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APPLICATION NUMBER:
NDA 21-799

MICROBIOLOGY REVIEW(S)

MICROBIOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 21-799

REVIEWER : Kalavati Suvarna
CORRESPONDENCE DATE : 10-13-04
CDER RECEIPT DATE : 10-19-04
REVIEW ASSIGN DATE : 10-19-04
REVIEW COMPLETE DATE : 07-11-05

SPONSOR: United Research Laboratories, Inc.
Mutual Pharmaceutical Company, Inc.
1100 Orthodox Street,
Philadelphia, PA 19124.

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Anti-parasitic

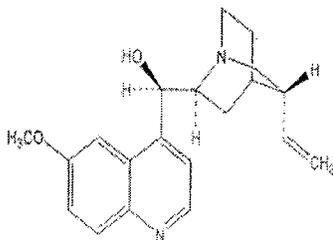
INDICATION: Treatment of uncomplicated *P. falciparum* malaria.

DOSAGE FORM: Oral capsule

PRODUCT NAMES:

- a. **PROPRIETARY:** None
- b. **NONPROPRIETARY:** Quinine sulfate
- c. **CHEMICAL:** Cinchonan-9-ol, 6'-methoxy-, (8 α ,9R)-, sulfate (2:1) (salt), dehydrate
CAS Number: 6119-70-6

STRUCTURAL FORMULA:



Molecular weight: 782.96

Empirical Formula: (C₂₀H₂₄N₂O₂)₂ · H₂SO₄ · 2H₂O

SUPPORTING DOCUMENTS: IND 67, 012

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY	3
2. INTRODUCTION AND BACKGROUND.....	5
2.1. Biology of the parasite.....	6
3. PRECLINICAL MICROBIOLOGY	7
3.1. Mechanism of Action.....	7
3.1.1. Binding of quinine with heme or hemazoin	7
3.1.2. Effect of quinine on heme polymerase, hemazoin content, and accumulation of hemoglobin within <i>Plasmodium</i>	8
3.1.3. Effect of quinine on degradation of heme <i>in vitro</i>	13
3.1.4. Effect of quinine on electrical conductivity of parasitized erythrocytes	14
3.1.5. Effect of heme-quinine complex on lipid peroxidation	15
3.1.6. Effect of quinine on nucleic acid and protein synthesis and on glycolysis in <i>P. falciparum</i>	15
3.1.7. Ability of quinine to inhibit tumor necrosis factor-alpha (TNF- α)	16
3.2. Activity <i>In Vitro</i>	19
3.2.1. <i>In vitro</i> activity against laboratory strains of <i>P. falciparum</i>	19
3.2.2. <i>In vitro</i> activity against clinical isolates of <i>P. falciparum</i> from different geographical regions	21
3.2.3. Activity of metabolite against <i>P. falciparum</i>	23
3.2.4. Effect of quinine on the different erythrocytic stages of <i>P. falciparum</i>	24
3.3. Activity <i>In Vivo</i>	25
3.3.1. <i>P. berghei</i>	25
3.3.2. <i>P. yoelii</i>	25
3.3.3. <i>P. chaubaudi</i>	25
3.3.4. <i>P. cynomolgi</i>	27
3.4. Drug Resistance	27
3.4.1. <i>In vivo</i>	28
3.4.2. Correlation between <i>in vitro</i> susceptibility and genotyping.....	29
3.5. Cross-Resistance	33
3.5.1. <i>In vitro</i>	33
3.5.2. <i>In vivo</i>	35
3.5.3. Clinical significance of cross-resistance to mefloquine.....	35
3.6. Drug Combination.....	36
3.6.1. <i>In vitro</i>	36
3.6.2. <i>In vivo</i>	38
4. CLINICAL MICROBIOLOGY	38
4.1. Description of clinical studies	39
4.2. Interpretive criteria	40
5. DISCUSSION.....	45
6. THE LABEL.....	48
6.1. Sponsor's Proposed Label	48
6.2. Comments	48
6.3. FDA's version of the label.....	50
7. REFERENCES	50
8. RECOMMENDATIONS.....	55

1. EXECUTIVE SUMMARY

The subject of this NDA is quinine sulfate (2x 648 mg capsule administered thrice a day orally for 7 days) for the treatment of uncomplicated *Plasmodium falciparum* malaria.

Mechanism of Action:

Quinine is an alkaloid derived from the cinchona bark. It inhibits nucleic acid and protein synthesis and eventually glycolysis in *P. falciparum*. Quinine can interact with hemazoin in parasitized erythrocytes at a concentration of 0.02 µg/ml. At a higher concentration (18.7 µg/ml), quinine inhibits heme polymerase activity in lysates of parasitized erythrocytes. This concentration is 27 fold greater than that required for inhibition of parasite growth. *In vitro*, quinine-hemazoin complex can lead to lipid peroxidation in liposomes. However, quinine did not have an effect on degradation of heme or accumulation of hemoglobin up to a concentration of 2.3 µg/ml. The drug was also shown to inhibit release of tumor necrosis factor-alpha from macrophages, a property that may be important for preventing progression of disease to severe malaria. However, the precise mechanism by which quinine exhibits its antimalarial activity is not well understood.

Activity in vitro:

The life cycle of *P. falciparum* consist of the exo-erythrocytic (hepatic) and erythrocytic stages. Quinine and its major metabolite, 3-hydroxyquinine, exhibit activity against the erythrocytic stages of *P. falciparum*. The activity of quinine was measured against laboratory strains (n = 13) and several clinical isolates (n = 129) from Thailand, Bangladesh and Africa. The antiparasitic activity was measured by incorporation of [³H]-hypoxanthine or by microscopic observations. A majority of these studies were done by incubating the asynchronous parasites with the drug for 24 to 72 hours. The results expressed as 50% inhibitory concentration (IC₅₀) show the quinine IC₅₀ values against the laboratory strains or clinical isolates to be ≤0.68 µg/ml. The metabolite was less active than the parent drug. The activity of other metabolites such as 2'-quininone, O-desmethylquinine and 10, 11-dihydroxydihydroquinine against *P. falciparum* was not examined *in vitro*. Activity of quinine or its metabolite against gametocytes, hepatic stages, and sporozoites of *P. falciparum* was not examined *in vitro*. Quinine is less active than quinidine (the optical isomer of quinine) against *P. falciparum*.

Activity in vivo:

The activity of quinine was measured against the erythrocytic stages of *P. berghei*, *P. yoelii* and *P. chabaudi* in mice and *P. cynomolgi* in monkeys. The activity of quinine against the hepatic stages of *Plasmodium* species was not examined.

In mice infected intraperitoneally with *P. berghei* (chloroquine-resistant or -sensitive strain), the suppressive dose for 50% reduction in parasitemia was 35.44 ± 13.77 mg/kg oral quinine for 4 days. A higher suppressive dose (169 ± 77.11 mg/kg or 285.85 ± 23.22 mg/kg for 4 days) was required in mice infected with *P. berghei* strain resistant to mefloquine or quinine. However, in mice infected intraperitoneally with the *P. yoelii* strain (chloroquine-, quinine- and mefloquine-resistant), the suppressive dose was same as for the *P. berghei* sensitive strain (47.33 ± 2.31 mg/kg quinine for 4 days). The activity of quinine in mice infected intravenously with *P. chabaudi* (chloroquine-resistant or -sensitive strain) was similar to that observed with the *P. berghei* strains. Recrudescence at day 28 was not examined. Also, no information was available on parasite clearance times.

In monkeys (n = 2) infected intravenously with *P. cynomolgi*, quinine at a dose of 31.6 mg/kg/day for 7 days cleared parasitemia and cured the monkeys. No recrudescence was observed during an additional 30 day follow-up period after monkeys were splenectomized.

Resistance:

Studies *in vivo* show that there is a potential for resistance development by *P. falciparum* to quinine. Cases of *P. falciparum* with clinical resistance to quinine have been reported in areas of South America, Southeast Asia, and Bangladesh.

Cross-resistance:

Cross-resistance between quinine and mefloquine was observed in some strains and isolates of *P. falciparum*. However, mefloquine was successful in the treatment of patients who showed RI or RII response to quinine therapy. Data on effectiveness of mefloquine in patients with RIII response to quinine therapy was not available.

Drug combination

The activity of quinine in combination with quinidine was additive against *P. falciparum in vitro*. The *in vitro* activity of quinine in combination with artemisinin varied from antagonistic to synergistic against different strains of *P. falciparum*.

A combination of quinine with clindamycin was synergistic against *P. falciparum in vitro* and against *P. chaubaudi in vivo*. The combination of quinine with chloroquine was additive against the chloroquine-resistant *P. falciparum* strain and antagonistic against the chloroquine-sensitive *P. falciparum* strain *in vitro*. However, the response was reversed when the activity of this combination was measured against chloroquine-resistant and chloroquine-sensitive *P. chaubaudi* strains *in vivo*.

Clinical Microbiology

Eleven randomized studies evaluated the efficacy of quinine (10 mg/kg TID for 7 days) in 382 patients with *P. falciparum* malaria. These patients were from different geographical areas such as Philippines, Thailand, Congo, Venezuela, Bangladesh, Vietnam, and Gabon. The parasitological evaluations were performed using Giemsa stained thin and/or thick smears. Absence of parasitemia was observed in 79% to 100% of the patients treated with quinine. The studies also show quinine to be effective in reducing parasitemia in patients from Bangladesh and Thailand, regions known to be resistant to chloroquine and sulfa-pyrimethamine. A study showed that clearance of gametocytes was slower compared to clearance of asexual parasite in quinine treated patients. The 3 day regimen with the 10 mg/kg dose of quinine was evaluated only in combination with other drugs.

Attempts were made to distinguish recrudescence from new infection using baseline and post-treatment blood samples in patients failing treatment using the polymerase chain reaction (PCR) assay in one study. However, details of the method and results were not included. Of the 10 patients who developed parasitemia after initial clearance with quinine treatment, 1 patient was classified as a new infection. The rate of recrudescence (RI resistance) in the other studies was 16%. Based on limited data, it is difficult to conclude whether the patients had a recrudescence or new-infection. Therefore, all patients developing parasitemia after treatment were considered as failures.

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Quinine in combination with tetracycline or clindamycin was more effective than quinine alone in two studies performed in Thailand by the same investigator. The parasitological cure rates in patients treated with quinine plus tetracycline was >95% and in those treated with quinine plus clindamycin was 88%. Five additional studies evaluated the combination of quinine with tetracycline or quinine with clindamycin but did not have a quinine monotherapy arm for comparison. It appears that quinine in combination with tetracycline or clindamycin may be useful in areas that have known quinine resistance.

2. INTRODUCTION AND BACKGROUND

The subject of this NDA is quinine sulfate capsules for the treatment of uncomplicated *Plasmodium falciparum* malaria. The sponsor has proposed a dosage of two 648 mg quinine sulfate capsules thrice a day, for 7 days.

Quinine is available in the US since pre-1938 and has been approved for the treatment of malaria in several countries outside the US such as Australia, France, Denmark, Germany, Netherlands, New Zealand and Sweden.

Quinine is an alkaloid derived from the cinchona bark. It is rapidly absorbed following oral administration, with bioavailability of approximately 76 to 88%. The CSF concentrations are 2 to 7% of the plasma concentration. Approximately, 69 to 95% of quinine is bound to proteins. Following a single dose administration of 8.7 mg/kg quinine sulfate to healthy subjects, the mean maximum plasma concentration (C_{max}) was 3.24 ± 0.69 $\mu\text{g/ml}$. The mean time to maximum plasma concentration (T_{max}) was 2.80 ± 0.82 hours and the area under the concentration versus time curve (AUC) was 27.99 $\mu\text{g}\cdot\text{h/ml}$. The plasma elimination half-life for quinine is about 10 to 11 hours in healthy subjects. The T_{max} , C_{max} , and AUC in malaria patients administered a single dose of 10 mg/kg quinine sulfate was approximately twice that observed in healthy subjects. The Mutual Pharma's quinine sulfate product was found to be equivalent to the product manufactured by the Government Pharmaceutical Company (GPO, Bangkok, Thailand) and used in clinical studies cited in the NDA.

Table 1: Pharmacokinetic Parameters [(Mean \pm SD (Range))] of quinine in healthy subjects and patients with uncomplicated *P. falciparum* malaria after a single dose^a of oral quinine sulfate

Pharmacokinetic parameter	Healthy subjects* (N=23)	Uncomplicated <i>P. falciparum</i> malaria patients* (N = 15)
Tmax (h)	2.80 \pm 0.82	5.9 \pm 4.7 (3.5 - 8.4)
Cmax ($\mu\text{g/ml}$)	3.24 \pm 0.69	8.4 (7.3 - 9.4)
AUC ₀₋₁₂ ($\mu\text{g}\cdot\text{h/ml}$)	27.99	73.0

*The healthy subjects got a fixed dose (~ 8.7 mg/kg based on the mean BW) whereas the patients got a 10mg/kg dose of oral quinine sulfate

There are 4 primary metabolites of quinine, 3-hydroxyquinine, 2'-quininone, O-desmethylquinine, and 10, 11-dihydroxydihydroquinine. 3-Hydroxyquinine is the major metabolite, with peak levels and AUCs approximately 10% of the parent drug. The activity of this metabolite against *P. falciparum* was measured *in vitro*. The activity of metabolites other than 3-hydroxyquinine were not tested *in vitro* or *in vivo*.

2.1. Biology of the parasite:

The four *Plasmodium* species that cause malaria in humans are *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. The life cycle of the *Plasmodium* species involves 2 phases (Figure 1), an asexual phase in a human host and a sexual phase in an insect vector (female mosquitoes of the genus *Anopheles*). Human infection is initiated by the bite of an infected mosquito and sporozoites are inoculated into the blood stream. Within 2 hours, the sporozoites migrate to the liver and penetrate the hepatocytes. After 1 to 2 weeks, the sporozoites undergo nuclear division to produce thousands of merozoites that are released by the rupture of the host cell. This part of the life cycle is called the **exoerythrocytic or hepatic schizogonic cycle**. The merozoites invade erythrocytes and initiate the **erythrocytic cycle**, which takes about 24 to 72 hours. Within the red blood cells (RBCs), the merozoites mature into trophozoites and schizonts that multiply asexually to form several merozoites. These merozoites can invade other erythrocytes upon rupture of the RBCs. The rupture of infected RBCs is accompanied by fever. Some merozoites mature into male microgametocytes or female macrogametocytes. The gametocytes are ingested by the mosquito during a blood meal. Maturation of gametocytes occurs within the female mosquito. The gametocytes then undergo fertilization to form a zygote and after 12 to 48 hours elongate into an ookinete. The ookinete penetrates the gut and develops into an oocyst. Formation of the oocyst takes approximately 2 days after the mosquito has taken a blood meal. The oocyst enlarges, undergoes nuclear division and ruptures to release thousands of sporozoites. These sporozoites migrate to the salivary gland and are inoculated into humans the next time the mosquito draws a blood meal.

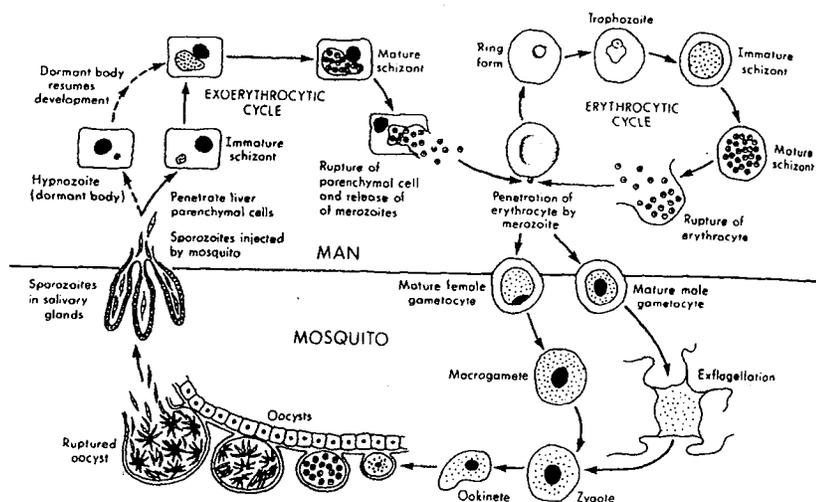


FIGURE 1 Life cycle of the malaria parasite. (Reproduced with permission from D. J. Krogstad and M. A. Pfaller, Prophylaxis and treatment of malaria, *Curr. Clin. Top. Infect. Dis.* 3:56-73, 1983.)

Patients with *P. vivax* or *P. ovale* malarias often suffer from **relapse**. This is thought to be due to activation of dormant parasites (**hypnozoites**) in the liver. In the case of *falciparum* malaria, recurrence is generally thought to be due to the presence of residual erythrocytic forms, a phenomenon called as **recrudescence**.

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3. PRECLINICAL MICROBIOLOGY

3.1. MECHANISM OF ACTION

The mechanism of action of quinine is thought to be due to inhibition of hemazoin formation/degradation, thereby killing the parasite by preventing detoxification of heme. Chloroquine, an approved antimalarial, is thought to have a similar mechanism of action. The ability of quinine to bind with heme or hemazoin and its effect on heme polymerase, hemazoin, and haemoglobin levels, glutathione-dependent degradation of heme, electrical conductivity of erythrocytes, lipid peroxidation, nucleic acid synthesis, protein synthesis, glycolysis, and release of tumor necrosis factor-alpha (TNF- α) from macrophages, were examined.

Plasmodium requires amino acids for synthesis of protein. One of the sources of amino acid is haemoglobin (Hb), the protein found in abundance in the erythrocyte. The digestion of Hb by aspartic, cysteine, and metallo proteases in the food vacuole of the parasite results in release of heme which is toxic to the parasite. Detoxification of heme can occur by polymerization to hemozoin or degradation of heme mediated by hydrogen peroxide within food vacuoles, or glutathione dependent degradation in the parasite's cytoplasm. Antimalarial agents such as chloroquine can interfere with this detoxification process.

3.1.1. Binding of quinine with heme or hemazoin

The ability of quinine to bind with oxidized heme (haemin) was examined *in vitro*¹. The binding was measured spectrophotometrically using benzene-soluble complexes of hemin with quinine or other antimalarials. Controls consisted of antimalarial drugs or hemin alone. The formation of a complex between quinine and hemin leads to loss of the typical ultraviolet absorption peak of quinine at 335 nm and increase in peaks α , β , and γ at 602, 490, and 408 nm, respectively, indicating formation of a quinine hemichrome coordination complex (Table 2). Similar observation was made with mefloquine and other experimental antimalarial drugs. However, no hemichrome complex was observed with the antimalarially inactive 9-epimer of quinine, epiquinine.

Table 2: Benzene-soluble complexes of antimalarials and hemin.

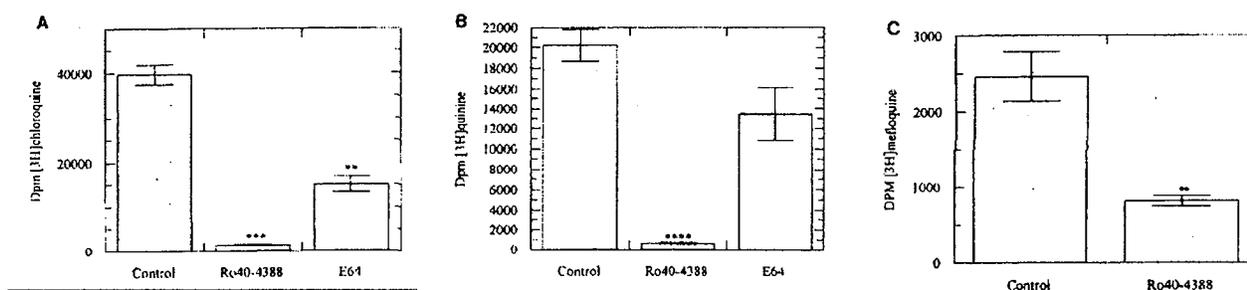
Drug	Wavelength of peak (nm)			O.D. γ peak	E(mM)*			[Drug] [Haemin]
	α	β	γ		α	β	γ	
Quinine	602	490	408	4.1	7.9	9.6	79.0	0.8
Cinchonine	605	490	409	2.9	9.6	11.3	92.6	1.3
RO 21,0960	605	492	408	2.0	5.8	6.4	58.4	1.2
Epiquinine	—	—	408	0.1	—	—	—	—
Mefloquine	608	495	408	3.2	9.8	11.7	102.0	1.3
WR 177,602	611	496	410	2.8	8.4	10.3	80.8	1.1
WR 122,455	608	495	410	2.7	5.5	6.3	52.1	0.8

* As haemin. Haemin was measured as the pyridine haemochrome [14].

The binding of quinine, chloroquine or mefloquine with hemazoin was examined in the presence or absence of Ro40-4388 (inhibitor of malaria plasmepsin I), and E64 (a nonselective inhibitor of cysteine proteinase)². Ring stages of the HB3 or K1 strains of *P. falciparum* were incubated with radiolabelled drug in the presence or absence of proteinase inhibitors Ro40-4388 (300 nM) or E64 (10 μ M) for 24 hours. The hemazoin was purified and radioactivity measured in the scintillation

counter. Incubation of the ring stage with radiolabelled quinine, chloroquine or mefloquine results in binding of the drug with hemazoin (Figure 2). However, the proteinase inhibitor Ro40-4388 reduced binding of quinine and chloroquine with hemazoin by >95%. The cystiene proteinase E64 reduced binding of quinine with hemazoin by 40% and the binding of chloroquine with hemazoin by 60%. The results suggest that quinine and other antimalarials (chloroquine and mefloquine) bind with hemazoin (heme polymers) in parasitized erythrocytes. The authors suggest that quinine and other antimalarial drugs may compete for the same binding sites in heme as the proteinase inhibitors Ro40-4388 or E64 since their activities are antagonistic in combination.

Figure 2. (A) Incorporation of [3 H] chloroquine into hemozoin in the presence or absence of an inhibitor of plasmepsin I, Ro40-4388 (300 nM), or of cysteine proteinase, E64 (10 μ M). Data represent means \pm standard deviations of five separate experiments; each experiment was performed in triplicate with 2×10^9 parasitized erythrocytes. ** $P < 0.05$, *** $P < 0.005$. (B) Incorporation of [3 H] quinine into hemozoin in the presence or absence of Ro40-4388 (300 nM), or E64 (10 μ M). Data represent means \pm standard deviations of five separate experiments; each experiment was performed in triplicate with 2×10^9 parasitized erythrocytes. **** $P < 0.0001$. (C) Incorporation of [3 H] mefloquine into hemozoin in the presence or absence of Ro40-4388 (300 nM). Data represent means \pm standard deviations of four separate experiments; each experiment was performed in triplicate with 3×10^9 parasitized erythrocytes. ** $P < 0.05$.



