AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EXPERIMENTAL THERAPEUTICS

Reversal of Chloroquine Resistance of *Plasmodium vivax* in *Aotus* Monkeys

Nicanor Obaldia III,[a,c,e] Wilbur K. Milhous,[b,c*] Dennis E. Kyle[b,d]

[a]Promed, S.A., Panama City, Republic of Panama
[b]Walter Reed Army Institute of Research, Silver Spring, Maryland, USA
[c]College of Public Health, University of South Florida, Tampa, Florida, USA
[d]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, USA
[e]Gorgas Memorial Institute of Health Studies, Panama City, Republic of Panama

ABSTRACT Chloroquine-resistant (CQR) vivax malaria has emerged as a threat to the malaria elimination agenda. The objective of this study was to assess if a combination of chloroquine (CQ) and prochlorperazine was able to reverse CQ resistance of the *Plasmodium vivax* AMRU-1 strain from Papua New Guinea in infected *Aotus* monkeys. For this purpose, in two independent experimental drug efficacy trials, a total of 18 *Aotus* monkeys infected with blood obtained from donor animals were randomly assigned to treatment and control groups and orally administered CQ at 10 mg/kg or prochlorperazine at 20 mg/kg, alone or in combination, for five consecutive days. Reversal of CQR was achieved in animals that received the drug combination, whereas neither drug alone produced cures. This same drug combination reverses CQR in *P. falciparum* and could be an alternative for treatment in humans with chloroquine-resistant *P. vivax* infections.

KEYWORDS *Plasmodium vivax*, chloroquine resistance, resistance reversals, *Aotus* monkeys, animal models

Each year, approximately 216 million new cases of malaria and more than 400,000 deaths occur worldwide (1). Malaria is caused by infection with a protozoan parasite of the genus *Plasmodium*, of which five species, including *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*, are transmitted to humans by the bites of infected female *Anopheles* mosquitoes. It is estimated that between 2010 and 2016 the incidence rate of malaria decreased 18% globally; nonetheless, during 2014 to 2016, significant increases were noted, especially in the region of the Americas, where *P. vivax* infections represent 64% of all cases detected (1). Even if *P. falciparum* infections are eliminated, it is predicted that *P. vivax* will remain an important cause of morbidity and mortality outside Africa (2, 3).

The emergence of chloroquine-resistant (CQR) *P. vivax* is a newly emerging problem of antimalarial drug resistance (4). Since the first description of resistant *P. vivax* in Papua New Guinea (5), other resistant isolates have been confirmed in Oceania, Asia, and South America (6–10). CQR *P. vivax* has been treated with artemisinin combination therapies (ACTs) with success (11), and tafenoquine (WR238605), a promising primaquine analog that has been demonstrated to be a potent blood schizonticide for the treatment of chloroquine-resistant *P. vivax* infections in *Aotus* monkeys (12), has been submitted for approval by regulatory agencies as a single-dose radical cure treatment (prevention of relapses) in patients 16 years of age and older (13, 14).

The mechanism of CQ resistance in *P. vivax* is not clearly defined. In *P. falciparum*, it is known that mutations in the chloroquine resistance transporter *pfcrt* are the primary determinant of CQ resistance, with mutations in the multidrug resistance gene (*pfmdr1*)

Received 18 April 2018 **Returned for modification** 31 May 2018 **Accepted** 14 June 2018

Accepted manuscript posted online 25 June 2018

Citation Obaldia N III, Milhous WK, Kyle DE. 2018. Reversal of chloroquine resistance of *Plasmodium vivax* in *Aotus* monkeys. Antimicrob Agents Chemother 62:e00582-18. https://doi.org/10.1128/AAC.00582-18.

Address correspondence to Nicanor Obaldia III, nobaldia@gorgas.gob.pa.

* Present address: Wilbur K. Milhous, Clemson University, College of Science, Clemson, South Carolina, USA.

