Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

From control to eradication of malaria: the end of being stuck in second gear?

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ARTICLE INFO

Article history:
Received 19 January 2010
Received in revised form 27 March 2010
Accepted 1 April 2010
Available online 20 May 2010

Keywords:
Malaria
Plasmodium falciparum
Anopheles mosquito
Antimalarial drugs
Vaccine
Vector control
Hemozoin
Matrix metalloproteinases

ABSTRACT

More than 2 billion people are at risk of malaria, which primarily affects poor populations in tropical and subtropical areas, including Southern Asia. As malaria incidence has been reduced strongly in some parts of endemic regions by combinations of interventions, including artemisinin-based therapies and insecticide-treated bed nets, a new goal has been established recently by charity foundations which support research on malaria: the worldwide eradication of the pathology. Doing away with control approaches which have been applied for the last 50 years and more focus on elimination objectives will deeply change priorities in the area of malaria treatment, chemoprevention, vector control, vaccine research and health system assessment. In this review, actual knowledge on pathogenesis and pharmacology is discussed, and new drugs, vaccines and insecticides are described.

1. Introduction

Each year, among 500 million cases of clinical malaria, 1 million people die; moreover, 2.37 billion people are estimated to be at risk of infection by *Plasmodium falciparum* (*P. falciparum*), the most deadly among *Plasmodia* parasites in humans[1]. To fight against malaria different strategies have been adopted during years, including: control, whose goal is to exclude new illnesses and deaths caused by malaria without blocking transmission; elimination, whose aim is to obtain disappearance of malaria cases and transmission in a localized area; and eradication, which expands elimination objectives globally to obtain disappearance of malaria parasites worldwide[2]. In the first half of the past century, malaria diffusion has been restricted to tropical and subtropical areas, while it has been eradicated from temperate areas, such as Western Europe and the United States, where economic development occurred and public health measures became disposable. As a consequence of these successes, a first Global Malaria Eradication Program was launched by the World Health Organization in 1955[3]. Use of chloroquine for treatment/chemoprevention and dichlorodiphenyltrichloroethane (DDT) for vector control played a key role in the program, which gained several successes in some areas, including India and Sri Lanka. Unfortunately, during following two decades, mutations in *P. falciparum* chloroquine resistance transporter gene conferring chloroquine resistance originated and spread from at least five independent foci worldwide; the first mutation originated in Southern Asia, and rapidly diffused towards Africa (Figure 1), while the others spread independently in South America (two foci), Papua New Guinea and Melanesia[4]. Additionally, DDT-resistant mosquitoes emerged. Finally, in 1969 the Global Malaria Eradication Program was abandoned. Therefore, sulphadoxine-pyrimethamine treatment replaced chloroquine-based therapy, but soon triple or quadruple mutant *P. falciparum* dihydrofolate reductase alleles conferred pyrimethamine resistance, which can be traced again to a few origin, Southern Asian parasites sharing a common ancestor with Africans (Figure 1)[4]. Such a situation prompted to enhance efforts to find new tools to control malaria, especially in Asian areas, leading to deliver artemisinin derivative drug-based therapies and to improve insecticide-based measures. Recently,

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due to large-scale disposability of antimalarial tools, a new hope for global eradication spread in the malaria community. In 2007, the Bill and Melinda Gates Foundation announced that malaria elimination would be prosecuted as primary goal[5]. Because of the perceived failure of the initial Global Malaria Eradication Program, many concerns spread among malaria experts. However such an intent was rapidly endorsed by the World Health Organization and the Roll Back Malaria association. As a consequence of this renewed interest in malaria eradication, what will change are not the contents of research agenda, but priorities in each research area, including treatment, chemoprevention, vector control, vaccines, as it has recently been suggested by Greenwood[2]. Here actual knowledge on malaria will be described, focusing on parasite life cycle and pathogenesis, on pharmacology and vector control, and on new targets and vaccines which are going to be disposable to fight against malaria and possibly reach the goal of eradication which raised such a large amount of hopes and concerns in the malaria community.

