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Safety of 8-aminoquinolines given to people with G6PD deficiency: protocol for systematic review of prospective studies

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ABSTRACT

Introduction: A single dose or short course of primaquine given to people infected with malaria may reduce transmission of *Plasmodium falciparum* through its effects on gametocytes. Primaquine is also known to cause haemolysis in people with variants of glucose-6-phosphate dehydrogenase (G6PD) deficiency. The objective of this systematic review was to assess the risk of adverse effects in people with G6PD deficiency given primaquine or other 8-aminoquinoline (8AQ) as a single dose or short course (less than 7 days).

Methods and analysis: We will search the following databases: Cochrane Infectious Diseases Group Specialized Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE and LILACS. Prospective cohort studies, randomised and quasi-randomised trials that evaluated 8AQs for whatever reason in adults or children with a known G6PD deficiency will be included. Two authors will independently assess each study for eligibility, risk of bias and extract data.

Ethics and dissemination: This systematic review will be published in a peer-reviewed journal. Brief reports of the review findings will be disseminated directly to the appropriate audiences and the WHO Technical Expert Group in Malaria Chemotherapy. As no primary data collection will be undertaken, no additional formal ethical assessment and informed consent are required.

Protocol registration in PROSPERO: The protocol is registered with PROSPERO, registration number CRD42013006518.

Strengths and limitations of this study

- The research team will provide an independent analysis, based on long experience in research synthesis.
- The team will evaluate the risk of bias in relation to the primary studies, and interpret the findings of the review in the light of these assessments.
- The quality of the adverse effects data in the studies may be low and prone to high risk of bias, since the reporting and description of adverse events are generally poor.

INTRODUCTION

Primaquine (PQ) has been the most commonly used 8-aminoquinoline (8AQ) antimalarial drug. Over the last 60 years, PQ has been used to treat the liver stages (hypnozoites) of *Plasmodium vivax* and *Plasmodium ovale* malaria to prevent relapses, and as a single-dose or short-course gametocytocidal drug with the goal of reducing transmission of falciparum malaria.[1] However, PQ still carries a reputation of being an 'unsafe' drug,[2] with side effects falling into three main categories[2 3]

- the drug can cause a dose-dependent acute haemolytic anaemia in individuals who have glucose-6-phosphate dehydrogenase (G6PD) deficiency[2 4–6]
- it can result in an increased level of methaemoglobin, which is usually mild and well tolerated[7]
- PQ can cause abdominal pain when taken on an empty stomach.[1 8]

G6PD deficiency is a complicated disorder (see box 1). It is common with an estimated 400 million people worldwide carrying a mutation in the G6PD gene[9–11] and also widespread in malaria endemic countries where PQ is potentially useful for malaria control and elimination.[11] The estimated proportion of individuals carrying a G6PD deficiency gene varies from 5% to as much as 33% in different parts of sub-Saharan Africa and Asia.[6 11] Malaria and G6PD deficiency share the same geographical distribution[9 11] and some authorities think that G6PD deficiency may be protective against malaria.[9 11–14]

Box 1 G6PD classification

There are many variants of glucose-6-phosphate dehydrogenase (G6PD) deficiency.[9 11] One classification of G6PD deficiency is based on enzyme activities and clinical manifestations[39]:

- Class I—severely deficient, associated with congenital non-spherocytic haemolytic anaemia;
- Class II—severely deficient (1–10% residual activity), associated with acute haemolytic anaemia;
- Class III—moderately deficient (10–60% residual activity);
- Class IV—normal activity (60–150%);
- Class V—increased activity (>150%).

