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TABLE OF CONTENTS

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[Intervention Protocol]

Methylene blue for treating malaria

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Editorial note: This protocol will not be progressed to the review stage as there has been no progress with the review in 30 months, and it no longer meets Cochrane's methodological standards.

ABSTRACT

Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

To assess the efficacy and safety of methylene blue for treating people with malaria.

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BACKGROUND

Description of the condition

Malaria is a vector-borne disease caused by infection with *Plasmodium* parasites, and is endemic in tropical and subtropical regions worldwide. According to the World Health Organization (WHO), approximately 91 countries are exposed to the disease, which resulted in 212 million cases (149 million to 304 million) of malaria occurred worldwide and 429,000 (235,000 to 639,000) deaths from malaria globally in 2015 (WHO 2016). Most of the severe and fatal infections are caused by *Plasmodium falciparum*, which is the most dangerous of the four *Plasmodium* species that are infective to people, and mainly affects children living in sub-Saharan Africa (WHO 2016).

If untreated, malaria can progress rapidly into severe, life-threatening manifestations, particularly if the person is infected with *P. falciparum*. Falciparum malaria is therefore a medical emergency that requires immediate treatment (White 2014).

The development of synthetic antimalarial drugs in the first half of the 20th century was one of the cornerstones for the success of malaria control programmes in various parts of the world in the 1950s and 1960s. However, re-emergence of the disease has occurred since then due to antimalarial drug resistance (Krogstad 1996).

The rediscovery of artemisinin derivatives (in traditional Chinese medicine named "qinghaosu") in the 1990s, was expected to stop the emergence of malaria in Africa and other parts of the world. These 'new' drugs were highly effective and well-tolerated. Given together with longer-acting second drugs as artemisinin-based combination therapies (ACTs), they became the first-line in malaria therapy, and contributed to a decrease in the number of malaria cases and deaths (Eastman 2009; WHO 2016). Artemisinin resistance has emerged in recent years (Dondorp 2009), and is now spreading throughout Southeast Asia (Ashley 2014). To maintain the progress of global malaria eradication efforts, new antimalarial drugs are urgently needed (malERA 2011). Moreover, limited availability of new substances is expected within the next years (Klein 2013; Wongsrichanalai 2013); therefore, it has been suggested to repurpose or to utilize drugs that are already licensed but not licensed as antimalarial drugs (Hobbs 2011). Among these drugs, which should ideally be immediately available, effective, and affordable (Olliaro 2003), methylene blue is a promising candidate.

Description of the intervention

Paul Ehrlich discovered that dyes that target certain microorganisms and leave the surrounding tissue unharmed could be used as drugs. In 1891, methylene blue was discovered to fit into this category for malaria treatment (Krafts 2012). It has high affinity for *Plasmodium* parasites and low toxicity to patients (Gensini 2007). Ehrlich's students continued to trial methylene blue, but it was not sufficiently effective to supplant the standard treatment with quinine (Krafts 2012). Since then, methylene blue has been approved for the treatment of methaemoglobinaemia, prevention of urinary tract infections in the elderly, treatment and prevention of ifosfamide-induced neurotoxicity, and intraoperative visualization of nerve tissues, endocrine glands, and fistulae (Schirmer 2003; Schirmer 2011). Methylene blue is a tricyclic phenothiazine drug with a characteristic blue colour. It is transformed to a colourless compound, leucomethylene blue, and excreted in the urine as a mixture of methylene blue and leucomethylene blue. The usual daily oral dose is 200 mg (Schirmer 2011). Methylene blue's half-life in humans is five to 10 hours (Schirmer 2011; Suwanarusk 2015). The bioavailability of methylene blue after oral administration is 72%, with peak plasma concentrations after two hours and an elimination half-life of 18 hours (Walter-Sack 2009).

When administered in high intravenous doses, severe gastrointestinal symptoms have been reported in adults (Schirmer 2011). It may also precipitate serotonin syndrome, especially when given together with serotonin reuptake inhibitors (Ng 2010). Furthermore, it can cause haemolytic anaemia in patients with glucose-6-phosphate dehydrogenase (G6PD) enzymatic deficiency (Gallo 2009). In sheep the LD50 value (lethal dose 50%, which is the dose at which 50% of a group will die) was found to be 42 mg/kg body weight (Schirmer 2011), and in rats 1250 mg/kg (Suwanarusk 2015). Contraindications include the concomitant use of serotonin reuptake inhibitors (Schirmer 2011), while patients with homozygous G6PD deficiency might require monitoring due to reduced haemoglobin level (Müller 2013).