TABLE 1 Detailed activity of prochlorperazine (PCP) and chloroquine (CQ) against infections of the chloroquine-resistant AMRU-1 strain of *Plasmodium vivax* in *Aotus* monkeys

Expt no. and group no. (mean ± SD days from final drug to clearance)	Monkey ID	Drug regimen		Response of parasitemia to drug			No. of days from final drug to:		No. of days negative
		Drug[a]	Daily dose (mg/kg)	None	Suppressed	Cleared	Clearance	Recrudescence	
Expt 1									
Group 1 (22 ± 19)	12914	CQ	10	X			8		55
	12911			X			43	52	11
	12906			X			14		49
Group 2 (11 ± 17)	12894	CQ	10			X	1		63
	12900	PCP	20			X	1		63
	12940			X			31		35
Control (14 ± 0)	12910	Vehicle		X			14		49
	12943			X			14		49
Expt 2									
Group 1 (13 ± 11)	12865	CQ	10	X			24		76
	12866				X		19	30	11
	12904				X		12	20	8
Group 2 (7 ± 0)	12882	PCP	20			X	7		93
	12870					X	7	30	23
	12876					X	7	14	7
Group 3 (2 ± 0)	12875	CQ	10			X	2		98
	12903	PCP	20			X	2		98
	12880					X	2		78
Control	12869	Vehicle		X			7		

[a]CQ, chloroquine; PCP, prochlorperazine.

playing a secondary role. Although *P. vivax* has homologs of *pfcrt* and *pfmdr1*, mutations in either gene have not been confirmed to confer CQR in vivax malaria. One of the hallmarks of CQR in *P. falciparum* is the reversal of resistance *in vitro* by verapamil, desipramine, and a series of other drugs (15–18). The reversal of CQR in falciparum malaria also has been confirmed *in vivo* with desipramine and prochlorperazine (PCP) in the *Aotus* model (15, 19). In this study, we aimed to determine if coadministration of CQ and PCP could reverse CQR in *P. vivax in vivo*.

(This research was presented at the Vivax Malaria Research: 2002 and Beyond Meeting, 3 to 8 February 2002, Bangkok, Thailand [20].)

RESULTS

For these studies, we used the CQR AMRU-1 strain of *P. vivax* (named after the Army Malaria Research Unit in Australia) (5), which was previously shown to infect *Aotus* monkeys and to be refractory to treatment with CQ at doses that clear and cure CQ-sensitive *P. falciparum* and *P. vivax* infections (12, 21, 22). We conducted two experiments and had experimental groups with CQ treatment alone (10 mg/kg/day), PCP alone (20/mg/kg/day), and CQ-PCP combinations (CQ at 10 mg/kg/day and PCP at 20 mg/kg/day), as well as untreated controls (Table 1). All treatments were given orally (*per os*) once a day for 5 days. The drug doses used in this study were based upon those in previous studies of PCP reversal of CQ resistance in *Aotus* monkeys infected with *P. falciparum* (22).

P. vivax AMRU-1 infected *Aotus* monkeys treated with CQ alone demonstrated marked resistance to CQ, with parasitemia in 5 of 6 animals not responding to 5 days of treatment (Fig. 1a and Fig. 2a). Only one *Aotus* monkey infected with CQR AMRU-1 had suppression of parasitemia during CQ treatment, yet the infection recrudesced 3 days after clearance. These results confirm that the AMRU-1 strain of *P. vivax* is CQR *in vivo* in *Aotus* monkeys.

Treatment of *P. vivax*-infected *Aotus* monkeys with PCP alone (20 mg/kg/day) did not significantly affect parasitemia during treatment, although parasitemia began to decrease on day 9 (4 days after treatment ended) in all 3 animals treated with 20 mg/kg/day of PCP alone (Fig. 1b). Interestingly, the untreated control animal (Fig. 1d)

