Is primaquine useful and safe as true exo-erythrocytic merontocidal, hypnozoitocidal and gametocidal antimalarial drug?

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Abstract

The main objective of this paper is to make available in a single document, a sequence of events that have been published on the biology of malaria parasites and their interaction with the human host, looking for arguments for effective and save treatment: what we know and what we would like to know about the effects of primaquine in order to justify its use in clinical and public health practice. The practicioner should be aware that the antimalarial activity, hemolytic and methemoglobinemic side effects, and detoxification of primaquine are all thought to depend on various biotransformation products of the drug. In spite of the universal use during over six decades, their site and mechanism of formation and degradation and their specific biologic effects remain very poorly understood in human beings. The mature gametocytes of *P. falciparum* are naturally resistant to chloroquine and other blood merontocides, but they are usually eliminated with a single dose of 1.315 mg/kg *per os* (p.o.) of primaquine phosphate (equivalent to 0.75 mg-base). Rather than empirically, related with relapses frequency, dosage schedules should only be determined through consideration of the kinetics and dynamics of the drug and its effect on sporozoites, pre and exo-erythrocytic merontes, hypnozoites and gametocytes of *P. vivax*. Where medical care services are not available or not capable to detect glucose 6- phosphate dehydrogenese- (G-6-PD) deficiencies and deleterious effects of the drug, we recommend not to use primaquine. Both, *P. vivax* primary clinical attack and *P. vivax* relapses, as and when they occur should be treated with a course of 10 mg/kg chloroquine-base p.o. Prevention of relapses is probably related to strain characteristics of *P. vivax* hypnozoites populations envolved. If well informed and

Resumen

El objetivo principal de este ensayo es poner a disposición en un documento único, una secuencia de eventos que han sido publicados sobre la biología de los parásitos del paludismo y su interacción con el huésped humano, buscando argumentos para el tratamiento eficaz y seguro: ¿qué sabemos y qué nos gustaría saber sobre los efectos de la primaquina para justificar su uso en la práctica clínica y de salud pública? El profesional debe estar atento a que tanto la actividad antipalúdica, como los efectos deletéreos hemolíticos y metahemoglobinémicos y de detoxificación de la primaquina dependen de varios productos de biotransformación de la droga. No obstante el uso universal durante seis décadas, el sitio y mecanismo de formación y degradación y sus efectos biológicos específicos en los seres humanos aún permanecen poco comprendidos. Los gametocitos maduros de *Plasmodium falciparum* son naturalmente resistentes a la cloroquina y a otras drogas merontocidas sanguíneas, pero son usualmente eliminados con dosis única de 1.315 mg/kg *per os* (p.o.) de fosfato de primaquina (equivalente a 0.75 mg base). En relación con la frecuencia de recaídas, en vez de empíricamente, los esquemas de tratamiento deberían determinarse considerando la farmacodinamia de la droga y su efecto sobre los esporozoitos, merontes pre-exo-eritrocíticos, hipnozoitos y gametocitos de *P. vivax*. Donde no hay servicios de atención médica disponibles, o éstos no están capacitados para identificar deficiencias de glucosa -6- fosfato dehidrogenasa (G6PD) ni efectos deletéreos de la droga, recomendamos que no se use primaquina. Tanto los ataques clínicos primarios como las recaídas de las infecciones por *P. vivax* como y cuando se presenten deben tratarse con 10 mg/kg de cloroquina base p.o. La prevención de las recaídas por *P. vivax* está proba-

qualified medical care workers decide to use primaquine in the absence of enzime defficiencies and are able to follow-up the clinical, toxicological and parasitic results, a daily dose of 0.25 mg/kg primaquine-base during 14 days could be administered safety for possible prevention of *P. vivax* relapses.

blemente relacionada con la cepa y las características de las poblaciones de hipnozoitos de *P.vivax* involucrados. Si los trabajadores de salud bien informados y calificados deciden usar primaquina en ausencia de deficiencias de enzimas y están dispuestos a hacer el seguimiento clínico, toxicológico y parasitológico, pueden administrar con seguridad una dosis diaria de 0.25 mg/kg de primaquina-base durante 14 días para la posible prevención de las recaídas por *P. vivax*.