In different animal models, the efficacy of methylene blue monotherapy was equivalent to artesunate and the combination with artesunate was synergistic (Ohrt 2014). In rodents, there was also synergistic activity of methylene blue together with quinine and pyrimethamine but not with chloroquine (Garavito 2012). In a murine model of cerebral malaria, methylene blue was effective and superior to dihydroartemisinin (Dormoi 2013). Up to now, the few clinical studies in humans have revealed promising antimalarial activity in mono- and combination therapy (Akoachere 2005; Bountogo 2010; Coulibaly 2015; Garavito 2012; Zoungrana 2008).

How the intervention might work

It has been hypothesized that methylene blue is active against *Plasmodium* parasites in various ways; with a pleiotropic effect and no unique target encoded in the parasite genome (Schirmer 2003). Possible mechanisms include increased concentrations of oxidants and toxic products targeting both erythrocytic stages and gametocytes (Adjalley 2011; Coulibaly 2009; Ohrt 2014; Schirmer 2003), and competitive inhibition in glutathione reductase sensitizing the parasite to the action of other antimalarials, such as chloroquine (Schirmer 2003), resulting in a strong gametocytocidal effect.

The gametocytocidal effect is invariably seen as beneficial because it may have a public health benefit in decreasing parasite transmission and, it is hypothesized, because targeting the transmission stages would reduce the rate at which parasite drug resistance spreads (Hastings 2006). This is why the WHO considers the gametocytocidal effect as a property with the "potential to delay or prevent the development of resistance" (WHO 2001).

Methylene blue displays in vitro activity against *P. falciparum*, synergistic activity when combined with artemisinin and related endoperoxides, but antagonistic effects with chloroquine and other quinolone antimalarials (Akoachere 2005). Similar results were detected for *Plasmodium vivax* (Suwanarusk 2015).

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It is important to highlight that methylene blue has been associated with haemolysis in G6PD-deficient subjects. G6PD deficiency can be common in some malaria-endemic areas, with associated reactions to drugs ranging from mil and transient to severe and life-threatening. However, in areas of high malaria transmission, the clinical benefit may outweigh the risk of haemolysis in patients with G6PD-deficiency, as suggested for other situations of low haemolysis risks (WHO 2015).

Why it is important to do this review

Malaria is a life-threatening disease and combating the incidence of malaria is part of the third target of the Sustainable Development Goals to end the epidemics of AIDS, tuberculosis, malaria, and neglected tropical diseases (UN 2016). This Cochrane Review aims to contribute directly to improving the management of one of the most important targets in this global effort. In light of the alarming increase in antimalarial drug resistance and cross-resistance, which includes the current first-line drugs, and the high burden of malaria worldwide, the introduction of additional effective drugs becomes a public health imperative (WHO 2016). Methylene blue is a potential candidate due to its low toxicity, pharmacokinetics, little potential of inducing resistance, and low cost (Schirmer 2003; Suwanarusk 2015). Furthermore, being a synthetic compound, methylene blue allows large-scale unlimited production regardless of supply or location of natural resources (Krafts 2012).

OBJECTIVES

To assess the efficacy and safety of methylene blue for treating people with malaria.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCTs) that evaluate the use of methylene blue for treating malaria.

Types of participants

Adults and children diagnosed with malaria by any microscopy or molecular method, or rapid diagnostic tests. We will exclude studies in which participants were diagnosed solely by clinical presentation and without laboratory confirmation.

Types of interventions

Intervention

Any scheme that includes methylene blue for treating malaria (including combination of methylene blue with other antimalarial drugs).

Control

No drug or placebo or any therapeutic regimen that does not include methylene blue. Any co-interventions should be identical in both the control and intervention groups.

Types of outcome measures

The description of these outcomes is based on that proposed by the WHO 2009.

Primary outcomes

- Clinical and parasitological treatment response, according to the criteria of the WHO's protocol for assessing and monitoring antimalarial drug efficacy (WHO 2003). We have based the outcomes approach on a previously published Cochrane Review (Zani 2014).
- Polymerase chain reaction (PCR)-unadjusted total failure: the sum of early treatment failure and late treatment failures without PCR adjustment, divided by the population in the group. In the calculation, we will include as denominators missing or indeterminate PCR; or new infections as failures.
- PCR-adjusted total failure: the sum of early treatment failures and late treatment failures due to PCR recrudescence, divided by the population in the group.

