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Therapeutic principles of primaquine against relapse of *Plasmodium vivax* malaria

J K Baird*

Eijkman-Oxford Clinical Research Unit, Eijkman Institute of Molecular Biology, Jakarta, Indonesia; Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, United Kingdom

*Corresponding author: jkevinbaird@yahoo.com

Abstract. *Plasmodium vivax* causes tens of millions of clinical attacks annually all across the malarious globe. Unlike the other major cause of human malaria, *Plasmodium falciparum*, *P. vivax* places dormant stages called hypnozoites into the human liver that later awaken and provoke multiple clinical attacks in the weeks, months, and few years following the infectious anopheline mosquito bite. The only available treatment to prevent those recurrent attacks is primaquine (hypnozoitocide), and it must be administered with the drugs applied to end the acute attack (blood schizontocides). This paper reviews the therapeutic principles of applying primaquine to achieve radical cure of acute vivax malaria.

1. Introduction

Human malaria remains an important problem of clinical and public health for the several billion-people exposed to risk of infection in the Americas, Africa, and Asia.[1] Among the seven species of plasmodia known to infect humans naturally, two dominate in terms of geographic distribution and numbers infected: *Plasmodium falciparum* and *Plasmodium vivax*, historically known as malignant or benign tertian malarias, respectively. That antiquated terminology has been proven dangerously misleading because vivax malaria is not benign and inconsequential but pernicious and often leads to complications associated with death, i.e., severe anemia or thrombocytopenia, acute respiratory distress, hepatic dysfunction, kidney injury, seizures and coma, multiple organ dysfunction, or shock. [2-4] A diagnosis of vivax malaria should be considered a clinical emergency and managed with prompt and effective therapy of the acute attack with assurance of recovery free of complications, along with treatment of latency by applying the only available hypnozoitocidal therapy, primaquine. [5] The most important obstacle to delivering that treatment is its toxicity in patients having an inherited deficiency of glucose-6-phosphate dehydrogenase (G6PD). This is the most common human genetic disorder affecting approximately 400 million people and occurring at a prevalence of about 8% in malaria endemic countries.[6]

2. *Plasmodium vivax* Biology

Several dozen mosquito species in the genus *Anopheles* naturally transmit malaria to humans via blood feeding by females. During feeding on humans, typically a dozen or more parasites in the infectious form of sporozoites will be injected. Each of those parasites finds its way to the liver within a few minutes and invades a hepatocyte where they initiate development into one of two forms: 1) an

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actively developing liver schizont, or 2) a quiescent form called a hypnozoite. The liver schizont becomes mature within a week or so, swelling and rupturing its host hepatocyte and releasing thousands of parasites called merozoites; these quickly invade red blood cells and, within 48 hours, burst that host cell with 8-32 new merozoites and provoke the febrile and other systemic symptoms of an acute primary attack of malaria.[7] Hypnozoites remain dormant for highly variable periods that often depend on geographic origin of the infecting strain, i.e., less than a month or for 8 or more months.[8,9] The period between infectious bite and primary attack is called pre-patency, while the period between the patent primary attack and first relapse is called latency.

The numbers of hypnozoites deposited in the liver with each infectious mosquito bite cannot be directly measured, and latency in vivax malaria cannot now be diagnosed. Nonetheless, it is estimated that about 5 hypnozoites occur per infectious bite [10] and that more than 80% of incidence attacks of acute vivax malaria derive from them.[10,11] The hypnozoite reservoir of renewed clinical attacks thus represents an important source of endemic vivax malaria morbidity and mortality. That reservoir can be diminished or eliminated only with appropriate radical cure of each case of vivax malaria. That requires using primaquine as hypnozoitocide.

3. Complexity in Primaquine Activity

Primaquine is one of the few antimalarial drugs known with broad activity against most stages of most species of plasmodia.[12] The blood schizontocides quinine or chloroquine have no activity against liver schizonts or hypnozoites, and chloroquine has activity against the gametocytes of *P. vivax* but not those of *P. falciparum*. Primaquine kills all liver stages of all species, as well as gametocytes, and it has blood schizontocidal activity against *P. vivax* but not *P. falciparum.* It is thus important to define therapeutic intent of primaquine treatment and therefore its dose and schedule. Table 1 summarizes these. The focus of this review is hypnozoitocidal therapy at the widely recommended daily dose of 0.5mg/kg for 14 days combined with a suitable partner blood schizontocidal therapy to achieve radical cure of vivax malaria.[5,13]

Table 1. Therapeutic effects of primaquine in vivax or falciparum malaria.

