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Reinventing Primaquine for Endemic Malaria

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Reinventing Primaquine for Endemic Malaria

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Abstract

After sixty years of continuous use, primaquine remains the only therapy licensed for arresting transmission and relapse of malaria. The US Army developed primaquine for soldiers in a wartime crisis setting. The dosing strategies suited to that narrow population were adopted without modification or validation for the broader population of humans exposed to risk of malaria. The poor suitability of these strategies in populations exhibiting greater vulnerability to hemolytic toxicity among glucose-6-phosphate dehydrogenase deficient patients has not been addressed. Primaquine requires chemotherapeutic reinvention delivering less threatening doses by leveraging unexplored co-drug synergies.

Keywords

Primaquine; G6PD deficiency; hemolytic toxicity; malaria elimination

New Tools for New Strategy

Malaria remains a threat to several billion people exposed to risk in endemic zones. Global strategy for coping with this threat aimed for minimizing the burden of disease through indefinitely sustainable interventions. A few years ago, however, the World Health Organization (WHO) formally endorsed calls for adopting strategy for malaria elimination. Interventions could no longer count sustained low-level transmission of malaria as a success – cessation of transmission now stands as the benchmark of success against endemic malaria. This new strategic thinking sparked understanding of the inadequacy of available chemotherapeutics tools and strategies in coping with diverse populations of parasites deeply embedded within the human and mosquito populations in endemic zones. Obsession with treatment of the acute attack attended neglect of chemotherapeutic attack of the many silent forms of malaria – most out of diagnostic reach and thus not challenged by conventional approaches to malaria chemotherapy [1]. Eliminating malaria will likely require a profound shift in chemotherapeutics strategic thinking that brings treatments to bear against the silent malarias that dominate endemic zones.

Consideration of the development and use of the 8-aminoquinoline drug primaquine demonstrates the conspicuous neglect of the silent malarias as a chemotherapeutics problem, and points to likely avenues of work that solve the problem. Modern antimalarials emerged from drug discovery programs aimed principally at soldiers at war. In describing the wartime efforts of the US government in antimalarial drug development during the 1940s, Shannon [2] wrote, "*The primary purpose of the investigations has been, at all times, to*

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satisfy the specific needs of the armed services." This perspective dominated antimalarial drug development through the remainder of the 20th century. Newer antimalarials like mefloquine and the artemisinins derived from the strategic needs of two military camps pitted against one another in the war in Vietnam: the US Army and the Army of the People's Republic of China [3]. Creation of the Medicines for Malaria Venture in the 1990s, a public-private consortium committed to development of drugs relevant to residents of endemic zones, acknowledged the poor suitability of therapeutics intended for soldiers and travelers.

A Military Drug

Primaquine derived from military strategic urgency. The onset of war in the Pacific denied Allied forces access to quinine against acute malaria – 95% of that global supply came from plantations in Java firmly in the hands of the Imperial Japanese armed forces in 1942. The Allies mobilized the production of atabrine, an inferior synthetic antimalarial for prevention and treatment. The predecessor of primaquine, pamaquine, was withdrawn for therapy against relapse of *P. vivax* in 1943 because atabrine exacerbated its already notorious toxicity [4]. The United States government feared not only the threat that relapse posed to victory in the Pacific, but also reintroduction of the then vanishing endemic vivax malaria by returning servicemen harboring latent tissue stages [1]. Despite this urgency, improving upon pamaquine required 8 years of effort.

That effort naturally focused upon the specific requirements of the US Army. After discovery of glucose-6-phosphate dehydrogenase deficiency (G6PDd) as the basis of hemolytic sensitivity to 8-aminoquinolines in 1956 [5], the Army sought regimens of therapy not threatening the most vulnerable troops, principally African-Americans having the A-variant of G6PDd. That variant exhibits mild and self-limiting hemolytic sensitivity to primaquine [6], whereas other variants, like Mediterranean B-, pose far greater risk of threatening complications following primaquine therapy [7].