Key words: malaria/therapy; primaquine; *plasmodium vivax*/infection; Mexico

Palabras clave: paludismo/terapia; primaquina; *plasmodium vivax*/infección; México

Plasmodia are obligatory intracellular parasites able to invade and asexually reproduce inside human parenchyma liver cells and erythrocytes. The infection begins when the sporozoite invades the hepatocyte. The resulting hepatic meronte produces thousands of merozoites that are able to invade red blood cells (RBC) and a cascade of pathological, clinical and immune responses and manifestations are initiated. The behaviour of the plasmodia in the liver is responsible for nonrelapsing malaria (*Plasmodium falciparum* and *P. malariae*) or relapsing malaria (*P. vivax* and *P. ovale*).[1] Knowledge of relapse characteristics of local or regional *P. vivax* strains is important for several obvious reasons, foremost among them being: a) the interpretation of malariometric data in endemic areas and during epidemic situations and b) the development of antimalarial chemotherapeutic policies as well as the interpretation of the results regarding drugs efficacy. Contacos *et al.*, studying human infections representatives from tropical zone documented different patterns of relapse activity of *P. vivax*. They described *vivax* infections representatives from temperate zone: a) with short periods of latency between primary attack and many relapses (six-ten); b) with long or shorter period of latency between primary attack and a some (four) frequent relapses; and c) with shorter latency between primary attack and fewer relapses (two).[2] Two theories try to explain the relapsing malaria: a) the resulting pre-erythrocytic merozoites may invade red blood cells, thereby initiating the erythrocytic cycle, or other hepatocytes, thereby continuing the exoerythrocytic cycle,[3] and b) the hypnozoite theory that postulates the existance of mixed populations of genetically distinct sporozoites, some of which develop immediately to merontes and some of which remain "dormant" in the liver for a longer time.[4]

The hypnozoite theory takes as controlling factors, the survival of the parasite and ecological interactions within its natural environment. Varying proportions of sporozoites produce hypnozoites, exhibiting varying periods of dormancy, ranging from less than 1 month (within the wide range of the tropical zone strains) to approximately 7-20 months or more for the temperate zone strains, before activation to merogony and resultant relapse at the observed intervals. The actual proportions of each sporozoite/hypnozoite type within the strains are unknown. Their distribution among the major strain groupings appears reasonably distinct and, to some extent, defines each grouping. The sequences of sporozoite as seen in non-relapsing malarias and early primary parasitemias are: to pre-erythrocytic meronte, to hepatic merozoites release, to erythrocyte merontes and to gametocytes. Conversely, sequences of sporozoite as seen in relapsing malarias and relapses are: to hypnozoite, to pre-erythrocytic meronte, to hepatic merozoites, to erythrocytic merontes and gametocytes. These differences are easily inferred from the summary of observed relapses. It is difficult to explain these phenomena by a simple cyclic merogony mechanism.[5,6] Additional work is needed to determine the biomolecular mechanisms envolved in the establishment and activation of hypnozoites and elucidation of true relapse.

Relapsig malaria

Hypnozoites of different strains of the human and non-human relapsing malaria parasite, have been detected among maturing pre-erythrocytic merontes in liver biopsies of chimpanzees infected by intravenous inoculation of sporozoites. *Plasmodium vivax* and *P. cynomolgi bastianellii* hypnozoites were detected by the immunoperoxidase technique.[7-10] Attempts have been made to demonstrate the site of invasion into tissue cells and their growth there, and to correlate the appearance and loss of hypnozoites with parasitaemic relapses. Hypnozoites decrease in numbers over-all and growing merontes were demonstrated in the liver.[11] The continued presence of hypnozoites of *Plasmodium simiovale* was also confirmed in rhesus monkeys.[12,13]

Non-relapsing *P. knowlesi* sporozoites inoculation into Rhesus monkey result in numerous nearly mature exo-erythrocytic (EE) merontes, but no hypnozoites were detected by indirect immunofluorescence.[14]

Hypnozoites are present in other relapsing *Plasmodia* infecting other hosts, they were demonstrated in tissue sections from four *Takydromus tachydromoides* (Sauria: *Lacertidae*) naturally infected with *Plasmodium sasai*.[15] Swiss mice experimentally infected with *P. yoelii yoelii* 264 BY sporozoites did not show blood parasites (85%) after a short (on day 4 - 5) incubation period. The hepatic parenchymal cells from the animals without primary infection manifestation (following 2 months of the infection) or with the disease self-arrested (on day 26 of postinfection) were the first to display mononuclear parasites similar to the hypnozoites. Rodent malaria parasites, as *P. vivax* does, might cause infection with prolonged incubation and true relapses. *Plasmodium yoelii* is a promising accessible model for studying the persistence phenomenon of malarial parasites in the liver.[16]