We will exclude missing or indeterminate PCR or new infections from the numerator and the denominator.

We will remove participants who do not satisfy the inclusion criteria after randomization from all calculations. A summary table of these definitions is shown in Table 1, Additional tables section.

Secondary outcomes

- Fever clearance time.
- Parasite clearance time.
- Gametocyte carriage at day seven or day 14.
- Gametocyte development (negative at baseline and positive at follow-up).
- Change in haemoglobin from baseline.

Adverse events

- Occurrence of serious adverse events: defined as fatal, life-threatening, or leading to hospitalization.
- Adverse events that lead to discontinuation of the drug.
- Occurrence of haemolysis in patients with G6PD deficiency (haemolysis defined a major haemolysis (Delta haemoglobin > 6 g/dL) and minor haemolysis (Delta haemoglobin < 2.5 g/dL (Grattagliano 2004)).
- Other haematological and biochemical adverse effects (for example, neutropenia or liver toxicity).
- Other adverse events.

Search methods for identification of studies

We will attempt to 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 using the search terms and strategy described in Appendix 1: the Cochrane Infectious Diseases Group Specialized Register; the Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE (PubMed); Embase (Elsevier); LILACS; and African Index Medicus. We will also search the WHO International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/en/) and ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home) for trials in progress, using 'methylene blue' and 'malaria' as search terms.

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Searching other resources

We will search the reference lists of included trials to find additional articles of relevance.

In addition, we will contact experts from the main regional societies, ministries of health, the Medicines for Malaria Venture, and the WHO for information about ongoing and unpublished trials.

Furthermore, we will also search relevant proceedings from 1990 onwards for trial information: the Multilateral Initiative on Malaria (MIM) Pan-African Malaria Conference, the American Society of Tropical Medicine and Hygiene Annual Meeting, the American Society of Tropical Medicine and Hygiene Annual Meetings, the ASEAN Congress of Tropical Medicine and Parasitology (ACTMP), and the International Congress on Infectious Diseases.

Data collection and analysis

We will use the Early Review Organizing Software (EROS) designed ad-hoc to select and extract data, and to assess the risk of bias of the included trials (Ciapponi 2011).

Selection of studies

Two review authors will independently screen articles identified by the literature search by title and abstract according to the inclusion criteria. We will resolve any discrepancies by consensus of the review author team. The full text of any articles that potentially fulfil the inclusion criteria will be obtained and assessed. We will list all articles excluded after full-text assessment in the 'Characteristics of excluded studies' table. We will illustrate the study selection process in a PRISMA diagram.

Data extraction and management

Two review authors will independently extract data and will assess the methodological quality of each included trial. We will resolve any discrepancies by consensus between the whole review author team.

Data will be extracted on the following: trial characteristics, trial population characteristics, details about the interventions, funding sources, and outcomes of interest. When we need more information, we will contact the trial authors. We will extract data from the published reports, including the mean and standard deviation values for continuous outcomes, and the number of events and people at risk for dichotomous data. Whenever possible, we will extract the data based on an intention-to-treat analysis.

Another important characteristic to be extracted from the included trials will be whether the participant was tested for G6PD, and if the trial authors excluded such participants.

We will contact the trial authors if necessary to obtain missing or supplementary information.

Assessment of risk of bias in included studies

Two review authors will independently assess the risk of bias of each included trial using the Cochrane 'Risk of bias' tool. We will assess the risk of bias on each of the following criteria: blinding (of participants, personnel, and outcome assessors), allocation concealment, random sequence generation, incomplete outcome data, and selective outcome reporting (Higgins 2011). Regarding the risk of bias, we will assess this as either low, high, or unclear risk (due to either lack of information or uncertainty over the potential for bias) (Atkins 2004). Any disagreements will be resolved by discussion between the review authors.

Measures of treatment effect

We will calculate mean differences (when studies use the same measure) or standardized mean difference (SMD) values (when the included trials use different measurement scales) and 95% confidence intervals (CIs) for continuous outcome measures. When necessary, effect estimates from P values, t statistics, or other available statistics will be calculated. For those trials that only provide change scores, we will perform separate analyses to trials that only provide final values. We will combine both values using the generic inverse variance method (Higgins 2011).

Unit of analysis issues

We will take into account the level at which randomization occurred. If more than one comparison from the same trial is eligible for inclusion in the same meta-analysis, we will either combine groups to create a single pair-wise comparison or appropriately reduce the sample size so that the same participants do not contribute multiply (splitting the 'shared' group into two or more groups). While the latter approach offers some solution to adjusting the precision of the comparison, it does not account for correlation arising from the same set of participants being in multiple comparisons (Higgins 2011).

