REVIEWS

The Malaria Problem: short communication.

Charles Ebikeme, Victoria Valdivia Giménez
All Res. J. Biol, **2010**, 1, 4-11

All your results are good results!

THE ALL RESULTS JOURNALS

Issue 1, Vol. 1, **2010**, 4-11

The Malaria Problem: short communication

Charles Ebikeme*[a] and Victoria Valdivia Giménez[b]

a) Centre de Résonance Magnétique des Systèmes Biologiques (RMSB), UMR5536 CNRS, Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux, France ; b) Instituto de Investigaciones Químicas, C.S.I.C-Universidad de Sevilla, c/Américo Vespucio, 49, Isla de la Cartuja, 41092 Sevilla, Spain

Corresponding author e-mail: charles@rmsb.u-bordeaux2.fr

Keywords: Malaria, negative results, phase 1 drugs, drug resistance

Introduction

Over a decade ago the world decided to approach malaria in a new way. The goal is the eradication of Malaria – and that, by 2015, will no longer be a major cause of mortality nor a barrier to social and economic development and growth anywhere in the world (www.rollbackmalaria.org). Malaria is the world's most prevalent infectious disease. Almost 40% of the world's population is at risk.[1] In 2006, 247 million malaria cases caused around a million deaths,[2] of which children and pregnant women were disproportionately affected. The African continent feels the greatest burden from this neglected disease, with 45 countries in the sub-Sahara being endemic for malaria and accounting for 86% of cases worldwide.[2]

Malaria is caused by protozoan parasites of the genus *Plasmodium*. Over 100 recognised species of *Plasmodium* exist, infecting a wide range of vertebrate hosts including primates, rodents, birds and reptiles. The deadliest human malaria parasite is *Plasmodium falciparum*, resulting in the most severe clinical symptoms of the disease and causing 90% of all malaria deaths. Along with *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* are the four main species that are known to infect humans. There are significant differences between the species; ranging from liver stage progression, parasite replication time within the host, and the timing in appearance of gametocytes in the bloodstream.[3,4] Each species causes a unique set of complications in terms of disease. *P. falciparum* infections are responsible for the most severe form of malaria and can result in cerebral malaria and pregnancy-related malaria. Malaria caused by *P. vivax* is becoming an increasing problem,[5] accounting for as much as 40% of the total disease burden.[6] Relapse can also be a problem with *P. vivax* infections. The development of dormant hypnozoite forms in the liver can last up to 20 years and cause subsequent reinfections in the blood.[7]

Plasmodium parasites are transmitted by female mosquitoes of the genus *Anopheles*, of which only 60 species are able to transmit the disease. Vector control remains a central aspect of any malaria eradication strategy. So much so that in some highly endemic countries vector control measures has led to reductions in deaths from malaria.[2] Historically, emphasis was put on reducing the numbers of mosquitoes by combinations of environmental hygiene and insecticide spraying. None was more important than dichlorodiphenyl-trichloroethane (DDT). DDT use today is rare, highlighting the great importance that mosquito resistance has to any and future vector control strategies. The development of resistance to DDT was a primary cause of the collapse of previous malaria control programmes in the latter half of the late century.[8] Currently, there is a near-complete dependence on pyrethroids for vector control, and the development of new techniques for vector control need to be increased.[9]

This article gives a brief overview of the current state of antimalarial drug development, paying close attention to the history and current problems of drug treatment, and highlighting possible future drugs in phase I clinical trials.

Antimalarials and Resistance

Antimalarials present the most important part of any integrated approach needed to combat the disease. Antimalarials have direct benefits to patients and a general decrease in disability-adjusted life years (DALYs) for the population in general. Today, parasite resistance to all but one class of antimalarials exists in most endemic countries.[10] Resistance has prompted the wide-scale shift in first-line treatments against malaria, under recommendation by the World Health Organisation (WHO). However, many countries continued to use ineffective mono-therapy treatments, due to, in part, the disparity in costs between the more conventional chloroquine and sulfadoxine-pyrimethamine based therapies and the recommended Artemisinin combination drugs.[11] However, the increase of international funding commitments,[10,12] resulting in increased malaria control programmes has gone some way to rectify this problem.

Drug resistance is the major cause of malaria treatment failure. However, influencing the rapid rise of drug resistance are factors such as non-compliance or non-adherence to drug regimen, nutritional status of patients, incorrect drug usage, counterfeit drugs, and misdiagnosis of patients.[13] Further clouding the issue is the distinction

between and outcome of drug resistance, treatment failure, and reinfection.[14] The mechanisms, molecular and biochemical, underlying resistance of *Plasmodium* species to the various antimalarial drugs has been greatly studied to date.[15,16] The genetic events that confer antimalarial drug resistance are specific for each drug and consist of mutations or single gene copy number mutations in genes related to drug targets (Table I).

