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MINIREVIEW

Chloroquine Resistance in *Plasmodium vivax*

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Emerging resistance to chloroquine (CQ) by *Plasmodium vivax* threatens the health of the hundreds of millions of people routinely exposed to the risk of infection with this organism. CQ has been the first-line therapy for vivax malaria since 1946 (32, 115). *Plasmodium falciparum* developed resistance to CQ in the 1950s (110), and today it occurs globally (91). Resistance by *P. vivax* was unknown until 1989, when Australians repatriated from Papua New Guinea failed routine treatment (94). Subsequent reports affirmed that finding, and CQ-resistant *P. vivax* (CRPV) was reported from Indonesia (8, 35, 99, 100, 111). Reports from Myanmar (76, 82) and India (56, 107) followed. CRPV appeared in travelers from Guyana, South America (88). However, studies in Thailand (38, 72, 103), the Philippines (10), and Vietnam (105) revealed only CQ-sensitive *P. vivax*. Surveys in Indonesia revealed a low frequency of CRPV in the west (~10%) (15, 16, 49, 50, 51, 53, 75) and a higher risk in the east (~45%) (9, 18, 52, 81, 102, 106). This minireview summarizes the present state of knowledge of CRPV as a scientific, clinical, and public health problem. It examines the genesis of CQ therapy for *P. vivax* and the laboratory and clinical data underpinning the diagnosis of CRPV. The available data showing the global distribution of CRPV are listed. Finally, the clinical data on alternative therapies against CRPV are reviewed.

VIVAX MALARIA AND ANTIMALARIAL THERAPY

Four species in the genus *Plasmodium* routinely infect humans. *P. vivax* infects 80 million people annually and accounts for most cases of malaria occurring outside Africa (79). It rarely causes death but inflicts debilitating fever, chills, nausea, vomiting, and myalgia. The prevalence of *P. vivax* typically ranges from <1 to 25% in areas of Asia and the Americas where the organism is endemic, but it is resurging and now threatens to reencroach where it had been eradicated (7, 28, 31). The chemotherapeutic management of vivax malaria therefore represents an issue of importance to global health.

The life cycle of plasmodia defines the chemotherapeutic strategies. These parasites pass through a complex life cycle marked by forms of distinct morphology, function, location, clinical consequence, and susceptibility to antimalarial agents. Figure 1 illustrates the four families of antimalarial drugs defined on the basis of their activities against specific stages in the life cycle. CQ is a blood schizonticide against both *P. vivax* and *P. falciparum*. Its activity as a gametocytocide within therapeutic ranges is nonexistent against *P. falciparum* gametocytes but is potent against *P. vivax* gametocytes. CQ alone exerts no known sporonticidal or tissue schizonticidal activity.

Relapse is an important aspect of the *P. vivax* life cycle bearing upon chemotherapy and its assessment. Relapse refers to clinical malaria caused by parasites in the bloodstream originating from dormant liver stages called hypnozoites seeded by sporozoites from infectious anopheline mosquitoes (Fig. 1). Relapse may occur weeks to years following the primary episode of parasitemia and clinical disease. Tissue schizonticides, like primaquine, prevent relapse by killing the stages of the organism in the liver. When a parasitemia reappears after blood schizonticidal therapy, it may be a relapse from the liver, a reinfection by a mosquito, or a recrudescence originating from asexual blood-stage parasites that survived therapy (Fig. 2). The emergence of CQ-resistant *P. vivax* favors the last possibility.

CQ THERAPY

Development. The first treatment of humans with CQ occurred in 1936 in four syphilis patients in Dusseldorf, Germany, given *P. vivax* (32). An accounting of the lost records of that trial describes CQ as being "too toxic for practical use in humans." The Germans investigated sontochin, a methylated analog, which American forces obtained in liberated Algeria in May 1943. Patents for sontochin and CQ were discovered in the United States, and both compounds went to clinical trials. That work, detailed by Wiselogle (114), proved that CQ was more effective and better tolerated.

The early nomenclature of CQ includes SN-7618 and "resochin," identifiers used by the American and German developers, respectively. The name CQ was formally registered in the United States in March 1946. A month later Loeb et al. (71) published the seminal paper on the activity of CQ against falciparum and vivax malaria. They recommended 1.5 g of base over 48 h for the treatment of acute falciparum or vivax malaria and 0.3 g of base weekly for prophylaxis. These remain the standards for treatment and prophylaxis.

