

Primaquine and relapses of *Plasmodium vivax*. Meta-analysis of controlled clinical trials

*Primaquina y recurrencias de malaria por Plasmodium vivax.
Metanálisis de estudios clínicos controlados*

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ABSTRACT: *Background:* Primaquine (PQ) is used against relapses of vivax malaria (RVM) but several aspects about dosage are unknown, as the total effective dose (TD) in a number of days. *Objective:* To compare PQ regimens against RVM in randomized or non-randomized controlled clinical trials (CCTs). *Methodology:* Meta-analysis. Information was sought until 31 December, 2012 in Lilacs, SciELO, PubMed (Medline), Cochrane Library, Cochrane Infectious Diseases Group, Embase. Experimental studies or CCT were used, always with concurrent control group. No matter whether or not the design was randomized, close label, supervised. It is not required that the study established difference between relapse and reinfection by molecular evidence. Inclusion and exclusion criteria for articles were applied and meet the inclusion criteria constituted adequate quality to be left in the meta-analysis. *Results:* 23 ECC with or without random allocation of treatment met the selection criteria. Include four schemes of TD (TD mg-number of days): 210-14 = 210 mg in 14 days; 210-7 = 210 mg in 7 days, 45 to 150 mg in 3 to 10 days, 0 (not PQ). If PQ is absent, recurrences occur 34.48% versus 19.66% with PQ 210-14 (significant difference), 210-14 showed effectiveness equal to that of 210-7. Treatments 210-7 and 210-14 were statistically better than 45 to 150 effectiveness. *Conclusions:* The use of PQ is necessary to reduce recurrences and TD 210 mg given at 7 or 14 days is which is more effective but more studies are required to treatment 210-7.

Keywords: Primaquine. Malaria. *Plasmodium vivax*. Recurrence. Effectiveness. Meta-Analysis.

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RESUMEN: *Antecedentes:* La primaquina (PQ) se usa contra recurrencias del paludismo vivax (RPV), pero se desconocen varios aspectos posológicos, como la dosis total (DT) eficaz en determinado número de días. *Objetivo:* Comparar regímenes de PQ contra RPV en estudios clínicos controlados (ECC) aleatorios o no aleatorios. *Metodología:* Metanálisis. Se buscó información hasta el 31 de diciembre de 2012 en Lilacs, SciELO, PubMed (Medline), *Cochrane Library*, *Cochrane Infectious Diseases Group*, *Embase*. Se usaron estudios experimentales o ECC, siempre con grupo control concurrente. Se incluyeron estudios con o sin asignación aleatoria, “close label” o “open label”, con tratamiento supervisado o no supervisado. No se exigió que el estudio estableciera diferencia entre recaída y reinfección mediante pruebas moleculares. Se aplicaron criterios de inclusión y exclusión a los artículos y satisfacer los de inclusión constituyó calidad adecuada para dejarlos en el metanálisis. *Resultados:* Se completaron 23 ECC, que reunieron los criterios de selección. Evaluamos 4 esquemas de dosis total (DT) dada en determinados días (DT mg-número de días): 210-14 = 210 mg en 14 días; 210-7 = 210 mg en 7 días; 45 a 150 mg en 3 a 10 días; 0 (no PQ). La ausencia de PQ llevó a la recurrencia de 34,48% frente a 19,66% con PQ 210-14 (diferencia significativa); 210-14 mostró eficacia igual a la de 210-7. Cada esquema 210-7 y 210-14 es mejor estadísticamente que 45 a 150. *Conclusiones:* El uso de PQ es necesario para reducir las recurrencias y la DT 210 mg dada en 7 ó en 14 días es la que mejor eficacia tiene pero se requieren más estudios con el esquema 210-7.

Palabras clave: Primaquina. Malaria. *Plasmodium vivax*. Recurrencia. Eficacia. Metanálisis.

INTRODUCTION

A unique feature of malaria by *Plasmodium vivax* and by *Plasmodium ovale* is the reappearance and recurrence of the parasitemia, with or without associated symptoms, weeks after having cured the disease with the total elimination of the blood parasites and the symptoms.

In cases of malaria by *P. vivax*, the malarial recurrence includes the resurgence and relapse and both of them are a result of the current plasmodial infection and they should be distinguish from reinfection (resulted from a new infection). The relapse is due to an asexual parasitemia resulted from liver hypnozoites of *P. vivax* and which occurs 4 to 5 weeks (usually for a limit of 4 weeks or 28 days) after the beginning of the treatment. Reinfection is an infection by another parasite clone that happens at any moment after the beginning of the treatment for the primary attack and it can only be proven through genetic analysis of the parasites, which is not yet available for the routine¹.

Primaquine (PQ) is the only antimalarial available in the routine to be administered to those suffering from *vivax* or *ovale* malaria with the objective of avoiding recurrences¹. Belonging to the group of the 8-aminoquinolines (8-AQ). There are many derivatives of 8-AQ with methoxy group in position 6 and substitutions in the side chain (radical) of position eight; the basic structure is the same with a change in the radical. It is the only 8-AQ in daily use to destroy the dormant liver stage parasites (hypnozoites) of *P. vivax*, against which it reaches maximum activity (+++). There is a slight effect (+) against the active liver stage parasites

(trophozoites, schizonts, merozoites) of *P. vivax* and *P. falciparum*. It removes gametocytes and sporozoites of *P. vivax* and *P. falciparum*, with maximum power (+++). It has a very slight action on erythrocytic asexual forms of *P. vivax* (+,-) and null (0) effect on the *P. falciparum*. The curative action (elimination of dormant liver stage parasites DLSP: hypnozoites) depends on:

- an amine group in position eight of the quinoline nucleus;
- a methoxy group in position 6.

The alkylation of the amine group in position eight with a lateral chain of five carbons and a terminal amine confers it with great power¹⁻⁶.

PQ seems more efficient when it occurs simultaneously with the blood schizonticides² and, from all antimalarials, this is the one which has the wider activity spectrum³. The PQ has proven highly useful for simple primary and secondary chemoprophylaxis⁴ or against recurrences⁵⁻⁸.

