

## AMPLIFICATION OF QUININE CARDIAC EFFECTS BY THE RESISTANCE-REVERSING AGENT PROCHLORPERAZINE IN FALCIPARUM MALARIA

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**Abstract.** The use of reversing agents to overcome drug resistance is a potential new treatment strategy for both malaria and cancer. Laboratory studies have raised questions about the safety of this therapeutic approach, but data in humans are lacking. We therefore assessed the toxic potential of reversing agent therapy in Thai patients receiving quinine (17 mg/kg given over 4.5 hr) for falciparum malaria by serial measurements of the QT<sub>c</sub> interval, an electrocardiographic (ECG) marker of the effect of quinine. Six patients were randomly assigned to receive intravenous quinine alone while another six received one intramuscular injection of 12.5 mg of the reversing agent prochlorperazine (PC; compazine®, stemetil®) 2.5 hr after the quinine infusion had begun. Compared with baseline values at 2.5 hr, there was prolongation of the QT<sub>c</sub> interval 30, 60, 90, and 120 min after PC was injected ( $P < 0.05$ ) but no further lengthening with quinine alone ( $P > 0.2$ ). Prochlorperazine alone did not lengthen the QT<sub>c</sub> interval in six healthy volunteers. Neither total nor free quinine plasma levels increased after PC was injected, suggesting that ECG changes may have been due to PC-induced intracellular accumulation of quinine. Although only minor quinine ECG effects were amplified by the reversing agent PC in this study, resistance-reversing therapy could potentiate more serious drug effects. The possibility that more serious toxic effects could be produced by this therapeutic approach should be investigated further.

Infection with drug-resistant strains of *Plasmodium falciparum* is a growing public health problem in most malaria-endemic areas. Mechanisms of resistance remain poorly understood, but some resistant parasites can rapidly release antimalarial compounds, thereby reducing drug accumulation.<sup>1</sup> Greater drug efflux is one of several features resistant malaria parasites share with drug-resistant cancer cells, in which the efflux is mediated by a transport protein, P-glycoprotein.<sup>2,3</sup>

A new, as yet unproven approach to malaria treatment involves reversing resistance by using calcium antagonists and other reversing agents to inhibit the parasite's drug efflux pump.<sup>4</sup> However, the rationale for reversing agent therapy is perhaps compromised by the fact that normal human tissues contain P-glycoprotein,<sup>3,5</sup> which may pump harmful substances out of cells. If so, a reversing agent could concentrate a co-administered antimalarial drug not only within the parasite but also within normal host tissue. For example, three different reversing agents increased

intracellular concentrations of chloroquine in cultured hepatocytes.<sup>6</sup> It has also been observed that when mice were given the anticancer compound vincristine with the reversing agent verapamil, vincristine tissue levels were increased, with more deaths following the combination than in mice given vincristine alone.<sup>7</sup>

We therefore investigated the possible toxicity of reversing agent treatment by measuring delays in myocardial repolarization in patients receiving quinine for falciparum malaria. Such delays, manifested as a prolonged QT<sub>c</sub> interval on the electrocardiogram (ECG), occur commonly at therapeutic plasma quinine levels but are not associated with cardiac or other serious adverse effects.<sup>8-10</sup> A prolongation of the QT<sub>c</sub> interval is therefore an objective, safe measure of quinine effect. Serial QT<sub>c</sub> interval determinations were made in patients receiving quinine alone and compared with values obtained in patients receiving a combination of quinine and prochlorperazine (PC; compazine®, stemetil®). Prochlorperazine is commonly used to control vomiting

in patients with malaria and has reversing agent properties.<sup>11</sup> Our intent was to determine if PC would amplify the electrocardiographic effects of quinine.

#### PATIENTS AND METHODS

Volunteer patients with uncomplicated falciparum malaria who required parenteral therapy were entered into the study after giving written informed consent. Prospective patients who by history were likely to have taken antimalarials that would still be present in the blood were excluded. Study subjects were randomly assigned to receive one of two regimens by a computer-generated random numbers list. The first regimen (Q) was a constant rate infusion of quinine dihydrochloride given as 7 mg/kg of body weight for 30 min followed by 10 mg/kg for 4 hr.<sup>12</sup> The second regimen (QP) included an identical infusion of intravenous quinine but, in addition, 12.5 mg of PC was given intramuscularly 2.5 hr after the quinine infusion had begun. A control group of healthy Thai male volunteers received a single intramuscular injection of 12.5 mg of PC alone.