Clinical, immunological and epidemiological implications of relapsing malaria

The phenomenon of malarial relapse is only theoretically explained. A latent stage for *Plasmodium* spp. in the liver, for which there is now extensive morphological and experimental confirmation, best explains both the relapse phenomenon and the long prepatent periods seen with some strains of *P. vivax*. These latent stages (hypnozoites) have been detected in three relapsing malarias and have been found to persist in the liver as uninucleate parasites for up to 229 days after sporozoite inoculation. They have been found in *in vitro* cultures of two species of *Plasmodium*, and their ultrastructure has been partially described.[17,18]

Silbermann and Stuiver[19] treated 215 patients with falciparum malaria. In 8 patients (4%) who had not returned to any malarial area, malaria attacks recurred after 6-20 weeks. Curiously, these were now caused by different species: *P. vivax* (4 patients) and *P. ovale* (4 patients). After proper management of malignant tertian malaria caused by *P. falciparum*, patients were considered cured. Yet, in a small number of patients attacks of malaria recur after different time intervals. The explanation is that these were delayed primary attacks of benign tertian malaria. Consequently the patients must have been infected by two different species of malaria at a time. In *P. vivax* and *P. ovale* hypnozoites occur and dormant stages in the liver are not susceptible to blood merozoitocides. Once the blood is (re)invaded,

the patient suffers a delayed primary attack or a relapse. In endemic areas, physicians are aware that definite cure of falciparum malaria does not prevent future attacks of vivax malaria.

The longevity of specific human memory T-cell responses is largely unknown. However, a knowledge of the duration of memory is important for understanding immunity to an organism and for planning vaccine intervention. To address this, Zevering *et al.*,[20] have examined T-cell memory to malaria by determining T-cell responses by subjects recently exposed to peptides spanning the circumsporozoite (CS) proteins of two species, *P. falciparum* and *P. vivax*. Responses to vivax CS peptides by exposed Thai subjects were more frequent than responses by nonexposed individuals, permitting identification of determinants seen by vivax-induced responses. In contrast, *falciparum*-exposed subjects were largely indistinguishable from nonexposed controls in responsiveness to *falciparum* CS determinants. These data provide the average life-spans of certain malaria-specific T cells and are consistent with, but do not prove, the hypothesis that antigenic persistence (in the form of *P. vivax* hypnozoites) correlates with persistence of human T-cell memory.

Anopheles stephensi mosquitoes infected with *P. vivax* were put in environments with temperatures of 30, 26 or 13 °C for 5 d, and sporozoites were inoculated into HepG2-A16 cell monolayers (hepatoma cells). On day 7 post-inoculation EE merontes and hypnozoites were observed using immunoperoxidase staining technique. The sporozoite developing rate of 30 °C and 13 °C group was significantly lower than that of 26 °C group (33%, 35% and 75%, respectively). The proportion of hypnozoites in the total number of EE forms was the highest in the 13 °C group (62.5%). It is suggested that the low temperature affected the viability of tachysporozoites or the phenotype of sporozoites and resulted in heightened hypnozoite rate. This is parallel with long incubation period of vivax malaria in the regions of high latitude. When the sporozoites are cryopreserved the proportion of hypnozoite increased. Cryopreservation does not inactivate all of the tachysporozoites, and resistance to ultralow temperature of bradysporozoites is greater. Aging of sporozoites decreased their developing rate and the EE merontes were found to grow sluggishly and asynchronously, indicating that the size of EE merontes and the age of sporozoites are in negative correlation. Meantime, proportion of the hyponozoites decreased significantly.[21]

The hypnozoites of different isolates of *P. vivax* from China in cultured materials were observed employing immunoperoxidase staining method. The percentages of hypnozoites among EE stages in three provinces, and

the latitude are in positive correlation. The equation is y= -6.68 + 2.05x. When the difference in latitude of *P. vivax* isolate source was more than 5 degrees, a significant difference in the percentage of hypnozoites in various geographic isolates was found. The results showed that in the regions north to the Yangtze River, the proportion of hypnozoite in the liver stage of *P. vivax* was larger than those in the Southern China, being consistent with the clinical manifestations.[22]