Therapeutic Activity	*Plasmodium vivax*	*Plasmodium falciparum*
Blood Schizontocide	Yes, at 0.25mg/kg daily dosing for 14 days but not practiced	No activity
Gametocytocide	Yes, but by primaquine anti-relapse or chloroquine therapy	Yes, 0.25mg/kg single dose
Liver Schizontocide	Yes, 0.5mg/kg daily within 48hr of infection (causal prophylaxis)	Yes, 0.5mg/kg daily within 48hr of infection (causal prophylaxis)
Hypnozoitocide	Yes, 0.5mg/kg daily for 14 days	Not applicable

4. Suitable Blood Schizontocidal Partner in Radical Cure

The suitability of partner blood schizontocides for radical cure of vivax malaria with primaquine hinges upon two key factors: 1) likelihood of drug resistance to the blood schizontocide, and 2) evidence of the safety and efficacy of primaquine when combined with a given blood schizontocidal therapy. Chloroquine has been the first line therapy for acute vivax malaria since 1946, but resistance to it appeared around 1990 and now dominates among strains found on the Indonesian archipelago and Southwestern Pacific, with confirmed cases all across the natural range of P. vivax.[14,15] Many artemisinin combined therapies (ACTs) have been evaluated against vivax malaria and most show excellent safety and efficacy at the same doses administered against *P. falciparum.*[16] Nonetheless, the pairing of blood schizontocides with hypnozoitocide also requires evidence of good safety and efficacy of the latter [17], and this has thus far been achieved only with dihydroartemisinin-piperaquine (DHA-PP) and artemether-pyronaridine (ATM-PYR) co-administered with primaquine (0.5mg/kg daily X 14d).[18,19]

Important drug-drug interactions (DDI) among the methoxy quinolones (a chemical class inclusive of quinine, the 8- and 4-aminoquinolines, and most other known antimalarials) impacting both efficacy and toxicity are known. The most remarkable may be both the profound potentiation of therapeutic activity of 8-aminoquinolines by quinine and the exacerbation of 8-aminoquinoline toxicity by the 4-aminoquinoline called quinacrine (or atabrin or mepacrine).[17] The recent demonstration of the likely dependence of primaquine therapeutic activity and probably toxicity as well upon metabolism by cytochrome P-450 2D6 isozyme [20-22], taken with the known inhibition of that metabolism by some blood schizontocides further underscores the crucial importance of proof of safety, tolerability, and efficacy of primaquine when it is combined with partner blood schizontocides for radical cure of vivax malaria.

5. Primaquine Toxicity

In non-pregnant G6PD-normal and otherwise healthy vivax malaria patients aged over 6 months, relatively high daily doses of primaquine (≥0.5mg/kg X 14d) taken with a snack or meal are remarkably well tolerated and non-toxic.[5,13] The predictable elevation of methemoglobin levels to typically between 4%-8% of hemoglobin very rarely exceeds 20%, the usual threshold of symptomatic (cyanosis, shortness of breath) methemoglobinemia.[23] Nonetheless, even relatively low daily doses of primaquine invariably provoke a potentially serious and threatening acute hemolytic anemia in patients having G6PD deficiency.[24]

G6PD deficiency is enormously diverse, with many dozens of single nucleotide polymorphisms (SNPs) occurring all along the length of this gene's 13 exons and introns of about 20 thousand base pairs.[25] This diversity also appears in G6PD activity phenotype, i.e., the extent to which any given SNP or set of them impacts the biochemical performance of G6PD enzyme, typically expressed as a percentage of normal activity. Among the five classes of G6PD abnormalities identified and defined by WHO [26], only two of those are typically considered in practice and the context of primaquine therapy: Class II and Class III. These are separated by a threshold of 10% of normal G6PD activity, i.e., Class II are considered "severely deficient" and Class III as "moderately deficient". In fact, both are invariably sensitive to primaquine and therefore contraindicated, but it may be true that the clinical consequences of accidental primaquine exposure may be a greater danger to patients with Class II versus Class III variants of G6PD deficiency.[26]

An important complicating factor in G6PD deficiency to be considered in practice is the X-linked nature of the disorder and its implications in interpreting and managing G6PD deficiency in male versus female patients. Having only a single X chromosome, males may be either normal or hemizygous for G6PD deficiency – they are either completely deficient (hemizygous) or normal. Females, on the other hand, possess two X chromosomes and may be normal, homozygous, or heterozygous for the abnormality. Homozygous females are effectively the same as hemizygous males with respect to phenotype, but heterozygous females exhibit extreme variability in enzyme activity phenotype due to the phenomenon of lyonization of X-linked traits like G6PD deficiency (Table 2). Lyonization involves the random inactivation of one of the X chromosomes in each cell at some point during embryonic development and results in mosaicism of the phenotype among cells, i.e., the red blood cell population has both completely normal and completely deficient cells represented by proportions ranging from 0% to 100% with a presumed median of 50%. Heterozygous females thus represent a subpopulation of highly variable vulnerability to primaquine toxicity and this has important clinical implications with regard to screening and therapy.