3.1.2. Effect of quinine on heme polymerase, hemazoin content, and accumulation of hemoglobin within *Plasmodium*

The ability of quinine and other antimalarials to inhibit heme polymerase I (HPAI) activity in the chloroquine-sensitive NYU-2 strain of *P. berghei* or chloroquine-resistant *P. berghei* strain derived from NYU-2 was examined *in vivo*³. The basis for classifying the strain as chloroquine-resistant was not specified. Swiss mice were infected intraperitoneally with 10^6 parasitized erythrocytes. Parasitemia was determined by examining 1000 erythrocytes in Geimsa stained blood smear. The sponsor has stated that the mice were treated with antimalarials (chloroquine, quinacrine, amodiaquine, ampyroquine, quinine, mefloquine) intraperitoneally when parasitemia values reached between 800 and 2000 parasites per 1000 erythrocytes. It is unclear if the parasitized erythrocytes and normal erythrocytes were counted separately. The parasitemia per μ l of blood was not reported. Control animals received solvent used to dissolve the drugs i.e., saline or mixture of ethanol:saline (1:2). Six hours after administration of the drug, mice were killed and blood sample collected and lysed. The lysate was incubated in the presence of acetate (acetate inactivates heme polymerase II) and exogenous heme at 37°C for 4 hours and the increase in the amount of hemazoin (polymerized heme) reflective of HPAI activity determined using a spectrophotometer. The HPAI activity was expressed as nanomoles of heme polymerized per hour per ml of packed erythrocytes, normalized to represent a parasitemia of 1,000 parasites per 1,000 erythrocytes. The results in Table 3 show that in the absence of drug, HPAI activity of

the chloroquine-resistant *P. berghei* strain was less than that of the chloroquine-sensitive *P. berghei* strain. Quinine (6 μ M or 1.4 μ g/ml) and mefloquine (3 μ M) do not inhibit HPAI while chloroquine, quinacrine, amodiaquine and amopyroquine inhibit the HPAI activity of both strains.

Table 3: Effect of treatment with antimalarial drugs on HPAI

Drug ^a	Dose ^b	HPAI activity ^c (no. of expts.) in:	
		CS <i>P. berghei</i>	CR <i>P. berghei</i>
None	0	541 \pm 42 (12)	284 \pm 19 (16)
Chloroquine	3	51 \pm 19 (8)	124 \pm 11 (6)
Quinacrine	3	47 \pm 34 (3)	138 \pm 22 (3)
Amodiaquine	4	47 \pm 8 (3)	163 \pm 18 (3)
Amopyroquine	5	87 \pm 27 (3)	156 \pm 11 (3)
Quinine	6	487 \pm 71 (5)	275 \pm 13 (3)
Mefloquine	3	525 \pm 132 (4)	254 \pm 11 (3)

^a The drugs were injected intraperitoneally 6 h before the mice were killed to obtain blood for the measurement of heme polymerizing activity.

^b The dose is given in micromoles of drug injected per mouse.

^c For CS *P. berghei*, pertinent data from a previous report (6) were recalculated to express heme polymerizing activity as a function of parasitemia instead of as a function of preformed hemozoin to facilitate comparisons with CR *P. berghei*.

^d Means \pm SD are shown. Activity is expressed as nanomoles of FP polymerized per hour per milliliter of packed erythrocytes normalized to represent a parasitemia of 1,000 parasites per 1,000 erythrocytes.

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The effect of quinine and other antimalarials on heme polymerase activity and level of hemozoin and free-heme was examined by the same authors in another study⁴. For this, mice were infected intraperitoneally with the chloroquine-sensitive NYU-2 strain of *P. berghei*. Six days after infection, the mice were treated with quinine or other antimalarial drugs. The blood was collected 6 hours after administration of drug and lysate of parasitized erythrocytes prepared for analysis of hemozoin and free heme content. The lysate was also used to measure heme polymerase activity as described in the previous study. The results in Table 4 show that quinine (6 μ M = 1.4 μ g/ml) did not have an effect on heme polymerase activity, amount of hemozoin, or amount of free heme. Similar observations were made with mefloquine. However, inhibition of heme polymerase and increase in free heme was observed with chloroquine (3 μ M). When higher concentrations of quinine were tested, a 50% inhibition of heme polymerase was observed (80 μ M = 18.7 μ g/ml; see Figure 3). Quinine (6 μ M = 1.4 μ g/ml) was also shown to reverse the effect of chloroquine when administered in combination, thereby suggesting that the mechanism by which the two drugs exhibit antiplasmodial activity is different (Table 5).

Table 4: Specificity of the control of heme polymerase by antimalarial drugs.

Drug	Dose ^a	FP ^b	Hemozoin ^c	Heme Polymerase ^d
None	None	40 \pm 16 (16) ^e	1,055 \pm 247 (16)	238 \pm 40.5 (9)
Quinine	6	69 \pm 17 (5)	1,510 \pm 416 (5)	273 \pm 38.6 (5)
Mefloquine	3 - 5	70 \pm 24 (4)	913 \pm 149 (4)	286 \pm 65.3 (4)
Primaquine	3	58 \pm 28 (4)	1,088 \pm 479 (4)	263 \pm 34.6 (4)
WR 88685	4 - 6	57 \pm 27 (3)	1,490 \pm 127 (3)	207 \pm 37.4 (3)
WR 54470	5 - 6	41 (2)	1,425 (2)	239 (2)
Chloroquine	3	123 \pm 39 (11)	798 \pm 111 (11)	36.6 \pm 12.9 (9)
Quinacrine	3	129 \pm 22 (3)	697 \pm 107 (3)	33.2 \pm 24.5 (3)
Amodiaquine	4	186 \pm 29 (3)	930 \pm 121 (3)	33.1 \pm 5.8 (3)
Amopyroquine	5	236 \pm 23 (3)	1,151 \pm 134 (3)	61.7 \pm 19.2 (3)
SN 10274	3 - 4	141 \pm 39 (3)	1,018 \pm 215 (3)	78.1 \pm 22.6 (3)
SN 11438	4	150 \pm 38 (3)	1,068 \pm 51 (3)	47.0 \pm 9.7 (3)
WR 29623	6	157 \pm 58 (3)	1,125 \pm 105 (3)	50.8 \pm 19.6 (3)

^a The dose is given in μ moles of drug injected per mouse.

^b Nanomoles of nonhemozoin FP per ml of packed, parasitized erythrocytes, corrected for differences in parasitemia as follows: the amount of FP per ml of packed erythrocytes was divided by the number of parasites per 1000 erythrocytes and multiplied by 1000.

^c Nanomoles of preformed hemozoin FP per ml of packed, parasitized erythrocytes corrected for parasitemia as described for nonhemozoin FP.

^d Nanomoles of FP incorporated into hemozoin per hour per μ mole of FP in preformed hemozoin.

^e Means \pm S.D. and number of experiments are shown.

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Figure 3: Inhibition of heme polymerase by antimalarial drugs in vitro. The incubation mixture consisted of 0.2 ml of lysate prepared from a 24 percent suspension of washed, parasitized erythrocytes, 50 μ M pepstatin A, 100 μ M trans-epoxysuccinyl-L-leucylamido (4-guanidino)-butane, 150 μ M heme, 75 μ M sodium acetate (pH 5.0), and varying concentrations of chloroquine (\bullet), amodiaquine (o), quinine (x), and mefloquine (Δ) in a total volume of 2 ml. The incubation was conducted at 37°C for 4 hours.

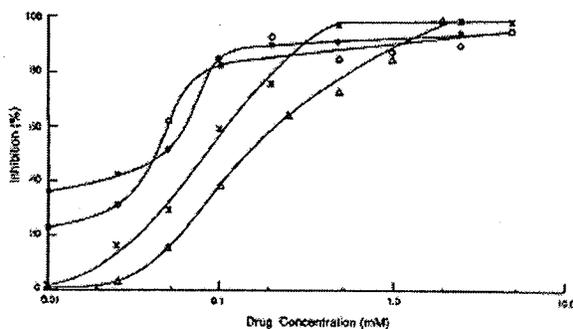


Table 5: Reversal of the effect of chloroquine on heme polymerase by quinine.

Time (Hours)	Chloroquine ^a	Chloroquine Quinine ^b	Chloroquine Quinine (twice) ^c	Chloroquine Mefloquine ^d
	(Heme Polymerase Activity) ^e			
0	272 \pm 20 ^f	267 \pm 17	254 \pm 27	254 \pm 11
2	106 \pm 2	340 \pm 30	324 \pm 46	312 \pm 35
4	71 \pm 6	175 \pm 14	376 \pm 67	---
6	58 \pm 6	129 \pm 36	370 \pm 41	348 \pm 29

^a 3 μ moles per mouse were injected intraperitoneally at 0 hour.

^b 3 μ moles of chloroquine and 6 μ moles of quinine per mouse were injected simultaneously intraperitoneally at 0 hour.

^c 3 μ moles of chloroquine and 6 μ moles of quinine per mouse were injected simultaneously intraperitoneally at 0 hour plus another 6 μ moles of quinine were injected intraperitoneally 2 hours later.

^d 3 μ moles of chloroquine and 4.8 μ moles of mefloquine per mouse were injected simultaneously intraperitoneally at 0 hour.

^e Nanomoles of FP incorporated into hemozoin per hour per μ mole of FP in preformed hemozoin.

^f Means \pm S.D. for three experiments are shown for each treatment.

The effect of quinine and other antimalarials on the level of hemozoin in 5 strains of *P. falciparum* was examined *in vitro*⁵. For this, trophozoites were incubated in the presence of 10 μ M drug for 4 hours and content of hemozoin and membrane associated heme determined spectrophotometrically at 345 nm. Hemozoin content was also determined in control infected cells prior to drug exposure and after incubation without drug. Quinine (10 μ M = 2.3 μ g/ml) and other antimalarials inhibited hemozoin formation but had no effect on membrane associated heme compared to chloroquine which inhibited hemozoin formation and increased level of membrane associated heme (Figures 4 and 5). The inhibition of parasite was also measured by uptake of radiolabeled hypoxanthine (Table 6). The inhibition of growth correlated with inhibition of hemozoin formation.

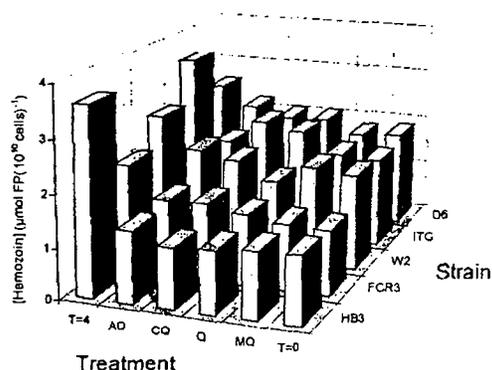


Figure 4: Effect of drugs on the levels of hemazoin in different strains, as affected by various drugs. Results shown here and in Figure 4 are representative of several identical experiments (at least twice for each strain and up to six times for some strains). Cultures at the trophozoite stage of various strains were incubated at 4 hour under culture conditions in the presence of 10 mM of amodiaquine (AQ), chloroquine (CQ), quinine (Q), and mefloquine (MQ). Infected cells were then processed for the determination of hemozoin levels and parasite viability. Control at the beginning (T=1) and end of the incubation (T= 4) were similarly analyzed. Inhibition of parasite growth (as % of untreated controls) are given in Table 6)

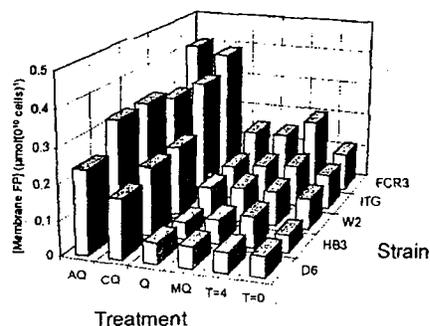


Figure 5: Effect of drugs on the levels of membrane-associated heme (FP) in different strains, as affected by various drugs. The same cultures used for the determination of hemazoin and parasite viability depicted in Figure 3 were used for the determination of membrane-associated heme.

Table 6: Inhibition of parasite growth by various drugs and are related in data presented in Figures 4 and 5.

Inhibition of parasite growth by various drugs ^a				
Strain	CQ	AQ	Q	MQ
FCR3	82.3	74.8	47.4	71.3
HB3	77.8	72.5	75.8	70.1
W2	46.9	52.5	57.1	63.5
D6	53.2	50.7	75.1	68.9
ITG	53.1	63.9	84.8	68.4

^aexperimental details are described in the legend to Figure 4

The effect of quinine and other antimalarials on accumulation of hemoglobin was examined⁶. For this, synchronized cultures of *P. falciparum* in the trophozoite stage (15-20% parasitemia) were exposed to different antimalarials for 3 or 4 hour at 37°C. The parasites were lysed and cytoplasmic fraction recovered for electrophoresis in 10% SDS-PAGE gel. A mixture of 0.1% *O*-dianisidine chloride and 20 mM hydrogen peroxide in 40 mM citric acid was used to stain heme. The staining of free heme and heme in association with monomers of hemoglobin (molecular mass 16.5 – 17 kDa) from normal erythrocyte lysates is shown in Figure 6. Untreated parasitized erythrocytes served as controls and did not show any stained bands, suggesting that hemoglobin is degraded. In the presence of chloroquine (CQ) and amodiaquine (AQ) inhibition of hemoglobin degradation (stainable bands of heme) was observed. Cells exposed to quinine and mefloquine do not show the stainable band of heme, suggesting that hemoglobin is degraded as in untreated controls.

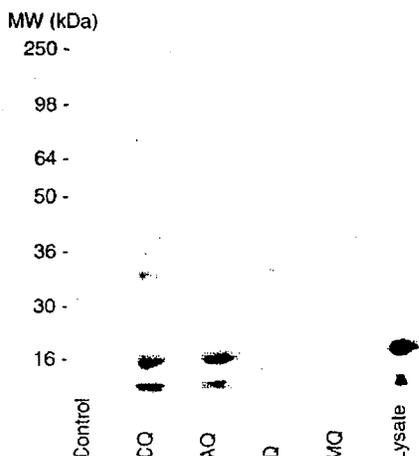


Figure 6: Effect of drug treatment on the accumulation of hemoglobin in parasite lysate. Cultures were treated with 10 mM of different drugs for 3 hours and free parasite were obtained. Samples of parasite cytoplasm and of lysate of normal erythrocytes were run on SDS-PAGE (10% polyacrylamide) omitting dithiotretol from the running buffer in order to avoid destruction of heme. After electrophoresis, gels were fixed for 20 minutes with 12.5% trichloroacetic acid and washed for 20 minutes in distilled water and stained for heme. The band at the running front is heme. CQ = chloroquine, AQ = amodiaquine, Q = quinine, MQ = mefloquine.

In addition, the hemoglobin content was determined by measuring the absorption spectrum of the parasite lysate at 412 nm and the inhibition of parasite growth was measured by uptake of radiolabeled hypoxanthine. In contrast with chloroquine (see Figures 7a and 8a), the antimalarial activity of quinine and mefloquine was not associated with accumulation of haemoglobin (Figures 7b, 7c, 8b, and 8c).

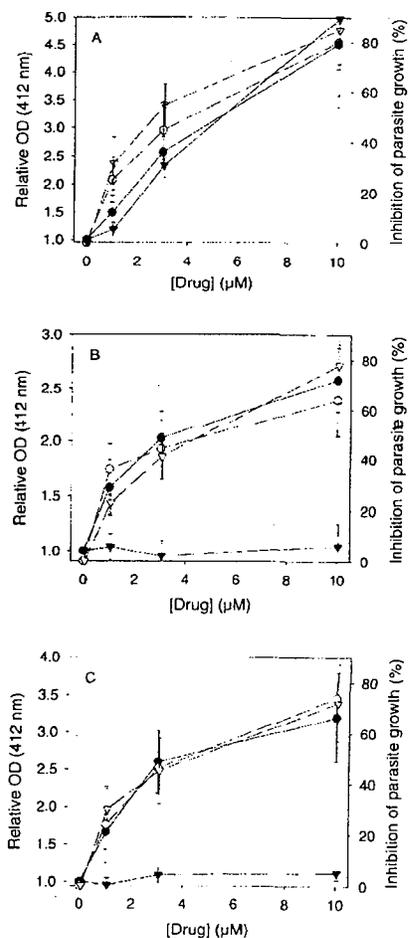


Figure 7: Dose-dependent effect of drugs on hemoglobin accumulation in parasite lysate. Full lines and filled symbols describe relative hemoglobin concentration (in absorbance units compared to zero drug concentration); broken lines and empty symbols depict % inhibition of parasite growth. (A) chloroquine (circles) and amodiaquine (inverted triangles); (B) chloroquine (circles) and quinine (inverted triangles); (C) chloroquine (circles) and mefloquine (inverted triangles);

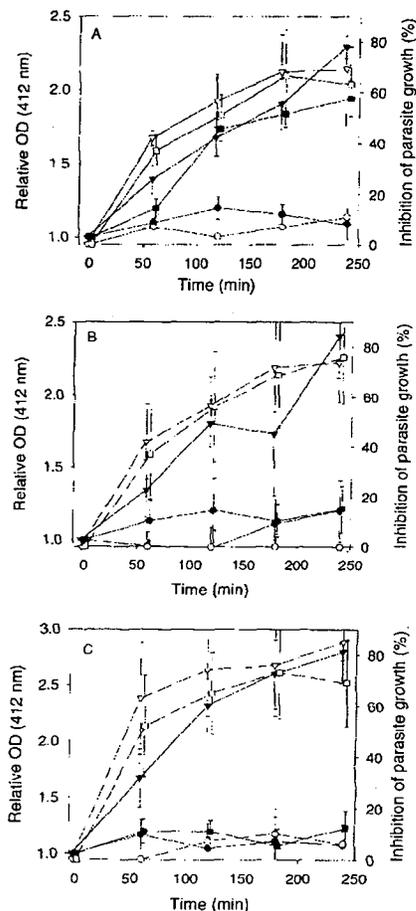


Figure 8: Time dependence of drug action on hemoglobin accumulation. Full lines and filled symbols describe relative hemoglobin concentration (in absorbance units compared to time zero); broken lines and empty symbols depict % inhibition of parasite growth. (A) Control (circles), chloroquine (inverted triangles) and amodiaquine (squares); (B) control (circles), chloroquine (inverted triangles) and quinine (squares); (C) control (circles), chloroquine (inverted triangles), and mefloquine (squares).

3.1.3. Effect of quinine on degradation of heme *in vitro*

Heme can be degraded in the presence of reduced glutathione (GSH). The effect of quinine and other antimalarials on inhibition of GSH-dependent degradation of heme was examined⁷. For this, free heme and GSH in HEPES buffer pH 7.0 with or without bovin serum albumin (BSA) were incubated in the presence or absence of drug at 37°C and the degradation of heme followed spectrophotometrically at 396 nm at 10 to 60 second intervals. Also, ghosts of erythrocytes obtained by hypotonic lysis were incubated with heme for 7 minutes at 37°C. The heme loaded ghosts were incubated with GSH in the presence or absence of drug at 37°C. At different time points, the membranes were pelleted and absorbance of heme determined spectrophotometrically. The results in Table 7 show that although quinine has minimal effect on GSH dependent degradation of heme in aqueous solution, it has no effect on GSH dependent heme degradation in membranes. The authors suggest that this may be due to quinine being a weak base and less efficient at dissolution of heme from membrane.

Quinine
Mutual Pharmaceuticals

Table 7: Constants of inhibition (K_i) of GSH-mediated destruction of heme *in vitro* by various drugs in HEPES buffer, HEPES buffer with BSA, and in membranes (erythrocyte ghosts).

Drug	HEPES	HEPES-BSA	Ghost
Chloroquine	1.66 ± 0.31	1.13 ± 0.07	0.85 ± 0.08
Amodiaquine	0.88 ± 0.16	0.55 ± 0.08	0.68 ± 0.13
Quinine	7.32 ± 0.47	14.41 ± 1.60	No inhibition
Mefloquine	1.44 ± 0.02	7.26 ± 0.57	>50
Halofantrine	0.95 ± 0.11	0.79 ± 0.14	0.95 ± 0.15
Pyronaridine	0.72 ± 0.05	2.69 ± 0.37	0.89 ± 0.13
Q2-93	0.3 ± 0.1	0.3 ± 0.1	ND
Q1-17	1.0 ± 0.15	0.77 ± 0.08	ND
8AC6	9.0 ± 0.5	16.7 ± 2.4	ND

3.1.4. Effect of quinine on electrical conductivity of parasitized erythrocytes

The electrical conductivity of erythrocytic membranes infected with *P. falciparum* trophozoites from a patient with cerebral malaria was examined before and after quinine (10 mg/kg at 8 hours interval) therapy⁸. The parasitemia before therapy was 27%. The membrane conductivity of parasitized erythrocytes was higher than normal erythrocytes prior to quinine therapy (Figure 9). The conductivity reduced after quinine therapy. The reduction in conductivity correlated with reduction in parasite density. The authors have suggested that quinine may block ion transport to parasite. However, ion transport in the parasite was not specifically analyzed.

Figure 9

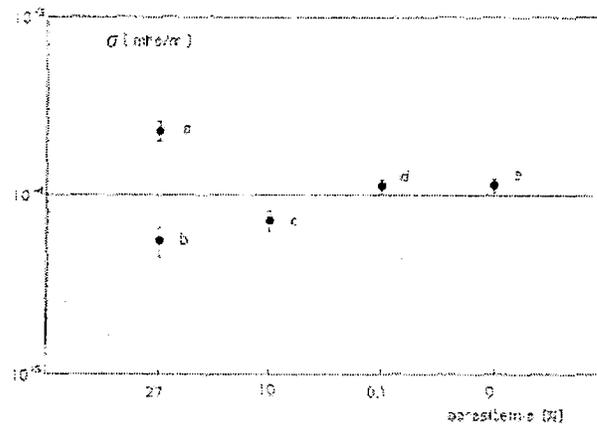


Figure. Electrical conductivity (σ) of *P. falciparum*-infected erythrocyte membranes as a function of parasitaemia: a, membrane conductivity before quinine therapy; b, membrane conductivity after the first dose of quinine; c, d, membrane conductivity during quinine therapy; e, membrane conductivity after stopping quinine.