The total dosis (TD) of 210 mg given in 14 days (210-14), for an adult weighting 60 kg (0.25 mg/kg/day for 14 days), is recommended by the WHO as the standard in order to avoid recurrences⁷. Blocking the transmission of *P. vivax* through the use of drugs is achieved with the usual PQ dose, which produces, in 4 to 20 hours, the complete loss of transmission to the anopheline⁸.

The clinical and parasitary cure, which include avoiding recurrences of the infection by *P. cynomolgi* and *P. vivax* is the role of the TD of the 8-AQ^{9,10}.

There are several recent reviews on the use of PQ to avoid the recurrences^{3,11,12}, as well as meta-analysis on the theme^{5,6,13-15}. The 2 meta-analysis of Cochrane^{6,13} assessed random and semi-random controlled clinical trials (CCT) in humans who compared treatments with chloroquine only against chloroquine added with PQ, given as 210-14, and other PQ schemes. The meta-analysis by John et al.¹⁵ included 87 studies published since 1950 under regimens of PQ anti-recurrences and they were based on what was found in Medline and Cochrane Central Register of Controlled Trials. The meta-analysis by Carmona-Fonseca⁵ was based exclusively on descriptive studies. Then why to conduce this study? Because, regarding the review by Cochrane, we found 23 studies instead of 9; we analyzed 5 treatment schemes instead of 3 (0: zero mg of PQ; 75-5: 75 mg of PQ distributed into 5 days; 210-14: 210 mg of PQ distributed into 14 days); we worked with 6,350 patients instead of 3,423. In relation to the meta-analysis made by John et al.¹⁵, it included segments of < 30 days (that measure the response of the acute attack); it included 7 studies without schizonticide, the TD categories used include one which contains the standard TD used in the standard procedure (14 days) or in 7 days, along with higher TD such as 315-14 (315 mg of PQ distributed into 14 days), 420-14 (420 mg of PQ distributed into 14 days), etc, and it does not discriminate the results among these values; because it includes Prospective and Retrospective studies; because it includes many studies without control group, it is to say Descriptive and solo CCT studies, without differentiating the recurrences in relation to the kind of study.

The objective of this review was to compare the effectiveness of PQ in avoiding the recurrences of malaria *vivax*, according to which it is administered with different dosing schemes (mg/kg-days of use) and according to which random and non-random CCTs are shown.

MATERIALS AND METHODS

STUDY CLASS AND SPECIFIC QUESTIONS TO SOLVE WITH THE META-ANALYSIS

A methodology was used for the meta-analysis¹⁶. The questions to be answered were:

- Is PQ effective in preventing the recurrences of *vivax* malaria?
- Does the effectiveness of PQ in preventing the recurrences of *vivax* malaria depend on the applied TD when given a fixed time?
- Does the effectiveness of PQ in preventing the recurrences of *vivax* malaria depend on the time (number of days) in which a given TD is effective?
- Does the effectiveness of PQ in preventing recurrences of *vivax* malaria depend on the place where the infection occurs?

INFORMATION SOURCES, KEYWORDS, DATES, LANGUAGES AND PATIENTS

The information was searched for in Lilacs, SciELO, PubMed (Medline), Cochrane Library, Cochrane Infectious Diseases Group, Embase. We searched for information up to December 31, 2012, in every language. The bibliography of all written literature was used in order to search for articles.

For the search in PubMed, the following terms restricted to major topics were used (Restrict to MeSH Major Topic): “primaquine” and “malaria, *vivax*”; the filter “clinical trial” was used; the articles found were reviewed and the ones which were not CCT, comparing different PQ schemes in sick people, were discarded. Also searched in PubMed:

- review articles with “primaquine, malaria *vivax*, recurrences, human”;
- meta-analysis (primaquine, malaria *vivax*, recurrences, human).

The search in Embase used the words primaquine, malaria and *vivax*. In SciELO, SciELO Public Health and SciELO Brazil, Peru, Venezuela and Colombia the word “primaquina” was used. In Lilacs, the search was made with “*primaquina*”, “*vivax*” and “*recurrencia*”. In Cochrane Library and in Cochrane Infectious Diseases Group the search was with “*primaquina*”.

In Google Académico the words “*primaquina*”, “*recurrencia*”, “*vivax*” were searched.

INCLUSION/EXCLUSIÓN CRITERIA OF THE ARTICLES; ASSESSMENT OF THE REPORTS

The design had to be experimental or CCT, always with a control group. It did not matter if the design was random, closed/blind, supervised. Every article had to inform with clarity and precision these data in order to be considered quality appropriate:

- number of included patients, without demanding minimum sample size;
- which treatment groups were used;

- size of each group;
- PQ doses administered and in how many days, having been used as the minimum of the schemes or different doses;
- the minimum follow-up time, after day 1 of the treatment of the acute malarial episode, was 60 days;
- number, proportion or rate of recurrence in each group;
- informing if the subjects were in malarial transmission areas right after receiving treatment;
- establishing the diagnosis of malaria with thick smear (microscopy), with polymerase chain reaction or any laboratorial diagnostic test and informing that the species was *P. vivax* only, no matter the parasitemia found; i) receiving any schizontocide (usually chloroquine) in addition to PQ;
- it was not required that the study would differ relapse from reinfection through molecular testing.

The extraction of data from each article was conducted by the author who used an Excel chart with the ten dates previously stated.

STATISTICAL ANALYSIS

The incidence of recurrences was calculated according to the protocol analysis¹⁷. The comparison of proportions was analyzed with the χ^2 test of Mantel-Hanszel (χ^2 M-H) of the EpiTable module of the EpiInfo 6.0 software. The SPSS 10.0 software was used in order to compare measures of central tendencies between groups, using the nonparametric test of Kruskal-Wallis (K-W) for independent groups.

The meta-analysis was made with the program of Joaquín Primo¹⁶, with the random effects model.

It was considered as statistically significant a probability of $p < 0.05$.

ETHICAL ASPECTS

The investigation project did not require ethical endorsement.