Dealing with missing data

We will perform a complete-case analysis. We will avoid making assumptions about the outcomes of participants lost to follow-up. Where possible, the study authors will be contacted for missing data. If we consider that the missing data render the result uninterpretable, we will exclude the data from the meta-analysis and clearly state the reason for exclusion.

We will remove from all calculations any participants who do not satisfy the inclusion criteria after randomization.

For the PCR-unadjusted total failure outcome: we will include missing or indeterminate PCR or new infections as failures in the numerator and in the denominator.

For the PCR-adjusted total failure outcome: we will exclude missing or indeterminate PCR or new infections from the numerator and the denominator.

The potential effects of missing data will be explored through a series of sensitivity analyses.

Assessment of heterogeneity

We will calculate the I^2 statistic value as a measure of the proportion of the overall variation in the proportion that was attributable to between-study heterogeneity (Higgins 2011).

We will appraise the extent of clinical heterogeneity among the included trials by comparing the distribution of participants characteristics and study factors. Since the most likely cause of heterogeneity will be the failure rate in the control group, we will take this into consideration for this parameter. Other factors that could be associated to heterogeneity are randomization, allocation concealment, blinding of outcome assessment, loss to follow-up, treatment type, type of control group, co-interventions and

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different types of outcome measurements. We will assess these variables by subgroup analysis if the I^2 statistic value is greater than 30%. Also, we will consider a low P value for the Chi^2 test (< 0.1) as sufficient reason to explore causes of heterogeneity.

We will describe statistical heterogeneity of intervention effects by calculating the I^2 statistic and using the Chi^2 test. Thresholds for the interpretation of I^2 statistic can be misleading, since the importance of inconsistency depends on several factors. We will interpret it as follows.

- 0% to 30%: might not be important.
- 30% to 60%: may represent moderate heterogeneity.
- more than 60%: may represent substantial or considerable heterogeneity.

Assessment of reporting biases

We will make a funnel plot to assess reporting biases when performing an analysis on 10 or more studies. Several explanations may account for funnel plot asymmetry, including true heterogeneity of effect with respect to trial size, poor methodological design (and hence bias of small trials), and publication bias. Therefore we will interpret results according to these possible explanations. However, given the topic we do not expect to have this number of studies for analysis.

Data synthesis

Where we consider studies to be sufficiently homogenous in terms of participants, interventions, and outcomes, we will pool data in a meta-analysis using Review Manager 5 (RevMan 5) (RevMan 2014). Both the fixed-effect model and the random-effects model will be used and compared in order to assess the degree of statistical heterogeneity. We assume that clinical heterogeneity is very likely to impact on our review results given the nature of the interventions included; therefore we will primarily report the random-effects model results, regardless of statistical evidence for heterogeneity. We will apply DerSimonian-Laird weights for the random-effects model where we find heterogeneity between studies (DerSimonian 1986). We will calculate all effects using inverse variance methods.

For continuous data reported as change scores in some included trials and as final values in other included trials, we will analyse these data separately. Also we will combine these values using the generic inverse variance method (Higgins 2011).

Certainty of the evidence

The certainty of the evidence will be assessed using the GRADE approach. We will prepare a 'Summary of findings' table to present the results of meta-analysis or narrative synthesis, or both, for the major comparisons of the review and for the primary outcomes. We will provide a source and rationale for each assumed risk cited in the table(s), and we will use GRADEpro Guideline Development Tool (GDT) software (GRADEpro 2015). The certainty of the evidence considers the within-study risk of bias (methodological quality), the directness of the evidence, heterogeneity of the data, precision of effect estimates, and risk of publication bias based on the methods described in Chapter 11 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Schünemann 2011). If meta-analysis is not possible, we will present the results narratively in the 'Summary of findings' table.

Subgroup analysis and investigation of heterogeneity

We plan to perform the following subgroup analyses.

- Country or region.
- Comparator drug.
- Population by age range (under 18 years old, 18 to 65 years old, over 65 years old). For the age group of under18 years old, we will subdivide into under five years old and over five years old.

Sensitivity analysis

We will perform a sensitivity analysis to investigate the robustness of the results to the risk of bias components by including only the studies that concealed the allocation and losses to follow-up or had low incomplete outcome data (less than 10%). Also, we will analyse the effect of missing data by examining the following.

