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Targeting *Plasmodium falciparum* transmission with primaquine: same efficacy, improved safety with a lower dose?

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Malaria transmission is declining worldwide, leading to a growing interest in strategies to reach elimination and eradication. Insecticide and drug resistance threaten these efforts, driving an interest in the use of gametocytocidal drugs to curb the spread of artemisinin resistance and accelerate the path to malaria elimination. Primaquine is the only marketed drug that can kill mature *Plasmodium falciparum* gametocytes, which can otherwise contribute to ongoing transmission for long periods of time. While primaquine has been widely used in Asia and the Americas, African countries have little experience with this drug and are reluctant to use primaquine due to a fear of hemolytic side effects. We discuss the underlying knowledge base and motivation to use primaquine as a *P. falciparum* transmission blocker, revealing that while primaquine implementation can benefit from further study, there remains an overall need for improved transmission-blocking drugs.

Over the past 12 years, the successes of malaria control programs have contributed to a worldwide decrease in malaria deaths by 42% [1]. This decline in endemicity has led to a global move toward malaria elimination [2], which is challenging in that it requires that the entire parasite reservoir, both in vectors and in humans, be targeted. There is a continuous search for new tools and strategies that target every stage of the complex parasite lifecycle.

Chemotherapeutic tools are attractive for malaria elimination, as there is no vaccine that is commercially available. Most of the antimalarial drugs that are currently available focus on curing the blood stages of infection to prevent disease, death and low birth weight. Provided that drug-resistant mutations are not present, effective blood schizonticides are able to cure the clinical symptoms for all five species of *Plasmodium* parasites responsible for human malaria [3]. Schizonticidal drugs are unable to target some of the asymptomatic stages of the parasite lifecycle, leaving reservoirs of infection that need to be targeted in malaria elimination settings. Specifically, important sources of ongoing transmission in low-endemic settings include the gametocytes of *Plasmodium falciparum* malaria, which can circulate for long periods of time, as well as the dormant hypnozoites of *P. vivax* and *P. ovale*, which can cause parasitological relapse weeks, months, and occasionally years later [3,4]. This review focuses on targeting the gametocytes of *P. falciparum* malaria, the parasite lifecycle stage responsible for ongoing transmission from the human to the mosquito.

The 8-aminoquinoline scaffold represents the only class of drugs under consideration for the clearance of mature *P. falciparum* gametocytes. A recent interest in gametocytocidal drugs arises from their potential to stop the spread of artemisinin-resistant parasites [5], and their ability to target challenging reservoirs of

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infection in malaria elimination settings [6]. While artemisinin-based combination therapies (ACTs) are able target young gametocytes, reducing infectivity [5], mature gametocytes – those responsible for the transmission and potential spread of artemisinin-resistant parasites – remain. The addition of an 8-aminoquinoline to an ACT is therefore a promising drug regimen that, by targeting mature *P. falciparum* gametocytes, can be used to curb the spread of artemisinin resistance in addition to stopping transmission in low-endemic elimination settings [5].

Primaquine, developed in the 1940s as a wartime prophylaxis, is the only 8-aminoquinoline drug that is commercially available [7]. While this drug is typically used for the radical cure of *P. vivax* malaria through a 7- to 14-day course of treatment targeting the dormant liver stage hypnozoites, a single dose of primaquine is sufficient to clear mature *P. falciparum* gametocytes [8]. The safety profile of primaquine depends on the total dose given to the patient, so it is critical to distinguish between the two indications for use. This review focuses on the safety and efficacy of single-dose primaquine as a *P. falciparum* gametocytocide.

Primaquine causes dose-dependent hemolysis in individuals that are deficient in glucose-6-phosphate dehydrogenase (G6PD), an enzyme that provides NADPH as reducing power to cells through the pentose phosphate pathway [9]. The other gametocytocidal drugs under consideration, the 8-aminoquinoline tafenoquine and thiazine dye methylene blue, face the same caveats [10,11]. Tafenoquine, a long-acting derivative of primaquine, is not recommended for use in G6PD-deficient (G6PDd) individuals [11]. Methylene blue, available since 1891, has seen limited use for the clinical cure of malaria because it temporarily turns the urine green and the sclera blue [12]. Despite renewed interest in methylene blue [8], there are few studies that examine its efficacy and safety as a gametocytocidal drug. The fact that primaquine is FDA approved, available on the market and has been studied and used for 60 years drives current academic and programmatic interests in using this drug as a gametocytocide.