The horizontal line represents the membrane conductivity of normal erythrocytes. The vertical bars indicate the error estimated from the fitting procedure used to extract the membrane electrical characteristics from the measured conductivity of the erythrocyte suspension.

Fresh blood was obtained by venipuncture from a patient with cerebral malaria. Separation of erythrocytes was carried out by centrifugation at 3000 rpm for 10 min. Plasma and buffy coat were removed, red cells were washed 3 times in isotonic phosphate-buffered saline (5 mM NaH_2PO_4 , pH 7.4, 0.15 M NaCl), and then resuspended at a haematocrit of 0.30.

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3.1.5. Effect of heme-quinine complex on lipid peroxidation

The induction of lipid peroxidation by heme-quinine complex *in vitro* was examined⁹. Liposomes prepared with phospholipids from rat liver microsomes were used. The liposome in Tris-HCL buffer pH 7.4 was mixed with heme-quinine complex and incubated at 37°C with continuous agitation. Heme-mefroquine complex and heme with primaquine were used as controls. It appears that mefloquine is same as mefloquine. The measurement of lipid peroxidation was based on the concentration of malondialdehyde. The results in Figure 10 show that heme-quinine complex increased lipid peroxidation in liposomes.

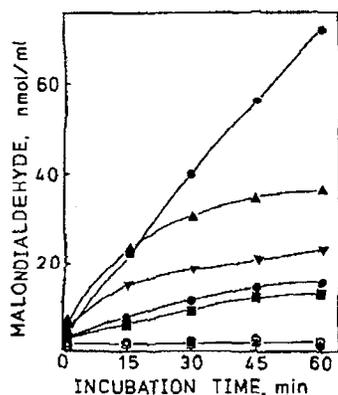


Figure 10: Time course of phospholipid peroxidation induced by the combination of heme with quinine, mefloquine or primaquine. The reactions were initiated by the addition of 25µM heme (⊙), heme (25µM)-quinine (100µM) complex (●), heme (25µM)-mefroquine (100µM) complex (▼), heme (25µM)-mefroquine (375µM) complex (▲), combination of 25µM heme and 100µM primaquine (■), 100µM quinine (○), 100µM mefloquine (Δ) or 100µM primaquine (□).

3.1.6. Effect of quinine on nucleic acid and protein synthesis and on glycolysis in *P. falciparum*.

The effect of quinine on nucleic acid and protein synthesis and on glycolysis in synchronized cultures of *P. falciparum* was examined¹⁰. For effect on nucleic acid inhibition, parasitized erythrocytes (3-4%) in RPMI medium were incubated in the presence of quinine (2.5 µg/ml), chloroquine (0.5 µg/ml) or other antimalarial drugs at 37°C for different time period. [³H]-hypoxanthine was added and incubation continued for an additional 20 hours. Drug-free cultures were used as controls. For effect on protein synthesis, [³H]-leucine was added instead of [³H]-hypoxanthine. For the effect on glycolysis, production of lactate was measured enzymatically based on the method of Holloway (1991)¹¹. The results in Table 8 show that inhibition of nucleic acid and protein synthesis of parasites occurred within 4 hours of exposure to quinine followed by inhibition of lactate production (after 9 hours of exposure).

Table 8

Pharmacodynamic Parameter Estimates *In Vitro* for the Rates of Action of Four Antimalarial Drugs on [³H]Hypoxanthine Uptake, [³H]Isoleucine Uptake, and Lactate Production of Synchronized Cultures of *P. falciparum* Clone ITO4

Drug	Inhibition of [³ H]hypoxanthine incorporation			Inhibition of [³ H]isoleucine incorporation			Inhibition of lactate production		
	T ₅₀ (SD) (hr)	s (SD)	E _{max} (SD) (%)	T ₅₀ (SD) (hr)	s (SD)	E _{max} (SD) (%)	T ₅₀ (SD) (hr)	s (SD)	E _{max} (SD) (%)
Chloroquine	1.8 (0.3)	1.3 (0.2)	58 (1.1)	1.8 (0.2)	1.1 (0.1)	57 (0.4)	11.8	NA	16 (0.4)
Quinine	3.5 (0.1)	1.9 (0.1)	93 (2.9)	4.0 (0.3)	1.9 (0.3)	60 (1.0)	9.3 (0.8)	3.0 (0.8)	38 (3.2)
Artemisinin	4.3 (0.2)	3.1 (0.4)	97 (4.8)	7.0 (0.2)	4.2 (0.6)	73 (0.4)	7.5 (0.3)	2.3 (0.2)	50 (0.1)
NA-artelinate	4.7 (0.1)	2.9 (0.2)	96 (4.2)	7.4 (0.2)	4.3 (0.4)	70 (1.4)	9.7 (0.5)	3.0 (0.5)	57 (0.1)

Note: Drug concentrations used were chloroquine, 500 ng/ml; quinine, 2500 ng/ml; artemisinin, 500 ng/ml; and sodium artemisinate, 500 ng/ml. E_{max} is the maximum inhibition observed, T₅₀ is the drug exposure time to reach 50% of maximum inhibition for each drug, and s is a parameter determining the steepness of the drug exposure time-effect relationship (see Materials and Methods). Values represent the mean (±SD) of three identical experiments, NA, Not available.

3.1.7. Ability of quinine to inhibit tumor necrosis factor-alpha (TNF- α)

The ability of quinine to inhibit release of TNF- α from human alveolar macrophages was examined¹². Macrophages (1×10^6 /ml) were incubated with $1 \mu\text{g/ml}$ of lipopolysaccharide (LPS) for 1 to 4 hours in the presence or absence of quinine. The supernatants were tested for the presence of TNF- α using a cytotoxicity assay with L929 cells. Neutral red was used to stain viable cells. The activity expressed as units per ml was defined as reciprocal of the dilution of the supernatant at which 50% cytotoxicity was observed. The authors stated that $200 \mu\text{M}$ ($46.8 \mu\text{g/ml}$) of quinine correlated with a cytotoxicity of 0.3 U/ml and did not have a significant effect on the experiment. However, the data were not shown. The results in Figures 11 and 12 shows that quinine inhibits TNF- α release from human alveolar macrophages. The inhibition correlated with decrease in TNF- α mRNA levels (Figure 13).

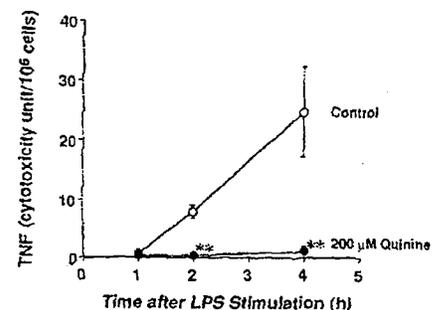


Figure 11: Time course of TNF release induced by LPS, $1 \mu\text{g/ml}$, either in the presence or absence of $200 \mu\text{M}$ quinine. The amount of TNF is expressed as the average cytotoxicity unit \pm SE, which was obtained from supernatants of 10^6 cells stimulated for 1, 2, and 4 hours with $1 \mu\text{g/ml}$ LPS ($n = 5$ to 8). Quinine was applied 5 minutes before LPS stimulation. ** $P < 0.01$, compared with the average cytotoxicity unit obtained from control samples (LPS alone) incubated for the same duration.

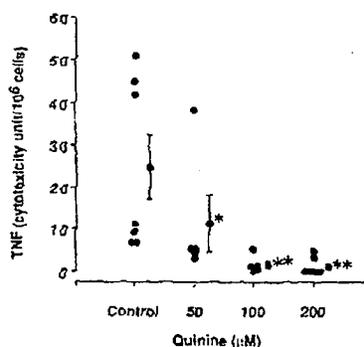


Figure 12: Dose-dependent effect of quinine on TNF activities in supernatants of macrophages stimulated with $1 \mu\text{g/ml}$ LPS for 4 hours. Data are expressed as individual data plots and mean \pm SE of cytotoxicity unit obtained from supernatants of 10^6 macrophages ($n = 5$ to 8). Quinine was applied 5 minutes before LPS stimulation. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively, compared with the average cytotoxicity unit in the absence of quinine.

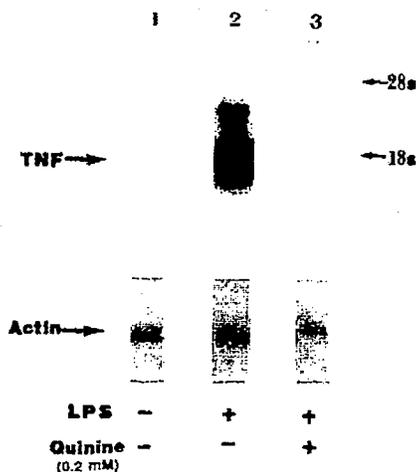


Figure 13: Representative Northern blot analysis of TNF- α mRNA. Alveolar macrophages, except those of control, were stimulated with LPS for 2 h in the presence or absence of quinine. Electrophoresed RNAs from these macrophages were hybridized with the radiolabeled TNF- α cDNA. Upper panel: Lane 1, control (no stimulation with LPS); lane 2, LPS, $1 \mu\text{g/ml}$; lane 3, LPS, $1 \mu\text{g/ml}$, and $200 \mu\text{M}$ quinine. The 18S and 28S indicate ribosomal RNAs, used as internal size markers. Lower panel: The expression of β -actin mRNA in each sample.

In addition to inhibition of TNF- α , the effect of quinine on phagocytosis of latex beads by LPS activated macrophages was examined. The calmodulin inhibitor W-7 was used as a comparator. Quinine did not have an effect on phagocytosis of latex beads. A reduction in phagocytosis (86%) was observed with W-7 (Table 9).

Table 9

*Effects of quinine and W-7 on phagocytosis of latex beads of LPS-activated macrophages**

	Phagocytosis (%)
Control	95.0 ± 1.6
200 μM quinine	93.6 ± 1.4
500 μM W-7	13.6 ± 3.9†

* Data are expressed as mean ± SE (n = 5).

The inhibition of TNF-α production in macrophages by quinine was examined in another study¹³. Chloroquine and artemether were used for comparison. *P. falciparum* lysates were mixed with human peripheral blood mononuclear cells at a 1:20 or 1:200 ratio and exposed to the antimalarial drugs. The supernatant was collected and TNF-α concentration determined by an enzyme linked immunosorbent assay. Both quinine (≥ 2.4 μg/ml) and chloroquine inhibit TNF-α production in macrophages (Figure 14).

Figure 14

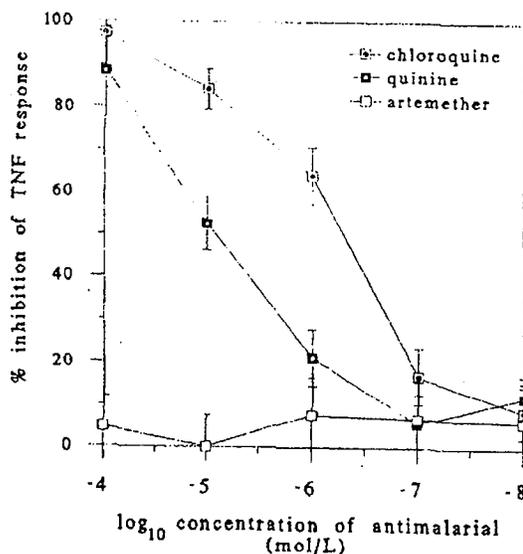


Figure. Inhibition by different antimalarial drugs of tumour necrosis factor production by human mononuclear cells stimulated with lysed erythrocytic forms of *P. falciparum*. (Mean of 7 experiments; bars represent standard error.)

In summary, the exact mechanism of action of quinine is not well understood. Available data suggests that quinine at a concentration of 2.5 μg/ml inhibits nucleic acid and protein synthesis as well as glycolysis in *P. falciparum* (Table 10). Quinine (0.02 μg/ml) can interact with hemazoin in parasitized erythrocytes. At a concentration of 18.7 μg/ml, quinine inhibits heme polymerase activity in lysates of parasitized erythrocytes. However, this concentration is 27 fold higher than that required for inhibition of parasite growth, suggesting that it may not be the primary target for the action of quinine. Quinine-hemazoin complex was shown to lead to lipid peroxidation in liposomes in a biochemical assay. However, quinine did not have an effect on degradation of heme or accumulation of hemoglobin. The drug was also shown to inhibit release of tumor necrosis factor-alpha from alveolar macrophages.

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Table 10: Summary of studies examining the mechanism of action of quinine.

Mechanism evaluation conditions	Heme binding	Hemozoin binding	Heme polymerase	Hemozoin content	Membrane associated heme	Haemoglobin accumulation	GSH dependent degradation of heme in membrane	GSH dependent degradation of heme in solution	Lipid peroxidation	Nucleic acid synthesis	Protein synthesis	Glycolysis	TNF-alpha release from macrophages
<i>Biochemical assay</i>													
	Yes*	NA	NA	NA	NA	NA	No change (1.4 µg/ml)	Weak inhibition (1.4 µg/ml)	Increase (23 µg/ml)	NA	NA	NA	Decrease (11.5 µg/ml)
<i>In vitro</i>													
Ring stage	NA	Yes (0.02 µg/ml)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Trophozoite stage	NA	NA	NA	Decrease (2.3 µg/ml)	No change (2.3 µg/ml)	No (2.3 µg/ml)	NA	NA	NA	NA	NA	NA	NA
Synchronous cultures	NA	NA	NA	NA	NA	NA	NA	NA	NA	Decrease (2.5 µg/ml)	Decrease (2.5 µg/ml)	Decrease (2.5 µg/ml)	NA
<i>In vivo</i>													
Mice	NA	NA	No change (1.4 µg/ml)	No change (1.4 µg/ml)	NA	NA	NA	NA	NA	Decrease (0.7 µg/ml)	NA	NA	NA
Mice	NA	NA	Inhibition (18.7 µg/ml)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

* concentration of quinine not specified

NA = information not available

3.2. ACTIVITY *IN VITRO*

The activity of quinine and its metabolite, 3-hydroxyquinine, was measured against the erythrocytic forms of *P. falciparum* after exposure to the drug for 24 to 72 hours either by microscopic examination (method of Rieckmann) or by incorporation of [³H]-hypoxanthine (method of Desjardins or Webster). A description of these 3 methods is given below. The results were expressed as the concentrations required for inhibition of growth of the parasite by 50%, 90% and 99% (IC₅₀, IC₉₀, and IC₉₉ values, respectively). Please note that no studies demonstrating the activity of quinine or its metabolite against the hepatic forms of *Plasmodium* species were available.

Rieckmann microtechnique method¹⁴: Blood with varying parasitemia (5 µl) was incubated in the presence of different concentrations of drug in RPMI 1640 medium supplemented with sodium bicarbonate (2 mg/ml), gentamycin sulphate (4 µg/ml) at 38-39°C for 24 to 30 hours in a candle jar. Thick blood smears were prepared and stained with giemsa stain and number of schizonts per 500 white blood cells (WBCs) in the control and drug treated samples determined. The percentage of ring forms that mature to schizont provides a useful endpoint for measuring drug activity. The IC₅₀ value defined as the concentration of drug required for 50% inhibition of schizont maturation was calculated.

Desjardins semiautomated microdilution method¹⁵: Parasitized erythrocytes (0.25 to 0.5%) were incubated in the presence or absence of drug in RPMI 1640 medium supplemented with 10% heat inactivated human plasma at 37°C for 24 hours in 5% CO₂. [³H]-hypoxanthine was added and cultures were incubated for an additional 18 hour period. The antiplasmodial activity was determined based on incorporation of [³H]-hypoxanthine. The IC₅₀ values were obtained by regression analysis.

Webster microdilution method¹⁶: The Webster method was essentially similar to that of Desjardins except that concentration of parasitized erythrocytes (0.3 to 0.9%) was slightly different and incubation was performed in a candle jar instead of 5% CO₂. Also, incubation after addition of radiolabel was 20 to 24 hours instead of 18 hours.

3.2.1. *In vitro* activity against laboratory strains of *P. falciparum*:

The *in vitro* activity of quinine against erythrocytic stages of 13 strains of *P. falciparum* was measured in 7 studies. Six of the strains were classified as chloroquine-resistant, one strain as mefloquine-resistant, and one as quinine-resistant. The basis for classifying the *P. falciparum* strain as chloroquine-, mefloquine-, or quinine-resistant was not specified. For one of the strains (Smith strain), the authors state that it was originally obtained from a patient who failed therapy with antimalarial drugs and has been used extensively in human volunteer studies where its resistance to chloroquine, quinine and pyrimethamine have been documented. However, additional details on cut-off values for resistance were not provided. The activity was measured using the method of Rieckmann or Desjardins (see Table 11). The quinine IC₅₀ values against the 13 strains ranged from 0.026 to 0.68 µg/ml. The activity of quinine against the mefloquine or chloroquine resistant *P. falciparum* strains was similar to that of the sensitive strains.

The variation in quinine IC₅₀ against culture adapted *P. falciparum* strains [FCR3-FMG (Gambia), UPA-PFL3 (Uganda), UPAS (passaged in Saimiri monkey), T1 (Thailand), T23 (Ivory

Quinine

Mutual Pharmaceuticals

Coast), D3 and 7G8] was measured¹⁷. The authors have stated that different lines of the strain with different IC₅₀ values were obtained after *in vitro* cultivation with drug pressure (drug not specified) or after thawing frozen stock culture and culturing without drug pressure. These lines were identified as parent strain x1, 2, 3, etc. Parasitized erythrocytes (0.2 to 0.3%) in RPMI medium supplemented with AB serum and [³H]-hypoxanthine were incubated in the presence of quinine or other cinchona alkaloids at 37°C for 72 hours in 5% CO₂. The IC₅₀ values were calculated based on the uptake of hypoxanthine. The results in Table 12 show that the quinine IC₅₀ against lines from the same strain varied 2 to 5 fold. In two studies, quinine was less active than quinidine against *P. falciparum* strains.

Table 11: *In vitro* activity of quinine against the laboratory strains of *P. falciparum*.

Study	Method	Modification	Strain	Resistant to drug*	Quinine	Other antimalarial agent
					Mean ± SE (range) IC ₅₀ in µg/ml	Mean IC ₅₀ ± SE in µg/ml
Nontprasert (1996) ¹⁸	Desjardins (1979)	<2% parasitized erythrocytes and 10% heat inactivated human sera	D6	mefloquine	0.096 (0.05 - 0.12)	none
Sharma (2000) ¹⁹	Rieckmann (1978)	none	MRC P.f 20	none	0.27	CQ = 0.49
			MRC P.f 76	chloroquine	0.69	CQ = 1.55
Fivelman (1999) ²⁰	Desjardins (1979)	none	D10	none	0.07 ± 0.001	CQ = 0.015 ± 0.001
Rahman (1997) ²¹	Details not given [³ H]-hypoxanthine uptake	none	RSA11	Chloroquine	0.123 ± 0.011	CQ = 0.14 ± 0.001
			NF54	none	0.042 ± 0.001	CQ = 0.09 ± 0.02
			H1	none	0.21 ± 0.001	CQ = 0.02 ± 0.001
			EAT/LON/1/G30	Chloroquine	0.14 ± 0.046	CQ = 0.24 ± 0.05
			K1	Chloroquine	0.073 ± 0.01	CQ = 0.28 ± 0.02
Carvalho (1991) ²²	Rieckmann (1978)	1% parasitized erythrocytes; incubation for 72 hours	BHz 26/86	Chloroquine	0.03	CQ = 0.05
Wesche and Black (1990) ²³	Rieckmann (1978)	none	FCQ-27/PNG	none	0.14	Quinidine = 0.056
Druilhe (1988) ¹⁷	Desjardins (1979)	Incubation for 72 hours; radiolabel added at start of experiment; strains freeze thawed and culture adapted and tested multiple times	18 strains	none	0.027 - 0.095	Quinidine = 0.007 - 0.043
			10 strains	Quinine [^]	0.1 - 0.68	Quinidine = 0.022 - 0.12
Desjardins (1979) ¹⁵	Desjardins (1979)	none	Uganda I	none	0.026 ± 0.005 (0.024 - 0.048)	CQ = 0.009 ± 0.001 MQ = 0.007 ± 0.001
			Smith	Chloroquine#	0.11 ± 0.009 (0.090 - 0.128)	CQ = 0.182 ± 0.023 MQ = 0.008 ± 0.001
Range of quinine IC₅₀ (µg/ml) for 13 strains					0.026 - 0.68	

* stated by author, basis not specified;

[#] Smith strain of *P. falciparum* was originally obtained from a patient who failed therapy with antimalarial drugs and has been used extensively in human volunteer studies where its resistance to chloroquine, quinine and pyrimethamine have been documented. However, details of the resistance cut-off values not provided.

[^] strains with quinine IC₅₀ > 100 ng/ml

SE = standard error

CQ = chloroquine; MQ = mefloquine;

Table 12: Activity of alkaloids against cultured quinine (Qn) susceptible and resistant lines.

<i>P. falciparum</i> line ^a	EC ₅₀ (ng/ml) ^b				
	Qn	Qd	Cn	Qn-Cn-Qd ^c (ΣFIC)	Qn-Cn-Qd/Qn
Qn susceptible					
FCR3x4	52	26	26	15 (0.48)	0.28
FCR3x5	42	43	19	40 (1.32)	0.95
FCR3x6	88	40	72	78 (1.30)	0.88
FCR3x8	80	22	18	15 (0.56)	0.18
FCR3x9	90	32	34	50 (1.19)	0.55
UPAx2	26	20	28	26 (1.06)	1
UPAx3	82	26	34	47 (1.25)	0.57
UPAx4	45	10	15	12 (0.75)	0.26
UPAx5	45	17	22	27 (1.13)	0.60
UPASx1	45	22	27	33 (1.15)	0.73
UPASx1	35	12	29	23 (1.12)	0.65
UPASx2	30	32	34	44 (1.37)	1.46
UPASx1	42	7.4	12.5	15.5 (1.23)	0.36
UPASx2	95	27	35	53 (1.34)	0.55
D3	46	11	25	18 (0.91)	0.39
7G8	27	8	12	14 (1.14)	0.51
FCPS44x1	34	19	34	30 (1.10)	0.85
FCPS44x2	35	19	32	37 (1.39)	1.18
Qn resistant					
FCR3x1	160	120	92	64 (0.53)	0.40
FCR2x2	680	115	92	58 (0.40)	0.085
FCR3x3	540	88	78	93 (0.80)	0.172
FCR3x7	180	64	80	68 (0.76)	0.377
UPAx1	150	58	26	56 (1.15)	0.373
UPASx2	100	22	60	36 (0.86)	0.36
UPASx3	280	80	130	25 (0.19)	0.089
UPASx4	212	31	32	25 (0.55)	0.117
T1x3	105	62	48	39 (0.60)	0.371
T1x4	115	37	40	57 (0.98)	0.495

^a Qn-susceptible lines were those for which EC₅₀ < 100 ng/ml; Qn-resistant lines were those for which EC₅₀ ≥ 100 ng/ml.