Standard versus effective therapy. The genesis of recommended therapy constitutes a critical factor now, 60 years later, in defining resistance by *P. vivax*. What was the minimally effective dose? Loeb et al. (71) provided no data, instead publishing the "Statement Approved by the Board for the Coordination of Malarial Studies." Most et al. (80) published the first clinical data in November 1946: several hundred American

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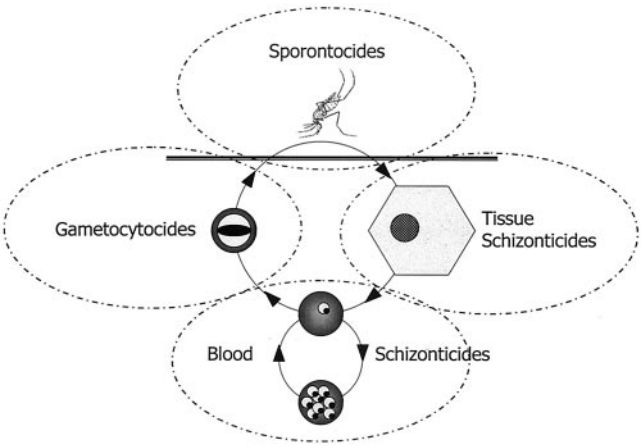

FIG. 1. Schematic representing the life cycle of plasmodia and the four families of antimalarial agents. Sporontocidal agents kill forms in the mosquito, including infectious sporozoites. Tissue schizonticides kill parasites developing (schizonts) or quiescent (hypnozoites) in the liver. Blood schizonticides kill the asexual blood forms (trophozoites and schizonts) that cause clinical malaria. The gametocytocides kill or sterilize the sexual forms (gametocytes) that infect mosquitoes.

soldiers were treated with 1.0 g over 12 h, 1.5 g over 96 h, or 2.0 g over 7 days. They recommended the use of 1.5 g over 48 h, a regimen not represented in their work.

In 1947, Gordon et al. (58) reported that only 0.8 g (for 6 days) had good efficacy against vivax malaria in 39 subjects. Berliner et al. (20) described total doses from 0.3 to 0.6 g as consistently curing blood-stage *P. vivax* (McCoy strain) in 10 subjects. However, total doses <0.3 g often failed. Others soon affirmed the sensitivity of *P. vivax* to substandard regimens down to a 0.3-g total adult dose. Hoekenga (65) described 0.6- or 0.45-g single-dose regimens in Honduras in 1952. Among 100 subjects receiving 0.6 g, only 1 failed the treatment. Among 120 subjects receiving 0.45 g, 5 failed the treatment. In 1950, Butts (27) reported on 202 patients in Central America treated with 0.08 to 1.56 g. Failures occurred only among those receiving <0.3 g. Wilson and Edeson (113) reported on similar findings from Malaysia; among 62 subjects treated with single doses of 0.3 to 0.6 g, none failed. According to Harinasuta as late as 1992 (as cited by Looareesuwan et al. [73]), *P. vivax* in Thailand remained sensitive to treatment with a single 0.35-g dose. The available data suggest a baseline sensitivity compatible with blood-stage cure of *P. vivax* with ≥0.3 g of CQ base.

The data from areas of endemicity led to reasoned recommendations for substandard treatments in "immune" populations (22). There was no need (or reliable evidence) to invoke immunity as the basis of efficacy. These regimens had superior efficacies in nonimmune people as well.

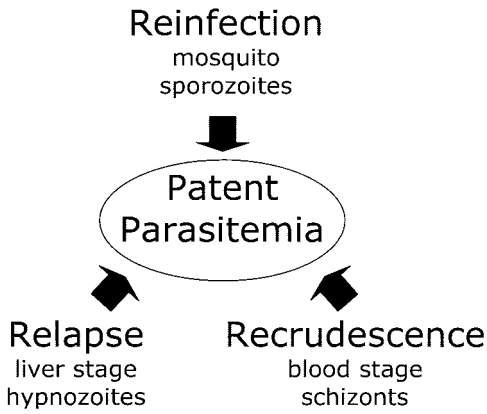

FIG. 2. The three paths to recurrent parasitemia for malarias caused by *P. vivax* and *P. ovale*. Sporozoites from mosquitoes reinfect the livers of humans, which yields merozoites that infect blood. Some sporozoites develop to quiescent hypnozoites in the liver and later cause relapse. Subpatent trophozoites in blood cells mature to schizonts that rupture and release merozoites that infect the blood and cause a recrudescence.