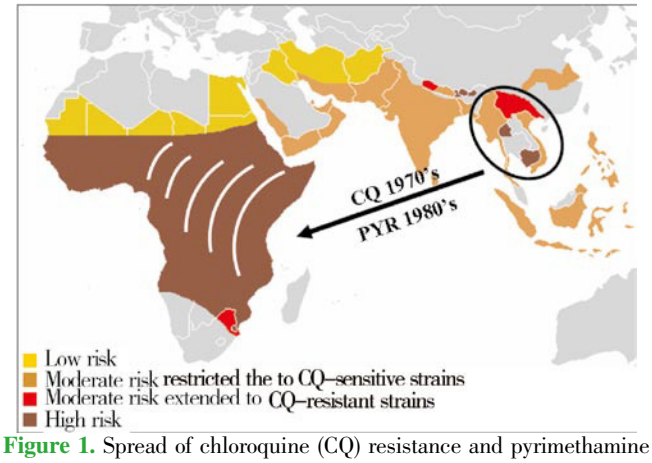

Figure 1. Spread of chloroquine (CQ) resistance and pyrimethamine (PYR) resistance from Southern Asia to Africa.

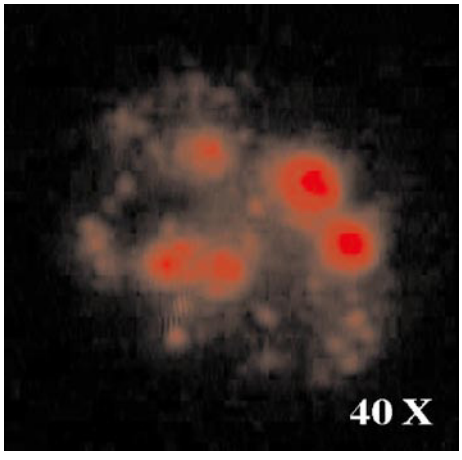

Figure 2. Fluorescent microscopy image of monocyte phagocytosis of trophozoite-parasitized red blood cells marked with PKH26 dye.

2. *Plasmodium* parasite life stages and hemozoin production

Plasmodium parasite is the pathogenic agent of malaria. In humans, four *Plasmodium* species with different disease pattern exist: *P. falciparum*, *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*) and *Plasmodium malariae* (*P. malariae*). A fifth species, *Plasmodium knowlesi* (*P. knowlesi*), which is usually responsible of simian malaria, has also been correlated to humans in Malaysia[6]. Among these species, *P. falciparum* is the most virulent, with worldwide diffusion; *P. vivax*, even thought less deadly, is common in subtropical areas outside Africa, especially in Southern Asia regions[7]. *Plasmodium* life cycle is notably complex, occurring either in mosquito vector, where sexual reproduction occurs, or in human host, involving asexual replication. As current and new antimalarial drugs target specific parasite stages in human host, while antivector control measures are generally directed to mosquitoes killing, present section will discuss *Plasmodium* stages in humans only.

After female *Anopheles* mosquito's bite, occurring while it takes its blood meal, 15-20 sporozoites are injected into the skin, where they stay for prolonged time before reaching the blood stream. During this period, some of them are degraded by phagocytes at the bite site, others enter the lymphatic system, where following removal occurs. However, survivor parasites finally find a blood vessel and rapidly enter the blood circulation, through which they travel from the dermis to the liver[8]. Different destiny of sporozoites in the skin is correlated to presence or absence in the parasite of so-called "sporozoite proteins essential for cell traversal"; indeed, sporozoites lacking those proteins fail to reach blood capillaries[9].

After cell traversal, sporozoites invade the liver, where they transmigrate through Kupffer cells and several hepatocytes before choosing a final one for homing. Liver invasion occurs through specific interaction between the surface-bound circumsporozoite protein (CSP) and heparin sulphate proteoglycans on hepatic cells[10]. Another molecule involved during liver invasion is thrombospondin-related anonymous protein (TRAP), which binds to the surface of hepatocytes using the conserved amino acid motif Trp-Ser-Pro-Cys-Ser-Val-Thr-Cys-Gly [11]. Both CSP and TRAP have been studied for vaccine application, as it will be described in dedicated section. In the hepatocyte, sporozoite forms the parasitophorous vacuole, a specialized compartment where it develops into schizont form containing 10 000-30 000 merozoites[12]. Molecular biology in liver stages is still quite unknown, but lipid delivery from hepatocyte seems to be crucial for parasite development as suggested by evidences on so-called "up-regulated in infective sporozoites gene 3", a parasite gene essential for sporozoite intrahepatocytic development which has been reported to bind liver-fatty acid binding protein in vitro[13]. Time of parasite permanence in the liver is the crucial factor which differentiates *P. falciparum* and *P. malariae* from *P. vivax* and *P. ovale:* while in the first two species the schizont development and rupture occur rapidly and merozoites are released into the blood stream 1-2 weeks after hepatocyte invasion, *P. vivax* and *P. ovale* can stay in liver cells as dormant hypnozoites for months or years before turning into schizont form, whose rupture causes long-term relapses after initial infection. For that reason, the number of infections by *P. vivax*, which is extremely diffused in Asia, is generally significantly underestimated in affected community[14].