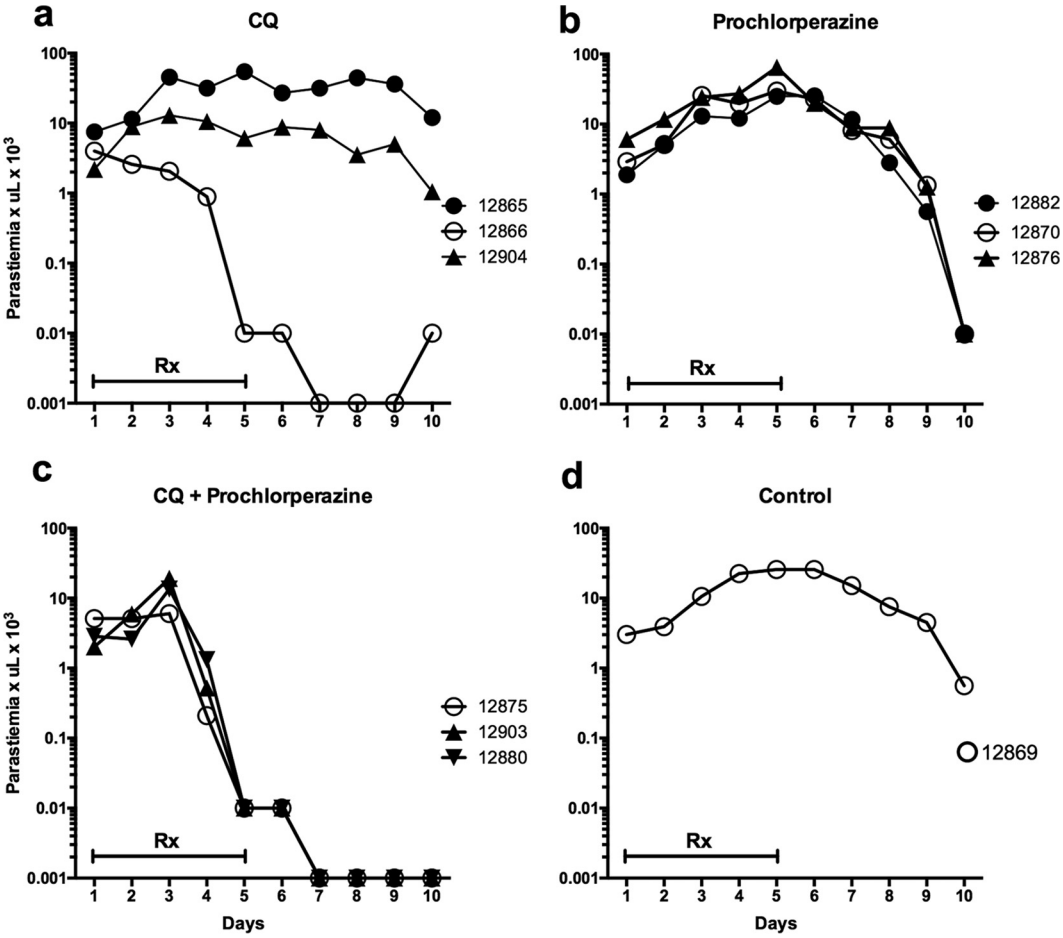

FIG 1 Parasitemia courses of *Aotus* monkeys infected with the chloroquine-resistant AMRU-1 strain of *P. vivax* treated with chloroquine (CQ) at 10 mg/kg (a), prochlorperazine (PCP) at 20 mg/kg (b), or CQ and PCP in combination (c). Parasitemia in an untreated control (d). Negative parasitemia data are plotted at 0.001. Rx, day of drug dosing.

in the same experiment also showed a decrease in parasitemia by day 10 of the study. In the second experiment (Fig. 2), the untreated controls maintained high levels of parasitemia through day 10. The untreated controls and the animals treated with CQ or PCP alone were treated with mefloquine as rescue therapy on day 10, as stipulated in the Institutional Animal Care and Use Committee (IACUC) protocol.

Five of 6 animals treated with the combination of CQ and PCP exhibited a marked decrease in parasitemia (Fig. 1c and 2b). The first decreases in parasitemia were observed on days 3 and 4 of the study, and parasitemia declined rapidly thereafter until clearance was observed on day 6 or 7. In the animals that cleared infection following treatment with CQ-PCP, no recrudescence was observed for up to days 65 to 98 of the study, and they were considered cured (Table 1). The treatment with CQ-PCP combinations thus produced cures, whereas treatment with CQ or PCP alone was ineffective against CQR *P. vivax*.