By indirect immunoperoxidase staining, different forms of EE stage of *P. vivax* (Southern China isolates) are revealed in d8 cultured material. The mature merontes are elongated in shape measuring 42-48 microns in diameter, immature merontes 14-28 microns and hypnozoites 4-7 microns. Exo-erythrocytic merontes are stained dark-brown only after conjugated by monoclonal antibody (McAb) 4B2 specific against erythrocytic stages of *P. vivax* while hypnozoites are only stained after conjugated by McAb 2F2 against sporozoite. These results show that the antigenic components of these two forms of EE plasmodia are quite different. The ratio of EE meronte and hypnozoite found within hepatoma cells culture is 1.5 to 1. Referred to the clinical manifestations of the isolate, among 5 volunteers not radically cured, two had long incubation period (283 d and 304 d, respectively) and three relapsed 235, 260 and 365 days after the primary attack. These data are unanimous with the comparatively large ratio of hypnozoites in the cultured material.[23]

Acquired immunity against the recombinant circumsporozoite protein of *P. falciparum* (rPfCS) or *P. vivax* (rPvCS) was studied in the Brazilian Amazon. Cellular responsiveness, evaluated by proliferative assays, was detected in about 45% of individuals who had recovered from recent acute malaria infections. Peripheral blood mononuclear cells of individuals whose last malaria infection was by *P. vivax* responded more to the rCS proteins than those who had *P. falciparum*. Since in *P. vivax* infections hypnozoites in the liver retain CS antigen, this stage may have contributed to the increased cellular response. The unexpected result was that in primoinfections by *P. falciparum* or *P. vivax* the proliferative response did not correspond to the rPfCS and rPvCS, respectively. In malaria-exposed individuals, there is a positive correlation between the intensity of the responses to the two rCS proteins. These results suggest that cross-reactive epitopes exist in the CS protein of *P. falciparum* and *P. vivax*.[24]

Kirchgatter and del Portillo[25] compared paired *P. vivax* isolates from primary attacks and relapses in Brazil using the merozoite surface protein 1 gene, PvMSP1, as a genetic marker. Samples from primary attacks contained genetically mixed parasites harboring the 2 major PvMSP1 allelic forms. Polymerase chain reaction revealed the presence of these 2 forms in the relapse parasites of 2/4 patients, demonstrating that the activation of hypnozoites is not clonal. DNA sequences from paired primary/relapse samples demonstrated that the parasites from the primary attack are identical to those in relapse samples in which the same allele forms were detected in both infections.

Malaria chemoprophylaxis and chemotherapy

The outcomes produced by the different characteristics of the relapse patterns of different strains, variants and mutants of plasmodia populations infecting man is a complex system of possibilities and uncertitud. These events are modified through their journey throughout the mosquito vector and the vertebrate host. Since the early observations in 1891 that methylene blue had some chemotherapeutic effect against human malaria, analogs replacing one of the methyl groups with a dialkilaminoalkil chain, increased both antimalarial activity and toxicity.

The hypothesis led in the 1920s to the first antimalarial synthetic agent: pamaquine. This drug would cure relapsing malaria when administered at maximum tolerated doses with quinine, but the combination was found to be too toxic to be used in clinical practice in the 1940s. Pentaquine emerged in 1948 and appear to be less toxic and because administration with quinine in experimental *P. vivax* infections in man yielded radical cure. Upon treatment of US Army personnel with naturaly acquired infections, there was a drastic drop in relapse rate. No further advantage was found in large series of pentaquine analogs prepared; but further studies led to the recognition of primaquine as the least toxic and most effective 8-aminoquinoline tested.[26]

For both, chemoprophylaxis and radical cure of vivax malaria, even after analysing a hugge amount of relevant documents published during the last 50 years it is almost impossible to establish a standard dosage within reasonable predictable limits. There are sociopolitical-economic-ecologic and public health pressures derived from the need for change from insecticides depending strategies for malaria vector control to bioreliant methods for Integrated Vector and Environmental Management. So, the development and improvement of modern and practical diagnostic methods and antimalarial effective and safe medicaments become a great challenge to the national and international scientific community.

The ideal plasmocidal agent should be selective and potent against sporozoites, tissue merontes, hypnozoites, hepatic merozoites, erythrocytic merontes, blood

merozoites, gametocytes and even esporogonic forms of the parasite; the plasmocidal action should be quick and complete; it should be effective for both, preventing clinical and pathologic manifestations and complications and avoiding recrudescences and relapses; it should be inocuous for the treated person and it should not produce neither deleterious effects nor allergies. Lastly, it should be able to prevent parasite drug resistance. It should be stable and its pharmacodynamic should reach quickly and maintain the minimum inhibitory concentrations in blood plasma, biological fluids and tissues for total elimination of parasites. Its mode of action should be equally effective regardless the administration route; it should be produced at industrial scale, with a rigurous quality control and quality assurance, and low cost. Certainly, primaquine is far from matching this requirements.