Preinfusion ECGs were performed and repeated at 30-min intervals. The QT<sub>c</sub> interval was measured without knowledge of the patient's drug regimen from the lead showing greatest T-U distinction at a paper speed of 50 mm/sec. The QT<sub>c</sub> intervals were corrected for rate<sup>13</sup> and the mean of five measurements was recorded. Patients were questioned every hour about quinine side effects. Clinical status and vital signs were assessed every 30 min.

For the measurement of quinine concentrations, plasma was obtained from each patient at 0, 2.5, 3.0, 3.5, 4.0, and 4.5 hr after drug administration. The amount of free (non-protein bound) quinine was determined by ultrafiltration using a micropartition system with YMT membranes (Amicon Division, Beverly, MA). Plasma and ultrafiltrate samples were assayed for quinine by high-performance liquid chromatography (HPLC) using fluorescence detection.<sup>14</sup> Plasma specimens for PC analysis detection were taken every half-hour for 2 hr after the drug was given, protected from light, and stored at -70°C. The PC levels were measured by HPLC with electrochemical detection.<sup>15</sup>

Clinical and laboratory findings on admission were compared using the Student's *t*-test for nor-

mally distributed values and the Mann-Whitney U test for values not normally distributed. The QT<sub>c</sub> intervals at 3.0, 3.5, 4.0, and 4.5 hr were compared using the paired Student's *t*-test to the QT<sub>c</sub> interval at 2.5 hr (the time when PC was administered to the QP group). The Spearman's rank test was used to correlate QT<sub>c</sub> intervals with plasma PC levels. Two-tailed tests of significance were calculated in all cases.

#### RESULTS

The initial mean  $\pm$  SD falciparum parasitemia was  $16,413 \pm 4,423/\text{mm}^3$  of blood in the Q group and  $16,637 \pm 4,487$  in the QP group ( $P > 0.1$ ). There were no significant differences between treatment groups in the initial QT<sub>c</sub> interval, age, body weight, or hematologic indices. Biochemical parameters were also comparable except that mean serum albumin levels were higher in the Q group than in the QP group ( $4.4 \pm 0.1$  and  $3.9 \pm 0.1$  g/dl, respectively;  $P < 0.05$ ). Individual albumin levels, however, were all within the normal range.

The QT<sub>c</sub> interval increased to a median of 106% of the preinfusion value 2.5 hr after beginning treatment with quinine (range 103–111%). At 2.5 hr, half the malaria patients received intramuscular PC and half did not; the quinine infusion was continued in both groups. Compared with baseline values at 2.5 hr, there was statistically significant further QT<sub>c</sub> prolongation 30, 60, 90, and 120 min after injection of PC in the QP group ( $P < 0.05$ ; Figure 1a). The QT<sub>c</sub> interval did not change in the Q group ( $P > 0.2$ ; Figure 1a) and became significantly shorter 60, 90, and 120 min after injection of PC in the six healthy volunteers who received this drug alone ( $P < 0.05$ ; Figure 1a).

The prolongations of myocardial repolarization in the QP group could not be explained by a PC-quinine interaction that affected either total or free quinine plasma levels (Figures 1b and c, respectively). The concentration-time curves for total and free quinine in the QP patients were similar to those in the Q group, and quinine concentration curves differed from the QT<sub>c</sub> curves (Figure 1). Similarly, plasma levels of PC did not correlate with QT<sub>c</sub> intervals during the 2-hr period following injection of PC ( $P > 0.2$ ; Table 1).

No cardiovascular or other side effects occurred in either the 12 malaria patients or the