DISCUSSION

Reversal of CQ resistance *in vivo* using resistance reversers was demonstrated for the first time in 1988 by Bitonti et al. when CQ was administered in combination with desipramine to *Aotus* monkeys infected with CQR *P. falciparum* (16). Additional studies have shown that it is possible to achieve *in vivo* reversal of CQ resistance by the coadministration of prochlorperazine and chloroquine, as evidenced by infection cure (22). Historic use of inexpensive antihistamine drugs, such as chlorpheniramine and promethazine, for the treatment or prevention of CQ-associated pruritus or as anti-

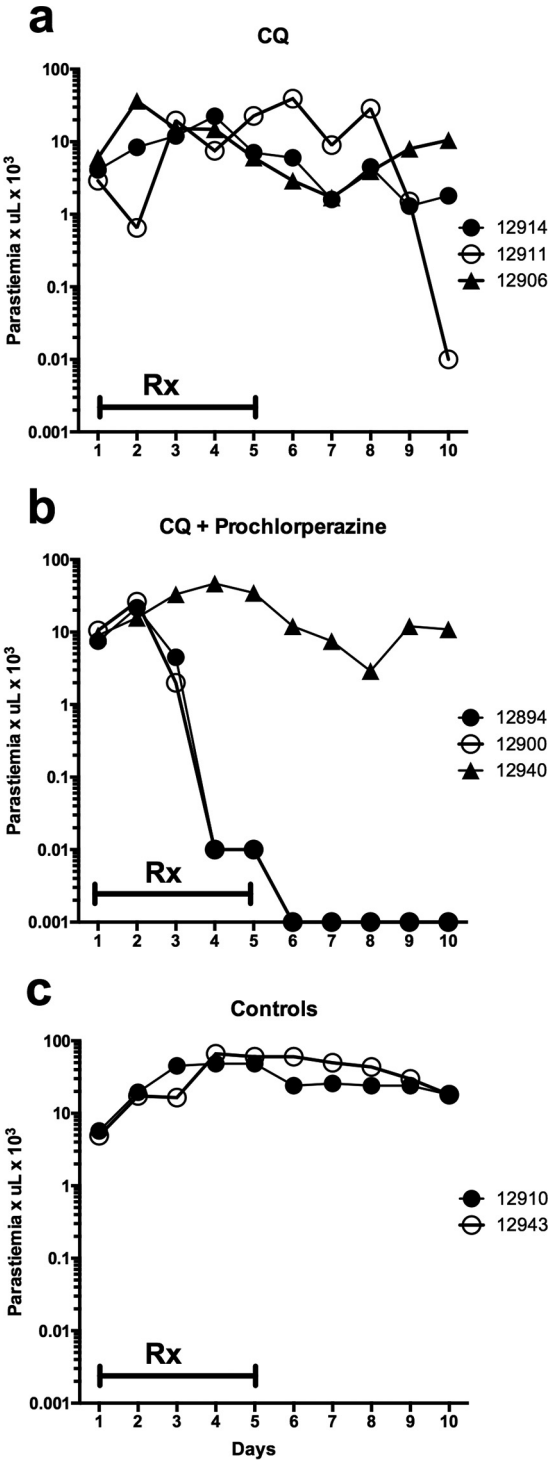

FIG 2 Parasitemia courses of *Aotus* monkeys infected with the chloroquine-resistant AMRU-1 strain of *P. vivax* treated with chloroquine at 10 mg/kg alone (a) or in combination with prochlorperazine (PCP; 20 mg/kg) (b) and controls (c). Negative parasitemia data are plotted at 0.001. Rx, day of drug dosing.

emetics suggest that the combination is safe and effective when used at standard dosages (18). Recent clinical trials have already demonstrated successful cure rates of 81 to 96% in patients with CQ-resistant uncomplicated malaria (23). Although not widely adopted for use, the potential remains for a combination of CQ with a resistance reversal drug to increase therapeutic responses in areas with CQR falciparum malaria.

Notwithstanding that CQR vivax malaria was first reported in 1989 (5), and these resistant infections have become quite prevalent in Indonesia (24), there remains a lack of understanding of the mechanism of CQR in *P. vivax*. Putative resistance genes *pvcrt-0* and *pvmdr1* have been investigated, yet the data are not yet convincing that either plays a central role in CQR in *P. vivax*. In *P. falciparum*, the *in vitro* reversal of resistance with verapamil phenotype was used to map the *pfcrt* locus and to identify the mutations that confer CQR in *P. falciparum*. In this study, we aimed to assess if the phenotypic reversal of CQR by PCP *in vivo* with *P. falciparum* (22) could be achieved with CQR *P. vivax*. Our data clearly show evidence for reversal of CQR in *P. vivax* by PCP combined with CQ. Although 1 of 6 animals treated with the combination did not respond as expected, we did not measure the blood levels of CQ or PCP, and thus the lack of response could be due to inadequate drug exposure.