^b Means ± standard deviations: for Qn-susceptible lines, 52.1 ± 23.4 (Qn), 21.8 ± 10.4 (Qd), 28.2 ± 13.3 (Cn), 32.0 ± 17.5 (Qn-Cn-Qd), and 0.65 ± 0.33 (Qn-Cn-Qd/Qn); for Qn-resistant lines, 252.2 ± 198.8 (Qn), 67.7 ± 33.4 (Qd), 67.3 ± 33.2 (Cn), 52.1 ± 21.1 (Qn-Cn-Qd), and 0.28 ± 0.15 (Qn-Cn-Qd/Qn).

^c Combination of equal parts.

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3.2.2. *In vitro* activity against clinical isolates of *P. falciparum* from different geographical regions:

The *in vitro* activity of quinine against 129 isolates of *P. falciparum* from Thailand, Bangladesh and Africa was measured in 4 studies. The activity was measured by the schizont maturation method of Rieckmann or uptake of radiolabelled hypoxanthine using a modified Desjardins method or the Webster method. Some of these isolates were classified as resistant to chloroquine (IC₉₉ values >200 nM), quinine (IC₉₉ values >2000 nM, i.e., >0.68 µg/ml or IC₅₀ >0.1 µg/ml), or mefloquine (IC₉₉ values >200 nM). The quinine IC₅₀ against the 129 isolates of *P. falciparum* ranged from 0.06 to 0.29 µg/ml and was similar to that observed against the laboratory strains (Table 13).

Table 13. *In vitro* activity of quinine against *P. falciparum* isolates from different geographical regions.

Study	Isolate (Region)	Resistant to Drug	Method	Modification	Quinine		Other antimalarial agent	
					Mean (range) IC ₅₀ in µg/ml	Mean (range) IC ₉₀ in µg/ml	Mean (range) IC ₅₀ in µg/ml	Mean (range) IC ₉₀ in µg/ml
Nontprasert (1996) ¹⁸	S1 (Thailand)	NS	Desjardins (1979) ¹⁵	<2% parasitized erythrocytes and 10% heat inactivated human sera	0.13 (0.11 - 0.25)	NS	None	None
	S2 (Thailand)	NS			0.11 (0.06 - 0.15)	NS	None	None
	S3 (Thailand)	NS			0.2 (0.13 - 0.26)	NS	None	None
	S4 (Thailand)	NS			0.15 (0.11 - 0.29)	NS	None	None
Noedl (2003) ²⁴	44 isolates (Bangladesh)	chloroquine, quinine, mefloquine ¹	Webster (1985) ¹⁶	none	0.1	0.21	CQ = 0.06 MQ = 0.03	CQ = 0.1 MQ = 0.05
	22 isolates (Thailand)	chloroquine, quinine, mefloquine ²			0.12	0.24	CQ = 0.06 MQ = 0.04	CQ = 0.1 MQ = 0.07
Druilhe (1988) ¹⁷	16 isolates (Thailand)	None	Desjardins (1979) ¹⁵	Incubation for 72 hours; radiolabel added at start of experiment; strains freeze thawed and culture adapted and tested multiple times	NS (0.02 - 0.093)	NS	Quinidine = 0.015 - 0.04	NS
	9 isolates (Thailand)	Quinine ³			NS (0.1 - 0.25)	NS	Quinidine = 0.019 - 0.12	NS
Sowunmi (1990) ²⁵	34 isolates (Africa)	NS	Rieckmann (1978) ¹⁴	none	0.071	0.39 [#]	Quinidine = 0.027	Quinidine = 0.09 [#]
Range of quinine IC₅₀ (µg/ml) for 129 isolates					0.06 - 0.29			

NS = not specified

¹ chloroquine IC₉₉ values >200 nM against 37 isolates; quinine IC₉₉ values >2000 nM against 13 isolates; mefloquine IC₉₉ values >200 nM against 26 isolates

² chloroquine IC₉₉ values >200 nM against 21 isolates; quinine IC₉₉ values >2000 nM against 5 isolates; mefloquine IC₉₉ values >200 nM against 18 isolates

³ isolates with quinine IC₅₀ >100 ng/ml were considered resistant; basis of this interpretive criteria not specified

[#] IC₉₉ value

Quinine
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3.2.3. Activity of metabolite against *P. falciparum*:

The activity of the metabolite, 3-hydroxyquinine, against *P. falciparum* was investigated *in vitro*²⁶. A modified version of the Desjardins method was used to measure activity. The metabolite of quinine was less active than the parent compound against *P. falciparum* (Table 14).

As stated previously, quinine is an alkaloid derived from cinchona bark. Dihydroquinine is a natural impurity found in quinine preparations during the extraction process. The activity of dihydroquinine against *P. falciparum* was measured. The dihydroquinine IC₅₀ values against *P. falciparum* ranged from 0.019 to 0.073 µg/ml and were lower than quinine.

Table 14: *In vitro* activity of 3-hydroxyquinine against *P. falciparum*.

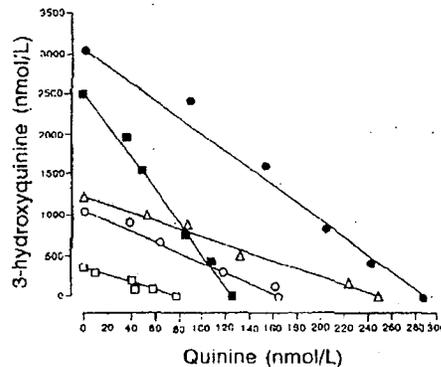
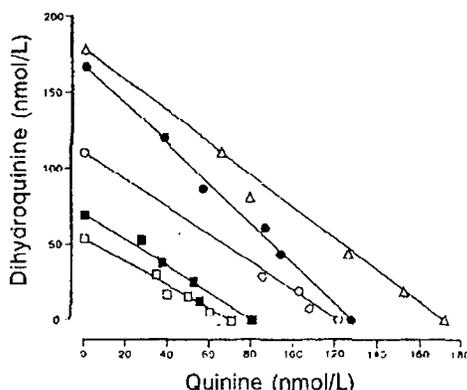
Study	Isolate/Strain	Method	Modification	Quinine Mean (range) IC ₅₀ in µg/ml	3-hydroxyquinine Mean (range) IC ₅₀ in µg/ml	Dihydroquinine# Mean (range) IC ₅₀ in µg/ml
Nontprasert (1996) ¹⁸	S1 (Thailand)	Desjardins (1979) ¹⁵	human sera used instead of plasma	0.13 (0.11 – 0.25)	0.39 (0.35 – 0.42)	0.058 (0.041 – 0.078)
	S2 (Thailand)			0.11 (0.06 – 0.15)	0.85 (0.80 – 1.07)	0.041 (0.036 – 0.070)
	S3 (Thailand)			0.2 (0.13 – 0.26)	0.29 (0.26 – 0.45)	0.034 (0.026 – 0.036)
	S4 (Thailand)			0.15 (0.11 – 0.29)	1.03 (0.87 – 1.04)	0.073 (0.042 – 0.10)
	D6-mefloquine resistant*			0.096 (0.05 – 0.12)	0.174 (0.13 – 0.25)	0.019 (0.017 – 0.021)

#dihydroquinine = natural impurity

* stated by author, basis not specified

The activity of quinine in combination with dihydroquinine or 3-hydroxyquinine was not synergistic or antagonistic (Figure 15). The authors have stated that the FIC indices for quinine in combination with dihydroquinine or 3-hydroxyquinine against *P. falciparum* were 0.85 - 1.25, and 0.81 - 0.93, respectively.

Figure 15: Isobolograms of quinine in combination with dihydroquinine and 3-hydroxyquinine at the 50% inhibitory concentration against 5 strains of *P. falciparum* *in vitro*.



Studies that examined the activity of metabolites other than 3-hydroxyquinine against *P. falciparum* were not included in the submission.

Quinine.

Mutual Pharmaceuticals

3.2.4. Effect of quinine on the different erythrocytic stages of *P. falciparum*:

The effect of quinine and artemisinin compounds on the inhibition of the ring, trophozoite and schizont forms of *P. falciparum*, using 3 isolates from Vietnam and one from China was examined²⁷. For this, synchronized cultures (> 90% rings) were allowed to develop into trophozoites (incubation period, 20 to 24 hours) and schizonts (incubation period, 36 hours). The parasite cultures with the different stages of *Plasmodium* were incubated with different drugs (concentration equals IC₉₀ value for the drug) for 10 hours. The actual concentration of the drug was not specified. The percent inhibition of parasite growth was measured by incorporation of [³H]-hypoxanthine added to the cultures at the same time as the drug. The authors have stated that the activity against all isolates was similar, however, data for only one isolate were shown. Quinine was more active against the schizont than the ring and trophozoite stages of *P. falciparum* (Figure 16). Among the artemisinins, artemether was the least active against the various erythrocytic stages of *P. falciparum*. Artemisinin inhibited > 90% rings and schizonts within 10 hours while dihydroartemisinin (DHA) inhibited growth (> 80%) of all 3 stages within 2 hours of exposure.

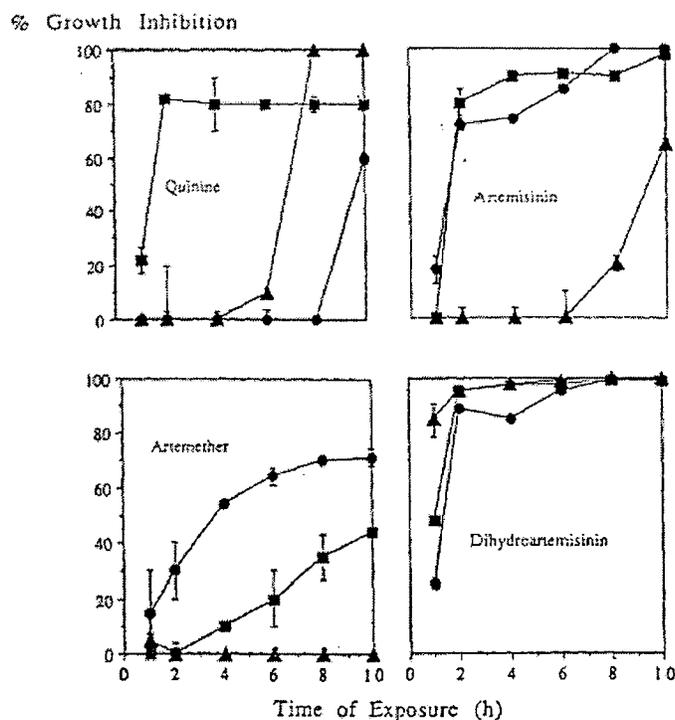


Figure 16: Stage-specific activities of quinine, artemisinin, and DHA on culture-adapted parasites of *Plasmodium falciparum*. Synchronized cultures of rings (0 h; ●), trophozoites (20-24 h; ▲) and schizonts (36 h; ■) were exposed to the IC₉₀ concentration of each drug for up to 10 h. The data are presented as percentage growth inhibition ± S.D. with time of drug exposure for the 3 asexual stages compared with untreated controls.

Drug	IC ₅₀ values (M)
DHA	0.8 - 3.0 × 10 ⁻⁹
Artemisinin	2.0 - 30.0 × 10 ⁻⁹
Artemether	7.0 - 30.0 × 10 ⁻¹²
Quinine	3.0 - 10.0 × 10 ⁻⁸

Overall *in vitro* studies show that quinine and its metabolite 3-hydroxyquinine are active against the erythrocytic stages of *P. falciparum*. Quinine was more active against the schizont stage than the trophozoite or ring stages. The activity of quinine against the gametocyte stage of *P. falciparum* was not examined *in vitro*. The activity of quinine against laboratory strains (n = 13) and clinical isolates (n = 129) from different geographical areas was similar *in vitro*. However, the metabolite was less active than the parent drug. The activity of metabolites other than 3-hydroxyquinine was not examined *in vitro*. Quinine was less active than its isomer, quinidine, and other antimalarials such as chloroquine and mefloquine. A 2 to 5 fold variation was observed in

the quinine IC₅₀ values against strains that were freeze thawed and culture adapted. Please note that methods to evaluate *in vitro* susceptibility of *P. falciparum* to antimalarial drugs are not standardized and different methods were used to determine *in vitro* activity.

3.3. ACTIVITY *IN VIVO*:

The activity of quinine was measured against the erythrocytic forms of *P. berghei*, *P. yoelii*, *P. chaubaudi*, and *P. knowlesi* in mice or monkeys. No studies were done to demonstrate the activity of quinine against the hepatic stages of *Plasmodium* species.

3.3.1. *P. berghei*:

The activity of quinine and quinidine against a chloroquine-sensitive and 3 resistant strains of *P. berghei* was examined²⁸. The criteria used to classify strains as resistant were based on the dose of the drug required to suppress parasitemia in mice. Resistant parasites collected from infected mice treated with chloroquine (128 mg/kg), mefloquine (64 mg/kg), or quinine (400 mg/kg) for 4 days were used for infection. Swiss mice were infected by intraperitoneal inoculation of 2.5×10^7 parasitized erythrocytes. Quinine or quinidine was administered orally at the time of infection for 4 days. Thin blood smears were prepared using pooled blood samples from each of the dose groups on day 5. The doses required for the 50% and 90% reduction of parasite count (SD₅₀ and SD₉₀, respectively) were calculated by regression analysis. The results in Table 15 show that quinidine was more effective than quinine against the 4 *P. berghei* strains. The activity of quinine against the chloroquine-resistant and chloroquine-sensitive strains of *P. berghei* was similar. However, approximately 4-fold higher concentration of quinine was required against the mefloquine-resistant strain, suggesting cross-resistance between quinine and mefloquine.

3.3.2. *P. yoelii*:

The activity of quinine and quinidine against a multi-resistant strain of *P. yoelii* was examined²⁸. The strain was resistant to chloroquine, quinine, and mefloquine based on studies in mice [chloroquine-resistant (64 mg/kg), quinine-resistant (400 mg/kg), and mefloquine-resistant (64 mg/kg)]. The study design was same as that described for *P. berghei* above. The quinine SD₅₀ against the *P. yoelii* strain was 47.33 ± 2.31 mg/kg (Table 15). Quinidine was more active than quinine against the *P. yoelii* strain. A *P. yoelii* sensitive strain was not used as control in this experiment.

3.3.3. *P. chaubaudi*:

The activity of quinine against a chloroquine-resistant strain AS (3QC) and chloroquine-sensitive strain ES317 of *P. chaubaudi* was examined²¹. For this, 5 male TO mice were infected intravenously with 10^7 parasitized erythrocytes of both strains AS (3QC) and ES317 of *P. chaubaudi*. The drugs were administered intraperitoneally or subcutaneously at the time of infection for 4 days. The quinine SD₅₀ against the chloroquine-resistant and -sensitive *P. chaubaudi* strains were similar (Table 15).

Table 15: The activity of quinine against *Plasmodium* species in animal models.

Study	Animal	Strain	Resistant to drug*	Inoculum of parasitized erythrocytes (route)	Drug route/ duration	Activity (mg/kg)#			
						Quinine SD ₅₀	Quinine SD ₉₀	Quinidine SD ₅₀	Quinidine SD ₉₀
Kazim (1991) ²⁸	Swiss Mice	<i>P. berghei</i>	None	2 to 2.5 x 10 ⁷ (IP)	Oral for 4 days; day of infection	35.44 ± 13.77	142.07 ± 1.15	12.56 ± 5.58	42.91 ± 18.07
						26.36 ± 5.03	165.53 ± 48.09	24.81 ± 6.55	139.03 ± 37.94
						169.35 ± 77.11	489.36 ± 16.72	37.28 ± 12.86	168.07 ± 17.20
						285.58 ± 23.22	462.11 ± 21.81	73.92 ± 15.29	227.63 ± 17.33
						47.33 ± 2.31	243.01 ± 54.91	29.03 ± 4.70	169.64 ± 35.49
Rahman (1997) ²¹	TO Mice	<i>P. chaubaudi</i> AS (2QC)	Chloroquine, quinine and mefloquine ⁴	10 ⁷ (IV)	IP or SC for 4 days; day of infection	37.97 ± 1.35	NS	NT	NT
						38.48 ± 3.45	NS	NT	NT

¹ strain resistant to chloroquine dose of 128 mg/kg x 4 days in mice

² strain resistant to mefloquine dose of 64 mg/kg x 4 days in mice

³ strain resistant to quinine dose of 400 mg/kg x 4 days in mice

⁴ strain resistant to chloroquine dose of 64 mg/kg x 4 days, quinine dose of 400 mg/kg x 4 days, and mefloquine 64 mg/kg x 4 days in mice

⁵ basis for resistance not specified

IP = intraperitoneal; IV = intravenous; SC = subcutaneous

Parasitemia recorded using giemsa stained thin blood smear and SD₅₀ and SD₉₀ values determined by plotting the log dose versus percent reduction of parasitemia in drug treated groups

NS = not specified

NT = not tested

Quinine

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3.3.4. *P. cynomolgi*:

The activity of quinine against the *P. cynomolgi* was measured in rhesus monkeys inoculated intravenously with 5×10^8 parasitized RBCs²⁹. Other antimalarial drugs were used as comparators. Four days after infection, different doses of drug were administered orally, once daily for 7 days. Blood smears (thin and thick) were prepared daily after the initial dose for 15 days and every other day thereafter. If no parasite was observed in 200 high power fields using thick blood smear, the smear was considered as negative. After 30 days, monkeys with negative blood smears were splenectomized and their blood smears examined for parasites every other day for 30 days. Recrudescence after splenectomy is indicative of previously subpatent malaria. Peak parasitemia (average 5.28×10^5 parasite/ μ l) was achieved between days 4 to 6 of infection in the control animals and the parasitemia was maintained for 30 days. None of the control animals died due to malaria. The 2 infected monkeys treated with the 31.6 mg/kg/day quinine were cured at 30 days after splenectomy (Table 16). The time to parasite clearance in animals treated with quinine was not specified. A lower dose of chloroquine (10 mg/kg/day) was required to cure the infected monkeys.

Table 16: Curative efficacy of quinine on asexual form of erythrocytic stage of *P. cynomolgi* in rhesus monkey (7-day treatment test).

Name and compound number	Drug dose in mg/kg/day producing antimalarial effect†								Minimum curative dose (mg/kg/day)	Maximum tolerated dose (mg/kg/day)
	31.6	100	31.6	10.0	3.15	1.0	0.316	0.1		
Chloroquine (WR 1544)				C	MS	SS	I	I	10.0	31.6
				C	MS	I	I	I		
				C						
Amodiaquin (WR 2977)				C	MS	MS	I		10.0	
				C	C	SS	I			
				MS						
Primaquine (WR 2975)				MS	MS	MS	MS	MS	Not curative	10.0
				MS	MS	MS	MS	MS		
Plasmochin (WR 4234)				C	MS	MS	MS	MS	31.6	
				C	MS	MS	MS	SS		
Quinine				C	MS				31.6	100
				C	NT‡					
WR 30090	C	C	I	I					100	>316
	C	MS	I	I						
Endochin (WR 7295)			I	I	I				Ineffective	
			I	I	I					

C = Cure, no recrudescence within 30 days after splenectomy

MS = Marked suppression, parasitemia cleared for at least 2 successive days but recrudescence prior to day 30

SS = Slight suppression, parasitemia suppressed temporarily to below 1,000 parasites/ μ l

I = ineffective, parasitemia not different from vehicle control

NT = no test, due to reasons such as death of monkey due to toxicity or intercurrent disease or inadequate baseline parasitemia

3.4. DRUG RESISTANCE

The sponsor did not provide publications that describe development of resistance of *P. falciparum* to quinine *in vitro* or *in vivo*.

3.4.1. In vivo:

A publication by Glew (1978)³⁰ that describes development of quinine resistance by *P. falciparum* *in vivo* was identified by an independent literature search. In this study, Aotus monkeys were infected intravenously with the Panama II strain of *P. falciparum* (1 to 5 x 10⁶ parasitized erythrocytes). Monkeys were administered quinine orally, when parasitemia reached 15,000 parasites/ μ l. The parasite count was determined daily using Giemsa stained blood smear. The response to quinine therapy was determined (Table 17). The Panama II strain was then passaged serially six times *in vivo* under quinine pressure (quinine 125 mg/kg for 8, 10 or 14 days) over a 6 month period to select for a quinine-resistant strain.

Of the 12 monkeys infected with the original strain, a quinine dose of 125 mg/kg for 14 days cured 8 monkeys of infection (Table 17). Recrudescence (RI) was observed in the remaining 4 monkeys at 28 days after initiation of therapy. An RII (marked reduction of parasitemia and no clearance by day 7) or RIII (no marked reduction in parasitemia within first 48 hours) response was observed with lower doses of quinine. After 6 passages in monkeys over a 6 month period (1 month per passage), the strain became uniformly resistant to quinine (Figure 17). For monkeys infected with the resistant strain, treatment with quinine 125 mg/kg for 14 days resulted in RIII response in 4 of the 12 monkeys and RII response in 5 of the 12 monkeys (Table 18). The RII and RIII responses were also observed in 2 of 4 monkeys that received a higher dose (160 mg/kg) of quinine. These results suggest a potential for development of resistance to quinine. The authors did not provide the results for development of resistance after each passage in the monkeys. Therefore, the timing of development of resistance cannot be determined.

Table 17

Response to quinine therapy of infections due to original strain of *P. falciparum* (Panama II) in Aotus monkeys

Daily dose (mg/kg)	Quinine treatment		Response* (numbers of monkeys)			
	Schedule	Duration (days)	S	RI	RII	RIII
20	QD†	14	-	-	-	3
40	QD	14	-	-	3	-
80	QD	14	-	1	1	1
125	QD	14	8‡	4	-	-

* S, susceptible; RI, quinine resistance with clearance of asexual parasitemia followed by recrudescence; RII, resistance with marked reduction of parasitemia, but no clearance; RIII, resistance with no marked reduction of asexual parasitemia.³⁰

† QD, once daily.
 ‡ Two monkeys died unexpectedly but blood smears had been negative for parasites for 15 and 19 days.