Quinoline antimalarials have been widely used for the treatment of malaria. Among these are mefloquine, quinine, pyronaridine, halofantrine, primaquine, and chloroquinine. The cheapest and more widely available antimalarial was quinine, the first known effective anti-malarial drug – an extract from the bark of the tree *Cinchona calisaya* – was used as an antimalarial agent as early as 1632,[17] and by the 19th century it was still the only known antimalarial agent. For decades first-line treatment of malaria involved chloroquine (CQ), the first synthetic antimalarial compound introduced after the second world war[18] following US government-sponsored clinical trials which showed that CQ had prominent effects as an anti-malarial drug.[19] Today, CQ is given as treatment for both uncomplicated malaria or severe malaria.[20]

Parasite resistance to CQ is widespread. CQ-resistant *P. falciparum* (CRPF) emerged from four independent foci. Firstly, in Southeast Asia around the Thai-Cambodian border, where CRPF infections were identified in 1957 and spread quickly to Thailand.[21] Two other foci were identified in 1960 in South America, in Venezuela and in the Magdalena Valley, Colombia.[22] In 1976, two confirmed cases of CRPF infection were reported from Port Moresby in Papua New Guinea[72] and probably represent the emergence of the fourth independent focus of CRPF infection. In Africa, CRPF was first found in 1978.[23] Resistance spread from the African coastal areas inland and by 1983 had been observed in Sudan, Uganda, Zambia, and Malawi. In 1973, Thailand was the first country to replace CQ as a first-line drug. The spread of CQ resistance was the main factor causing the increased child mortality rates observed in Africa since the last decade of the last century.[24]

CQ resistance is multigenetic and results in a reduced parasite accumulation of the drug,[25,26] with reduced concentrations of the drug in the digestive vacuole of the parasite. CQ enters the food vacuole and targets the polymerisation of toxic haem, binding and thus preventing its polymerisation to haemozoin.[27,28] This results in an increase in toxic haem leading to enhanced oxidative stress, membrane damage and eventually parasite death.[29] One mechanism of resistance is associated with polymorphisms in a 36 kb segment of the parasite's chromosome 7, which contains a polymorphic gene encoding a unique 330 kDa protein, *cg2*.[30] However, association of *cg*2 with chloroquine resistance in field isolates is incomplete.[31] Genetic crosses identified a role of the *P. falciparum* chloroquine-resistance transporter (PfCRT), a carrier protein located in the membrane of the digestive vacuole of the blood-stage parasite.[32,33,34] Multiple polymorphisms in the gene are associated with chloroquine resistance both *in vitro* and *in vivo*. However, PfCRT is not the sole molecular determinant of chloroquine resistance. Mutations in the homolog of the major multidrugtransporter *P. falciparum* multidrug resistance gene (PfMDR) seems to modulate the extent of chloroquine resistance conferred by mutations in PfCRT.[35] Furthermore, the pfmdr1 gene has been shown to be involved in mefloquine resistance and cross-resistance to halofantrine.[36,37]

Sulfadoxine-pyrimethanine (SP), a class of antifolates, is another drug used to treat uncomplicated malaria.[20] Its widespread use in many countries as a first-line antimalarial treatment was prompted by the emergence of CQ resistance. However, resistance developed rapidly; SP was introduced in Thailand in 1967 and resistance was reported within the same year.[23,38] Antifolates (including pyrimethamine-sulfadoxine, chlorproguanil-dapsone, and proguanil-atovaquonel) represent the more traditional second-line treatment option for malaria, but again, resistance is widespread.[39] These classes of drugs have a mode of action through either inhibiting the formation of dihydropteroate catalyzed by dihydropteroate synthase (DHPS) by competing for the active site of DHPS;[40] or by inhibition of dihydrofolate reductase (DHFR), thus preventing the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate by DHFR. Mechanisms of resistance seem to be associated with several point mutations in the respective genes.[41,42] A specific combination of these mutations is heavily associated with treatment failure.[43] However, treatment outcomes become increasingly more difficult to predict as the level of mutation falls off .[14]

In 1972, Chinese scientists discovered *Qing hao su* – sesquiterpere lactone artemisinin – isolated from the leaves of the sweet wormwood *Artemisia annua*. Artemisinin-based combination therapies (ACTs) are now generally considered as the best current treatment for uncomplicated falciparum malaria for a number of reasons.[2] The most important being that no real mode of resistance has yet been implicated with Artemisinin itself. Most likely because a shorter half-life allows rapid clearance from the body, which avoids persistence of the drug at sub-lethal concentrations, and as a result will avoid the emergence of resistant parasites. Currently, several treatment options are available – as mandated by the WHO[2] – artemether-lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, and artenusate + sulfadoxine-pyrimethamine. It is hoped that by combining antimalarial drugs with different modes of action parasite resistance can either be prevented or its onset delayed considerably, allowing completion of full dose regimens to end in high cure rates and an eventual decrease of disease transmission, benefiting patients and the larger community. Today, more potent derivatives of its active chemical have been developed. These include artemether, artemotil, and artesunate. The problem of the appearance of resistance to artemisinin is one that health professionals, policy makers and research scientists are well aware of.[44] To that effect, ACTs are now the recommended strategy both for clinical care and for the avoidance of drug resistance.[2] To date, there has been no *in vivo* cases of resistance reported, however, *in vitro* susceptibility was found to vary with mutations in *pfmdr1* and *pfcrt* (the two genes proposed to modulate sensitivity to CQ).[45] Furthermore, cases of drug