RELAPSE

Assessment of the therapeutic effect of CQ requires an understanding of relapse, which is parasitemia originating from latent hypnozoites. The risk of relapse varies with geographic origin. Strains form tropical regions cause relapses more quickly and more often than strains from temperate regions. The rates from a variety of studies have been reviewed elsewhere (12). The risk of relapse with tropical *P. vivax* often exceeds 50% within a month of the primary attack, and multiple relapses are the rule. Among strains from temperate regions, the risk of relapse typically ranges from 5 to 25%, and multiple relapses are rare.

Early clinical trials often used the Chesson strain of *P. vivax*, isolated from an American soldier in New Guinea during the war (45). Quinine therapy against Chesson is carefully considered here because it provides a basis for understanding how relapse confounds gauging of the therapeutic response to CQ. Wiselogle (114) provided a comprehensive review of the activity of quinine against *P. vivax*. In the American clinical trials conducted during World War II, quinine served as the positive control for therapeutic activity. Shannon, in Wiselogle (114), explains the rationale as the fact that quinine is eliminated within 12 h, and thus, a relapse uninhibited by lingering drug is allowed. Shannon further explains the certainty that quinine cured the blood; subjects challenged by blood inoculation and given 12 g of quinine over 12 days were cured without recurrence.

The Chesson sporozoite-challenged subjects treated with quinine represent, as they did 60 years ago, the key to gauging the therapeutic response to CQ by *P. vivax*. Figure 3 illustrates the relapse rates after quinine treatment. Relapse occurred no sooner than 17 days after the primary attack (5 days after quinine treatment). The median time to relapse was 22 days after the primary attack, and 60% had relapses by 30 days. However, a relapse before 17 days is possible; people harboring multiple broods of hypnozoites accumulated over months or years of exposure may have a relapse at any time. Figure 1 nonetheless suggests that a relapse before 17 days is unlikely among patients experiencing a primary attack after a brief exposure.

RELAPSE AFTER CQ TREATMENT

In the 1940s recurrent parasitemia after effective CQ therapy defined the timing and the risk of relapse for CQ-sensitive

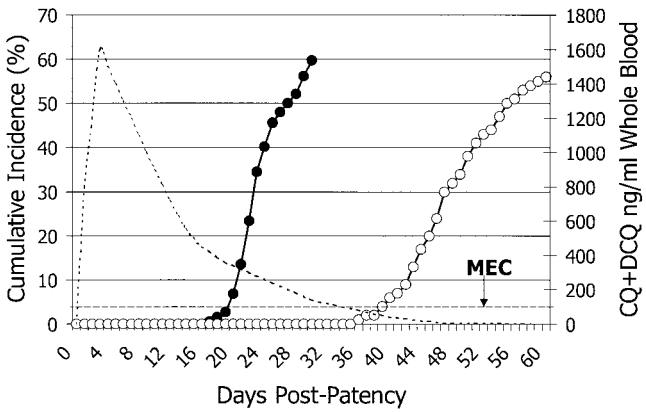

FIG. 3. Cumulative incidence of relapses (left *y* axis) of *P. vivax* infections after blood schizonticidal therapy with quinine (solid points) or CQ (hollow points) during clinical trials conducted during the 1940s. Data were gathered from various sources (5, 33, 41, 58, 80, 92, 112, 114). The dotted curve represents hypothetical CQ drug levels (right *y* axis) declining to below the MEC of approximately 100 ng/ml, marked by the hashed line parallel to the *x* axis.

P. vivax. Figure 3 illustrates those data. The risk of relapse begins after 35 days, and the rate climbs to 58% by 60 days. What explains the dramatic difference with relapse after quinine treatment? Neither drug alone affects liver stages. The explanation for the difference between the two plots constitutes the rational basis for a clinical diagnosis of resistance.

Figure 3 reveals that the challenge of a relapse commences at 17 days and that >60% of patients have relapses by 35 days (after quinine treatment). The CQ lingering in the bloodstream for up to 35 days after therapy prevents the recurrent parasitemias of relapse. CQ-sensitive parasites attempting development in the bloodstream at 35 days encounter drug levels below the minimal effective concentration (MEC) and relapse occurs. If this interpretation is accurate, standard therapy should result in drug levels close to the MEC at 35 days. Data from other sources confirm this. Berliner et al. (20) measured the MECs for *P. vivax* in the 1940s. They noted complete cure required ≥10 ng/ml of plasma. Coatney et al. (34) measured CQ levels after a 1.3-g regimen on day 35 and found them to be 8, 9, and 19 ng/ml. These data represent the basis of the widely cited MEC for *P. vivax* being 10 ng/ml and corroborate the explanation given for the delayed relapses after CQ therapy.