When merozoites are delivered from the liver into the blood stream, erythrocyte invasion rapidly occurs, involving several steps: attachment, mediated by the co-ligand complex formed by major merozoite surface proteins

(MSP) -1 and -9, which bind erythrocyte band 3 protein[15]; reorientation of the apical end of merozoite towards the erythrocyte surface through merozoite apical membrane antigen-1 transmembrane protein[16]; and penetration, involving two distinct, redundant and hierarchically organized parasite transmembrane protein families, namely the erythrocyte binding antigens and the *P. falciparum* reticulocyte-binding homologs, which binds glycophoryns and other unknown receptors[17]. In *P. vivax*, reticulocyte invasion is mediated through interaction with the Duffy blood group antigen, the erythrocyte receptor for the chemokine interleukin-8. Generally African people lack this antigen; for this reason *P. vivax* is more common in tropical areas outside Africa, like Southern Asia and Malaysia[18].

Once merozoites invade red blood cells, they develop into several asexual parasite stages, namely ring, trophozoite and schizont form, with different structure and specialized stage-specific features[19]. As erythrocytes lack standard intracellular organelles, Plasmodia need an alternative source of food to prosecute fast-growing objective: the abundant hemoglobin solves this obstacle, being catabolized in a large digestive vacuole (food vacuole[20]. As a consequence of hemoglobin digestion, parasites expand rapidly their surface, and at the same time produce hemozoin, a crystalline polymer where hemoglobin-derived ferriprotoporphirine IX is sequestered; moreover, hemozoin is able to peroxidate membrane polyunsaturated fatty acids through heme autocatalysis[21]. After 48 hours (or 72 hours for *P. malariae*), the erythrocytes rupture releasing new merozoites, which invade new erythrocytes and so on. In some random cases, parasites turn into gametocyte form, ready to be transferred to *Anopheles* mosquito when it takes new blood meal. Once in the vector, sexual cycle will start.

Erythrocyte rupture is followed by some of clinical symptoms of malaria, such as fever, chill, headache, abdominal and back pain, nausea, diarrhoea, and sometimes vomiting. Some of the complications in severe malaria are anemia, respiratory distress, cerebral malaria[22]. It has been suggested that hemozoin could be related to these complications. Free hemozoin and hemozoin-containing trophozoites are avidly phagocytosed by circulating monocytes; as an average, each monocyte ingests 8-10 trophozoites. Figure 2 shows a monocyte fed with throphozoite-parasitized red blood cells marked with PKH26 fluorescent dye (Sigma-Aldrich, St. Louis, MO); image was obtained by fluorescent microscopy (magnification: 40×).

As a consequence, several hemozoin-fed monocyte functions are dramatically impaired, including antigen presentation, oxidative burst, bacterial killing, coordination of erythropoiesis[23-26]. Moreover, hemozoin-fed monocytes produce higher amounts of cytokines, such as tumor necrosis factor alpha (TNFalpha), interleukin-1 beta (IL-1beta), monocyte inflammatory protein-1 alpha (MIP-1alpha)[27, 28], which according to Clark theory are responsible for fever in cerebral malaria[29]. Recently, a mechanism involving matrix metalloproteinase-9 (MMP-9) has been proposed to explain such an excessive release of cytokines. It has been shown that in monocyte cultures the expression and activity of MMP-9 after phagocytosis of hemozoin is enhanced by several cytokines, including TNFalpha and IL-1beta [30,31]. This evidence is enforced by a model in vivo of cerebral malaria, where higher levels of MMP-9 were found in brain of cerebral malaria-sensitive *Plasmodium berghei* infected mice; interestingly, immunohistochemistry showed that excessive metalloproteinase was produced by cells of monocytic lineage[32]. As MMP-9 gene transcription is under control of TNFalpha, while active MMP-9 is able to shed the soluble form of TNFalpha into the extracellular environment by cleavage of its membrane-bound precursor, a pathological loop involving TNFalpha and MMP-9 is established, leading to abnormal levels of cytokine production[30]. TNFalpha is also the soluble mediator promoting monocyte degranulation, which is upregulated after phagocytosis of malarial pigment, according to recent data on hemozoin-dependent higher release of monocyte lysozyme, an enzyme stored in gelatinase granules together with MMP-9[33]. Recent studies focused on what component of hemozoin (peroxidated lipids or heme core) is responsible of enhancement of MMP-9 and related cytokines, such as TNFalpha and IL-1beta. Data from monocyte cultures showed that lipids attached to hemozoin may play a crucial role either in enhanced MMP-9 expression and activity or in TNFalpha and IL-1beta production, while 15(S,R)-hydroxy-6,8,11,13-eicosatetraenoic acid, a peroxidation product of hemozoin, may be involved[31, 34]. On the other side, heme core may concur in activation, as it has been showed that lipid-free synthetic pigment beta-hematin is able to perform cleavage of the proform of MMP-9 *in vitro*[35]. Certainly more studies are required, but relationship between hemozoin and matrix metalloproteinases appears to be a promising research field for new therapies in complicated malaria, also taking in account that drugs against these enzymes are already disposable because of their role in other diseases, like cancer and neuroinflammation.

