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ORIGIN AND SPREAD OF ANTIMALARIAL RESISTANCE IN AFRICA.

Clinical Science

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ABSTRACT

The Western Cambodia region is infamous for its malaria parasites. Twice already in the 1950s and the 1960s they have developed resistance to key drugs, and the underlying mutations spread inevitably around the world, forcing public health sector to find new ways to contain the disease. It is now happening again. Over the years, artemisinin, the most powerful drug available for management of malaria, has reported in a substantial number of individuals in Cambodia, Myanmar, Vietnam, Laos, and border regions of Thailand. Researchers and public health experts worry that history may repeat itself and the resistant parasites spread globally. Recent discovery of drug resistance-associated genes, pfcrt, pfmdr1, dhfr, dhps, and k13 and applications of microsatellite markers flanking the genes have revealed of the evolution of resistant parasites to all classes of antimalarials and the geographical distribution of drug resistance. Here, we review our recent knowledge of the Origin and spread of parasite resistance to the previously used drugs (chloroquine, sulfadoxine/pyrimethamine) and artemisinin combination therapy. Though efforts to prevent and eliminate resistance so far are still unsuccessful, but, new advances into the genes other than the threat insight should help scientists identify and track resistant parasites including finding better ways to contain them.

KEYWORDS

Malaria, Resistance, chloroquine, sulfadoxine, pyrimethamine, artemisinin

INTRODUCTION

Since 1940s, the use antimalarials has played a tremendous role in malaria control programs. However, the use of antimalarials worldwide has initiated high selective pressure on Plasmodium falciparum causing the emergence and spread of resistance with increased malaria incidences and related deaths1. Chloroquine and sulphadoxine-pyrimethamine (SP) have become greatly ineffective as monotherapy for the management of Plasmodium falciparum infection in many parts of the world1,2. To date, not all molecular targets of known antimalarials that are currently in use are defined. Drug resistance can be mediated by a direct catalytic mechanism, or it can be due to amplification of the gene encoding the target enzyme or transporter that pumps the drug out of the parasite3. Additionally, resistance can be mediated by processes that mitigate toxicity induced by the drug. ACT is now the recommended regimen for treatment of all P. falciparum infections in malaria endemic zones4, 5. So far ACTs are very effective, fast-acting, tolerable and safe. They are available and are usually administered for three days in various formulations. The various combinations are effective against all the asexual parasites stages and early stages of the sexual phase and, thereby, reduce transmission5. Unfortunately, artemisinin resistance in Plasmodium falciparum has now emerged and is spreading rapidly throughout Southeast (SE) Asia. Since it was first detected 10 years ago in Pailin, Western Cambodia artemisinin resistance has since become prevalent in other Cambodian provinces Thailand Myanmar border areas and Southern Vietnam and is emerging in Southern Laos and Central Myanmar. Artemisinin resistance threatens the efficacy of all ACTs, as its spread from SE Asia to Africa would slow malaria control and elimination efforts worldwide. However, to prevent the spread of artemisinin-resistant P. falciparum, the WHO and other partners initiated an artemisinin resistance containment project for the Greater Mekong Subregion in 2009.The goal was to identify and prevent artemisinin-resistant parasites from spreading outside of documented hotspot regions along the Thai-Cambodian border by ensuring proper diagnosis and reported malaria cases. Subsequently, the WHO, along with other partners, have initiated the Global Plan for Artemisinin Resistance Containment and Emergency Response to artemisinin resistance in the Greater Mekong Subregion6, [7,8].

Sulphadoxine-pyremathamine (SP) Resistance

Sulfadoxine and pyrimethamine inhibit the P. falciparum enzymes dihydropteroate synthase (Pfdhps) and dihydrofolate reductase (Pfdhfr), respectively, which function in the folate pathway. Resistance to these antimalarials is conferred by dominant mutations in catalytic sites and/or amplification of the pfdhps and pfdhfr genes. Multiple mutations in pfdhps and pfdhfr confer resistance to sulfadoxine– pyrimethamine in endemic regions, and parasites with quintuple mutations (pfdhfrN51I, C59R, S108N and pfdhpsA437G, K540E) have been linked to sulfadoxine–pyrimethamine resistance in Africa. Sulfadoxine–pyrimethamine has been partnered with the artemisinin-based compound artesunate. Double, triple and quadruple mutations in pfdhfr and pfdhps genes led to replacement of this drug combination with a second ACT composed of the artemisinin-based compound artemether and the partner drug lumefantrine in North East India2 -[9].