RATIONALE FOR IN VIVO DIAGNOSIS

Biological versus clinical resistance. A single dose of >0.3 g of CQ or a level in blood plasma >10 ng/ml should achieve complete cure of blood-stage CQ-sensitive *P. vivax*. An infection that persists in the face of these exposures to drug may be classified biologically resistant. However, no one recommends that vivax malaria be treated with just 0.3 g of CQ. Standard therapy delivers five times that dose, and failure of that regimen constitutes clinical resistance. However, the tidy separation of biological and clinical resistance is difficult with *P. vivax*. Clinicians wonder if the standard 1.5-g CQ regimen eliminates parasitemia. If not, infection survived the towering drug concentrations following therapy (approximately 200 ng/ml of plasma). This is clinical resistance. In contrast, a parasite emerging from the liver and penetrating waning levels of drug above 10 ng/ml of plasma is biologically resistant. Although spared the immediate spike of drug soon after therapy, relapsing parasites would nonetheless have been killed if they were sensitive (Fig. 3).

Recrudescence versus reinfection and relapse. Molecular biological assays classify genotypes of *P. vivax* and distinguish reinfection from relapse (26, 40, 67). However, distinguishing recrudescence from reinfection or relapse represents the critical need in analyzing the therapeutic response. The methods of genetic analysis available at present do not allow this. A genetic match between isolates causing primary and secondary parasitemias does not prove recrudescence because hypnozoites often derive from the genotype of the reference isolate causing parasitemia (40, 67). Mismatch is also ambiguous: it may be a reinfection or a relapse originating from hypnozoites of a distinct genotype or a recrudescence of a minority genotype not originally detected. No method available at present allows the unambiguous classification of *P. vivax* parasitemias originating from recrudescence, relapse, or reinfection. Multilocus genotyping may ultimately bring clarity to this issue (6).

Sensitive versus resistant. The detection of CRPV does not require relapse, reinfection, and recrudescence to be distinguished (13). A recurrent parasitemia, regardless of origin, should not occur within 35 days of standard CQ therapy (Fig. 1). Parasitemia before 35 days penetrated an ordinarily lethal exposure to CQ. Parasitemia clearing within 4 days and not reappearing in 35 days may be classified as sensitive.

Clinicians and health policy makers want to know when "resistant" parasites survived therapy or merely penetrated waning levels of drug. The question is not academic. If parasites routinely survive a therapeutic intervention, it must be changed. If parasites instead relapse only after successful therapy, the problem may be attacked with primaquine. The classification explained here allows no such distinction. The analysis nonetheless provides key insights that help guide treatment decisions.

Parasites classified as resistant either persisted for 4 days or cleared but reappeared within 35 days. Persistent parasitemia after 4 days represents the highest grade of clinical resistance, whereas parasitemia recurring late (e.g., 30 days) is probably a relapse in which the isolates penetrated drug levels close to the MEC. The day of recurrence offers a scale upon which relative resistance may be gauged. Parasites penetrating higher drug levels soon after therapy are empirically more resistant than parasites that penetrate lower drug levels later. Clinicians and health policy makers may consider the proportion classified as resistant and the median day of recurrence indicators of the degree of resistance in the parasite population.

EVIDENCE SUPPORTING IN VIVO DIAGNOSIS

Recurrent parasitemia within 35 days of CQ therapy supports a provisional diagnosis of resistance. Confirmation requires proof of adequate compliance to and absorption of therapy by reliable supervision or, ideally, by determination of the levels of drug in blood. Figure 3 illustrates data derived from clinical trials with supervised dosing and ascertainment of drug levels. Counterfeit drug, poor compliance, and emesis

may prevent normal drug levels from being achieved. An unambiguous diagnosis of CRPV infection requires the demonstration of parasitemia with ordinarily effective drug levels (>10 ng/ml of plasma).

The blood challenge experiments of Berliner et al. (20) provided a definitive MEC for CQ-sensitive *P. vivax*. After CQ treatment the relapse pattern with the plasma drug levels (Fig. 3) corroborated the estimate of the MEC. Two issues must be considered today when the MEC is applied to present assessments of CQ treatment effectiveness: (i) the analytical methods of the 1940s and (ii) plasma CQ levels versus whole-blood CQ levels.

Most laboratories have adopted the extraction and high-pressure liquid chromatography procedures described by Patchen et al. (86). This method quantifies CQ and its major metabolite, desethylchloroquine (DCQ), in whole blood collected onto filter paper. This medium spares the need for centrifugation and a cold chain, a key advantage in the setting of the rural tropics.