Some important G6PD variants, with information about haemolysis, are:

- *G6PD A—variant* has 10–20% of the enzyme activity (Class II) and is prevalent in Africa but also occurs in other populations.[11] Primaquine sensitivity studies on 'mild' cases of A-variant individuals of African origin found that primaquine-induced haemolysis is usually self-limiting and ends a few days after stopping treatment.[11 40–42] The current WHO recommendations for adults with 'mild' G6PD deficiency are for 8 weekly 45 mg doses of primaquine.[1 29 42] In addition to preventing the severity of haemolysis, another important benefit of extending the dosing schedule is the chance for affected individuals with G6PD deficiency to have the opportunity to discontinue the primaquine before the severity of haemolysis becomes too serious.[11]
- *Mahidol variant* is important in Myanmar and Thailand and results in 5–32% normal level enzyme activity.[43] One small study that investigated the effects of primaquine for *Plasmodiumvivax* in individuals with G6PD deficiency (n=22) in Thailand who were given 15 mg primaquine for 14 days with standard chloroquine treatment.[44] No serious adverse effects were reported and no patient required transfusion. Buchachart and colleagues concluded that standard primaquine therapy would be safe in Thailand, even for those who are G6PD deficient.[44] However, although WHO recommended that primaquine should be used for *P. vivax* radical cure in this region, they also recommended prior G6PD testing.[1] Recht cautioned that the evidence base for these recommendations is relatively scanty and inconclusive.[11 31] Mahidol variants are heterogeneous, and even if a handful of studies have reported no serious adverse reactions to primaquine, these studies may not have included patients with severe G6PD variants.[11]
- *Mediterranean variant* is widely seen in the Mediterranean region and Middle East. It reduces enzyme activity to 1–10% (Class II) of normal levels, thereby causing one of the most severe forms of G6PD deficiency. In addition, primaquine-induced haemolysis in the Mediterranean variant is not self-limiting like A-variant.[11 45] WHO guidelines state that primaquine should not be given to individuals with such a severe deficiency.[1 29]

Epidemiology

G6PD deficiency has X-linked inheritance, making the deficiency more variable in women.[11 15–18] Women can be homozygous for G6PD deficiency (when a woman inherits the two deficient alleles of the G6PD gene one from eachparent) or heterozygous (when a woman inherits one normal and one abnormal gene). Men can only be normal or hemizygous for G6PD deficiency (because men have only one copy of the G6PD gene).[11 15–18] Heterozygous women have two populations of erythrocytes, one G6PD deficient and the other with normal G6PD function.[11 15–18] However, the ratio of normal to deficient cells can vary due to the phenomenon of lyonisation.[19] Lyonisation is a random process and the resulting proportions of normal and deficient cells may deviate significantly from the expected 50:50 ratio,[15 19] leading some heterozygotes to have virtually normal expression, and others with expression levels comparable with female homozygotes (ie, entirely deficient). At the population level, G6PD deficiency is more commonly expressed in men, though in populations with high frequencies of deficiency, homozygous inheritance can be common, and the prevalence of affected heterozygotes may be of public health concern.[11]

Haemolysis

Most individuals with G6PD deficiency have no clinical manifestations and remain asymptomatic until they are exposed to a haemolytic trigger, such as fava bean, PQ or a severe infection.[11] G6PD deficiency-induced haemolysis is usually characterised clinically by fatigue, back pain, anaemia and jaundice.[9 20] It can also lead to a severe clinical syndrome called blackwater fever, which is haemoglobinuria coupled with acute renal failure.[21] Although the biomedical pathway leading to PQ-induced haemolysis remains unclear, the severity of the clinical symptoms depends on the degree of enzyme deficiency (which is variant dependent), the total dose, the time course of exposure and pre-existing factors like concurrent infection, age and haemoglobin (Hb) concentration.[9 22]

Transmission

PQ kills *Plasmodium falciparum* gametocytes and appears to reduce or prevent infectiousness to mosquitoes in laboratory experiments, although direct comparative evidence for this effect is limited.[23 24] The WHO has recommended PQ for many years as a gametocytocidal drug, with the intention of reducing *P. falciparum* transmission in a community, although how widely it is actually used is not clear. The drug is usually given as a single dose. In some of the older studies, the drug was used in mass treatment campaigns alone[25] or together with a schizonticide,[26] and increasingly the partner drug for the asexual stages is an artemisinin-based combination therapy (ACT), which itself has effects on some gametocytes.[27 28] The potential reduction in transmission is important, but the drug does not benefit the individual directly.