Atovaquone (of the naphthoquinone drug class) is partnered with proguanil in the drug Malarone (GlaxoSmithKline). Owing to its high cost as well as the emergence of resistance to atovaquone, Malarone is mainly used by travelers rather than resident populations of endemic countries. P. falciparum cytochrome b (PfCytB), which is a mitochondrial electron donor, is the target of atovaquone. Mutations in pfcytb that lead to changes in its catalytic activity render resistance to atovaquone. In combination, atovaquone and proguanil (in its inactive prodrug form) dissipate mitochondrial membrane potential. This synergistic action is lost in parasites with mutated pfcytb; in particular, clinical failure of atovaquone is associated with pfcytbY268S/C/N. Notably, parasites with mutations in pfcytb (albeit not Y268S/C/N) show reduced transmission to mosquitoes. This has led to the suggestion that although atovaquone resistance may arise, it will not easily spread, and thus, atovaquone–proguanil may be useful for prophylaxis in elimination strategies (where it is significant to contain transmission to vectors)[10].

Chloroquine (CQ) Resistance

Chloroquine targets the polymerization of free haem within the food vacuole of the parasite. In the food vacuole, haemoglobin that has been taken up from the host is digested into amino acids, which are used for parasite protein synthesis, and into Fe2+containing haem48. Fe2+-containing haem is oxidized to Fe3+-containing protoporphyrin IX (FPIX), which is toxic to the parasite and thus converted to haemozoin (the black pigment of malaria). Chloroquine disrupts haemozoin formation. The major chloroquine- resistance mechanism is drug efflux via the P. falciparum chloroquine- resistance transporter (encoded by pfcrt) located at the food vacuole. An SNP, K76T, in pfcrt was universally associated with chloroquine resistance in Africa and globally, K76T and other additional mutations in pfcrt are associated with the development of chloroquine resistance[11,12,13,14,15].

Plasmodium falciparum multidrug resistance (Pfmdr)

Mutations and amplification in broad, xenobiotic efflux pumps (such as P. falciparum multidrug resistance protein (pfmdr1), which encodes a protein that is also located in the membrane of the food vacuole) confer measurable levels of resistance to many antimalarials. Mutations in and/or amplification of pfmdr1 confer resistance to partner drugs such as mefloquine and lumefantrine and thus limit effective treatment with ACTs. Indeed, widespread amplification of pfmdr1 in southeast Asia led to replacement of artesunate and

mefloquine by dihydroartemisinin (DHA; the active metabolite of all artemisinins) and PPQ. Although PPQ is structurally related to chloroquine, it is effective against parasites that harbour some resistance mutations in pfcrt and is therefore of value even in the presence of chloroquine resistance. Notably, the introduction of the pfcrtC101F mutation into chloroquine-resistant P. falciparum parasites reduced chloroquine transport and thus rendered them chloroquine sensitive but PPQ resistant. Together, these studies provide molecular evidence that different alleles of pfcrt differently affect resistance to chloroquine and PPQ. Artemether–lumefantrine and artesunate– amodiaquine also interact with pfcrt and pfmdr1, but each ACT selects different resistance alleles in these genes, suggesting that artesunate–amodiaquine is effective in treating parasites resistant to a partner drug such as lumefantrine. Parasite genetic profiles responsive[16171819]

Origin of CQ resistance to Africa

Mutations in pfcrt usually occurred as a result of changes of amino acid at position 72-76 resulting to a geographic region-restricted evolution of P.falciparum resistance to CQ. Other polymo20rphism out of these positions have been described through genotyping of pfcrt position 72-76 and microsatellite haplotyping flanking of this region however no clear geographical association has been reported[14].