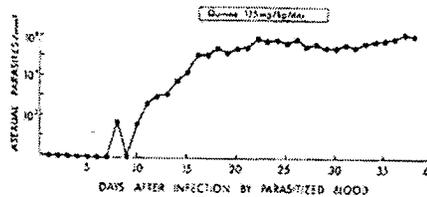


Figure 17: RIII resistance to quinine therapy (125 mg/kg for 14 days) in an Aotus monkey infected with the resistant strain of *P. falciparum* (PanamaII).

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Response to quinine therapy of infections due to a resistant strain of *P. falciparum* (Panama II) in Aotus monkeys

Daily dose (mg/kg)	Quinine treatment		Response*			
	Schedule	Duration (days)	S	RI	RII	RIII
125	QD†	14	-	3	5	4
160	BID†	14	2	-	1	1

* See Table 1.
 † QD, once daily; BID, one-half of total daily dose administered twice daily.

Table 18

Quinine
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3.4.2. Correlation between *in vitro* susceptibility and genotyping:

The sponsor provided 5 publications that describe the correlation between *in vitro* susceptibility of strains and/or isolates to quinine and mutations in the *pfmdr1* gene (encodes for multidrug resistant transporter) or genes of other transporters.

P. falciparum is known to have 49 transporters based on its genome sequence. These transporter genes lie within a 113 kb (99 kb coding region and 14 kb non coding region) DNA region. To identify the genes that contribute to quinine resistance in *P. falciparum*, DNA sequences of the 49 transporters were amplified from 97 culture adapted *P. falciparum* isolates and analyzed for single nucleotide polymorphisms (SNPs)³¹. The 97 isolates were obtained from malaria patients in different geographical areas, namely, Africa, Asia, Americas and Papua New Guinea. In addition, sequences of the 49 transporter genes from 4 strains (Hb3 strain from Central America, Dd2 strain from South-east Asia, D10 strain from Papua New Guinea, and 7G8 strain from South America) were used as controls. The SNPs and polymorphic microsatellite (MS) regions in the culture adapted isolates were identified after alignment of DNA sequences of the isolates with DNA sequences of the control strains. The authors identified 67 MS and 164 SNPs in 42 of the 49 transporter genes. The presence of SNPs was correlated with the *in vitro* susceptibility of the isolates to quinine and chloroquine. Fifteen SNPs from 6 transporter genes (*pfcr*, G2, G7, G25, G30 and G49) and 4 SNPs from *pfmdr1*, G47, and G70 were associated with increase in the IC₅₀ values for CQ and Q against the 97 isolates (Table 19). These associations were not uniform for isolates from all geographical areas and the clinical significance of such a finding is not known.

Table 19: SNPs from 11 *P. falciparum* putative transporter encoding genes associated with *in vitro* susceptibilities to chloroquine and quinine.

Gene	Predicted products	Locus	AA change	Informativeness Index ^a				CQ (P-value)				QN (P-value)				Comments
				All	Asia	Africa	Americas	All	Asia	Africa	Americas	All	Asia	Africa	Americas	
<i>Pfcr</i>	Putative transporter 10 TM segments with CEGA motif	72	C-S	0.61	0	0	0.94	0.097	NI	NI	0.19	0.25	NI	NI	0.17	No association
		74	M-I	0.86	0.58	0.86	0	<u>9.6E-10</u>	3.1E-7	<u>2.5E-7</u>	NI	<u>7.6E-8</u>	<u>1.6E-4</u>	<u>0.001</u>	NI	CO/QN Africa and Asia
		75	N-E	0.86	0.58	0.86	0.37	<u>6.6E-10</u>	3.1E-7	<u>2.5E-7</u>	NI	<u>5.1E-8</u>	<u>1.6E-4</u>	<u>0.001</u>	NI	CO/QN Africa and Asia
		76	K-T	0.89	0.58	0.84	0.86	<u>1.7E-18</u>	3.1E-7	<u>1.1E-7</u>	<u>4.9E-4</u>	<u>5.3E-8</u>	<u>1.6E-4</u>	<u>0.0077</u>	0.28	CO/all areas; QN/Asia and Africa
		220	A-S	0.85	0.58	0.86	0.68	<u>2.3E-18</u>	3.1E-7	<u>2.5E-7</u>	<u>4.9E-4</u>	<u>1.8E-9</u>	<u>1.6E-4</u>	<u>0.001</u>	0.28	CO/all areas; QN/Asia and Africa
<i>Pfmdr1</i>	ABC transporter	271	F-Q	0.86	0.58	0.86	0	<u>9.6E-10</u>	3.1E-7	<u>2.5E-7</u>	NI	<u>7.6E-8</u>	<u>1.6E-4</u>	<u>0.001</u>	NI	CO/QN Africa and Asia
		326	N-D/S	0.87	0.58	0.83	0.39	<u>1.2E-9</u>	3.1E-7	<u>6.7E-7</u>	NI	<u>2.3E-8</u>	<u>1.6E-4</u>	<u>0.0011</u>	0.26	CO/QN Africa and Asia
		358	I-ITL	0.9	0.77	0.21	0.39	<u>3.7E-6</u>	0.0012	NI	NI	<u>1.8E-5</u>	0.044	NI	NI	CO/Asia
		371	R-I	0.87	0.58	0.86	0.18	<u>8.5E-10</u>	3.1E-7	<u>2.5E-7</u>	NI	<u>6.2E-8</u>	<u>1.6E-4</u>	<u>0.001</u>	NI	CO/QN Africa and Asia
		1034	S-C ^b	0.63	0.48	0.1	0.88	0.0066	NI	NI	<u>0.0073</u>	0.015	NI	NI	0.12	CO/Americas, may be geographical
G2	ABC transporter	1042	N-D	0.8	0.79	0	0.37	0.001	0.19	NI	NI	<u>3.7E-4</u>	0.064	NI	NI	May be geographical
		191	Y-H	0.86	0.7	0.36	0.99	<u>2.8E-7</u>	0.0075	NI	0.086	<u>6.6E-7</u>	0.067	NI	<u>0.028</u>	Weak CO/Asia; weak QN/Americas
G7	ABC transporter	437	A-S	0.85	0.58	0.47	0.97	<u>12E-7</u>	0.076	NI	<u>0.031</u>	<u>4.3E-6</u>	0.3	NI	<u>0.029</u>	Weak CO/QN, Americas
G25	Sulphate permease	1390	&1	0.74	0.4	0.87	0.92	<u>1.8E-6</u>	NI	<u>0.01</u>	0.32	<u>0.0032</u>	NI	0.18	0.37	CO/Africa
G30	GTPase	Intron	C-G	0.85	0.22	0.94	0.37	<u>9.2E-8</u>	NI	<u>0.0048</u>	NI	<u>2.6E-5</u>	NI	0.011	NI	CO/QN/Africa
		241	L-V	0.89	0.54	0.75	0.37	<u>0.0033</u>	0.075	0.4	NI	<u>7.1E-5</u>	<u>0.0058</u>	0.068	NI	CO/Asia, low, may be geographical
G49	ABC/ATPase	146	Q-E	0.82	0.88	0	0	<u>7.0E-5</u>	0.018	NI	NI	<u>9.3E-6</u>	<u>0.025</u>	NI	NI	Weak CO/QN/Asia, may be geographical
		1046	L-I	0.82	0.78	0.36	0.92	0.076	0.23	NI	<u>0.021</u>	0.35	0.12	NI	0.14	Weak QN/Americas, may be geographical
		1116	L-I	0.88	0.5	0.73	0.8	<u>1.6E-7</u>	0.0053	0.24	0.16	<u>6.5E-6</u>	0.029	0.17	0.24	Weak CO/QN/Asia, may be geographical
G54	Membrane protein	141	Y-Y	0.9	0.87	0.87	0.65	0.063	0.041	0.19	0.089	<u>0.0089</u>	<u>0.0047</u>	0.35	0.21	QN/Asia
		144	T-T	0.9	0.87	0.87	0.65	0.063	0.041	0.19	0.089	<u>0.0089</u>	<u>0.0047</u>	0.35	0.21	QN/Asia
G55	ABC transporter	-	&2	0.91	0.79	0.92	0	0.051	0.38	<u>0.0049</u>	NI	0.3	0.35	0.056	NI	CO/Africa
G70	Choline transporter	105	E-K	0.85	0.85	0.55	0	0.014	0.059	0.32	NI	<u>1.5E-5</u>	0.017	0.16	NI	QN/Asia

P-values are emphasized if significant association was determined by permutation analysis (bold) or linear regression/ANOVA analysis (P < 0.05, underlined); see *Experimental procedures* for these two independent statistical strategies. Some strong P-values for 'All' regions may reflect geographical subdivision (indicated in the comments column) rather than CQ or QN associations, as confirmed by direct geographical association tests (data not shown); in these cases, only weak or non-significant P-values were obtained for isolates from individual continents.
a. Informativeness Index (I, see *Experimental procedures*) was used to exclude SNPs with low minor allele frequency; loci with I < 0.5 are denoted non-informative (NI). Cases with marginal I-values of 0.5-0.7 should also be viewed with care because of relatively low minor allele frequency or parasito sample sizes. &1 represents a trinucleotide insertion and &2 indicates a microsatellite polymorphism 700 bp downstream of the stop codon.
b. Indicates a singleton third allele.

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In another study³², the association of *pfmdr1* gene mutations with *in vitro* susceptibility of 61 *P. falciparum* isolates from Thailand, Myanmar, Vietnam, and Bangladesh was examined. The genetic mutations within the *pfmdr1* gene were characterized by PCR. The isolates were categorized into 4 groups depending on the mutations at codon position 86, 184, 1034, and 1042 (Table 20). In addition, increase in copy number of the *pfmdr1* gene was determined by PCR using dilutions of parasite DNA and the *ldh* gene (single copy lactate dehydrogenase gene) as control. The *in vitro* susceptibility of the isolates to quinine and other antimalarials was measured using the [³H]-hypoxanthine uptake and method of Webster (1985)¹⁶. The cut-off values for resistance to the different antimalarials were as follows: mefloquine IC₅₀ >20 ng/ml or IC₉₀ >80 ng/ml; quinine IC₅₀ >500 ng/ml or IC₉₀ >1,000 ng/ml; chloroquine IC₅₀ >80 ng/ml. Please note the basis for choosing the cut-off value was not specified in the publication.

Table 20: Patterns of mutations observed in *pfmdr1*.

Category	Codon at position ^a				No. of isolates
	86	184	1034	1042	
I	Asn	Tyr	Ser	Asn	17
II	Tyr	Tyr	Ser	Asn	23
III	Asn	Phe	Ser	Asn	15
IV	Asn	Phe	Cys	Asp	10
	Asn	Phe	Cys	Asn	
	Asn	Phe	Ser	Asp	

^a The mutated amino acids (in boldface) and the number of isolates in each category are shown.

No association was observed between the quinine IC₅₀ values and mutations in the *pfmdr1* gene (Table 21).

Table 21: Median IC₅₀s and percent resistance for isolates in 4 categories.

Category	Mefloquine		Quinine		Chloroquine		Median IC ₅₀ (interquartile range) ^b	
	% R ^c	Median IC ₅₀ (interquartile range)	% R	Median IC ₅₀ (interquartile range)	% R	Median IC ₅₀ (interquartile range)	Artemisinin	Artesunate
I (n = 17)	100	60.68 (49.64-69.90)	35	188.62 (104.44-271.88)	76	56.40 (47.08-73.92)	1.74 (1.10-2.04)	4.51 (2.47-4.84)
II (n = 23)	0	15.79 (11.43-19.56)	4	94.68 (77.79-107.83)	91	88.19 (73.09-107.83)	0.73 (0.54-1.40)	1.43 (1.06-2.69)
III (n = 15)	80	59.51 (30.87-73.96)	31	148.87 (70.07-239.88)	81	70.92 (19.38-92.08)	2.57 (1.80-3.85)	2.57 (1.80-3.85)
IV (n = 10)	20	19.44 (10.01-24.70)	11	184.36 (141.28-233.41)	89	70.10 (63.69-98.97)	0.97 (0.80-1.30)	0.85 (0.64-0.93)
<i>P</i> value for difference ^d	<0.001	<0.001	0.266	0.002	<0.001	0.007	<0.001	0.011

^a % R, percentage of resistant isolates.

^b For artemisinin and artesunate, there are no established *in vitro* cut offs for resistance and sensitivity.

^c *P*-values were determined by chi-square analysis for percentage of resistant isolates and analysis of variance for IC₅₀s.

An increase in copy number of *pfmdr1* was observed in all isolates with high quinine IC₅₀ values (median IC₅₀ values of 289.31 ng/ml; see Table 22). However, the number of isolates tested was small (n = 8). Also, the correlation between *pfmdr1* gene amplification and treatment failure with quinine was not examined.

Table 22: Median IC₅₀s and percent resistance for isolates categorized by *pfmdr1* gene amplification.

Category	Mefloquine		Quinine		Chloroquine		Median IC ₅₀ (interquartile range)	
	% R ^a	Median IC ₅₀ (interquartile range)	% R	Median IC ₅₀ (interquartile range)	% R	Median IC ₅₀ (interquartile range)	Artemisinin	Artesunate
II and IV, no amplification (n = 35)	6.1	16.86 (11.13-20.45)	15.2	112.01 (80.98-143.74)	100	84.37 (68.22-107.12)	0.82 (0.64-1.09)	1.32 (0.83-2.13)
I and III, no amplification (n = 24)	87.5	53.01 (34.15-62.79)	16.7	144.35 (71.96-199.15)	91.7	69.06 (50.87-86.92)	1.18 (0.74-1.92)	2.48 (1.71-4.60)
I and III, amplification (n = 8)	100	72.13 (64.77-99.21)	37.5	289.31 (253.44-360.07)	100	57.55 (43.51-64.92)	1.89 (1.28-2.76)	5.63 (3.33-8.33)
P value for difference ^b	<0.0001	<0.0001	0.3280	0.0010	0.3654	0.0029	0.0008	<0.0001

^a % R, percentage of resistant isolates.
^b P values were determined by Fisher's exact chi-square test for percentage of resistant isolates and the Wilcoxon rank sum test for IC₅₀s.

In another study³³, the relationship between *in vitro* susceptibility, genotype and copy number of *pfmdr1* gene was analyzed using isolates from patients in the Amazon region in Brazil. These patients participated in a clinical trial with quinine/tetracycline. However, additional details on treatment dose, duration, and clinical outcome were not included. Blood samples were obtained from patients with confirmed *P. falciparum* infection. Of the 46 samples collected, 26 were successfully cultured *in vitro*. Strain W2 (chloroquine resistant strain) and D6 (mefloquine resistant chloroquine sensitive strain) were used as controls. *In vitro* activity was measured using the method of Desjardins¹⁵. All isolates had chloroquine IC₅₀ value >100 nM (range: 103 - 620 nM) and were considered resistant. In the case of quinine, the isolates had IC₅₀ >30 nM i.e. >0.07 µg/ml (range: 74 - 280 nM or 0.17 - 0.66 µg/ml) and were classified by the authors as having reduced susceptibility to quinine. Sequence analysis of the *pfmdr1* gene was performed using PCR. The *pfmdr1* gene of all isolates showed Asn, Phe, Cys, Asp and Tyr at positions 86, 184, 1034, 1042 and 1246, respectively. These mutations have been associated with chloroquine resistance in *P. falciparum* strains and isolates from South America. Isolates with high quinine IC₅₀ did not differ in sequences (Table 23). The *pfmdr1* gene copy number was analyzed using southern blot in 12 of the 26 isolates. All isolates carried a single copy of *pfmdr1* gene. Overall, mutations in *pfmdr1* gene that correlated with high quinine IC₅₀ values could not be determined in this study.

Table 23

Plasmodium falciparum multidrug resistance (*pfmdr1*) point mutations and gene copy analysis*

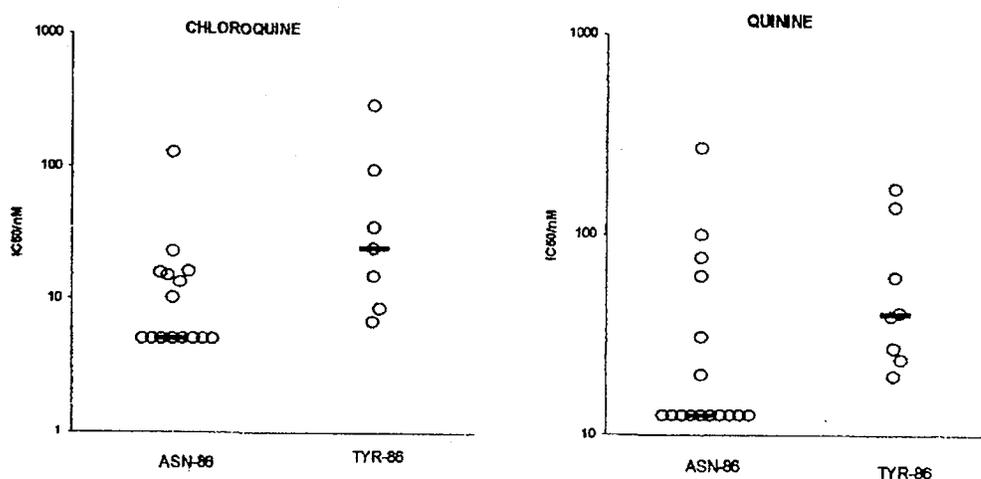
Isolates	Origin	CQ M/S	Amino acids					<i>pfmdr1</i> gene copy number
			86	184	1034	1042	1246	
HBB3	S. America	S	Asn	Phe	Ser	Asp	Asp	
768	S. America	R	Asn	Phe	Cys	Asp	Tyr	
306	S. America	R	Asn	Phe	Cys	Asp	Tyr	
312	S. America	R	Asn	Phe	Cys	Asp	Tyr	
IEC 55/84	S. America	R	Asn	Phe	Cys	Asp	Tyr	
IEC 56/84	S. America	R	Asn	Phe	Cys	Asp	Tyr	
IEC 54/84	S. America	R	Asn	Phe	Cys	Asp	Tyr	
IEC 64/84	S. America	R	Asn	Phe	Cys	Asp	Tyr	
Px101	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px102	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px103	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px104	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px105	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px106	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px107	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px108	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px109	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px110	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px111	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px112	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px113	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px114	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	1
Px115	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px116	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px117	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px118	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px119	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px120	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px121	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px122	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px123	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px124	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px125	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
Px126	Peixoto	R	Asn	Phe	Cys	Asp	Tyr	ND
W7	Indochina	R	Tyr	Tyr	Ser	Asn	Asp	1
D6	Africa	R	Asn	Tyr	Ser	Asn	Asp	1

* The amino acid for each gene mutation was predicted by direct DNA sequence analysis. The five point mutations from the HBB3, 768, 306, 312, IEC55/84, IEC56/84, IEC54/84, and IEC64/84 strains were described by Fries and others.¹¹ The genomic DNAs from the isolates were digested with *Eco*RI and the Southern blots were probed with a *pfmdr1* polymerase chain reaction (PCR) fragment of the *pfmdr1* gene corresponding to bases 3073-3740 of the coding region simultaneously with the circumsporozoite protein gene.¹¹ CQ = chloroquine; R = resistant; S = sensitive; ND = not determined.

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In another study³⁴, the association between *pfmdr1* mutation and quinine susceptibility was analyzed in fresh field isolates from Gambia. Sequence polymorphisms in the *pfmdr1* gene were detected by PCR. *In vitro* susceptibility was measured using the Desjardins method¹⁵. The increase in quinine IC₅₀ of the isolates correlated with presence of the Tyr-86 mutation of the *pfmdr1* gene (Figure 18). The authors have stated that the results suggest cross-resistance between chloroquine and quinine. Here again, the correlation between *pfmdr1* mutation and clinical outcome with quinine was not examined.

Figure 18: *In vitro* activities of chloroquine and quinine of the Gambian isolates with the asn-86 and tyr-86 *pfmdr1* mutations. Median IC₅₀s are shown.



In another study³⁵, the relationship between transporter genes and *in vitro* susceptibility of *P. falciparum* isolates from Thai-Burma border was examined. *In vitro* susceptibility was measured by the method of Desjardins¹⁵. PCR and sequencing were used to identify SNPs in the *pfmdr1* gene or genes for other transporters. Weak associations were observed between the *pfmdr1*-184 mutation and *in vitro* susceptibility to quinine (Table 24). Also, association was seen between mutation in the G7 gene and activity of quinine against *P. falciparum in vitro*.

In summary, a study showed that *P. falciparum* with resistance to quinine can be selected *in vivo*. Variable results were obtained in the 5 studies that evaluated the correlation between *in vitro* susceptibility of strains and/or isolates from different regions and mutations in the *pfmdr1* gene. Some studies showed an association between mutations in the *pfmdr1* gene and reduced susceptibility to quinine *in vitro* while others did not. No attempt was made to correlate mutation changes or copy number changes in *pfmdr1* gene with clinical outcome of patients treated with quinine.

Table 24: Association between SNPs in 10 putative transporter genes and IC₅₀s for eight antimalarial drugs.