Knowledge of molecular biology of *Plasmodium* and biochemistry of hemozoin catabolism is useful to define targets for chemoprevention, therapy and vaccination, as it will be discussed in following sections.

3. Current and new drugs

Antimalarial drugs are essential tools for chemoprevention and treatment of clinical malaria. The ideal drug must be fast acting, cost-effective, and with low side effects. Current antimalarial drugs are grouped in seven classes: 4-aminoquinolines, arylaminoalcohols, antifolates, 8-aminoquinolines, artemisinins, inhibitors of the respiratory chain and antibiotics, as resumed in Figure 3.

4-aminoquinolines inhibit hemozoin formation in the food vacuole by complexing with ferriprotoporphirine IX. Most diffused drugs from this class are chloroquine and amodiaquine. Cheap and safe chloroquine was the favourite drug chosen for the Global Eradication Program during past century, but today use of chloroquine for *P. falciparum* has been largely reduced because of diffused resistant strains, while drug is still useful against other strains, including *P. vivax*. Amodiaquine is an alternative drug effective against low chloroquine-resistant parasites, but it can lead to severe hepatic side effects if used for prolonged times[36].

Also arylaminoalcohols are chiral molecules generally administered as the racemate form, and inhibit hemozoin formation through a not well understood mechanism; while 4-aminoquinolines act inside the food vacuole, arylaminoalcohols are effective outside, blocking hemoglobin access into the digestive organelle. The most used drugs from this family are quinine and mefloquine. Generally, it is recommended to use quinine in association with tetracycline, doxycycline or clindamycin, while mefloquine must be combined with artesunate. Multiple

reversible side effects are associated with these two drugs, including arrhythmia and hypoglycaemia for quinine and insomnia and psychological disorders for mefloquine. Other less currently used arylaminoalcohols are halofantrine and lumefantrine. Halofantrine shows dangerous cardiac side effects, while lumefantrine is safer but less active, even though recently a more effective formula combinig lumefantrine with arthemeter has been introduced[37].

Antifolate drug class includes inhibitors of either dihydropteroate synthase, like sulfadoxine and sulfonedapsone, or dihydrofolate reductase, such as pyrimethamine, cycloguanil and chlorcycloguanil. During 1970s, sulphadoxine-pyrimethamine treatment replaced chloroquine-based therapy due to chloroquine-resistance insurgence However, prolonged use of sulfadoxine-pyrimethamine combination may induce Stevens-Johnson syndrome as severe side effect[38].

