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Review: Improving the Therapeutic Index of 8-Aminoquinolines by the Use of Drug Combinations: Review of the Literature and Proposal for Future Investigations

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Review: Improving the Therapeutic Index of 8-Aminoquinolines by the Use of Drug Combinations: Review of the Literature and Proposal for Future Investigations

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Abstract. Because 8-aminoquinolines affect critical survival stages of *Plasmodium* parasites, treatment and control of malaria could be markedly improved by more widespread use of these drugs; however, hemolytic toxicity, which is widely prevalent in G6PD-deficient patients, severely constrains this use. Primaquine was approved more than 50 years ago after extensive clinical testing. Review of the mid-20th century literature in the light of present understanding of pharmacokinetics and metabolism suggests that manipulation of these factors might dissociate 8-aminoquinoline efficacy from toxicity and lead to an improved therapeutic index.

INTRODUCTION

The 8-aminoquinoline primaquine is a key antimalarial drug for both *Plasmodium falciparum* and *P. vivax*. Primaquine is a tissue schizontocide that kills early liver stages of *P. falciparum* and *P. vivax*, thereby providing causal prophylaxis against both infections.[1] Antischizontocidal activity can be achieved with a single dose of 30 mg primaquine within 48 hours of sporozoite inoculation. Primaquine is also the only clinical agent that kills *P. vivax* dormant stages (hypnozoites) and *P. falciparum* stage 5 gametocytes. The World Health Organization (WHO) recommendations to eliminate *P. vivax* hypnozoites and prevent *P. vivax* relapse are 0.25–0.50 mg/kg per day for 14 days (for persons with normal glucose 6 phosphate dehydrogenase [G6PD] levels).[2] The recommended regimen to kill *P. falciparum* gametocytes and prevent transmission is one dose of 0.75 mg/kg.[2]

The antigametocyte and antirelapse indications are being increasingly emphasized. For *P. falciparum* infection in Myanmar treated with standard blood schizontocidal regimens, patients were randomized to one dose of primaquine (75 mg base/kg) or no treatment.[3] The incidence of gametocytemia was 66 gametocyte-person-weeks per 1,000 weeks of follow-up in the non-primaquine group versus 5 gametocyte-person-weeks in the primaquine group. In the same study, there were 69 patients who had recurrences of *P. falciparum* infection, but 330 patients had recurrences of *P. vivax*, including 235 patients who, on the basis of light microscopy, apparently presented only with *P. falciparum*. A 2011 study on the Thai–Myanmar border confirmed that, because *P. vivax* may be unrecognized in *P. falciparum* infection, the "most commonly transmitted parasite after treatment for *falciparum* malaria, paradoxically, was not *P. falciparum*, but *P. vivax*."[4]

The use of primaquine for any of these indications is limited, however, by its hemolytic toxicity in G6PD-deficient patients. At least 200 G6PD genetic variants are known[5] and are classified according to the phenotype. Classes I–IV are, respectively, completely enzyme deficient with chronic nonspherocytic hemolytic anemia (class I), severely deficiency with < 10% of normal activity (class II), mildly/moderately deficient with 10% to 60% of normal activity (class III), and very mildly deficient or not deficient with 60% to 100% of normal activity (class IV).[6] G6PD deficiency is X-linked. The hemizygous male with the common African variant G6PD A- is typically mildly/moderately deficient with 10–60% of normal activity.[6] In contrast, the hemizygous male with G6PD Mediterranean variant would be severely affected with < 10% of normal activity.[6] WHO recommendations for primaquine dosing reflect the need to diminish or avoid the drug depending on the severity of G6PD deficiency. Although daily primaquine is recommended for patients who are G6PD normal, weekly primaquine is recommended for patients with mild to moderate G6PD deficiency, and primaquine is contraindicated for persons with severe G6PD deficiency.[2]

It is postulated that the efficacy and toxicity of 8-aminoquinolines, such as primaquine, are ultimately related to the generation of reactive oxygen intermediates.[7]

Primaquine and similar 8-aminoquinolines were developed in the 1940s to 1950s. The literature detailing this work suggests that the efficacy and toxicity of 8-aminoquinolines may be modified by coadministration of blood schizonticidal agents. Some of the agents that were used in these combinations are now known to affect enzymes that metabolize primaquine and other 8-aminoquinolines. We review this literature to suggest methods by which changing 8-aminoquinoline pharmacokinetic/pharmacodynamic properties might be attempted. The ultimate aim was to identify a partner drug that will increase the therapeutic index and thereby, mitigate the therapeutic constraints of hemolytic toxicity in G6PD-deficient patients.