Pharmacologists and basic scientists are striving to overcome the hemolytic issues associated with 8-aminoquinoline drugs. The mechanism of action of these compounds is mysterious and has proven challenging to establish [13], and it is unclear whether the efficacy and hemolytic toxicity of 8-aminoquinolines can be separated. Both primaquine and tafenoquine are understood to be prodrugs, of which active metabolite(s) remain to be identified and their corresponding pharmacokinetic parameters understood. The exploration of new chemical scaffolds is also underway, with two promising transmission-blocking candidates in the drug development pipeline. Both KAE609 and OZ439 are currently undergoing Phase II clinical trials [14]. With time and luck, new drugs may be able to replace single-dose primaquine. Until then, primaquine is available as an imperfect but potentially important tool for global malaria strategies.

Most debates about primaquine as a *P. falciparum* gametocytocide are centered on the optimal dose to be used, as both the gametocytocidal efficacy and hemolytic toxicity in G6PDd individuals are dose dependent [5]. An understanding of the safety profile is complicated by the fact that there are hundreds of biochemical genetic variants of G6PDd that vary in severity [9]. The WHO classifies G6PD deficiency in five categories according to levels of enzyme activity [15]. The African A– variant of G6PDd is considered to be mildly primaquine-sensitive (class III) and has been widely studied in US troops and African American prisoners in Illinois in the 1960s [5,8]. Other variants are not as well-studied, but include the most severe (class II) Mediterranean variant of G6PD deficiency, as well as heterogeneous Asian variants, most of which are considered to be severe [16]. Based on geographical overlap, G6PDd is speculated to be genetically selected for protection against severe malaria, as the prevalence of this X-linked trait varies by historical *P. falciparum* transmission intensity, ranging from <1 to 32.5% in endemic countries [16–18]. These geographical patterns allow for a certain degree of predictability of the risks of primaquine use in G6PDd individuals within a given locale. However, there is debate over the acceptable level of risk and benefit for primaquine. How should single-dose primaquine be used operationally? Is the current evidence base sufficient to inform widespread use? Are population-level efficacy and the risk of serious adverse events predictable [8,19,20]? What constitutes an acceptable loss of hemoglobin or hematocrit? The severity of primaquine-induced side effects can range from minor side effects to life-threatening acute hemolytic anemia or, in rare cases, renal failure [5,8]. What level of risk is acceptable for large-scale rollout [21]?

There are two WHO recommendations on single-dose primaquine as a *P. falciparum* gametocytocide. By examining their underlying knowledge base and need, it becomes clear that while recommendations to use primaquine are driven by potential benefits, the risks are less clearly defined. Indeed, neither WHO recommendation is supported by the statement 'Strong recommendation, high quality evidence' typical of WHO recommendations. The 1973 recommendation states the use of a 0.75 mg/kg dose of primaquine with G6PD testing, where available, to clear *P. falciparum* gametocytes [22]. The basis for this recommendation was a single infectivity study where a 45-mg single dose of primaquine was given to three adult males with *P. falciparum* malaria, effectively blocking transmission in direct feeding assays and clearing the gametocytes seen on thick blood films [23]. While this WHO recommendation implies that a 0.75 mg/kg single dose of primaquine should be used with caution, as it may cause clinically significant hemolysis in G6PDd individuals, G6PD testing has prohibitive cost and infrastructure requirements that are rarely available in actual field settings [22]. In October 2012, global malaria strategies drove the issue of a new WHO recommendation, to forgo G6PD testing and give a much lower 0.25 mg/kg dose of primaquine with an ACT to all *P. falciparum* cases in areas threatened by artemisinin resistance or approaching malaria elimination [24]. Formal dose-finding studies were not conducted, as this dose was derived from pooled

efficacy studies of single-dose primaquine and plasmoquine, its more toxic predecessor, over a span of approximately 80 years [22]. Analyzed doses ranged from 0.065 to >0.75 mg/kg with various blood schizonticide partner drugs, using non-standardized assays to measure infectivity [22]. The most common infectivity assay was membrane feeding, the gold standard, because assessments of gametocyte density and prevalence cannot differentiate between infectious and non-infectious gametocytes that persist after primaquine treatment [5,22]. Analysis revealed that single low doses of primaquine (as low as 0.065 mg/kg) rendered *P. falciparum* gametocytes non-infectious to mosquitoes, an effect further enhanced by the concurrent use of an ACT [22]. The safety of the 0.25 mg/kg single dose was assumed and was based on few adverse events reported over the past 60 years despite the widespread use of multiple 0.75 mg/kg doses without G6PD testing [5,22]. However, pharmacovigilance was noted to be weak, and authors expressed the need for safety studies on single-dose primaquine to determine hemolytic risks in G6PDd individuals [5,22].