The results of this study demonstrate for the first time a phenotypic similarity between CQR in *P. falciparum* and *P. vivax*, namely, the ability of PCP coadministration to overcome CQR in an *in vivo* model of disease. As noted from previous studies in the *Aotus* model, PCP is the most potent drug demonstrated to reverse CQR in *P. falciparum in vivo*, and PCP produces a similar effect *in vitro* (22). Interestingly, an *ex vivo* phenotypic study with CQR *P. vivax* was not able to demonstrate a reduction in CQ 50% inhibitory concentrations (IC_{50}s) with several CQ reversal agents, including verapamil; unfortunately, this study did not include PCP, so we do not know if synergy happens *in vitro* as well (25).

The data presented in this study demonstrate that a combination of CQ and PCP reverses CQ resistance of the *P. vivax* AMRU-1 strain in infected *Aotus* monkeys. Neither drug effects cure when used alone. This drug combination could be an alternative for treatment in humans with CQ-resistant *P. vivax* infections, and the resistance reversal phenotype may assist with efforts to identify the molecular basis for CQR in *P. vivax*.

MATERIALS AND METHODS

Animals. Twenty male and female *Aotus lemurinus lemurinus* karyotype VIII and IX monkeys (26), consisting of 18 experimental and two *P. vivax*-infected blood donors, were maintained in the animal facility of the Gorgas Memorial Institute in Panama City, Panama. The animals were housed and cared for as described previously (19, 27). The weight of the monkeys when inoculated with parasites ranged from 749 to 1,002 g.

Parasites. The CQ-resistant *P. vivax* AMRU-1 strain was originally isolated from an Australian soldier infected in Papua New Guinea in 1989 (5) and was successfully adapted by serial passage to Panamanian *Aotus l. lemurinus* monkeys, which served as a suitable model for drug evaluation studies (12, 21).

Parasitemia determination and follow up. Giemsa thick blood smears were prepared from all animals by a prick in the marginal ear vein and were examined daily beginning the day after inoculation, until parasitemia was cleared and for at least 7 days thereafter. Blood films were then examined twice a week up to 100 days after treatment when, if negative, infection was considered cured. Parasitemia was enumerated by the Earle-Perez technique and expressed as number of parasites/microliter (28). Parasitemia outcomes were defined as described previously (29–31): suppressed, if parasitemia persisted but reduced to less than one-fiftieth of control; cleared, if parasitemia became negative by 12 days after patency and remained negative for 7 days (undetectable by microscopy after 5 min of examination); and cured, if parasitemia cleared and remained negative for 100 days after end of treatment. If parasitemia reached >150,000 parasites/μl, hematocrit (HTO %) dropped to 50% below baseline, or platelets reached <50,000/μl prior to day 28 postinfection (p.i.), the animals were rescue treated with a single oral dose of 20 mg/kg of mefloquine (MQ).

Drug preparation and administration. Stock solutions of CQ (WR1544BM;AR20613) and prochlorperazine (PCP) (WR280001AC;BN3106) in distilled water were prepared at appropriate concentrations of drug base and maintained at 4°C during the course of treatment. CQ and PCP doses are expressed as mg/kg of base drug. Drugs were administered over 5 days in equally divided doses once a day by gastric intubation in a volume of 7 ml, followed by a 7-ml rinse with distilled water.