REVIEW OF THE LITERATURE

8-aminoquinolines evaluated in humans. The first 8-aminoquinolines to be extensively clinically investigated were pamaquine and pentaquine. Primaquine was developed in the 1940s[7] and is the only 8-aminoquinoline that has been in clinical use since that time except for quinocide in the former Soviet Union.[8] Tafenoquine can be mentioned as a prominent clinical agent in present development.[7] For patients without the threat of hemolytic anemia caused by G6PD deficiency, primaquine dosing is limited by gastrointestinal side effects. Primaquine replaced pamaquine and pentaquine because of primaquine's superior therapeutic index with respect to radical cure versus

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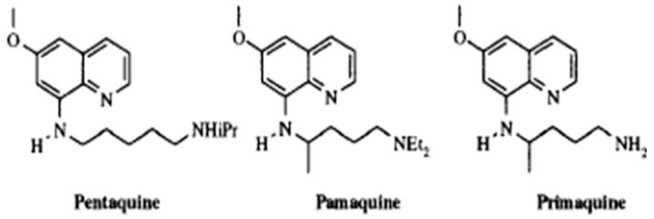

FIGURE 1. Clinical 8-aminoquinolines.

gastrointestinal adverse events.[9] The structures of pamaquine, pentaquine, and primaquine are shown in Figure 1.

Augmentation of antirelapse efficacy of 8-aminoquinolines by coadministration of blood-stage antimalarials. Clinical experiments in the 1940s and 1950s investigated the efficacy of 8-aminoquinolines alone and combined with blood schizonticidal drugs in preventing relapse (Table 1). Study subjects were challenged with the Chesson strain of *P. vivax* through the bites of infected mosquitoes. After the subject had been febrile and had parasitemia for a few days, drug was administered to eliminate blood-stage parasites. The patient was then observed for recurrence of parasites. The rationale of these experiments was as follows: if a curative dose of a blood schizonticidal drug had been administered, any recurrence of parasites constituted relapse from dormant hypnozoites in the liver. The investigators validated this approach by showing that blood stage-induced infections in human volunteers (thereby lacking liver stages) consistently and completely cleared their infections after standardized blood schizontocidal therapies without recurrences over long periods of follow-up or risk of reinfection.

Alving and others[10] treated patients with quinine alone, pentaquine alone, or the combination. In all cases, blood-stage parasites disappeared within 6 days. Subsequent parasite recurrence rates were 92% for quinine alone (data not shown), 60% for pentaquine alone, and 17% for the combination. The similarity of parasite clearance times and recurrence times between the groups led Alving and others[10] to assume that, if recurrence of parasites after quinine alone was because of relapse, recurrence of parasites in the pentaquine groups would also be because of relapse. In this case, administration of quinine with pentaquine reduced the relapse rate of pentaquine alone.

Experiments with a sequential design were performed for quinine and other 8-aminoquinolines. When quinine, which has a short half-life, was administered several days before or after an 8-aminoquinoline, the effect on liver stages was as if each drug had been separately administered. In a small experiment, all patients given pamaquine followed by quinine relapsed, whereas some patients in whom pamaquine was administered concurrently with quinine did not relapse.[11] When quinine was administered before primaquine, relapse was seen in 79% of patients, whereas only 5% relapsed when the two drugs were administered at the same time.[12] Another group of patients received the long half-life drug chloroquine (1 g) on the first day of primaquine therapy. This group also showed less relapse (26%) compared with the group in which primaquine was administered after quinine.

Modification of 8-aminoquinoline toxicity by coadministration of a blood schizonticide. The side effects of 8-aminoquinoline are gastrointestinal reactions, methemoglobinemia, and in persons with G6PD deficiency, mild to severe intravascular hemolysis.[13] Because methemoglobin formation is easily studied and quantified, the occurrence of this side effect in patients treated with 8-aminoquinolines alone can be compared with methemolgobinemia in patients treated with 8-aminoquinolines combined with blood schizonticides (Table 1).