- Missing or indeterminate PCR are included as failures in the numerator and in the denominator for the PCR-adjusted outcome.
- New infections are included in the numerator as successes and in the denominator for the PCR-adjusted outcome.
- Exclusions after enrolment are included as failures in the numerator and denominator for both PCR-adjusted and unadjusted outcomes.
- Exclusions after enrolment are included as successes in the numerator and denominator for both PCR-adjusted and unadjusted outcomes.

We will report where the analysis alters the quantitative result. Also a sensitivity check will be conducted by using the fixed-effect model to reveal differences in results with the random-effects approach.

We will also test the robustness of results by repeating the analysis using different measures of effect size (risk ratio, odds ratio) and different statistical models (fixed-effect and random-effects models).

The PRISMA statement for reporting systematic reviews and meta-analysis of interventional studies will be followed (Liberati 2009; Moher 2010).

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REFERENCES

Additional references

Adjalley 2011

Adjalley SH, Johnston GL, Li T, Eastman RT, Ekland EH, Eappen AG, et al. Quantitative assessment of Plasmodium falciparum sexual development reveals potent transmission-blocking activity by methylene blue. *Proceedings of the National Academy of Sciences of the United States of America* 2011;**108**(47):E1214-23.

Akoachere 2005

Akoachere M, Buchholz K, Fischer E, Burhenne J, Haefeli WE, Schirmer RH, et al. In vitro assessment of methylene blue on chloroquine-sensitive and -resistant Plasmodium falciparum strains reveals synergistic action with artemisinins. *Antimicrobial Agents and Chemotherapy* 2005;**49**(11):4592-7.

Ashley 2014

Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. *New England Journal of Medicine* 2014;**371**(5):411-23.

Atkins 2004

Atkins D, Eccles M, Flottorp S, Guyatt GH, Henry D, Hill S, et al. Systems for grading the quality of evidence and the strength of recommendations I: critical appraisal of existing approaches The GRADE Working Group. *BMC Health Services Research* 2004;**4**(1):38.

Bountogo 2010

Bountogo M, Zoungrana A, Coulibaly B, Klose C, Mansmann U, Mockenhaupt FP, et al. Efficacy of methylene blue monotherapy in semi-immune adults with uncomplicated falciparum malaria: a controlled trial in Burkina Faso. *Tropical Medicine & International Health* 2010;**15**(6):713-7.

Ciapponi 2011

Ciapponi A, Glujovsky D, Bardach A, García Martí S, Comande D. EROS: a new software for early stage of systematic reviews. In: Tavares F, Leao L , editors(s). HTAi 2011 Conference; 2011 June 27-29; Rio de Janeiro, Brazil. Rio de Janeiro, Brazil: Health Technology Assessment International, 2011.

Coulibaly 2009

Coulibaly B, Zoungrana A, Mockenhaupt FP, Schirmer RH, Klose C, Mansmann U, et al. Strong gametocytocidal effect of methylene blue-based combination therapy against falciparum malaria: a randomised controlled trial. *PLOS One* 2009;**4**(5):e5318. [DOI: 10.1371/journal.pone.0005318]

Coulibaly 2015

Coulibaly B, Pritsch M, Bountogo M, Meissner PE, Nebié E, Klose C, et al. Efficacy and safety of triple combination therapy with artesunate-amodiaquine-methylene blue for falciparum malaria in children: a randomized controlled trial in Burkina Faso. *Journal of Infectious Diseases* 2015;**211**(5):689-97.

DerSimonian 1986

DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials* 1986;**7**(3):177-88.

Dondorp 2009

Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in Plasmodium falciparum malaria. *New England Journal of Medicine* 2009;**361**(5):455-67.

Dormoi 2013

Dormoi J, Briolant S, Desgrouas C, Pradines B. Efficacy of proveblue (methylene blue) in an experimental cerebral malaria murine model. *Antimicrobial Agents and Chemotherapy* 2013;**57**(7):3412-4.

Eastman 2009

Eastman RT, Fidock DA. Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews. Microbiology* 2009;**7**(12):864-74.

Gallo 2009

Gallo V, Schwarzer E, Rahlfs S, Schirmer RH, van Zwieten R, Roos D, et al. Inherited glutathione reductase deficiency and Plasmodium falciparum malaria--a case study. *PLOS One* 2009;**4**(10):e7303.