In 2010, the WHO[1] malaria treatment guidelines made a new recommendation (box 2, additional recommendations in the second edition of the guidelines) for PQ. The guidelines recommend the addition of a single dose of PQ (0.75 mg/kg) to ACT for uncomplicated falciparum malaria as an antigametocyte medicine,

Box 2 MEDLINE search strategy

1. antimalarials
2. 8-aminoquinolone
3. primaquine
4. 1 OR 2 OR 3
5. g-6-pd
6. glucose 6 phosphate dehydrogenase
7. g6pd deficiency
8. glucosephosphate dehydrogenase deficiency
9. 5 OR 6 OR 7 OR 8
10. 4 AND 9
11. haemoly*
12. hemoly*
13. 11 OR 12
14. primaquine-sensitiv*
15. 13 OR 14
16. Primaquine*
17. sensitive*
18. 16 AND 17
19. 15 OR 18
20. 19 AND 4

particularly as a component of a pre-elimination or an elimination programme. No evidence was provided to underpin this recommendation, although annex 4 to the guidelines contains expert opinion on the topic. In 2010, 20 countries outside of Africa had already included this recommendation for single-dose PQ in their national treatment guidelines for uncomplicated *P. falciparum*,[29] although a dose of 0.5 mg/kg was sometimes recommended rather than 0.75 mg/kg.

Recent developments

The emergence of artemisinin resistance in *P. falciparum* in the Mekong area has had led to interest in PQ's potential value, potentially at lower doses, for interrupting *P. falciparum* transmission[28 30] to prevent the transmission of drug resistant strains. The threat of adverse events, however, remains in the background. A recent narrative summary of the literature on 8AQs[31] highlighted the adverse effects seen in higher doses, and also noted that in the past it had been used routinely in large malaria control programmes without apparent problems, for example, the use of quinocide and plasmocide in Russia.[32] Drawing on the published review, a WHO policy update was issued in October 2012[33] recommending that:

> In: (1) areas threatened by artemisinin resistance where single dose primaquine as a gametocytocide for *P. falciparum* malaria is not being implemented, and
>
> (2) elimination areas which have not yet adopted primaquine as a gametocytocide for *P. falciparum* malaria:
>
> A single 0.25 mg/base/kg primaquine dose should be given to all patients with parasitologically confirmed *P. falciparum* malaria on the first day of treatment in addition to an ACT, except for pregnant women and infants <1 year of age.

This new guideline was based on the results of the safety literature review,[31] the report of the evidence review group,[6] and other publications suggesting that the lower dose of 0.25 mg/kg would (1) still be effective at blocking transmission, and (2) have no adverse effects, even in those who are G6PD deficient.[28 30 34]

Independent assessments

PQ at >0.5 mg/kg (and probably lower doses) acts on gametocytes and reduces transmission of malaria from mosquitoes in individual patients. The overall predicted infectiousness, estimated by the area under the curve of gametocyte prevalence and density, is reduced by about half after a single dose.[24] There remains a debate about whether this will result in meaningful reduction in transmission on a population basis, where there may be large numbers of asymptomatic or untreated infectious persons, poor timing of PQ treatment compared to timing of gametocyte presence or short-drug half life.

The evidence on safety is less clear. The WHO guideline[33] was based on an evidence review group report[6] that summarised existing published literature, but it did not use standard synthesis approaches to critically appraise the evidence.