TABLE 1
Modification of 8-aminoquinoline efficacy and toxicity by coadministered drugs

8-AQ	Dose	Coadministered drug	Dose	Number subjects/patients	Number relapse (%) or percent MetHgb (mean)	Ref.
Efficacy against *P. vivax* relapse						
Pentaquine	60 mg per day × 14 D	None	NA	5	3 (60%)	10
Pentaquine	60 mg per day × 14 D	Quinine	2 g per day × 14D	47	8 (17%)	10
Pamaquine	15 mg per day × 28 D	Quinine	2 g per day × 8 D after 8AQ	5	5 (100%)	11
Pamaquine	15 mg per day × 28 D	Quinine	2 g per day × 28 D with 8AQ	5	3 (60%)	11
Primaquine	15 mg per day × 14 D	Quinine	2 g per day × 14 D before 8AQ	19	15 (79%)	12
Primaquine	15 mg per day × 14 D	Quinine	2 g per day × 14 D with 8AQ	19	1 (5%)	12
Primaquine	15 mg per day × 14 D	Chloroquine	1 g with first day of 8-AQ	19	5 (26%)	12
Toxicity: MetHgb formation						
Pamaquine	30 mg per day × 5-7 D	None	NA	29	4.4	14
Pamaquine	30 mg per day × 5-7 D	Quinacrine	Not stated	6	12.2	14
Pentaquine	60 mg per day × 14 D	None	NA	10	4.5	15
Pentaquine	60 mg per day × 14 D	Quinacrine	5 g over 14 days	5	12.5	15
Pentaquine	60 mg per day × 14 D	Quinine	2 g per day × 14 D	82	4.3	15
Pamaquine	31.5 mg per day × 14 D	None	NA	12	4.4	16
Pamaquine	31.5 mg per day × 14 D	Quinine	2 g per day × 14 D	27	4.5	16
Primaquine	15 mg per day × 14 D	None	NA	10	6.1	16
Primaquine	15 mg per day × 14 D	Quinine	2 g per day × 14 D	33	6.1	16
Primaquine	22.5 mg per day × 14 D	None	NA	16	10.2	16
Primaquine	22.5 mg per day × 14 D	Quinine	2 g per day × 14 D	41	6.8	16
Primaquine	30 mg per day × 14 D	None	NA	24	11.2	16
Primaquine	30 mg per day × 14 D	Quinine	2 g per day × 14 D	8	8.4	16
Primaquine	60 mg per day × 14 D	None	NA	8	14.7	16
Primaquine	60 mg per day × 14 D	Quinine	2 g per day × 14 D	4	8.8	16
Primaquine	120 mg per day × 14 D	None	NA	5	20.1	16
Primaquine	120 mg per day × 14 D	Quinine	2 g per day × 14 D	6	9.9	16

Note that 8-aminoquinoline doses refer to milligrams of base. Quinine doses refer to grams of sulphate. Chloroquine dose refers to grams of base. Quinacrine dose refers to grams of the hydrochloride. 8-AQ = 8-aminoquinoline; D = day; MetHgb = methemoglobin; PQ = primaquine.

Quinacrine given with an 8-aminoquinoline increased the amount of methemoglobinemia compared with the amount that was seen when the 8-aminoquinoline alone was administered. In patients being treated with pamaquine alone or pamaquine plus quinacrine, the percent methemoglobinemia after 5–7 days of therapy was 4% for the pamaquine alone group versus 12% for the combination group.[14] When pentaquine was administered alone or with quinacrine, the percent methemoglobin also increased from 4% to 12%.[15]

In contrast, the combination of quinine and 8-aminoquinolines did not lead to an increase in percent methemoglobin. No change between the methemoglobin value for the 8-aminoquinoline alone versus the combination with quinine was seen for pentaquine[15] and pamaquine,[16] and for primaquine, combination with quinine led to an apparent decrease in methemoglobin in a dose-related fashion.[16] In the same work, chloroquine seemed to have a similar effect in malaria patients but not in normal volunteers.

We were able to find one study in which hemolysis rather than methemoglobin formation was the endpoint. Although this study used pentaquine at a high dose (120 mg/day for 14 days), it is of interest that, in four patients given this dose alone, there was a mean loss of hemoglobin of 3.2%, whereas in four patients given this dose plus quinine, the loss of hemoglobin was much diminished at 0.1%.[17]

These results may be explained on a pharmacokinetic basis (see below).

