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Antimalarial Agents: Mechanism of Chloroquine Resistance

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INTRODUCTION

The increasing prevalence of antimalarial resistance has emphasized our lack of understanding about this phenomenon. Many *Plasmodium falciparum* isolates from South America, Southeast Asia, and Africa are now resistant to chloroquine and to pyrimethamine-sulfadoxine (Fansidar) (2–4, 21). In contrast, all *P. vivax*, *P. ovale*, and *P. malariae* isolates are chloroquine susceptible (2). We examine here the present understanding of chloroquine resistance.

MECHANISM OF CHLOROQUINE RESISTANCE

Studies by many investigators, beginning with those of Fitch (8, 9), have shown that chloroquine-resistant *P. falciparum* accumulates less chloroquine than susceptible parasites (12, 18). This observation suggests that chloroquine resistance in *P. falciparum* results from either decreased uptake or increased excretion of the drug by the resistant parasite (24a). However, the mechanisms responsible for this difference in chloroquine accumulation and for chloroquine resistance have been unknown. The available epidemiologic data suggest that chloroquine resistance in Africa may have been imported from Southeast Asia in the late 1970s. However, it is not clear whether there are any potential epidemiologic links between chloroquine resistance in Southeast Asia and the resistance which appeared earlier in South America. It is possible that most, or all, chloroquine resistance has been selected from one or a few independent genetic events in South America and/or Southeast Asia.

One can envision several mechanisms by which the parasite might become resistant to clinically achievable concentrations of chloroquine. These include (i) a defective concentrating mechanism so that the parasite vesicle concentrates no more chloroquine than the vesicles of mammalian cells (i.e., loss of the non-weak base effect) (18a, 18b), (ii) a permeability barrier which reduces chloroquine accumulation within the vesicle (analogous to the altered porins observed in members of the family *Enterobacteriaceae*) (16), (iii) an enzyme which inactivates chloroquine (analogous to β-lactamases or aminoglycoside-modifying enzymes) (22), and (iv) an altered target site (an acid vesicle system which functions normally even when intravesicular pH is raised above 6.0).

Studies which demonstrate that susceptible and resistant parasites initially accumulate chloroquine at the same rate (28 to 29 fmol/10^6 parasitized erythrocytes per min) (18) suggest that the chloroquine-concentrating mechanism is the same in susceptible and resistant parasites. Chloroquine released from resistant parasites migrates to the same position on a thin-layer chromatogram as chloroquine which has not been exposed to parasitized erythrocytes (15). This result suggests that the resistant parasite does not have a chloroquine-inactivating enzyme. Studies with simple monoprotic weak bases, such as NH_4Cl, indicate that the growth of both chloroquine-susceptible and -resistant parasites is inhibited by raising intravesicular pH (19). These studies suggest that the acid vesicle system of the parasite does not function normally when its pH has been raised and that the acid vesicle system of the resistant parasite has not adapted to function at an intravesicular pH ≥6.0.

Although the studies described above did not define the mechanism of chloroquine resistance, our recent studies (performed in collaboration with investigators at the Walter Reed Army Institute of Research) demonstrate that resistant *P. falciparum* parasites have a mechanism for releasing chloroquine (an efflux process) (18). This efflux is either absent or greatly reduced in the susceptible parasite (Fig. 1). The fact that the initial rate of chloroquine accumulation is the same in resistant and susceptible parasites (18) suggests that efflux causes the observed difference in steady-state chloroquine accumulation and that it may be the only significant difference between resistant and susceptible parasites. Although there may be more than one mechanism of chloroquine resistance in *P. falciparum*, the rapid efflux phenotype has now been observed in isolates from Southeast Asia, South America, and Africa. Thus, this mechanism of resistance is present in each of the three continents with chloroquine-resistant *P. falciparum*.

These studies suggest that chloroquine resistance in *P. falciparum* has some similarities to multi-drug resistance in mammalian cancer cells (1, 6, 10) (Table 1). Those cells rapidly release anticancer drugs to which they are resistant, such as daunomycin and vinblastine. Calcium-channel

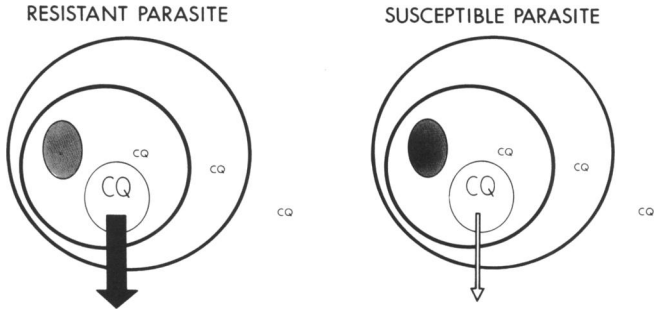

FIG. 1. Efflux of chloroquine from resistant and susceptible parasites. The resistant *P. falciparum* parasite releases chloroquine 40- to 50-fold more rapidly than the susceptible parasite (18). This difference in their rates of chloroquine release is consistent with the differences in their steady-state accumulations of chloroquine and in their chloroquine 50% effective doses. These data suggest that the critical difference between resistant and susceptible parasites may be the rate at which they release chloroquine.