resistance induced experimentally are few and of a moderate level, and even then, have been proven to be transient.[46] The mode of action of artemisinin and its derivatives is proving to be complicated and has not been completely elucidated, however, it seems to stem from alkylation of molecules by radicals produced from the reductive cleavage of the intact peroxide by ferroheme ferrous-protoporphyrin IX.[31,47]

ACTs represent the present best hope for treatment of malaria. If the history of the malaria parasite has taught us anything then it's that onset of parasite resistance is a distinct inevitability, which brings up the question of where will the next generation of antimalarials come from? Charles Ledger *"gave quinine to the world"* even before the causative agent or the disease was known, the wars in Europe and Vietnam led to the development of chloroquine, mefloquine and halofantrine, and ancient China has brought the blue-green herb that is currently the first line of defence against the world's most prevalent disease. The next generation of antimalarials are some way off from becoming a clinical reality. Most antimalarial drugs developed thus far have been identified and developed using conventional drug discovery techniques.[48] The future will bring many progressive leaps in the kind of techniques employed by researchers to increase the beneficial output of new compounds. Pharmacogenetic-pharmacokinetic relations and parameters, pathogen and host genomic and proteomic information, as well as randomised trials and replication will all prove fruitful when applied to antimalarial drugs, not only in understanding the resistance factors at play but also in understanding the clinical success and failure of present and future antimalarial treatments.[49]

Drugs in Phase I Clinical Trials

Aminoquinoline antimalarial (AQ-13) has been shown to be effective *in vitro* against *P. falciparum* malaria parasites resistant to CQ and other antimalarials, as well as being active in a model of human infection with *P. vivax*, CQ-resistant *P. falciparum* in the squirrel monkey, a model of human infection with CQ-resistant *P. falciparum*, and in two *in vivo* monkey models of human malaria (*P. cynomolgi* in the rhesus monkey Macaca mulatta). Its performance in human subjects is being investigated in Phase 1 (safety/toxicity and pharmacokinetic) studies.[50,51,52] Furthermore, AQ-13 has proven to be of similar safety to that of CQ in preclinical studies performed by SRI International (IND 55,670). The trial on AQ-13 was appropriately designed as a randomized controlled Phase I study, allowing the assessment of safety and physiological outcomes after treatment as compared to an existing and widely used drug, CQ. A key limitation inherent to such studies is the small number of participants studied. This means that the study cannot prove that AQ-13 is safe, or even as safe as CQ, but rather simply that the findings do not raise immediate safety concerns.[53]

With malaria parasites often being resistant to CQ and SP, chlorproguanil-dapsone is a potential alternative. The objective of the clinical trial was to compare chlorproguanil-dapsone with other antimalarial drugs for treating uncomplicated falciparum malaria.[54] Recent trials have shown that chlorproguanil-dapsone (with 1.2 mg chlorproguanil) as a single dose had fewer treatment failures than chloroquine (1 trial), but more treatment failures and people with parasitaemia at day 28 than sulfadoxine-pyrimethamine (3 trials). Two trials compared the three-dose chlorproguanil-dapsone (with 2 mg chlorproguanil) regimen with sulfadoxine-pyrimethamine in new attendees. There were fewer treatment failures with chlorproguanil-dapsone by day 7 (1 trial). Neither trial reported total failures by day 28. A further trial was carried out in participants selected because they had previously failed sulfadoxine-pyrimethamine. Adverse event reporting was inconsistent between trials, but chlorproguanil-dapsone was associated with more adverse events leading to discontinuation of treatment compared with sulfadoxine-pyrimethamine (1 trial). It was also associated with more red blood cell disorders. Randomized controlled trials that follow up to day 28, record adverse events, and use an intention-to-treat analysis are required to inform any policy decisions. In all, it seems that there is insufficient data about the effects of the current standard chlorproguanil-dapsone regimen (three-dose, 2 mg chlorproguanil). However, chlorproguanil-dapsone has been withdrawn in 2008 following demonstration of post-treatment haemolytic anaemia in G6PD deficient patients in a Phase III trial of chlorproguanil-dapsone and chlorproguanil-dapsone-artesunate versus artemether-lumefantrine.[55,56] Significant reductions of haemoglobin levels in patients with G6PD deficiency have been observed with both CD and CDA.