Early analytical methods did not discriminate between CQ and DCQ (25, 77). The 10-ng/ml MEC represents the sum of the plasma CQ and DCQ concentrations. In vitro studies with CQ-sensitive *P. falciparum* consistently reveal that CQ and DCQ have roughly equal antimalarial activities (54, 57, 108). In gauging adequate exposure to drug in the bloodstream, the sum of the CQ and DCQ concentrations is the operative statistic. The ratio of the CQ concentration to the DCQ concentration has no clear bearing on the interpretation of this question, except that a very low DCQ concentration relative to the CQ concentration (e.g., the ratio of the CQ concentration to the DCQ concentration > 10) suggests contamination of the sample with CQ dust.

The level of CQ-DCQ in whole blood corresponding to 10 ng/ml of plasma must be estimated to obtain a MEC in whole blood. Several studies measured CQ and DCQ concentrations in whole blood and plasma (2, 21, 47, 59, 96). The ratio of the concentration in whole blood to the concentration in plasma ranged from 5 to 10 (median, 8), yielding an estimated MEC in whole blood of 75 to 150 ng/ml. These values agree with those occurring 30 to 35 days after therapy, when relapse commences (Fig. 1). Rombo et al. (95) deduced an MEC of 90 ng/ml of whole blood for *P. vivax* among subjects taking CQ prophylaxis. A 100-ng/ml MEC for CQ-DCQ in whole blood against CQ sensitive *P. vivax* was adopted (13).

Recurrent parasitemia within 35 days of therapy with ≥100 ng of CQ-DCQ per ml in whole blood demonstrates resistance to CQ. A test duration of 28 days was recommended (13) to accord with the standard World Health Organization test for *P. falciparum*. The in vivo assessment represents a direct and often convenient means of detecting resistance.

OTHER APPROACHES TO DIAGNOSING RESISTANCE

Experimental animals. Some nonhuman primate host-adapted strains of *P. vivax* have been evaluated. Collins and colleagues (36, 37) studied two strains of *P. vivax* acquired from patients in Indonesia who failed CQ therapy. The therapeutic profiles of CQ among well-characterized strains in humans provide a basis for the classification of wild isolates as sensitive or resistant in animal models. Collins et al. (36) discussed the rationale for classifying the CQ susceptibilities of strains of *P. vivax* in *Aotus* or *Saimiri* monkeys. They point to variations in the minimal therapeutic doses for strains known to be sensitive to CQ in humans: Vietnam Palo Alto (>18 mg), Achiote (10 mg), and Chesson (9 mg). CQ-resistant Indonesian strain CDC I failed to be eradicated with 15 mg and was classified as "possibly even more resistant than the Vietnam Palo Alto strain." The other strain from Indonesia failed to be eradicated with 30 mg and was classified as resistant (37).

In vitro methods. The diagnosis of resistance to *P. falciparum* in vitro has been standard procedure since the 1970s. Although *P. vivax* has not been cultivated continuously, it develops for periods sufficient to assess the therapeutic response. Methods for doing so have been described since the 1970s (24, 55, 90, 93), and there is renewed interest in these techniques (60, 68, 98, 103, 104). No standard criteria for classifying in vitro responses as sensitive or resistant yet exist. However, many isolates have been characterized in Thailand, where the clinical responses to CQ treatment remain uniformly sensitive. This provides a baseline for in vitro sensitivity: ~50 ng/ml consistently inhibits development by 50%. In vitro testing for CRPV may prove useful among well-equipped laboratories.

Molecular probes. No genetic mutations have been linked to resistance to CQ by *P. vivax*. Nomura et al. (83) investigated mutations in the *P. vivax* ortholog of the *crt* gene of *P. falciparum*, which has been linked to CQ resistance. The mutations incriminated in *P. falciparum crt* did not occur among CRPV isolates, and no other mutations in that gene correlated with the phenotype. The genetic determinants of resistance to CQ apparently differ between *P. vivax* and *P. falciparum*. Continuous cultivation allowed the search for *crt* mutants in *P. falciparum*, and similar progress for *P. vivax* may be difficult; but genomic analyses (46) may ultimately yield genetic determinants of resistance.

Prophylaxis and cross-sectional studies. Among 94 study subjects taking supervised CQ prophylaxis (5 mg/kg of body weight weekly) in Indonesian New Guinea for 18 or 52 weeks, 29% developed *P. vivax* infections (11, 48), a rate indistinguishable from that for a placebo group (48). Among 41 subjects in the same region evaluated in two other studies (8, 81), 61% developed vivax malaria. Vivax malaria occurring during supervised prophylaxis proves resistance to CQ.