Garavito 2012

Garavito G, Bertani S, Quiliano M, Valentin A, Aldana I, Deharo E. The in vivo antimalarial activity of methylene blue combined with pyrimethamine, chloroquine and quinine. *Memórias do Instituto Oswaldo Cruz* 2012;**107**(6):820-3.

Gensini 2007

Gensini GF, Conti AA, Lippi D. The contributions of Paul Ehrlich to infectious disease. *Journal of Infection* 2007;**54**(3):221-4.

GRADEpro 2015 [Computer program]

McMaster University (developed by Evidence Prime) GRADEpro GDT. Version accessed 10 March 2017. Hamilton (ON): McMaster University (developed by Evidence Prime), 2015. Available at gradepro.org.

Grattagliano 2004

Grattagliano I, Russmann S, Palmieri VO, Jüni P, Bihl F, Portincasa P, et al. Low membrane protein sylfhydrils but not G6PD deficiency predict ribavirin-induced haemolysis in hepatitis C. *Hepatology* 2004;**39**(5):1248-55.

Hastings 2006

Hastings IM. Gametocytocidal activity in antimalarial drugs speeds the spread of drug resistance. *Tropical Medicine & International Health* 2006;**11**(8):1206-17.

Higgins 2011

Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

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Hobbs 2011

Hobbs C, Duffy P. Drugs for malaria: something old, something new, something borrowed. *F1000 Biology Reports* 2011;**3**:24.

Klein 2013

Klein EY. Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread. *International Journal of Antimicrobial Agents* 2013;**41**(4):311-7.

Krafts 2012

Krafts K, Hempelmann E, Skórska-Stania A. From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy. *Parasitology Research* 2012;**111**(1):1-6.

Krogstad 1996

Krogstad DJ. Malaria as a reemerging disease. *Epidemiologic Reviews* 1996;**18**(1):77-89.

Liberati 2009

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology* 2009;**62**(10):e1-34.

malERA 2011

malERA Consultative Group on Drugs. A research agenda for malaria eradication: drugs. *PLoS Medicine* 2011;**8**(1):e1000402.

Moher 2010

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. International Journal of Surgery 2010;**8**(5):336-41.

Müller 2013

Müller O, Mockenhaupt FP, Marks B, Meissner P, Coulibaly B, Kuhnert R, et al. Haemolysis risk in methylene blue treatment of G6PD-sufficient and G6PD-deficient West-African children with uncomplicated falciparum malaria: a synopsis of four RCTs. *Pharmacoepidemiology and Drug Safety* 2013;**22**(4):376-85.

Ng 2010

Ng BK, Cameron AJ. The role of methylene blue in serotonin syndrome: a systematic review. *Psychosomatics* 2010;**51**(3):194-200.

Ohrt 2014

Ohrt C, Li Q, Obaldia N, Im-Erbsin R, Xie L, Berman J. Efficacy of intravenous methylene blue, intravenous artesunate, and their combination in preclinical models of malaria. *Malaria Journal* 2014;**13**:415.

Olliaro 2003

Olliaro PL, Taylor WR. Antimalarial compounds: from bench to bedside. *Journal of Experimental Biology* 2003;**206**(Pt 21):3753-9.

RevMan 2014 [Computer program]

Nordic Cochrane Centre, The Cochrane Collaboration Review Manager 5 (RevMan 5). Version 5.3. Copenhagen: Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Schirmer 2003

Schirmer RH, Coulibaly B, Stich A, Scheiwein M, Merkle H, Eubel J, et al. Methylene blue as an antimalarial agent. *Redox Report* 2003;**8**(5):272-5.

Schirmer 2011

Schirmer RH, Adler H, Pickhardt M, Mandelkow E. "Lest we forget you--methylene blue...". *Neurobiology of Aging* 2011;**32**(12):2325.e7-16.

Schünemann 2011

Schünemann HJ, Oxman AD, Higgins JPT, Vist GE, Glasziou P, Guyatt GH, on behalf of the Cochrane Applicability and Recommendations Methods Group and the Cochrane Statistical Methods Group. Chapter 11: Presenting results and 'Summary of findings' tables. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Suwanarusk 2015

Suwanarusk R, Russell B, Ong A, Sriprawat K, Chu C S, PyaePhyo A, et al. Methylene blue inhibits the asexual development of vivax malaria parasites from a region of increasing chloroquine resistance. *Journal of Antimicrobial Chemotherapy* 2015;**70**(1):124-9.

