Primaquine challenge study

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1 Trial Overview

Plasmodium vivax malaria is the main cause of symptomatic malarial illness outside of sub-Saharan Africa. There were an estimated 6.5 million cases of *P. vivax* malaria in 2019. The main obstacle to the elimination of *P. vivax* are the dormant liver forms – hypnozoites – which cause relapsing infections. The only widely available treatment for relapsing vivax malaria (radical cure) is the 8-aminoquinoline drug primaquine. Primaquine is not used commonly because of concerns of haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most common enzyme deficiency in humans. Practical primaquine regimens with acceptable safety in G6PD deficiency would be of substantial utility, especially in the absence of reliable G6PD testing. This study aimed to determine whether radical curative primaquine regimens (total dose circa 375 mg base) could be safely administered to healthy volunteers with hemizygous G6PD deficiency over 2-3 weeks, and to assess the comparative safety of ascending dose regimens in relation to single high dose primaquine (45 mg base).

This exploratory phase 1-type study aimed to characterise the haemolytic effect of primaquine in healthy male volunteers with G6PD deficiency. It has two parts:

1. Part 1: adaptive trial of ascending dose primaquine regimens;
2. Part 2: cohort study of single high dose primaquine (45 mg base).

In the first part volunteers are given ascending dose primaquine regimens, whereby the speed of dose escalation and the total duration was varied according to pre-specified rules and perceived safety by study investigators; in the second part volunteers were re-challenged with a single 45 mg base dose. Part 2 of the study allows us to assess the relative safety of ascending doses with respect to the currently recommended dose regimen for individuals with G6PD deficiency (45 mg weekly over 8 weeks).

1.1 Primary objective of part 1

The primary objective of part 1 is to characterise the safety and tolerability of ascending dose primaquine regimens in healthy male volunteers with hemizygous G6PD deficiency (regimens of up to 20 days).

1.2 Primary objective of part 2

The primary objective of part 2 is to characterise the safety and tolerability of single dose 45 mg base primaquine in healthy male volunteers with hemizygous G6PD deficiency.

1.3 Secondary objectives: parts 1&2

The secondary objectives are:

1. To compare haemolysis following single high dose primaquine and ascending dose primaquine regimens;
2. To characterise the predictors of haemolysis in G6PD deficiency;
3. To characterise the markers of response to haemolysis in G6PD deficiency (white count, liver, renal and bone marrow);
4. To characterise increases in methaemoglobin in G6PD deficiency following primaquine administration;
5. To determine the primaquine and carboxyprimaquine exposures in G6PD deficient individuals.

2 Study Methods

2.1 Trial design

Part 1 is a phase 1 single-centre, open label, non-randomised, adaptive trial of ascending dose primaquine regimens in male hemizygote G6PD deficient volunteers. Part 2 is a phase 1 single-centre, open label, non-randomised, single-arm, trial of high dose primaquine in male hemizygote G6PD deficient volunteers.

2.2 Sample size

There was no fixed sample size calculation for this trial. This is an intensive phase 1 type trial in healthy volunteers and sample size is determined primarily by available resources (number of volunteers who can be in the trial at any given time and the number of healthy G6PD deficient volunteers willing to take part in the study).

2.3 Framework

The primary analyses are descriptive. The goal of this phase 1 trial is to gather initial data on haemolytic responses to primaquine administration in G6PD deficiency in order to design a feasible primaquine ascending dose regimen which can be safely trialled in *P. vivax* malaria patients with G6PD deficiency. All baseline values (e.g. haemoglobin) are defined as the mean of the screening and day 0 values.

3 Statistical Principles

All volunteers who received any dose of primaquine will be included in the safety analyses. Volunteers who received fewer than 5 days of an ascending dose regimen and left the trial for reasons not related to safety (i.e. haemolysis) will not be included in the analyses looking at determinants of haemolysis. Missing haemoglobin or reticulocyte values during follow-up will not be imputed. Effect estimates will be reported as mean point estimates with 95% confidence intervals.

4 Trial population

4.1 Screening and enrolment

We will show a CONSORT diagram summarizing the number of patients screened; the reasons for exclusion; the number of enrolled in each part of the trial.

Level of withdrawal will be tabulated and cover the following aspects:

- Discontinuation of primaquine for any reason (part 1);
- Withdrawal from study follow-up (parts 1 & 2);
- Withdrawal from entire study and requests that data is not used (parts 1& 2)

4.2 Baseline characteristics

The following key baseline characteristics will be summarized:

- Age, sex and weight;
- G6PD genotype and enzyme activity concentration;
- Thalassaemia genotype (α and β) and haemoglobin E genotype;
- Haematological parameters (haemoglobin, reticulocyte count, methaemoglobin; differential white count, platelet count);
- Liver enzyme levels (AST, ALT, bilirubin) and renal function (serum creatinine).