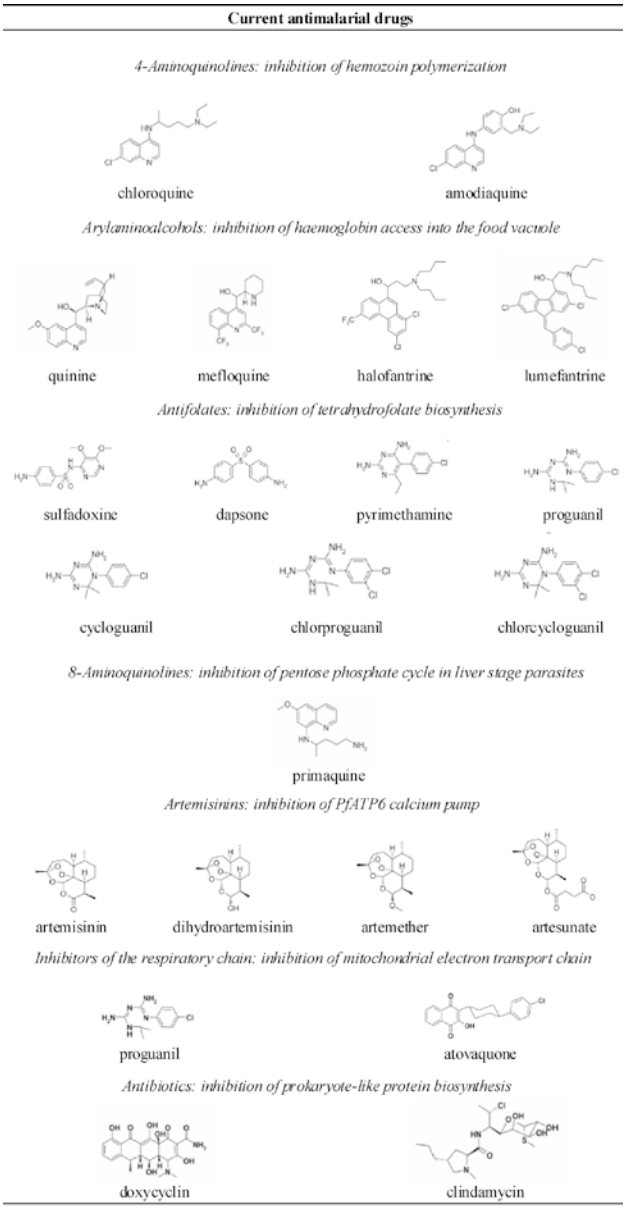

Figure 3. Classification of current antimalarial drugs according to chemical structure.

Among 8-aminoquinolines, the only available drug is primaquine, which acts against sexual-stage and pre-erythrocytic stage of *Plasmodium* parasites. Primaquine is the official antimalarial drug used for *P. vivax* therapy, but it can also be used for *P. falciparum* chemoprevention. Use on glucose-6-phosphate dehydrogenase-deficient patients, who are frequent in Africa and Asia, must be avoided, because of hemolytic risk[39].

Artemisinins are a big family of antimalarial drugs derived from artemisinin lactone, the active principle of *Artemisia annua* plant. Most common derivatives are artemether and artesunate, both modified from dihydroartemisinin, the hemiacetale form of artemisinin[40]. These drugs are directed not only against late ring stages, like other antimalarials, but also act against early rings, being the most active and rapid antimalarial drug disposable by now. Mechanism of action of artemisinins is not well understood, and several targets have been proposed, including some food vacuole proteins or an endoplasmic reticulum adenosine-triphosphatase calcium pump. No severe side effects have been reported for artemisinins. Due to their low cost, efficacy and safety, artemisinins are currently the most used antimalarial drugs and are also recommended for association with other antimalarial classes. Indeed, artemisinin-based combination therapy is currently the standard therapy adopted in a large number of countries[41].

Inhibitors of the respiratory chain are well tolerated drugs, used for chemoprevention and treatment of uncomplicated *P. falciparum* malaria. Among these drugs, the most common one is atovaquone, which has been combined with proguanil to contrast emerging resistance. It targets the ubiquinone binding side of the cytochrome bc1 complex, blocking electron transport[42].

Finally, antibiotics like doxycycline and clindamycin have antimalarial therapeutical abilities, targeting prokaryote-like proteins and blocking development of the apicoplast during exo-erythrocytic schizogony, leading to impaired parasite maturation. Unfortunately, they are effective only after second parasite intraerythrocytic cycle; as a consequence, fever persists four days instead of two if antibiotics are the only drug used for treatment. Thus, in acute malaria they must be associated with faster acting drugs, such as arylaminoalcohols or artemisinins[43].

Although antimalarial drugs number is apparently elevated, resistance represents a serious emergency. Resistance to 4-aminoquinolines is generated when a mutation occurs on gene coding for a food vacuole membrane-associated transport protein (*P. falciparum* chloroquine resistance transporter) which transfers the drug out of the organelle. Resistances to chloroquine emerged in the past century, during the Global Eradication Program, and today 80% of *P. falciparum* strains are resistant to this drug[4]. Other strains are still sensitive, even though cloroquine-resistant *P. vivax* have been found in several regions, including South Eastern Asia[44]. Amodiaquine is effective against low chloroquine-resistant parasites, but amodiaquine-resistance occurs in several Asian areas[45]. Also resistance for arylaminoalcohols has been related to a membrane-associated transport protein (*P. falciparum* multidrug resistance-1), which promotes enter of arylaminoalcohols into the vacuole, where they are not effective. Clinical resistance for quinine and mefloquine occurs in several Asian countries[46]. Among antifolates, multiple mutations in *P. falciparum* dihydrofolate reductase gene are responsible for drug resistance: triple mutant form does not respond to