RESULTS

In PubMed, 33 writings were collected (“Primaquine”[Majr] AND “Malaria, Vivax”[Majr] and “clinical trial” as filter), but only 14 of them were actually CCT on the comparison of PQ doses in treated patients with vivax malaria. In Embase, the search with the bibliographic

list of other publications allowed us to find 12 other studies, but these writings did not refer to the theme, or they would not fulfill the inclusion criteria or were new in relation to PubMed. In SciELO Public Health (Subject: Ciencias de la salud (List of journal by subjects) it was searched in “All indexes” and “Entire collection” and 5 articles were found, but none of them were CCT. In SciELO Brazil Peru, Venezuela and Colombia no references on CCT of PQ were found. In Lilacs, four references were found, though only one was CCT. In Cochrane Library there were six references and only two on recurrences of vivax malaria^{6,13}. In Cochrane Infectious Diseases Group, we found the same as in Cochrane Library. In PubMed we found review articles and meta-analysis ones; in both cases, many references were obtained^{5,6,13-15}. The search in Scholar Google yielded 94 results; the titles in the writings were compared to the ones obtained in all other sources, but it resulted in no new reference which would meet the inclusion criteria.

In short, there were 23 CCTs with or without random treatment allocation, which met the selection criteria¹⁸⁻⁴⁰: from PubMed 14 references were included, from Lilacs 1 and with the system’s search procedures (search in physical archives of the *Biblioteca Médica-Universidad de Antioquia*, n = 4, articles from the author’s library and the ones obtained before with other objectives, n = 4) 8 were obtained. The studies were published in a lapse of 34 years, between 1978 and 2012: 23% between 1978 and 1999; 58% between 2000 and 2009; 19% between 2010 and 2012. The 23 studies assessed a total of 12 schemes (TD-days in which they were applied) of PQ.

The PQ schemes may be analyzed under different perspectives:

- according to the daily dose used (mg/kg/day) during a certain number of days (1 to 14 days);
- according to the TD given in a certain number of days, i.e., the daily doses administered in “X” number of days;
- a given amount of mg/kg/day once/week for a determined number of weeks.

The relations between daily doses (mg/kg/day) and TD in “X” days are:

- 0.25 mg/kg/day for 14 days: it the standard procedure, here called 210-14, referring to what in an adult weighting 60 kg corresponds to 210 mg of TD in 14 days;
- 0.50 mg/kg/day for 7 days: is the standard TD but administered in 7 days; scheme 210-7;
- 1.17 mg/mg/day for 3 days: the standard TD given in 3 days; scheme 210-3;
- 0.25 mg/kg/day for 3, 5, 7, 9 or 10 days: each one corresponds to the same daily doses of the standard scheme but with lower TD than the standard one, supplying between 45 and 150 mg of TD in 3 to 10 days (including here a study which administered 45 mg in a single dose (meaning, in 1 day); scheme 45 to 150 (in 3 to 10), noting that here it is included a study which gave 150 in 5 days, i.e., 30mg per day;
- 0.75 mg/kg/day for 1 day a week and for 8 weeks²⁶ (a report): equivalent to 360 mg of TD, which is 71% higher than the standard TD (210 mg) but with very low daily doses [6,4 mg/day instead of 15 mg/day in an adult of 60 kg]); is included in the group 210-14;

- 0.375 mg/kg/day for 14 days: $0.375 \text{ mg} \times 60 \text{ kg} \times 14 \text{ days} = 315 \text{ mg}$ of TD. Equivalent to 1.5 times (50% more) the standard TD;
- 0.50 mg/kg/day for 14 days: $0.50 \text{ mg} \times 60 \text{ kg} \times 14 = 420 \text{ mg}$ of TD. Corresponding to twice the standard TD;
- 0.25 mg/kg/day for 3 to 10 days: $0.25 \text{ mg} \times 60 = 15 \text{ mg} \times 3 \text{ days} = 45 \text{ mg}$ of TD; if they are 4, 5, etc. days, corresponding to 60, 75, etc. mg of TD. Here we include a study which administered 150 mg of TD in 5 days, i.e., 30 mg/day instead of the standard 15 mg/day. They are all lower TDs than the standard ones. The TD 150-10, 150-5, 135-9, 105-7, 75-5 and 45-3 group in a category called 45 to 150 in 3 to 10 days;
- did not use PQ: TD 0 mg.

We proceeded into grouping the studies according to their objectives (to compare the anti-recurrence effectiveness of TDs of PQ given a fixed amount of time). The result were these 4 schemes of treatment in relation to the TD of PQ and the days in which this TD was used TD: 210 mg in 14 days; 210 mg in 7 days; 45 to 150 mg in 3 to 10 days; and 0 mg (not used). Among these 4 schemes, 5 comparisons are made:

- 210-14 *versus* 210-7;
- 210-14 *versus* 45 to 150-3 to 10;
- 210-14 *versus* 0;
- 210-7 *versus* 45 to 150-3 to 10; y
- 45 to 150-3 to 10 *versus* 0.

The scheme 210-3 was observed in 2 studies²²⁻²³ and are included in the general analysis but not in the meta-analysis because each one of the studies do not have a pair to compare to. The studies with TD of 315 (1 study) and 420 (2 studies) given in 14 days group up with the ones of 210 in 14 days.

The number of patients per PA scheme was as follows: with 210-14 there were 1,587 patients; with 210-7 there were 232; with 210-3 there were 101; with 45 to 150 in 3 to 10 days there were 2,380; with 0 (without PQ) there were 2,151 patients. In total, 6,451 patients were evaluated.

Children and adults were evaluated and dosed according to their body weight. The standard dose corresponds to 0.25 mg/day for a weight of 60 kg, implying in 15 mg/day. When it comes to children, the amount is lower, but in this work, they are similar to the ones of adults when using the same expected TD. Thus, expressions such as 210x14 refer to studies with children and adults who have always had the standard TD based in 0.25 mg/kg/day.

The total number of patients included in the analysis of the 23 articles is 6,451, divided into: 1587 in 210-14, 232 in 210-7, 101 in 210-3, 2380 with 45 to 150 and 2151 in PQ = 0.