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TABLE 1. Parallels between chloroquine resistance in *P. falciparum* and multi-drug resistance in mammalian cancer cells[a]

Characteristic	Effect on:	
	P. falciparum	Mammalian cancer cells
Drug efflux from resistant cells	Enhanced efflux of chloroquine	Enhanced efflux of daunomycin and vinblastine
Effect of resistance phenotype on drug accumulation	Less accumulation of chloroquine	Less accumulation of daunomycin and vinblastine
Effect of calcium-channel blockers	Inhibition of efflux, resulting in greater accumulation of chloroquine	Inhibition of efflux, resulting in greater accumulation of daunomycin and vinblastine

[a] Based on data from references 10, 18, and 20.

blockers inhibit drug efflux from resistant cells and increase their accumulation of (and susceptibility to) daunomycin and vinblastine. In *P. falciparum*, calcium-channel blockers, daunomycin, and vinblastine inhibit the efflux (and enhance the accumulation) of chloroquine from (by) the resistant parasite (18). Viewed from this perspective, these results suggest that calcium-channel blockers, daunomycin, and vinblastine may inhibit chloroquine efflux by binding to a site in the resistant parasite similar in function to the P-glycoprotein of multi-drug-resistant mammalian cancer cells (5, 7, 11, 13, 17, 23–26). This analogy is consistent with the effects of calcium-channel blockers and chloroquine against otherwise chloroquine-resistant *P. falciparum*. The seminal studies of Martin et al. (20) showed that the combination of verapamil (a calcium-channel blocker) plus chloroquine inhibited the growth of otherwise resistant *P. falciparum*. Taken together with the ability of calcium-channel blockers to inhibit chloroquine efflux, these results suggest that inhibition of chloroquine efflux produces increased chloroquine accumulation, which enhances the effect of chloroquine against the resistant parasite.

Alternatively, one could hypothesize that chloroquine efflux from resistant *P. falciparum* is dependent on intracellular calcium and is unrelated to proteins similar in function to the P-glycoprotein of mammalian cancer cells. Chloroquine efflux is inhibited by several calcium-channel blockers and by daunomycin (which may function as a calcium-channel blocker in some systems) (14). The mechanism by which alterations in intracellular calcium might influence chloroquine efflux is not clear, nor does this hypothesis explain the inhibitory effect of vinblastine on chloroquine efflux.