Cross-sectional analyses of CQ levels may help gauge endemic resistance. Collection of blood on dried filter paper and stained blood films (and later analysis in a laboratory) allow assessment of hundreds of people with just a single day on-site, whereas in vivo assessments require at least 1 month on-site. This approach requires caution, however. A patient reporting to a clinic with concentrations in blood greater than the MEC may have recently self-administered drug, and even sensitive *P. vivax* may take 4 days to clear. Nonetheless, the proportion of patients infected with *P. vivax* and having concentrations in blood greater than the MEC provides an estimate of the risk of resistance (14).

GEOGRAPHIC RANGE OF RESISTANCE

Oceania. The data from Oceania come from just six infections acquired in Papua New Guinea, and no new report has appeared in the past 10 years. Nonetheless, the risk of thera-

peutic failure is considered high on the basis of the weight of the data from Indonesian New Guinea (see below and Table 1). Moreover, unpublished data (99a) revealed therapeutic failure rates from 0 to 33% in the late 1980s. Surveys of therapeutic responses to CQ throughout Oceania are needed.

East Asia. Six East Asian nations have reported data on CRPV: Indonesia, Malaysia, Myanmar, Thailand, Vietnam, and Philippines. The data from Indonesia span 1992 to 2002 and reveal a high risk in the eastern archipelago. The risk in the far eastern province of Papua is highest. Among 41 subjects taking supervised prophylaxis, 61% developed vivax malaria (8, 81), as did 29% (the same rate as the placebo group) in two other studies (11, 48). Among 88 subjects treated up to 1995, 41% had a response consistent with resistance (8, 81). Among 282 subjects evaluated since then, 39% were infected with resistant isolates (9, 11, 48, 52, 102, 106). However, all 38 subjects evaluated on a small, isolated island in Papua had infections with sensitive organisms (52). In western Indonesia two separate surveys at Nias, near northern Sumatra, found 9 inadequate responses among 49 subjects (18%) (16, 50). A survey of 54 subjects in Indonesian Borneo revealed that 22% of the isolates were CRPV (53). Surveys at Lombok (west of Bali) and central Java in the early 1990s found no resistance among isolates from 20 and 14 subjects, respectively (15, 49). However, the site in central Java was reevaluated in 2001, and among 77 subjects, 14 (18%) were infected with CRPV (75). A traveler to Flores in the Lesser Sundas archipelago had CRPV infection (61).

CPRV is endemic in Malaysia, Myanmar, and Vietnam. A traveler to Sabah in Malaysian Borneo (1) presented with vivax malaria in Sweden with ordinarily protective CQ levels in blood. Among a series of 60 patients hospitalized in Kuala Lumpur (1983 to 1992), 6 were described as infected with CRPV (66). CRPV has been confirmed in Myanmar: Myat-Phone-Kyaw et al. (82) presented two case reports, and a survey of 50 patients (76) revealed that 14% were infected with CRPV. A study of 23 subjects in Vietnam in 1995 revealed no CRPV (105), but recently, 28 of 113 (25%) subjects had recurrent *P. vivax* infections by day 28 after treatment (87).

There is no evidence of CPRV from Thailand, Cambodia, Laos, China, the Korean Peninsula, or the Philippine archipelago. However, Thailand represents the only one of these nations in which adequate surveys have been conducted (38, 72, 103): 1,046 subjects were reliably evaluated, and only 4 (0.4%) had recurrent parasitemia within 28 days after infection (and these responded to a second round of CQ therapy). No reports of CQ efficacy against *P. vivax* were found from Cambodia, Laos, China, or the Koreas. At Palawan in the Philippines, Baird et al. (10) evaluated 21 *P. vivax* infections and none recurred within 28 days.

South Asia. Only seven cases of CRPV have been reported from India (42, 44, 56, 89, 69). The only other study from southern Asia comes from Iran: Hamedi et al. (60) evaluated 39 subjects, and none had a recurrent parasitemia within 28 days.

South America. Phillips et al. (88) reported on the presence of CPRV in three travelers to Guyana repatriated to Canada. Baird et al. (17) evaluated 32 subjects in Guyana with vivax malaria, and none had recurrent parasitemia within 28 days. Likewise, Castillo et al. (30) and Villalobos-Salcedo et al. (109) evaluated 44 and 73 subjects in Columbia and Brazil, respectively, and none had recurrent parasitemia within 28 days. Machado et al. (74) evaluated 30 subjects infected near Belem in Brazil; the infection was cleared from all subjects by day 4, and none had a recurrence by day 30. However, Soto et al. (101) described three cases of CRPV infection among 27 subjects in Colombia, and Alecrim et al. (4) described a single case from Amazonia. Ruebush and colleagues (97) confirmed four cases of CRPV infection among 177 (2%) infected subjects evaluated in the Amazon region of Peru. CRPV apparently occurs in the New World but at a low frequency (risk, probably <5%).