Gene	Predicted product	Position	Amino acid change ^a	Nucleotide change ^c	N	Freq. common variant ^d	P value							
							CO	ON	AO	AS	LUM	DHA	DON	MFO
<i>pfmdr</i>	ABC transporter	86	N-Y	AAT-TAT	108	104	0.357	0.646	0.992	0.576	0.809	0.843	0.717	0.079
		184	Y-F	TAT-TTT	105	61	0.405	0.040	0.374	0.025	0.134	0.970	0.568	0.020
		1034	S-C	AGT-TGT	107	107	NI	NI	NI	NI	NI	NI	NI	NI
		1042	N-D	AAT-GAT	108	98	0.778	0.063	0.635	0.144	0.000	0.270	0.763	0.000
G2	ABC transporter	1246	D-Y	GAT-TAT	106	105	NI	NI	NI	NI	NI	NI	NI	NI
		191	Y-H	TAT-CAT	107	107	NI	NI	NI	NI	NI	NI	NI	NI
		437	A-S	GCA-TCA	105	90	0.149	0.795	0.540	0.995	0.304	0.093	0.426	0.768
G7	ABC transporter	1390	&1	NA	104	96	0.061	0.015	0.622	0.042	0.350	0.258	0.712	0.246
G25	Sulfate transporter	Intron	G-A	G-A	101	77	0.771	0.838	0.224	0.702	0.138	0.624	0.071	0.579
G30	GTPase	Intron	C-G	C-G	108	104	0.289	0.870	0.485	0.106	0.638	0.091	0.977	0.272
G47	Glycine transporter	241	L-V	TTA-GTA	105	94	0.386	0.085	0.315	0.389	0.284	0.702	0.995	0.799
G49	ABC/ATPase	146	O-E	CAA-GAA	108	66	0.696	0.937	0.711	0.606	0.299	0.117	0.145	0.988
		1046	K-I	AAA-ATA	108	78	0.260	0.319	0.638	0.316	0.401	0.831	0.557	0.817
		1116	L-I	TTA-ATA	108	96	0.063	0.714	0.435	0.293	0.514	0.002	0.187	0.867
G54	Membrane protein	141	Y-Y	TAC-TAT	102	54	0.209	0.904	0.194	0.848	0.165	0.751	0.443	0.965
		144	T-T	ACG-ACA	102	54	0.209	0.904	0.194	0.848	0.165	0.751	0.443	0.965
G55	ABC transporter	Intron	&2	NA	107	60	0.227	0.212	0.475	0.414	0.161	0.608	0.429	0.938
G70	Choline transporter	105	F-K	GAA-AAA	102	52	0.535	0.292	0.866	0.229	0.497	0.865	0.195	0.557

^a P1ert is not included in the table because it was monomorphic in all but one isolate examined. Drug abbreviations are as described in the text. We used *t* tests to compare IC₅₀s in parasites with polymorphisms, and significant results (*P* < 0.05) are shown in bold. Both polymorphism data and IC₅₀s are listed in the supplemental material online. Comparisons that were significant in Mu et al.'s southeast Asian data set (14) are shown underlined (CO and ON columns only).

^b &1 is a 3-bp indel, &2 is a dinucleotide microsatellite in an intron. We found four alleles. For this analysis, alleles were grouped into those that were < 194 and > 196. Some loci showed insufficient variation (< 2 alleles with one allelic state); these are labeled NI.

^c NA, not applicable.

^d Frequency of common variant.

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3.5. CROSS-RESISTANCE:

Cross-resistance between quinine and mefloquine was observed in *Plasmodium* species *in vitro* and *in vivo*.

3.5.1. In vitro:

The development of cross-resistance between mefloquine and quinine was examined in 2 strains of *P. falciparum in vitro*³⁶. The K1 and W2mef strains of *P. falciparum* were exposed to increasing concentrations of mefloquine to select for isolates with reduced susceptibility to mefloquine. *In vitro* susceptibility of isolates was measured. However, details of the method used for susceptibility testing were not included in the publication. A correlation was observed between mefloquine and quinine IC₅₀ values (Table 25). Expression of Pgh1A, the protein product of *pfmdr1*, was measured by western blotting and the protein signal quantified using a phosphoimager. The correlation between Pgh1 expression and *in vitro* susceptibility to quinine and mefloquine was variable. For example, in the K1mef strain, an increase in quinine IC₅₀ value by 2.3 fold correlated with a 2-fold increase in Pgh1 expression while in the W2mef strain, a 1.5-fold increase in quinine IC₅₀ value resulted in a 2.6 fold increase in Pgh1 expression. These results should be interpreted with caution as only 2 strains were tested and the clinical significance of this finding is not known.

Table 25: Summary of drug sensitivity of isolates selected after mefloquine drug pressure.

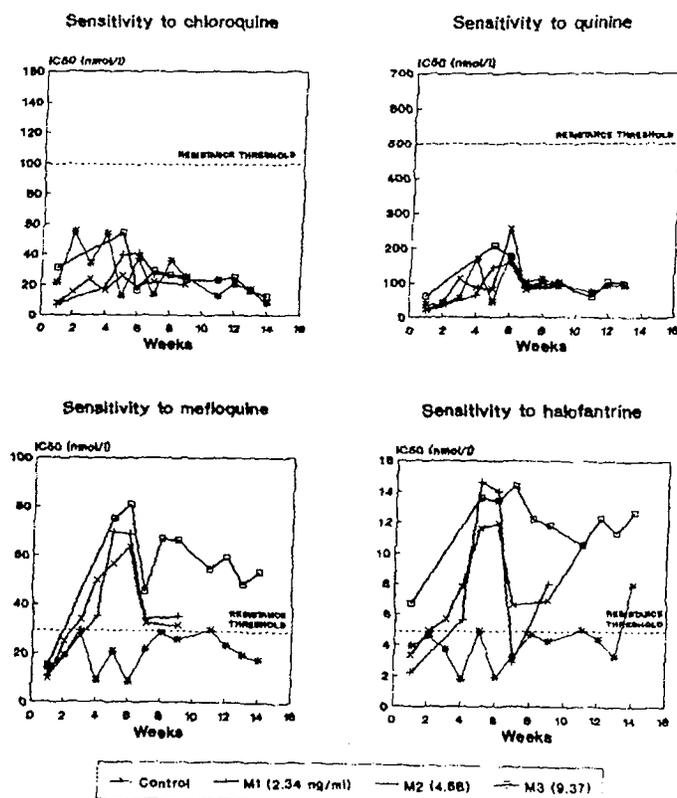
Strain	IC ₅₀ , ng/ml					Pgh1 expression*
	Mefloquine	Halofantrine	Chloroquine	Quinine	Amodiaquine	
K1	22.4	7.8	446.9	127.1	19.9	1 ± 0.1
K1mef	50.1	12	246.1	293.3	16.2	2 ± 0.3
K1mef ²	91.2	13.2	316.3	288.4	19.9	2.6 ± 0.4
W2mef	58.88	12.9	194.98	239.9	12.9	1.9 ± 0.4
W2mef ²	51.29	12.9	123.89	190.6	12.9	2.6 ± 0.5
W2mef ³	83.18	26.8	98.8	309	15.1	4.2 ± 0.6

*These figures are from five independent experiments and are normalized to Pgh1 expression in strain 3D7.

In another study³⁷, the FCN strain of *P. falciparum* was exposed to mefloquine concentration of 4.68 ng/ml and then re-exposed to mefloquine 2.34 ng/ml or 9.37 ng/ml. After 11 weeks of exposure, the mefloquine IC₅₀ values increased above threshold for resistance (30 – 40 nmol/L). The basis for this threshold was not specified. The parasites were then cultured in drug free media for 4 weeks and IC₅₀ determined. For *in vitro* susceptibility, parasite cultures were exposed to drug and [³H]-hypoxanthine in RPMI medium and incubated in a candle jar at 37°C for 48 hours. Incorporation of radiolabel was measured and IC₅₀ determined.

The results in Figure 19 show that isolates with high mefloquine IC₅₀ values also showed increase in halofantrine IC₅₀ and quinine IC₅₀ values. However, the quinine IC₅₀ values were not above the threshold for quinine resistance (quinine IC₅₀ = 600 nmol/L). Please note that the basis for the resistance thresholds was not specified. This study suggests that there is no cross-resistance between mefloquine and quinine against the *P. falciparum* FCN strain *in vitro*.

Figure 19: Evolution of the drug sensitivity profile of the FCN1 strain of *P. falciparum* after 11 weeks of mefloquine pressure. IC₅₀ = 50% inhibitory concentration.



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In another study³⁸, the correlation between the *in vitro* activity of quinine and mefloquine or halofantrine against clinical isolates from Africa was examined. The *in vitro* activity was measured using a modified method of Desjardins¹⁵. The incubation period was modified. Cultures were incubated for 42 hours before addition of [³H]-hypoxanthine. The results in Table 26 show that the activity of quinine correlated with the activity of mefloquine ($r = 0.086$) or halofantrine ($r = 0.028$). However, the correlation was not significant. Thus, the clinical significance of the *in vitro* correlation between quinine and mefloquine is not known.

Table 26. Correlation of *in vitro* responses of African isolates of *Plasmodium falciparum* to quinoline compounds, halofantrine, and artemisinin.

Drug pair		R*	P†
Chloroquine	Quinine	0.651	<0.001
Chloroquine	Mefloquine	-0.405	0.029
Chloroquine	Halofantrine	-0.352	0.037
Quinine	Mefloquine	0.086	NS
Quinine	Halofantrine	0.028	NS
Mefloquine	Halofantrine	0.863	<0.001
Artemisinin	Chloroquine	-0.399	0.018
Artemisinin	Quinine	-0.065	NS
Artemisinin	Mefloquine	0.424	0.022
Artemisinin	Halofantrine	0.569	<0.001

* Spearman's rank-order correlation coefficient (n = 56).

† NS = not significant.

3.5.2: *In vivo*:

The *in vivo* cross-resistance between quinine and mefloquine was examined using a mefloquine-resistant *P. berghei* strain in mice (see section 3.3.1). There was a 4-fold increase in the SD₅₀ value for quinine against the mefloquine-resistant strain compared to the sensitive strain, suggesting a likelihood of cross-resistance between these drugs (Table 15).

3.5.3. Clinical significance of cross-resistance to mefloquine:

The clinical significance of cross-resistance between quinine and mefloquine was examined³⁹. Twenty-four patients who showed either a RI (n = 22) or RII (n = 2) response to treatment with 600 mg quinine administered orally every 8 hours for 7, 10 or 14 days alone or in combination with standard dose of sulfadoxine-pyrimethamine were treated with mefloquine (1000 to 1250 mg). All patients had a successful parasitological outcome with mefloquine at 28 days. Evaluation of patients with RIII responses to quinine would be useful to better understand the clinical significance of decrease in the *in vitro* susceptibility of clinical isolates to mefloquine and quinine.

In summary, mefloquine appears to be effective in the treatment of patients with RI and RII responses to quinine therapy even though a correlation between quinine and mefloquine IC₅₀ values against some clinical isolates has been observed *in vitro*. However, data evaluating the effectiveness of mefloquine in patients with RIII responses to quinine therapy were not available.

3.6. DRUG COMBINATION:

The activity of quinine in combination with other antimalarials (for example, clindamycin, chloroquine, artesiminin, artesunate or quinidine) was examined *in vitro* or *in vivo*.

3.6.1. *In vitro*:

The activity of quinine in combination with other antimalarial drugs was measured using chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* in 4 studies. The criteria for classifying the strain as chloroquine-resistant were not specified. The checkerboard titration using either the Desjardins radiolabelled hypoxanthine uptake assay or the Reickman's schizont maturation method was used to measure activity. The fractional inhibitory concentration (FIC) was provided in one study and was calculated as follows:

$$\text{FIC} = (\text{IC}_{50} \text{ of drug A+B} / \text{IC}_{50} \text{ of drug A}) + (\text{IC}_{50} \text{ of drug A+B} / \text{IC}_{50} \text{ of drug B})$$

A FIC value of >1.0 but <2.0 represents additivity, a value of <1.0 represents synergy, and a value of >2.0 represents antagonism between the two drugs that were used in combination. The activity of quinine plus chloroquine was additive against the chloroquine-resistant strain and antagonistic against the chloroquine-sensitive strain (Table 27). The activity of quinine in combination with clindamycin was synergistic against both the falciparum strains. The activity of quinine in combination with artemisinin varied from antagonistic to synergistic against different strains. The activity of quinine in combination with quinidine was additive.

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Table 27: *In vitro* activity of quinine in combination with other antimalarials against chloroquine resistant and sensitive strains of *P. falciparum*.

Study	Method	Modification	Strain/isolate	Resistant to drug*	Drug combination	Activity	FIC
Rahman (1997) ²¹	Details not given [³ H]-hypoxanthine uptake	none	NF54 NF54 K1 K1	none none chloroquine chloroquine	Quinine + chloroquine Quinine + clindamycin Quinine + chloroquine Quinine + clindamycin	antagonism synergism additive synergism	NA NA NA NA
Gupta (2002) ⁴⁰	Rieckmann (1978) ¹⁴	none	F-32 FCR-3 K1	none none chloroquine	Quinine + artemisinin (at IC ₅₀) Quinine + artemisinin (at IC ₉₀) Quinine + artemisinin (at IC ₉₉) Quinine + artemisinin (at IC ₅₀) Quinine + artemisinin (at IC ₅₀) Quinine + artemisinin (at IC ₉₉) Quinine + artemisinin (at IC ₅₀) Quinine + artemisinin (at IC ₉₀) Quinine + artemisinin (at IC ₉₉) Quinine + artesunate Quinine + artesunate Quinine + quinidine	additive to antagonistic synergism synergism to additive synergism to antagonistic synergism to additive synergism synergism to additive synergism to additive synergism to additive synergism synergism additive	1.15 – 29.42 0.21 – 0.60 0.04 – 1.28 0.76 – 3.79 0.09 – 0.53 0.01-0.42 0.88 – 1.68 0.32 – 1.32 0.12 – 1.10 NA NA NA
Fivelman (1999) ²⁰	Desjardins (1979) ¹⁵	none	D10 RSA11	none chloroquine	Quinine + artesunate Quinine + artesunate	synergism synergism	NA NA
Wesche and Black (1990) ²³	Rieckmann (1978) ¹⁴	none	FCQ-27/PNG	none	Quinine + quinidine	additive	NA

FIC = fractional inhibitory concentration = (IC₅₀ of drug A+B/IC₅₀ of drug A) + (IC₅₀ of drug A+B/IC₅₀ of drug B).

FIC <1 = synergism; FIC >1 and <2 = additive; FIC >2 = antagonism

NA = not available

* Stated by author; interpretive criteria not specified.

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3.6.2. In vivo:

The activity of quinine in combination with chloroquine or clindamycin was examined in mice using the NF54 (chloroquine-sensitive) and K1 (chloroquine-resistant) strains of *P. chaubaudi*²¹. Mice were infected by intravenous inoculation of parasitized erythrocytes (10⁷) and administered different doses of the drugs by the subcutaneous or intraperitoneal route on the day of infection and for 3 additional days. Blood smears were prepared on day 5 to determine parasite count and SD₅₀ values calculated. The ratio of the SD₅₀ values of the drug combination relative to the SD₅₀ values of individual drugs were determined and plotted as an isobologram. The raw data was not provided. The combination of quinine with clindamycin was synergistic *in vivo* and similar to that observed *in vitro* (Tables 27 and 28). However, unlike the observation *in vitro*, quinine in combination with chloroquine was antagonistic against the chloroquine-resistant strain K1 and additive against the chloroquine-sensitive strain NF54.

Table 28: Drug combination response against *P. chaubaudi* strain *in vivo*.

Study	Animal/ <i>Plasmodium</i> species	Inoculum of parasitized erythrocytes (route)	Drug route/ duration	Strain	Resistant to drug*	Drug combination	Activity [#]
Rahman (1997) ²¹	Mice/ <i>P. chaubaudi</i>	10 ⁷ (IV)	IP or SC for 3 days	NF54	none	Quinine + chloroquine	Additive
				NF54	none	Quinine + clindamycin	Synergism
				K1	chloroquine	Quinine + chloroquine	Antagonism
				K1	chloroquine	Quinine + clindamycin	Synergism

* stated by author; interpretive criteria not specified

[#] based on 4 day suppressive test

4. CLINICAL MICROBIOLOGY:

The sponsor included data from 11 publications that describe randomized studies evaluating the efficacy of oral quinine for the treatment of uncomplicated *P. falciparum* malaria (Table 29). The comparators in these studies were chloroquine, mefloquine, sulfadoxin-pyrimethamine, artesunate or a combination of these antimalarials with or without quinine. Of the 11 studies, only one study by Watt (1988)⁴¹ was blinded. The study was conducted in the Philippines and compared the efficacy of quinine to chloroquine in 20 patients with *P. falciparum* malaria and did not evaluate recrudescence. In addition, there were 5 studies that evaluated the efficacy of quinine in combination with tetracycline, chloroquine, clindamycin or sulfadoxin-pyrimethamine for the treatment of uncomplicated *P. falciparum* malaria (Table 30). These publications were identified by searching OLDMEDLINE, MEDLINE®, EMBASE®, JICST-Eplus and Biosis Previews for the period 1951 to date of NDA submission. A total of 382 patients from Philippines, Thailand, Congo, Venezuela, Bangladesh, Vietnam, and Gabon received oral quinine for 7 days. Please note that the DeVries study (2000)⁴² was an extension of the Bich study (1996)⁴³.

The quinine dose used in these studies was 10 mg (sulfate salt)/kg TID for 7 days. Two studies used a 650 mg quinine sulfate TID for 5 or 6 days and one study used 3 doses of 12 mg/kg. Studies that evaluated the 3 day regimen of quinine sulfate were only tested in combination with other drugs.

4.1. Description of clinical studies:

Inclusion criteria in the clinical studies were uncomplicated *P. falciparum* malaria diagnosed by Giemsa stained thin and/or thick smears and/or a specified *P. falciparum* parasite density. The parasite densities in these studies ranged from 32 to 569,722 parasites/ μ l. Patients with severe malaria or who are unable to take oral drugs or who used antimalarials 2 to 7 days prior to study start, and patients with mixed infections were excluded. Patients underwent parasitological evaluations up to 28 days after initiation of therapy in 9 of the 11 studies. In the remaining 2 studies, parasitological evaluations were performed until parasite clearance or until day 63. The parasitological response at day 28 was defined as:

Sensitive (S) = Clearance of parasite within 7 days and no recrudescence within 28 days

Resistant (RI) = Clearance of parasite within 7 days but recrudescence by day 28

Resistant (RII) = Marked reduction of parasitemia and no clearance by day 7

Resistant (RIII) = No marked reduction in parasitemia within first 48 hours

The primary endpoint of these studies was parasitological cure (i.e., a sensitive response). Clinical outcome was not measured. The cure rates at day 28 ranged from 79 to 100% in these studies (Table 29). The recrudescence rate or level of RI resistance was up to 16%. The level of RII and RIII resistance was $\leq 3\%$ and $\leq 6\%$, respectively. The fever clearance time (FCT) and parasite clearance time (PCT) were determined in 7 of the 11 studies. The FCT was defined as the interval from the start of treatment until body temperature returned to normal and remained in the normal range for 24 to 48 hours. The FCT in patients treated with quinine ranged from 4 to 152 hours. The PCT was defined as the interval from start of treatment to first of 2 or 3 consecutive negative blood smears. The PCT for quinine treated patients ranged from 24 to 128 hours.

Gametocyte counts using Giemsa stained thick smear was determined in the study by Pukrittayakamee (2004)⁴⁴. The results of the gametocyte clearance time were shown graphically for the different treatment groups (Figure 20). The median gametocyte clearance time (200 hours) was longer than the median asexual parasite clearance time (80 hours) in patients treated with quinine.

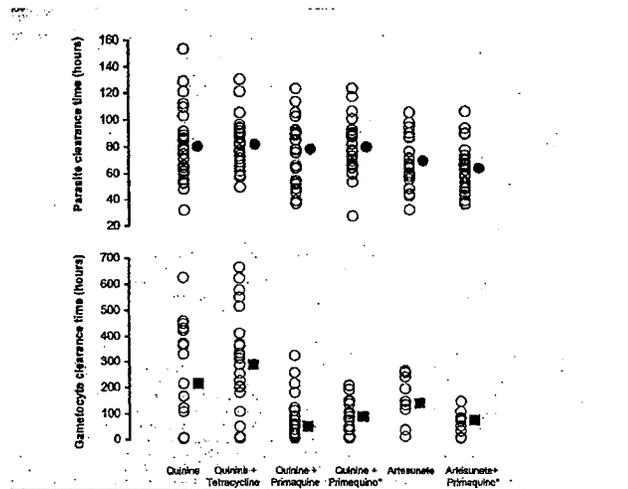


Figure 20: Parasite clearance time and gametocyte clearance time in the six treatment groups of patients with *P. falciparum* malaria. Medians are shown as squares. Primaquine was given at 0.25 or 0.50 (*) mg/day.

Besides microscopic examination, genotyping of the merozoite surface protein 1 (MSP1), merozoite surface protein 2 (MSP2), and glutamine rich protein (GLURP) using PCR was performed in a study by McGready (2000)⁴⁵. The genotyping of baseline and post-treatment isolate was used to differentiate recrudescence from new infection. However, details of the method used for performing the assay, its validation for the purpose of detection of new infections, and actual results were not included in the publication. Based on the genotypic analysis in this study, 9 patients who received quinine had recrudescence and 1 patient had a new infection. For the purpose of this review, all patients who developed parasitemia at day 28 were considered failures due to limited information regarding genotyping and phenotyping.

Quinine in combination with tetracycline or clindamycin was more effective than quinine alone in two studies performed in Thailand by the same investigators^{44, 46}. Five additional studies evaluated the efficacy of quinine in combination with tetracycline, chloroquine, sulfadoxin-pyrimethamine or clindamycin (Table 30). These studies did not have a quinine monotherapy arm. The parasitological cure rates for quinine plus tetracycline, and quinine plus clindamycin were >95%, and 88%, respectively. The efficacy of quinine (12 mg/kg, 3 doses) with clindamycin (5 mg/kg, 3 doses) or doxycycline (2 mg/kg, 3 doses) was evaluated in one study where the combinations with either clindamycin or doxycycline were more effective than quinine alone⁴⁷. However, please note that the dose of quinine in this study was lower than most other studies. The combination of quinine with tetracycline may be useful in areas where quinine resistance is reported.

4.2. Interpretive criteria:

There are no standardized methods for measuring *in vitro* susceptibility of antimalarial drugs against *Plasmodium* and no interpretive criteria have been established.

Table 29: Parasitological and clinical outcome quinine alone or in combination with other antimalarial drugs in the treatment of uncomplicated falciparum malaria.

