PLASMODIUM VIVAX INFECTIONS IN U.S. ARMY TROOPS: FAILURE OF PRIMAQUINE TO PREVENT RELAPSE IN STUDIES FROM SOMALIA

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Abstract. Different strains of Plasmodium vivax vary in their sensitivity to primaquine, the only drug that prevents relapses. Described are the clinical data and relapse pattern for 75 soldiers treated for vivax malaria since returning from Somalia. Following their initial attack of malaria, 60 of the 75 cases received a standard course of primaquine (15 mg base daily for 14 days). Twenty-six of the 60 soldiers subsequently relapsed for a failure rate of 43%. Eight soldiers had a second relapse following primaquine therapy after both the primary attack and first relapse. Three of these soldiers had received a higher dosage of primaquine (30 mg base daily for 14 days) after their second attack. The apparent ineffectiveness of primaquine therapy in preventing relapses suggests the presence of primaquine-resistant P. vivax strains in Somalia.

The majority of malaria infections diagnosed in the United States are caused by Plasmodium vivax. Relapses occur with P. vivax infections because delayed developmental forms of the parasite, called hypnozoites, are present in the liver. Hypnozoites can mature months to years later to cause clinical disease. After maturing and multiplying in the liver, the parasite enters a red blood cell for further development. Clinical disease is produced when parasites are released from red blood cells. A radical cure, defined as the complete elimination of parasitemia from the body, is achieved for P. vivax infections by the use of both blood-stage antimalarial drugs and primaquine, which is the only available drug that can destroy hypnozoites. Determining drug-resistant patterns in P. vivax strains is difficult because the parasite cannot be cultured in vitro and a standard microbiologic definition of resistance cannot be developed. Primaquine resistance for P. vivax infections is defined clinically when a patient relapses after receiving a standard regimen of primaquine (15 mg base daily for 14 days). Primaquine-resistant strains have been reported from Papua New Guinea, Southeast Asia, and Central and South America.

The deployment of more than 20,000 troops to Somalia represented the first major U.S. military operation in a malarious area since Vietnam. Mefloquine was used for malaria prophylaxis by all Army personnel for whom it was contraindicated. Initially, primaquine was not included in the terminal prophylaxis regimen because the risk of P. vivax infections was reported to be low.

In Somalia, 48 cases of malaria were diagnosed among U.S. military personnel. Forty-one patients were infected with P. falciparum, six with P. vivax, and one with P. malariae. Several months after returning to the United States, the number of P. vivax infections increased unexpectedly in soldiers who had deployed. Primaquine was then added to the terminal prophylactic regimen of all returning soldiers. Later, it became apparent that a number of soldiers were experiencing relapses after a failed radical cure with the standard primaquine regimen and the recommended treatment dosage of primaquine for soldiers was doubled (30 mg base daily for 14 days). We will describe the epidemiologic data of 75 soldiers who were diagnosed with P. vivax malaria after returning from Somalia and the problems associated with their chemoprophylactic and treatment regimens.

METHODS

A standardized protocol for the management of suspected malaria patients was instituted at Fort Drum, New York in May 1993. This installation had provided more than 60% of the Army soldiers who served in Somalia. All soldiers seeking medical attention who had a fever or history of fever and who had been to Somalia were admitted. The soldier was discharged after 48 hr if he became afebrile and had at least four sets of negative thick and thin blood smears. For the purposes of the present study, a case was confirmed as malaria when one of the authors (BTW), an expert malaria microscopist, verified the diagnosis on a blood smear. Polymerase chain reaction (PCR) techniques to detect P. vivax, P. falciparum, P. ovale, and P. malariae were used on blood collected on filter paper from seven cases. Parasite DNA was extracted from dried blood dots on filter paper using QIAamp columns according to the manufacturer's instructions (Qiagen Inc., Chatsworth, CA) or by boiling in the presence of Chelex-100 (Bio-Rad Laboratories, Richmond, CA). All samples were subjected to amplification and detection of malarial 18s rRNA genes as previously described. In addition, a portion of the P. vivax circumsporozoite gene and the K1-14 sequence of P. falciparum were amplified and detected as described.