Experimental design: experiment 1. Each of eight *Aotus* monkeys (749 to 950 g) were randomized by weight and sex into two groups of three monkeys each, and two controls and were inoculated intravenously (i.v.) in the saphenous vein with 5×10^4 parasitized erythrocytes of the AMRU-1 strain of *P. vivax*, obtained from the femoral vein of a donor monkey as described previously (12). When parasitemia reached $\sim 5 \times 10^3$ parasites/μl of blood, the animals were treated for 5 days as follows: group 1, comprised of *Aotus* 12914, 12911, and 12906, received chloroquine (CQ) at 10 mg/kg; group 2, comprised of *Aotus* 12894, 12900, and 12940, was treated with PCP at 20 mg/kg plus CQ at 10 mg/kg; and the control group, consisting of *Aotus* 12910 and 12943, received the drug vehicle only.

Experimental design: experiment 2. Each of 10 *Aotus* monkeys (753 to 1,002 g) were randomized by weight and sex into three groups of three monkeys each and one control and were inoculated i.v. in

the saphenous vein with 5×10^6 parasitized erythrocytes of the AMRU-1 strain of *P. vivax*, as above. When parasitemia reached $\sim 5 \times 10^3$ parasites/μl of blood, the animals were treated for 5 days as follows: group 1, comprised of *Aotus* 12865, 12866, and 12904, received CQ at 10 mg/kg; group 2, comprised of *Aotus* 12882, 12870, and 12876, was treated with PCP at 20 mg/kg; group 3, consisting of *Aotus* 12875, 12903, and 12880, received PCP at 20 mg/kg plus CQ at 10 mg/kg; and the control group, consisting of *Aotus* 12869, received the drug vehicle only.

Ethical statement. The experimental protocol "Reversal of chloroquine resistance with the coadministration of prochlorperazine and chloroquine against infections of chloroquine resistant (CQR) AMRU-1 strain of *Plasmodium vivax* in *Aotus lemurinus lemurinus* monkeys" was approved and registered at the Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES) – Institutional Animal Care and Use Committee (CIUCAL) under accession number 1999/01. It was conducted in accordance with the Animal Welfare Act, the *Guide for the Care and Use of Laboratory Animals* (32), and the laws and regulations of the Republic of Panama. The general animal use protocol for the contract "Evaluation of Drug and Vaccine Candidates in the Human Malaria/*Aotus* Monkey Model" (award DAMD17-96-C-6051), was approved by the U.S. Army Medical Research and Materiel Command (Fort Detrick, Maryland, USA).

ACKNOWLEDGMENTS

This work was done with the financial support of the U.S. Army Medical Research and Materiel Command (award DAMD17-96-C-6051). The views, opinions, and/or findings contained herein are those of the authors and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation.

We gratefully acknowledge the following individuals: Gloria Cisneros, Frank Durham, William Otero, and Lionel Martinez for technical assistance and Maritza Brewer for secretarial assistance. At Promed S.A., we thank Ginés Sánchez and Gladys Calvino for administrative assistance and Jose Camilo Marín, Temistocles Lao, Roberto Rojas, and the animal caretakers for the care and handling of animals. We also acknowledge the support of the Sistema Nacional de Investigación (SNI) of the Secretaria Nacional de Ciencia Tecnología e Innovación (SENACYT) and the Gorgas Memorial Institute, Republic of Panama in the publication of this article.

N.O. III, W.K.M., and D.E.K. planned and conducted the experiments, analyzed the data, and wrote the paper.