Mefloquine, a quinolinemethanol antimalarial, is effective as therapy and prophylaxis for all species of malaria infecting humans, including multi-drug resistant *P.falciparum*. Mefloquine is a chiral molecule with two asymmetric carbon centres, which means it has four different stereoisomers. The drug is currently manufactured and sold as a racemate of the (R,S)- and (S,R)-enantiomers by Hoffman-LaRoche, a Swiss pharmaceutical company. Mefloquine's clinical utility has been impaired by its association with neuropsychiatric side effects.[57] The pharmacological basis of these side effects are not known but two of the most reported hypotheses relate to its action on (i) the adenosine receptor[58] and (ii) its effect on the cholinesterase enzyme.[59] For both these mechanisms, there is a significant stereoselective activity of the two enantiomers.[60] Studies show that the (-) isomer is 50-100 fold more potent towards adenosine receptors compared with the (+) isomer.[61] In addition, (-)-mefloquine has considerably more anti-cholinesterase activity.[62] It has therefore been hypothesised that (+)-mefloquine may have a better central nervous system (CNS) safety profile compared with either the racemate or (-)-mefloquine.[63] The Phase I clinical trial consisted of a randomized, ascending dose, double-blind, active and placebo-controlled, parallel group study in healthy male and female volunteers designed to investigate this hypothesis and to describe the comparative pharmacokinetics of the racemate and the single enantiomer.[64]

Ferroquine (FQ)+Artesunate(AS),[65] a unique organometallic compound, is a novel antimalarial drug designed to overcome the chloroquine (CQ) resistance problem currently in phase I clinical trials. FQ has been

revealed to be equally active on CQ-sensitive and CQ-resistant *P. falciparum* laboratory strains and field isolates, and is also curative on rodent malaria parasites. AS, a class of the artemisinin group of drugs that treat malaria, is a semi-synthetic derivative of artemisinin that is water-soluble and may therefore be given by injection.[66] Ferroquine and artesunate combination therapies have been the subject of Phase I clinical trials.[67] Combinations of the two drugs were used to assess the safety of different doses of ferroquine with artesunate in adult African patients with uncomplicated malaria and to assess activity in reducing parasitemia and the pharmacokinetics of ferroquine and its metabolites.

Future Prospects

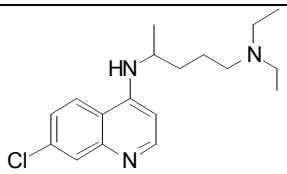
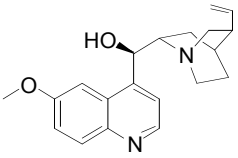
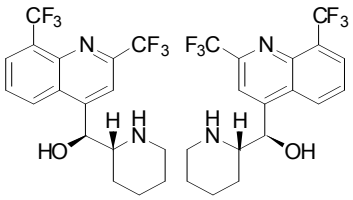
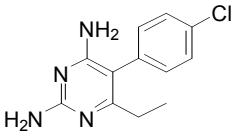
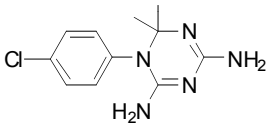
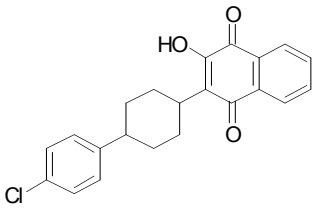
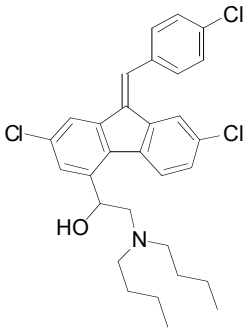
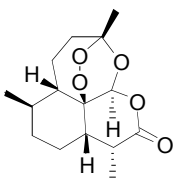
Malaria is preventable and curable, however, in the absence of quick and effective treatment, symptoms of the disease progress and death results.[68] Accurate diagnosis is needed in all cases as well as accurate surveillance in all endemic areas.[69] To curb the incidence of treatment failure, the spread of resistance, WHO recommends confirmation of malaria through parasite-based diagnosis in all patients prior to commencing treatment. Prompt parasitologic confirmation by microscopy or alternatively by rapid diagnostic tests (RDTs) are recommended in all patients suspected of malaria before treatment is started. Thus, diminishing unnecessary use of ACTs and provide critical and accurate surveillance data to manage programmes and monitor impact. However, misdiagnosis and over-diagnosis of malaria still occurs.[70] One reason is simply due to the fact that early symptoms of malaria are non-specific.

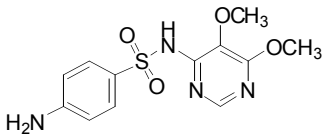
Bringing research agendas and control programmes together is one of the greatest challenges to fighting any infectious disease. For malaria endemic countries, strengthening the existing health systems is crucial. Prevention, diagnosis and treatment needs to be followed up with accurate surveillance. The last few years have seen a great development in new treatments and new strategies, mainly because of the failure of old regimes due to parasite resistance.