The studies were carried out in 12 different countries, from 3 continents: America: 7 countries (Brazil, Colombia, Costa Rica, El Salvador, Mexico, Nicaragua, Peru), Africa: 1 country (Ethiopia), Asia: 4 countries (India, Pakistan, Thailand, China) (Table 1).

The follow-up time varied from 2 and 15 months, with an average of 6.7 months. The follow-up with less than 4 months was observed in 24% of the evaluations; the 4-month

Table 1. Clinical controlled studies included in the review about the effectiveness of primaquine regimens in order to prevent recurrences of malaria by *P. vivax*.

Author, year of publication	Country of the study	Months of follow-up	PQ scheme (TD-days)*				
			210-14	210-7	210-3	45 to 150-3 to 10	0
Da Silva, 2003	Brazil	6		91		97	
Abdon, 2001	Brazil (Belem, Para)	6	40	39		40	
Pinto, 1998	Brazil (Para)	3		39		105	
Villalobos, 2000	Brazil (Rondonia)	2	31			30	
Solari, 2000	Peru	2	28	28			
Álvarez, 2006	Colombia	6	64			127	
Carmona, 2009	Colombia	4	66		63**		
Carmona, 2010	Colombia	4		35	38**		
Bergonzolli, 2000	C. Rica/Nicaragua	6	32			95	
Cedillos, 2012	El Salvador	7.5				147	144
Gomez, 1965	Mexico	9	363			389	
Yeshiwondim, 2010	Ethiopia	5	132				108
Adak, 2001	India	12				220	224
Gogtay, 1999	India (Bombay)	6	63			62	60
Rajgor, 2003	India (Bombay)	6	103				101
Kim, 2012	India (Calcutta)	15	42			59	50
Yadav, 2002	India (Orissa)	12				759	723
Leslie, 2008	Pakistan	11	55				71
Leslie, 2004	Pakistan (Afghan)	9	383				212
Rowland, 1999	Pakistan (Afghan)	12	100			250	350
Pukrittayakamee, 1994	Thailand (Bangkok)	3	18				21
Walsh, 2004	Thailand (Bangkok)	2	12				10
Li, 1999	China (Yunnan)	9	55				77
Total		6.8 [#]	1587	232	101	2380	2151

*In Table 2 there are information on the exposed and the recurrences in each treatment, in each country; **These were reviewed but they did not enter the meta-analysis' calculations because each one of them does not have a pair to compare to; [#]Mean follow-up: 6.8 months (median: 6.0). Minimum: 2, maximum: 15 months.

one in 8% of them, the one of 5 months in 4%, 6 months in 28%, 8 months in 4%, 9 months in 12%, 11 months in 4%, 12 months in 12% and 15 months in 4%. It is possible that “long” length follow-ups, when remaining in endemic areas, may have generated new infections, which did not differ from true relapses.

There is a significant difference in the follow-up months in relation to the place (grouped in 5 places: East Africa, Asia, Central America-Mexico, India-Pakistan, South America): (p (Kruskal-Wallis) = 0.007): the shortest follow-up is in Asia (4.13 months) and the longer one in India-Pakistan with 10.38 months.

For the 210-14 scheme, the proportion of recurrences is “high” with 4 months (19.00%) or with 9 months (17.11%) of follow-up and it is “low” with 5 months (3.03%) or with 11 months (1.82%), with 2 months it is 12.86% and with 3 months it is 9.57%. There is no correlation between the percentage of recurrences and the months of follow-up: $\rho = 0.066$; $p = 0.788$). Something similar happens with TD 0 of PQ, but TD 45 to 150 shows evident stability in the proportion of recurrences in relation to time of follow-up: the proportion varies between 16.19 and 28.16% for 2 to 15 months of follow-up, but, also, if the value 16.19% is discarded, the range is between 21.68 and 28.16% for 6 to 15 months.

RECURRENCE ACCORDING TO THE TREATMENTS

The comparison by meta-analysis (model of random effects) of the 5 treatment schemes of treatment with PQ (Table 2; Figure 1) shows the following:

- the schemes 210-14 and 210-7 (2 studies) have equal anti-recurrence effectiveness, with 5.88 and 5.97% of recurrences in each one of them, in this same order ($p = 0.960$ for RR of DerSimonian-Laird);
- the schemes 210-14 and 45 to 150 in 3 to 10 days (7 studies) allow very different recurrence proportions: 13.52 and 28.24% (reason: 2.1), respectively, and there is a significant statistical difference between them ($p = 0.019$);
- the schemes 210-7 and 45 to 150 (3 studies) cause 6.19 and 18.01% of recurrence (reason: 2.91), with significant difference ($p = 0.001$);
- the schemes 210-14 and 0 (9 studies) associate to recurrence of 19.66 and 34.48%, in this order (reason: 1.4), with no significant difference ($p = 0.020$);
- the schemes 45 to 150 and 0 (5 studies) produce 20.20 and 29.40% recurrences (reason: 1.5), without significant difference ($p = 0.228$).

In short:

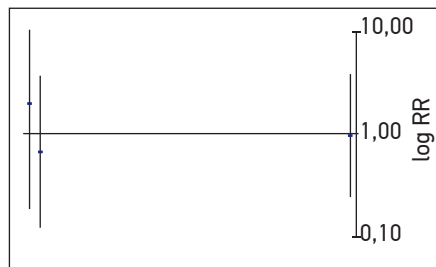
- it is not completely strange that 210-14 and 210-7 are similar, since they have the same TD though with very different number of days;
- the RR is statistically significant in the cases of schemes 210-14 *versus* 45 to 150 ($p = 0.019$), 210-0 ($p = 0.020$) and 210-7 *versus* 45 to 150 ($p = 0.001$). It should be noted that when controlling the heterogeneity of the comparison 210-14 *versus* 45 to 150

Table 2. Five comparisons of four schemes of primaquine administration in controlled clinical trials.