REFERENCES

1. WHO. 2017. World malaria report 2017. WHO, Geneva, Switzerland.
2. Vitor-Silva S, Siqueira AM, de Souza Sampaio V, Guinovart C, Reyes-Lecca RC, de Melo GC, Monteiro WM, Del Portillo HA, Alonso P, Bassat Q, Lacerda MV. 2016. Declining malaria transmission in rural Amazon: changing epidemiology and challenges to achieve elimination. Malar J 15:266. https://doi.org/10.1186/s12936-016-1326-2.
3. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, Kaslow DC, Noor A, Okumu F, Steketee R, Tanner M, Wells TNC, Whittaker MA, Winzeler EA, Wirth DF, Whitfield K, Alonso PL. 2017. malERA: an updated research agenda for malaria elimination and eradication. PLoS Med 14:e1002456. https://doi.org/10.1371/journal.pmed.1002456.
4. Wang W, Liang Y, Jie Z, Wu S, Wang H. 2015. Global extent of chloroquine-resistant *Plasmodium vivax*. Lancet Infect Dis 15:630. https://doi.org/10.1016/S1473-3099(15)00016-X.
5. Rieckmann KH, Davis DR, Hutton DC. 1989. *Plasmodium vivax* resistance to chloroquine? Lancet ii:1183–1184.
6. Whitby M, Wood G, Veenendaal JR, Rieckmann K. 1989. Chloroquine-resistant *Plasmodium vivax*. Lancet ii:1395.
7. Whitby M. 1997. Drug resistant *Plasmodium vivax* malaria. J Antimicrob Chemother 40:749–752. https://doi.org/10.1093/jac/40.6.749.
8. Soto J, Toledo J, Gutierrez P, Luzz M, Llinas N, Cedeno N, Dunne M, Berman J. 2001. *Plasmodium vivax* clinically resistant to chloroquine in Colombia. Am J Trop Med Hyg 65:90–93. https://doi.org/10.4269/ajtmh.2001.65.90.
9. Khan A, Godil FJ, Naseem R. 2016. Chloroquine-resistant *Plasmodium vivax* in Pakistan: an emerging threat. Lancet Glob Health 4:e790. https://doi.org/10.1016/S2214-109X(16)30251-0.
10. Baird JK. 2004. Chloroquine resistance in *Plasmodium vivax*. Antimicrob Agents Chemother 48:4075–4083. https://doi.org/10.1128/AAC.48.11.4075-4083.2004.
11. Grigg MJ, William T, Menon J, Barber BE, Wilkes CS, Rajahram GS, Edstein MD, Auburn S, Price RN, Yeo TW, Anstey NM. 2016. Efficacy of artesunate-mefloquine for chloroquine-resistant *Plasmodium vivax* malaria in Malaysia: an open-label, randomized, controlled trial. Clin Infect Dis 62:1403–1411. https://doi.org/10.1093/cid/ciw121.
12. Obaldia N, 3rd, Rossan RN, Cooper RD, Kyle DE, Nuzum EO, Rieckmann KH, Shanks GD. 1997. WR 238605, chloroquine, and their combinations as blood schizonticides against a chloroquine-resistant strain of *Plasmodium vivax* in *Aotus* monkeys. Am J Trop Med Hyg 56:508–510. https://doi.org/10.4269/ajtmh.1997.56.508.
13. Walsh DS, Looareesuwan S, Wilairatana P, Heppner DG, Jr, Tang DB, Brewer TG, Chokejindachai W, Viriyavejakul P, Kyle DE, Milhous WK, Schuster BG, Horton J, Braitman DJ, Brueckner RP. 1999. Randomized dose-ranging study of the safety and efficacy of WR 238605 (tafenoquine) in the prevention of relapse of *Plasmodium vivax* malaria in Thailand. J Infect Dis 180:1282–1287. https://doi.org/10.1086/315034.
14. MMV. 28 November 2017. GSK submits US regulatory application for single-dose tafenoquine for *Plasmodium vivax* malaria. MMV, Geneva, Switzerland. https://www.mmv.org/newsroom/press-releases/gsk-submits-us-regulatory-application-single-dose-tafenoquine-plasmodium.
15. Martin RE, Marchetti RV, Cowan AI, Howitt SM, Broer S, Kirk K. 2009. Chloroquine transport via the malaria parasite's chloroquine resistance transporter. Science 325:1680–1682. https://doi.org/10.1126/science.1175667.
16. Bitonti AJ, Sjoerdsma A, McCann PP, Kyle DE, Oduola AM, Rossan RN, Milhous WK, Davidson DE, Jr. 1988. Reversal of chloroquine resistance in malaria parasite *Plasmodium falciparum* by desipramine. Science 242:1301–1303. https://doi.org/10.1126/science.3057629.
17. Milhous W, Kyle D. 1998. Introduction to the modes of action and mechanisms of resitance to antimalarials. In Sherman IW (ed), Malaria: parasite biology, pathogenesis and protection. ASM Press, Washington, DC.
18. Oduola AM, Sowunmi A, Milhous WK, Brewer TG, Kyle DE, Gerena L, Rossan RN, Salako LA, Schuster BG. 1998. *In vitro* and *in vivo* reversal of