Chemoprophylaxis

Alving *et al.*[27] demonstrated that prophylaxis against malaria induced by the bites of infected mosquitoes does not reach a maximum until 12 h after ingestion of a single dose of 180 mg of primaquine. Carson *et al.*[28] showed that plasma levels of primaquine (and other 8-aminoquinolines) do not correlate with the therapeutic effect; in some instances, high plasma levels in some individuals receiving an 8-aminoquinoline either alone or together with proguanil did not result in high antimalarial activity. Controlled hemolysis studies in G-6-PD-deficient volunteers, suggested that haemolysis does not begin until 24-72 h after the initial ingestion of primaquine,[29] and apparently both anti-malarial and haemolysis effects of primaquine are dose-related.

Physicians should be prepared to provide prophylactic medications for travelers to malarious areas and to treat patients with malaria. Chloroquine hydrochloride is the suppressive agent of choice for treatment of mild infections due to all species of malaria except for those due to chloroquine-resistant strains of *P. falciparum*. For treatment of severe infections with *P. falciparum*, quinine is the suppressive agent of choice. Chloroquine is also the clinical prophylactic agent of choice for most travelers. Primaquine is not recommended to prevent infection with *P. vivax* or *P. ovale*. For clinical prophylaxis of chloroquine-resistant strains of *P. falciparum*, no completely satisfactory regimen is presently available.[30,31]

Malaria chemotherapy

The diagnosis of malaria should include the species involved and in case of *P. falciparum* infection the parasitaemia index: the percentage of the infected red cells. *P. vivax*, *P. ovale* and *P. malariae* infection are treated with chloroquine, in case of *P. vivax* and *P. ovale* malaria followed by primaquine. Mefloquine and halofantrine are indicated for chloroquine-resistant *vivax* infections. Advice on management and treatment is different for mild and severe *P. falciparum* infections. Parasitaemias of ≥ 5% of infected erythrocytes impose urgent medical attention; mild infections may be treated on an outpatient basis. In severe infections quinine has to be started immediately, while frequent checks of vital functions and blood parameters are indicated. New treatment options are the use of artemisinine (preparations) or atovaquone, both efficacious and low in adverse effects and toxicity.[30,32]

Quinine-resistant *P. falciparum* was first reported in 1910 from Brazil. Today this parasite is resistant in most endemic areas to the widely used blood merontocide, chloroquine. Many strains are resistant also to antifols (e.g. pyrimethamine, proguanil) and some are also no longer eliminated by quinine. These polyresistant parasites have an enhanced ability to resist also new drugs such as mefloquine and halofantrine. There are indications that *P. vivax* is also becoming resistant to chloroquine in Papua-New Guinea where primaquine resistance of the hypnozoites also exists. The modes of action of antimalarials and mechanisms by which parasites become resistant to them are discussed. Future developments include the search for radically new compounds, for drugs that reverse chloroquine resistance and for new strategies to impede the progress of this problem.[33]

McCall and Pearce[34] made a review of patient treatment for all cases of malaria in Queensland in 1992 35% (n= 341) of *P. falciparum* infections were treated with chloroquine, even though most of these were acquired in countries where chloroquine or chloroquine/antifolate-resistant *P. falciparum* malaria existed. *Plasmodium vivax* infections were treated with chloroquine in 58% of cases. In 50% primaquine was administered but only 57% of these received the dose recommended for the eradication of hypnozoites. Current patterns of malaria treatment are basically similar in any of the malaria-receptive zones elsewhere. Simple but comprehensive guidelines for malaria treatment may assist medical practitioners in the provision of prompt, effective treatment, and help to prevent the reestablishment of endemic malaria in Australia.

