1059

Reprint Series
2 December 1988, Volume 242, pp. 1301–1303

SCIENCE

Reversal of Chloroquine Resistance in Malaria Parasite *Plasmodium falciparum* by Desipramine

ALAN J. BITONTI,* ALBERT SJOERDSMA, PETER P. MCCANN, DENNIS E. KYLE, A. M. J. ODUOLA, RICHARD N. ROSSAN, WILBUR K. MILHOUS, AND DAVID E. DAVIDSON, JR.

Reversal of Chloroquine Resistance in Malaria Parasite *Plasmodium falciparum* by Desipramine

ALAN J. BITONTI,* ALBERT SJOERDSMA, PETER P. MCCANN, DENNIS E. KYLE, A. M. J. ODUOLA, RICHARD N. ROSSAN, WILBUR K. MILHOUS, DAVID E. DAVIDSON, JR.

Desipramine and several other tricyclic antidepressant drugs reverse chloroquine resistance in *Plasmodium falciparum* in vitro at concentrations observed in the plasma of human patients treated for depression. Reversal of resistance is associated with increased chloroquine accumulation in the parasite, probably because of inhibition of a putative chloroquine efflux pump. When owl monkeys (*Aotus lemurinus lemurinus*) infected with chloroquine-resistant *Plasmodium falciparum* were treated with chloroquine plus desipramine, their parasitemias were rapidly suppressed. Desipramine was found to be one of the most effective compounds yet described for the reversal of chloroquine resistance both in vitro and in vivo.

CHLOROQUINE, A 4-AMINOQUINOline introduced for the treatment of malaria over 40 years ago (*1*), is highly effective against susceptible strains of *Plasmodium falciparum*; however, chloroquine resistance, which was first reported in 1961 (*2*), now occurs in most geographic regions where malaria is endemic. It is now known that resistant plasmodia accumulate less chloroquine than do susceptible strains (*3*), and recent work shows that this reduced accumulation may be due to rapid efflux of the drug from the resistant parasite. Verapamil, a calcium channel blocker, inhibits this process (*4*) and reverses chloroquine resistance in *P. falciparum* in vitro (*5*). Other calcium antagonists reverse chloroquine resistance in vivo (*6*).

The knowledge that some tricyclic psychotropic drugs have weak intrinsic antimalarial activity (*7*) and are calcium antagonists (*8*) prompted us to study one class of these drugs in combination with chloroquine. We report here that desipramine (Norpramin), as well as other tricyclic antidepressant compounds, reverses chloroquine resistance in two *P. falciparum* strains in vitro at concentrations that occur in the plasma of patients undergoing treatment for depression (*9*). Owl monkeys (*Aotus lemurinus lemurinus*) infected with chloroquine-resistant *P. falciparum* and treated with desipramine plus chloroquine showed rapidly suppressed parasitemias, demonstrating the potential clinical use of this chemotherapeutic approach.

Three strains of *P. falciparum* were used: chloroquine-susceptible West African clone D-6 (*10*) [chloroquine IC_{50} (50% inhibitory concentration) = 7 ng/ml], chloroquine-resistant FCR-3 (*11*) (chloroquine IC_{50} = 70 ng/ml), and the multidrug-resistant Indochina clone W-2 (*10*) (chloroquine IC_{50} = 160 ng/ml). The parasites were maintained in human erythrocytes (type 0+; 6% hematocrit) in vitro in RPMI 1640 medium and 10% human serum (*12*). Drug testing was carried out by following [^{3}H]-hypoxanthine incorporation to measure growth rates of *P. falciparum* in the semiautomated microdilution technique (*13*) in which hematocrits were 1% and starting parasitemias were 0.5%. *P. falciparum* was incubated with test compounds for 48 hours.

Evidence for marked synergism between desipramine and chloroquine against chloroquine-resistant *P. falciparum* (FCR-3) was obtained when we used isobologram analysis (*5*). Quantitative analysis of the efficacy of the desipramine-chloroquine combination was done by constructing dose-response curves for chloroquine in the presence of several fixed concentrations of desipramine. The presence of 20 to 500 ng of desipramine per milliliter caused a shift of the dose-response curves to the left, showing that desipramine produced reversal of chloroquine resistance in the multidrug-resistant Indochina W-2 clone (Fig. 1A). Similar results were obtained with the FCR-3 strain. Desipramine did not change the response of the chloroquine-sensitive West African clone D-6 to chloroquine (Fig. 1B). Desipramine alone at 500 ng/ml did not inhibit the growth of *P. falciparum* (FCR-3) by more than 20 to 30% (IC_{50} > 4000 ng/ml). A number of antidepressant drugs were analyzed in this manner, and the concentrations of drug that would lower the chloroquine IC_{50} by 50% and 80% were determined (Table 1).