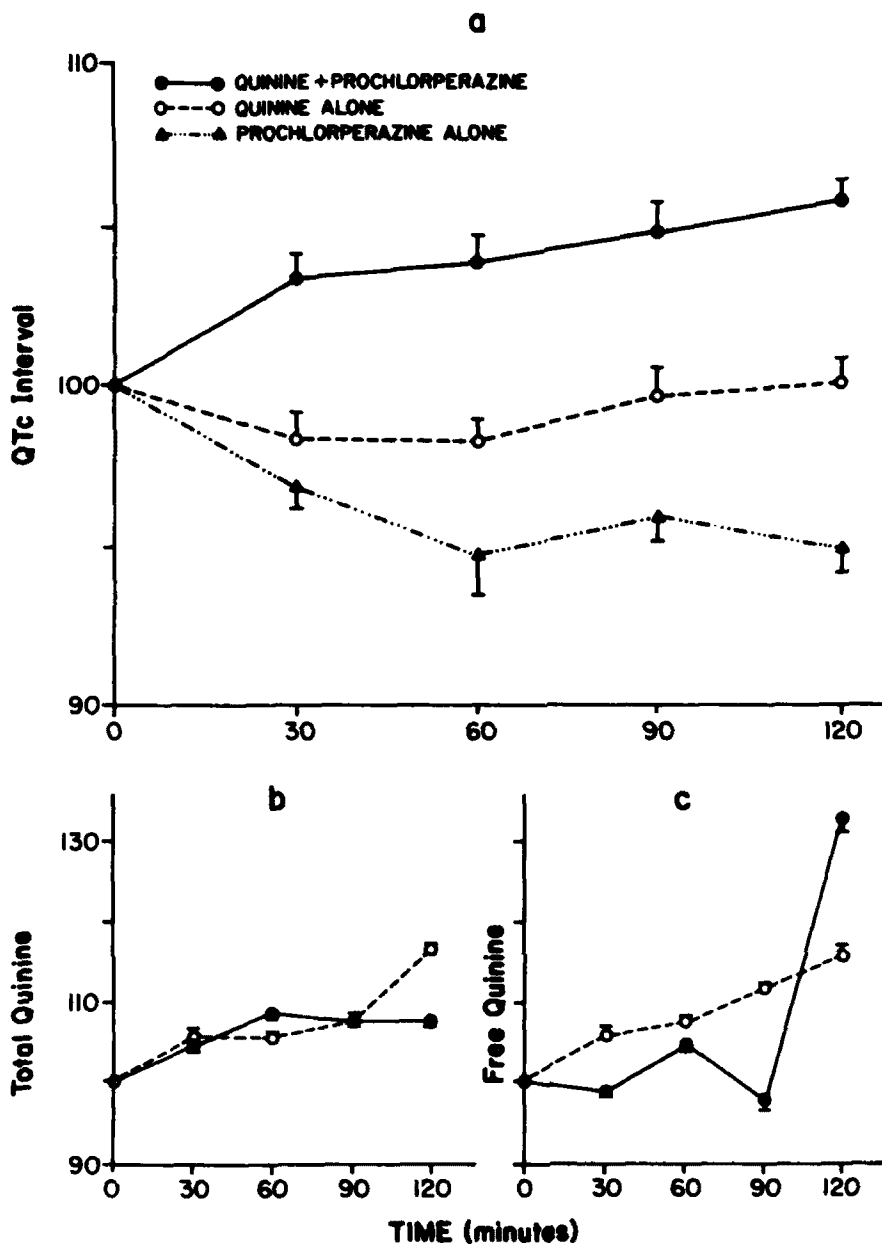


FIGURE 1. a, Changes in the  $QT_c$  interval after treatment with either quinine and prochlorperazine (group QP), quinine alone (group Q) or prochlorperazine alone (group P). b, total quinine plasma levels. c, free quinine plasma levels. Bars are the percentage of a baseline value and 95% confidence interval determined after 2.5 hr of quinine infusion (groups QP and Q) or immediately before injection (group P). Note prolongation of the  $QT_c$  interval in the group QP only (a) that is not explainable by changes in quinine levels (b and c).

six healthy volunteers. The  $QT_c$  intervals lengthened but even our highest recorded value (360 msec) was well below the upper limit of normal values (440 msec). Standard treatment with oral quinine (650 mg per os every 8 hr) and tetracycline (250 mg per os four times a day) was begun as soon as patients were able to tolerate oral medication and produced clinical and parasitologic cures in every case.