UN 2016

United Nations. Global Sustainable Development Report 2016. https://sustainabledevelopment.un.org/content/documents/2328Global%20Sustainable%20development%20report%202016%20(final).pdf (accessed 15 September 2017).

Walter-Sack 2009

Walter-Sack I, Rengelshausen J, Oberwittler H, Burhenne J, Mueller O, Meissner P, et al. High absolute bioavailability of methylene blue given as an aqueous oral formulation. *European Journal of Clinical Pharmacology* 2009;**65**(2):179-89.

White 2014

White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. *Lancet* 2014;**383**(9918):723-35.

WHO 2001

World Health Organization. Antimalarial drug combination therapy. Report of a WHO Technical Consultation. 4-5 April 2001. http://apps.who.int/iris/bitstream/10665/66952/1/WHO_CDS_RBM_2001.35.pdf (accessed 1 March 2017).

WHO 2003

World Health Organization. Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. 2003. http://apps.who.int/iris/bitstream/10665/68453/1/WHO_HTM_RBM_2003.50.pdf (accessed 1 March 2017).

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WHO 2009

World Health Organization. Methods for surveillance of antimalarial drug efficacy. November 2009. www.who.int/malaria/publications/atoz/9789241597531/en/ (accessed 1 March 2017).

WHO 2015

World Health Organization. Guidelines for the treatment of malaria. Third edition. April 2015. www.who.int/malaria/publications/atoz/9789241549127/en/ (accessed 1 March 2017).

WHO 2016

World Health Organization. World Malaria Report 2016. http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1 (accessed 10 September 2017).

Wongsrichanalai 2013

Wongsrichanalai C, Sibley CH. Fighting drug-resistant Plasmodium falciparum: the challenge of artemisinin resistance. *Clinical Microbiology and Infection* 2013;**19**(10):908-16.

Zani 2014

Zani B, Gathu M, Donegan S, Olliaro PL, Sinclair D. Dihydroartemisinin-piperaquine for treating uncomplicated Plasmodium falciparum malaria. *Cochrane Database of Systematic Reviews* 2014, Issue 1. Art. No: CD010927. [DOI: 10.1002/14651858.CD010927]

Zoungrana 2008

Zoungrana A, Coulibaly B, Sié A, Walter-Sack I, Mockenhaupt FP, Kouyaté B, et al. Safety and efficacy of methylene blue combined with artesunate or amodiaquine for uncomplicated falciparum malaria: a randomized controlled trial from Burkina Faso. *PLOS One* 2008;**3**(2):e1630.

ADDITIONAL TABLES

Table 1. Primary outcomes summary

Participants	PCR-unadjusted		PCR-adjusted	
	Numerator	Denominator	Numerator	Denominator
Exclusions after enrolment	Excluded	Excluded	Excluded	Excluded
Missing or indeterminate PCR	Included as failures	Included	Excluded	Excluded
New infections	Included as failures	Included	Excluded	Excluded

APPENDICES

Appendix 1. Search strategy

Cochrane

ID Search Hits

#1 MeSH descriptor: [Methylene Blue] explode all trees

#2 Methylthioninium Chloride:ti,ab,kw (Word variations have been searched)

#3 Methylene Blue:ti,ab,kw (Word variations have been searched)

#4 Urolene Blue:ti,ab,kw (Word variations have been searched)

#5 Chromosmon:ti,ab,kw (Word variations have been searched)

#6 Swiss Blue:ti,ab,kw (Word variations have been searched)

#7 #1 or #2 or #3 or #4 or #5 or #6

#8 MeSH descriptor: [Malaria] explode all trees

#9 malaria*:ti,ab,kw (Word variations have been searched)

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#10 Paludi*:ti,ab,kw (Word variations have been searched)

#11 Remittent Fever*:ti,ab,kw (Word variations have been searched)

#12 Marsh fever*:ti,ab,kw (Word variations have been searched)

#13 blackwater fever*:ti,ab,kw (Word variations have been searched)

#14 black water fever*:ti,ab,kw (Word variations have been searched)

#15 MeSH descriptor: [Plasmodium] explode all trees

#16 plasmodi*:ti,ab,kw (Word variations have been searched)

#17 Haemamoeb*:ti,ab,kw (Word variations have been searched)

#18 #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17

#19 #7 and #18

MEDLINE (PubMed)