The Origin and spread of CQ resistance was mediated by a lineage of the CVIET (amino acid no 72 to 76 in Pfcrt with mutations) type1. Initially the CQ mutant P. falciparum was discovered in Thai-Cambodian border in the late 1950s. This was regarded as the origin of one of the most significant routes for CQ resistance "Southeast Asia to Africa". The mutant parasite migrated to Thailand by 1959 and reached to the neighboring countries of the Thai-Cambodian border by 1962. CQ resistance had expanded to all the regions of Southeast Asia by mid 1970s. It first appeared in east coast of Africa (Tanzania and Kenya) in late 1970s, about 20 years after the emergence of resistance. It proceeded to East African countries by early 1980s, then central part and Western part of Africa by mid 1980s. CQ resistance became a serious burden to the health sector in many parts of West African countries by early 1990s. The hypothesis that CQ resistance originated in the late 1950s in Southeast Asia then to east coast of Africa in mid 1970s has been supported strongly by the recent findings showing that almost all the CQ mutant parasites found in Africa share the same lineage (CVIET)21. However, in other studies several pfcrt genotypes apart from CVIET type have been pronounced in Africa. The commonly distributed pfcrt genotype in South America and the Pacific known as SVMNT was described in Tanzania with a prevalence of 19%. In Democratic Republic of Congo, a common isolate (SVIET) previously described in West Papua was found22. To date it is still unclear whether these non-Southeast Asian pfcrt types migrated from non-African regions or they were generated indigenously[2324].

Origin and Spread of SP resistance to Africa.

The first field trial of pyrimethamine monotherapy for the treatment of P. falciparum was carried out in African children in 1951. At that time CQ was effective and thus pyrimethamine was mainly used for mass drug administration or prophylaxis of malaria. However, resistance to pyrimethamine appeared during or shortly after trials of mass eradication or prophylaxis in many endemic regions. SP was initially used in Thailand in the late 1960s as first-line for the management of P. falciparum infection. After that, SP monotherapy or in combination with other antimalarial(s) was widely introduced in many endemic regions in Southeast Asia and South America in the 1970s, and after some delay in Africa. Resistance to SP was first reported at the Thai-Cambodia border in 1960s. After that, a resistant lineage generated in the region appears to have accumulated mutations (CNRNI →CIRNI or CNRNL →CIRNL): amino acids at positions 50, 51, 59, 108 and 164 in dhfr with mutations and spread to other regions in Southeast Asia in the presence of SP pressure. Microsatellite analyses of flanking dhfr have shown that all these genotypes have the same evolutionary origin in Southeast Asia1 [25].

Distribution of dhfr genotypes is strongly associated with high level of SP resistance in the field. The dhfr quartet (CIRNL) mutant is the predominant genotype in Thailand, while SP resistance is most serious. The CIRNI type of dhfr triple mutant has been predominant in Cambodia and Vietnam, meanwhile the CNRNL type of triple mutant is mainly distributed in Myanmar. In the Malay Peninsula, both types of triple mutants have been observed. The dhfr double mutant (CNRNI) is predominant in Laos. In addition, three genotypes (CNCNI, CICNI and CICNL) have also been found in Southeast Asia with a prevalence of less than 5%, dhfr single mutants (CNCNI) have multiple origins in many endemic regions. However, it remains unknown whether the CICNI and CICNL types evolved from the common lineage of a major resistant genotype in Southeast Asia, or independently evolved from a distinct lineage[25].

In Africa, the CIRNI type of dhfr triple mutant is predominant in many endemic regions. All triple mutants found in 12 African countries (South Africa, Benin, Cameroon, The Comoros, Congo, Gabon, Ghana, Guinea, Ivory Coast, Mali, Senegal, and Uganda) have identical or very similar microsatellite haplotype to that observed in the dhfr triple mutant from Southeast Asia. This indicates that the Southeast origin triple mutant migrated to Africa and spread out many endemic regions within the continent. To date it still remains unclear when the pyrimethamine resistant parasite migrated to Africa, although a study indicates that the Asian origin triple mutant in Kenya arrived by at least late 1980s[2627].

Recent findings indicate strong evidence for the indigenous evolution of the dhfr triple mutant (CIRNI) in Kenya and Ghana. In these countries, two unique microsatellite haplotypes, which are distinct from the Southeast Asian origin haplotype, are shared in both the dhfr double (CNRNI and CICNI) and triple mutants observed in the respective countries, suggesting indigenous evolution of the dhfr triple mutant from the double mutant in Africa. Similar indigenous evolution of dhfr triple mutants has been also found in Cameroon.In Africa, dhfr double mutants (CNRNI and CICNI) have multiple and indigenous lineages, a situation which is in sharp contrast to Southeast Asia where all double mutants show a single lineage. Interestingly, no Southeast Asian double mutant has been described in Africa so far. This could be due to the early migration of the Southeast Asian triple mutant from Southeast Asia. Alternatively, double mutants originating from Southeast Asia could have been wiped out during selection in the presence of SP pressure[282930].