Pharmacokinetics and metabolism of the 8-aminoquinolines. *Pamaquine and pentaquine.* Sparse information is available for these old drugs. After oral dosing of pamaquine in humans, peak serum levels are achieved at approximately 3 hours, and then, they decline with a half-life of approximately 2 hours.[18] Zubrod and others[18] also commented that the "pathway of metabolism of the drug is not known. [There is e]vidence for the presence of two metabolic intermediates."[18] These two metabolites were not identified but would likely represent products of cytochrome P450 or oxidative deamination pathways. Armer and others[19] found that the activity of pentaquine against coccidian parasites was inhibited by monoamine oxidase inhibitors but not cytochrome P450 inhibitors,[19] suggesting that one of the products of this pathway may mediate the drug's antiparasitic effect. The importance of the monoamine oxidase metabolic pathway is becoming increasingly recognized for primaquine as well (see below).

Primaquine. Absorption is linear over the doses of 15–45 mg.[13] After a 45 mg dose, the time of maximum concentration is 3 hours and the maximum concentration is 153 ng/mL.[13] The mean elimination half-life of primaquine was 4–7 hours in several studies. Primaquine itself is generally believed unlikely to be the clinically active form of the drug, because it is extensively metabolized and less active than some of its metabolites or derivatives (for example, 4-hydroxy primaquine and 5-hydroxy,6-demethyl primaquine) in *in vitro* models.[20]

The predominant contributors to primaquine metabolism by human liver microsomes were cytochrome P450 (CPY) 1A2, 2B6, 2D6, and 2E1.[21] Elsewhere, primaquine is noted to inhibit CYPs 1A2, 2D6, and 3A4 and induce CYP 1A2.[22] In healthy volunteers, the maximum concentration of primaquine was increased by a mean of 23% and area under the curve (AUC) of 19% when administered with grapefruit juice,[23] suggesting inhibition of intestinal CYP450-mediated metabolism and/or interaction with transport by P-glycoprotein and organic anion-transporting polypeptides.[24]

There is a large body of evidence that primaquine is converted by oxidative deamination to the carboxylic acid metabolite carboxyprimaquine, which is the predominate species circulating in plasma after oral administration in man.[23] Monoamine oxidase (MAO) in mitochondria converts primaquine to primaquine aldehyde, which can then be converted to primaquine alcohol or primaquine carboxylic acid (carboxyprimaquine).[25] Strong evidence for the pathway primaquine—primaquine aldehyde—carboxyprimaquine was seen in *in vitro* cell models.[26] Recently, we showed MAO-A (but not MAO-B) isoenzyme-mediated metabolism of primaquine with the concurrent appearance of masses consistent with carboxyprimaquine formation (Pybus B and others, unpublished data).

It is evident that primaquine is a substrate for cytochrome P450-mediated oxidation and also monoamine oxidase-mediated metabolism. Both efficacy and hemolysis seem to depend on metabolic activation of the parent drug, but firm evidence as to which metabolites mediate efficacy and toxicity is lacking at present.

Determination of metabolic pathways that lead to efficacy is difficult, because there is no *in vitro* model of hypnozoites within liver cells with which to evaluate efficacy; however, evaluation of pathways that lead to toxicity in the sense of methemoglobin formation can be performed. Formation of methemoglobin by primaquine increased at least 10 times by coincubating red cells with human liver microsomes, and this metabolic conversion could also be accomplished by recombinant human CYPs 1A2, 3A4, 2D6, 2B6, and 2E1.[27] Inhibitors of CYPs 2B6, 2D6, and 3A4 caused significant inhibition of the methemoglobin formation mediated by the microsomes.

Transport of 8-aminoquinolines into and out of liver cells seems not to have been investigated, but quinidine is a recognized inhibitor of human P-glycoprotein and organic cation transporter 1 (quinine also inhibits this transporter) and is a substrate for organic cation transporter 2.[28] Intrahepatic or intraparasitic 8-aminoquinoline levels might be modified by changes in 8-aminoquinoline transport by coadministered drugs.