Search	Query
#21	Search **(#19 AND #20)**
#20	Search **(((Randomized Controlled Trial[pt] OR Controlled Clinical Trial[pt] OR Randomized Controlled Trials[Mesh] OR Random Allocation[Mesh] OR Double-Blind Method[Mesh] OR Single-Blind Method[Mesh] OR Clinical Trial[pt] OR Clinical Trials[Mesh]) OR (Clinical Trial[tw]) OR ((Singl*[tw] OR Doubl*[tw] OR Trebl*[tw] OR Tripl*[tw]) AND (Mask*[tw] OR Blind*[tw])) OR (Placebos[Mesh] OR Placebo*[tw] OR Random*[tw] OR Research Design [mh:noexp]) NOT (Animals [Mesh] OR NOT Human[Mesh]))**
#19	Search **(#7 AND #18)**
#18	Search **(#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17)**
#17	Search **Haemamoeb*[tiab]**
#16	Search **plasmodi*[tiab]**
#15	Search **Plasmodium[Mesh]**
#14	Search **black water fever*[tiab]**
#13	Search **blackwater fever*[tiab]**
#12	Search **Marsh fever*[tiab]**
#11	Search **Remittent Fever*[tiab]**
#10	Search **Paludi*[tiab]**
#9	Search **malaria*[tiab]**
#8	Search **Malaria[Mesh]**
#7	Search **(#1 OR #2 OR #3 OR #4 OR #5 OR #6)**

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(Continued)

#6	Search **Swiss Blue[tiab]**
#5	Search **Chromosmon[tiab]**
#4	Search **Urolene Blue[tiab]**
#3	Search **Methylene Blue[tiab]**
#2	Search **Methylthioninium Chloride[tiab]**
#1	Search **Methylene Blue[Mesh]**

Embase

No.	Query
#21	#19 AND #20
#20	'randomized-controlled-trial'/de OR 'randomization'/de OR 'controlled-study'/de OR 'multicenter study'/de OR 'phase-3-clinical-trial'/de OR 'phase-4-clinical-trial'/de OR 'double-blind-procedure'/de OR 'single blind-procedure'/de OR random$:ab,ti OR crossover$:ab,ti OR 'cross over $':ab,ti OR factorial$:ab,ti OR placebo$:ab,ti OR volunteer$:ab,ti OR (singl$:ab,ti OR doubl$:ab,ti OR trebl$:ab,ti OR tripl$:ab,ti AND (blind$:ab,ti OR mask$:ab,ti)) NOT ('animals'/exp NOT ('humans'/exp AND 'animals'/exp))
#19	#7 AND #18
#18	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
#17	haemamoeb*:ab,ti
#16	plasmodi*:ab,ti
#15	'plasmodium'/exp
#14	'black water fever$':ab,ti
#13	'blackwater fever$':ab,ti
#12	'marsh fever$':ab,ti
#11	'remittent fever$':ab,ti
#10	paludi*:ab,ti
#9	malaria*:ab,ti
#8	'malaria'/exp
#7	#1 OR #2 OR #3 OR #4 OR #5 OR #6
#6	'swiss blue':ab,ti

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(Continued)

#5	chromosmon:ab,ti
#4	'urolene blue':ab,ti
#3	'methylene blue':ab,ti
#2	'methylthioninium chloride':ab,ti
#1	'methylene blue'/exp

LILACS

(MH Azul de Metileno OR Chromosmon OR Cromosmon OR Impaludismo OR Maleita OR ((Methyl$ OR Metileno OR Swiss OR Metiltionina OR Suíço OR Suizo) AND (Cloruro OR Cloreto OR Chloride OR Blue OR Azul))) AND (MH Malaria OR Malaria OR Paludi$ OR Malárica OR MH Plasmodium OR plasmodi$ OR ((blackwater OR Marjales OR Remitente OR Remittent OR Mangue) AND (Febre OR Fiebre OR Fever)))

African Index Medicus

Database :

AIM

Search on :

Methyl$ [Key Word]

References found :

9 [refine]

Displaying:

1 .. 9 in format [**Detailed**]

WHAT'S NEW

Date	Event	Description
13 June 2022	Amended	This protocol will not be progressed to the review stage as there has been no progress with the review in 30 months, and it no longer meets Cochrane's methodological standards.

HISTORY

Protocol first published: Issue 10, 2017

CONTRIBUTIONS OF AUTHORS

All authors made substantial contributions to the conception and design of this protocol, drafted the protocol, and revised the content. All authors read and approved the final version of the protocol.

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DECLARATIONS OF INTEREST

MC declares no conflict of interest known.
TW declares no conflict of interest known.
MFR declares no conflict of interest known.
AC declares no conflict of interest known.

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