Failure of Combined Chloroquine and High-Dose Primaquine Therapy for *Plasmodium vivax* Malaria Acquired in Guyana, South America

Elizabeth J. Phillips, Jay S. Keystone, and Kevin C. Kain

From the Tropical Disease Unit, Department of Medicine, University of Toronto, Toronto, and The Toronto Hospital, Toronto, Ontario, Canada

The presence of chloroquine-resistant *Plasmodium vivax* malaria in the New World has been suspected but not confirmed. We report the cases of three patients who acquired vivax malaria in Guyana, South America, and for whom standard chloroquine therapy (25 mg/kg) failed despite therapeutic blood levels. The optimal treatment of chloroquine-resistant *P. vivax* malaria is unknown, but recent studies suggest that a combination of chloroquine (25 mg/kg) and high-dose primaquine (2.5 mg/kg over 48 hours) is effective therapy. Two of our patients had recurrences of *P. vivax* malaria 6–8 weeks after receiving directly observed therapy with this combination. These cases confirm the presence of chloroquine-resistant *P. vivax* in Guyana and emphasize the need for better treatment regimens for chloroquine-resistant and primaquine-resistant *P. vivax* malaria.

Plasmodium vivax infection is usually treated with a combination of chloroquine, to eradicate blood-stage parasites, and primaquine, to prevent relapses caused by liver hypnozoites. The recurrence of *P. vivax* infection in a previously treated individual who does not live in an area of endemicity can be caused by recrudescence due to chloroquine-resistant blood-stage parasites or by a relapse due to primaquine-resistant hypnozoites. Chloroquine-resistant *P. vivax* (CRPV) was first described in 1989 in Papua New Guinea [1]. Over the last five years, preliminary reports have suggested that CRPV is also present in South America [2–4]; however, these cases were not well documented.

See the editorial by Turner on pages 1174–5.

We report the cases of three patients who acquired *P. vivax* infection in Guyana, South America, and for whom at least one course of standard chloroquine therapy (25 mg/kg) failed. Furthermore, *P. vivax* infection recurred in two of these patients following directly observed therapy with a combination of chloroquine (25 mg/kg) plus high-dose primaquine (2.5 mg/kg over 48 hours), which has recently been shown to be effective in treating CRPV from Irian Jaya [5].

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This study was approved by the Ethical Review Committee of the Toronto Hospital, and all participants gave informed consent.

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Reprints or correspondence: Dr. Kevin C. Kain, Tropical Disease Unit, EN G-224, The Toronto Hospital, 200 Elizabeth Street, Toronto, Ontario, Canada, M5G 2C4.

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Case Reports

Case 1. A 36-year-old man who traveled to Guyana to work for 9 months was treated with several unknown antimalarial regimens for recurrent fever. Two weeks after returning home to Canada, he again developed fever; malaria smears confirmed *P. vivax* infection. He was treated with chloroquine (25 mg/kg) and primaquine (15 mg base/d for 14 days). On day 11 of primaquine therapy, he developed recurrent fever. Malaria smears were positive for *P. vivax;* the levels of chloroquine in simultaneously collected whole blood (chloroquine = 274 ng/mL; desethylchloroquine = 139 ng/mL) were in the range considered to be therapeutic (>90 ng/mL) [6].

The patient was admitted to the hospital to receive directly observed therapy with chloroquine (25 mg/kg) plus high-dose primaquine (2.5 mg/kg base over 48 hours) [5]. His glucose-6-phosphate dehydrogenase (G6PD) level was normal, and he tolerated this combination therapy well. Levels of chloroquine in whole blood were again determined on days 3 (chloroquine = 1,182 ng/mL; desethylchloroquine = 424 ng/mL) and 14 (chloroquine = 797 ng/mL; desethylchloroquine = 352 ng/mL) of therapy and were in the therapeutic range. Malaria smears were negative on days 4, 7, and 28.

Six weeks after receiving directly observed therapy, the patient presented with recurrent fever; malaria smears were again positive for *P. vivax.* He was treated with halofantrine (24 mg/kg over 24 hours; repeated at day 7) and primaquine (30 mg base/d for 28 days). Follow-up blood films were negative for *P. vivax* on days 2, 7, and 28, and he remained asymptomatic 6 months later.

Case 2. A 33-year-old man experienced two febrile episodes while traveling in urban and rural areas of Guyana for 6 months; these episodes were treated with 4–7-day courses of oral quinine. On the day before he left Guyana to return to Canada, he again developed fever and was treated with chloroquine and primaquine. In Canada, chloroquine levels were measured on day 7 of therapy and were in the therapeutic range (chloroquine = 281 ng/mL; desethylchloroquine = 164

ng/mL). Two weeks later he again developed fever, and malaria smears were positive for *P. vivax*. Chloroquine levels determined at presentation to the hospital were subtherapeutic (chloroquine = 13 ng/mL; desethylchloroquine = 22 ng/mL), and he was retreated with chloroquine (25 mg/kg).