Desipramine increased the accumulation of [^{3}H]chloroquine by approximately ten times in clone W-2 and approximately three

Table 1. Reversal of chloroquine resistance with antidepressant drugs. The parasites were incubated with serial twofold dilutions of chloroquine known to produce well-defined dose-response curves along with fixed concentrations of antidepressant drugs. [^{3}H]Hypoxanthine incorporation was used as an indicator of antimalarial effects in vitro. The values represent the mean concentrations of antidepressant drugs that cause either a 50% or an 80% decrease in the IC_{50} of chloroquine in at least two experiments in which the results were within 30% of the mean.

Drug	Concentration of drugs needed for reversal of chloroquine resistance (ng/ml)			
	West African FCR-3 strain		Indochina W-2 clone	
	50%	80%	50%	80%
Desipramine	40	150	35	120
Protriptyline	30	80	50	160
Imipramine	30	130	50	150
Nortriptyline	40	120	80	200
Doxepin	70	150	35	140
Amoxapin	120	320	360	>500
Maprotiline	165	500	280	>500
Mianserin	350	>500	>500	>500
Trazodone	>500	>500	>500	>500

A. J. Bitonti, A. Sjoerdsma, P. P. McCann, Merrell Dow Research Institute, Cincinnati, OH 45215.
D. E. Kyle, A. M. J. Oduola, W. K. Milhous, D. E. Davidson, Jr., Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC 20307.
R. N. Rossan, Gorgas Memorial Laboratory, Panama, APO Miami, FL 34002-0012.

*To whom correspondence should be addressed.

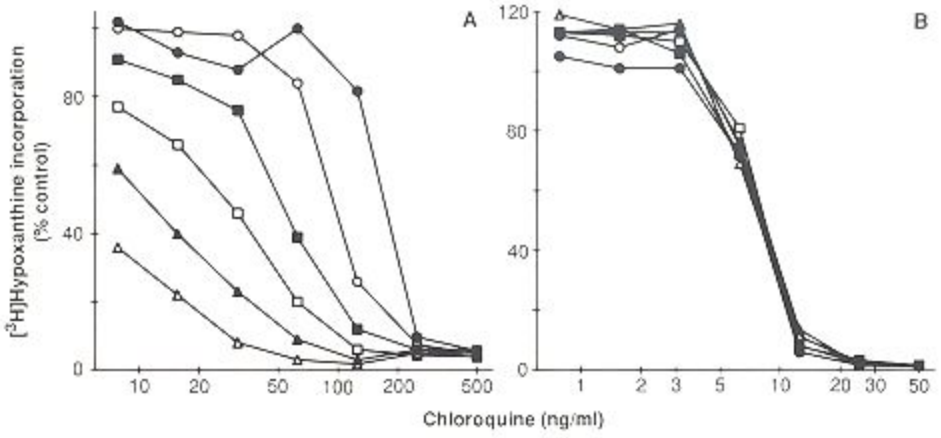

Fig. 1. Desipramine increases sensitivity to chloroquine in resistant *P. falciparum* clone W-2 (**A**) but not in susceptible *P. falciparum* clone D-6 (**B**). Dose-response curves for chloroquine were done in the absence (●) or presence of 20 (○), 50 (■), 100 (□), 200 (▲), or 500 (△) ng of desipramine per milliliter. Incubations at each drug concentration were run in duplicate.