pyrimethamine, but is still sensitive to chlorcycloguanil, while all dihydrofolate reductase inhibitors are ineffective against quadruple mutant form, which has been found in 67% of South Eastern Asian isolates[47]. Regarding 8-aminoquinolines, although resistance to primaquine has been reported from South Eastern Asia, it is rare. Such cases are managed with a higher dose of primaquine[48]. Clinical resistance for artemisinins is not yet relevant, but a mutation in *P. falciparum* adenosine-triphosphatase-6 gene has been described[49]. Additionally, partial artemisinin-resistant *P. falciparum* malaria has recently emerged on the Cambodia-Thailand border: in two open-label, randomized trials, *P. falciparum* showed lower susceptibility to artesunate in western Cambodia as compared with northwestern Thailand, with slow parasite clearance *in vivo* but not *in vitro*[50, 51].

Resistance to inhibitors of respiratory chain is almost worldwide diffused. Resistance to atovaquone has been contrasted by combination with proguanil, but recently a case of treatment failure in a patient who was given a second-line atovaquone-proguanil treatment has been described[52]. Interestingly, no antibiotic-resistant parasite strains have been found up to now, but since their association with other classes is recommended, advantage is limited. As a consequence, today the only drugs which guarantee almost worldwide an effective therapy are artemisinins. Thus, artemisinin-based combination therapy is currently the standard treatment recommended. However, malaria community is clearly worried about first mutations observed in artemisinin-targeted parasites, and research on new drugs is becoming urgent day by day.

Several new drugs or drug combinations are being tested in clinical trials, some with promising results, while others showed severe side effects. The black mark is that few of all these drugs in trial are really innovative: generally, new drugs are modified form or new artemisinin-based combinations of previously described antimalarial classes, while drugs with new targets are still lacking. Table 1 resumes what drugs are in clinical trials or have been dropped out. Major details have been reviewed by Schlitzer[53].

Table 1
Classification of new antimalarial drugs according to different stage of clinical trial.

	New antimalarial drugs
In advanced stages of clinical studies	Piperaquine/dihydroartemisinin
	Pyronaridine/artesunate
	Tafenoquine
Clinical data are available for	Dapsone/chlorproguanil/artesunate
	Fosmidomycin
	Azithromycin
	Pafuramidine
Beginning clinical evaluation	AQ-13
	Tert-butyl-isoquine
	Ferroquine
	T3
Dropouts	Artemisone
	OZ-277
	GW844520

Briefly, advanced data are disposable for combined therapies piperaquine/dihydroartemisinin and pyronaridine/artesunate. Both piperaquine and pyronaridine have been developed during past century in China, where resistances emerged, sometimes cross-resisting also with chloroquine. Combination with dihydroartemisinin or artesunate seems to be effective and well tolerated[54, 55]. Tafenoquine, a primaquine derivative, has been shown to be highly effective against *P. vivax*[56]. Association of dapsone/chlorproguanil/artesunate has been formuled to avoid extension of quadruple mutant of dihydrofolate reductase gene, and the combination is now in phase III clinical trial[57]. Fosmidomycin is an antibiotic which is absent in humans. It showed high antimalarial activity in early-phase of clinical trials. Additionally, antimalarial fosmidomycin/artesunate combination and more potent fosmidomycin are in preclinical trial[58]. Another tested antibiotic is azithromycin, which acts against *P. vivax*[59]. Unfortunately, up to now studies comparing azithromycin with other antimalarial drugs such as doxycyclin, quinine or mefloquine displayed discouraging results[60, 61] while combination with artesunate is effective in Asia but is not recommended for acute malaria treatment in resistant areas of Africa[62]. Pafuramidine molecule is obtained through modification of diamidine, the drug used historically against sleeping sickness. Pafuramidine shows antimalarial properties, even though its action mechanism is unclear, and a phase II clinical trial in Thailand showed high efficacy against *P. vivax* and *P. falciparum* without side effects[63]. Three aminoquinolines, namely AQ-13, ferroquine and tert-butyl-isoquine are beginning clinical evaluation; mechanism is supposed to be the same as chloroquine[64-66]. Additionally, another promising drug, T3, is entering in clinical trial. This new drug belongs to bis-cationic compounds and blocks phospholipids biosynthesis, which is essential for parasite to produce membrane[67]. Finally, some new drugs have been abandoned or neglected, such as GW844520, an inhibitor of respiratory chain which displayed cardiotoxicity during animal studies, OZ-277, a synthetic peroxide, and artemisone, an artemisinin-derived molecule[53]. Almost 40 years ago anticancer drugs such as methotrexate and trimetrexate, were shown to be effective against the malaria parasite, but perceived toxicity prevented their development as antimalarials; interestingly, due to emerging artemisinin resistance, possible use at lower doses of such drugs has been recently reconsidered[68].