A questionnaire eliciting information on illnesses, personal protective measures taken against arthropod vectors, use of chemoprophylactic drugs, and activities in Somalia was administered to all soldiers treated for malaria. In addition, patient interviews and pharmacy record and patient chart reviews were completed to obtain information on symptoms, drug compliance, and history of present illness. For this report, a soldier was classified as having received a standard radical curative course of therapy if he stated that he had completed a 14-day regimen of primaquine after his malaria attack and if there was evidence from a chart review or pharmacy records that primaquine was prescribed.
RESULTS

Case description. After returning from Somalia, 75 male soldiers had confirmed *P. vivax* infections during the period of observation (May 1993 through February 1994). The PCR analyses corroborated the microscopic diagnoses of *P. vivax* and no sequences of *P. ovale* were detected. The soldiers’ ages ranged from 18 to 39 years (median = 20) and the length of time served in Somalia ranged from 31 to 153 days (median = 73). Standard oral malaria treatment regimens of chloroquine or quinine and doxycycline were used to treat the acute illnesses. All soldiers were returned to full duty.

Epidemiology. Seventy-seven percent of the cases were from one military unit (total number of soldiers in this group = 499). The attack rates for *P. vivax* by race-ethnic group in this unit were 14.6% for Hispanics (n = 41), 14.3% for Asians (n = 7), 13.1% for whites (n = 360), and 3.5% for blacks (n = 85). Whites were four-fold more likely to be infected with *P. vivax* than blacks (odds ratio = 4.1; 95% confidence interval = 1.2–12.4). The attack rates in this unit also varied by geographic region. For soldiers (n = 128) operating in Jilib, a town along the Juba river, the attack rate was 29%. For soldiers (n = 127) working in Bandar Salaam, an abandoned rice plantation 8 km south of Jilib, the attack rate was 11%. For soldiers (n = 120) in the port city of Kismayo, 100 km southeast of Jilib, the attack rate was less than 1%.

Mefloquine chemoprophylaxis. Ninety-six percent of the soldiers reported taking at least two doses before arrival in Somalia, and 95% reported that they had not missed a dose while in Somalia. Mefloquine was administered to the troops under direct observation by the military command structure in Somalia. After returning home to the United States, only 63% reported full compliance with the terminal prophylactic regimen by taking four doses of mefloquine; 23% reported missing only one dose during this period. Terminal prophylaxis with mefloquine was not supervised. For the soldiers in this study, the time from the last dose of mefloquine to the first symptoms ranged from 11 to 242 days (median = 69 days; n = 73; Figure 1a).

Primquine terminal prophylaxis. In May 1993 following the recognition of *P. vivax* infections at Fort Drum, primquine terminal prophylaxis was recommended for all soldiers who had returned from Somalia. Twenty-nine soldiers had their first hospitalization for malaria before the recommendation to use primquine terminal prophylaxis was made. One soldier did not receive his primquine terminal prophylaxis because he was training at another site when the medication was distributed. Forty-five of 75 cases received primquine terminal prophylaxis before their first confirmed attack, but several weeks after completing their mefloquine terminal prophylaxis. For the soldiers receiving primquine terminal prophylaxis, the primary attack occurred from six to 197 days after the start of the regimen (median = 61 days; n = 45; Figure 1b). Eighteen of the 37 soldiers who had lived in Jilib and had a *P. vivax* infection received primquine terminal prophylaxis before their primary attack. Assuming that the remaining soldiers (n = 109) in this unit took their medication, the failure rate for primquine terminal prophylaxis in this group was 16%.

Radical cure using primquine. For this report, 60 of the 75 cases were classified as having received a standard radical curative course of primquine after their first confirmed clinical attack of malaria. Figure 2 outlines the primquine usage for the 75 cases. Twenty-six soldiers relapsed after their attempted radical cure for a failure rate of 43% (26 of 60). The interval from the primary attack to the first relapse among those treated with primquine ranged from 22 to 193 days, with a median time of 75 days (Figure 3). An additional seven soldiers had a relapse, but we were unable to verify that they had received a standard radical curative course of primquine after their primary attack. For five of these seven soldiers, we could not find documentation that primquine had been prescribed. One soldier stated that he did not take his primquine and one could not remember if he had taken it.

Eight soldiers had a second relapse. All had received a
standard radical curative nurse of primaquine after both the primary attack and first relapse. The time from the first to the second relapse ranged from 31 to 127 days, with a median of 107 days. Of the 33 soldiers who had at least one relapse, 18 soldiers received a higher dose of primaquine (30 mg base daily for 14 days) after their first relapse. Three of these soldiers relapsed for a second time, giving a 17% failure rate for the high-dose regimen.