The unique biochemical aspects of the parasite continue to be exploited in the hope of drug design. Parasite Genome Initiatives are ongoing efforts of full genomic sequencing to facilitate full understanding of how parasites develop, survive and reproduce in their respective hosts, of parasite-host and parasite-immune system interactions and of the factors that determine behaviour, pathogenicity, drug resistance and antigenic variation. Parasite genome sequencing together with effective genetic manipulation (gene knock-out and gene silencing) provides a valuable means of mimicking loss of function attributable to therapeutic intervention (albeit with the caveat that pharmacological agents cannot mimic the zero activity state produced by conventional gene knock-out experiments).

Already, this year has seen significant advancements in the world of malaria research. GlaxoSmithKline has made available, in the public domain, thousands of compounds – confirmed-hit structures – to the general scientific community. The genome for *Artemisia annua* – the herb producing the active ingredient in the most effective treatment for malaria – has been mapped.[71] The further highlighting of genes and markers that could pave the way for higher yield varieties is another step in the road to complete eradication of this debilitating disease.

Table I: Antimalarial compounds, targets, and resistance.

Structure	Molecule	Frequency of Resistance[a]	Resistance gene
	Chloroquine	++++[b]	*cg2/pfcrt/pfmdr1*
	Quinine	++	*pfcrt /pfmdr1/ pfnhe*
	Mefloquine	+++	*pfmdr1*/*other*
	Pyrimethamine	++++	*pfdhfr*
	Cycloguanil	++	*pfdhfr*
	Atovaquone	+	*pfcytb*
	Lumefantrine	++	*pfcrt*/*pfmdr1*
	Artemisinin	+	*pfcrt*/*pfmdr1*/ *Pf*ATPase6

	Sulfadoxine	++++	*pfdhps*

a P.B. Bloland. In: WHO/CDS/CSR/DRS/2001.4, **2001**
b Resistance frequency: (+) presence of resistance; (−) absence of resistance (WHO. In: *Roll Back Malaria*. WHO/CDS/RBM/2001.33, **2001**).

References

1) Greenwood, B., & Mutabingwa, T. (2002). Malaria in 2002. *Nature*, *415*(6872), 670-672. doi:10.1038/415670a

2) WHO (2008) World Malaria Report.

3) Krotoski, W. A., Krotoski, D. M., Garnham, P. C., Bray, R. S., Killick-Kendrick, R., Draper, C. C., Targett, G. A., et al. (1980). Relapses in primate malaria: discovery of two populations of exoerythrocytic stages. Preliminary note. *British Medical Journal*, *280*(6208), 153-154.

4) Wells, T. N. C., Alonso, P. L., & Gutteridge, W. E. (2009). New medicines to improve control and contribute to the eradication of malaria. *Nature Reviews. Drug Discovery*, *8*(11), 879-891. doi:10.1038/nrd2972.

5) Mueller, I., Galinski, M. R., Baird, J. K., Carlton, J. M., Kochar, D. K., Alonso, P. L., & del Portillo, H. A. (2009). Key gaps in the knowledge of Plasmodium vivax, a neglected human malaria parasite. *The Lancet Infectious Diseases*, *9*(9), 555-566. doi:10.1016/S1473-3099(09)70177-X.

6) Price, R. N., Tjitra, E., Guerra, C. A., Yeung, S., White, N. J., & Anstey, N. M. (2007). Vivax malaria: neglected and not benign. *The American Journal of Tropical Medicine and Hygiene*, *77*(6 Suppl), 79-87.

7) Cogswell, F. B. (1992). The hypnozoite and relapse in primate malaria. *Clinical Microbiology Reviews*, *5*(1), 26-35.

8) Greenwood, B. (2002). The molecular epidemiology of malaria. *Tropical Medicine & International Health: TM & IH*, *7*(12), 1012-1021.

9) Kilama, W., & Ntoumi, F. (2009). Malaria: a research agenda for the eradication era. *Lancet*, *374*(9700), 1480-1482. doi:10.1016/S0140-6736(09)61884-5.

10) WHO (2009) World Malaria Report.

11) Attaran, A., Barnes, K. I., Curtis, C., d'Alessandro, U., Fanello, C. I., Galinski, M. R., Kokwaro, G., et al. (2004). WHO, the Global Fund, and medical malpractice in malaria treatment. *Lancet*, *363*(9404), 237-240. doi:10.1016/S0140-6736(03)15330-5.

12) Nafo-Traoré, F. (2004). Response to accusations of medical malpractice by WHO and the Global Fund. *Lancet*, *363*(9406), 397. doi:10.1016/S0140-6736(04)15443-3.

13) Baird, J. K. (2005). Effectiveness of antimalarial drugs. *The New England Journal of Medicine*, *352*(15), 1565-1577. doi:10.1056/NEJMra043207.

14) Olliaro, P. (2005). Drug resistance hampers our capacity to roll back malaria. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *41 Suppl 4*, S247-257. doi:10.1086/430785.