Standard chemotherapy for the malaria acute attack with chloroquine

A single dose of 10 mg/kg of chloroquine-base administered *per os* (p.o.) is sufficient to eliminate the clinical symptoms and parasitaemia (both asexual parasites and

gametocytes) in case of *P. vivax, P. ovale* and *P. malariae* infections. The administration of a total dose of 25 mg of chloroquine-base per kg body weight, administered in three consecutive days clears only the asexual forms of susceptible *P. falciparum*.[35]

Chloroquine kills early protogametocytes of *P. falciparum* while hemoglobin digestion is still taking place.[36] Mature *P. falciparum* gametocytes do not appear at all when chloroquine is administered alone as soon as the asexual forms become patent at the begining of the primary attack.[37] The administration of a total dose of 25 mg of chloroquine-base per kg body weigth, administered in three consecutive days eliminates both asexual forms and gametocytes of *P. vivax, P.ovale* and *P. malariae*.[36,38]

Anti-*P. falciparum* gametocytes chemotherapy with primaquine

The mature gametocytes of this parasite species are naturally resistant to chloroquine and other blood merontocides. *Plasmodium falciparum* mature gametocytes are usually eliminated with a single dose of 1.315 mg/kg p.o. of primaquine phosphate (equivalent to 0.75 mg-base). They are generally destroyed in 72 hours by such a single dose of primaquine, but they could reappear in the blood stream as long as blood merontes are in progress. Rieckman *et al.*[39] showed gametocytocidal and sporontocidal effects of primaquine and no effect of sulfadiazine with pyrimethamine in a chloroquine-resistant strain of *P. falciparum*.

Gametocytes from the Honduras I/CDC clone HB-3 of *P. falciparum* were exposed to different concentrations of primaquine diphosphate. It could be shown that the drug acts specifically on the mitochondria of the cells by destroying their internal structure and causing these organelles to swell.[40]

Acute intravascular haemolysis could be induced even by a single dose of 45 mg primaquine-base in individuals with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Reeve *et al.*[41] calls the attention on the need to discontinue the administration of single dose of primaquine in populations exhibiting between 3-5% G-6-PD deficiencies.

Warning. A RBC G-6-PD level should be obtained before administration of primaquine.

Anti-relapse *P. vivax* infections treatment with primaquine

In endemic areas, it is extremely difficult to differenciate between *P. vivax* relapse and reinfection. Consequently,

there are no more than clinical impressions about relapse rates in different countries.

The so-called "radical" or "anti-relapse" treatment has been empirically prescribed to prevent true relapses of *P. vivax* and *P. ovale*. Primaquine, an 8-aminoquinoline has been reported by WHO[42] as the treatment of choice for vivax infections since 1950. This medicament has been advocated as the only tissue merontocidal drug currently available for such a treatment.[35,43]

Warning. A RBC G-6-PD level should be obtained before administration of primaquine.

Doses

*   Daily dose of 0.438 p.o. of primaquine phosphate (equivalent to 0.25 mg-base) per 1 kg body weight, once a day during 14 consecutive days.
*   For certain infections, particularly those from Southeast Asia, it would be necessary to administer a double daily dose of 0.876 p.o. of primaquine phosphate (equivalent to 0.50 mg-base) per 1 kg body weigth, once a day during 14 consecutive days.
*   For other infections, particularly those from Latinamerican and Caribbean countries, the daily administration of 0.25 mg/kg-base has been reduced to 3 to 5 days.
*   The usual pediatric dose is 0.667 mg/kg p.o. of primaquine phosphate (equivalent to 0.39 mg-base) per 1 kg body weigth per day during 14 consecutive days.[44]

Primaquine phosphate treatment (0.5 mg/kg/day, p.o., 14 days) significantly increases values for serum sodium, potassium, and uric acid, while calcium levels are decreased in male *Macaca fascicularis*. Serum values returned to baseline (pretreatment) levels by 30 days following primaquine treatment.[45]

After five decades, we agree with Coatney *et al.*:[46] "the occasional occurrence of haemolysis is sufficient justification for close observation of patients and ready accessibility to well equipped medical facilities"; with Alving et al.:[27] "primaquine should never be given unsupervised to a medically unsophisticated population", and Motulsky et al.,[47] "G-6-PD –deficiency affects more than 100 million males and about twice as many females". This genetically X-linked deficiency is quite prevalent, occurring in probably 200-300 million people of all races. As with haemoglobin, there are many molecular variants of G-6-PD. Individuals with this deficiency are healthy, unless stressed by certain drugs such as primaquine or serious intercurrent

illnesses such as acute hepatitis, pneumonia, or diabetic acidosis.[48] Although the frequency of severe primaquine-induced haemolysis is low, the large number of individuals potentially at risk calls for careful supervision of primaquine administration.

Warning. A G-6-PD level should be obtained before administration of primaquine.

