wheel of unmet effects can be reversed and activity of drug can be enhanced. Compounds such as Epigallocatechin-3-gallate (EGCG) and quercetin- 3, 3’-dimethylether rhamnoglucoside are reported to be an
MDR inhibitor and has an anti-malarial activity which is additive when combined with artemisinin.

- Today, with the scarcity of natural products, the drug discovery of new moieties will become limited soon. Hence there is necessity to enhance the immunity of the host or population possibly by immunomodulators or more effectively by subsequent research in development of vaccines. Immunomodulator like Picroliv and vaccine under study such as RTS, S and RTS, S/AS01 has been reported to activate the host immune system.

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