Author, year (Country)	No. of patients	Age range in years	Study design	Quinine dose and duration	Comparator dose and duration	Diagnostic procedure	Mean or median baseline parasitemia (range)	Mean or median FCT [range] in hours	Mean or median PCT [range] in hours	Parasitological response			
										S	RI	RII	RIII
Watt <i>et al.</i> , 1988 (Philippines) ⁴¹	20	16-49	R, DB	Q5 (648 mg quinine sulfate every 8 hours 5 days)	CQ (1500 mg over 3 days)	Asexual parasites per 200 white blood cells; parasitemia based on patients' actual white blood count	Q5 = 8710 ± 2754 (1500 - 26381) CQ = 9333 ± 3020 (1323 - 33900)	Q5 = 43.2 ± 20.0	Q5 = 60.3 ± 12.5	10/10# (100%)	NR	NR	NR
Pukrittayakamee <i>et al.</i> , 2004 (Thailand) ⁴⁴	142	14-62	R, OL	Q7 (10 mg/kg quinine sulfate TID for 7 days)	-Q7 (10 mg/kg every 8 hours) + T7 (4 mg/kg qid) for 7 days; -A7 = 200 mg day 1, 100 mg for 6 days	Giemsa stained blood smear; parasites per 1,000 RBCs or 200 WBCs determined	Q7 = 9004 (234 - 116,054) Q7T7 = 14,066 (630-231,104) A7 = 64, 449 (321 - 569,722)	Q7 = 63 ± NR [7 - 152] Q7T7 = 33 ± NR [8 - 117] A7 = 34 ± NR [7 - 180]	Q7 = 80 ± 26 [NR] Q7T7 = 81 ± 19 [NR] A7 = 69 ± 19 [NR]	21/25 (84%) 22/22 (100%) 19/21 (90%)	4/25 (16%) 0 (0%) 2/21 (9.5%)	0 (0%) 0 (0%) 0 (0%)	0 (0%) 0 (0%) 0 (0%)
Mueller <i>et al.</i> , 2004 (Congo) ⁴⁶	98	N/A	R, OL	Q7 (500 mg quinine sulfate 3 times daily for 7 days)	As (5 gm) or (9 gm) for 7 days	Giemsa stained thick blood smear	Q7 > 2000 A(5g) > 2000 A (9g) > 2000	Q7 = NR A (5g) = NR A (9g) = NR	Q7 = NR A (5g) = NR A (9g) = NR	27/43 (79%) 11/32 (34%) 9/30 (30%)	NR NR NR	NR NR NR	NR NR NR
Ache <i>et al.</i> , 2002 (Venezuela) ⁴⁹	165	N/A Mean 28.5	R, OL	Q7 (10 mg/kg quinine sulfate every 8 hours for 7 days)	-CQ3 (25 mg/kg for 3 days); -CQ4 (40 mg/kg for 4 days); -SP1 (single dose)	Thick blood smear; parasites per 500 WBCs and mean count of 6000 WBCs/μl	Q7 = 2195 ± 968.7 CQ3 = 1860 ± 594.1 CQ4 = 1929.6 ± 962.8 SP1 = 1799.2 ± 763.6	Q7 = NR CQ3 = NR CQ4 = NR SP1 = NR	Q7 = NR CQ3 = NR CQ4 = NR SP1 = NR	48/48 (100%) 0 (0%) 40/52 (77%) 41/53 (77%)	0 (0%) 12/12 (100%) 12/52 (23%) 12/53 (23%)	0 (0%) 0 (0%) 0 (0%) 0 (0%)	0 (0%) 0 (0%) 0 (0%) 0 (0%)

R = randomized; DB = double blind; OL = open label; Q = quinine; CQ = chloroquine, A = artesunate, T = tetracycline, M = mefloquine, SP = sulfadoxine/pyrimethamine, As = Artemisia tea # no follow-up; parasite clearance by day 6;

Sensitive (S) = Clearance of parasite within 7 days and no recrudescence within 28 days

Resistant (RI) = Clearance of parasite within 7 days but recrudescence by day 28

Resistant (RII) = Marked reduction of parasitemia and no clearance by day 7

Resistant (RIII) = No marked reduction in parasitemia within first 48 hours

PCT = parasite clearance time

FCT = fever clearance time;

SP = Artemisia tea

Table 29 continued.

Author, year (Country)	No. of patients	Age range in years	Study design	Quinine dose and duration	Comparator dose and duration	Diagnostic procedure	Mean or median baseline parasitemia (range)	FCT [range] in hours	PCT [range] in hours	Parasitological response			
										S	RI	RII	RIII
Rahman <i>et al.</i> , 2001 (Bangladesh) ⁵⁰⁺	413	12-60	R, OL	Q7 (10 mg/kg quinine sulfate every 8 hours for 7 days)	Q3 (30 mg/kg every 8 hours for 3 days followed by single dose of SP (2.5mg/kg); - CQ3 (25 mg/kg over 3 days); - M1 (20 mg/kg for 1 day)	Blood smear (no details given)	Q7 = 10940 (500-250000) Q3SP1 = 11500 (480-154,560) CQ3 = 15000 (500-149,000) M1 = 11500 (560-97000)	Q7 = 33.9 ± 27.6 [NR] Q3SP1 = 35.1 ± 24.7 [NR] CQ3 = 33.5 ± 29.0 [NR] M1 = 25.5 ± 26.5 [NR]	Q7 = 54.5 ± 21.8 [NR] Q3SP1 = 56.4 ± 27.1 [NR] CQ3 = 68.9 ± 35.5 [NR] M1 = 57.1 ± 29.1 [NR]	40/49 (82%) 96/145 (66%) 34/149 (23%) 51/70 (73%)	5/49 (10%) 32/145 (22%) 32/149 (21%) 9/70 (13%)	1/49 (2%) 5/145 (3%) 24/149 (16%) 3/70 (4%)	3/49 (6%) 12/145 (8%) 59/149 (40%) 7/70 (10%)
Pukrittayakamee <i>et al.</i> , 2000 (Thailand) ⁴⁶	161	15-64	R, OL	Q7 (10 mg/kg quinine sulfate TID for 7 days)	-Q7 (10 mg/kg every 8 hours) + C7 (5 mg/kg qid) for 7 days; -Q7 (10 mg/kg every 8 hours) + T7 (4 mg/kg qid) for 7 days	Giemsa stained blood smear; parasites per 1,000 RBCs or 200 WBCs determined	Q7 = 9,493 Q7C7 = 17,155 Q7T7 = 9,352	Q7 = 56 ± NR [4-152] Q7C7 = 47 ± NR [8-120] Q7T7 = 36 ± NR [8-117]	Q7 = 77 ± 25 [NR] Q7C7 = 79 ± 20 [NR] Q7T7 = 77 ± 23 [NR]	46/53 (87%) 60/60 (100%) 47/48 (98%)	7/53 (13%) 0 (0%) 1/48 (2%)	0 (0%) 0 (0%) 0 (0%)	0 (0%) 0 (0%) 0 (0%)
McGready <i>et al.</i> , 2000 (Thailand) ⁴⁵	108	15-37	R, OL	Q7 (10 mg/kg quinine sulfate every 8 hours for 7 days)	M2 (total 25 mg/kg over 2 days) + A3 (total dose of 12 mg/kg over 3 days)	Blood smear (details not given); PCR genotyping of the MSP-1, MSP-2, and GLURP genes to identify recrudescence from re-infection	Q7 = 19,086 (79-149,389) M2A3 = 11,651 (32-241,127)	Q7 = NR M2A3 = NR	Q7 = NR M2A3 = NR	27/41 (67%) 64/65 (98%)	9 [^] ; 1 [§] 1 [§]	NR NR	NR NR

R = randomized; OL = open label; NR = not reported; FCT = fever clearance time; PCT = parasite clearance time
 Q = quinine; CQ = chloroquine, A = artesunate, T = tetracycline, M = mefloquine, SP = sulfadoxine/pyrimethamine, C = clindamycin
 Resistant (RI) = Clearance of parasite within 7 days but recrudescence by day 28
 Resistant (RII) = Marked reduction of parasitemia and no clearance by day 7
 Resistant (RIII) = No marked reduction in parasitemia within first 48 hours
[^] recrudescence, pre- and post genotype for MSP1, 2 and GLURP same on day 63;
[§] new infection, pre- and post genotype for MSP1, 2 and GLURP different on day 63
 Please note that the details on genotyping were insufficient for review.
 + area with CQ and SP failure

Table 29 continued.

Author, year (Country)	No. of patients	Age range in years	Study design	Quinine dose and duration	Comparator dose and duration	Diagnostic procedure	Mean or median baseline parasitemia (range)	FCT [range] in hours	PCT [range] in hours	Parasitological response			
										S	RI	RII	RIII
De Vries <i>et al.</i> , 2000 (Vietnam) ⁴²	221	7-64	R, OL	Q7 (10 mg/kg quinine sulfate TID for 7 days)	-A1 (20 mg/kg single dose) + Q3 (10 mg/kg tid for 3 days); -A1 (20 mg/kg single dose) + Q5 (10 mg/kg tid for 5 days)	Giemsa stained thick and thin blood smears; 100 high power fields examined	Q7 = 16, 157 (12,642 - 20, 646) A1Q3 = 16, 123 (12, 611 - 20, 611) A1Q5 = 23, 202 (17, 888 - 30, 091)	Q7 = 62 ± NR [NR] A1Q3 = 41 ± NR [NR] A1Q5 = 42 ± NR [NR]	Q7 = 62 ± NR [NR] A1Q3 = 41 ± NR [NR] A1Q5 = 42 ± NR [NR]	S	RI	RII	RIII
										56/69 (81%)	11/69 (16%)	1/69 (1%)	1/69 (1%)
										46/74 (38%)	28/74 (38%)	0	0
Bich <i>et al.</i> , 1996 (Vietnam) ⁴³	118	9-64	R, OL	Q7 (10 mg/kg quinine sulfate TID for 7 days)	-A1 (20 mg/kg single dose) + Q3 (10 mg/kg tid for 3 days); -A1 (20 mg/kg single dose) + D3 (4 mg/kg for 3 days)	Giemsa stained thick and thin blood smears; 100 high power fields examined	Q7 = 29,636 ± 39,720 (91,058 - 190,133) A1Q3 = 21,321 ± 21,877 (1,060 - 111,294) A1D3 = 46,088 ± 91,523 (1,152 - 461,386)	Q7 = 66 ± 24 [24 - 128] A1Q3 = 43 ± 14 [16 - 72] A1D3 = 41 ± 19 [16 - 136]	Q7 = 66 ± 24 [24 - 128] A1Q3 = 43 ± 14 [16 - 72] A1D3 = 41 ± 19 [16 - 136]	S	RI	RII	RIII
										36/44 (82%)	7/44 (16%)	1/44 (2%)	0 (0%)
										23/32 (72%)	9/32 (28%)	0	0
Metzger <i>et al.</i> , 1995 (Gabon) ⁴⁷	108	15-70	R, OL	Q1.5 (12 mg/kg every 12 hours; 3 doses)	-Q1.5 (3 doses of 12 mg/kg) + C3 (5mg/kg bid for 3 days); -Q1.5 (3 doses of 12 mg/kg) + D3 (2 mg/kg bid for 3 days)	Giemsa stained thick smears	Q1.5 = 5515 (450 - 68,000) Q1.5C3 = 10, 642 (200 - 115,000) Q1.5D3 = 7807 (1,000 - 140,000)	Q1.5 = NR Q1.5C3 = NR Q1.5D3 = NR	Q1.5 = NR Q1.5C3 = NR Q1.5D3 = NR	S	RI	RII	RIII
										14/37 (38%)	23/37 (62%)	0	0
										33/36 (92%)	3/36 (8%)	0	0
Segal <i>et al.</i> , 1974 (Thailand) ⁵¹	47	15-53	R, OL	Q6 (549 mg quinine base every 8 hours for 6 days)	W6 (600 mg every 8 hours for 6 days)	Giemsa stained blood film; 400 high power fields examined	Q6 = 13500 (1240-91,880) W6 = 20,400 (3,840 - 87,814)	Q6 = 65.1 ± NR [21 - 103] W6 = 66.3 ± NR [21 - 113]	Q6 = 65.1 ± NR [21 - 103] W6 = 66.3 ± NR [21 - 113]	S	RI	RII	RIII
										21/22 (95%)	1/22 (4%)	NR	NR
										23/25 (92%)	2/25 (8%)	NR	NR

R = randomized; OL = open label; FCT = fever clearance time; PCT = parasite clearance time;

Q = quinine; A = artemisinin; D = doxycycline; W = WR 33063

Resistant (RI) = Clearance of parasite within 7 days but recrudescence by day 28

Resistant (RII) = Marked reduction of parasitemia and no clearance by day 7

Resistant (RIII) = No marked reduction in parasitemia within first 48 hours

Table 30: Parasitological and clinical outcomes of quinine in combination with other drugs in the treatment of uncomplicated falciparum malaria.

Author, year (Country)	No. of patients	Age range in years	Study design	Quinine (Q) combination dose and duration	Comparator dose and duration	Diagnostic procedure	Mean or median baseline parasitemia (range)	FCT [range] in hours	PCT [range] in hours	Parasitological response			
										S	RI	RII	RIII
Vannianton <i>et al.</i> , 1996 (Thailand) ⁵²	36	14 - 51	R, OL	Q7 (10 mg/kg every 8 hours) + T7 (4 mg/kg qid) for 7 days	Q7 (10 mg/kg every 8 hours for 7 days) + CQ3 (total 25mg/kg over 3 days)	Thick and thin blood smears	Q7T7 = 19489 (10,710 - 35,480) Q7CQ3 = 18197 (9,550 - 34,670)	Q7T7 = 41 ± 27 Q7CQ3 = 51 ± 33	Q7T7 = 83 ± 21 Q7CQ3 = 80 ± 25	17/18 (95%) 11/18 (61%)	1/18 (5%) 7/18 (39%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)
Loareesuwan <i>et al.</i> , 1994 (Thailand) ⁵³	93	16 - 67	R, OL	Q7 (600 mg salt every 8 hours) + T (250 mg 4 times daily) for 7 days	M1 (1250 mg for 1 day) + T7 (250 mg every 6 hours for 7 days)	Blood smears; 200 high power fields	Q7T7 = 12,638 (200-367,770) M1T7 = 10,607 (267 - 183,300)	Q7T7 = 61.8 ± 42.1 [4 - 156] M1T7 = 47.8 ± 32.7 [4 - 128]	Q7T7 = 73.9 ± 23.7 [23 - 128] M1T7 = 65.0 ± 22.8 [26-127]	45/46 (98%) 44/47 (94%)	1/46 (2%) 3/47 (6%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)
Karbwang <i>et al.</i> , 1994 (Thailand) ⁵⁴	60	15 - 35	R, OL	Q7 (600 mg as sulfate salt every 8 hours) + T (250 mg every 6 hours) for 7 days	A5 (200mg loading dose and 100mg every 12 hours for 5 days)	Field stained blood smear; parasites per 1,000 RBCs or 200 WBCs determined	Q7T7 = 35,188 (351 - 175,010) A5 = 50,206 (504 - 292,560)	Q7T7 = 55.4 ± NR [4 - 104] A5 = 31.3 ± NR [4 - 67]	Q7T7 = 73.2 ± NR [36 - 135] A5 = 36.5 ± NR [24-52]	30/30 (100%) 29/30 (97%)	0 (0%) 1/30 (3%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)
Kremsner <i>et al.</i> , 1988 (Brazil) ⁵⁵	95	14 - >40	R, OL	Q3 (15 mg sulfate salt/kg every 12 hours) + C3 (10 mg/kg every 12 hours) for 3 days	-Q3 (15 mg sulfate salt/kg every 12 hours for 3 days) + SP (500/25 mg for 2 days); -AM 2 (10mg/kg initially and 7.5 mg/kg at 24 and 48 hours later)	Giemsa stained thin and thick blood smear; parasite count in 100 high power fields	Q3C3 = 6989 (500 - 56,200) Q3SP2 = 3630 (500 - 58,500) AM2 = 5360 (500 - 68,000)	Q3C3 = NR Q3SP2 = NR AM2 = NR	Q3C3 = 86.4 ± NR Q3SP2 = 78.2 ± NR AM2 = 82.7 ± NR	36/40 (90%) 9/30 (30%) 1/25 (4%)	4/40 (10%) 19/30 (63%) 6/25 (24%)	0 (0%) 2/30 (7%) 7/25 (28%)	0 (0%) 0 (0%) 5 (44%)
De Souza <i>et al.</i> , 1985 (Brazil) ⁵⁶	89	18 - 55	R, OL	Q3 (600 mg as sulfate salt every 8 hours for 3 days) + SP1 (25/500 mg on day 1)	M1 (1000 mg on day 1)	Blood smear (no details given)	Q3SP1 = 19626 M1 = 19580	Q3SP1 = NR M1 = NR	Q3SP1 = NR M1 = NR	46/50 (92%) 49/49 (100%)	4/50 (8%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)

R = randomized study; OL = open label; NR = not reported; FCT = fever clearance time; PCT = Parasite clearance time;
 Q = quinine; CQ = chloroquine, A = artesunate, T = tetraacycline, C = clindamycin, M = mefloquine, SP = sulfadoxine/pyrimethamine, AM = artemether
 Sensitive (S) = Clearance of parasite within 7 days and no recrudescence within 28 days
 Resistant (RI) = Clearance of parasite within 7 days but recrudescence by day 28
 Resistant (RII) = Marked reduction of parasitemia and no clearance by day 7
 Resistant (RIII) = No marked reduction in parasitemia within first 48 hours

5. DISCUSSION:

The sponsor has requested approval of quinine sulfate capsule (2 x 648 mg/kg TID for 7 days) for the treatment of uncomplicated *P. falciparum* malaria.

Quinine is an alkaloid derived from the cinchona bark. 3-Hydroxyquinine is the major metabolite of quinine in the body.

Mechanism of action:

Quinine can interact with hemazoin in parasitized erythrocytes. Inhibition of nucleic acid and protein synthesis in *P. falciparum* parasitized erythrocytes was observed within 4 hours of exposure to quinine and inhibition of glycolysis within 9 hours. A concentration of quinine (18.7 µg/ml) that was 27-fold higher than that required for inhibition of parasite growth *in vitro* was shown to inhibit heme polymerase activity in lysates of parasitized erythrocytes. *In vitro*, quinine-hemazoin complex can lead to lipid peroxidation in liposomes. However, quinine did not have an effect on degradation of heme or accumulation of hemoglobin. The drug was also shown to inhibit release of tumor necrosis factor-alpha from macrophages, which may prevent progression of the disease. However, the precise mechanism by which quinine exhibits antiparasitic activity is not well understood.

Activity *in vitro*:

Quinine and its metabolite, 3-hydroxyquinine, was active against the erythrocytic stages of *P. falciparum*. The activity of quinine was measured against laboratory strains (n = 13) and several clinical isolates (n = 129) from Thailand, Bangladesh, and Africa. Please note that methods to evaluate *in vitro* susceptibility of *P. falciparum* to antimalarial drugs are not standardized. The antiparasitic activity was measured by incorporation of [³H]-hypoxanthine or by microscopic observations. A majority of these studies were done by incubating the asynchronous parasites with the drug for 24 to 72 hours. The results expressed as 50% inhibitory concentration (IC₅₀) show the quinine IC₅₀ values against the laboratory strains or clinical isolates to ≤ 0.68 µg/ml. The metabolite (IC₅₀ ≤ 1.03 µg/ml) was less active than the parent drug. The activity of metabolites other than 3-hydroxyquinine, was not examined *in vitro*. Quinine was less active than its isomer, quinidine, and other antimalarials such as chloroquine and mefloquine.

Quinine was more active against the schizont stage than the trophozoite or ring stages. The *in vitro* activity of quinine against the gametocyte stage of *P. falciparum* was not examined. The quinine IC₅₀ values against strains that were freeze thawed and culture adapted were 2 to 5 fold higher than that against fresh cultures.

No studies were done to demonstrate the *in vitro* activity of quinine against the hepatic stages of the *Plasmodium* species.

Activity *in vivo*:

The activity of quinine was measured against the erythrocytic forms of *P. berghei*, *P. yoelii*, *P. chabaudi*, and *P. cynomolgi* in mice or monkeys.

In mice infected intraperitoneally with *P. berghei* (chloroquine-resistant or -sensitive strain) and treated at the time of infection with oral quinine or quinidine, a 50% reduction in parasitemia was observed at a dose of $\leq 35.44 \pm 13.77$ mg/kg, and $\leq 24.81 \pm 6.55$ mg/kg, respectively. A 4-fold higher concentration of quinine was required against the mefloquine-resistant strain, suggesting cross-resistance between quinine and mefloquine. Higher concentrations were also required against a quinine-resistance strain. However, details of how the quinine-resistant strain was derived were not provided.

The quinine SD_{50} in mice infected with a multi drug-resistant *P. yoelii* strain was similar to that observed with the *P. berghei* chloroquine-resistant strain.

In another study, the intraperitoneal or subcutaneous quinine doses for suppression of parasitemia in mice infected intravenously with *P. chabaudi* chloroquine-resistant and chloroquine-sensitive strains were similar to that seen in the *P. berghei* and *P. yoelii* murine models.

Recrudescence was not examined in any of the studies using the mouse model. Also, no information was available on parasite clearance times.

Oral quinine (31.6 mg/kg for 7 days) administered 4 days after infection was effective in curing 2 monkeys infected with *P. cynomolgi*. No recrudescence was observed in the 30 day follow-up period after splenectomy. A lower dose of chloroquine (10 mg/kg for 7 days) was required to achieve the same effect. The time to parasite clearance was not specified in this study.

The activity of quinine against hepatic stages of the *Plasmodium* species was not examined *in vivo*.

Resistance:

A potential for resistance development by *P. falciparum* to quinine was examined *in vivo*. Serial passage of the erythrocytic forms of *P. falciparum* Panama II strain in Aotus monkeys treated with subcurative doses of quinine resulted in a strain with uniform resistance to quinine. Monkeys infected with the resistant strain showed RII and RIII type responses when treated with 125 mg/kg dose of quinine for 14 days.

In patients with uncomplicated malaria, the level of RI, RII, and RIII resistance reported in patients treated with quinine (10 mg/kg TID for 7 days) from different areas of South America, Southeast Asia and Bangladesh was $\leq 16\%$, $\leq 3\%$, and $\leq 6\%$, respectively. In the absence of genotyping and phenotyping, it is unclear whether the recrudescence is due to resistance.

Cross-resistance:

Cross-resistance between quinine and mefloquine was observed in 2 strains of *P. falciparum* when exposed to mefloquine pressure. In contrast, no cross-resistance was observed between the 2 drugs against strains selected after exposure of the FCN strain of *P. falciparum* to mefloquine pressure in another study. Although, a positive correlation was observed between the *in vitro* activity of quinine and mefloquine or halofantrine, suggesting a likelihood of development of cross-resistance between the two drugs, mefloquine was successful in the treatment of patients who showed RI or RII response to quinine.

Drug combination

The *in vitro* activity of quinine in combination with other drugs against *P. falciparum* was measured. A combination of quinine with clindamycin was synergistic against *P. falciparum*. The activity of quinine in combination with quinidine was additive against *P. falciparum*. The activity of quinine in combination with artemisinin varied from antagonistic to synergistic against different strains. The combination of quinine with chloroquine was additive against the chloroquine-resistant *P. falciparum* strain and antagonistic against the chloroquine-sensitive *P. falciparum* strain.