In a controled study, Bunnag *et al*.[49] administered on admission a single dose of 300 or 450 mg chloroquine-base to *P. vivax* malaria Thai patients (n=167). All patients in both groups showed a rapid parasite clearance times (67.1 and 58.1 h respectively). The patients, then were allocated at random (double blind) to receive a daily dose of 15 mg or 22.5 mg primaquine-base for 14 d. Relapses in both groups occurred within 6 months. The relapse rate in the primaquine 15 mg group was significantly higher than that in the 22.5 mg group (17.5% *vs*. 2.4%).

Warhurst[50] questioned the effectivity of primaquine against mature gametocytes and hypnozoites. Together with Doherty *et al*.[51] he assesed the frequency of relapse after treatment with conventional 14 days of daily doses of 0.25 mg/kg primaquine-base. Patients were travellers returning to London, UK from Africa, Indian subcontinent, Sout Asia, and South and Central American countries. The authors feel it is time to revise recommended treatment with primaquine. The following options are stated:

a) To continue with current practice and to treat relapses with chloroquine followed by primaquine in higher dose (0.25 mg/kg primaquine-base twice daily)

b) Not to use primaquine at all, but to treat relapses as and when they occur with a further course of chloroquine, and

c) to treat all cases with primaquine for the first attack but with a higher dose (0.25 mg/kg primaquine-base twice daily), once the RBC concentration of G-6-PD is known to be adequate. This recommendation was previously made by Luzzi *et al*.[52]

Primaquine mode of action and toxicity

Although hypothesis regarding possible mechanisms of action of primaquine and its metabolites and of several other classes of tissue merontocides and hypnozoiticides have been proposed, they have not been proven correct, since none have been subject to critical clinical trials according to different parasite strains and hypnozoites populations characterization, and reliable *in vitro* and *in vivo* tests are still to be developed.

Primaquine mode of action could be based in the capacity of primaquine to bind to the parasite's DNA and modify its properties. Primaquine and other 8-aminoquinolines have been shown to induce mitochondrial lesions in the EE forms of *P. fallax* in tissue cultures,[53,54] in EE forms of *P. Berghei berghei*[55] and *P. yoelii*.[56] This led to the suggestion that its mode of action may be related to electron transport and to the oxidation reduction of ubiquinones in the parasite.[57]

Frischer *et al*.[58] have explored the feasibility of studying primaquine metabolism in cultured human cells. The biotransformation of primaquine can be investigated *in vitro* in serum-supplemented liquid cultures of partially synchronized and exponentially growing human erythroleukemic K562 cells. Further, these cells can be replaced by cells present in normal bone marrow. The availability of reproducible, quantitative, and practical new tools for the study of primaquine metabolism *in vitro* raises a number of challenging questions and may improve understanding of the mode of action, toxicology, and pharmacogenetics of 8-aminoquinolines.

Most antimalarial drugs are eliminated by hepatic metabolism, but the influence of malaria infection on the hepatic elimination of these drugs has been exceptionally examined. The elimination of primaquine was measured in isolated perfused rat livers of malaria-infected Sprague-Dawley rats. There was no significant difference in the volumes of distribution of primaquine between the infected and control animals. There was an inverse linear correlation between primaquine clearance and the percentage parasitaemia ($r= 0.722$, $p< 0.05$). These results suggest that the extent to which primaquine elimination had been compromised was related to the severity of malaria infection, and that in severe infections reduced efficiency of elimination raises the possibility of drug toxicity.[59]

Parkhurst *et al*.[60] and Nora *et al*.[61] described high-performance liquid chromatographic methods for the simultaneous determination of primaquine and its metabolites from plasma and urine samples obtained after oral administration of primaquine diphosphate. Following partial deproteinization with acetonitrile, samples were chromatographed by direct injection into a cyano column with UV detection at 254 nm. Levels as low as 100 ng/ml per 20-ml injection were quantitated. Preliminary pharmacokinetic analysis is reported for two human volunteers after oral doses of 60 mg and 90 mg. Two apparent plasma metabolites and two possible urinary metabolites of primaquine are also reported.