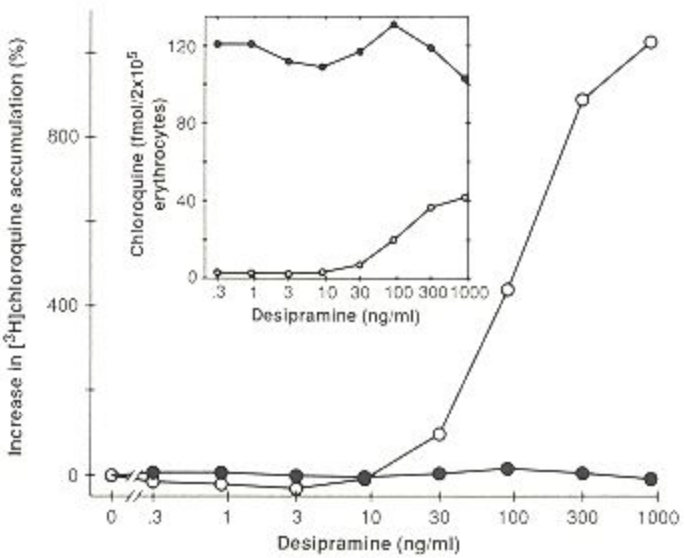

Fig. 2. Desipramine increases accumulation of [^{3}H]chloroquine by resistant *P. falciparum* clone W-2 (○) but not in susceptible *P. falciparum* clone D-6 (●). Parasitized erythrocytes (2×10^5 in 0.1 ml) were incubated in RPMI 1640 medium containing 10% human serum with 5 n*M* [^{3}H]chloroquine (15.3 Ci/mmol) and several concentrations of desipramine for 60 min at 37°C. Erythrocytes were then centrifuged through oil and prepared for scintillation counting as described (*23*). The inset shows the actual amounts of chloroquine accumulated in the susceptible D-6 and resistant W-2 clones. In the absence of desipramine, clone W-2 took up 3.7 fmol of chloroquine per 2×10^5 parasitized erythrocytes whereas clone D-6 took up 113 fmol of chloroquine per 2×10^5 parasitized erythrocytes. Similar results were obtained in three separate experiments.

times in FCR-3, but had no effect on chloroquine accumulation by the chloroquine-susceptible clone D-6 (Fig. 2). Thus, there is a direct correlation between the degree of chloroquine resistance and the effects of desipramine on chloroquine accumulation, since clone W-2 is more resistant to chloroquine inhibition than is the FCR-3 strain (20-fold resistance versus 10-fold resistance, respectively). Although desipramine increased chloroquine accumulation by clone W-2 back toward the level of accumulation measured in clone D-6 (Fig. 2, inset), full restoration of chloroquine accumulation did not occur during the 60 min of incubation.

Although it is clear that exposure of *P. falciparum* to desipramine caused an increased chloroquine accumulation as was shown before with verapamil (*4*), the molecular mechanism was not elucidated. However, there is a parallel between the action of desipramine in drug-resistant *P. falciparum* and the action of verapamil in some multidrug-resistant cancer cells. The multidrug-resistant phenotype in cancer cells is linked to the expression of a 150- to 170-kD protein in the plasma membrane, which is known as the P-glycoprotein (*14*). This protein is thought to act as a putative efflux pump and to mediate an outward transport process which limits intracellular levels of many drugs to sublethal levels. Drugs that reverse multidrug-resistance in cancer cells generally bind to the P-glycoprotein (*15*) and may directly inhibit its function. Alternative mechanisms for the synergism between desipramine and chloroquine may include: (i) interference with the function of calmodulin (*8*), which seems to be involved in *P. falciparum* merozoite invasion of erythrocytes (*16*), (ii) interaction with membrane phospholipids (*17*), and (iii) lysosomotropic effects (*18*).

We next evaluated the effects of desipramine plus chloroquine on owl monkeys (*Aotus lemurinus lemurinus*) (*19*) that had been inoculated with 5×10^6 trophozoites of the Vietnam Smith strain of *P. falciparum*. Five days after inoculation they were treated with chloroquine alone or chloroquine plus desipramine for 3 days. In the monkeys that received chloroquine alone the parasitemias persisted whereas in the monkeys treated with chloroquine and desipramine the parasites became undetectable (Table 2). Recrudescence occurred after the 3-day course of

Table 2. Clearance of chloroquine-resistant *P. falciparum* in owl monkeys by chloroquine plus desipramine. The monkeys were inoculated with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Five days after inoculation, parasitemias were confirmed in the test animals and drug treatments were begun. Treatments (per kilogram of body weight, administered by gavage) were as follows: monkey 1, no treatment; monkey 2, 20 mg of chloroquine once per day for 3 days; monkey 3, 20 mg of chloroquine once per day for 5 days; and monkeys 4 and 5, 20 mg of chloroquine once per day for 3 days and simultaneously, 25 mg of desipramine, three times per day for 3 days. Monkeys 4 and 5 received an initial 50 mg of desipramine, but this dose was not well tolerated by monkey 4 and we decreased the desipramine dose to 25 mg/kg for the remaining treatments for both monkeys. Parasitemias were enumerated daily in Giemsa-stained blood films. The data show the number parasites ($\times 10^{-3}$) per microliter of blood.

Day	Monkey 1 (control)	Chloroquine		Chloroquine + desipramine	
		Monkey 2	Monkey 3	Monkey 4	Monkey 5
		Before treatment			
0	2	1	2	1	0.9
		Treatment			
1	35	33	51	22	3
2	28	43	57	10	2
3	131	24	92	0.3	0.06
		After treatment			
4	444	22	228	0.09	<0.01
5	321	75	117	<0.01	0
6	419	43	197	0	0
7	649	434	302	0	0
8	568	265	321	<0.01	0
Day of recrudescence				Day 8	Day 14

treatment with chloroquine plus desipramine; 7 days of treatment with chloroquine is usually necessary to cure owl monkeys infected with chloroquine-susceptible *P. falciparum* (*20*). Therapy was stopped in this initial experiment after 3 days because of the striking reduction in parasitemia.