15) Escalante, A. A., Smith, D. L., & Kim, Y. (2009). The dynamics of mutations associated with anti-malarial drug resistance in Plasmodium falciparum. *Trends in Parasitology*, *25*(12), 557-563. doi:10.1016/j.pt.2009.09.008.

16) Hayton, K., & Su, X. (2004). Genetic and biochemical aspects of drug resistance in malaria parasites. *Current Drug Targets. Infectious Disorders*, *4*(1), 1-10.

17) Baird, J. K., Sustriayu Nalim, M. F., Basri, H., Masbar, S., Leksana, B., Tjitra, E., Dewi, R. M., et al. (1996). Survey of resistance to chloroquine by Plasmodium vivax in Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *90*(4), 409-411.

18) Andriantsoanirina, V., Ménard, D., Tuseo, L., & Durand, R. (2010). History and current status of Plasmodium falciparum antimalarial drug resistance in Madagascar. *Scandinavian Journal of Infectious Diseases*, *42*(1), 22-32. doi:10.3109/00365540903289670.

19) Solomon, V. R., & Lee, H. (2009). Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. *European Journal of Pharmacology*, *625*(1-3), 220-233. doi:10.1016/j.ejphar.2009.06.063.

20) White, N. J. (1996). The treatment of malaria. *The New England Journal of Medicine*, *335*(11), 800-806.

21) Harinasuta, T., Suntharasamai, P., & Viravan, C. (1965). Chloroquine-resistant falciparum malaria in Thailand. *Lancet*, *2*(7414), 657-660.

22) Moore, D. V., & Lanier, J. E. (1961). Observations on two Plasmodium falciparum infections with an abnormal response to chloroquine. *The American Journal of Tropical Medicine and Hygiene*, *10*, 5-9.

23) Campbell, C. C., Chin, W., Collins, W. E., Teutsch, S. M., & Moss, D. M. (1979). Chloroquine-resistant Plasmodium falciparum from East Africa: cultivation and drug sensitivity of the Tanzanian I/CDC strain from an American tourist. *Lancet*, *2*(8153), 1151-1154.

24) Snow, R. W., Trape, J. F., & Marsh, K. (2001). The past, present and future of childhood malaria mortality in Africa. *Trends in Parasitology*, *17*(12), 593-597.

25) White, N. (1999). Antimalarial drug resistance and combination chemotherapy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *354*(1384), 739-749. doi:10.1098/rstb.1999.0426.

26) Mu, J., Ferdig, M. T., Feng, X., Joy, D. A., Duan, J., Furuya, T., Subramanian, G., et al. (2003). Multiple transporters associated with malaria parasite responses to chloroquine and quinine. *Molecular Microbiology*, *49*(4), 977-989.

27) Egan, T. J., Mavuso, W. W., Ross, D. C., & Marques, H. M. (1997). Thermodynamic factors controlling the interaction of quinoline antimalarial drugs with ferriprotoporphyrin IX. *Journal of Inorganic Biochemistry*, *68*(2), 137-145.

28) Leed, A., DuBay, K., Ursos, L. M. B., Sears, D., De Dios, A. C., & Roepe, P. D. (2002). Solution structures of antimalarial drug-heme complexes. *Biochemistry*, *41*(32), 10245-10255.