Country	Treatment-1		Treatment-2		Weight (%)	RR**	95%CI	p-value
	Recurrences	Exposed	Recurrences	Exposed				
	210-14 ^a	5.88%*	210-7 ^b	5.97%*				
Peru ³¹	2	28	3	28	54.5	0.67	0.12 – 3.69	0.65692
Brazil ¹⁸	2	40	1	39	65.5	1.95	0.18 – 20.64	0.58564
Total	4	68	4	67	RRdl	0.96	0.24 – 3.86	0.95976
	210-14 ^a	11.58%*	45 to 150-3 to 10 ^c	23.10%*				
India ²⁵	1	63	16	62	13.0	0.06	0.01 – 0.45	0.00683
India ³⁹	16	42	16	59	26.3	1.40	0.80 – 2.48	0.32276
Brazil ³²	2	31	8	30	17.4	0.24	0.06 – 1.05	0.07290
Brazil ¹⁸	2	40	8	40	17.2	0.25	0.06 – 1.11	0.07951
Colombia ²⁰	12	68	26	71	26.1	0.48	0.27 – 0.88	0.03598
C. Rica-Nicaragua ²¹	4	32	4	31	15.8	0.97	0.27 – 3.54	0.96422
Mexico ³⁶	37	363	80	389	23.2	0.50	0.34 – 0.71	0.00040
Total	74	639	158	682	RRdl	0.50	0.28 – 0.90	0.01953
	210-14 ^a	19.66%*	0 ^d	34.48%*				
India ²⁵	1	63	7	60	6.6	0.14	0.02 – 1.07	0.06215
India ³⁹	16	42	15	50	13.9	1.27	0.72 – 2.25	0.49985
India ⁴⁰	6	103	13	101	12.1	0.45	0.18 – 1.14	0.10834
Pakistan ³⁰	32	100	178	350	14.4	0.63	0.46 – 0.85	0.00721
Pakistan ²⁷	74	100	86	212	15.1	1.82	1.49 – 2.23	0.00002
Pakistan ²⁶	1	55	22	71	7.0	0.06	0.01 – 0.42	0.00546
Thailand ³³	3	25	8	10	11.1	0.15	0.05 – 0.45	0.00841
Thailand ²⁹	2	87	4	30	8.4	0.17	0.03 – 0.89	0.04541
Ethiopia ³⁸	4	132	9	108	10.9	0.36	0.12 – 1.15	0.09306
Total	139	707	342	992	RRdl	0.43	0.21 – 0.88	0.01995
	210-7 ^b	6.19%*	45 to 150-3 to 10 ^c	18.01%*				
Brazil ²⁴	8	116	22	116	63.6	0.36	0.17 – 0.78	0.01428
Brazil ¹⁸	1	39	8	40	9.1	0.13	0.02 – 0.98	0.05272
Brazil ²⁸	3	39	17	105	27.3	0.48	0.15 – 1.53	0.22490
Total	12	194	47	261	RRdl	0.36	0.19 – 0.66	0.00094
	45 to 150-3 to 10 ^c	20.20%*	0 ^d	29.40%*				
El Salvador ³⁵	35	147	60	144	17.9	0.57	0.40 – 0.81	0.00861
Pakistan ³⁰	128	250	178	350	20.3	1.01	0.86 – 1.18	0.95167
India ²⁵	16	62	7	60	10.7	2.21	0.98 – 4.99	0.08019
India ¹⁹	59	220	134	224	19.4	0.45	0.35 – 0.57	0.00000
India ³⁴	49	759	62	723	17.7	0.75	0.52 – 1.08	0.13782
India ³⁹	16	59	15	50	13.9	0.90	0.50 – 1.64	0.78124
Total	303	1457	456	1551	RRdl	0.79	0.54 – 1.16	0.22791

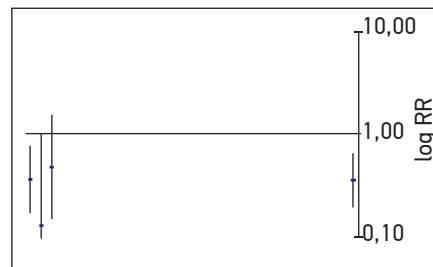
^a0.25 mg/kg/day for 14 days; ^b0.50 mg/kg/day for 7 days; ^c0.25 mg/kg/day for 3, 5, 10 days; ^d0 mg/kg/day for 0 days; *Percentage of recurrence in each PQ scheme: (total Recurrences/total Exposed)x100; **RR: relative risk = (%recurrences Treatment-1)/(%recurrences Treatment-2); RR < 1, Treatment-1 has less recurrences than Treatment-2; RR > 1, the contrary; RR = 1, both treatments have the same recurrences. RRdl: relative risk for DerSimonian-Laird; 95%CI: 95% confidence interval.

210-14 versus 210-7



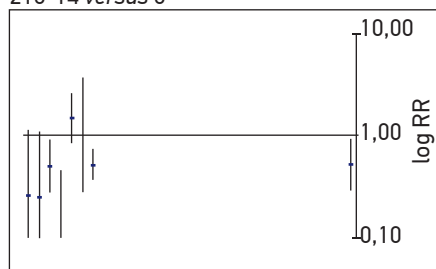
RR (DerSimonian-Laird) = 0.96
 95%CI 0.24 – 3.86; p = 0.95976
 No difference.

210-14 versus 45 a 150-3 a 10



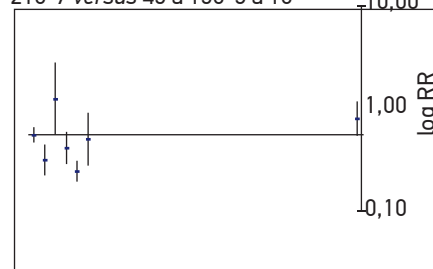
RR (DerSimonian-Laird) = 0.50
 95%CI 0.28 – 0.90; p = 0.01953
 Better: 210-14.