#### DISCUSSION

The rationale for the use of reversing agents in malaria centers on similarities between multidrug resistance in *P. falciparum* infection and that in tumor cells, where resistance is mediated by a transport protein product of the multidrug-resistance (*mdr1*) gene.<sup>2,3</sup> There is evidence both for and against a role for *mdr*-like genes in re-

TABLE I

Relationship between plasma prochlorperazine (PC) levels and QT<sub>c</sub> intervals in six patients receiving PC and quinine

	Minutes after PC			
	30	60	90	120
Median plasma PC level, ng/ml (range)	4.3 (2.1-5.9)	3.8 (2.3-5.4)	4.2 (2.1-5.4)	4.0 (1.9-4.6)
QT <sub>c</sub> interval (% of value before injection of PC)	105	108	107	107
Correlation coefficient (r)*	0.77	0.14	0.09	0.43
Significance of correlation (P)	0.2	0.5	0.8	0.2

\* Spearman's rank correlation between the QT<sub>c</sub> interval and the plasma PC level.

sistant *P. falciparum* infection.<sup>16, 17</sup> The possible harmful effects of reversing agent therapy are of concern in both malaria and neoplasia, but are difficult to assess in cancer patients. When compared with individuals being treated for malaria, oncology patients are generally older, are receiving more toxic therapeutic agents, and have multiple medical problems.

The results of our study extend observations from in vitro and animal work, which suggest that reversing agent therapy may be unsafe.<sup>6, 7</sup> Prochlorperazine clearly amplified the delays in myocardial repolarization that commonly accompany quinine treatment. The mechanism(s) by which ECG changes were potentiated by PC cannot be stated with certainty. Quinine levels did not increase after PC was injected (Figure 1b). Serum albumin levels were lower in the QP group, but more biologically active unbound drug was not the explanation for QT<sub>c</sub> interval lengthening in these patients. The QP group generally had lower, not higher, levels of free quinine (Figure 1c);  $\alpha_1$  acid glycoprotein, not albumin, is the major protein that binds quinine.<sup>18</sup>

It is unlikely that PC by itself produced the ECG changes. The QT<sub>c</sub> interval lengthening is a prominent toxic effect of some phenothiazines, particularly piperidine phenothiazines such as thioridazine.<sup>19</sup> Piperazine phenothiazines, such as PC, are not noted for cardiac effects. The PC levels in plasma were not correlated with QT<sub>c</sub> interval duration (Table 1) and PC given alone to healthy Thai controls shortened rather than lengthened myocardial repolarization (Figure 1a).

How did the administration of PC with quinine lead to lengthening of the QT<sub>c</sub> interval? One possibility is that PC slows the extrusion of quinine from cardiac tissue, perhaps by inhibition of an export pump such as P-glycoprotein.

Prochlorperazine reversed quinine resistance in cultured falciparum parasites (Kyle D, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, unpublished data), presumably by inhibition of the parasite drug pump. Another possibility is that PC potentiates quinine effects on cardiac cell membranes. Quinine, like quinidine, is thought to lengthen the duration of myocardial repolarization by influencing action potentials of cells in the cardiac conduction system,<sup>20</sup> and has been shown to alter cell membrane electrical conductivity.<sup>21</sup>

We measured a benign ECG effect of quinine as a marker of potential toxicity, and observed that quinine co-administered with PC prolonged the QT<sub>c</sub> interval. The theoretical concern that inhibition of P-glycoprotein might increase the toxicity of drugs to normal tissues<sup>22</sup> is now supported by clinical evidence. Although methods may be found for overcoming the toxicity of individual reversing agents,<sup>23</sup> concerns about their potentiation of the effects of co-administered compounds should be investigated further. Such concerns are timely since clinical trials of reversing-agent therapy have now begun.<sup>24, 25</sup>