29) Loria, P., Miller, S., Foley, M., & Tilley, L. (1999). Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *The Biochemical Journal*, *339 (Pt 2)*, 363-370.
30) Su, X., Kirkman, L. A., Fujioka, H., & Wellems, T. E. (1997). Complex polymorphisms in an approximately 330 kDa protein are linked to chloroquine-resistant P. falciparum in Southeast Asia and Africa. *Cell*, *91*(5), 593-603.
31) Olliaro, P. (2001). Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacology & Therapeutics*, *89*(2), 207-219.
32) Wellems, T. E., Walker-Jonah, A., & Panton, L. J. (1991). Genetic mapping of the chloroquine-resistance locus on Plasmodium falciparum chromosome 7. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(8), 3382-3386.
33) Fidock, D. A., Nomura, T., Talley, A. K., Cooper, R. A., Dzekunov, S. M., Ferdig, M. T., Ursos, L. M., et al. (2000). Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Molecular Cell*, *6*(4), 861-871.
34) Travassos, M. A., & Laufer, M. K. (2009). Resistance to antimalarial drugs: molecular, pharmacologic, and clinical considerations. *Pediatric Research*, *65*(5 Pt 2), 64R-70R. doi:10.1203/PDR.0b013e3181a0977e.
35) Warhurst, D. C. (2001). A molecular marker for chloroquine-resistant falciparum malaria. *The New England Journal of Medicine*, *344*(4), 299-302.
36) Volkman, S. K., Wilson, C. M., & Wirth, D. F. (1993). Stage-specific transcripts of the Plasmodium falciparum pfmdr 1 gene. *Molecular and Biochemical Parasitology*, *57*(2), 203-211.
37) Peel, S. A., Bright, P., Yount, B., Handy, J., & Baric, R. S. (1994). A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (pfmdr) of Plasmodium falciparum in vitro. *The American Journal of Tropical Medicine and Hygiene*, *51*(5), 648-658.
38) Wernsdorfer, W. H., & Payne, D. (1991). The dynamics of drug resistance in Plasmodium falciparum. *Pharmacology & Therapeutics*, *50*(1), 95-121.
39) Gregson, A., & Plowe, C. V. (2005). Mechanisms of resistance of malaria parasites to antifolates. *Pharmacological Reviews*, *57*(1), 117-145. doi:10.1124/pr.57.1.4.
40) Hyde, J. E. (2007). Drug-resistant malaria - an insight. *The FEBS Journal*, *274*(18), 4688-4698. doi:10.1111/j.1742-4658.2007.05999.x.
41) Wu, Y., Kirkman, L. A., & Wellems, T. E. (1996). Transformation of Plasmodium falciparum malaria parasites by homologous integration of plasmids that confer resistance to pyrimethamine. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(3), 1130-1134.
42) Triglia, T., Wang, P., Sims, P. F., Hyde, J. E., & Cowman, A. F. (1998). Allelic exchange at the endogenous genomic locus in Plasmodium falciparum proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. *The EMBO Journal*, *17*(14), 3807-3815. doi:10.1093/emboj/17.14.3807.
43) Nzila, A. M., Mberu, E. K., Sulo, J., Dayo, H., Winstanley, P. A., Sibley, C. H., & Watkins, W. M. (2000). Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in Plasmodium falciparum: genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. *Antimicrobial Agents and Chemotherapy*, *44*(4), 991-996.
44) Yeung, S., Pongtavornpinyo, W., Hastings, I. M., Mills, A. J., & White, N. J. (2004). Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices. *The American Journal of Tropical Medicine and Hygiene*, *71*(2 Suppl), 179-186.
45) Afonso, A., Hunt, P., Cheesman, S., Alves, A. C., Cunha, C. V., do Rosário, V., & Cravo, P. (2006). Malaria parasites can develop stable resistance to artemisinin but lack mutations in candidate genes atp6 (encoding the sarcoplasmic and endoplasmic reticulum Ca2+ ATPase), tctp, mdr1, and cg10. *Antimicrobial Agents and Chemotherapy*, *50*(2), 480-489. doi:10.1128/AAC.50.2.480-489.2006.
46) Peters, W., & Robinson, B. L. (1999). The chemotherapy of rodent malaria. LVI. Studies on the development of resistance to natural and synthetic endoperoxides. *Annals of Tropical Medicine and Parasitology*, *93*(4), 325-329.
47) Meshnick, S. R. (2002). Artemisinin: mechanisms of action, resistance and toxicity. *International Journal for Parasitology*, *32*(13), 1655-1660.
48) Rathore, D., McCutchan, T. F., Sullivan, M., & Kumar, S. (2005). Antimalarial drugs: current status and new developments. *Expert Opinion on Investigational Drugs*, *14*(7), 871-883. doi:10.1517/13543784.14.7.871.
49) Kerb, R., Fux, R., Mörike, K., Kremsner, P. G., Gil, J. P., Gleiter, C. H., & Schwab, M. (2009). Pharmacogenetics of antimalarial drugs: effect on metabolism and transport. *The Lancet Infectious Diseases*, *9*(12), 760-774. doi:10.1016/S1473-3099(09)70320-2.
50) Deng, H., Liu, H., Krogstad, F. M., & Krogstad, D. J. (2006). Sensitive fluorescence HPLC assay for AQ-13, a candidate aminoquinoline antimalarial, that also detects chloroquine and N-dealkylated metabolites. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *833*(2), 122-128. doi:10.1016/j.jchromb.2005.12.011.
51) De, D., Krogstad, F. M., Cogswell, F. B., & Krogstad, D. J. (1996). Aminoquinolines that circumvent resistance in Plasmodium falciparum in vitro. *The American Journal of Tropical Medicine and Hygiene*, *55*(6), 579-583.
52) De, D., Krogstad, F. M., Byers, L. D., & Krogstad, D. J. (1998). Structure-activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines. *Journal of Medicinal Chemistry*, *41*(25), 4918-4926. doi:10.1021/jm980146x.
53) Mzayek, F., Deng, H., Mather, F. J., Wasilevich, E. C., Liu, H., Hadi, C. M., Chansolme, D. H., et al. (2007). Randomized dose-ranging controlled trial of AQ-13, a candidate antimalarial, and chloroquine in healthy volunteers. *PLoS Clinical Trials*, *2*(1), e6. doi:10.1371/journal.pctr.0020006.
54) Bukirwa, H., Garner, P., & Critchley, J. (2004). Chlorproguanil-dapsone for treating uncomplicated malaria. *Cochrane Database of Systematic Reviews (Online)*, (4), CD004387. doi:10.1002/14651858.CD004387.pub2.
55) Fanello, C.I., Karema, C., Avellino, P., Bancone, G., Uwimana, A., Lee, S. J., d'Alessandro, U., Modiano, D. (2008) High Risk of Severe Anaemia after Chlorproguanil-Dapsone+Artesunate Antimalarial Treatment in Patients with G6PD (A-) Deficiency. *Plos One*, *3* (12), 4031-4036.