ALTERNATIVE THERAPIES

Mefloquine. Some authorities recommend mefloquine for therapy for CRPV (29, 78). No clinical data yet support that recommendation. Mefloquine proved effective against CQ-resistant *P. falciparum* and was effective against CQ-sensitive *P. vivax* (3, 43, 62). Good efficacy against CRPV seems a reasonable supposition, and Collins et al. (37) demonstrated that mefloquine had good efficacy against an Indonesian CRPV strain in *Aotus* monkeys. However, work by Nomura et al. (83) points to different mechanisms of resistance between the two species, and caution is warranted.

Indirect evidence suggests that mefloquine may be efficacious against CRPV. Ohrt et al. (85) demonstrated the complete efficacy of mefloquine for prophylaxis against the CRPV strain known to occur in northeastern Indonesian New Guinea. However, they also found that daily doxycycline had complete efficacy against CRPV, and Taylor et al. (106) showed doxycycline monotherapy to have only 33% efficacy against CRPV. Clinical trials of mefloquine against CRPV are needed.

Halofantrine, CQ plus doxycycline, or primaquine. Taylor et al. (106) evaluated CQ and doxycycline combined in Indonesia and found 71% efficacy. This was superior to the 29 and 33% efficacies of the respective monotherapies against vivax malaria but was inferior to the 91% efficacy of the combination against *P. falciparum*. Baird et al. (9) evaluated halofantrine monotherapy and CQ combined with primaquine against *P. vivax* in Indonesian New Guinea. CQ combined with primaquine (10 mg/kg over 2 weeks or 2.5 mg/kg over 48 h) provided superior efficacy in 79 patients (87%) relative to the efficacy of CQ monotherapy in 50 patients (30%). Halofantrine monotherapy cured all 19 subjects treated, although there was one recurrence on day 28 after the end of treatment.

Phillips et al. (88) used CQ (25 mg/kg over 2 days) and primaquine (2.5 mg/kg over 2 days) in three patients with CRPV infections acquired in Guyana. They described this therapy as inadequate because two patients had recurrent parasitemia after 6 weeks. However, the abbreviated primaquine regimen was not intended to prevent relapse but to clear the bloodstream, and it apparently achieved this in all three patients. The best combination of CQ plus primaquine may be 0.5 mg of primaquine/kg daily for 14 days or 1.0 mg of primaquine/kg daily for 7 days. This regimen clears the bloodstream of CRPV and would prevent relapse. It should not be used for patients likely to be infected with *P. falciparum* as well, because it has no efficacy against that type of infection (19).

TABLE 1. Summary of recently reported CQ sensitivity or resistance by *P. vivax*

Region	Country	Area	Yr	No. of infections examined	No. of resistant infections[a]	Use of drug[b]	Reference
Oceania	Papua New Guinea	East New Britain	1989	2	2	Rx	94
		Not specified	1989	1	1	Rx	111
		Madang/Lae	1991	1	1	Rx	35
		New Britain	1992	1	1	Rx	35
		Madang and Lae	1992	2	2	Rx	99
Southeast Asia	Indonesia	Arso, Papua	1992	24	16	Px	8
		Arso, Papua	1992	1	1	Rx and Px	8
		Nias, Sumatra	1992	1	1	Rx and Px	100
		Arso, Papua	1993	46	10	Rx	81
		Arso, Papua	1993	17	9	Px	81
		Arso, Papua	1995	50	35	Rx	9
		Arso, Papua	1995	54	12	Px	11
		Arso, Papua	1995	40	15	Px	48
		Menoreh Hills, Java	1996	14	0	Rx	15
		Nias, Sumatra	1996	21	3	Rx	16
		Nabire, Papua	1996	34	18	Rx	18
		Arso, Papua	1997	49	42	Rx	102
		Western Lombok	1997	20	0	Rx	49
		Western Borneo	1998	54	12	Rx	53
		Northern Sulawesi	1998	11	1	Rx	51
		Lesser Sundas	1998	1	1	Rx	61
		Gag Island, Papua	1999	38	0	Rx	52
		Jayapura, Papua	2001	17	5	Rx	106
		Nias, Sumatra	2002	28	6	Rx	50
		Menoreh Hills, Java	2002	77	14	Rx	75
	Malaysia	Sabah, Borneo	1996	1	1	Rx and Px	1
		Urban composite	1998	60	6	Rx	66
	Myanmar	Mine Pyin and Pwint Phyu	1993	2	2	Rx	82
		Mingaladon	1995	50	7	Rx	76
	Thailand	Bo Rai	1991	77	0	Px	23
		Chantaburi	1995	57	0	Rx	103
		Urban composite	1999	886	4	Rx	72
		Sa Kaeo	2002	26	0	Rx	38
	Vietnam	Na Trang	2000	23	0	Rx	105
		Not specified	2002	120	28	Rx	87
	Philippines	Palawan	1996	21	0	Rx	10
South Asia	Iran	Bandar Abbas	2002	39	0	Rx	60
	India	Bombay	1995	2	2	Rx	56
		Bombay	1995	7	1	Rx	89
		Calcutta	1995	1	1	Px and Rx	107
		Mathura	1996	1	1	Rx	44
		Bombay	2000	1	1	Rx	69
		Bombay	2003	1	1	Rx	42
South America	Guyana	Interior	1996	3	3	Rx	88
		Interior	2002	32	0	Rx	17
	Colombia	Llanos Orientales/Uraba	2001	27	3	Rx	101
		Cali	2002	44	0	Rx	30
	Brazil	Manaus	1999	1	1	Rx	4
		Rondonia	2000	73	0	Rx	109
		Belem	2003	30	0	Rx	74
	Peru	Amazonia	2003	177	4	Rx	97