The safety of the primaquine regimens the Army wished to apply were evaluated in healthy adult volunteers with A-G6PDd, and very few of them [8]. The Army used 15mg primaquine daily for 14 days when G6PDd status was not known, or 45mg weekly for 8 weeks in G6PDd patients. The prolonged dosing of 14 days mitigated risk to unscreened G6PDd patients, and the 8 weeks of weekly dosing seemed safe and effective in A-G6PDd patients [6]. Further, the US Army determined that the 45mg weekly primaquine already in use for chemoprophylaxis (along with 300mg chloroquine in the same tablet) [9] also killed gametocytes of *P. falciparum* [10]. By the 1960s the US Army had its practical chemotherapeutic solutions against hypnozoites and gametocytes (Table 1). The WHO, at that time heavily reliant upon military expertise with antimalarials, readily adopted these treatment strategies for global use [11].

An Incompletely Developed Drug

Therapies aimed at clinically silent forms like hypnozoites and gametocytes vanished from the malaria chemotherapy research and development agenda. The inadequacy of primaquine to chemotherapeutics in endemic zones escaped attention against a backdrop of global strategy uncommitted to elimination/eradication. The perceived success of sustained low-level transmission likely underpins the failure to acknowledge the importance of chemotherapeutic attack of the silent malarias. The risks imposed by primaquine to unscreened G6PDd patients abetted the neglect of its broad application against parasite populations considered relatively non-threatening to patients and strategic aims.

The hasty development of primaquine failed to consider dose- and toxicity-minimizing strategies in broader populations than American soldiers. The US Army primaquine

regimens met their requirements and further development ceased around 1965. It is important to acknowledge that those treatment strategies were never adapted, much less optimized, to use in resource-limited settings presenting a diversity of G6PDd variants and patients far beyond the US Army experience. Administration of primaquine causing severe and fatal events indeed occurs [12-15], and providers in endemic zones are rationally reluctant to apply regimens of unknown safety. The broad lack of contemporary evidence demonstrating efficacy exacerbates this problem – an act of certain risk yielding uncertain benefit may be frank recklessness.

G6PD deficiency is the most prevalent genetic abnormality of humanity, and it is the most diverse [16]. Mutations resulting in impaired enzyme activity occur all along the length of that relatively large gene (approx. 20 kilobases of 13 exons and 12 introns; encoding 515 amino acids). Many dozens of clinically significant G6PDd variants occur, but primaquine sensitivity phenotype is known in only three: African A- (mild); Mahidol (moderate); and Mediterranean B- (severe). Stark differences in primaquine sensitivity between A- and B- variants emphasize the risks imposed with implementation of dosing strategies intended for use by the US Army. Among A- patients, primaquine hemolyzes older but not younger red blood cells. One A- G6PDd subject recovered and maintained normal red blood cell counts despite continuous daily dosing (30mg) for 4 months [6]. In contrast, B- subjects given a single 45mg dose typically hemolyzed 25% of their red blood cells, and even reticulocytes remain exquisitely sensitive to subsequent primaquine dosing [17-19]. G6PDd variants occur in the real world that may seriously threaten patients exposed to primaquine therapies safely administered to otherwise healthy African-American men. The real world also includes vulnerable small children, pregnant women, and patients with acute malaria – no regimen of primaquine has been systematically evaluated for safety in these groups. Experts have long acknowledged this very significant problem [20], but no research agenda progressed to redress it.

Beyond the very modest demonstrations of safety and efficacy of the US Army primaquine regimens in African-American men, the community of science has yet to optimize primaquine therapies in far broader human populations. Less threatening doses and dosing regimens adjusted to use with contemporary antimalarials requires specific explorations of such. Recognizing this avenue of investigation as effectively unexplored begins a process of reinvention for primaquine therapies.

Reinventing Primaquine

The view of primaquine as dangerous and ineffective must be tempered with understanding of the failure to fully develop its potential usefulness. The drug, as developed by the US Army a lifetime ago, left critical avenues of investigation unexplored. Those gaps, relatively unimportant to soldiers and travelers, are vitally important to chemotherapy of patients in endemic settings. The community of science should undertake what amounts to full development of primaquine with strategy focused sharply upon reducing the risk of harm in G6PDd patients by demonstrating good efficacy at the lowest possible exposure to drug by fully leveraging co-drug synergism against the parasite.