56) WHO | QSM/MC/IEA.117. 4 March 2008. Antimalarial chlorproguanil-dapsone (LapDap™) withdrawn following demonstration of post-treatment haemolytic anaemia in G6PD deficient patients in a Phase III trial of chlorproguanil-dapsone-artesunate (Dacart™) versus artemether-lumefantrine (Coartem®) and confirmation of findings in a comparative trial of LapDap™ versus Dacart ™. *Information Exchange System* Alert No. 117. http://www.who.int/medicines/publications/drugalerts/Alert_117_LapDap.pdf.

57) Bernard J, Le C. J., Sarrouy J., Renaudineau J., Geffray L., Dufour P., et al. (1987)Toxic encephalopathy caused by mefloquine?. *Presse Med*,*16*(33), 1654-1655.

58) Shepard R.D., Fletcher A. (1998). Use of (+)-mefloquine for the treatment of malaria with reduced side-effects. *Int Patent Appl WO*, 9839003.

59) Ngiam T.L., Go ML. (1987). Stereospecific inhibition of cholinesterases by mefloquine enantiomers. *Chem Pharm Bull* (Tokyo), *35*(1), 409-412.

60) Barraud de Lagerie, S., Comets, Gautrand, C.,Fernandez, C., Auchere, D., Singlas, E., Mentre, F., Gimenez, F. (2004). Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar(GF1210918) in mice. *British Journal of Pharmacology*, *141*, 1214-1222.

61) Fletcher, A., Shepherd, R. (2003). Use of (+)-mefloquine for the treatment of malaria. *US patent* 6664397.

62) Speich, R., Haller, A. (1994). Central anticholinergic syndrome with the antimalarial drug mefloquine. *NEJM* , *331*, 57–58.

63) Pham Y.T., Nosten F., Farinotti R., White N.J., Gimenez F. (1999) Cerebraluptake of mefloquine enantiomers in fatal cerebral malaria. *Int J Clin Pharmacol Ther, 37*(1), 58-61.

64) Tansley, R. (2009). Study to Investigate the Safety, Tolerability and Pharmacokinetics of AD 452 [(+)-Mefloquine] Compared With Racemic Mefloquine. http://clinicaltrials.gov/ct2/show/NCT00931697.

65) Daher, W., Biot, C., Fandeur, T., Jouin, H., Pelinski, L., Viscogliosi, E., Fraisse, L., et al. (2006). Assessment of Plasmodium falciparum resistance to ferroquine (SSR97193) in field isolates and in W2 strain under pressure. *Malaria Journal*, *5*, 11. doi:10.1186/1475-2875-5-11.

66) Batty, K. T., Thu, L. T. A., Davis, T., Ilet, K. F., Mai, T. X., Hung, N. C., Tien, N. P., Powell, S. M., Thien, H. V., Binh, T. Q., Kim, N. V. (1998). A pharmacokinetic and pharmakodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. *Br. J. Clin. Phar, 45*, 123-129.

67) Supan, C. (2007) A Comparative Safety and Activity Study With Ferroquine Associated With Artesunate Versus Amodiaquine Associated With Artesunate in African Adult Patients With Uncomplicated Malaria. http://clinicaltrials.gov/ct2/show/NCT00563914?term=Ferroquine+%2B+Artesunate&rank=2.

68) Kokwaro, G. (2009). Ongoing challenges in the management of malaria. *Malaria Journal*, *8 Suppl 1*, S2. doi:10.1186/1475-2875-8-S1-S2.

69) Brooker, S., Kolaczinski, J. H., Gitonga, C. W., Noor, A. M., & Snow, R. W. (2009). The use of schools for malaria surveillance and programme evaluation in Africa. *Malaria Journal*, *8*, 231. doi:10.1186/1475-2875-8-231.

70) Chandler, C. I. R., Jones, C., Boniface, G., Juma, K., Reyburn, H., & Whitty, C. J. M. (2008). Guidelines and mindlines: why do clinical staff over-diagnose malaria in Tanzania? A qualitative study. *Malaria Journal*, *7*, 53. doi:10.1186/1475-2875-7-53.

71) Graham, I. A., Besser, K., Blumer, S., Branigan, C. A., Czechowski, T., Elias, L., Guterman, I., et al. (2010). The genetic map of Artemisia annua L. identifies loci affecting yield of the antimalarial drug artemisinin. *Science (New York, N.Y.)*, *327*(5963), 328-331. doi:10.1126/science.1182612.

72) Grimmond, T. R., Donovan, K. O., & Riley, I. D. (1976). Chloroquine resistant malaria in Papua New Guinea. Papua and New Guinea Medical Journal, 19(3), 184-185.