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## REFERENCES

- Krogstad DJ, Gluzman IY, Kyle DE, Oduola AMJ, Martin SK, Milhous WK, Schlesinger PH, 1987. Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. *Science* 238: 1283-1285.
- Fojo AT, Akiyama S, Gottesman MM, Pastan I, 1985. Reduced drug accumulation in multiply drug-resistant human KB carcinoma cell lines. *Cancer Res* 45: 3002-3007.
- Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I, 1987. Expression of a multidrug resistance gene in human tumors and tissues. *Proc Natl Acad Sci USA* 84: 265-269.
- Martin SK, Oduola AMJ, Milhous WK, 1987. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. *Science* 235: 899-901.
- Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham M, 1987. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissue. *Proc Natl Acad Sci USA* 84: 7735-7738.
- Watt G, Long GW, Grogl M, Martin SK, 1990. Reversal of drug-resistant falciparum malaria by calcium antagonists: potential for host cell toxicity. *Trans R Soc Trop Med Hyg* 84: 187-190.
- Horton JK, Thimmaiah KN, Houghton JA, Horowitz ME, Houghton PJ, 1989. Modulation by verapamil of vincristine pharmacokinetics and toxicity in mice bearing human tumor xenografts. *Biochem Pharmacol* 38: 1727-1736.
- White NJ, Looareesuwan S, Warrell DA, 1983. Quinine and quinidine: a comparison of EKG effects during the treatment of malaria. *J Cardiovasc Pharmacol* 5: 173-175.
- White NJ, Looareesuwan S, Warrell DA, Warrell MJ, Chanthavanich P, Bunnag D, Harinasuta T, 1983. Quinine loading dose in cerebral malaria. *Am J Trop Med Hyg* 32: 1-5.
- White NJ, Looareesuwan S, Warrell DA, Warrell MJ, Chanthavanich P, Bunnag D, Harinasuta T, 1982. Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria. *Am J Med* 73: 564-572.
- Ganapathi R, Grabowski D, Turinic R, Valenzuela R, 1984. Correlation between potency of calmodulin inhibitors and effects on cellular levels and cytotoxic activity of doxorubicin (adriamycin) in resistant P388 mouse leukemia cells. *Eur J Cancer Clin Oncol* 20: 799-806.
- Davis TME, Supanaranond W, Plakritayakamee S, Karbwang J, Molunto P, Mekton S, White NJ, 1990. A safe and effective consecutive-infusion regimen for rapid quinine loading in severe falciparum malaria. *J Infect Dis* 161: 1305-1308.
- Moss AJ, 1986. Prolonged QT-interval syndromes. *JAMA* 256: 2985-2987.
- Edstein MD, Prasitthipayong A, Sabcharoen A, Chongsuphaisiddhi T, Webster HK, 1990. Simultaneous measurement of quinine and quinidine in human plasma, whole blood, and erythrocytes by high-performance liquid chromatography. *Ther Drug Monit* 12: 493-500.
- Fowler A, Taylor WB, Bateman DN, 1986. Plasma prochlorperazine assay by h.p.l.c. with electrochemical detection. *J Chromatogr* 380: 202-205.
- Foote SJ, Kyle DE, Martin RK, Oduola AMJ, Forsyth K, Kemp DJ, Cowman AF, 1990. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. *Nature* 345: 255-258.
- Wellems TE, Panton LJ, Gluzman IY, do Rosario VE, Gwadz RW, Walker-Jonah A, Krogstad DJ, 1990. Chloroquine resistance not linked to mdr-like genes in a *Plasmodium falciparum* cross. *Nature* 345: 253-255.
- Mihaly GW, Hyman KM, Smallwood RA, 1987. High-performance liquid chromatographic analysis of quinine and its diastereoisomer quinidine. *J Chromatogr* 415: 177-182.
- Burda CD, 1968. Electrocardiographic abnormalities induced by thioridazine (Mellaril). *Am Heart J* 76: 153-156.
- Mason JW, Winkle RA, Rider AK, Stinson EB, Harrison DC, 1977. The electrophysiologic effects of quinidine in the transplanted human heart. *J Clin Invest* 59: 481-489.
- Aceti A, Bonincontro A, Cametti C, Celestino D, Leri O, 1990. Electrical conductivity of human erythrocytes infected with *Plasmodium falciparum* and its modification following quinine therapy. *Trans R Soc Trop Med Hyg* 84: 671-672.
- Anonymous, 1989. Multidrug resistance in cancer. *Lancet* ii: 1075-1076.
- Deloron P, Basco LK, Dubois B, Gaudin C, Clavier F, LeBras J, Verdier F, 1991. In vitro and in vivo potentiation of chloroquine against malaria parasites by an enantiomer of amlodipine. *Antimicrob Agents Chemother* 35: 1338-1342.
- Bjorkman A, Willcox M, Kihamia CM, Mahikwano LF, Phillips-Howard PA, Hakansson A, Warhurst D, 1991. Field study of cyproheptadine/chloroquine synergism in falciparum malaria. *Lancet* 336: 59-60.
- Warsame M, Wernsdorfer WH, Bjorkman A, 1992. Lack of effect of desipramine on the response to chloroquine of patients with chloroquine-resistant falciparum malaria. *Trans R Soc Trop Med Hyg* 86: 235-236.

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