Potential for drug–drug interaction by combination partners. Quinine is metabolized largely by CYP 3A4.[21] Elsewhere, quinine is noted to be a substrate of CYPs 1A2 and 2C19 and inhibit CYPs 2C8, 2C9, 2D6, and 3A4.[29] Chloroquine is reported to be metabolized by CYPs 2C8, 2D6, and 3A4[30] and inhibit CYP 2D6.[31] Quinacrine is metabolized by CYP 3A4[32] and results in a significant decrease of CYP 2E1 activity and expression.[33]

Alterations in pharmacokinetics/metabolism of the 8-aminoquinolines by coadministered blood schizonticides. Pamaquine exposure seems to be increased by the coadministration of quinacrine.[18] In four patients, pamaquine concentrations after coadministered drug were at least six times the level before quinacrine was administered, and the half-lives approximately quadrupled. Clayman and others[16] found a dose-related decrease in primaquine concentrations with coadministration with quinine from no apparent effect at 22.5 mg to a 50% reduction at 120 mg primaquine. Edwards and others[34] showed that the conversion of primaquine to a major but inactive metabolite, carboxyprimaquine, was diminished in the presence of quinine. The AUC for carboxyprimaquine

when primaquine alone was administered was 7,533 μg/L-hour versus 3,831 μg/L-hour when primaquine plus quinine was administered.[34] However, the AUC of primaquine was unchanged,[34] suggesting a shift to a different metabolic pathway.

DISCUSSION

The antirelapse activity of pentaquine, pamaquine, and primaquine in humans was increased by coadministration of quinine, and the antirelapse activity of primaquine was increased by chloroquine. Methemoglobin formation because of pentaquine or primaquine was increased by the coadministration of quinacrine but importantly, not by quinine. The overall data suggest that efficacy and toxicity of 8-aminoquinolines are modifiable by drug coadministration. Importantly, the quinine data suggest that efficacy and toxicity can be differentially modifiable. The likelihood that these pharmacodynamic changes reflect altered 8-aminoquinoline metabolism is supported by the changes in pamaquine exposure by quinacrine and carboxyprimaquine exposure by quinine.

Although active metabolites of primaquine have not been identified, *in vitro* studies indicate metabolism of the parent compound by CYPs and monoamine oxidases, especially monoamine oxidase-A (Pybus B and others, unpublished data), and perhaps other amine oxidases as well (Walker L unpublished data). The coadministered drugs quinine, chloroquine, and quinacrine are known substrates or inhibitors of a wide range of CYPs. Interaction of these coadministered drugs with amine oxidases and transporters has not been detailed. We hypothesize that alteration of primaquine pharmacodynamics and pharmacokinetics by drug combinations is based on competition between the 8-aminoquinoline and coadministered drugs for CYPs, other metabolizing enzymes such as oxidases, and/or transporters.

We have used methemoglobin formation as a surrogate marker for red cell toxicity, because methemoglobin seems to be a consistent hallmark response to 8-aminoquinolines, and these data are readily available in many clinical studies. However, hemolysis in G6PD deficiency is the toxicity that is limiting clinical use, and data are not available on drug interactions and direct hemolytic risk. There seems to be a link between hemolysis and methemoglobin formation, both resulting from the oxidative stress elicited in erythrocytes. However, the direct mechanistic link is unclear. Most drugs that cause hemolysis in G6PD-deficient persons also generate methemoglobinemia, although nitrite causes methemoglobinemia but not hemolysis in this patient population.[35]

The key reason that little is known about hemolytic toxicity in G6PD is the lack of preclinical models and the difficulty of conducting clinical studies. The Nonhemolytic 8-Aminoquinoline Consortium is now qualifying preclinical G6PD mouse models to solve this problem. These models include two qualified mouse models: a G6PD mutant and a severe combined immunodeficiency (SCID) mouse model in which G6PD-deficient human red cells are infused into the animal (unpublished data). A monkey model in which glutathione-depleted Rhesus red cells are infused back into the animal is also under development. Human studies for evaluation of safety of primaquine and tafenoquine in G6PD patients are also ongoing and paving the way for assessing drug combinations in the clinic in the future.

Drug combinations will not be of clinical value if efficacy and toxicity are increased in tandem. The quinine data lead us to think that 8-aminoquinoline efficacy and toxicity are caused by different 8-aminoquinoline metabolites and that efficacy may be increased without increasing toxicity. To investigate this hypothesis, we intend to coadminister primaquine with quinine or chloroquine and with known clinical inducers and inhibitors of cytochromes, oxidases, and transporters in preclinical efficacy and toxicity models. If we are correct, it may be possible to increase the therapeutic index of primaquine, and ultimately tafenoquine, with coadministration of other clinical agents, and thus may optimize the treatment of malaria with 8-aminoquinolines. With the new models and human capability now available, we hope to substantially impact malaria prevention, control, and elimination.

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