4. Vector control

Historically, consistent reduction of *Anopheles* mosquitoes in areas endemic for malaria always correlated with lower levels of human infections. Vector control thus plays a key role in recent malaria eradication program. Current strategies are based on use of insecticide-treated bednets and indoor residual spraying[69].

License for insecticide treatment on bednets is restricted to few pyrethroids, while spraying strategy is based on a larger range of insecticides, including cost-effective DDT, which was the pesticide chosen for Global Malaria Program during last century[3]. Unfortunately, pyrethroids and DDT share the same target, a voltage-gated sodium channel involved in neuronal signal transmission, facilitating increase of risk of insecticide-resistance to both insecticide typologies[70]. During the last decade, several pyrethroid-

resistant mosquitoes, including *Anopheles funestus* and *Anopheles gambiae*, were found in some African regions[71,72]. Additionally, mutations conferring resistance to DDT, sometimes cross-linking with pyrethroid-resistance, have been also described[73]. For this reason research of new insecticides is becoming urgent. At the moment, two different approaches are prosecuted[74]: first, molecular biology of mosquito and biochemistry of blood meal human host selection are studied, searching for specific attractants and repellent molecules; second, broad-based analysis of mosquito and parasite genome is performed to better understand mechanisms of parasite development in the vector and to produce genetically modified vaccines which would prevent transmission, as described in following section.

5. Vaccines

During the last decade, great investments and efforts were made to develop a vaccine able to reduce mortality and morbidity from malaria in young children in areas where malaria is endemic. Primary goal is to produce for 2015 a licensed vaccine with protective efficacy of 50% in severe malaria which persists for at least a year. Secondly, by 2025, vaccine will be improved to 80% efficacy and four-year persistence[75]. To achieve these objectives, the program of experimental vaccine research expanded towards two different but overlapping fields. First approach focuses on parasite life cycle knowledge, searching for antigens to be recognised by new vaccines; on the other hand, better understanding of protective immune mechanisms against malaria is prosecuted to provide a basis for rational vaccination design[76]. All designed vaccines, either those in clinical trial or those abandoned, can be classified in three cathegories: pre-erythrocytic stage, asexual-blood stage and transmission-blocking vaccines. Essential difference among these families is the vaccine preventing effect towards the transmission of infection and the clinical disease: pre-erythrocytic stage vaccines prevent infection and thereby disease; asexual-blood stage vaccines prevent disease but not infection; transmission-blocking vaccines do not provide any immediate direct benefit to the vaccinated individual, but will help to reduce transmission of the parasite in the community[74].

Pre-erythrocytic stage vaccines should prevent sporozoites invasion of blood and liver, protecting against clinical malaria. Radiation-attenuated sporozoites were the oldest application of this approach, and humans exposed to bites of irradiated mosquitoes with attenuated sporozoites were totally protected[77]. Unfortunately, deliver procedure was definitely impractical and such a vaccine was abandoned. However, a new interest grew up recently, as an American industry started to produce radiation-attenuated sporozoites of *P. falciparum* in a large-scale procedure, and now this vaccine is in phase 1/2a of clinical trial[74]. Another way to attenuate sporozoites is obtained by genetic manipulation. For example, use of sporozoites deficient of 6-cystein secretory proteins (essential for parassitophorous vacuole formation) gave totally protection against clinical malaria [78]. Recently, genetic-engineering shifted to subunit vaccine production, focusing mainly on two surface proteins, CSP and TRAP, which are necessary for the parasite to move through and invade the liver. Unfortunately, the most part of vaccines designed for these antigens were dropped out after phase 2 of clinical trials. Only the RTS,S/ASO$_2$ vaccine candidate gave high protection, even though it showed short-lasting immunity[79, 80].