Arresting transmission

The gametocytocidal dose of 45mg primaquine against *P. falciparum* was not the minimally effective dose. The Army sought confirmation that this dose, already in use for chemoprophylaxis in American soldiers in Vietnam, indeed prevented transmission. The data leading to the recommendation for a single 45mg dose of primaquine against transmission of *P. falciparum* came from just two studies and a total of 14 volunteers [10, 21]. A reporting of findings in another 17 volunteers given 15mg to 45mg primaquine

therapy was published in an obscure special issue of the journal *Military Medicine* [22]. Table 2 summarizes those essential findings. Remarkably, a 30mg single dose did almost as well as the 45mg dose in preventing oocyst formation in mosquitoes, as did a single 15mg dose. Similarly, although Burgess & Bray [21] firmly recommended the 45mg single dose, their data also shows 15mg or 30mg being as effective. None of these subjects (except two controls [22]) received blood schizontocidal co-therapy – the effects of any possible synergy from therapy of the acute attack are wholly absent. In this retrospect, the decision on anti-transmission therapy for falciparum malaria seems to have unnecessarily settled upon a relatively threatening dose. Optimizing gametocytocidal primaquine therapy in patients also receiving standard artemisinin-combined therapies (ACTs, which indeed kill immature gametocytes), both in terms of dose and timing, seems highly likely to yield a far less threatening therapy for G6PDd patients.

Arresting relapse

Primaquine as hypnozoitocide against *P. vivax* offers several avenues of potential improvements to safety. The activity of 8-aminoquinolines against hypnozoites is synergized by at least several co-drugs representing as many chemical families [23]. The phenomenon, recognized late in the clinical development of primaquine [24], was not exploited, i.e., by seeking out a partner drug that provided the lowest possible effective dose of primaquine. At that time chloroquine was already the primary therapy against acute *P. vivax* malaria. The chloroquine-primaquine pair provided adequate efficacy against relapse (albeit inferior to quinine-primaquine [24]), and this radical cure became the last chemotherapeutic innovation for vivax malaria for over sixty years.

During the 1980s the US Army explored options to primaquine for radical cure, and at least one investigator revisited the issue of co-drug synergy as a means of delivering less threatening doses [25]. However, the Army later abandoned radical cure, instead committing the best 8-aminoquinoline candidate (tafenoquine) to development as a chemoprophylactic [26]. Co-drug synergy against hypnozoites thus became irrelevant. Around 2004, however, the Army abandoned tafenoquine for prophylaxis after a very high rate (93%) of vortex keratopathy occurred among Australian soldiers taking the drug weekly for six months [27]. In 2008 the drug returned to clinical development for radical cure, this time by MMV and GlaxoSmithKline with the cooperation of the Army. In experiments not designed to detect the phenomenon, US Army researchers noted the minimally effective dose of tafenoquine decreased 10-fold when used with blood schizontocide against relapse in the *Plasmodium cynomolgi* model [28]. The reinvention of primaquine (and further development of tafenoquine) should include a deliberate search for co-drugs that provide maximal synergy against relapse and thus minimal dose and threat of hemolysis.

Arresting hemolysis

Another avenue may be considered in reinventing primaquine. During 1948 the developers of primaquine working at Stateville Penitentiary in Illinois conducted a series of experiments in a single prisoner volunteer that did not later appear in peer-reviewed literature (unlike much of their work). The findings, if verified, promise solution of the onerous primaquine-G6PDd problem. The experiments were described in a semi-annual report to US NIH sponsors of the development program [29]. A 60mg daily dose of pentaquine (a candidate 8-aminoquinoline) caused one subject to have, "…*developed severe acute hemolytic anemia after several days of treatment*…" He later suffered the same outcome with a 30mg daily dose of pamaquine. However, when treated again with a 60mg daily dose of pentaquine but with 500mg methylene blue daily, he was able to consume that dose "…*for fourteen days without the development of intravascular hemolysis*." The same report presents data demonstrating methylene blue synergy of the anti-relapse activity of

isopentaquine (another candidate drug) in Chesson *P. vivax* (Table 3). Further work on methylene blue has not yet been discovered in the archives of these studies obtained from the National Library of Medicine and the Archives of the National Science Foundation. The reinvention of primaquine should include trials of methylene blue as an adjunct for arrest of 8-aminoquinoline hemolytic toxicity in G6PDd patients.