A number of formal dose-finding studies that aim to determine the lowest efficacious single dose of primaquine are underway ([25] NCT01743820*, [26] NCT01838902, [27] NCT01935882*, [28] NCT01906788*, [29] NCT01849640*; *membrane feeding assay). A recent study showed that a combination of the ACT artemether lumefantrine and a 0.4 mg/kg dose of primaquine completely cleared gametocytes in Ugandan children ([30] NCT01365598) [31]. A lower dose of 0.1 mg/kg reduced gametocyte density but did not result in clearance, and the authors queried whether these gametocytes were infectious, suggesting that infectivity studies using this dose should be carried out. Indeed, two recent Cochrane reviews on single-dose primaquine expressed the need for infectivity studies of primaquine with ACT partner drugs [19,20]. Importantly, there remains a need to validate the efficacy of the WHO-recommended 0.25 mg/kg single dose of primaquine.

To complicate matters, primaquine efficacy against *P. vivax* liver stages has recently been linked to mutations in the cytochrome P450 2D6 (CYP 2D6) gene, whereby individuals with certain mutations are unable to metabolize primaquine into the active metabolite. This has been demonstrated through prophylactic failure of primaquine in *P. berghei* malaria infected CYP 2D knock-out mice, and radical treatment failure in humans with *P. vivax* malaria with CYP 2D6 mutations [32,33]. It will be important to monitor for primaquine treatment failure for *P. falciparum* gametocytes in people with CYP 2D6 mutations, and to explore whether other genotypes also affect primaquine activation. The potential link between the efficacy of primaquine against *P. falciparum* gametocytes and CYP 2D6 metabolism remains to be established.

There is also a need for formal dose-finding studies on single-dose primaquine in G6PDd populations to assess safety [5,20]. In two recently published studies, children in Tanzania were given a 0.75 mg/kg dose of primaquine with sulphadoxine pyrimethamine and artesunate. The first study entailed mass drug administration, where mean hemoglobin concentrations dropped most significantly in G6PD A– hemi/homozygotes (-2.5 g/dl, 95% CI: –1.2 to –3.8 g/dl) [34]. In this study, a 5-year-old with the wildtype G6PD B variant had severe anemia (Hb drop from 8.3 to 4.8 g/dl) that was asymptomatic and recovered after being given hematinic drugs [34]. The second study, which entailed the treatment of children with *P. falciparum* malaria, again did not result in symptomatic hemolysis. The most pronounced drop in hemoglobin, seen at day 7 (a 5.2% drop, 95% CI –8.4 to –1.8%), was highest in children of the A– variant of G6PDd although the sample sizes were too small to make statistical comparisons [35]. There is a need to explore the safety profile of different doses of primaquine in G6PDd individuals, with study results stratified by the genetic variant of G6PD deficiency and the level of residual enzyme activity.

The 2012 WHO recommendation was driven by need. Artemisinin resistance is a global health emergency, as there are no marketed blood schizonticides available to replace ACTs as first-line antimalarials. The spread of drug resistance is a fight against time with potentially devastating consequences that necessitated that the primaquine dose be determined through a collection of unstandardized efficacy studies [22]. The use of primaquine for *P. falciparum* elimination faces more complex risk-benefit arguments, because it would be the first time a drug would be used to benefit a population at potential risk to the individual. That is, primaquine prevents onward transmission of *P. falciparum* infection, which is altruistic, as it does nothing to alleviate symptoms or the risk of serious disease in the human host. What level of risk is acceptable when the benefit of adding single-dose primaquine to a standard ACT is altruistic? Furthermore, are there alternative tools we can use to accelerate to zero that are less risky?

Policy uptake of the 2012 WHO recommendation is driven by urgency, risk and benefit [5]. In the Greater Mekong Subregion, the epicenter of artemisinin resistance [36], single low-dose primaquine has been adopted as policy in Myanmar, Thailand and Vietnam [Hwang J, Pers. Comm.]. However, the actual level of policy implementation is unclear, as healthcare workers may be reluctant to prescribe primaquine due to safety concerns arising from use of the drug for the radical cure of *P. vivax* malaria [19,37]. In malaria elimination settings, policy uptake has been slower, with Botswana, Ethiopia, Namibia, South Africa, Swaziland, Zanzibar and Zimbabwe very recently incorporating primaquine in policy documents [Msellem M, Mudambo K, Hwang J, Pers. Comm.]. In 2013, Swaziland originally considered the nationwide rollout of primaquine but doctors raised safety concerns, prompting a safety monitoring study that was recently initiated [Pan S, Pers. Comm.].

