reduce clinical *P. falciparum* malaria in children, and newer whole-sporozoite vaccines have shown efficacy to prevent *P. falciparum* infection of adults in early field trials. The addition of antimerozoite vaccines that prevent clinical disease will be important new interventions as our existing tools for malaria control, including drugs and insecticides, lose their activity to increasingly resistant parasites and mosquitoes.

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References

1. Cohen, S. *et al.* (1961) Gamma-globulin and acquired immunity to human malaria. *Nature* 192, 733–737
2. Siddiqui, W.A. (1977) An effective immunization of experimental monkeys against a human malaria parasite, *Plasmodium falciparum*. *Science* 197, 388–389
3. Crosnier, C. *et al.* (2011) Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 480, 534–537
4. Srinivasan, P. *et al.* (2017) A malaria vaccine protects *Aotus* monkeys against virulent *Plasmodium falciparum* infection. *NPJ Vaccines* 2, art. no. 14.
5. Alanine, D.G.W. *et al.* (2019) Human antibodies that slow erythrocyte invasion potentiate malaria-neutralizing antibodies. *Cell* 178, 216–228.e21
6. Urusova, D. *et al.* (2019) Structural basis for neutralization of *Plasmodium vivax* by naturally acquired human antibodies that target DBP. *Nat. Microbiol.* 4, 1486–1496
7. Rawlinson, T.A. *et al.* (2019) Structural basis for inhibition of *Plasmodium vivax* invasion by a broadly neutralizing vaccine-induced human antibody. *Nat. Microbiol.* 4, 1497–1507
8. Cole-Tobian, J.L. *et al.* (2009) Strain-specific duffy binding protein antibodies correlate with protection against infection with homologous compared to heterologous *Plasmodium vivax* strains in Papua New Guinean children. *Infect. Immun.* 77, 4009–4017
9. Batchelor, J.D. *et al.* (2011) Dimerization of *Plasmodium vivax* DBP is induced upon receptor binding and drives recognition of DARC. *Nat. Struct. Mol. Biol.* 18, 908–914
10. Payne, R.O. *et al.* (2017) Human vaccination against *Plasmodium vivax* Duffy-binding protein induces strain-transcending antibodies. *JCI Insight* 2, e93683
11. Carias, L.L. *et al.* (2019) Identification and characterization of functional human monoclonal antibodies to *Plasmodium vivax* Duffy-binding protein. *J. Immunol.* 202, 2648–2660

Spotlight

Killing of *Plasmodium vivax* by Primaquine and Tafenoquine

Miles B. Markus[1,2,*]

Primaquine administration results in H_2O_2 accumulation in bone marrow, where gametocytes and asexual parasites are therefore killed. This finding, by Camarda *et al.*, supports the theory that the nonperipheral blood origin of recurrent *Plasmodium vivax* malaria is both hypnozoites (relapse source) and merozoites (recrudescence source), not hypnozoites only.

It is invariably assumed that the reason why patients who have been given primaquine (PQ) or tafenoquine (TQ) together with a blood schizontocidal drug often do not suffer any *Plasmodium vivax* malarial recurrences is that PQ and TQ kill hypnozoites (a term coined by me in 1978). The explanation for this good treatment outcome may not be so straightforward, however (see Box 1 for some of the remaining questions).

PQ's antimalarial mechanism has hitherto been unclear, but Camarda *et al.* [1] recently concluded that a two-step biochemical relay process is involved. PQ is first converted into hydroxylated metabolites via the CPR/CYP2D6 metabolic complex [1,2]. Thereafter, spontaneous oxidation of metabolites produces quinoneimine forms, with simultaneous generation of hydrogen peroxide (H_2O_2). The quinoneimines are then reduced back to the hydroxyl forms, perpetuating a catalytic cycle which results in H_2O_2 accumulation. Camarda *et al.* believe that it is the build-up of cytotoxic amounts of H_2O_2 that efficiently kills plasmodial parasites at sites of metabolic transformation. This will happen in the liver and bone marrow; perhaps elsewhere too, a possibility that remains to be clarified. These insights from *Plasmodium falciparum* infection [1] should improve our interpretation of biological and epidemiological aspects of *P. vivax* malaria, and lead to rational drug design.