While the summary is a comprehensive collection of the current literature, there is a need to appraise this in the light of the quality of the data collected.[34]

Rationale for this review

The unpublished review by Recht *et al*[35] compiled published and unpublished studies on the safety of 8AQs. This review is comprehensive but did not attempt to examine the accuracy or quality of the primary studies measuring risk. The existing Cochrane review of effectiveness of PQ for reducing transmission is currently being updated and expanded to include 8AQs other than PQ. There is a need for a systematic review to evaluate the safety of PQ and other 8AQs when used at low doses (0.25 mg/kg) in persons with G6PD deficiency so that guidelines can be based on the best possible evidence.

Aim

To assess the risk of haemolysis in people with G6PD deficiency given PQ or other 8AQ as a single dose or short course (less than 7 days).

OBJECTIVES

1. To assess the incidence of haemolysis (measured using Hb/red blood cell/haematocrit (HCT)/packed cell volume (PCV)) in:
   A. patients with G6PD deficiency who receive short course (<7 days) PQ (or other 8AQ) in comparison with those who receive placebo/no intervention;

B. patients given short course (<7 days) PQ (or other 8AQ) with G6PD deficiency, in comparison with those without G6PD deficiency.

2. To explore the effect of G6PD phenotypic variations and PQ dose on the incidence of haemolysis.

METHODS AND ANALYSIS

The protocol is registered with PROSPERO, registration number CRD42013006518.[36]

Criteria for considering studies for this review

Types of studies

Randomised, quasi-randomised trials and prospective cohort studies (containing non-randomised comparisons or uncontrolled single-arm cohorts).

Retrospective case series (where patients with haemolysis are identified, and then retrospectively examined for G6PD deficiency and PQ use) will be excluded. We will exclude in vitro studies.

Types of participants

Adults or children who have been tested for G6PD deficiency, using percentage enzyme activity, genotype, rapid fluorescent spot test or any other method as reported by the authors of the primary study.

We will not include studies that exclude patients with G6PD deficiency.

Types of interventions

Single dose or short course (up to 7 days) of 8AQ.

Types of outcome measures

Main outcomes

- Measure of change of Hb, HCT or PCV.
- Measures of change in Hb plus additional evidence of haemolysis, such as clinical (haematuria, jaundice) or laboratory (bilirubin, red cell morphology) measures.

Other outcomes

- Patients requiring a blood transfusion
- Patients requiring dialysis
- All-cause mortality.

Search methods for identification of studies

We will identify all relevant trials, regardless of language or publication status (published, unpublished, in press and in progress).

Electronic searches

The following databases will be searched: Cochrane Infectious Diseases Group Specialized Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, Scopus, Web of Science; EMBASE and LILACS (see box 2 for full MEDLINE search strategy).

Conference proceedings

We will search the following conference proceedings for relevant abstracts: the MIM Pan-African Malaria Conferences and the American Society of Tropical Medicine and Hygiene (ASTMH).

Searching other resources

We will contact researchers and other experts in the field of malaria chemotherapy, including those at WHO. We will check the reference lists of all studies identified by the above methods.

Data collection and analysis

Selection of studies

Two authors will evaluate the eligibility of studies using a pretested form, working independently to scan all abstracts and obtain full text of articles. In cases of discrepancy, agreement will be reached by discussion. If further clarification is necessary we will attempt to contact the authors for more information.

Data extraction and management

Two authors will independently extract data using a data extraction form. We will extract data on study characteristics including:

- *Study design*: Methods, site, year, outcomes.
- *Participants*: Age, sex, baseline parasitaemia, baseline Hb.
- *Intervention*: Drug dose and regimen, co-interventions.
- *Safety monitoring*: G6PD test used, frequency of safety monitoring, length of follow-up.

We will calculate and report the loss to follow-up in each group by extracting the number of individuals participating and the number analysed in each treatment group for each outcome.

For dichotomous outcomes, we will record the number of participants experiencing the outcome and the number of participants in each treatment group. For continuous outcomes, we will extract the arithmetic means and SDs for each treatment group together with the numbers of participants in each group. If the data have been reported using geometric means, we will record this information and extract SDs on the log scale. If medians have been used we will also extract ranges.