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LITERATURE CITED

1. **Ames, G. F.-L.** 1986. The basis of multidrug resistance in mammalian cells: homology with bacterial transport. Cell **47:** 323–324.
2. **Centers for Disease Control.** 1985. Revised recommendations for preventing malaria in travelers to areas with chloroquine-resistant *Plasmodium falciparum*. Morbid. Mortal. Weekly Rep. **34:** 185–195.
3. **Centers for Disease Control.** 1986. Need for malaria prophylaxis by travelers to areas with chloroquine-resistant *Plasmodium falciparum*. Morbid. Mortal. Weekly Rep. **35:**21–27.
4. **Centers for Disease Control.** 1987. Chloroquine-resistant *Plasmodium falciparum* in West Africa. Morbid. Mortal. Weekly Rep. **36:**13–14.
5. **Chen, C.-J., J. E. Chin, K. Ueda, D. P. Clark, I. Pastan, M. M. Gottesman, and I. B. Roninson.** 1986. Internal duplication and homology with bacterial transport proteins in the **mdr1** (P-glycoprotein) gene from multidrug-resistant human cells. Cell **47:**381–389.
6. **Cornwell, M. M., M. M. Gottesman, and I. H. Pastan.** 1986. Increased vinblastine binding to membrane vesicles from multidrug-resistant KB cells. J. Biol. Chem. **261:**7921–7928.
7. **Cornwell, M. M., I. H. Pastan, and M. M. Gottesman.** 1987. Certain calcium channel blockers bind specifically to multidrug-resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. J. Biol. Chem. **262:**2166–2170.
8. **Fitch, C. D.** 1970. *Plasmodium falciparum* in owl monkeys: drug resistance and chloroquine-binding capacity. Science **169:**289–290.
9. **Fitch, C. D.** 1973. Chloroquine-resistant *Plasmodium falciparum*: difference in the handling of ^{14}C-amodiaquin and ^{14}C-chloroquine. Antimicrob. Agents Chemother. **3:**545–548.
10. **Fojo, A., S.-I. Akiyama, M. M. Gottesman, and I. Pastan.** 1985. Reduced drug accumulation in multiply drug-resistant human KB carcinoma cell lines. Cancer Res. **45:**3002–3007.
11. **Fuqua, S. A. W., I. M. Moretti-Rojas, S. L. Schneider, and W. L. McGuire.** 1987. P-glycoprotein expression in human breast cancer cells. Cancer Res. **47:**2103–2106.
12. **Geary, T. G., J. B. Jensen, and H. Ginsburg.** 1986. Uptake of [^{3}H]chloroquine by drug-sensitive and resistant strains of the human malaria parasite *Plasmodium falciparum*. Biochem. Pharmacol. **35:**3805–3812.
13. **Gerlach, J. H., J. A. Endicott, P. F. Juranka, G. Henderson, F. Sarangi, K. L. Deuchars, and V. Ling.** 1986. Homology between P-glycoprotein and a bacterial hemolysin transport protein suggests a model for multidrug resistance. Nature (London) **324:** 485–489.
14. **Gibbs, C. L.** 1985. Acute energetic effects of daunomycin on heart muscle. J. Cardiovasc. Pharmacol. **7:**556–561.
15. **Gluzman, I. Y., P. H. Schlesinger, and D. J. Krogstad.** 1987. Inoculum effect with chloroquine and *Plasmodium falciparum*. Antimicrob. Agents Chemother. **31:**32–36.
16. **Hancock, R. E. W.** 1987. Role of porins in outer membrane permeability. J. Bacteriol. **169:**929–933.
17. **Kartner, N., D. Evernden-Porelle, G. Bradley, and V. Ling.** 1985. Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. Nature (London) **316:**820–823.
18. **Krogstad, D. J., I. Y. Gluzman, D. E. Kyle, A. M. J. Oduola, S. K. Martin, W. K. Milhous, and P. H. Schlesinger.** 1987. Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. Science **238:**1283–1285.

18a. **Krogstad, D. J., and P. H. Schlesinger.** 1986. A perspective on antimalarial action: effects of weak bases on *Plasmodium falciparum*. Biochem. Pharmacol. **35:**547–552.

18b. **Krogstad, D. J., and P. H. Schlesinger.** 1987. The basis of antimalarial action: non-weak base effects of chloroquine on vesicle pH. Am. J. Trop. Med. Hyg. **36:**213–220.

19. **Krogstad, D. J., P. H. Schlesinger, and I. Y. Gluzman.** 1985. Antimalarials increase vesicle pH in *Plasmodium falciparum*. J. Cell Biol. **101:**2302–2309.
20. **Martin, S. K., A. M. J. Oduola, and W. K. Milhous.** 1987.

Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. Science **235**:899–901.

21. **Miller, L. H., R. J. Howard, R. Carter, M. F. Good, V. Nussenzweig, and R. S. Nussenzweig.** 1986. Research toward malaria vaccines. Science **234**:1349–1356.
22. **Neu, H. C.** 1987. The biochemical basis of antimicrobial and bacterial resistance. Bull. N.Y. Acad. Sci. **63**:295–317.
23. **Riordan, J. R., K. Deuchars, N. Kartner, N. Alon, J. Trent, and V. Ling.** 1985. Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. Nature (London) **316**:817–819.
24. **Roninson, I. B., J. E. Chin, K. Choi, P. Gros, D. E. Housman, A. Fojo, D.-W. Shen, M. M. Gottesman, and I. Pastan.** 1986. Isolation of human **mdr** DNA sequences amplified in multidrug-resistant KB carcinoma cells. Proc. Natl. Acad. Sci. USA **83**: 4538–4542.

24a. **Schlesinger, P. H., D. J. Krogstad, and B. L. Herwaldt.** 1988. Antimalarial agents: mechanisms of action. Antimicrob. Agents Chemother. **32**:793–798.

25. **Scotto, K. W., J. L. Biedler, and P. W. Melera.** 1986. Amplification and expression of genes associated with multidrug resistance in mammalian cells. Science **232**:751–755.
26. **Ueda, K., M. M. Cornwell, M. M. Gottesman, I. Pastan, I. B. Roninson, V. Ling, and J. R. Riordan.** 1986. The **mdr1** gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem. Biophys. Res. Commun. **141**:956–962.