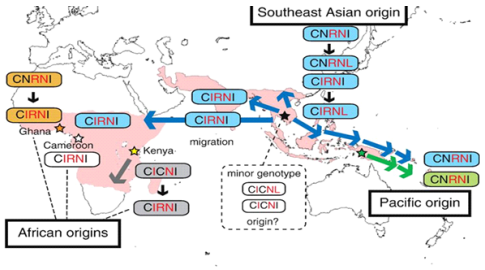

Fig.1. A diagram showing Evolution and spread of chloroquine and pyrimethamine resistance. Asia, the Pacific region, and Africa. A resistant lineage spreading to other regions in Asia to Africa1.[28]

ACT resistance

Currently, therapeutic efficacy studies (TES) are considered the gold standard for determining antimalarial drug efficacy. However, the WHO recommends that TES results be complimented using molecular marker studies. Therefore, it was desirable to identify a molecular marker for artemisinin resistance. Initial studies using a genome-wide association approach found two loci on P. falciparum chromosomes 10 and13 to be linked with artemisinin resistance. Artemisinin resistance is a heritable genetic trait, associated with three loci on chromosomes 10, 13 and 14, and non-synonymous single nucleotide polymorphisms (SNPs) in the propeller domain of a kelch gene on chromosome 13 (PF3D7_1343700). These "K13-propeller" polymorphisms are currently the best predictors of artemisinin resistance in mainland SE Asia, and sub-Saharan Africa with the most common mutation, C580Y, approaching genetic fixation in Western Cambodia31 32 33 34. A large multicenter clinical investigation by the Tracking Resistance to Artemisinin Collaboration (TRAC) has shown that the artemisinin resistance phenotype that is presently spreading through the Greater Mekong Subregion (GMS) is associated with K13 polymorphisms. An African parasite strain, selected under high artemisinin pressure for several years, was shown to survive drug exposure in a newly developed resistant ring-stage survival assay (RSA). Whole-genome sequencing revealed the M476I mutation in the β-propeller domain of PfKelch13 (which is encoded by PF3D7_1343700). The C580Y mutation of the PfKelch13 β-propeller was strongly associated with approximate 80% of resistant strains in southeast Asia, with R539T and I543T mutations showing second and

third place prevalence, respectively. Polymorphisms in pfkelch13 were rapidly mapped throughout southeast Asia and Africa (and, to a very limited degree, in Bangladesh and India). GWAS also established the presence of several artemisinin-resistant founder populations in Cambodia and Vietnam. The C580Y mutation arose independently in three different Cambodian founders along with polymorphisms in ferredoxin (pffd), apicoplast ribosomal protein S10 (pfarps10), multidrug resistance protein 2 (pfmdr2) and chloroquine-resistance transporter (pfcrt), which suggests that unexpected genetic interactions affect levels of resistance, parasite fitness and/or potential for transmission to mosquitoes 2 8 7 6 35.

CONCLUSION

Recent advances in detection of P. falciparum drug resistance using molecular markers has significantly promoted our understanding of the emergence, prevalence, and geographical spread of drug resistant lineages. In many endemic regions, the spread of parasites resistant to either CQ, SP and now ACT appears to have resulted due to migration of limited resistant lineages. Interestingly it is worth noting that the number of resistant lineages is restricted not only in CQ and SP, but also ACT although, resistance mechanisms to these antimalarials are relatively different. The underlying theory for this limited occurence of resistant lineages could be because resistant parasites have adapted the stress in the host, despite its advantage in surviving drug treatment, and reduced fitness.

However, as discussed in the review, multiple resistant lineages evolved independently in various endemic regions, despite the spread of resistant lineages. Generally, these indigenous lineages have been less predominant in parasite populations as compared with a migrated 'superior lineage' such as the Southeast Asian lineage in SP resistance. Further identification and tracking of genetic resistance markers would reveal more new independent lineages and more details of the routes of geographical spread of resistance than known so far. The information obtained would help to answer the significant querry on 'whether resistance parasites are generated frequently or not'.

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