chloroquine resistance in *Plasmodium falciparum* with promethazine. Am J Trop Med Hyg 58:625–629. https://doi.org/10.4269/ajtmh.1998.58.625.
19. Obaldia N, III, Otero W, Marin C, Aparicio J, Cisneros G. 2011. Long-term effect of a simple nest-box on the reproductive efficiency and other life traits of an *Aotus lemurinus lemurinus* monkey colony: an animal model for malaria research. J Med Primatol 40:383–391. https://doi.org/10.1111/j.1600-0684.2011.00489.x.
20. Obaldia N, Kyle D, Milhous W. 2002. Reversal of chloroquine resistance with the co-administration of prochlorperazine and chloroquine against infections of the chloroquine resistant (CQR) AMRU-1 strain of *P. vivax* in *Aotus lemurinus lemurinus* monkeys, p 56. Vivax Malaria Research: 2002 and Beyond Meeting, 3 to 8 February 2002, Bangkok, Thailand.
21. Cooper RD. 1994. Studies of a chloroquine-resistant strain of *Plasmodium vivax* from Papua New Guinea in *Aotus* and *Anopheles farauti* s.l. J Parasitol 80:789–795. https://doi.org/10.2307/3283259.
22. Kyle DE, Milhous WK, Rossan RN. 1993. Reversal of *Plasmodium falciparum* resistance to chloroquine in Panamanian *Aotus* monkeys. Am J Trop Med Hyg 48:126–133. https://doi.org/10.4269/ajtmh.1993.48.126.
23. Bagla P. 1997. Malaria fighters gather at site of early victory. Science 277:1437–1438. https://doi.org/10.1126/science.277.5331.1437.
24. Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. 2014. Global extent of chloroquine-resistant *Plasmodium vivax*: a systematic review and meta-analysis. Lancet Infect Dis 14:982–991. https://doi.org/10.1016/S1473-3099(14)70855-2.
25. Wirjanata G, Handayuni I, Prayoga P, Leonardo L, Apriyanti D, Trianty L, Wandosa R, Gobay B, Kenangalem E, Poespoprodjo JR, Noviyanti R, Kyle DE, Cheng Q, Price RN, Marfurt J. 2017. *Plasmodium falciparum* and *Plasmodium vivax* demonstrate contrasting chloroquine resistance reversal phenotypes. Antimicrob Agents Chemother 61:e00355-17. https://doi.org/10.1128/AAC.00355-17.
26. Ma NS, Rossan RN, Kelley ST, Harper JS, Bedard MT, Jones TC. 1978. Banding patterns of the chromosomes of two new karyotypes of the owl monkey, *Aotus*, captured in Panama. J Med Primatol 7:146–155. https://doi.org/10.1159/000459804.
27. Obaldia N, 3rd. 1991. Detection of *Klebsiella pneumoniae* antibodies in *Aotus l. lemurinus* (Panamanian owl monkey) using an enzyme linked immunosorbent assay (ELISA) test. Lab Anim 25:133–141. https://doi.org/10.1258/002367791781082603.
28. Earle W, Perez M. 1931. Enumeration of parasites in the blood of malarial patients. J Lab Clin Med 19:1124–1130.
29. Schmidt LH. 1978. *Plasmodium falciparum* and *Plasmodium vivax* infections in the owl monkey (*Aotus trivirgatus*). III. Methods employed in the search for new blood schizonticidal drugs. Am J Trop Med Hyg 27:718–737.
30. Schmidt LH. 1978. Plasmodium falciparum and *Plasmodium vivax* infections in the owl monkey (*Aotus trivirgatus*). II. Responses to chloroquine, quinine, and pyrimethamine. Am J Trop Med Hyg 27:703–717. https://doi.org/10.4269/ajtmh.1978.27.703.
31. Schmidt LH. 1978. *Plasmodium falciparum* and *Plasmodium vivax* infections in the owl monkey (*Aotus trivirgatus*). I. The courses of untreated infections. Am J Trop Med Hyg 27:671–702. https://doi.org/10.4269/ajtmh.1978.27.671.
32. National Research Council. 1996. Care and use of laboratory animals. National Research Council, National Academies Press, Washington, DC. https://www.nap.edu/catalog/5140/guide-for-the-care-and-use-of-laboratory-animals.