210-14 versus 0



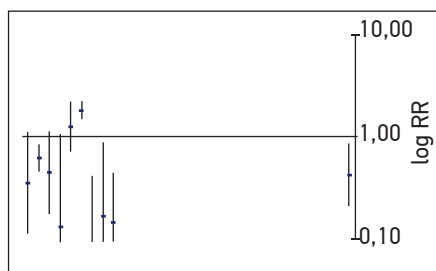
RR (DerSimonian-Laird) = 0.43
 95%CI 0.21 – 0.88; p = 0.01995
 Better: 210-14

210-7 versus 45 a 150-3 a 10



RR (DerSimonian-Laird) = 0.36
 95%CI 0.19 – 0.66; p = 0.00094
 Better: 210-7

45 a 150-3 a 10 versus 0



RR (DerSimonian-Laird) = 0.79
 95%CI 0.54 – 1.16; p = 0.22791
 Equals

Brief:
 The TD 210-14 and 210-7 statistically have the same capacity to prevent recurrences. Both TD are better than 45 to 150-3 to 10 and than using no PQ.
 The TD 45 to 150-3 to 10 have the same capacity of avoiding recurrences as not using PQ.

Figure 1. Forest's Graphics (random effects model).

- (see below), a significant difference between the 2 schemes is kept, always in favor of 210-14;
- the absence of difference between 45 to 150 *versus* 0 exists for before and after controlling heterogeneity (see below).

It is observed, finally, that the standard TD 210 mg in 3 days²³ lacked utility (53.85%) when compared to this same doses given in 7 or 14 days (14.71%), although the high daily doses were well tolerated.

The influence of the place (country) was evaluated as follows: within each PQ scheme all studies of each country are grouped and the proportions of the countries compared (Table 3). Within each one of the schemes using PQ (210-14, 201-7, 45 to 150) there is a significant difference due to the place (country) in the proportion of presented recurrence in 2 months of follow-up. Also, if PQ is not used, there is a significant difference between the proportions of recurrence by country.

Another important aspect related to the place (country) and the PQ schemes is that, in each scheme, the proportion of recurrences varies notoriously, even if PQ is not used. In 210-14 the lower proportion is observed in Ethiopia (3.03%) and Thailand (4.46%) and the higher one is in Pakistan (41.96%), which is a value at around 13 times the one in Ethiopia and Thailand. It should be noticed that in Brazil, Peru and Mexico there is a lower proportion than in India, a country usually pointed out as one of very few recurrences, while Colombia is the country in America with the highest recurrence proportion under the 210-14 scheme, which is standard. What happened in Pakistan is completely out of what was expected for any of the sites. In Thailand, the risk of recurrence is 3 times lower than in Colombia (17.74 *versus* 4.46%: $17.74/4.46 = 4.00$), whereas it is 0.26 times lower than in Brazil and 1.5 times lower than in India.

The scheme 45 to 150 (including 75-5), the official one in India, presents the lowest values in this country (12.73%) and in Costa Rica and Nicaragua (12.90%), with 51.12% for Pakistan. The value 12,73% for India with 45 to 150 is very similar to 11.06% with 210-14. With this scheme, the recurrence in India is the same as in Costa Rica and Nicaragua, and it is 0.5 times lower than in Brazil.

If PQ is not used (PQ = 0), the lowest proportion of recurrences is found in Ethiopia (8.33%) and the highest, once again, in Pakistan (45.18%).

India has 5 studies and their analysis in relation to the 3 schemes used it the following: 210-14 has recurrences of 10.58% (22/208); 75-5 of 12.73% (140/1100) and 0 of 19.95% (231/1158) ($p = 0.000$). The comparison of these proportions by pairs indicates that 210-14 does not differ from 75-5 ($p(\chi^2) = 0.388$) but it does from PQ = 0 ($p(\chi^2) = 0.003$); also, 75-5 differs from 0 ($p(\chi^2) = 0.000$). In short, in India, the three schemes behave differently, being similar in anti-recurrence effectiveness 210-14 and 75-0 and both of them are better than not using PQ.

Table 3. Proportion of recurrence by place (country) under the same primaquine scheme.

Position	Country	Exposed	Recurrences (%)	Reason: Maximum/Country*	RR**
	210-14				
1	Brazil	71	5.63	7.45	0.1343
2	Peru	28	7.14	5.87	0.1702
3	Colombia	124	17.74	2.37	0.4228
4	C. Rica/Nicaragua	32	12.5	3.36	0.2979
5	Mexico	363	10.19	4.12	0.2429
6	Ethiopia	132	3.03	13.85	0.0722
7	India	208	11.06	3.79	0.2635
8	Pakistan	255	41.96	1	1
9	Thailand	112	4.46	9.41	0.1063
	$p(\chi^2) = 0.000001$	1325	15.70#	2.67	0.3741
	210-7				
1	Brazil	222	6.76	3.38	0.2957
2	Colombia	35	22.86	1.00	1
	$p(\chi^2) = 0.00539$	257	8.95#	2.55	0.3915
	45 to 150-3 to 10				
1	Brazil	291	18.9	2.7	0.3697
2	Colombia	71	36.62	1.4	0.7163
3	C. Rica/Nicaragua	31	12.9	3.96	0.2524
4	El Salvador	147	23.81	2.15	0.4658
5	Mexico	389	20.57	2.49	0.4023
6	India	1100	12.73	4.02	0.2490
7	Pakistan	250	51.12	1	1
	$p(\chi^2) = 0.000001$	2279	20.54#	1.78	0.4017
	PQ = 0				
1	El Salvador	144	41.67	1.08	0.9222
2	Ethiopia	108	8.33	5.42	0.1844
3	India	1148	20.12	2.25	0.4454
4	Pakistan	633	45.18	1	1
5	Thailand	40	30.00	1.51	0.6640
	$p(\chi^2) = 0.000001$	2073	28.85#	1.57	0.6385

*Indicates how many times the proportion of recurrences of a country is contained in the maximum value observed for the countries. **RR: relative risk = the proportion of recurrences in a country with higher proportion (maximum), taken as not exposed to PQ, divided by the proportion of each country. It indicates the lowest risk there is in each country in relation to the maximum (always, the RR value of a specific country is lower than 1). #The values of these lines are mean measured for this scheme.