The activity of quinine in combination with chloroquine or clindamycin was examined in mice using the NF54 (chloroquine-sensitive) and K1 (chloroquine-resistant) strains of *P. chaubaudi*. A combination of quinine with clindamycin was synergistic. Quinine in combination with chloroquine was antagonistic against the chloroquine-resistant K1 strain of *P. chaubaudi* and additive against the chloroquine-sensitive NF54 strain of *P. chaubaudi*. This result was in contrast to that observed *in vitro* using the *P. falciparum* strain

Clinical Microbiology

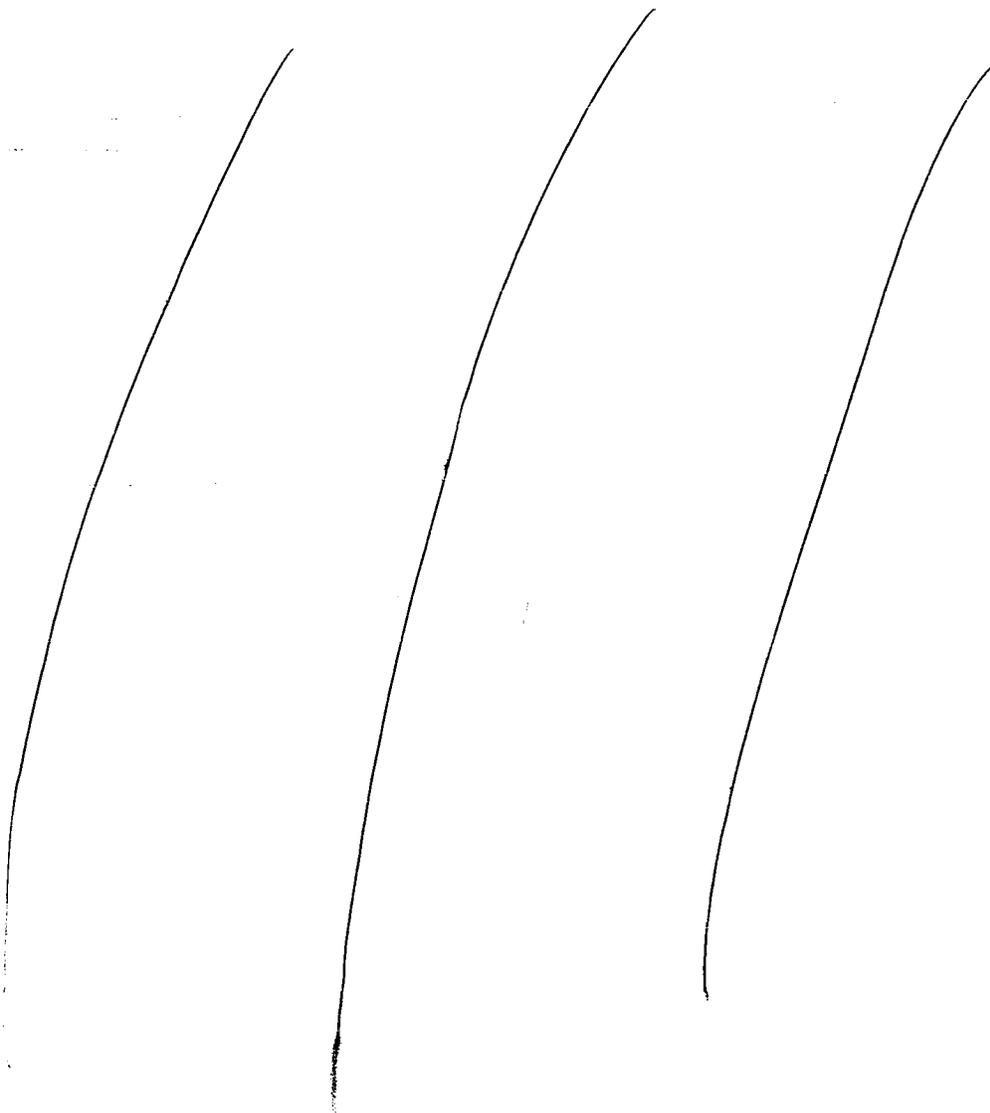
Eleven randomized studies evaluated the efficacy of quinine (10 mg/kg TID for 7 days) in 382 patients with *P. falciparum* malaria. These studies were conducted in Philippines, Thailand, Congo, Venezuela, Bangladesh, Vietnam, and Gabon. The cure rates at day 28 ranged from 79 to 100% in these studies (Table 29). The recrudescence rate or level of RI resistance was up to 16%. The level of RII and RIII resistance were $\leq 3\%$ and $\leq 6\%$, respectively. Only one study performed genotyping of the MSP1, MSP2 and GLURP genes to differentiate recrudescence from new infection. However, no details regarding methodology used, method validation, and raw data were included in the publication. Based on limited data, recrudescence cannot be differentiated from new infections or resistant parasites.

In a study that determined gametocyte counts, the median gametocyte clearance time (200 hours) was longer than the median asexual parasite clearance time (80 hours) in patients treated with quinine.

Studies that evaluated the efficacy of 3 day regimen of quinine monotherapy were not included. The 3 day regimen of quinine was examined in combination with other drugs. Quinine in combination with tetracycline or clindamycin was more effective than quinine alone in two studies performed in Thailand by the same investigator. Other studies that evaluated the combination of quinine with tetracycline or quinine with clindamycin did not have a quinine monotherapy arm for comparison. The parasitological cure rates in patients treated with quinine plus tetracycline was >95% and in those treated with quinine with clindamycin was 88%.

6. THE LABEL

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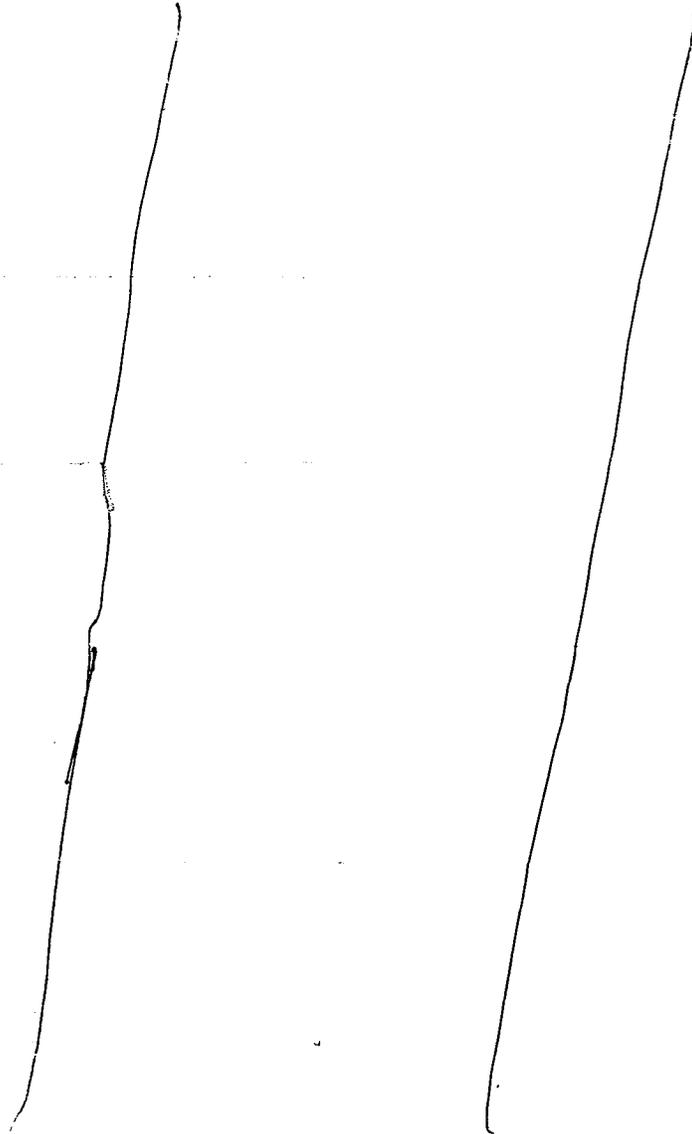


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7. REFERENCES

- (1) Warhurst DC. The quinine-haemin interaction and its relationship to antimalarial activity. *Biochem Pharmacol* 1981 December 15;30(24):3323-7.
- (2) Mungthin M, Bray PG, Ridley RG, Ward SA. Central role of hemoglobin degradation in mechanisms of action of 4-aminoquinolines, quinoline methanols, and phenanthrene methanols. *Antimicrob Agents Chemother* 1998 November;42(11):2973-7.

Quinine

Mutual Pharmaceuticals

-
- (3) Fitch CD, Chou AC. Regulation of heme polymerizing activity and the antimalarial action of chloroquine. *Antimicrob Agents Chemother* 1997 November;41(11):2461-5.
 - (4) Chou AC, Fitch CD. Control of heme polymerase by chloroquine and other quinoline derivatives. *Biochem Biophys Res Commun* 1993 August 31;195(1):422-7.
 - (5) Zhang J, Krugliak M, Ginsburg H. The fate of ferriprotophyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. *Mol Biochem Parasitol* 1999 March 15;99(1):129-41.
 - (6) Famin O, Ginsburg H. Differential effects of 4-aminoquinoline-containing antimalarial drugs on hemoglobin digestion in Plasmodium falciparum-infected erythrocytes. *Biochem Pharmacol* 2002 February 1;63(3):393-8.
 - (7) Famin O, Krugliak M, Ginsburg H. Kinetics of inhibition of glutathione-mediated degradation of ferriprotoporphyrin IX by antimalarial drugs. *Biochem Pharmacol* 1999 July 1;58(1):59-68.
 - (8) Aceti A, Bonincontro A, Cametti C, Celestino D, Leri O. Electrical conductivity of human erythrocytes infected with Plasmodium falciparum and its modification following quinine therapy. *Trans R Soc Trop Med Hyg* 1990 September;84(5):671-2.
 - (9) Sugioka Y, Suzuki M. The chemical basis for the ferriprotoporphyrin IX-chloroquine complex induced lipid peroxidation. *Biochim Biophys Acta* 1991 May 24;1074(1):19-24.
 - (10) ter Kuile F, White NJ, Holloway P, Pasvol G, Krishna S. Plasmodium falciparum: in vitro studies of the pharmacodynamic properties of drugs used for the treatment of severe malaria. *Exp Parasitol* 1993 February;76(1):85-95.
 - (11) Holloway PA, Krishna S, White NJ. Plasmodium berghei: lactic acidosis and hypoglycaemia in a rodent model of severe malaria; effects of glucose, quinine, and dichloroacetate. *Exp Parasitol* 1991 February;72(2):123-33.
 - (12) Maruyama N, Kakuta Y, Yamauchi K et al. Quinine inhibits production of tumor necrosis factor-alpha from human alveolar macrophages. *Am J Respir Cell Mol Biol* 1994 May;10(5):514-20.
 - (13) Kwiatkowski D, Bate C. Inhibition of tumour necrosis factor (TNF) production by antimalarial drugs used in cerebral malaria. *Trans R Soc Trop Med Hyg* 1995 March;89(2):215-6.
 - (14) Rieckmann KH, Campbell GH, Sax LJ, Mrema JE. Drug sensitivity of plasmodium falciparum. An in-vitro microtechnique. *Lancet* 1978 January 7;1(8054):22-3.
 - (15) Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob Agents Chemother* 1979 December;16(6):710-8.
 - (16) Webster HK, Boudreau EF, Pavanand K, Yongvanitchit K, Pang LW. Antimalarial drug susceptibility testing of Plasmodium falciparum in Thailand using a microdilution radioisotope method. *Am J Trop Med Hyg* 1985 March;34(2):228-35.

-
- (17) Druilhe P, Brandicourt O, Chongsuphajaisiddhi T, Berthe J. Activity of a combination of three cinchona bark alkaloids against *Plasmodium falciparum* in vitro. *Antimicrob Agents Chemother* 1988 February;32(2):250-4.
 - (18) Nontprasert A, Pukrittayakamee S, Kyle DE, Vanijanonta S, White NJ. Antimalarial activity and interactions between quinine, dihydroquinine and 3-hydroxyquinine against *Plasmodium falciparum* in vitro. *Trans R Soc Trop Med Hyg* 1996 September;90(5):553-5.
 - (19) Sharma P, Pillai CR, Devi SJ. In vitro schizontocidal activity of standard antimalarial drugs on chloroquine-sensitive and chloroquine-resistant isolates of *Plasmodium falciparum*. *Indian J Exp Biol* 2000 November;38(11):1129-33.
 - (20) Fivelman QL, Walden JC, Smith PJ, Folb PI, Barnes KI. The effect of artesunate combined with standard antimalarials against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* in vitro. *Trans R Soc Trop Med Hyg* 1999 July;93(4):429-32.
 - (21) Rahman NN. Evaluation of the sensitivity in vitro of *Plasmodium falciparum* and in vivo of *Plasmodium chabaudi* Malaria to various drugs and their combinations. *Med J Malaysia* 1997 December;52(4):390-8.
 - (22) Carvalho LH, Brandao MG, Santos-Filho D, Lopes JL, Krettli AU. Antimalarial activity of crude extracts from Brazilian plants studied in vivo in *Plasmodium berghei*-infected mice and in vitro against *Plasmodium falciparum* in culture. *Braz J Med Biol Res* 1991;24(11):1113-23.
 - (23) Wesche DL, Black J. A comparison of the antimalarial activity of the cinchona alkaloids against *Plasmodium falciparum* in vitro. *J Trop Med Hyg* 1990 June;93(3):153-9.
 - (24) Noedl H, Faiz MA, Yunus EB et al. Drug-resistant malaria in Bangladesh: an in vitro assessment. *Am J Trop Med Hyg* 2003 February;68(2):140-2.
 - (25) Sowunmi A, Salako LA, Laoye OJ, Aderounmu AF. Combination of quinine, quinidine and cinchonine for the treatment of acute falciparum malaria: correlation with the susceptibility of *Plasmodium falciparum* to the cinchona alkaloids in vitro. *Trans R Soc Trop Med Hyg* 1990 September;84(5):626-9.
 - (26) Shanker S, Toohey M, Munro R. In vitro activity of seventeen antimicrobial agents against *Gardnerella vaginalis*. *Eur J Clin Microbiol* 1982 October;1(5):298-300.
 - (27) Skinner TS, Manning LS, Johnston WA, Davis TM. In vitro stage-specific sensitivity of *Plasmodium falciparum* to quinine and artemisinin drugs. *Int J Parasitol* 1996 May;26(5):519-25.
 - (28) Kazim M, Puri SK, Dutta GP. Comparative evaluation of blood schizontocidal activity of quinine and quinidine against drug resistant rodent malaria. *J Commun Dis* 1991 December;23(4):254-6.
 - (29) Davidson DE, Jr., Johnsen DO, Tanticharoenyos P, Hickman RL, Kinnamon KE. Evaluating new antimalarial drugs against trophozoite induced *Plasmodium cynomolgi* malaria in rhesus monkeys. *Am J Trop Med Hyg* 1976 January;25(1):26-33.

Quinine

Mutual Pharmaceuticals

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- (30) Glew RH, Collins WE, Miller LH. Selection of increased quinine resistance in *Plasmodium falciparum* in Aotus monkeys. *Am J Trop Med Hyg* 1978 January;27(1 Pt 1):9-13.
 - (31) Mu J, Ferdig MT, Feng X et al. Multiple transporters associated with malaria parasite responses to chloroquine and quinine. *Mol Microbiol* 2003 August;49(4):977-89.
 - (32) Pickard AL, Wongsrichanalai C, Purfield A et al. Resistance to antimalarials in Southeast Asia and genetic polymorphisms in *pfmdr1*. *Antimicrob Agents Chemother* 2003 August;47(8):2418-23.
 - (33) Zalis MG, Pang L, Silveira MS, Milhous WK, Wirth DF. Characterization of *Plasmodium falciparum* isolated from the Amazon region of Brazil: evidence for quinine resistance. *Am J Trop Med Hyg* 1998 May;58(5):630-7.
 - (34) Duraisingh MT, Jones P, Sambou I, von Seidlein L, Pinder M, Warhurst DC. The tyrosine-86 allele of the *pfmdr1* gene of *Plasmodium falciparum* is associated with increased sensitivity to the antimalarials mefloquine and artemisinin. *Mol Biochem Parasitol* 2000 April 30;108(1):13-23.
 - (35) Anderson TJ, Nair S, Qin H et al. Are transporter genes other than the chloroquine resistance locus (*pfcr1*) and multidrug resistance gene (*pfmdr*) associated with antimalarial drug resistance? *Antimicrob Agents Chemother* 2005 June;49(6):2180-8.
 - (36) Cowman AF, Galatis D, Thompson JK. Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the *pfmdr1* gene and cross-resistance to halofantrine and quinine. *Proc Natl Acad Sci U S A* 1994 February 1;91(3):1143-7.
 - (37) Rojas-Rivero L, Gay F, Bustos MD et al. Mefloquine-halofantrine cross-resistance in *Plasmodium falciparum* induced by intermittent mefloquine pressure. *Am J Trop Med Hyg* 1992 September;47(3):372-7.
 - (38) Pradines B, Rogier C, Fusai T, Tall A, Trape JF, Doury JC. In vitro activity of artemether against African isolates (Senegal) of *Plasmodium falciparum* in comparison with standard antimalarial drugs. *Am J Trop Med Hyg* 1998 March;58(3):354-7.
 - (39) Harinasuta T, Bunnag D, Lasserre R. Quinine resistant *falciparum* malaria treated with mefloquine. *Southeast Asian J Trop Med Public Health* 1990 December;21(4):552-7.
 - (40) Gupta S, Thapar MM, Wernsdorfer WH, Bjorkman A. In vitro interactions of artemisinin with atovaquone, quinine, and mefloquine against *Plasmodium falciparum*. *Antimicrob Agents Chemother* 2002 May;46(5):1510-5.
 - (41) Watt G, Long GW, Padre LP, Alban P, Sangalang R, Ranoa CP. Chloroquine and quinine: a randomized, double-blind comparison of efficacy and side effects in the treatment of *Plasmodium falciparum* malaria in the Philippines. *Trans R Soc Trop Med Hyg* 1988;82(2):205-8.
 - (42) de Vries PJ, Bich NN, Van Thien H et al. Combinations of artemisinin and quinine for uncomplicated *falciparum* malaria: efficacy and pharmacodynamics. *Antimicrob Agents Chemother* 2000 May;44(5):1302-8.
 - (43) Bich NN, de Vries PJ, Van Thien H et al. Efficacy and tolerance of artemisinin in short combination

-
- regimens for the treatment of uncomplicated falciparum malaria. *Am J Trop Med Hyg* 1996 October;55(4):438-43.
- (44) Pukrittayakamee S, Chotivanich K, Chantra A, Clemens R, Looareesuwan S, White NJ. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. *Antimicrob Agents Chemother* 2004 April;48(4):1329-34.
- (45) McGready R, Brockman A, Cho T et al. Randomized comparison of mefloquine-artesunate versus quinine in the treatment of multidrug-resistant falciparum malaria in pregnancy. *Trans R Soc Trop Med Hyg* 2000 November;94(6):689-93.
- (46) Pukrittayakamee S, Chantra A, Vanijanonta S, Clemens R, Looareesuwan S, White NJ. Therapeutic responses to quinine and clindamycin in multidrug-resistant falciparum malaria. *Antimicrob Agents Chemother* 2000 September;44(9):2395-8.
- (47) Metzger W, Mordmuller B, Graninger W, Bienzle U, Kremsner PG. High efficacy of short-term quinine-antibiotic combinations for treating adult malaria patients in an area in which malaria is hyperendemic. *Antimicrob Agents Chemother* 1995 January;39(1):245-6.
- (48) Mueller MS, Runyambo N, Wagner I, Borrmann S, Dietz K, Heide L. Randomized controlled trial of a traditional preparation of *Artemisia annua* L. (Annual Wormwood) in the treatment of malaria. *Trans R Soc Trop Med Hyg* 2004 May;98(5):318-21.
- (49) Ache A, Escorihuela M, Vivas E et al. In vivo drug resistance of falciparum malaria in mining areas of Venezuela. *Trop Med Int Health* 2002 September;7(9):737-43.
- (50) Rahman MR, Paul DC, Rashid M et al. A randomized controlled trial on the efficacy of alternative treatment regimens for uncomplicated falciparum malaria in a multidrug-resistant falciparum area of Bangladesh--narrowing the options for the National Malaria Control Programme? *Trans R Soc Trop Med Hyg* 2001 November;95(6):661-7.
- (51) Segal HE, Chinvanthananond P, Laixuthai B et al. Preliminary study of WR 33063 in the treatment of falciparum malaria in northeast Thailand. *Am J Trop Med Hyg* 1974 July;23(4):560-4.
- (52) Vanijanonta S, Chantra A, Phophak N, Chindanond D, Clemens R, Pukrittayakamee S. Therapeutic effects of chloroquine in combination with quinine in uncomplicated falciparum malaria. *Ann Trop Med Parasitol* 1996 June;90(3):269-75.
- (53) Looareesuwan S, Vanijanonta S, Viravan C et al. Randomised trial of mefloquine-tetracycline and quinine-tetracycline for acute uncomplicated falciparum malaria. *Acta Trop* 1994 June;57(1):47-53.
- (54) Karbwang J, Na-Bangchang K, Thanavibul A, Bunnag D, Chongsuphajaisiddhi T, Harinasuta T. Comparison of oral artesunate and quinine plus tetracycline in acute uncomplicated falciparum malaria. *Bull World Health Organ* 1994;72(2):233-8.
- (55) Kremsner PG, Zotter GM, Feldmeier H, Graninger W, Rocha RM, Wiedermann G. A comparative trial of three regimens for treating uncomplicated falciparum malaria in Acre, Brazil. *J Infect Dis* 1988 December;158(6):1368-71.

-
- (56) de Souza JM, Sheth UK, de Oliveira RM, Roulet H, de Souza SD. An open, randomized, phase III clinical trial of mefloquine and of quinine plus sulfadoxine-pyrimethamine in the treatment of symptomatic falciparum malaria in Brazil. *Bull World Health Organ* 1985;63(3):603-9.

8. RECOMMENDATIONS

This NDA is approvable with respect to Microbiology pending an accepted version of the label.

Kalavati Suvarna
Microbiologist, HFD-590

CONCURRENCES:

HFD-590/Deputy Dir _____ Signature _____ Date

HFD-590/Micro TL _____ Signature _____ Date

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MICROBIOLOGIST

Shukal Bala
8/2/05 02:01:32 PM
MICROBIOLOGIST