Of special interest and significance is that the PQ-associated findings for bone marrow [1] correlate with the latest (8-year-old) explanation for recurrence of *P. vivax* malaria [3,4]. See Box 1 in reference [5] for the definitions of 'recurrence', 'recrudescence', and 'relapse', the meanings of which are central to understanding the discussion here. The recurrence hypothesis [3,4] is that (in addition to recrudescences thought to be initiated by parasites in the bloodstream) both hepatic hypnozoites and merozoites in organs and tissues are sources of clinical and parasitemic *P. vivax* malarial recurrences, as opposed to hypnozoites in the liver only. There is no logical reason why recurrent *P. vivax* malaria should necessarily have only one nonbloodstream origin (namely, hypnozoites). The PQ research results [1] and the bimodal *P. vivax* malarial recurrence concept [3,4] are directly related because the new information reported for PQ gives

Box 1. Open Questions

- How does the mechanism of action of tafenoquine (TQ) compare with that of primaquine (PQ)?
- Parasitologically, to what extent do PQ and TQ, in combination with blood schizontocides, kill nonbloodstream, extrahepatic, asexual *Plasmodium vivax* parasites, for example, in bone marrow (where *P. vivax* is prevalent [3,4])?
- Further to the previous question, do PQ and TQ differ in the extent to which they eliminate nonhepatic *P. vivax* parasites in different anatomical sites, when partnered with the same blood schizontocide?
- What is the mechanism underlying the apparent synergism between the 'anti-relapse' drugs PQ and TQ and the so-called 'blood schizontocides'?
- In untreated *P. vivax* malaria, what proportion of recurrences are purely hypnozoite-mediated relapses; and how many recurrences have, instead, sequestered/extravascular merozoites as the origin of parasite repopulation of the bloodstream? More importantly, how many instances of the latter type of recurrence (these being recrudescences, not relapses) are prevented by combination drug treatment, that is, which includes PQ or TQ, compared with the number prevented by blood schizontocidal monotherapy?
- Is temporary, drug-induced, erythrocytic-stage parasite dormancy a factor related to recrudescence of *P. vivax* malaria [3,5]?
- Can primatized mice be used for investigating any of the issues mentioned above?

rise to a question: does PQ do more in humans outside the peripheral circulation than just act as a hypnozoitocide and schizontocide hepatically? Does it also help to prevent *P. vivax* recurrences by killing noncirculating, extrahepatic asexual parasites – especially in the bone marrow, which is now known to be a habitat where *P. vivax* lives and flourishes [3,4]? Camarda *et al.* [1] refer to *P. vivax* occurrence in the context of PQ acting against asexual stages and gametocytes. It has long been recognized, on the grounds of parasitemic evidence, that PQ, like TQ, is able to inactivate extrahepatic asexual forms [6,7]. But it has been assumed that the killing takes place in the bloodstream. Is this primarily where asexual *P. vivax* stages are destroyed by 8-aminoquinoline drugs or is it mainly in, for example, bone marrow? Irrespective of where exactly it happens, Commons *et al.* [8] suspect that addition of PQ (i.e., combination therapy) when treating *P. vivax* malaria helps to reduce the occurrence of recrudescences through PQ's blood-stage schizontocidal ability. To summarize, the discovery by Camarda *et al.* that PQ leads to the generation of H_2O_2 in bone marrow [1] would seem to be compatible with the idea that PQ-associated asexual parasite killing in *P. vivax* infections occurs more widely than just in the bloodstream and liver. This has important implications for malaria parasite elimination in human populations.