HETEROGENEITY STUDY

- The evaluation of the heterogeneity of the studies indicates that the groups arranged for comparison have studies with little heterogeneity in the case of 210-14 *versus* 210-7 and 210-7 *versus* 45 to 150, though it is high in other cases: 210-14 *versus* 45 to 150; 210-14 *versus* 0; 45 to 150 *versus* 0. Thus, its conformation seems adequate in 2 comparisons and inadequate in 3 of those (Table 4). The problem of high heterogeneity in these comparisons is solved by eliminating one or 2 studies from each group, that is making a subgroup analysis which only combines studies that fulfill certain conditions or characteristics, in a way that those are more homogeneous, so: 210-14 *versus* 45 to 150: eliminates the study by Kim³⁹, in India, the only one in 7 to demonstrate RR higher than 1.00. The RR becomes significant for the comparison of 6 studies (RR = 0.43; 95%CI 0.28 – 0.66; p = 0.000);
- 210-14 *versus* 0: studies of Kim³⁹ and Leslie²⁷ are eliminated, the first one already commented and the second one carried out in Pakistan; the RR of those were 1.27 and 1.82, while in the other 7 studies the RR was < 0.640. The RR was kept significant for the comparison of 5 studies (RR = 0.24; 95%CI 0.13 – 0.42; p = 0.000);
- 45 to 150 *versus* 0: the studies by Gogtay²⁵, Rowland³⁰, in Pakistan, and Adak¹⁹, in India, are removed, with extreme RR in the group of 6 studies: 2.21; 1.01 and 0.45;

Table 4. Assessment on the heterogeneity of the studies (according to Primo¹⁶).

Comparison	Q	k	p(Q)	H	I ² (%)	95%CI (I ²)	I ²	H
				$\sqrt{[Q/(k-1)]}$	$100\%[Q - (k-1)]/Q$		$(H^2-1)/H^2$ †	
210-14 <i>versus</i> 210-7	0.52	2	0.46985	0.7211	91	just 1 gl	0.99	Low
210-14 <i>versus</i> 45 to 150-3 to 10	19.3	7	0.00365	17.944	69	32 – 86	0.99	High*
210-14 <i>versus</i> 0	88.8	9	0.00000	33.185	91	85 – 95	0.99	High*
210-7 <i>versus</i> 45 to 150-3 to 10	1.22	3	0.54265	0.7810	-64	0 – 83	1.02	Low
45 to 150-3 to 10 <i>versus</i> 0	39.9	6	0.00000	28.238	87	75 – 94	0.99	High*

Q of Cochran = χ^2 with k-1 degrees of freedom (gl); k: number of studies included in the analysis; p(Q): probability associated to Q; H of DerSimonian-Laird = $\sqrt{[Q/(k-1)]}$; I² = $100\%[Q - (k-1)]/Q$; Relation between H and I² = $(H^2 - 1)/H^2$; H: Heterogeneity (H < 1.2: low; H = 1.2 to 1.5: moderated; H > 1.5: high); *The heterogeneity is eliminated by excluding 1-2 studies in each group, so: 210-14 *versus* 45 to 150-3 to 10: eliminates the study by Kim³⁹. Keeps significant (RR = 0.43; 95%CI 0.28 – 0.66; p = 0.00013) - now, H = 1.19; 210-14 *versus* 0: eliminates the studies by Kim³⁹ and Leslie²⁷. Becomes more significant (RR = 0.28; 95%CI 0.15 – 0.55; p = 0.00018) - now, H = 1.61; 45 to 150 *versus* 0: eliminates the studies by Gogtay²⁵, Rowland³⁰ and Adak¹⁹. Keeps significant (RR = 0.69; 95%CI 0.54–0.87; p = 0.00231) - Now, H = 1.04.

the 3 studies have RR between 0.57 and 0.90 and the RR is kept significant for the comparison of 5 studies (RR = 0.69; 95%CI 0.54 – 0.87; p = 0.002).

QUESTIONS AND ANSWERS ON THE EFFECTIVENESS OF PRIMAQUINE AGAINST RECURRENCES OF *P. VIVAX*

In a brief way and based in the findings described, the questions asked for this systematic review may be answered as follows:

- Is PQ effective? Answer: yes. Indeed, the CCT in humans there is a clear benefit when comparing the schemes 210-14 and 201-7 to not using PQ or using it in lower doses than 210, which is the standard TD.
- Does the effectiveness of PQ depend on the applied TD when given a fixed time? Answer: yes. The comparison of TD in CCT in humans (Table 2) showed that the standard TD of 210, given in 7 or in 14 days, is associated to recurrence proportions of 8.88% (gross average between 6.19 and 11.58%) against 20.54% to 45 to 150 (gross average between 18.01 and 23.07%) and to 29.01% for TD 0 of PQ (gross average between 27.91 and 30.11%). It is clear that the higher the TD, the lower the recurrences. Yes, outside the meta-analysis procedure, we obtained the average of the proportion of recurrences in each treatment, the averages being 13.80 for 210-14 (219/1,587); 9.48% for 210-7 (22/232); 21.18% for 45 to 150 (204/2,380) and 30.68% for PQ = 0 (660/2,151). For 210-3 the average is 64.03% (89/139). The proportion of recurrences in general was 23.02% (1,494 between 6,489 patients), with 30.68% (660/2,151) in patients without PQ and 19.22% in patients with PQ ($p(\chi^2) = 0.000$). If we exclude the scheme 210-3 because of its exaggerated recurrences (higher to the group without PQ), it is found that the 4 remaining schemes have significant differences in the proportion of recurrences ($p(\chi^2) = 0.000$). The scheme 210-14 does not differ from 210-7 ($p(\chi^2) = 0.070053$). The scheme 45 to 150 differs significantly from not using PQ ($p(\chi^2) = 0.000$).

It is clear that the higher the TD, the lower the recurrences, with the new information that the time in which the TD is administered seems to influence it, for 210 mg in 3 days have very high recurrence, in 14 days it is 12.75% and the minimum is for 7 days with 7.17%.