[a] Classified as resistant by the reporting authors.
[b] Rx, standard treatment; Px, standard prophylaxis.

Malarone. Lacy et al. (70) evaluated Malarone (250 mg of atovaquone and 100 mg of proguanil daily for 3 days; GlaxoSmithKline, London, United Kingdom) in 16 subjects with *P. vivax* infections in Indonesian New Guinea. They also received 0.5 mg of primaquine/kg daily for 14 days. All subjects cleared the fever and parasitemia by day 3 and remained free of parasitemia for 28 days. Malarone combined with primaquine is the only available therapy with proven efficacy (>90%) against CRPV, but that finding is from the results of a study with just 16 patients.

Tafenoquine. Tafenoquine (GlaxoSmithKline) is an 8-aminoquinoline analog of primaquine that has potent schizonticidal activity against *P. vivax* and *P. falciparum* in tissue and blood and that is now in clinical trials. This drug has been demonstrated to be effective against CRPV in nonhuman primates (39, 84).

Sulfadoxine-pyrimethamine. Recent work by Hastings and Sibley (63) suggests that *P. vivax* may be susceptible to antifolate inhibitors of dihydrofolate reductase (DHFR). DHFR mutations in *P. vivax* are apparently responsible for the lack of activity among the antifolates. Hastings et al. (64) also found that quadruple mutations in dihydrofolate reductase corresponded to the therapeutic failure of sulfadoxine-pyrimethamine treatment among patients with *P. vivax* infections acquired in Indonesia. When quadruple *P. vivax dhfr* mutants do not occur, sulfadoxine-pyrimethamine may be useful against CRPV, but clinical trials are needed.

CONCLUSIONS

Infections with CQ-sensitive *P. vivax* were routinely cured with as little as 0.3 g of CQ base, even though 1.5 g has been the recommended therapy since 1946. The clinical failure of standard therapy therefore represents infection with an organism with a high degree of resistance. A persistent or recurrent parasitemia within 14 days of the start of treatment probably represents recrudescence by a highly resistant strain of *P. vivax*. A recurrent parasitemia between 15 and 35 days after the start of treatment with >100 ng of CQ-DCQ per ml is resistant to CQ, regardless of whether that parasitemia originates from a relapse, a reinfection, or a recrudescence. In general, the day of recurrence correlates inversely with degree of resistance, but isolates that cause recurrences after CQ and DCQ levels fall below the MEC (at about day 35) cannot be classified as sensitive or resistant. When 30 mg of CQ base against *P. vivax* in *Aotus* monkeys fails, the organism may be classified as resistant. When 50 ng of CQ base/ml fails to inhibit in vitro schizont development by more than 50%, the isolate may be classified as resistant. CRPV appears to be most common in eastern Indonesia, especially on the island of New Guinea. It appears sporadically elsewhere in Southeast Asia, typically among <15% of strains. No cases of CRPV infection have yet occurred in Thailand. The data supporting alternative therapies for CRPV are scanty. A small trial of Malarone combined with primaquine in Indonesian New Guinea may be the best available evidence of the good efficacy of this agent against CRPV.

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