

Short Communication

Is chloroquine making a comeback?

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Malaria remains one of the leading causes of disease and death in the tropics, mainly of children under 5 years of age. Chloroquine began as a first-line antimalarial in 1946; it became the corner stone of antimalarial chemotherapy for the next 40 years. It is one of the least expensive antimalarials available and is still in widespread use. Chloroquine can be taken both as prophylactic and as a treatment. For decades, chloroquine was a remarkably effective, safe, and inexpensive antimalarial. Optimism about effectiveness of chloroquine led public health professionals to predict the eradication of malaria by 2000. Despite much research during the last 40 years, the exact mechanism by which the chloroquine kills the malaria parasite remains controversial. Biochemical analyses suggest that this class of compounds enter acidic vacuoles of host cells, where they inhibit the growth of parasites by forming a complex with haematin. Unfortunately, *Plasmodium falciparum* gradually became resistant to chloroquine. After first appearing in Southeast Asia and South America in the late 1950s, resistance spread throughout Africa by the 1980s. Resistance to chloroquine was slow to develop, taking almost 20 years, despite extensive use of the drug, suggesting that mutations in several genes were required to produce the resistance phenotype. The mechanism of chloroquine resistance also is uncertain. Similar to the artemisinin derivatives, prospects for long-term chloroquine efficacy will be enhanced if it can be used in combination, such as chloroquine-azithromycin. With the right dosage and combination, the prospects for a chloroquine comeback are good.

Key words: Malaria, chloroquine, resistance, *Plasmodium*.

INTRODUCTION

Malaria is caused by infection with a single-cell parasite, *Plasmodium*. Four *Plasmodium* spp. cause malaria in human beings: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. *P. falciparum* is the most important because it accounts for the majority of infections and causes the most severe symptoms. Malaria remains one of the leading causes of morbidity and mortality in the tropics. According to the World Malaria Report (2011), there were 106 malaria-endemic countries in 2010. There were 216 million cases of malaria in 2010; 81% of these were in the World Health Organization (WHO) African region. An estimated 3.3 billion people were at risk of malaria. An estimated

655, 000 persons died of malaria in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO African region. Chloroquine, first synthesized in Germany, was not recognized as a potent antimalarial until the 1940s. By 1946, it was found to be far superior to other contemporary synthetic antimalarials (Coggeshall and Craige, 1949). It became the most important antimalarial chemotherapy drug for the next 40 years. It is one of the least expensive antimalarial drugs available and is still used widely. It can be administered as a prophylactic as well as a treatment. Chloroquine had an enormous impact on the treatment of malaria and has probably saved hundreds of millions of lives. It was hoped that chloroquine would have a pivotal role in the WHO malaria eradication programme, which begun in 1955. For decades, chloroquine was a very effective antimalarial, which led public health professionals to predict the

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eradication of malaria by 2000 (Pampana, 1963). By 1979, the equivalent of >500 million tablets of chloroquine were used each year (Kouznetsov, 1987). *P. falciparum* gradually became resistant to chloroquine, starting from Southeast Asia and South America in the late 1950s, resistance spread to Africa by the 1980s (Wellems et al., 1991). Meanwhile, alternative antimalarials were more expensive, and many countries continued to use chloroquine despite evidence that it was losing effectiveness. Recognition of this led to accusations of malpractice against the WHO and the World Bank, and a vigorous drive to replace chloroquine with more-effective artemisinin combination therapies (Attaran et al., 2004).

MODE OF ACTION

Despite much research during the last four decades, the exact mechanism by which chloroquine kills the malaria parasite remains controversial (Foley and Tilley, 1997; Foote and Cowman, 1994; Peters, 1997). The drug chloroquine inhibits DNA and RNA biosynthesis, rapidly degrades s-ribosome and dissimilates ribosomal RNA. Inhibition of protein synthesis is also observed, evidently as a secondary effect. Inhibition of DNA replication is proposed as a general mechanism of the antimicrobial action of chloroquine. Chloroquine accumulates in very high concentrations in the parasite food vacuole (Geary et al., 1990). Once inside a food vacuole, chloroquine is thought to inhibit the detoxification of heme. Chloroquine then becomes protonated (to CQ²⁺) as the digestive vacuole is known to be acidic (pH 4.7); chloroquine then cannot leave by diffusion. Chloroquine caps hemozoin molecules to prevent further bio-crystallization of heme, thus, leading to heme buildup. Chloroquine binds to heme (or FP) to form what is known as the FP-Chloroquine complex, which is highly toxic to the cell and disrupts membrane function. Action of the toxic FP-chloroquine and FP results in cell lyses and ultimately parasite cell auto digestion. Therefore, the parasite cell drowns in its own metabolic products.