Six weeks later the patient presented with recurrent fever, and malaria smears were again positive for *P. vivax*. He was admitted to the hospital to receive directly observed therapy with chloroquine and high-dose primaquine. His G6PD levels were normal, and he tolerated the treatment well. Follow-up malaria smears were negative on days 7 and 28. Eight weeks later he returned to the hospital with fever; malaria films were again positive for *P. vivax*. He was treated with halofantrine (24 mg/kg over 24 hours; repeated at day 7) and primaquine (30 mg base/d for 28 days) and remained well 6 months later.

Case 3. A 37-year-old man traveled to Guyana for 2 months. One week before leaving Guyana, he developed a high fever and was treated with chloroquine and primaquine. Six weeks later, fever recurred and malaria smears were positive for *P. vivax*. Chloroquine levels determined at presentation to the hospital were therapeutic (chloroquine = 105 ng/mL; desethylchloroquine = 22 ng/mL). He was admitted to the hospital to receive directly observed therapy with a combination of chloroquine (25 mg/kg) and high-dose primaquine (2.5 mg/kg over 2 days), and he tolerated the treatment well (the level of G6PD was normal). Cultures of specimens from oral ulcers were positive for herpes simplex virus on day 2 of treatment. Malaria smears were negative on days 4, 7, and 28, and he remained well at a follow-up visit 1 year later.

Discussion

This study is the first to confirm the presence of CRPV malaria in South America. Although previous reports have indicated that CRPV infection may occur sporadically in Brazil and Colombia [2, 3], these cases were poorly documented; antimalarial therapy was neither directly observed nor confirmed with drug levels, and the risk of reinfection could not be excluded in all cases. In addition, the long delay to the recurrence of vivax malaria in many patients has led other investigators to conclude that these cases were more likely relapses than chloroquine treatment failures [4].

A recent study suggested that CRPV may exist in Guyana [4]. However, this was a retrospective assessment of failures of chloroquine prophylaxis, and compliance with prophylactic regimens was not confirmed with drug levels. To our knowledge, our cases are the first to confirm *P. vivax* malaria that is resistant to treatment doses of chloroquine in the New World.

Recurrent *P. vivax* malaria was diagnosed and treated in Canada in all cases, thus eliminating reinfection as a confounder, and two of three patients whose chloroquine levels were considered to be therapeutic had recrudescences of vivax malaria. The evidence in case 2 strongly suggests that the patient had CRPV malaria, but this diagnosis is not definitive since it was not possible to confirm that a diagnosis of *P. vivax* malaria was made in Guyana.

In each episode of *P. vivax* malaria that occurred in Canada, the diagnosis was confirmed by PCR-based speciation [7]. In the first two cases, paired sequential *P. vivax* samples were available for molecular analysis. A portion of the circumsporozoite and merozoite surface protein-1 genes was amplified from each isolate and compared by sequencing or by single-strand conformational polymorphism analysis as previously described [7–9]. Single-strand conformational polymorphism or sequence analysis indicated that each of the paired isolates was identical for each of these polymorphic genes (data not shown).

These observations confirm that these were recrudescent infections, at least in the case of patient 1, who experienced a recurrence at day 11. In case 2, the initial *P. vivax* isolate was not available, and the paired isolates that were examined were collected 6 weeks apart. This period, combined with the observed sequence identity between these sequential isolates, would be consistent with either a late recrudescence or an early relapse.

Although combination chloroquine plus high-dose primaquine has been reported to be effective therapy for CRPV malaria [5], it failed to prevent the recurrence of vivax malaria in two of our three patients. Since recurrence occurred 6–8 weeks after the patients received directly observed therapy, it is probable that combination therapy eradicated the erythrocytic phase of the disease and that recurrence was caused by a failure of primaquine to radically cure hepatic hypnozoites. However, late recrudescences following chloroquine treatment cannot be ruled out for either of these cases since recurrences occurring after 30 days could represent either late chloroquine treatment failures or relapses secondary to primaquine-resistant hypnozoites [6].

Our cases confirm the presence of CRPV malaria in Guyana. They also emphasize the need for further study to define better treatment regimens for primaquine-resistant hypnozoites and CRPV malaria. With the emergence of chloroquine-resistant and primaquine-resistant vivax malaria in South America, future control and management of this infection will be more difficult.

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