Perhaps unsurprisingly, given the theory that PQ is a hypnozoitocide in humans, in rhesus monkeys a few sporozoite-initiated *Plasmodium cynomolgi* infections (that were not long-standing) did not recur after administration of PQ alone [9]. Nonetheless, we must be cognizant of the fact that, when PQ is used on its own in humans, it is very inefficient at preventing recurrences of *P. vivax* malaria. Baird and Rieckmann stated 16 years ago [6] that this 'is neither widely cited nor recognized'. They added: 'We are not aware of any hypothesis explaining this phenomenon.'. This is still the situation today. We should further note that, although PQ is relatively ineffective on its own in preventing recurrence of *P. vivax* malaria, blood schizontocides like chloroquine are not detectably very useful on their own either. By contrast, dual-drug therapy (PQ + a blood schizontocide) markedly improves efficacy.

We do not yet know how this works [7]. The presumed synergistic consequences of combination therapy are not restricted to PQ as the 8-aminoquinoline partner drug and *P. vivax* as the target: they also apply to TQ and the simian parasite *P. cynomolgi*. When TQ together with chloroquine was given to infected rhesus monkeys, the dose of TQ needed for radical cure was reduced by up to 10-fold, compared with when TQ was administered alone [10]. We know that the effects of PQ on the disposition of chloroquine are negligible, whereas chloroquine somehow has a significant influence on PQ pharmacokinetics, increasing PQ concentrations. We can probably extrapolate this to TQ. But the precise mechanism whereby PQ and TQ exert their apparently synergistic curative effect when combined with blood schizontocides remains shrouded in mystery.

We can, however, conclude that the putative synergy when PQ (and possibly TQ) is combined with a blood schizontocidal drug may enhance elimination in the bone marrow (hematopoietic or otherwise) of *P. vivax* parasites that would normally have been a source of recrudescences (mimicking hypnozoite-associated relapses). Perhaps *P. vivax* elsewhere, such as in the spleen [4], is also eradicated in the same way. Although we do not know what the overall, presumably synergistic, mechanism is, I offer what is at least a likely partial explanation for the recurrence-inhibiting effect. This is simply that H_2O_2 generated as a result of PQ administration [1] inactivates noncirculating asexual parasite stages.

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References

1.. Camarda, G. *et al.* (2019) Antimalarial activity of primaquine operates via a two-step biochemical relay. *Nat. Commun.* 10, 3226

2.. Marcsisin, S.R. *et al.* (2016) Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: current state of the art. *Pharm. Ther.* 161, 1–10

3.. Markus, M.B. (2018) Biological concepts in recurrent *Plasmodium vivax* malaria. *Parasitology* 145, 1765–1771

4.. Markus, M.B. (2018) New evidence for hypnozoite-independent *Plasmodium vivax* malarial recurrences. *Trends Parasitol.* 34, 1015–1016

5.. Markus, M.B. (2015) Do hypnozoites cause relapse in malaria? *Trends Parasitol.* 31, 239–245

6.. Baird, J.K. and Rieckmann, K.H. (2003) Can primaquine therapy for vivax malaria be improved? *Trends Parasitol.* 19, 115–120

7.. Baird, J.K. (2018) Tafenoquine for travelers' malaria: evidence, rationale and recommendations. *J. Travel Med.* 25 (1), tay110

8.. Commons, R.J. *et al.* (2018) The effect of chloroquine dose and primaquine on *Plasmodium vivax* recurrence: a WorldWide Antimalarial Resistance Network systematic review and individual patient pooled meta-analysis. *Lancet Infect. Dis.* 18, 1025–1034

9.. Schmidt, L.H. *et al.* (1982) Responses of sporozoite-induced and trophozoite-induced infections to standard antimalarial drugs. *Am. J. Trop. Med. Hyg.* 31, 646–665

10.. Dow, G.S. *et al.* (2011) Radical curative efficacy of tafenoquine combination regimens in *Plasmodium cynomolgi*-infected rhesus monkeys (*Macaca mulatta*). *Malar. J.* 10, 212