MECHANISM OF RESISTANCE

Resistance to chloroquine was slow to develop, taking almost 20 years, despite extensive use of the drug, suggesting that mutations in several genes were required to produce the resistance phenotype. The mechanism of chloroquine resistance is uncertain. Chloroquine-resistant parasites accumulate less chloroquine in the food vacuole than sensitive parasites (Fitch, 1970) and one assumption is that chloroquine resistance is not based on the mode of action of the drug but on the accessibility of the drug to the parasite food vacuole. Early studies indicated that chloroquine resistance was associated with an elevated level of drug efflux. Drug-resistant parasites

were reported to release pre-accumulated chloroquine almost 50 times faster than chloroquine-sensitive isolates (Verdier et al., 1985; Krogstad et al., 1985). Furthermore, verapamil was shown to reduce the apparent rate of drug efflux from chloroquine-resistant parasites (Krogstad et al., 1985). Since verapamil is known to reverse the P-glycoprotein-mediated efflux of drugs in multidrug-resistant tumor cells (Martin et al., 1987), this led to the proposal that efflux of chloroquine by a plasmodial P-glycoprotein is responsible for chloroquine resistance.

On the other hand, Bray et al. (1999) have suggested that chloroquine resistance is caused by reduced affinity of chloroquine for heme, thereby reducing chloroquine uptake. Another proposal is that chloroquine is transported actively through the parasite by the Na⁺/H⁺ exchanger (NHE) and that resistance to chloroquine is mediated by mutations in the NHE (Wunsch et al., 1998), but this suggestion has been disputed. Wellems et al. (1990) analyzed a cross between a chloroquine-resistant and a chloroquine-sensitive strain of *P. falciparum*, and identified a chloroquine-resistance locus within a 400-kb segment of chromosome 7 (Wellems et al., 1990, 1991). Su et al. (1997) mapped the putative chloroquine-resistance locus to a 36-kb region and identified the open reading frames of 8 potential genes within this region. Initially, chloroquine resistance was thought to be caused by *cg2*, a gene coding for a polymorphic protein located on the parasite periphery. However, recent transformation studies have ruled out *cg2* and suggest another gene, *pftcr*, within this region (Fidock et al., 2000).

STATUS OF RESISTANCE

Chloroquine, soon after introduction in the 1950s, quickly became the main drug of choice globally to treat uncomplicated *P. falciparum* infections, for instance, as part of the Global Malaria Eradication Campaign, launched by the WHO in 1955. However, *P. falciparum* eventually developed resistance to chloroquine and has spread to almost all the endemic countries today (Wellems and Plowe, 2001). Chloroquine-resistant parasites in Africa were thought by some to share the same origin as the Indo-China strains, but by others to have developed locally as a result of mass drug administration plus intrinsic entomological, epidemiological and parasitological factors that promoted local resistance selection (Diribe and Warhurst, 1985). Current molecular studies suggest the Asian origin of African isolates, but at least four different foci of chloroquine resistance have so far been identified (Warhurst et al., 1995). Resistance to chloroquine spread to almost all the countries thus limiting the effective use of this low cost antimalarial.

Although chloroquine resistance is widespread, resistance, in general, is not very potent (Valderramos et

al., 2010). *In vitro*, parasites are considered to be chloroquine-resistant, the 50% inhibitory concentration (IC₅₀) of the drug is >160 nmol/L (51 µg/L) (Ringwald, 2005). Recent reports from areas where malaria is endemic documented that the majority of chloroquine-resistant isolates of *P. falciparum* have IC₅₀ values <400 nmol/L (128 µg/L) (Bacon et al., 2007; Sibley and Ringwald, 2006; Hatabu et al., 2005; Borrmann et al., 2002; Moreno et al., 2001; Yang et al., 1997; Noedl et al., 2003). In contrast, typical trough levels are 80 to 125 nmol/L (25 to 40 µg/L). Thus, the majority of chloroquine-resistant isolates have IC₅₀ values that are only 3 to 5-fold higher than typical trough plasma concentrations. In accordance with these findings, increasing the dose and frequency of administration of chloroquine can increase plasma concentrations to levels higher than the IC₅₀ values of chloroquine-resistant parasites. Similar to the artemisinin derivatives, prospects for long-term chloroquine efficacy will be enhanced if it can be used in combination, such as chloroquine-azithromycin (Dunne et al., 2005). With the right combination therapy, the prospects for a chloroquine comeback are good.

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