**DISCUSSION**

Our observations suggest that there are primaquine-resistant (see Editor's footnote on page 231) *P. vivax* strains in Somalia. Cases of primaquine treatment failures have been reported in patients who visited Kenya, Sudan, and Ethiopia,13 but our data represent the largest number of reported cases from this region. It is possible that some of our observed primaquine prophylactic and therapeutic failures were due to noncompliance. Compliance with the primaquine regimen was self-reported and could not be verified by blood drug levels because of the short half-life of primaquine. Our findings need to be confirmed by directly observed primaquine therapy.

The decision to use primaquine as part of a traveler's terminal prophylaxis regimen is complex because the actual risk of exposure to relapsing forms of malaria is generally not known. Initially, the Army did not use primaquine for terminal prophylaxis because the risk of *P. vivax* infection was reported to be low. *Plasmodium falciparum* was the predominant species in Somalia.14 In retrospect, using data from local semi-immune adults may not adequately describe the risk of *P. vivax* to nonimmune persons. *Plasmodium vivax* infections in semi-immune individuals may be relatively mild and are easily self-treated with chloroquine, which is readily available in Africa. Medical care would not be sought and there would be an under-reporting of *P. vivax* cases. The risk was also underestimated because *P. vivax* infections are unusual in Africa. Most black Africans lack the Duffy group antigens that are needed for *P. vivax* to enter erythrocytes.15 This explains the lower attack rate among black soldiers in our study because most black Americans are of African descent. Somalis are unique in that as a racial group in Africa at least one-third of the population processes the Fy gene that allows expression of one of the Duffy antigens.16 Because of the homogeneity of Somali society,17 the potential for a reservoir of *P. vivax* in this population is heightened. The Army's experience in treating malaria in U.S. troops in Somalia also supported the view that *P. falciparum* would pose the greatest malarial risk. More than 85% of the U.S. cases treated in Somalia were caused by *P. falciparum*. In retrospect, it is possible that some of these cases were unrecognized mixed infections because acute *P. falciparum* infections have been reported to suppress the expression of *P. vivax* infections.18

Estimating the risk of infection before entering a malarious area is further complicated by the focal geographic distribution of the mosquitoes carrying *P. vivax*. In this study, the intensity of transmission in Somalia varied with attack rates of 29% to less than 1% within 100 km. Furthermore, using the length of exposure as a criteria for the use of primaquine may not be helpful. Primaquine is generally recommended for those with prolonged exposure, such as missionaries and Peace Corps Volunteers.19 However, the soldiers in this study were in Somalia less than three months. Our experience demonstrates the difficulties in estimating the risk of acquiring a *P. vivax* infection and indicate the need to use primaquine as part of a terminal prophylactic regimen among individuals returning from Somalia.

The current U.S. Army experience has documented significant problems in providing radical cures for patients with *P. vivax* malaria acquired in Somalia. The Centers for Disease Control and Prevention recommends that all *P. vivax* infections, both primary attacks and relapses, be treated with 15 mg of primaquine base daily for 14 days after verifying glucose-6-phosphate dehydrogenase status.20 This conventional treatment was developed during the Korean War for malaria acquired in Korea.21 It is now apparent that different strains of *P. vivax* vary in their sensitivity to primaquine and therefore in their likelihood to relapse. The Chesson strain found in Papua New Guinea has a 30% failure rate with the standard primaquine regimen.22 Strains from Southeast Asia and Central and South America have also been reported to relapse after standard regimens of primaquine.23 Most authorities agree that it is reasonable to treat patients who acquire their infections from these areas with a higher-dose regimen.24 To reduce side effects, a regimen of 15 mg daily of primaquine base for 28 days has been used instead of 30 mg base daily for 14 days under the assumption that it is the total cumulative dose of primaquine that is important in eliminating hypnozoites. Unfortunately, there are no data to support the longer duration regimen. Human and animal studies have only used total dose schedules over a shorter time interval (60 mg base daily for seven days versus 30 mg base daily for 14 days) in showing equivalent cure rates.24,25 Extrapolating these findings of using the same total dose over a longer time period has not been verified. Our experience indicates that *P. vivax* infections acquired in Somalia should be treated with a 30 mg base daily regimen for two weeks and, if possible, the therapy should be given under direct observation.

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