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Trends in Parasitology

Spotlight

How does primaquine prevent *Plasmodium vivax* malarial recurrences?

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Flannery *et al.* and Luiza-Batista *et al.* recently reported on liver and blood stages of *Plasmodium vivax* in humanized mice. The biology of *P. vivax* can be investigated using the mouse models, which will also facilitate drug research. This could lead to better treatment and control of *P. vivax* malaria.

Prospects for future research on *P. vivax*, using humanized mice, are looking brighter because of important advances made by Flannery *et al.* [1] and Luiza-Batista *et al.* [2]. The FRG huHep mouse model has made it possible to obtain novel *P. vivax* liver-stage information [1], whereas some features of the extrahepatic part of the life cycle of *P. vivax* have been found to be reproduced in the HIS-HEry mouse model [2]. I reflect herein mainly upon radical cure-related aspects of *P. vivax* malaria.

Liver-chimeric FRG huHep mice have livers populated with human primary hepatocytes. This mouse model has been used [1] *inter alia* to compare the size and structure of both hypnozoites [3] and schizonts over time and monitor changes in their abundance, to examine hypnozoites and liver schizonts microscopically following drug treatment, to correlate the sporozoite dose with the subsequent hepatic parasite load, and to quantify the overall liver-stage burden by *Plasmodium* 18S qRT-PCR. The model has also been used [1] to compare some experimental variables: two intravenous routes of sporozoite inoculation, *P. vivax* infection characteristics in humanized mice of two background strains, mosquito salivary gland versus midgut oocysts as the source of sporozoites, and infection differences between nine clinical isolates.

Of particular interest is the microscopically observed effect of primaquine on hypnozoites and liver schizonts [1]. Despite the value of non-human primate models for *P. vivax* studies [4], researchers are not normally able to see, directly, how drug treatment affects hypnozoites in simian hosts. Nonetheless, what was interpreted as microscopic evidence for the *in vivo* killing of *Plasmodium cynomolgi* hypnozoites by primaquine was mentioned 34 years ago [5]. Prior to publication in 1988, Professor Garnham discussed the research results with me, since I had worked with him while a PhD student at Imperial College London. Those limited findings regarding the effect of primaquine [5] do not, however, compare with the detailed microscopic examinations of the post-treatment appearance of hypnozoites (and schizonts) in humanized mouse livers that have now been carried out [1].

Administration of 60 mg/kg primaquine on days 0 to 1 post inoculation [1] resulted in an absence of hypnozoites and schizonts in the liver but not, at times thereafter, in immediate parasite clearance. For instance, on treatment days 3 to 4 post inoculation, the developmentally arrested schizonts were smaller than those in control mice, with parasites showing structural abnormalities [1]. Extended incubation after primaquine exposure revealed that arrested schizonts did not resume growth. Flannery *et al.* [1] point out that there is no functional FRG huHep mouse immune system (a possible explanation for some of their findings), but they nevertheless think that primaquine's mode of action against *P. vivax* liver stages may be more complicated than the conventional dogma that parasites are eliminated immediately. They suggest that primaquine 'may work by irreversibly inhibiting the biological processes needed for activation or the subsequent processes of schizogony and cytokinesis by the parasite'.

Importantly, testing of primaquine and chloroquine together in mice (a drug combination widely used to treat patients with *P. vivax* malaria) revealed enhanced killing of hypnozoites, although a confounding influence of a third drug included by Flannery *et al.* [1] is theoretically possible. Chloroquine also potentiates primaquine activity *in vitro* against both *P. cynomolgi* hypnozoites and schizonts in primary hepatocytes [6]. Analogous synergy resulting from this drug combination is likewise evident from *P. vivax* research *in vitro* [7].

To extrapolate, it seems feasible that the extent (at present unknown) of *in vivo* killing of noncirculating, extrahepatic asexual *P. vivax* parasites in humans by primaquine could be similarly enhanced when primaquine is given together with chloroquine. This is a possibility because primaquine-associated elimination of *P. vivax* erythrocytic schizonts in bone marrow (and perhaps elsewhere outside the peripheral circulation and liver as well) may for particular biochemical reasons take place [8]. It will be interesting to find out what happens in the HIS-HEry mouse model.

The question of whether the use of primatized mice harboring *P. vivax* or *P. cynomolgi* would enable determination of the effect of primaquine on noncirculating, extrahepatic merozoites, was in fact raised 3 years ago [8]. It had previously been speculated that *P. vivax* asexual stages might occur in the bone marrow of humanized mice (see reference 4 in [8]). Luiza-Batista *et al.* [2] have now answered both questions. They say that their mouse model for blood-stage infections, which includes parasite localization in the bone marrow, 'will provide a platform for drug screening *in vivo*'.

Box 1. Questions for future *Plasmodium vivax* research

- Do any merozoites from hepatic relapse schizonts move to the bone marrow or spleen?
- Do splenic merophores or merozoites in splenic dendritic cells (found long ago in rodent malaria) occur in *P. vivax* infections?
- Long-standing dogma is that the total (cumulative) dose of primaquine is what matters. Is this correct, now that time-related variations in apparent primaquine efficacy [1,7] have been demonstrated?
- Can erythrocytic schizonts be visible in murine bone marrow after treatment with primaquine (cf. persisting post-treatment schizonts in liver cells [1])? If so, that would be suggestive but insufficient evidence that primaquine failed to inactivate merozoites. Accordingly, it would be necessary to determine parasite viability by attempting to infect naïve mice, using post-treatment, parasite-containing bone marrow.
- Considering that the spleen is a major reservoir of *P. vivax* parasites, as synoptically reviewed elsewhere [9], is primaquine as effective (or ineffective) in inactivating non-phagocytosed parasites in the spleen as in bone marrow, where *Plasmodium*-killing hydrogen peroxide is known to accumulate [8]?
- How many noncirculating, extrahepatic merozoites are eliminated when primaquine is given alone, compared with combination therapy? In other words, is there a directly detectable, *in vivo* additive or synergistic effect on asexual parasites?

A theory proposed during the post-hypnozoite-discovery era is that noncirculating merozoites may be responsible for a proportion of relapse-like recurrences of human *P. vivax* malaria [9]. Investigation of whether primaquine kills asexual parasites in bone marrow in the HIS-HEry mouse model [2], especially when administered together with chloroquine, would be a follow-up on this hypothesis, which was put forward in 2011. If primaquine does indeed inactivate blood-stage parasites in murine bone marrow (Box 1), that could be interpreted as validating the hypothesis. It could then be assumed that primaquine acts similarly in humans, thus preventing some non-hypnozoite-mediated recurrences in *P. vivax* malaria patients. By definition, those would be prevented recrudescences, which have a merozoite origin, not prevented relapses, which have a hypnozoite origin.

Various recent studies have revealed the extent of the occurrence of noncirculating merozoites in chronic *P. vivax* malaria, thereby providing support for the above-mentioned hypothesis. The overall, noncirculating, erythrocytic merozoite reservoir is now known to be huge, whereas only a few hypnozoites are present in *P. vivax*-infected individuals [1]. Obaldía *et al.* [10] emphasize that the recent elucidation of concealed reservoirs of blood-stage *P. vivax* parasites 'fundamentally changes existing paradigms of *P. vivax* biology, pathogenesis, and epidemiology'.

Finally, in addition to drug testing, the two mouse models [1,2] are suitable for studying the 'new' biology of *P. vivax* (Box 1). We can therefore expect further progress in these two areas of *P. vivax* research. If the goal of eradicating malaria is to be achieved, it is of the utmost importance that such progress be made.

Declaration of interests

The author declares no competing interests.

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