Table 2. G6PD genotypes and phenotypes among males and females.

Genotype	Male	Female	Phenotype
Wild-type	XY	XX	Normal
Hemizygous	X*Y		Fully deficient
Heterozygous		X*X	Fully normal to fully deficient
Homozygous		X*X*	Fully deficient

6. G6PD Screening Before Primaquine Therapy

In many hospital settings offering laboratory services, G6PD screening or testing services may be available and applied to patients needing primaquine therapy against relapse of vivax malaria. Patients confirmed as G6PD-normal (>70% of normal enzymatic activity in a quantitative test) may be confidently prescribed even high-dose primaquine and strongly encouraged to complete the 14-day regimen. Likewise, patients identified as G6PD-deficient by qualitative or quantitative testing may be confidently excluded from daily primaquine therapy. However, a G6PD-normal test outcome by qualitative screening requires great caution with females. Because these tests typically perform poorly when G6PD levels exceed 30% of normal, significantly deficient and at-risk females may test as G6PD normal, i.e., primaquine eligible (Table 3). Females must be quantitatively tested in order to confidently include for primaquine therapy, otherwise clinical monitoring of them is indicated with or without qualitative screening.[27]

Clinical monitoring is an unproven method of managing primaquine therapy in patients of unknown or uncertain G6PD status. Nonetheless, clinical monitoring has been put into practice since the earliest days of 8-aminoquinoline therapy, even long before the discovery of G6PD deficiency as the basis of hemolytic sensitivity to these drugs (with the drug called plasmochin marketed by Bayer in 1926). Indeed, it is the very basis of the extended 14 days of dosing with both plasmochin and primaquine, i.e., relatively small doses over a relatively long period to allow observation of toxic effects and cessation of therapy before serious harm may be done.[28] That is the principle at work, but no work has proven it, and the onset of acute hemolytic anemia – typically not before the fourth dose and seldom after the seventh – tends to be sudden serious. Early clinicians using plasmochin complained that serious harm had been done by the drug before becoming overtly manifest.

Methemoglobinemia may not be a good clinical indicator of toxicity. It occurs in almost all patients taking daily primaquine and does not appear markedly higher in G6PD deficient patients. Elevation of methemoglobinemia is very slight after the first several doses and stabilizes at a peak level only after the 7^{th} to 9^{th} dose. In most patients experiencing onset of symptomatic hemolytic crisis, cyanosis, dyspnea, and hematuria occurs, sometimes with marked jaundice. Any of these signs should prompt cessation of primaquine therapy. It may be that onset of hematuria may be detected earlier using a simple urine dipstick sensitive to free hemoglobin in urine but this has not been evaluated in primaquine-induced acute hemolytic anemia.

Table 3. G6PD screening or testing in conjunction with primaquine therapy.

	Clinical	**Qualitative Screen**	**Quantitative Test**
Methods	Clinical monitoring	Visualization of NADP+ fluorescence or dye reduction	Spectrophotometric measurement of G6PD enzymatic activity
Readout	Cyanosis, dyspnea, hematuria	Absent or diminished visual signal in hemolysate on paper	% normal G6PD activity in hemolysate
Timing	Onset at days 3-5 of dosing	Prior to dosing	Prior to dosing
Indication of test positive for deficiency	Cease dosing	Do not initiate dosing	Do not initiate dosing
Sensitivity to enzymatic activity in ***hemizygous males***	Not known	<30% of normal enzymatic activity yields 100% diagnostic sensitivity	<70% of normal enzymatic activity yields 100% diagnostic sensitivity
Sensitivity to enzymatic activity in ***homozygous females***	Not known	<30% of normal enzymatic activity yields 100% diagnostic sensitivity	<70% of normal enzymatic activity yields 100% diagnostic sensitivity
Sensitivity to enzymatic activity in ***heterozygous females***	Not known	<30% of normal enzymatic activity yields 100% diagnostic sensitivity, but misclassifies 30%-80% activity as normal	<70% of normal enzymatic activity yields nearly 100% diagnostic sensitivity

7. Managing Threat of Relapse in Primaquine Ineligible

The relative proportion of patients eligible to safely receive high dose primaquine may be greatly diminished by a number of causes: 1) G6PD deficient; 2) G6PD unknown and beyond reach of clinical monitoring; 3) G6PD normal by qualitative screening, but female and beyond reach of clinical monitoring; 4) Pregnant or lactating; or 5) CYP2D6 impaired. In terms of the G6PD ineligible here, WHO has historically recommended a high weekly dose of primaquine (0.75mg/kg) for 8 weeks and good efficacy of it has been reported.[29] However, the original findings with safety of this regimen [30] was obtained from just 3 otherwise healthy African-American men with the relatively mild A-variant of G6PD deficiency (Class III). A recent study in 19 Cambodian men with Viengchan variant (Class II) and acute vivax malaria showed deeper hemolytic reactions to the first weekly dose (two required hospitalization and one transfusion).[31] The weekly 0.75mg/kg weekly dose regimen may not be considered proven safe without direct variant-specific evidence of such in patients suffering acute vivax malaria.