These will be summarised by their median (range) for the continuous variables and by their total numbers for the categorical variables.

5 Analysis

This analysis plan was written after graphical visualisation of the haematological data (daily haemoglobin and reticulocyte counts) from parts 1&2 (graphs made for the Data Safety Monitoring Board during the study). This is the first detailed trial of ascending dose primaquine regimens in G6PD deficient individuals, and a priori we did not know how best to summarise the data (e.g. expected day of nadir).

5.1 Primaquine exposure definitions

Part 1 Ascending dose regimens cannot be summarised by a single quantity as they 2-dimensional: they are defined by the rate of increase in the daily doses and the durations at each dose level. Graphical visualisation of the mean daily haemoglobin in the volunteers recruited in part 1 show that the most substantial haemolysis occurred between days 5 and 10. For this reason, we will summarise each ascending dose regimen by the total cumulative dose given by day 10 (including day 10). This will be in units of mg/kg (taking into account volunteer weight).

Part 1 The single 45 mg base dose will be summarised as the mg/kg single dose of primaquine (45mg divided by the volunteer weight).

Parts 1&2 We will summarise exposure to primaquine by the total area under the concentration time curve for the carboxy metabolite. If active metabolites of primaquine are identified in future studies, then their pharmacokinetic properties will be evaluated with respect to our pre-specified measures of haemolysis (next section). In particular, the urine concentrations of the urine 5,6 orthoquinone look to be a promising candidate.

5.2 Outcome definitions

Primary outcomes (haemolysis) The extent of haemolysis in each volunteer for parts 1&2 will be quantitated as follows:

1. Maximum absolute fall in haemoglobin between day 0 and day 28 (absolute difference with respect to baseline haemoglobin);
2. Maximum relative fall in haemoglobin between day 0 and day 28 (relative change (%) with respect to baseline haemoglobin);
3. Maximum increase in total bilirubin between day 0 and day 28 (absolute difference with respect to baseline total bilirubin);
4. Maximum increase in lactate dehydrogenase (LDH) between day 0 and day 28 (absolute difference with respect to baseline LDH);
5. Mean decrease in haemoglobin per day (g/day) between days 5 and day 10 (part 1 only: estimated from a linear model fit to daily haemoglobin measurements);
6. Mean decrease in haemoglobin per day (g/day) between days 1 and day 7 (part 2 only: estimated from a linear model fit to daily haemoglobin measurements).

Endpoints 5 and 6 were chosen following graphical visualisation of the daily data.

Secondary outcomes The following endpoints will be assessed:

1. Maximum fold-change relative to baseline for AST, ALT and creatinine;
2. Maximum absolute change in haptoglobin and LDH;
3. Peak reticulocyte count;
4. Peak methaemoglobin percentage;
5. Clinically significant changes in the differential white count.

5.3 Analysis methods

Multivariable model of haemolysis For each primary haemolysis outcome we will fit a multivariable linear model with the covariates: the summary primaquine exposure; the baseline reticulocyte count; and the baseline haemoglobin. Data from parts 1&2 will be analysed separately. The primary focus will be on absolute falls in haemoglobin adjusted for baseline haemoglobin. Additional covariates will also be tested in a secondary model: baseline G6PD enzyme activity; G6PD genotype; haemoglobin type (thalassaemia and/or haemoglobin E).

Markers of response to haemolysis Correlations between the secondary endpoints and the measures of haemolysis will be assessed. Longitudinal data will be plotted showing median (IQR) values over time for the different dosing groups. Changes in methaemoglobin will be compared to expected methaemoglobin changes in G6PD normal *P. vivax* patients who received similar primaquine doses.

Pharmacokinetic analyses We will fit a one-compartment population pharmacokinetic model to the carboxy-primaquine measurements from all volunteers. This will be used to summarise the individual exposure to primaquine as the area under the time concentration curve.

Tolerability Subjects who discontinued early their primaquine will be listed and brief clinical details provided:

- Reason why stopped;
- Were they able to climb a flight of stairs;

Adverse events (AEs) These will be confined to:

- Abdominal pain
- Nausea
- Vomiting
- shortness of breath in the ward
- shortness of breath climbing a flight stairs
- fatigue
- Rises in a given biochemical test graded 2 by the CTC.

AE grades: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

The relationship between the AE and primaquine dosage will be documented.

5.4 Statistical software

All analyses will be be in done in R. Data and code will be made openly available at publication.