Photooxidation of primaquine (1) and 5-hydroxy-primaquine (5) afforded a blue dye for which o-quinone

structure 4 was elaborated. Similar oxidation of N-ethoxyacetylprimaquine (10) afforded o-quinone (11). Tissue merontocidal activity of 4 and 11, and bisquinolylmethine 3 prepared earlier, showed that none of them had noteworthy antimalarial activity, but all three produced methemoglobin.[62]

Suspensions of washed human red blood cells were treated with nine synthetic putative metabolic derivatives of primaquine, and their individual effects on activity of the hexose monophosphate shunt (HMS) were quantitated by radiometric analysis of $14CO_2$ from [14C] glucose. The most potent HMS stimulant was 5-hydroxy-6-methoxy-8-aminoquinoline (5H6MQ), which caused 10-fold elevation of HMS activity at an estimated concentration of 0.004 millimolar primaquine (mM). Ten mM primaquine was required to achieve the same effect. Thus, 5H6MQ was approximately 2500-fold more reactive with the HMS than primaquine. Baord *et al.*[63] suggested that 5H6MQ-induced elevation of HMS activity was at least partially independent of glutathione redox reactions, hydrogen peroxide accumulation and reaction with oxyhemoglobin. The relevance of these observations suggests mechanisms of hemolytic toxicity of primaquine.

Carson and Frischer[48] reported G-6-PD- deficiency and related disorders of the pentose phosphate pathway. Powell *et al.*[64] demonstrated the antimalarial and hemolytic properties of 4.4-diaminodiphenyl sulfone (DDS) and Eppes *et al.*[65] confirmed the protective and hemolytic effects of 4.4'- DDS administered daily together with weekly chloroquine and primaquine against chloroquine-resistant *P. falciparum*. Carson,[29] reported hemolysis due to inherited erythrocyte enzyme deficiencies and he also studied the clinical, metabolic and molecular consequences of genetic disorders of the pentose phosphate pathway.[66] Carson *et al.*[28] have associated the toxicology of the 8-aminoquinolines and genetic factors in man.

Conclusions and recommendations

- Efforts should be done to improve the timely malaria parasitological diagnosis, assuring the highest sensitivity and specificity in order to establish immediate treatment of the acute attack. In most parts of the world, erythrocytic asexual forms and gametocytes of *P. vivax* are sensitive to a single dose of 10 mg chloroquine-base administered p.o. It is unnecessary, unsafe and costly to use something else. Efforts should be done to register the relapse rate after such a single dose in order to define informed policies and estrategies for aditional treatments.

- The antimalarial activity, hemolytic and methemoglobinemic side effects, and detoxification of primaquine are all thought to depend on various biotransformation products of the drug. Their site and mechanism of formation and degradation are not fully known and their specific biologic effects remain very poorly understood, particularly in human beings. Primaquine is apparently absorbed rapidly, but it is not know to what extend, or whether is subject to a modification by chemical or enzymatic action in the gut wall or liver of the human host.

- Rather than empirically related with relapses frequency, dosage schedules can only be determined through consideration of the kinetics and dynamics of the drug and its effect on sporozoites, pre and exo-erythrocytic merontes, hypnozoites and gametocytes. There is a commendable work done on the identification of primaquine metabolites, but it is not yet possible to demonstrate the substances responsible for its parasiticidal effect and their stability.

- Insufficient attention has been given to the alternative laboratory models suitable for curative drugs screening. The *Rhesus* and *Chimpanzee* systems are not suitable for screening purposes because of limited availability and high cost of the operation. The inability to grow exo-erythrocytic forms and hypnozoites *in vitro* is a major constraint to examine the influence of chemotherapeutic agents on these stages. The avian, rodent and non-human primates models should be critically evaluated in order to identify similarities and differences with malaria infections response in man.

- Since the early 1960s, chloroquine resistant *P. falciparum* populations have been spread all over the malarious areas of the world. Many alternative treatments quinine + pyrimethamine + sulphadoxine combination, quinine + tetracycline, mefloquine, artemisinine, artesunate, artemether, halofantrine, pyronaridine and others have been designed. Extense literature is available regarding their indications and toxicity. Combined administration of primaquine with quinine, mefloquine, antifolics and sulfonamides should be avoided.

- Exceptionally, a gametocydal single dose of 0.75 mg/kg of primaquine-base is recommended after the *P. falciparum* acute attack is properly managed

if and only when close clinical and parasitological follow-up of the patients is feasible. A RBC G-6-PD level should be obtained before administration of primaquine. A colorimetric practical field method could be very useful.

- Otherwise, where medical care services are not available or not capable to detect G-6-PD deficiencies and deleterious effects of the drug, we recommend not to use primaquine at all. Both, vivax primary clinical attack and vivax relapses, as and when they occur should be treated with a course of 10 mg/kg chloroquine-base p.o. If qualified medical care workers decide to use primaquine and are able to follow-up the clinical, toxicological and parasitological results, a daily dose of 0.25 mg/kg primaquine-base during 14 days could be administered for possible prevention of *P. vivax* relapses.

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