An alternative approach applying the same rationale as intermittent preventive therapy (IPT) against *P. falciparum* in hyper- to holo-endemic communities in Sub-Saharan Africa [32] may be useful in managing primaquine ineligible vivax malaria patients. In patients diagnosed with *P. vivax* but not receiving primaquine therapy and considered at very high risk of multiple recurrent attacks, may be much like the beneficiaries of IPT. It may thus be reasonable to employ chemo-preventive or chemotherapeutic strategies to avoid repeated clinical attacks by *P. vivax*, although no randomized trials have been done to assess this. Where applied, the strategy should consider the risk and timing of relapse. For example, chemo-prevention of presumptive intermittent suppressive therapy may not be reasonable where late relapse (>6mo) among a minority of patients (<30%) is the rule (e.g., the Korean Peninsula).

Table 4. Reasoned strategies for managing relapse threat in primaquine-ineligible patients with vivax malaria of high risk of multiple rapid relapses (<4mo).

	Chemo-Preventive	**Presumptive Intermittent Suppressive Therapy**
Indication for use	Patent *P. vivax* malaria where relapse is frequent and rapid but patient is ineligible for primaquine or it is not available	Patent *P. vivax* malaria where relapse is frequent and rapid but patient is ineligible for primaquine or it is not available
Class of drugs	Blood schizontocidal	Blood schizontocidal
Mode of action	Suppression	Presumptive cure
Drugs	Chloroquine, mefloquine, atovaquone-proguanil, doxycycline	Chloroquine, mefloquine, dihydroaretemisinin-piperaquine, artemether-lumefantrine
Dosing	Per standard chemoprophylaxis practice (daily or weekly) beginning the week following treatment of the acute attack and continuing for at least 2mo, preferably 4mo, and as much as 6mo.	Per standard therapeutic practice against the acute attack and then repeated monthly at least once, preferably 3 times, and as many as 5 times.
Contraindications	Per standard chemo-prevention practice for each distinct drug (33)	Per standard chemotherapy practice for each drug (33)

8. Therapeutic Principles for Primaquine Against Vivax Malaria

Acute vivax malaria constitutes a medical emergency demanding prompt diagnosis and an effective therapeutic strategy inclusive of neutralizing the threat of relapse in the weeks and months to follow.

Widespread resistance to chloroquine may demand an alternative blood schizontocide to partner with primaquine to achieve radical cure, and the safety and efficacy of primaquine with that alternative should be considered uncertain and possibly in doubt unless proven otherwise.

Every effort to ascertain G6PD status should be made in patients diagnosed with vivax malaria and primaquine withheld from those identified as G6PD deficient by any screening or testing procedure.

No regimen of primaquine should be considered proven safe in any patient with G6PD deficiency.

Where G6PD screening by qualitative testing is done in *P. vivax* patients, females cleared as normal phenotype should nonetheless be monitored clinically for onset of hemolytic anemia.

Where good clinical monitoring is available, patients should receive primaquine therapy against relapse without specific knowledge of G6PD status.

Where clinical monitoring is unavailable or unreliable, patients of unknown G6PD status should not be prescribed primaquine but should instead be managed using strategies to prevent relapse not involving 8-aminoquinoline therapies (e.g., presumptive intermittent suppressive therapy).

The failure of supervised high dose primaquine therapy to prevent relapse should be considered likely due to severely impaired or dysfunctional CYP2D6 activity and the patient provided alternative relapse avoidance strategies (e.g., presumptive intermittent suppressive therapy).

9. Summary

Plasmodium vivax causes an acute debilitating and potentially fatal febrile illness that may recur multiple times in the weeks, months, and few years following a single infectious mosquito bite. Those recurrences derive from latent hepatic hypnozoites that can only be treated with 8-aminoquinoline drugs, primaquine being the only one now available. That class of drugs invariably causes an acute hemolytic toxic reaction in patients deficient in G6PD enzyme activity. Point-of-care qualitative screening for G6PD deficiency would greatly mitigate the risk of harm with primaquine by safely excluding those most at risk of greatest harm, but would not relieve the requirement for clinical monitoring of females cleared for primaquine therapy by that method. Alternatives to primaquine for mitigating the risk of serious harm by allowing the parasite to freely relapse in patient not eligible for primaquine should be optimized and validated, especially in highly vulnerable pregnant women and their young infants.

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