To facilitate the use of primaquine for malaria elimination, stakeholders convened in 2012 and 2014 to identify critical barriers to rollout in sub-Saharan Africa, where 90% of G6PDd individuals are of the mildly primaquine-sensitive A– variant [8,38,39]. The rollout group chose to support sub-Saharan Africa, where *P. falciparum* malaria is responsible for approximately 90% of malaria cases, due to the region's limited experience with primaquine as well as a recent move toward

malaria elimination in the southern region of Africa. That is, malaria endemic countries in Asia and South America have higher levels of *P. vivax* transmission and have used primaquine for radical cure for many decades. In Asia, several countries including Bhutan, Indonesia, Malaysia and the Philippines use single high-dose primaquine (0.75 mg/kg) without G6PD testing for the treatment of *P. falciparum* gametocytes [Gueye CS, Pers. Comm.]. Regardless, the discussions and research efforts that have resulted from the low-dose primaquine meeting are informative for any country considering the use of primaquine as a *P. falciparum* gametocytocide.

In the stakeholder meeting, the main concern expressed by in-country program managers in Africa was a fear of hemolytic toxicity in G6PDd individuals. These fears can be allayed by safety studies that aim to define tolerability in G6PDd individuals. To further reassure in-country program managers, policy makers, stakeholders and health authorities, active safety monitoring should be conducted in countries that are implementing primaquine. Although active pharmacovigilance has not been achieved for any antimalarial to date, primaquine is a simple case because safety outcomes are predictable and can be monitored through observing hemoglobin levels within the first week after treatment. This type of pharmacovigilance system can also be applied to the rollout of other oxidative hemolytic drugs, including tafenoquine and methylene blue. If longer follow-up times are possible, it will be important to use pharmacovigilance to assess for the safety of primaquine in pregnancy outcomes, as primaquine use in pregnancy and breastfeeding are currently contraindicated due to a lack of data [8]. The main risk is in safety outcomes, as the G6PD status of the fetus is unknown. Therefore, if an individual takes primaquine before she realizes that she is pregnant, there is a theoretical risk that the fetus could hemolyze and develop hydrops fetalis.

Program managers in Africa also expressed the difficulties of administering weight-based doses to children with currently available primaquine tablet sizes, which are typically 15 mg each. To address these challenges, contemporary dose-finding studies to define the therapeutic range, which span from the lowest efficacious dose to the highest safe dose in vulnerable populations, are underway. While the therapeutic range can inform dosing regimens and guide the potential manufacture of smaller tablets, there remains a need to develop child-friendly pediatric formulations of primaquine. The therapeutic range can furthermore determine if G6PD testing is necessary and inform drug–drug interaction studies with ACT partner drugs. The latter is critical, as the 2012 WHO recommendation states that single-dose primaquine should be used in combination with an ACT [24]. A few recent studies have investigated for these interactions ([40] NCT01552330 with artesunate pyronaridine, and [41] NCT01525511 with dihydroartemisinin piperaquine), although results have not yet been reported. In settings where other diseases are highly endemic, in particular tuberculosis and HIV, drug–drug interaction studies are also needed [38].

While defining the therapeutic range, conducting active safety monitoring, and studying the potential for drug–drug interactions will provide the basic knowledge to support the programmatic use of primaquine for *P. falciparum* elimination, the translation of individual-level efficacy to population-level benefits will be challenging [19]. There are three strategies under consideration for primaquine use to target *P. falciparum* transmission. They are to add primaquine to the treatment of symptomatic cases, to identify and treat all confirmed malaria cases in mass screen and treat (MSaT) programs or to use primaquine as a component of mass drug administration (MDA) programs. MSaT and MDA campaigns are expected to be especially challenging, as these strategies administer drugs to people who are not apparently ill and may not be infected by malaria. While the efficacy of primaquine as a transmission blocker has not been proven at a population level [19,20], malaria transmission modeling based on data from low-endemic Southeast Asian studies suggests that in MSaT scenarios, the addition of primaquine to an ACT does not add substantially to transmission reduction [42]. In any case, many countries are planning to use primaquine as a transmission blocker, the results of which can be established retrospectively. Wherever rollout will take place, stakeholder buy-in is needed, and care must be taken to inform communities of the risks and benefits of primaquine programs.

In summary, global malaria strategies can greatly benefit from transmission-blocking drugs. At the moment, primaquine is the only commercially available transmission-blocking drug. Although this drug can play an important role in malaria strategies, ongoing research can hopefully further define and improve the safety profile for its use, and in the longer run, lead to the development of alternative drugs. Importantly, the potential use of primaquine as a transmission blocker introduces the topic of MSaT or MDA strategies that may prove to be crucial for elimination and eradication. Therefore, the interest and controversy generated by primaquine will likely shape the role of malaria transmission-blocking drugs both in the present and in the future.

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