Reinvented Drug for Reinvented Strategy

Reinvented primaquine would create opportunities for chemotherapeutic strategies aimed at the entrenched endemic malarias. Gametocytemia silently sowing infectious anopheline mosquitoes and transmission may be directly attacked on larger and far more aggressive scales. The stubborn and prevalent hypnozoite reservoir would finally be exposed to safe and sustained chemotherapeutic assault. Reinvented primaquine may also bring an end to inefficient and impractical species-specific treatment where both important species occur together – an ACT and primaquine against transmission and relapse (simultaneously) would provide radical cure without regard to diagnostic availability or sensitivity.

Primaquine was never developed for use in endemic zones. Its development left critical gaps in safety that rendered it ineffective or hazardous where most malaria occurs. Exploring and leveraging co-drug synergies against both transmission and relapse in order to minimize risk of harm to G6PDd patients can reinvent this vital drug. Despite 60 years of continuous use as the only treatment against malaria transmission and relapse, primaquine is an emerging drug in need of a wide range of development work in laboratory, clinical, and endemic settings.

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Abbreviations

WHO	World Health Organization
G6PDd	glucose-6-phosphate dehydrogenase deficiency
MMV	Medicines for Malaria Venture
GSK	GlaxoSmithKline (United Kingdom)
ACT	artemisinin combined therapies

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Table 1

The primaquine therapies.

Therapy	Intent	Dose*	Duration	G6PD Safety	Notes
Gametocytocidal	Anti-transmission	45 mg	Single	Otherwise healthy A-men	No proven clinical or public health benefit
Hypnozoitocidal	Anti-relapse	15 mg daily	5 days	Unknown	No known efficacy, no longer used
		15 mg daily	14 days	None, except cessation	Korean, Indian & American strains
		30 mg daily 45 mg weekly	14 days 8 weeks	None, except cessation Otherwise healthy A-men	Tropical Asian strains Efficacy not widely proven
Causal prophylactic	Prevention in travelers	30 mg daily	Up to 50 weeks	None, except cessation	Unlicensed indication

*All doses as subscribed for adults weighing 40 to 70 kg, mean of 60 kg.

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Table 2

Proportion of anopheline mosquitoes harboring oocyts of *P. falciparum* (Malayan or Ugandan strains) after feeding on chronically parasitemic volunteers given single doses of primaquine on Day 0.

Volunteer #	Blood Schizontocide	PQ dose	Hour on Day 0		Day Post-Therapy		
			0	12	1	7	14
3	300 mg CQ	0	70%	60%	nd	50%	10%
4	300 mg CQ	0	60%	nd	75%	25%	nd
6	None	45 mg	60%	80%	10%	0%	0%
7	None	45 mg	70%	65%	0%	0%	15%
8	None	45 mg	65%	nd	0%	0%	0%
9	None	30 mg	80%	40%	0%	0%	nd
10	None	30 mg	75%	40%	0%	nd	nd
11	None	30 mg	80%	75%	10%	0%	nd
12	None	30 mg	nd	35%	0%	20%	nd
14	None	15 mg	75%	25%	60%	0%	nd
15	None	15 mg	30%	nd	0%	0%	nd
16	None	15 mg	75%	nd	55%	0%	nd
17	None	15 mg	65%	nd	40%	0%	0%

Adapted from Rieckmann *et al*, [22].

Nd: No data

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Table 3

Methylene blue synergized isopentaquine efficacy against relapse by Chesson strain *P. vivax* in American prisoner volunteers.

Methylene blue*	Isopentaquine‡	Quinine§	Relapsed/Challenged (%)	Followup Duration
0	60	0	9/10 (90%)	< 195 d
0	60	2,000	4/10 (40%)	167 – 374 d
500	60	0	3/9 (33%)	123 – 289 d
500	60	2,000	0/3 (0%)	249 d

Adapted from unpublished data of Alving *et al.* [29]

*Milligrams of base & chloride salt of methylene blue, daily dose for 14 days.

‡Milligrams of base daily for 14 days

§Millgrams of the sulfate salt of quinine