Although the doses of desipramine used in these studies appear to be high in comparison to the doses used in humans, the plasma desipramine concentrations were assumed to be in the same range of those seen with conventional doses of desipramine in humans because of a marked difference in plasma kinetics between monkeys and humans. In rhesus monkeys, a single oral dose of 25 mg of desipramine per kilogram yielded peak plasma concentrations of 50 ng/ml with a half-life of about 5 to 6 hours (*21*). Thus, steady-state plasma concentrations with three times daily administration of 25 mg of desipramine per kilogram (total daily dose, 75 mg/kg) would probably have been somewhat higher than 50 ng/ml. In humans, 0.4 mg of desipramine per kilogram given orally three times daily (total daily dose, 1.2 mg/kg) yielded a mean steady-state concentration of 42.7 ng/ml with a half-life of 18 hours (*22*). Detailed pharmacokinetic and toxicology studies must be conducted before desipramine can be used for the clinical treatment of chloroquine-resistant malaria. However, our present data suggest that the dose needed to obtain clearance of parasites in humans may be a small fraction of the dose administered to monkeys in this study, making clinical use of desipramine for treatment of malaria a realistic possibility.

REFERENCES AND NOTES

1. R. F. Loeb *et al.*, *J. Am. Med. Assoc.* **130**, 1069 (1946).
2. D. V. Moore and J. E. Lanier, *Am. J. Trop. Med. Hyg.* **10**, 5 (1961).
3. P. B. Macomber *et al.*, *Science* **152**, 1374 (1966); C. D. Fitch, *ibid.* **169**, 289 (1970).
4. D. J. Krogstad *et al.*, *ibid.* **238**, 1283 (1987).
5. S. K. Martin, A. M. J. Oduola, W. K. Milhous, *ibid.* **235**, 899 (1987).
6. W. K. Milhous *et al.*, paper presented at the 3rd International Conference on Malaria and Babesiosis, Annecy, France (1987).
7. P. Dutta, L. Siegel, J. Pinto, S. Meshnick, *Clin. Res.* **34**, 202 (1986); J. E. Kristiansen and S. Jepsen, *Acta Pathol. Microbiol. Immunol. Scand. Sect. B* **93**, 249 (1985).
8. C. H. Reynolds and P. T. J. Claxton, *Biochem. Pharmacol.* **31**, 419 (1982).
9. J. Amsterdam, D. Brunswick, J. Mendels, *Am. J. Psychiatry* **137**, 6 (1980).
10. A. M. J. Oduola, N. F. Weatherly, J. H. Bowdre, R. E. Desjardins, *Exp. Parasitol.* **66**, 86 (1988).
11. J. B. Jensen and W. Trager, *Am. J. Trop. Med. Hyg.* **27**, 743 (1978).
12. W. Trager and J. B. Jensen, *Science* **193**, 673 (1976).
13. R. E. Desjardins, C. J. Canfield, J. D. Haynes, J. D. Chulay, *Antimicrob. Agents Chemother.* **16**, 710 (1979).
14. R. L. Juliano and V. Ling, *Biochim. Biophys. Acta* **455**, 152 (1976); W. T. Beck, *Biochem. Pharmacol.* **36**, 2879 (1987).
15. A. R. Safa *et al.*, *J. Biol. Chem.* **262**, 7884 (1987).
16. Y. Matsumoto, G. Perry, L. W. Scheibel, M. Aikawa, *Eur. J. Cell Biol.* **45**, 36 (1987).
17. K. Kanno and Y. Sasaki, *Biochem. Pharmacol.* **31**, 2977 (1982).
18. U. E. Honegger, A. A. Roscher, U. N. Wiesmann, *J. Pharmacol. Exp. Ther.* **225**, 436 (1983).
19. R. N. Rossan *et al.*, *Am. J. Trop. Med. Hyg.* **34**, 1037 (1985).
20. L. H. Schmidt, *Trans. R. Soc. Trop. Med. Hyg.* **67**, 446 (1973).
21. T. R. Insel, E. A. Lane, M. Sheinin, M. Linnoila, *Eur. J. Pharmacol.* **136**, 63 (1987).
22. B. Alexanderson, *Eur. J. Clin. Pharmacol.* **5**, 1 (1972).
23. P. Stahl, P. H. Schlesinger, E. Sigardson, J. S. Rodman, Y. C. Lee, *Cell* **19**, 207 (1980).

24. We thank R. C. Ursillo for discussions and review of the manuscript and J. A. Dumont and T. L. Bush for technical assistance.

29 July 1988; accepted 18 October 1988