- Does the effectiveness of PQ depend on the number of days in which a given TD is effective? Answer: Yes. The standard TD (0.25 mg/kg/day) given in 7 to 14 days seems to have the same effectiveness, but given in 3 days it is of no use, according to the data informed²²⁻²³. It was not possible to clearly capture the influence of time in the follow-up of the recurrence proportion.
- Does the effectiveness of PQ depend on the place where the infection occurs? Answer: Yes. The data indicated a variation in recurrence according to the place: the

higher proportion of recurrences occurs in Pakistan (39.65%) and the lowest one in Ethiopia (5.68%) and it is lower in America (6 countries), with 17.83%, than in Asia (3 countries), with 25.22%.

DISCUSSION

The CCTs analyzed here indicate that the absence of PQ in the treatment led to the recurrence of 34.48 against 19.66% with PQ 210-14, with significant advantage for the use of PQ; also, 210-14 has effectiveness equal to 210-7. From this last schemes, there are very few evaluations with CCT, which need to be urgently increased, since 7 days of treatment against 14 of them is an enormous advantage.

It is very important to recognize that each scheme 210-7 and 210-14 is statistically better than 45 to 150 in 3 to 10 days (including 75-5), which has zero PQ anti-recurrence capacity (PQ = 0). But 75-5 in India, and only there, could be useful, without being clear the reason why, because in this country the only significant difference is between 75-5 and 0, while it seems the same to give 210 rather than 75 or 210 rather than 0 mg of TD of PQ.

In the review by Cochrane⁶, with random CCTs, it was found that 75-5 lacks of preventive effects from the occurrences of *vivax* when compared with PQ = 0. This review also found that 210-14 produced significantly less recurrences than PQ = 0 and that the direct comparison of 210-14 and 75-5 showed that 210-14 was significantly lower.

The effectiveness of TD 210 given in 3, 5 or 7 days, was assessed, which involves giving out high daily doses of such. In 3 or in 5 days, it was of no use and the recurrences were very high (> 50%), in 4 or in 6 months²²⁻²³, but there were no significant toxic effects when the directions were strictly followed when using high daily doses⁴¹.

The influence of the place, according to this work, teaches that within each scheme using PQ (210-14, 201-7, 45 to 150) there are significant differences in the proportion of recurrences, and the same happens if PQ is not used. This seems to show that, among other points, the sensitivity of the hypnozoite clones of each country to PQ is very different and that the tendency of hypnozoites recurring also differs. Other variables which may act refer to, for example, the real intake of drugs and the existence of genetic and ethnic factors that are expressed in people with different capability to metabolize drugs.

The interest is in finding that with 210-14, Brazil, Peru and Mexico have lower proportions of recurrence than India, which supports the use of 210 mg in those countries.

The meta-analysis on the effectiveness of PQ against *vivax* recurrences, according to the longitudinal descriptive field studies⁵, showed that the TD of 210 and 75 mg had the same effectiveness, but 75 mg had only been evaluated in India, where *P. vivax* seems to be more

sensible to PQ than in other places. The meta-analysis of the CCTs presented here show that 210-14 is better than 45 to 150 in 3 to 10 days (including TD 75) and that 45 to 150 is similar to using no PQ. The comparison of the questions which guided both analysis teaches the following about the effectiveness of PQ against recurrences of *vivax* malaria:

- Is PQ effective? In both studies the answer is affirmative and both demonstrate that when PQ is not used, the risk of recurrence increases significantly.
- Does the effectiveness of PQ depend on the applied TD when given a fixed time? The answer with descriptive studies showed that 75 mg had the same effectiveness of 210 in 7 or in 14 days, but with CCT the effectiveness of 210 in 7 or 14 days is frankly better than in the case of 45 to 150 mg in 3 to 10 days.
- Does the effectiveness of PQ depend on the number of days in which a given TD is effective? The TD 210 in 7 or 14 days seems to have the same effectiveness, but given in 3 days it has no use, according to the informed data.
- Does the effectiveness of PQ depend on the place where the infection occurs? The answer is yes in both studies.

It has already been noticed that the 2 meta-analysis by Cochrane^{6,13} only included random and semi-random CCT in humans who compared treatments with chloroquine against chloroquine plus PQ, given as 210-14, and other PQ schemes; which included 9 studies instead of the 23 CCT (randomized and non-randomized) which we have added; in Cochrane 3 schemes were analyzed while here we analyzed 5; they worked with 3,423 patients and here there were 6,350. While in theory the CCT can have advantages on the non-randomized CCTs, these advantages have to be shown in practice; besides that, many effects of the non-randomized units to the treatment groups may be controlled in this analysis. Given the shortage of schemes used, going from 3 to 5 is an important advance of this report and the same happens when going from 3,423 to 6,350 patients. It is convenient to reinforce the similarities of the results between the review of Cochrane⁶ and the present study: that one found that 75-5, in relation to the use of PQ, lacked of preventive effects on the recurrences of *vivax* and this one reports that it was found that 210-14 produced significantly less recurrences than PQ = 0 and the comparison 210-14 *versus* 75-5 showed that 210-14 was significantly better.

A strength of the present study is the high level of patients involved, as well as the relatively high number of studies analyzed, whose quality, when meeting the inclusion criteria previously determined for the acceptance of the study, may be judged as good. However, it should be noted that the quantity of studies gathered in each comparison is low, varying between 2 (210-7 *versus* 210-14) and 9 (210-14 *versus* 0). Perhaps the main explanation for this low quantity of studies is due to the hegemony the standard scheme 210-14 has, despite the questioning and the low interest of researchers to test other procedures.

CONCLUSION

It is concluded that:

- the use of PQ is necessary to reduce the frequency of recurrences;
- the standard TD is 0,25 mg/kg/day for 14 days is the best effectiveness in preventing the recurrences of *vivax* malaria and it should be maintained;
- studies in different countries, regions (tropical *versus* subtropical) and continents must be carried out in order to assess the idea resulted from this work, also present in meta-analysis of descriptive studies⁵, that this standard TD given in 7 days has the same effectiveness of the one given out in 14 days, but the studies on the earlier are few and are mostly restricted to Brazil.

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