Assessment of risk of bias

Two authors will independently asses the methodological quality of all studies and discuss any differences of opinion. We will use the EPOC criteria[37] to assess for bias:

- Was the allocation sequence adequately generated?
- Was the allocation adequately concealed?
- Were baseline outcome measurements similar?
- Were baseline characteristics similar?
- Were incomplete outcome data adequately addressed?
- Was knowledge of the allocated interventions adequately prevented during the study?
- Was the study free from selective outcome reporting?
- Was the study free from other risks of bias?

We will categorise these judgements as 'low' risk of bias, 'high' risk of bias or 'unclear'. Where our judgement is unclear we will attempt to contact the authors for clarification.

Assessment of adequacy of monitoring and reporting bias

Monitoring and reporting bias will be assessed using a specifically designed tool. The tool will review:

- the adequacy of laboratory monitoring: the tests conducted, the timing of the tests, the validity of the tests;
- the completeness of the reporting of the tests;
- the independence of data analysis from the study sponsor.

Dealing with missing data

The primary analysis will be a complete case analysis. If data from the trial reports are insufficient, unclear or missing, we will attempt to contact the authors for additional information.

Assessment of heterogeneity

We will assess for heterogeneity by assessing the forest plots for overlapping CIs, reporting the I^2 statistic with a level of 50% to denote moderate levels of heterogeneity and applying the χ^2 test with a p value of 0.10 to indicate statistical significance.

Data analysis

The primary analysis will group studies by study design as follows.

Randomised or quasi-randomised controlled trials:

- where G6PD deficient individuals are allocated to treatment with PQ/8AQ or without PQ/8AQ;
- where individuals with G6PD deficiency are allocated to different doses of PQ/8AQ.

Non-randomised controlled studies:

- where individuals with G6PD deficiency are treated with PQ/8AQ or without PQ/8AQ;
- where individuals with G6PD deficiency are treated with different doses of PQ/8AQ;
- where individuals with G6PD deficiency and non-deficient individuals are treated with the same dose of PQ/8AQ.

Non-randomised uncontrolled studies:

- single-arm studies where individuals with G6PD deficiency are treated with one specific PQ/8AQ dose.

Where studies report a control group, we will compare dichotomous outcomes using risk ratios and continuous outcomes using mean differences. All results will be presented with 95% CIs. In the absence of heterogeneity we will use a fixed-effect model to combine studies, and where we detect moderate heterogeneity and it is still reasonable to combine trials we will use a random-effects model.

We will assess the quality of evidence using the GRADE approach.[38]

Subgroup analysis and investigation of heterogeneity

Where there are sufficient data we will stratify our analysis by the degree of G6PD deficiency, using standard classifications based on phenotypes.

Where data allow we will also stratify the analysis by total dose (low <0.4 mg/kg/day, medium 0.4–<0.6 mg/kg/day or high ≥0.6 mg/kg/day dose).

We explore for other potential causes of heterogeneity by additional subgroup analyses by age, gender, weight, type of malaria, level of parasitaemia and intensity of malaria transmission.

Sensitivity analysis

When there are sufficient data, we will conduct sensitivity analyses to investigate the robustness of the estimates for the primary outcomes by excluding trials at high risk of bias.

We will also explore the potential effects of participant withdrawals by conducting a sensitivity analysis, where excluded trial participants are first added back into the analysis as having experienced the outcome and second as not experiencing the outcome.

ETHICS AND DISSEMINATION

This systematic review will be published in a peer-reviewed journal. It will also be presented at national and international conferences in the fields of malaria and at the Cochrane colloquium. Brief reports of the review findings will be disseminated directly to the appropriate audiences and the WHO Technical Expert Group in Malaria Chemotherapy. As no primary data collection will be undertaken, no additional formal ethical assessment and informed consent are required.

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Competing interests None.

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