Among asexual blood-stage vaccines, favourite candidate antigens are parasite proteins involved during red blood cells invasion by merozoites, such as MSP for *P. falciparum*. Although translation to humans of promising results obtained from rodent models was difficult, few vaccines containing MSPs reached phase 1 or 2 trials[81]. Another strategy adopted to design asexual blood-stage vaccines focuses on red blood cells antigens involved during merozoite invasion or after erythrocyte infection. For example, Duffy antigen, the human receptor of *P. vivax* on the reticulocyte surface, is a good candidate to block invasion; however, due to numerous polymorphisms of *P. vivax* Duffy binding protein in Asia, a polyvalent vaccine should be useful[82]. On the other side, specifical sub-set of surface proteins expressed by *P. falciparum*-infected erythrocytes involved during sequester in placenta can be used to design specific vaccines for pregnant women. An example is given by VAR2CSA, which binds placental chondroitinsulphate A[83]. Finally, asexual blood-stage vaccine research investigates also the inflammatory responses which block parasite mediators of disease related to severe complications of malaria, even though any vaccine would be directed on clinical symptoms but would not abolish infection[74].

In a malaria eradication perspective, efficacy of either pre-eryhtrocytic or asexual blood-stage vaccines is a crucial point. While all of them achieve reduction of clinical malaria, only those with high efficacy could prevent the transmission of infection and stop gametocyte production. For this reason, the 30-years old idea of a malaria vaccine able to block transmission of infection by feeding mosquitoes with effective antibodies was reborn during recent years. Target antigens to prevent sporogenic development in the vector are sexual-stage specific molecules involved during or after fertilization, such as p25, p28, p48/45 and p230 *P. falciparum* or *P. vivax* proteins[84, 85]. Interestingly, some of these gamete surface molecules (p48/45 and p230) are also expressed in gametocyte in the blood[85]. Studies on these antigens allowed to compare nature and duration of naturally-acquired sexual- and asexual-stage immunity. Preliminar investigation on transmission-blocking vaccines showed good activity results. Indeed, after feeding mosquitoes with gametocyte-containing blood and sera from vaccinated animals, a reduction of vector infection was observed[86]. Phase 1 human trials also started, displaying vaccine activity, though at lower levels than those obtained from animal tests[87]. Up to now, only in one study p25 vaccine was associated with systemic adverse events[88].

6. Conclusions

Large-scale availability of antimalarial tools described in previous sections lead to unexpected success in malaria control, and research progress helped to prosecute elimination perspectives in several endemic areas. Such an encouraging premise has renewed interest in malaria

community towards a global eradication program. As recently proposed[2], priorities of different research areas (chemoprevention, treatment, vector control, vaccines, health systems) must be updated according to new directives designed for eradication goal.

Administration of antimalarial drugs for chemoprevention can be performed through two different procedures, such as intermittent preventive treatment, which requires drug administration at specified times to individuals at risk when they have access to health systems, and mass drug administration, which is performed once at the same time towards whole population at risk to prevent transmission. In a perspective of eradication, minor priority will be given to intermittent preventive treatment, while mass drug administration must be certainly improved. Regarding treatment, the development of drugs for uncomplicated malaria is still a high priority, especially considering new alarm of artemisinin resistance which could dramatically stop efficacy of present antimalarial therapies. However, to block transmission instead of controlling clinical cases, major attention must be directed towards research of drugs which can be effective against *Plasmodium* sexual stages, while eradication of *P. vivax* will probably require enhanced research on drugs able to kill hypnozoites safer then primaquine. Moreover, as infection transmission blocking is a primary goal of eradication program, vector control will play a key role in the new list of research priorities, aimed to develop new insecticides for insecticide-treated bednets and indoor residual spraying, and to improve other alternative methods, such as repellents, to contrast residual day- and outdoor-biting mosquitoes. Among vaccines, those with low efficacy against clinical malaria should be studied with reduced priority, while more investments are needed for research on transmission blocking vaccines. Finally, special attention will be reserved to health systems, either focusing on improvement of access to therapy for all economical categories of people, which remains a high priority, or ameliorating cost-efficacy of interventions and combinations, which becomes a new priority to be enhanced. Time will demonstrate if eradication of malaria is possible. By the way, adjustments to research priorities will be useful to assess further elimination programs. Please, pay attention: war is in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Present work was adapted and extended from a lecture delivered by Dr. Mauro Prato at the Second Reunion of Young Internal Medical Doctors of Piemonte (North Western Italy, 2009) and was supported by the Compagnia di San Paolo-IMI grants to MP in the context of the Italian Malaria Network. Thanks are due to Prof. Paolo Arese for counselling.

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